

Examining tutor-tutee interactions and their effect on song learning in Zebra finches



By Darshan Rajesh Dhanajkar

20181039

Indian Institute of Science Education and Research (IISER) Pune

Biology Department

A thesis submitted to

Indian Institute of Science Education and Research Pune

in partial fulfillment of the requirements for the BS-MS Dual Degree Program

Thesis supervisor: Dr. Raghav Rajan

(Indian Institute of Science Education and Research (IISER) Pune)

TAC Expert: Dr. Gaurav Das,

(NCCS Pune.)

Certificate

This is to certify that this dissertation Analysis of father-son interactions and its effect on juvenile song learning in male zebra finch .Here 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 Darshan Rajesh Dhanajkar at Indian Institute of Science Education and Research under the supervision of Dr Raghav Rajan, Assistant Professor, Biology Division, IISER Pune during the academic year 2022-2023.



Name of your Guide

Dr Raghav Rajan

Committee:

Name of your Guide : Dr. Raghav Rajan

Name of Your TAC: Dr Gaurav Das

This thesis is dedicated to Aai ani Pappa

Declaration

I hereby declare that the matter embodied in the report entitled " Analysis of father-son interactions and its effect on juvenile song learning in male zebra finch " are the results of the work carried out by me at the Department of Biology, IISER Pune under the supervision of Dr Raghav Rajan and the same has not been submitted elsewhere for any other degree.

Darshan

Darshan Rajesh
Dhanajkar

Date: 10/04/2022

List of Tables

Table 1: Description of different behaviors	23
Table 2: Amount of tutor's singing and Song similarity value data	27
Table 3 Results of unpaired t-test for the song analysis part.	39

List of Figures

Figure 1 Timeline of various stages of song learning in Zebra finches	11
Figure 2 Song Spectrogram for male zebra finches during song learning	12
Figure 3 Early Experimental Setup	16
Figure 4 Modified Experimental Setup	17
Figure 5 Snapshot of Song Tutoring	19
Figure 6 Box plot: Song Similarity, Consistency, Linearity for experimental and control birds	26
Figure 7 Plot of Amount of tutor's singing vs Song similarity values	27
Figure 8 Raster and histogram plots of "Focus" behavior	29
Figure 9 Raster and histogram plots of "Self cleaning"and "weird body movement (Stretching) "behavior	30
Figure 10 Raster and histogram plots of "Beaks Cluttering"and "Quivering" behavior	31
Figure 11 S1.Snapshot of Pecking sequence	39
Figure 12 S2.Snapshot of Tail Wagging	39
Figure 13 S3.Snapshot of Quivering Behavior	40
Figure 14 S4.Snapshot of Stretching Behavior	41
Figure 15 Raster and Average activity pattern of 'Quivering' Behavior	42

Contents

Abstract	8
Acknowledgments	9
Contributions	10
Chapter 1 Introduction.....	11
Chapter 2 Methods.....	15
2.1 Birds.....	15
2.2 Setup:.....	15
2.3 Experimental Design and Tutoring Session:	18
2.4 Analyses:.....	20
2.4.1 Audio Analysis:	20
2.4.2 Video Analysis (For checking Behavioural Aspect)	23
Chapter 3: Results	25
<i>3.1 Lesser Song similarity, linearity, and consistency in experimental birds than normally reared birds</i>	<i>25</i>
3.2 Behavior Analysis:.....	27
3.2.1 Amount of tutors singing doesn't correlate with Juvniles song learning	27
3.2.2 Some singing events found accompanied by juveniles' focused, quivering, beaks clattering, stretching and self-cleaning behaviors.	28
3.2.4 Juvenile's 'Self Cleaning and Weird Body Movement (Stretching)' activity deepens after tutors' singing begins.	28
3.2.5 An increase in "Beak Cluttering" activity after the tutor's singing begins, as well as an increase in "Quivering" behaviour activity of the Juvenile before and after the tutor's singing begins::.....	29
Chapter 4: Discussion	32
Chapter 5: Conclusion.....	35
References	36
Key Terms	37

Abstract

In neuroscience, zebra finches are used for studying bird vocalizations, which helps us draw parallels with human speech learning (Doupe & Kuhl, 2003). In this songbird species, only the male zebra finch sings as a part of its courtship ritual (Sossinka Böhner 1980). This song is learnt by the juvenile from its father. (Slater et al., 1988) and (Derégnaucourt et al., 2013) explored the effects of different tutors in place of the father as well as the influence of male and female siblings on song learning of young zebra finch. These investigations center on the tutor's preferences and how presence of other birds affect learning in young juvenile zebra finches. Further, different birds show different accuracy of song copying. Thus, it is possible some interaction between the tutor and juvenile influence this copying of song. It's difficult to tutor zebra finch bird using playback through a speaker. One of the reasons for less accurate song copying by the juvenile could be absence of interaction with the tutor which is present in live tutoring. Thus, it is important to quantify interaction between tutor and juvenile during song learning. This might provide reason for difference in learning between live and playback tutoring.

We analyzed the father-son interaction recordings of zebra finches and identified some behaviors. We didn't detect much correlation between the father's singing and the juvenile's total song learning. However, we observed some singing events accompanied by juveniles' quivering, stretching, and self-cleaning behaviors. It appeared that the father's singing was inspired by the little juvenile's "activities."

Acknowledgments

I'd like to thank Dr.Raghav Rajan for his advice, suggestions, and help with my project at Rajan Lab. I'd like to express my gratitude to Dr.Gaurav Das for being my thesis expert and for providing valuable inputs during the mid-year report discussion.

I am grateful to the Rajan Lab members for providing the necessary project support. Thank you to Shikha for assisting me with asking appropriate questions throughout the project. I'd like to thank Shreelekha for assisting me with data collection and modifying the setup during the early stages of the experiments. Further, I'd like to thank Ninad, Samyuktha, Manali, Anand, Sonam, Dhanya and Sheetal for having seemingly unrelated conversations that turned out to be useful along the way. I would like to thank Mr.Prakash Raut for taking care of birds.

I would feel amiss if I were to omit acknowledging Kartik, Ritesh, and Prajwal for all of the late-night conversations that helped me get through difficult times.

Finally, I'd like to express my heartfelt appreciation to my parents for their unwavering love and support throughout this journey and being there through my years of study and the process of research and writing this thesis. This achievement would not have been possible without their assistance. Thank you very much.

Contributions

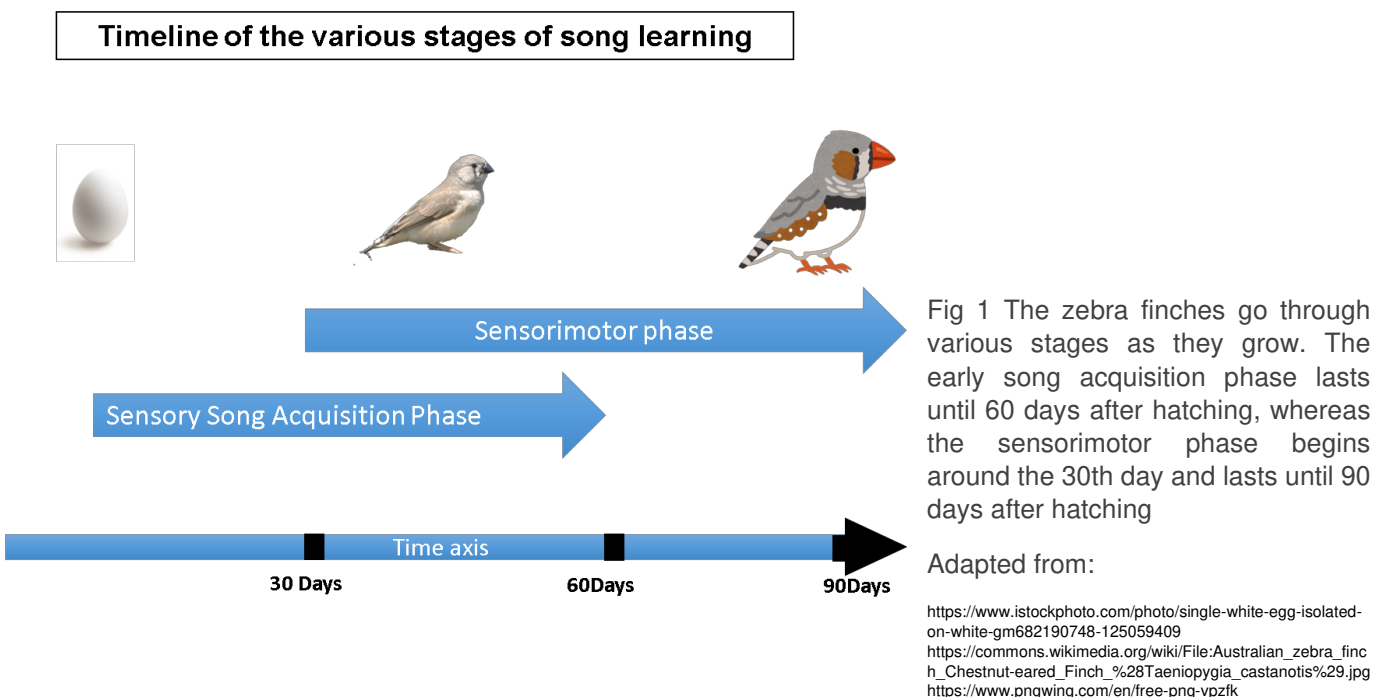
Contributor name	Contributor role
Raghav Rajan, Darshan Dhanajkar	Conceptualization Ideas
Raghav Rajan, Darshan Dhanajkar	Methodology
Raghav Rajan	Software
Raghav Rajan, Darshan Dhanajkar	Validation
Darshan Dhanajkar, Raghav Rajan, Shikha Kalra	Formal analysis
Darshan Dhanajkar, Raghav Rajan, Shikha Kalra	Investigation
Raghav Rajan	Resources
Darshan Dhanajkar, Shreelekha B.S.	Data Curation
Darshan Dhanajkar	Writing - original draft preparation
Darshan Dhanajkar, Raghav Rajan Shikha Kalra	Writing - review and editing
Raghav Rajan, Darshan Dhanajkar	Visualization
Raghav Rajan	Supervision
Raghav Rajan, Darshan Dhanajkar	Project administration
Raghav Rajan	Funding acquisition

Chapter 1 Introduction

Songbirds are frequently studied alongside human vocalizations. Songbird research can provide valuable insights into the neural and behavioural mechanisms that underpin vocal learning, allowing us to better understand human vocalisations. Songbirds are particularly useful model organisms for studying vocal learning because their song learning is similar to human speech learning. (Doupe & Kuhl, 2003)

The zebra finch is the most commonly used songbird for studying vocal development. due to several unique features of their behavior and biology that make them suitable for this area of research. Male zebra finches produce stereotypical songs that are learned and passed on to young zebra finches, providing an excellent model system to study the neural mechanisms underlying vocal learning, memory, and production (Fee & Scharff, 2010; Price, 1979). The relatively small and simple structure of the zebra finch brain facilitates researchers in studying the neural circuits involved in song production and learning. Zebra finches are highly social animals that live in groups and engage in courtship and mating rituals (Peter J.B. Slater, 1988; Zann, 1996). Because this social behaviour is necessary for vocal learning and communication, zebra finches are an excellent model for researching the social context of vocalization.

