Comparative Analysis of Introductory Notes across four Estrildid finches

End-semester Project Report

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Date: 18th November 2022
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A. Introduction

The Zebra finch song is an excellent model to study the mechanism behind learned, complex motor sequences. Usually, these songs are preceded by repeated short vocalisations called introductory notes or INs (Price 1979). Such gestures have been observed in other animals as well, and multiple hypotheses try to explain these introductory gestures. Introductory gestures could enhance the communicative aspect of the display and increase its detectability (Wiley and Richard 1982); they could serve as recognition signals to the main display by providing singals for species identity (Soha and Marler 2000), or they could be a motor preparatory function for when the brain 'prepares' parameters to generate the complex movement (Rajan and Doupe 2013).

INs in zebra finches are vocalisations of short duration and simple spectral composition. Initial, more variable INs converge acoustically to a stereotypical last IN, which signals the readiness of song sequence generation (Rajan and Doupe 2013). Previous studies in the lab have shown that Zebra finch INs are feedback-independent (Rao et al., 2019), and their features are shaped by both learning and biological predispositions (Kalra et al., 2021). It was also observed that INs have high beginning bout probability and self-transition probability in Zebra finches as compared to calls or song motif syllables, i.e., they occur at the beginning of a song bout and are usually repeated a number of times. (Fig. 1)
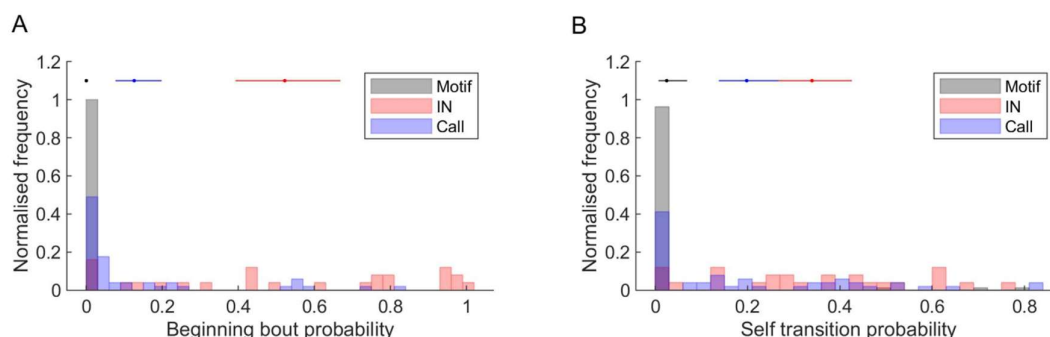
Figure 1. Bout beginning probability and self-transition probability distributions for identified motif syllables, introductory notes (INs) and calls (Borrowed from Kalra et al., 2021 Supplementary Material)

While Zebra finch songs are well-studied, only a few studies have looked at INs in other songbirds, in species such as Rufous-sided Towhees, Alder flycatcher, and Willow flycatcher (Richards 1981, Kroodsma 1984). Shiovitz 1975 analysed the song and IN structure of Indigo Buntings and, through playback experiments, showed that initial notes are effective in capturing the attention of male Indigos. But, these papers, which analyse song structure in depth, have not compared the structure and features of INs across several species.

Studies which have done a comparative analysis of song structure across a taxon have looked at broad, population-level features such as minimum and maximum frequency, duration,

inter-note interval, and how these features are affected by the ecology and phylogeny of the birds. Buskirk 1997 shows that, in American wood warblers (Parulinae), song structure correlates with habitat conditions, whereas note structure is unaffected by habitat and constrained by phylogenetic history. Similarly, it was shown that habitat and body size play an important role in shaping the song structure of primitive birds (Tinamidae) (Bertelli and Tubaro 2002). In contrast, Mejias et al., 2020 showed that Vireonids' song structure diversity is unaffected by habitat and is mainly driven by phylogenetically conserved morphological traits.

Through this project, we aim to compare and contrast IN structure and features of different Estrildid birds to understand better their function, and how they might be related to the song structure. This would help us contextualize the studies on motor preparatory function of INs in Zebra finches, i.e., whether we can generalize the results of INs in Zebra finches to other species or if they're Zebra finch-specific. To do this, I would first compare song structures across Estrildid species, identify and label INs and motif syllables in recorded songs and analyze them, along with Nandu T S, an MS-project student in the Rajan Lab.