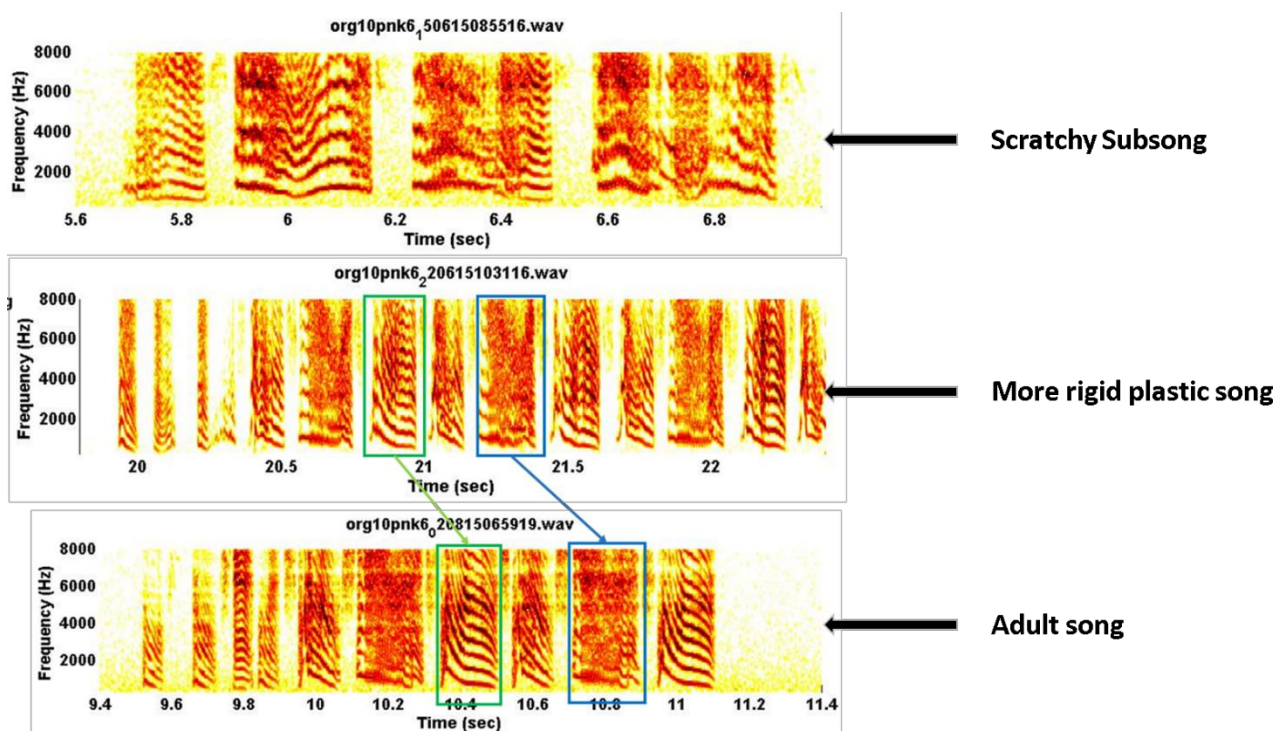
The male juvenile zebra finches learn the song by listening and imitating the tutor's song (CK Catchpole 2008). Young juvenile zebra finches learn the song from an adult



male, preferably their father in the natural case (Zann 1996) , or from another adult male if the father is absent.

Young zebra finches learn songs through sensory song acquisition and sensory-motor phases. The sensory phase lasts 60 days after birth, whereas the sensory-motor phase starts at 30 days and lasts 90 days. The bird is more sensitive to songs in the first 60 days after birth, which means that song exposure up to this point may affect and become fixed in the memory of the young juvenile. As the bird enters the sensorimotor phase, the bird song begins to solidify in the bird's memory. He will repeatedly practice the song until the bird song crystallizes around 90 days. (Kroodsma, 1980 ;Price, 1979; Zann 1996)

As the zebra finches mature, their scratchy subsong evolves and changes, resulting in a repetitive song pattern. Following subsong, the young zebra finches attempt to repeat and recite some of the formed song elements. The newly formed song elements are repeated and kept. These song elements combine to form a plastic song which is characterized by presence of variable syllables. This song slowly changes into a crystallized song. It has a limited repertoire of species-specific song



characteristics(Marler, 1990)

Fig 2.The image depicts the three distinct phases of young bird songs. The uppermost spectrogram shows a scratchy subsong that transitions to a more rigid plastic song (Middle

spectrogram) around 60 days after hatching. The adult crystallised song is shown in the lowermost spectrogram. (Photo credit: Shikha Kalra)

Cross-species song tutoring in songbirds has been studied in various research studies. For example, one study showed that male zebra finches were able to learn songs from a different species, the Bengalese finch, when raised together. However the young zebra finches learnt the structural components of Bengalese finches, but their temporal structure remains zebra finch specific (Makoto Araki 2016). Similarly, female Bengalese finches were able to learn songs from male zebra finches. This suggests that social interactions between different species can facilitate song learning and transfer of information. (Takahasi et al., 2010). One area where our understanding of song transmission could be improved is in determining the relative importance of different interactions in song learning. In order to answer this question, some studies examined the behaviors of zebra finches and discovered that social interactions with adult tutors during development improve song learning. (CK Catchpole, 2008; Eales, 1989; Baptista LF, 1986; Derégnaucourt S, 2013) Juvenile finches that interact with tutors visually and acoustically for the first few months after birth show more significant vocal learning than juveniles who are only passively exposed to the song (Eales 1989). However, there is still debate about how much interaction with the tutor is necessary and which behaviors may be important in juvenile learning.

A study by Sébastien Derégnaucourt (2012) studied the comparisons of different tutoring methods including playing tape recordings, live tutors, and operant conditioning with song as a reward, which can be used to teach the song to young zebra finches. They discovered, however, that birds tutored with playback tape recording learned less than birds tutored with operant conditioning, and birds tutored with operant conditioning learned less than birds tutored with live tutors.

However, recently the researchers tried to tutor the bird with the video playback. A study by Nikhil Phaniraj et al. 2022 and Zdzislaw Galoch et al. 2007, on tablet song tutoring attempted to address the effect of visual tutoring on song learning. In that study, they put an adult male's singing video on a tablet and kept it before the young juvenile zebra finch. However, the results did not show comparable song learning with normally reared birds. I hypothesize that some interactions between the tutor and tutee

are critical. In the tablet tutoring, these interactions would not have been there, resulting in poor learning. Also, when playing on a tablet, such behaviors cannot be in sync. So, to create an interactive model that teaches young birds how to sing, we need at least probabilistic data on how the male bird model should interact with the juvenile.

In this thesis, I investigate the role of various behaviors during father-son interactions. The goal was to determine whether the presence or pattern of certain behaviors was essential for accurate song learning. I hypothesized that some juvenile behaviors are responsible for father singing more and that Juveniles could have certain response to tutor's song. To put this to the test, I used juveniles from different nests who will be tutored by their fathers during a limited number of tutoring sessions(tutoring limited to 10 hours of tutoring over 10 days, 1 hour per day) . The tutoring session was recorded and later examined for different behavioral patterns.

Chapter 2 Methods

The Institute Animal Ethical Committee approved the experimental procedures in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, New Delhi).

2.1 Birds

35 days post-hatched young juveniles were used in the experiment. Before the start of the experiment, they were raised with their mother, the father was removed from the cage 7-10 days after hatching, to avoid exposure to tutor song. To avoid possible visual interaction with other birds, all adult male siblings or potential male tutors were removed from the same cage, and the cage was kept covered from the sides. The lights were programmed to turn on at 6 a.m. and turn off at 8 p.m. The experimental young birds came from various nests. We needed to keep many bird pairs for breeding so that we could get as many male birds as possible for our experiment because we only needed young male birds, and they had to be 35 days old. We kept track of the breeding and noted when eggs were laid or hatched so that we could begin the next experiment after 35 days.

2.2 Setup:

The setup was kept in a sound isolation box to minimize the outside noise. During the experiment, a microphone (AKG Acoustics C417PP omnidirectional condenser microphone; 44000 sampling rate) was attached to the cage, and a GoPro/VivoV20 camera was used to record video (60 frames per second, 1080p resolution).. We can't tell where the bird is from the center when we lay the white sheet on the floor. As a result, we added black-white checkboxes to the cage floors. There is also Deeplabcut (animal pose estimation software) that aids in identifying the animal and marking its movement. For using the Deeplabcut software, the black-white checkbox-like sheet is required for calibration. I intended to use it for calculating the distance between two birds. However, because we were initially more focused on

behavior, we did not analyze it for that purpose. It would be interesting to investigate further in the future.

Early Setup:

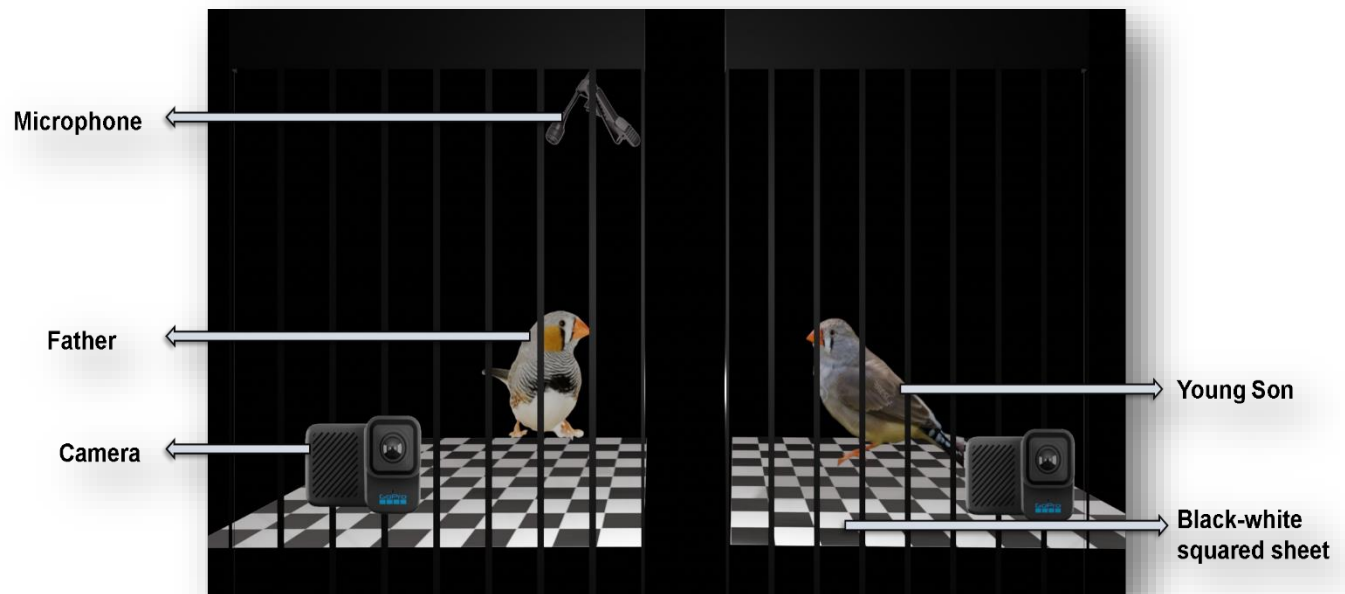


Figure 3 .Early two Cage setup where the father and son were in two separate cages [Father on left and juvenile zebra finch on the right side]

For the first set of birds (3 juveniles), we directly put the tutor cage in front of the tutee cage. The cage bars served as a barrier between the tutor and juvenile, and the two cameras recorded both the tutor and juvenile separately. To facilitate better analysis with BORIS (Behavior Analysis Software that has been used for experimental analysis), the setup was changed so that the Tutor and the juvenile could be seen in the recording window in the same frame. As a result, for the next batch of birds, the two-cage system was replaced with a similar large cage separated from the center by a plastic sheet. This sheet prohibited physical contact, but they could freely see and call/sing to each other. (Sarah Golücke 2019).