B. Methods

Bird species

The Estrildid birds have been selected based on the availability of good-quality, undirected songs from those species. The bird species we are working with are listed in Table 1, and their phylogenetic relationship is illustrated based on the classification by Olsson and Alstrom 2020.

Sl No	Common name	Scientific name	Sample size
1	Zebra finch	*Taeniopygia guttata castanotis*	8
2	Bengalese finch	*Lonchura striata*	5
3	Java finch	*Padda oryzivora*	5
4	Blue-capped cordonbleu	*Uraeginthus cyanocephalus*	8

Table 1. List of selected species
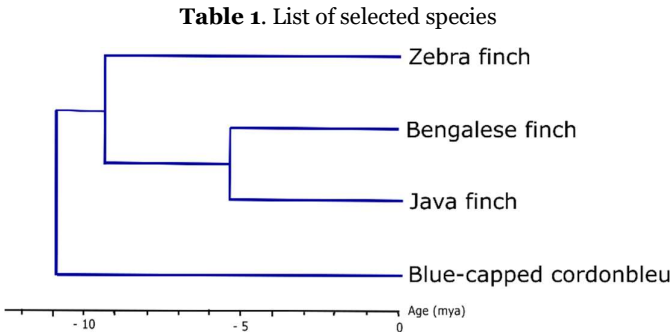
Figure 2. Phylogenetic relationship of selected species based on classification by Olsson and Alstrom 2020

Figure 3. Images of Java finch, Zebra finch, Bengalese finch and Blue-capped cordonbleu respectively

Recording songs

Songs from Java finches, Bengalese finches and Zebra finches were recorded in the lab and the following methods were used - the bird was isolated for a minimum of 24 hours within a soundproof recording box and a microphone was brought into the recording box and attached to the roof of the bird's cage to collect audio. The mike was connected to a mixer that was only used as an amplifier. On the PC, we use Alsamixer, a Linux-based graphical program used to configure the sound settings and adjust the volume settings. After setting up Alsamixer, we used the PyCBS, a python-based program that plots the RMS of input audio files. It allows us to choose different sound devices and can record in mono or stereo. Data was digitized at 44100Hz sampling rate. The Audio files were saved and exported in 'wav' format in the lab PC.

Labelling songs

Recordings of bird songs are cut up into 30 s or 60 s segments, screened for songs and labelled using *Screen Song Keyboard Modified* and *Auto Song Segment Label* (ASSL), Matlab programs that were developed by Dr Raghav Rajan. ASSL generates a spectrogram of the recording, identifies syllables (in a certain range of frequency and amplitude), and allows us to label syllables easily. After parsing through several song bouts, each unique syllable is given a specific label. Call syllables are labelled *k* or *l*, and song motif syllables are labelled with letters beginning from *a*. About 25-30 song bouts/files are labelled for each bird.

I have labelled songs of 2 Java finches, 4 Blue-capped Cordonbleus and 1 Bengalese finch. I also verified the labels of 2 Java finches and 3 Bengalese finches. Zebra finch songs and some Java finch and Bengalese finch songs had been previously labelled. Representative labelled songs are shown in fig. 4 below. Labelled song data with syllable name, onset time, offset time, frequency, amplitude and other information was saved as an OnsetOffset text file.