Modified Setup:

For the later set of birds (5 Juveniles), we used the big cage separated from centre by plastic sheet to record the experiment.

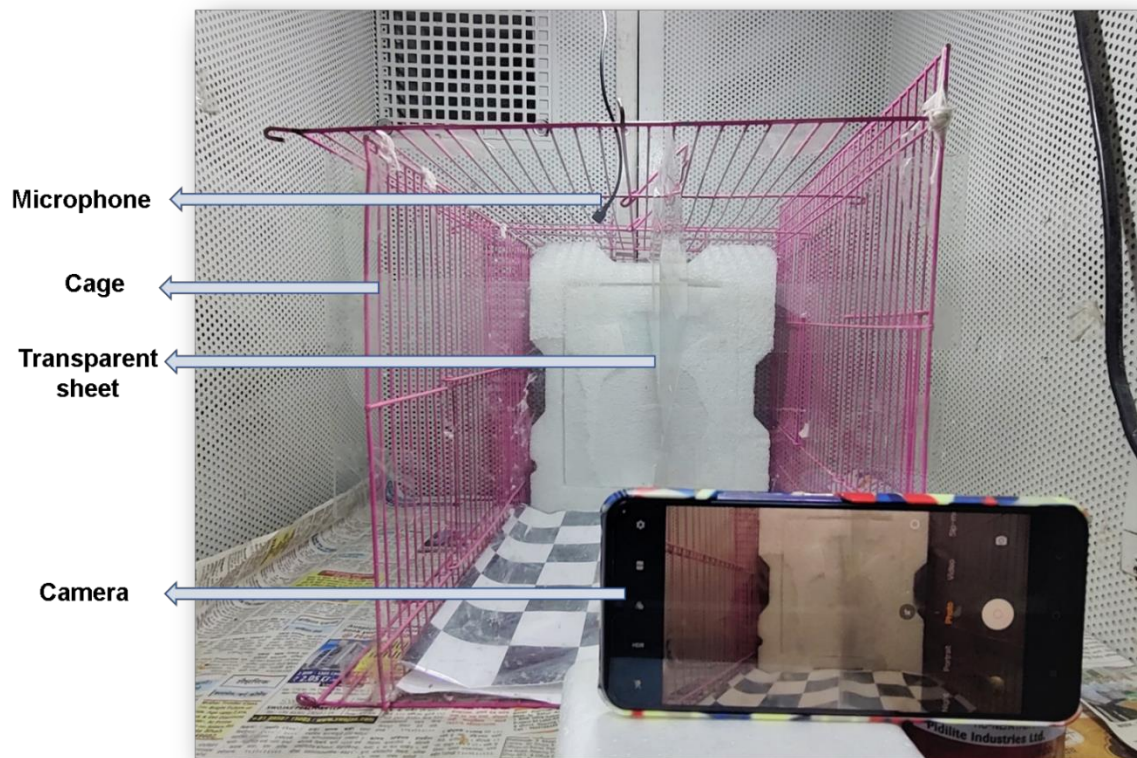
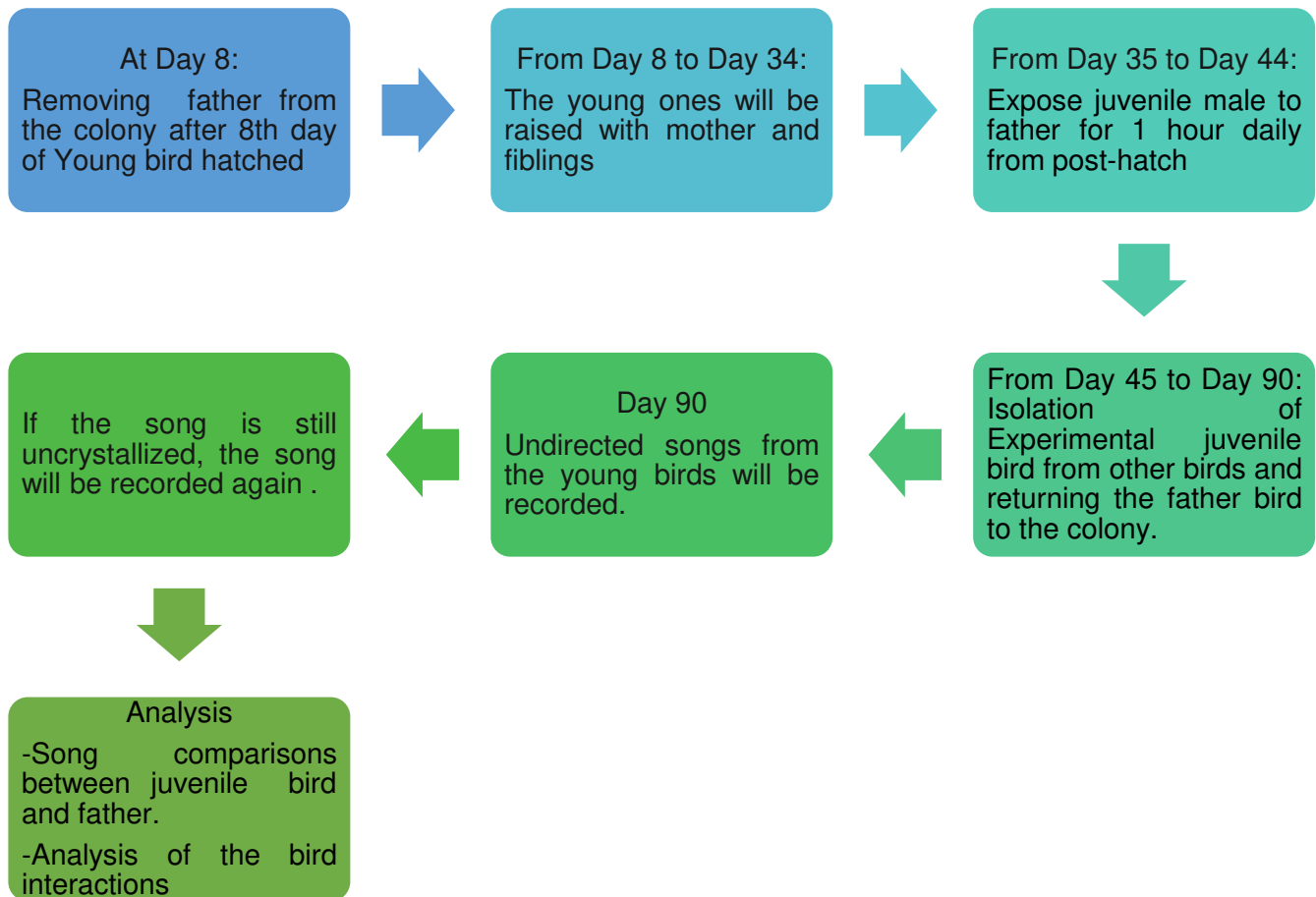


Figure 4. The modified setup where the thin plastic sheet separated the two compartments. The camera is placed right in front of the cage for recording,

2.3 Experimental Design and Tutoring Session:



The experimental session includes one hour of daily live tutoring for each individual. One session was conducted each day over the next ten days. During the session, the juveniles and their fathers were placed in two separate cage compartments. We removed the food and water cups from the setup because we only provided 10 hours of live tutoring, significantly less than what normally reared birds receive. We intended to remove other distractions from the setup, such as food and water, that could slow their interactions. They were visually isolated at the start of the experiment by turning off the lights. We would begin camera recording from inside the cage setup and microphone recording from the outside PC. Then we'd turn on the lights.

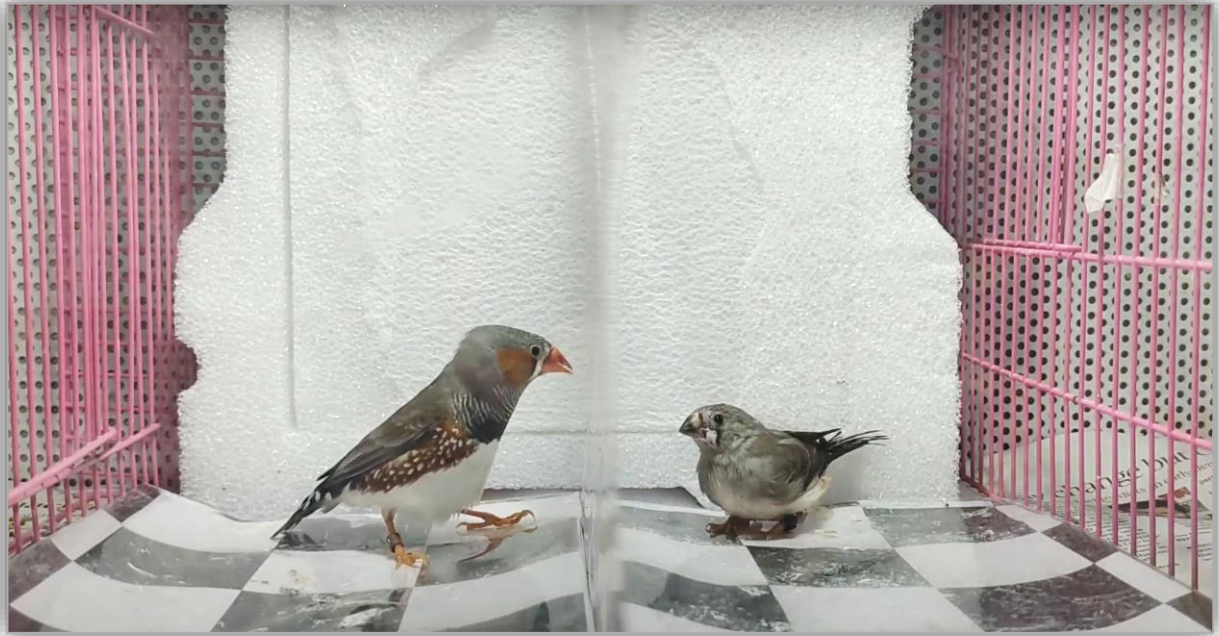


Figure 5. The tutoring by an adult male father to Juvenile Male zebra finch [Father on left side, Juvenile on right side]. The thin line between them is a plastic sheet separating them. The microphone is set at the top of the cage.

After 90 days, the juveniles were audio recorded for the day, and the songs were later screened for song elements. We return the birds to the Avian colony after recording the songs 90-120 days after hatching.

2.4 Analyses:

2.4.1 Audio Analysis:

The recorded experimental session is screened for songs, and some of them are labelled using Matlab-generated programs: Screen Song File Keyboard Modified and Auto Song Segment Labelling (ASSL). Raghav Rajan created and developed this. - Screen Song File Keyboard Modified is used to screen the song files from all the audio data in the session, whereas Auto Song Segment Labelling labels individual syllables within the songs in the given files. We needed two types of audio recordings to analyze for the purposes of our experiment.

a) Audios recorded during the experimental session

We used experimental session audio to determine the quantity of the tutor's song. We wanted to see if there was a link between amount of tutors singing and its effect on young birds' song learning. In addition, we used the father's song from the experimental session to compare with that of the young bird's crystallized song.

b) Audios recorded of Juveniles post 90-120 Days after hatching

Undirected songs were recorded from the Juveniles. Their songs were compared to those of their fathers. Sound Analysis Pro (SAP) software was used to compare the motifs of father and juvenile. (OFER TCHERNICHOVSKI*, A procedure for an automated measurement of song similarity 1999) In the following section of the analysis, we will review the values we used to compare the father and young juvenile's song.

2.4.1.1 Song Comparison Indexes

Normally, reared birds have a song that sounds more like their father. Furthermore, the young ones sing the song linearly, meaning that different syllables will follow a specific pattern. Even if we check this song pattern across different trials, the pattern will be consistent.

We wanted to see if the experimental young zebra fishes followed the same pattern as the normally reared ones. As a result, for the Song Analysis, we used a variety of

indexes such Song Linearity, and Song Consistency to compare with normally reared birds. (Scharff and Nottebohm, 1991) and to compare the juveniles learnt song with their father , I have used song similarity score which will be discussed below. (Note:All three indexes are independent of each other.)

A] Song Similarity Score:

The Song similarity score determines how similar the spectrogram of one motif is to another. Sound Analysis Pro (SAP) was the software we used to calculate song similarity (Ofer Tchernichovski, 2000). The Similarity score is calculated based on the percentage of similarity and accuracy. It will scan through the given song spectrum and look for similar parts in two spectrograms (to calculate the percentage of similarity) before comparing the smaller details piece by piece (Accuracy). This was used to calculate similarity between father's song and juvenile's song.

B] Song Linearity:

Song Linearity (Sl) measures the degree of branching of the song, i.e., whether the bird's song linearly follows a stereotypical sequence. I have provided one example to easily understand the concept.

# Different notes per song	I A B C	I A B C
Song	I A B C I A B C	I A B B C I A B B C
# Transition types per song	I A A B B C C I	I A A B B B B C C I

$$\frac{\text{\# Different notes per song}}{\text{\# Transition types per song}}$$

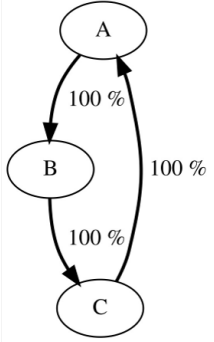
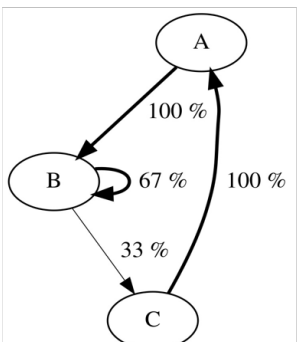
$$\frac{4}{4} = 1$$

$$\frac{4}{5} = 0.8$$

$$\text{Sequence Linearity} = \frac{\text{\# different notes per song}}{\text{\# transition types per song}}$$

C] Song Consistency:

Song Consistency measures how consistent the typical sequence appears for different song trials.

Typical transitions	AB BC CA	AB BB CA
Song	ABCABC	ABBBBCABBBBCABBBBC
All possible transitions	AB BC CA	AB BB BC CA
Transition Probabilities of syllables		

In the table above, we have presented the transition probabilities of syllables. The darker arrows indicate transitions that are more favorable compared to others. These darker arrows represent the typical transitions that will be taken into account when calculating sequence consistency. The song consistency will be calculated via:

$$Sc = \frac{\sum \text{typical transitions per song}}{\sum \text{total transitions per song}}$$

For 1st sequence, [AB,BC,CA] were found 5 times out of total 5 transitions.

So, $Sc=5/5=1$

For 2nd sequence, typical transitions [AB,BB,CA] found 11 times out of total 14 transitions

So, $Sc=11/14=0.78$

2.4.2 Video Analysis (For checking Behavioural Aspect)

BORIS Software is used for video analysis (Friard 2016). This software can identify and tag behavioral events across the timeline. The behaviors were classified into two categories. a) State events and b) point events

We can manually add the event to the timeline by pressing the corresponding code key. For example, pressing the 'b' key initiates the Beaks cluttering behavior, and repressing the same key terminates the behavior. The following behaviors had been labelled by me.

Table 1: Description of different behaviors

Behavior type	Behavior code	Description
State event	Beak Opening	•A yawning-like opening of the beak
State event	Beaks cluttering	•Activity of the fast movement of beaks without any calls or any singing
Point event	Calls	•Occurrence The sharp syllables apart from singing
State event	Continuous Calling	•If the calls of a juvenile or adult bird are continuous. Stop long calls after 2-3 seconds of silence.
State event	Eating	•Only if there is any leftover food in the cage
State event	Facing other cage	•Occurrence The sharp syllables apart from singing
State event	Flying	•flying around and clinging to the cage's side wall
State event	Focused	•Activity showing the timespan after/during the tutor's singing where the Juvenile stopped moving around cages and froze for a while (Behavior specific to juvenile)
Point event	jumping	•Activity of jumping
State event	Love(Seating besides one another)	•Activity where the subject is sitting beside other subject
State event	pecking	•Activity of pecking the screen between them or the cage bars
State event	Quivering	•Shaking the entire body after sitting or standing for an extended period
State event	Resting	•The bird's sitting posture, with no indication of sleep.
State event	running along cages	•Activity by any subject showing the fast jumping and moving along the screen/bars separating both the subjects.
State event	Self-cleaning	•Activity of the cleaning of body and wings by beak pecking
State event	Singing	•Activity of the Tutor to sing a song or juvenile singing a song
State event	Sleeping position	•Activity in resting position (Sleeping)
State event	Tail wagging	•Bird activity of rapidly flicking the tail left and right

State event	Weird body opening movement	•Activity Initially unfamiliar to us, but it's similar to human stretching. (Here, the bird extends his one wing and leg towards one side of the body)
-------------	-----------------------------	--

To quantify different behaviors, we analyzed video recordings at 3 different time points, namely 15 Seconds before the start of the tutor's song, during the tutor's song, and 15 seconds after the end of the tutor's singing.

To check for the relationship between the Song similarity and the amount of tutors singing, we calculated Linear Correlation Coefficient.

$$r = \frac{1}{(n - 1)} \sum \frac{(x_i - \bar{x})}{S_x} \frac{(y_i - \bar{y})}{S_y}$$

Where $\bar{x}$ and S_x are the mean and standard deviation of the x's of sample data and the $\bar{y}$, and S_y are the mean and standard deviation of the y's of sample data

Note: Some snapshots of the behaviours have been added in supplementary

Chapter 3: Results

3.1 Lesser Song similarity, linearity, and consistency in experimental birds than normally reared birds

The birds normally reared with the tutor have a more consistent and linear song. In our experiment, the juvenile had less presence of the tutor (10 hour of social presence) as compared to normal other birds (Usually 3 months). So, to check the effect of our experimental setup on song learning of young zebra finches, I compared the song similarity, linearity, and consistency of our experimental birds with the normally reared birds. I had used the social tutored birds data for these comparisons (Shikha Kalra, 2021)

We performed unpaired t-test analysis on different song indices values. We found the p-value of song similarity and song linearity to be 0.2328 and 0.1270.(Figure 6This shows that there was not significant difference between the experimental and control birds. However, for the song consistency part, there was a significant difference between the values of both the experimental and control birds' song linearity ($p=0.0371$, t-test). Overall, these results showed that song learning in my experimental paradigm (10 hours) was comparable to song learning with a social tutor for 30 days. Importantly, this paradigm gave a wide range of song copying accuracy and this was useful for examining the factors crucial for accurate song learning.