a)   Blue-capped cordonbleu

b)   Bengalese finch

c)   Java finch

d)   Zebra finch
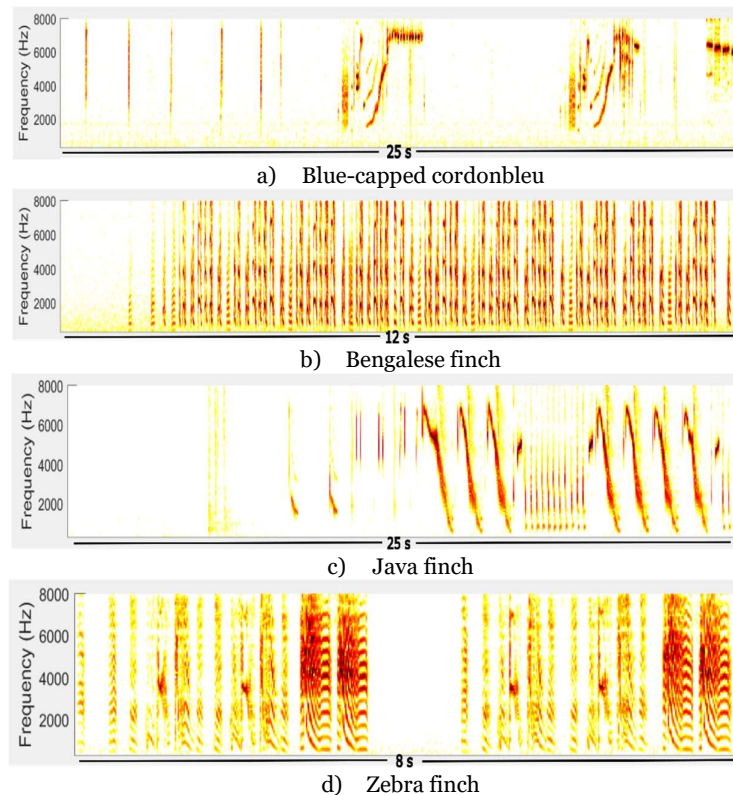
Figure 4. Song spectrogram of different species

Data Analysis

The Onset Offset files were processed to first insert 'Start' and 'End' syllables before the beginning and at the end of each song bout. Song bout is defined as a series of vocalizations separated by 2 seconds of silence (Sossinka and Bohner 1980). Then, the song bouts with less than 3 unique syllable types were excluded, because we wanted to focus on bouts with song motifs, and not those which only contained calls or other vocalizations. These modified files were then used to calculate transition probability matrices, plot transition probability diagrams, quantify different aspects of song structure and acoustic feature, create plots and do statistical tests on the data. All the code was written in Python and can be found on my Github profile, at this link - https://github.com/vasudha-kulkarni/IN-comparative-analysis.

Comparing song structures

We used the following measures to quantify and compare song structure across species – Sequence Linearity is a measure of the ordering of notes in a song, Sequence Consistency tells us how often a particular path (or motif sequence) is followed in the songs (Scharff and Nottebohm 1991) and Transition Entropy gives us an idea about the variability of a song (Sakata and Brainard 2006). These measures are calculated as –

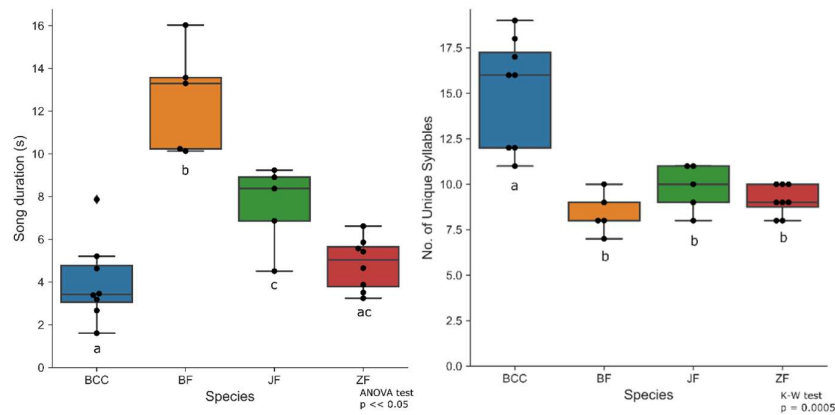$$Sequence\ Linearity = \frac{No.\ of\ unique\ syllables}{No.\ of\ unique\ transitions}$$

$$Sequence\ Consistency = \frac{Sum\ of\ typical\ transition\ probabilities}{Sum\ of\ all\ transition\ probabilities}$$

$$Transition\ entropy = \sum_i -p_i \cdot \log_2 p_i$$

C. Results

Differences in song structure

Different species have different song duration, with Bengalese finches (BF) having the longest song, whereas Zebra finch (ZF) song duration is not different from that of Java finch (JF) or Blue-capped cordonbleu (BCC) (Fig 5a). BCCs have a significantly higher number of unique syllables as compared to other species (Fig 5b). To understand if the differences were significant, we used ANOVA (where the data was distributed normally) and Kruskal-Wallis test (where it wasn't) and when the differences were significant, we used Tukey-Kramer and Dunn tests as post-hoc tests respectively. A summary of results of statistical test on these categorical comparisons are provided in Table 2.