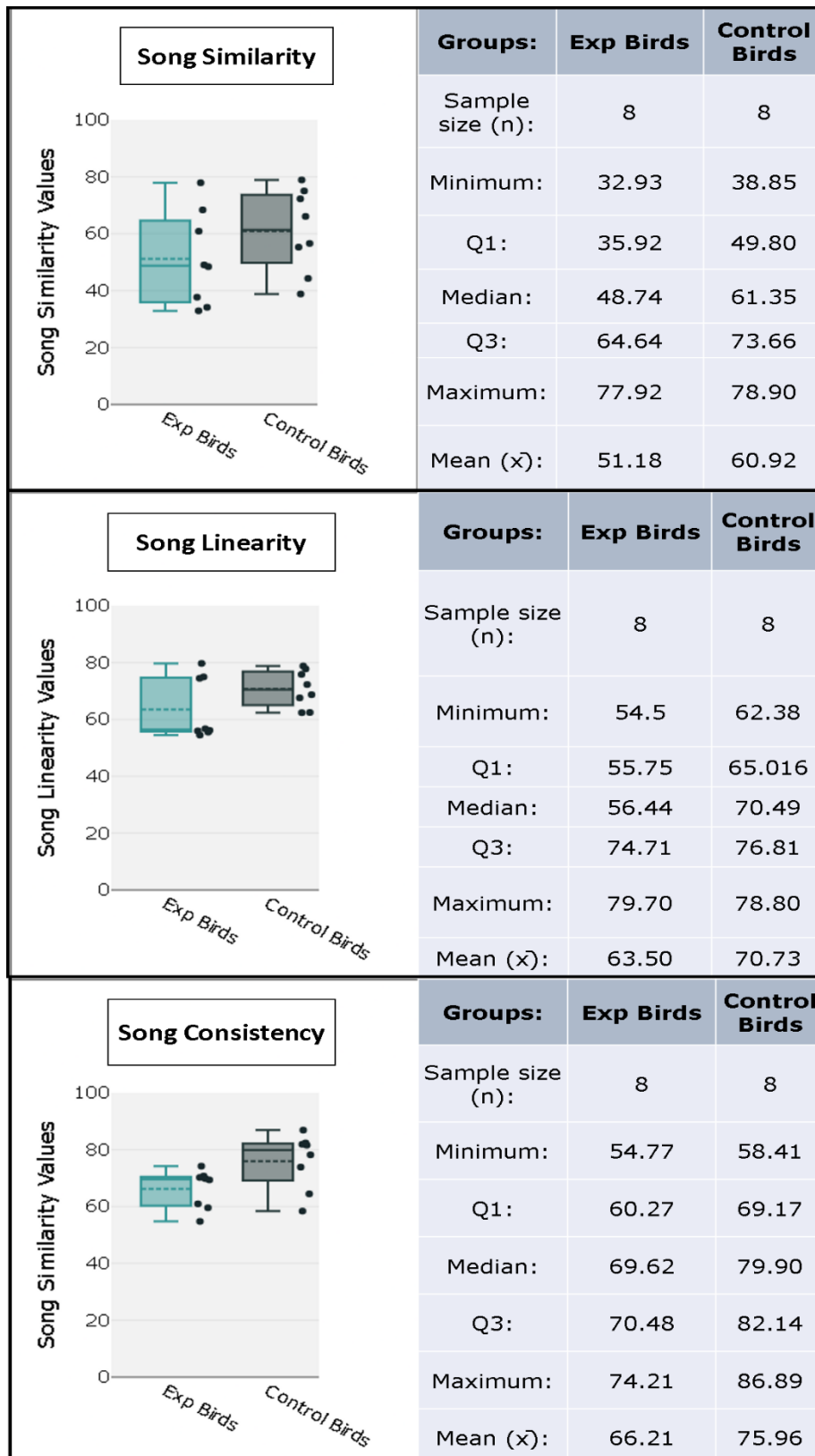


Figure 6. The three box plots demonstrate the comparison of experimental and control birds. Each point represents a bird. The first plot represents song similarity, the middle plot represents song linearity, and the final plot represents song consistency value.Box plots represent values for all birds (n=8 for experimentally tutored birds in this study and n=8 for socially tutored birds from Kalra et al. 2021).The table beside each box plot shows different box plot properties.

3.2 Behavior Analysis:

I wanted to know if there was any pattern that both birds follow around the singing event for the behavior analysis and if any specific juvenile behaviors could influence the adult tutor to sing more.

3.2.1 Amount of tutors singing doesn't correlate with Juvniles song learning

I wanted to check if there was a correlation between the amount of song sung by Tutor and the accuracy of song learning by the juvenile. I have plotted the amount of time singing by the tutors versus the song similarity value of the juvenile.

Table 2: Amount of tutor's singing and Song similarity value data		
BIRD ID	TOTAL DURATION IN MIN	SAP
P48	3.595716667	77.92
P47	0.283266667	68.38
P46	6.6139	60.89
P070	15.77035	49.04587
P44	18.80285	48.44
P069	23.47273333	37.7
B190B191	5.54835	34.14679
B123O97	2.507833333	32.92661

I also calculated the linear correlation value, which was -0.379. The P-Value for it is 0.354492. It showed no correlation between the amount of tutors' songs and song learning by Juvenile birds.

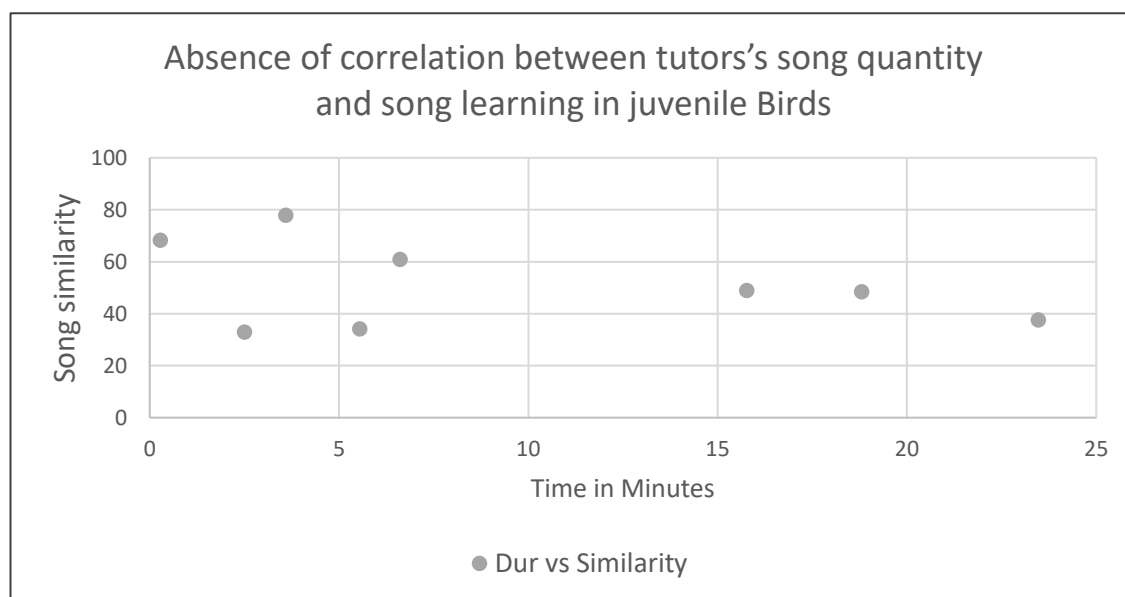


Figure 7. The plot of Amount of singing by the tutor on the X-axis and the Song similarity of the Juvenile on the Y axis

3.2.2 Some singing events found accompanied by juveniles' focused, quivering, beaks cluttering, stretching and self-cleaning behaviors.

To check whether young juvenile's particular behaviour occurs at specific time around the tutor's singing, I plotted the raster plots of particular behaviour and checked for peak or any patterns. I plotted, two graphs for each behavior: One showing the raster plot of occurrences of a behavior for all the singing events for a bird. The white bar graph represents the duration and occurrence of that particular behavior. The 2nd graph below raster plots is the graph of average activity of a behavior across the timeline. (15s before the start of tutor song to approx. 15s after the end of tutor song)

We have checked for the 'Focus', 'Self Cleaning', 'Weird Body movement/Stretching', 'Beaks cluttering' and 'Quivering' behaviors. We got the following results.

3.2.3 Increase in the 'Focus' behavior after Tutors singing onset

The Focus behavior is an attentive behavior shown by the Juvenile. This behavior was marked after the tutors singing onset and if the Juvenile was seen in as 'freez' or no movement. The behavior was marked till it does some other behavior like jumping, pecking, quivering, etc. We observe an increase in 'Focus' behavior just after the onset of tutor's song (Figure 8). This suggests juvenile tries to pay attention to tutor's song.

3.2.4 Juvenile's 'Self Cleaning and Weird Body Movement (Stretching)' activity deepens after tutors' singing begins.

The self cleaning represents the cleaning the body with the beak, pecking the tail/back/stomach, etc. Whereas the weird body movement represents the stretching of the body where the bird will open up his leg and wing sideways. As the tutor sang, we observed the decrease in both of these activities (Figure 9)

3.2.5 An increase in "Beak Cluttering" activity after the tutor's singing begins, as well as an increase in "Quivering" behaviour activity of the Juvenile before and after the tutor's singing begins::

Beak cluttering is the rapid close-open beak movement of the bird. This behavior was shown by the young bird when the Tutor sang the song. The Quivering is the whole body shaking movement of the bird. When plotted against the tutor's singing onset it showed the increase in the activity before and after the singing onset.

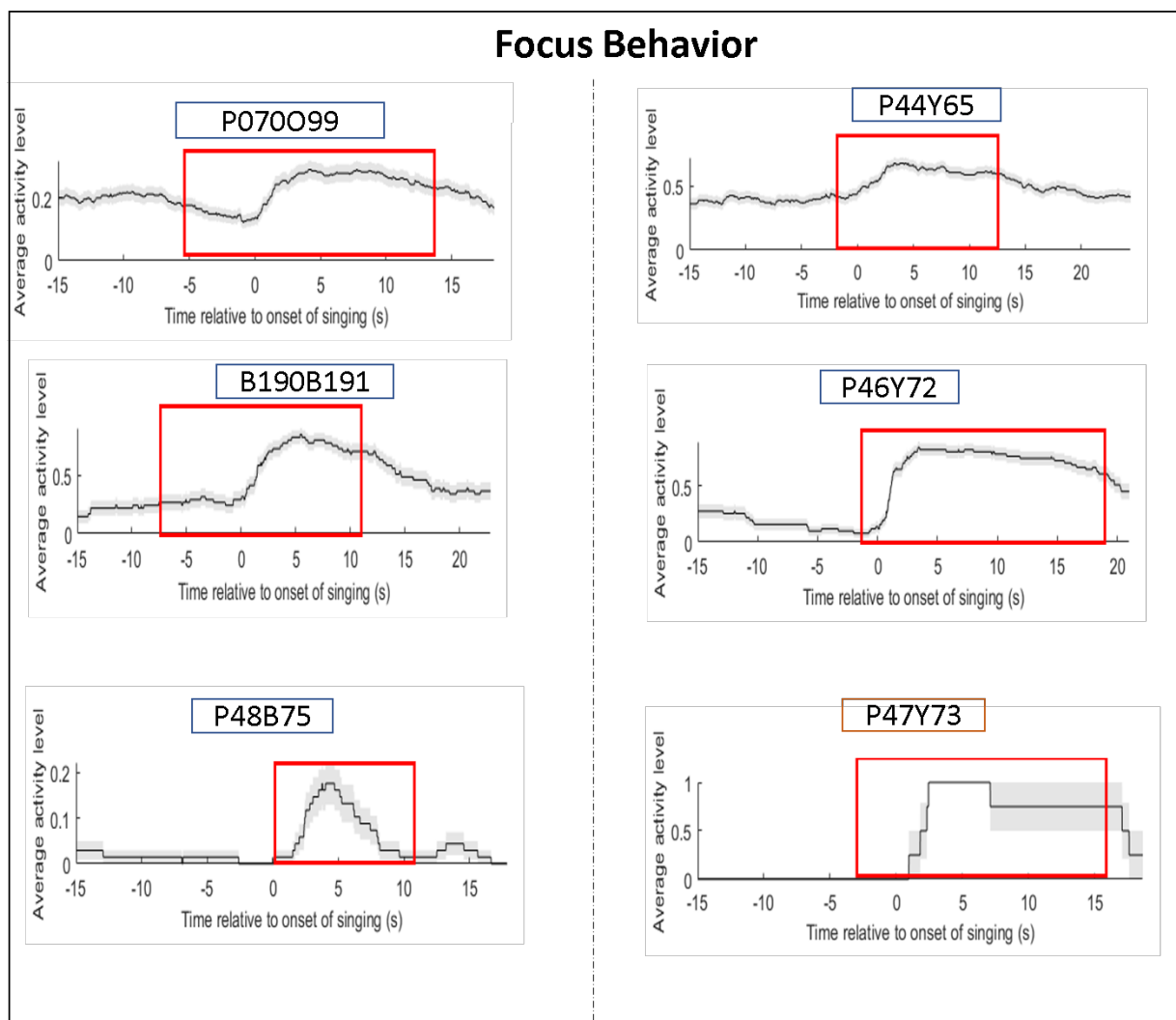


Figure 8 Focus behavior of a juvenile is observed at the onset of tutor song. The average activity patterns for a specific 'Focus' Behavior. Each subfigure represents a different bird. The red rectangle denotes an increase in the activity of the "Focus" behaviour around the start of singing.

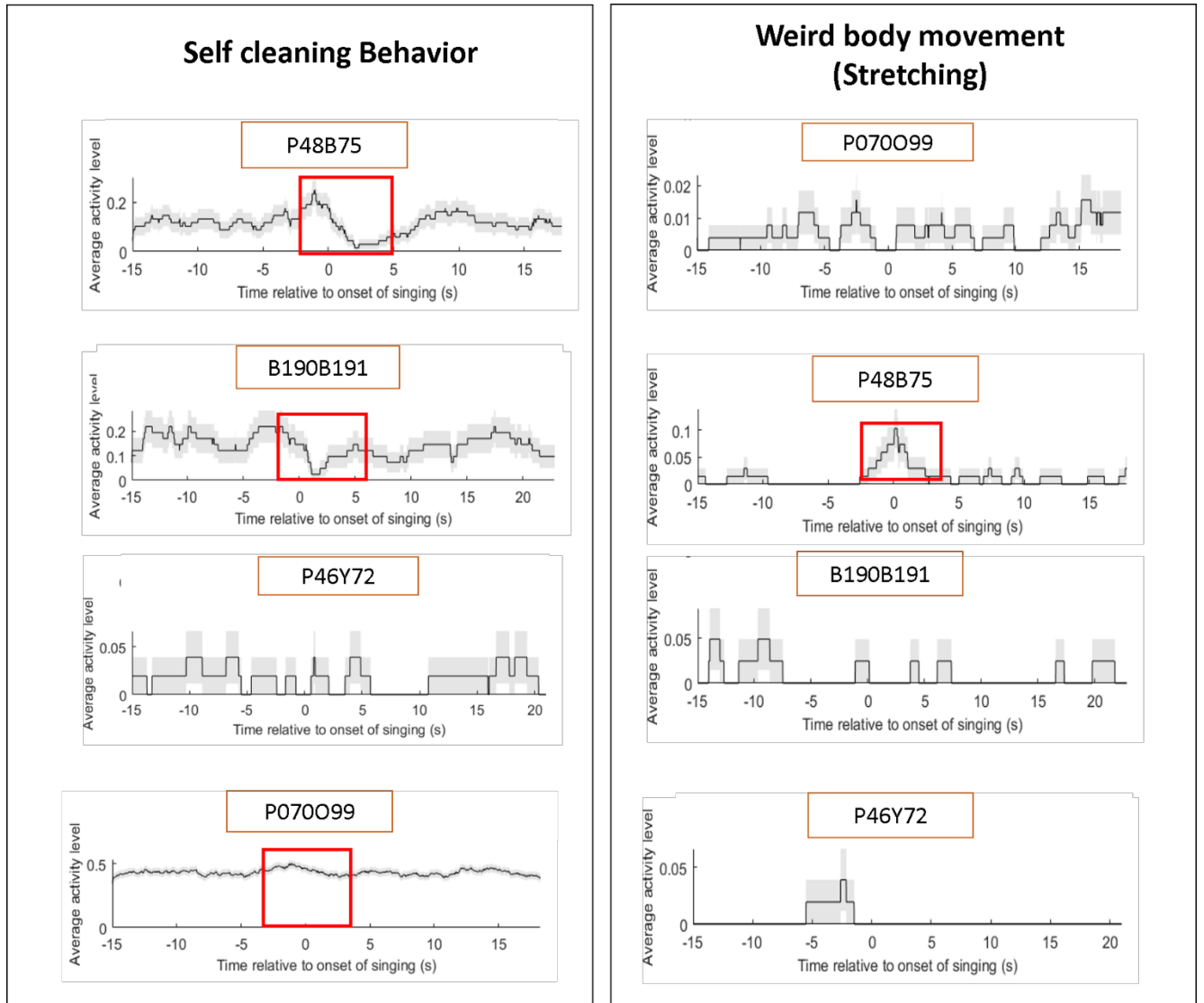


Figure 9 'Self cleaning' behavior show decrease after Tutors singing onset, implying a temporary stop of the above behaviors.

The figure average activity patterns for two behaviors. On the left panel, two subfigures represents behavioral pattern of 'Self cleaning' behavior of four birds. On the right panel, the two subfigures represent "weird body movement (Stretching)" behavior for four birds.

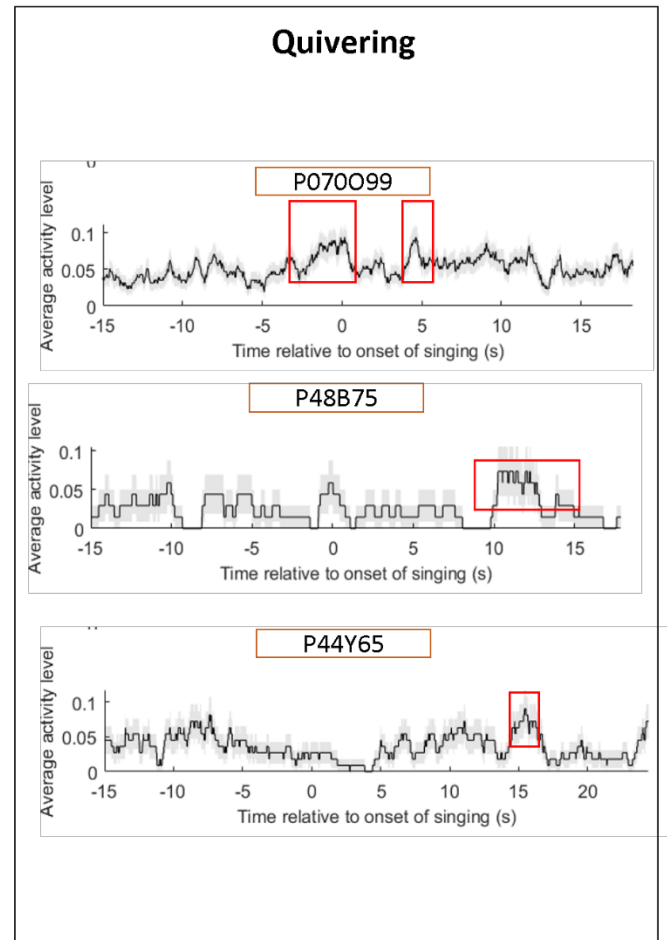
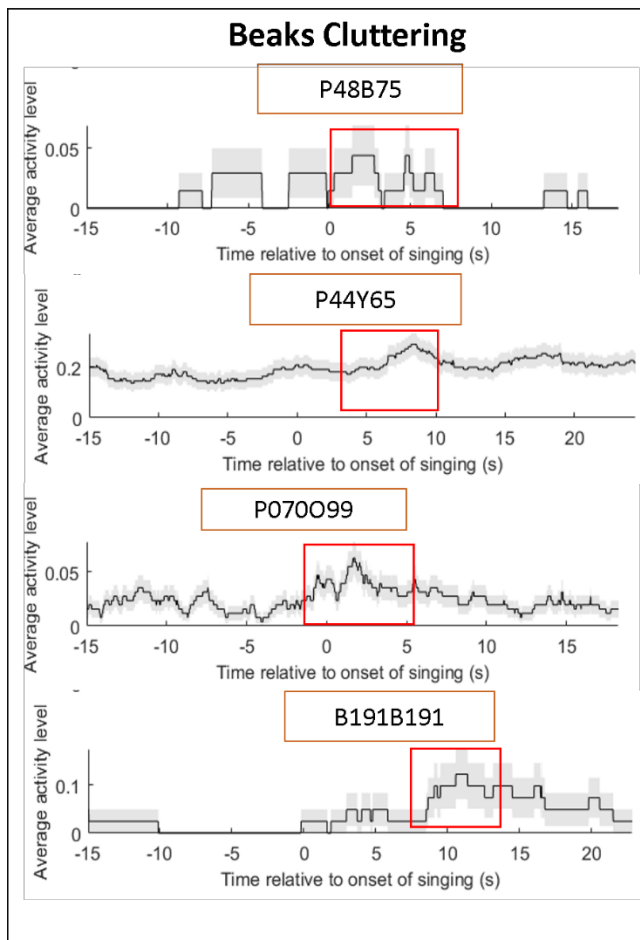


Figure 10 The 'Beaks cluttering' behavior shows increase in the activity after the singing onset whereas the quivering shows two peaks, one before and another after the onset of tutor's song. The figure depicts raster plots and average activity patterns for two behaviors. The red line represents the singing behavior onset. All the green dots represent singing behaviour offset. On the left panel, 4 subfigures represents behavioral pattern of 'Beaks Cluttering' behavior of 4 birds. On the right panel, the 3 subfigures represent 'Quivering' behavior for 3 birds.

Chapter 4: Discussion

The results show that zebra finches learn no less when separated from their parents and tutored for a shorter period than normally reared birds (Fig 6). We didn't find any correlation when plotted for the amount of songs tuttee exposed to with the song similarity score (Fig 7). It could imply that song learning does not vary with the number of songs a bird is exposed to. Some patterns such as several jumps, frequent quivering, frequent self-cleaning (Grooming) before the song initiation, and freeze movement of the Young juvenile bird after he started listening to the song were observed frequently (Fig 8-10). These behaviors were found in repetitive in nature.

Song Learning in the experimental and control birds

The t-test results revealed no significant differences in song similarity or consistency, but the experimental birds' song linearity was significantly lower than the control birds. This means that even though the young zebra finches learn the song. Their syllable pattern remained unstructured. Overall, it allows us to compare the song similarity score with the behavior analysis, but it does not allow us to compare the song linearity score.

The Influence of Tutor Singing on Juvenile Song Learning:

The results showed a bit negative correlation of $R = -0.3791$ and $R^2 = 0.1437$. The P-Value is 0.354492. The result is not significant at $p < 0.05$. It appears that there is no correlation between the amount of singing and the tutor's singing. It's possible that other behaviours influenced how much song was learned or that the young birds learned differently due to their innate nature. We even discovered that one of the bird with the least exposure to singing learned a good song, whereas the young bird with the most exposure learned an average song.

It is possible that young birds may or may not have paid attention to the tutor's song during the tutoring. According to Yining Chena,(2016) more attentive behavior by young juveniles leads to increased song learning. This could explain why birds' song learning styles differ.

The tutors singing and juvenile's response

The plots (Fig 8) indicated that the juvenile birds exhibited attentive behavior when the tutor sang, which suggests that they were actively processing and attempting to memorize the tutor's song. This attentive behavior may also indicate that the juvenile birds required some time to register the tutor's song in their memory. Through this attentive behavior, the juvenile birds strengthened their song memory, and once the song was established in their memory, they were free to play around. This process of strengthening song memory through attentive behavior is crucial for young birds' song learning, as it allows them to retain the tutor's song and incorporate it into their own repertoire. This process may take some time, as the juvenile birds need to listen to the tutor's song repeatedly and engage in attentive behavior to memorize the song. However, once the song is established in their memory, they can use it as a basis for their own creative expression, allowing them to develop their own unique song style. Overall, this process of attentive behavior and song memorization could be critical for the development of young birds' song-learning abilities.

Our observations of stretching and self-cleaning behaviors (Fig 9) revealed that, although these behaviors were frequently observed around the onset of singing, both behaviors temporarily ceased when the young bird began to listen to the tutor's song. After a period of attentive listening, the young bird resumed its previous behavior or started a new one. This pattern of behavior suggests capable of interrupting its body activity movement to concentrate on the song. This ability to interrupt its ongoing behavior to listen to the tutor's song highlights the importance of attentive behavior in young birds' song learning. Overall, our observations suggest that attentive behavior is a critical component of young birds' song learning, allowing them to focus on and internalize the tutor's song. By interrupting their ongoing behavior to listen to the song, young birds are able to strengthen their song memory and ultimately develop their own unique song style.

During our observation of young birds' responses to the tutor's singing, we noted that one of the most interesting behaviors exhibited by a juvenile bird was the "beaks clattering" movement. This behavior was characterized by the young bird rapidly moving its beaks in a way that appeared to imitate the tutor's beak movement. The young bird seemed to be excited and engaged by the tutor's song, and this movement

may have been an attempt to synchronize its own movement with the tutor's. This behavior bears some similarity to human infants' lip-syncing with their parent. Like young birds, human infants are also highly responsive to auditory stimuli and may attempt to imitate their parents' movements or sounds (P K Kuhl 1996). This behavior may be a way for young birds to practice and internalize the tutor's song, allowing them to better retain and reproduce the song in the future. Overall, the "beaks clattering" behavior exhibited by the young bird highlights the complex and dynamic nature of young birds' responses to the tutor's song. Through this behavior, the young bird may be attempting to synchronize its own movements with the tutor's, allowing it to better internalize and learn the tutor's song. This behavior may also reflect the young bird's excitement and engagement with the tutor's song, underscoring the importance of attentive and responsive behavior in young birds' song learning.

Chapter 5: Conclusion

We found no significant correlation between the amount of singing by the tutor and the accuracy of song copying by male juvenile zebra finches. The bird with the least exposure to tutor song produced a good copy of tutor's song, while the bird with the most exposure learned an average song.

The observations of the juvenile birds' responses to the tutor's song revealed their attentive behavior towards listening to tutor song. This process is crucial for young birds to learn song, as it enables them to retain and incorporate the tutor's song into their own song. The observations also revealed the interruption of ongoing behaviors, such as stretching and self-cleaning, when the young bird began to listen to the tutor's song. This pattern of behavior suggests the importance of attentive behavior in young birds' song learning. By interrupting their ongoing behavior to listen to the song, young birds are able to strengthen their song memory. One of the interesting behaviors exhibited by the young birds was the "beaks clattering" movement, which may have been an attempt to synchronize their movement with the tutor's song, and practice and internalize the tutor's song. Overall, the observations highlight the complex and dynamic nature of young birds' responses to the tutor's song, underscoring the importance of attentive and responsive behavior in young birds' song learning.

References

- A J Doupe, P K Kuhl. 1999. "Birdsong and human speech: common themes and mechanisms." *Annual review of Neuroscience*.
- Baptista LF, Petrinovich L. 1986. "Baptista LF, Petrinovich L. Song development in the white-crowned sparrow: Social factors and sex differences." *Animal Behaviour*.
- CK Catchpole, PJ Slater. 2008. *Bird Song: Biological Themes and Variations*. Cambridge, UK: Cambridge Univ Press.
- Derégnaucourt S, Poirier C, Kant AV, Linden AV, Gahr M. 2013. "Comparisons of different methods to train a young zebra finch (*Taeniopygia guttata*) to learn a song." *Journal of Physiology, Paris*.
- Eales, Lucy A. 1989. "The influences of visual and vocal interaction on song learning in zebra finches." *Animal Behaviour*.
- Friard, O., & Gamba, M. 2016. "BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. ." *Methods in Ecology and Evolution*.
- Kroodsmas, Donald E. Pickert, Roberta. 1980. "Environmentally dependent sensitive periods for avian vocal learning." *Nature*.
- Makoto Araki, M M Bandi , Yoko Yazaki-Sugiyama. 2016. "Mind the gap: Neural coding of species identity in birdsong prosody." *Science*.
- Marler, P. 1990. "Song learning: the interface between behaviour and neuroethology." *Philosophical Transactions of the Royal Society B: Biological Sciences* .
- Michale S Fee, Constance Scharff. 2010. "The songbird as a model for the generation and learning of complex sequential behaviors." *ILAR Journal*.
- neuroethology., Song learning: the interface between behaviour and. 1990 . "Marler P." *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*.
- Nikhil Phaniraj, Sanjana Joshi, Pradeepkumar Trimbake, Aditya Pujari, Samyuktha Ramadurai, Shikha Kalra, Nikhil Ratnaparkhi, View ORCID ProfileRaghav Rajan. 2022. "CineFinch: An animated female zebra finch for studying courtship interactions." (bioRxiv).
- Nottebohm, Constance Schraff and Fernando. 1991. "A Comparative Study of the Behavioral Deficits following Lesions of Various Parts of the Zebra Finch Song System: Implications for Vocal Learning. ." *The Journal of Neuroscience*.
- Nottebohm, Peter Marler and Mimi B. 1989. "The Neuroscience of Birdsong." *Annual Review of Neuroscience*.
- OFER TCHERNICHOVSKI*, FERNANDO NOTTEBOHM*, CHING ELIZABETH HO†, BIJAN PESARAN† & PARTHA PRATIM. 1999. "A procedure for an automated measurement of song similarity." *ANIMAL BEHAVIOUR*,.
- OFER TCHERNICHOVSKI*, FERNANDO NOTTEBOHM*, CHING ELIZABETH HO†, BIJAN PESARAN† & PARTHA PRATIM. 2000. "A procedure for an automated measurement of song similarity." *ANIMAL BEHAVIOUR*.

- P K Kuhl, A N Meltzoff. 1996. "Infant vocalizations in response to speech: vocal imitation and developmental change." *The Journal of the Acoustical Society of America*.
- Peter J.B. Slater, Lucy A. Eales, N.S. Clayton. 1988. "Song Learning in Zebra Finches (*Taeniopygia guttata*): Progress and Prospects." *Advances in the study of the Behavior*.
- Pfenning, A. R., Hara, E., Whitney, O., Rivas, M. V., Wang, R., Roulhac, P. L., ... & Jarvis, E. D. 2014. "Convergent transcriptional specializations in the brains of humans and song-learning birds. ." *Science* 346(6215), 1256846.
- Price, Philip H. 1979. "Developmental determinants of structure in zebra finch song." *Journal of Comparative and Physiological Psychology*.
- Sarah Golüke, Hans-Joachim Bischof, Jacob Engelmann, Barbara A. Caspers, Uwe Mayer. 2019. "Social odour activates the hippocampal formation in zebra finches (*Taeniopygia guttata*)." *Behavioural Brain Research* 41-49.
- Sébastien Derégnaucourt 1, Colline Poirier, Anne Van der Kant, Annemie Van der Linden, Manfred Gahr. 2012. "Comparisons of different methods to train a young zebra finch (*Taeniopygia guttata*) to learn a song." *journal of physiology paris*.
- Shikha Kalra, Vishruta Yawatkar, Logan S James, Jon T Sakata and Raghav Rajan. 2021. "Introductory gestures before songbird vocal displays are shaped by learning and biological predispositions." *Proceedings of the Royal Society B: Biological Sciences*.
- Yining Chena, Laura E. Mathesonb, and Jon T. Sakata. 2016. "Mechanisms underlying the social enhancement of vocal learning in songbirds." *PNAS*.
- Zann, RA. 1996. *The Zebra Finch: A Synthesis of Field and Laboratory Studies*. New York,: Oxford Univ Press.
- Zdzislaw Galoch, Hans-Joachim Bischof. 2007. "Behavioural responses to video playbacks by zebra finch males." *Behavioural Processes*.

Key Terms

Ethogram: An ethogram is a record of an animal's different behaviours

Motif: A stereotypical repetitive pattern of different syllables

Spectrogram: A visual representation of amplitude over time at various frequencies. In the spectrogram darker color of certain element represents higher power/amplitude over the other area.

Syllable: A unit element of song that can be labelled separately.

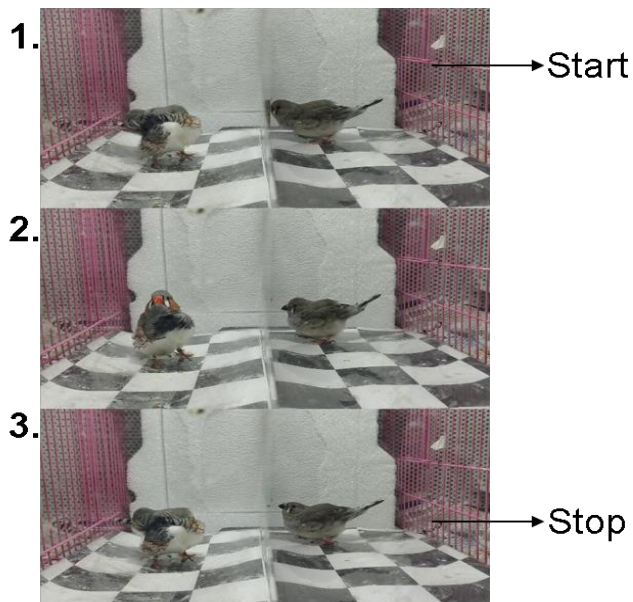
Supplementary Information

Table 3 Results of unpaired t-test for the song analysis part.

	Song Similarity	Song Linearity	Song Linearity
P-value	0.2328	0.1270	0.0371
Confidence interval: Mean(Experimental Birds - Control Blrds)	-9.7388	-7.2269	-9.7506018138
95% confidence interval of this difference:	From -26.4858203223 to 7.0081368348	From -16.7800081467 to 2.3262040117	From -18.8315201078 to -0.6696835197

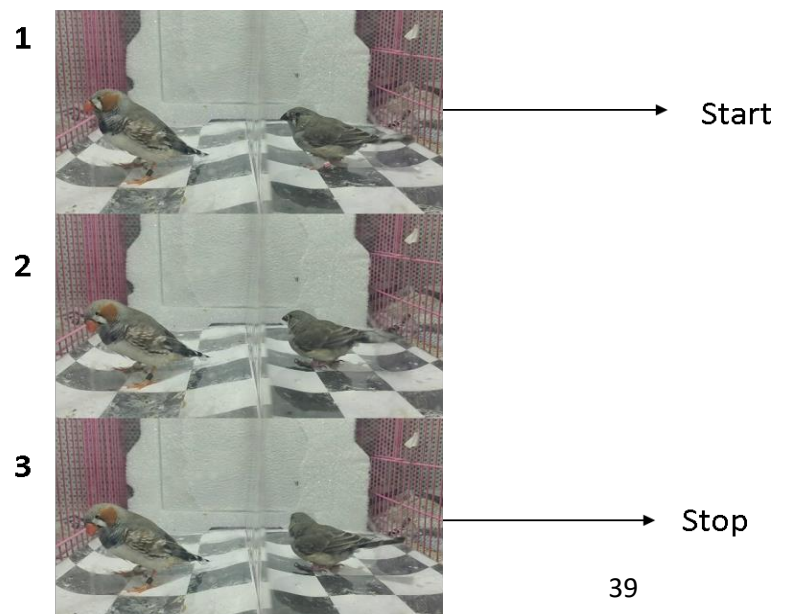
➤ Snapshots of some of some of the behaviors:

Pecking sequence



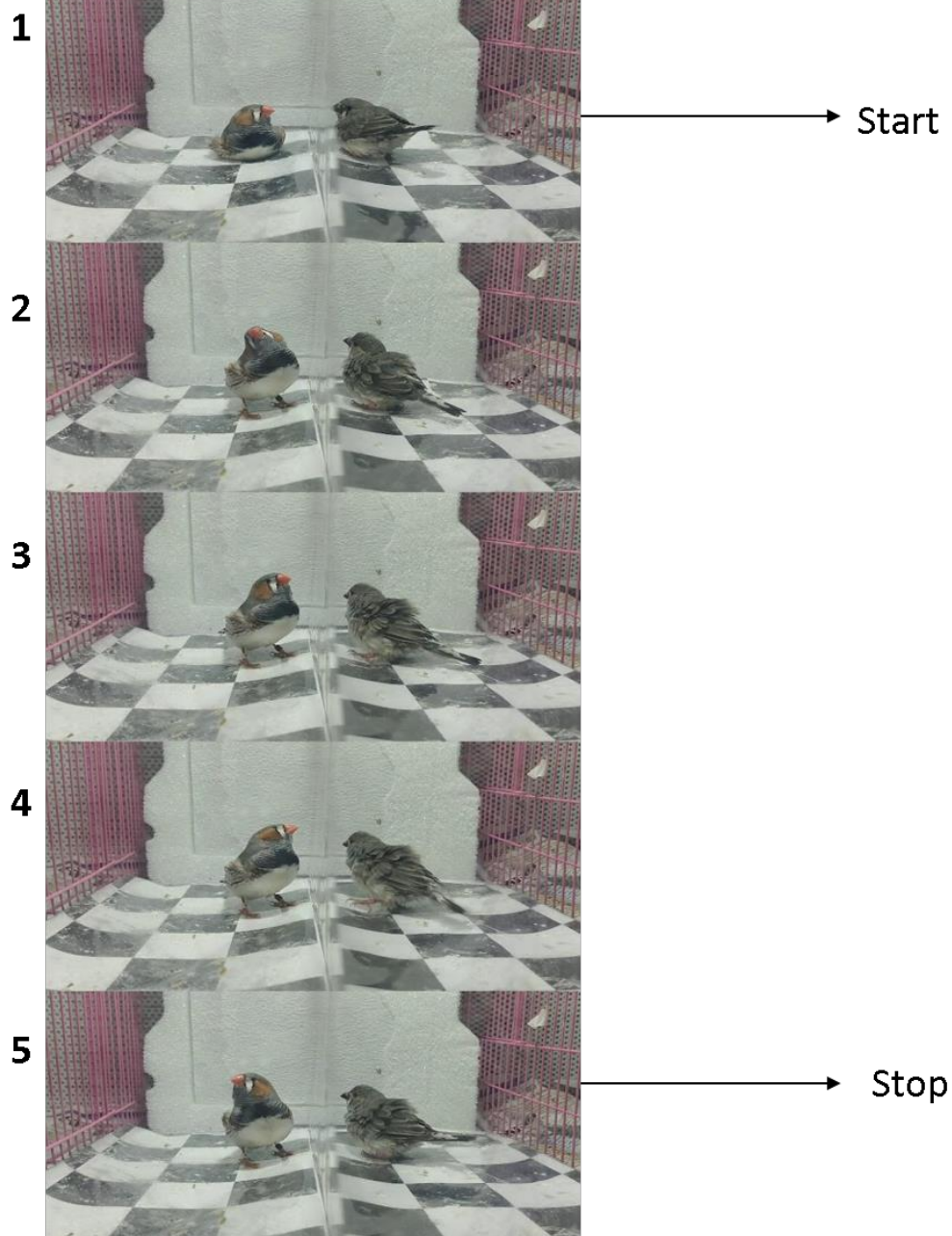
S1.Snapshot of Pecking sequence

Tail Wagging Behaviour Sequence



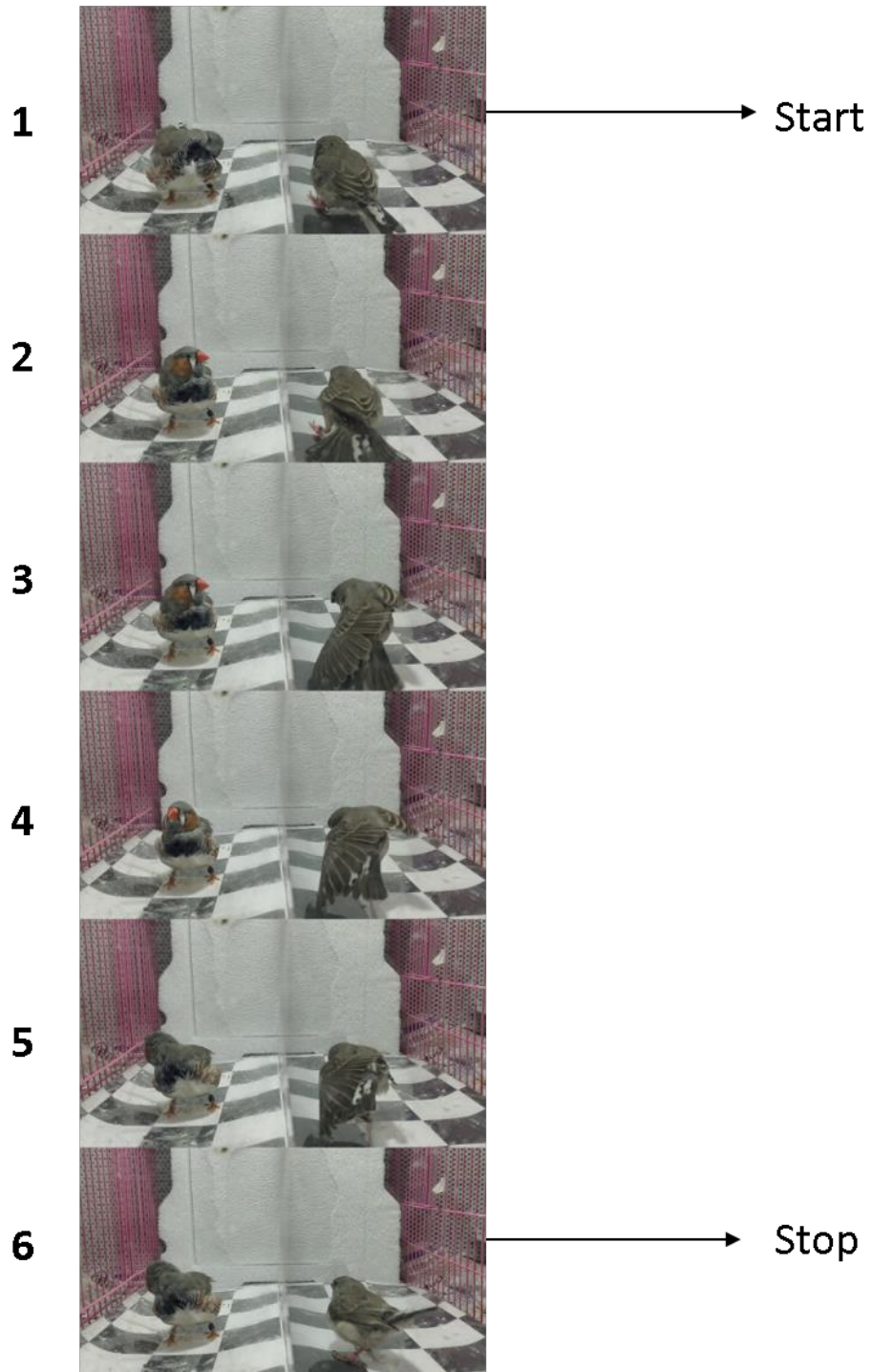
S2.Snapshot of Tail Wagging

Quivering Behaviour Sequence



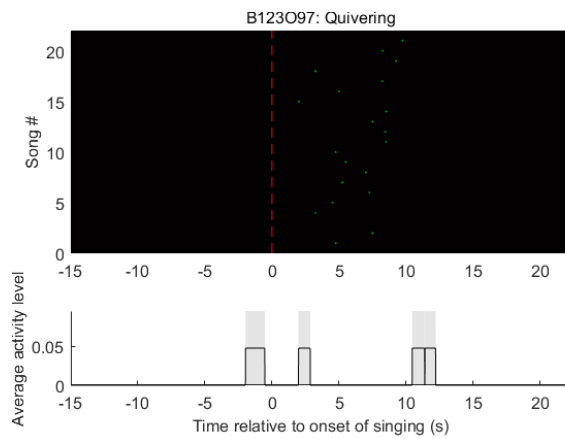
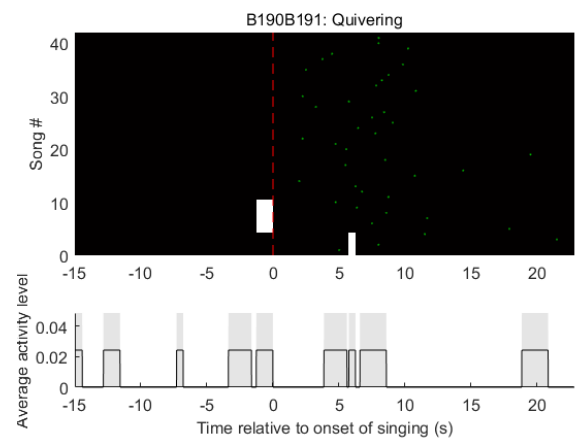
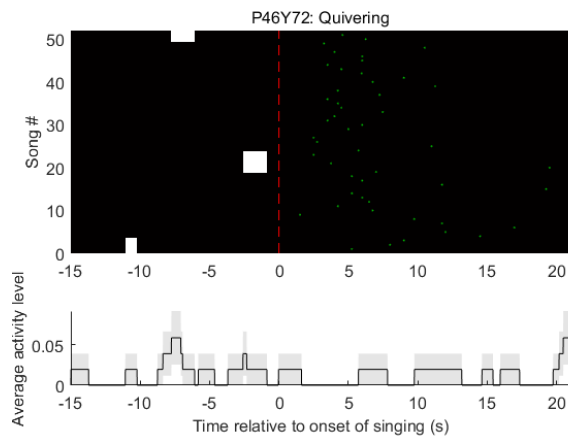
S3.Snapshot of Quivering Behavior

Stretching Behaviour Sequence



S4.Snapshot of Stretching Behavior

Raster Data of Quivering:



S5. Raster plot and average activity plots of different birds for 'Quivering' behavior