Figure 5. a) Mean song duration across species b) Average number of unique syllables across species

There are no significant differences in sequence linearity and sequence consistency across species (Fig. 6a, 6b). Transition entropy is significantly lower in BF, possibly because they have lesser number of unique syllables, and the calculation of transition entropy should be normalized for that.
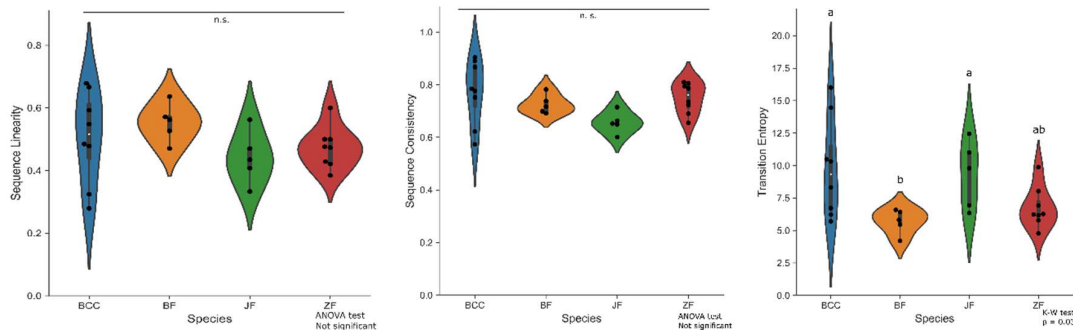

Figure 6. a) Sequence Linearity b) Sequence Consistency and c) Transition Entropy across species

Parameter	Test	Significance	BCC-BF	BCC-JF	BCC-ZF	BF-JF	BF-ZF	JF-ZF
Song duration	ANOVA	2.1992e-07	**0.001**	**0.013**	0.78	**1.5e-3**	0.001	0.075
No. of unique syllables	Kruskal-Wallis	0.000532	**2.1e-3**	**0.02**	**7.5e-3**	0.19	0.45	0.49
Sequence Linearity	ANOVA	n.s.	Not applicable					
Sequence Consistency	ANOVA	n.s.						
Transition Entropy	Kruskal-Wallis	0.030823	**0.018**	0.757	0.083	**0.016**	0.405	0.067
Average repeats of first syllable	Kruskal-Wallis	0.012303	**0.006**	**0.049**	**0.005**	0.48	0.78	0.613

Table 2. Summary of statistical analyses results of ANOVA and Kruskal-Wallis test for categorical comparison of means across species and p-values of pairwise comparisons through post-hoc tests

Initial Syllable Properties

Once we have characterized some of the features of song structures, we wanted to look more closely at the Starting syllables, that is, the syllables with which birds can start their songs, and Initial syllables which are the first 5 syllables of the song.
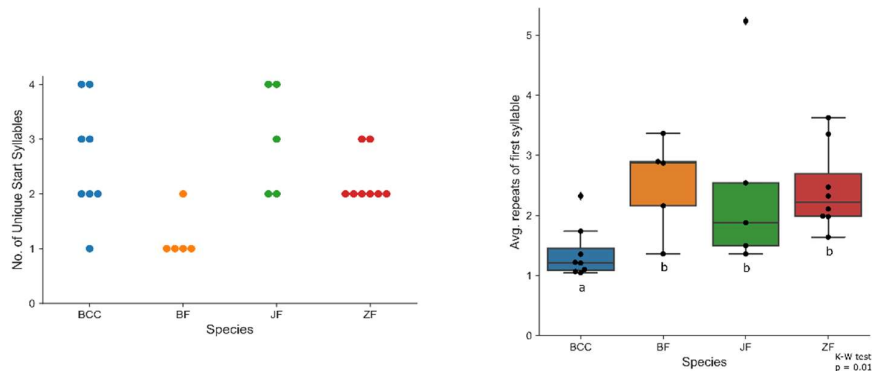
Figure 7. a) Number of unique starting syllables across species b) Average number of repeats of the first syllable

Although different species have different number of unique syllables, they can begin their song with only 1-4 different syllables (Fig. 7a). We also calculated the number of times the first syllable of the song repeats in the beginning – BCCs have significantly lesser average repeats of the first syllable, as compared to other species. In fact, the first syllable of the song

rarely repeats in BCCs, and hence the average number of repeats is close to 1, i.e., first syllable only occurs once (Fig. 7b).
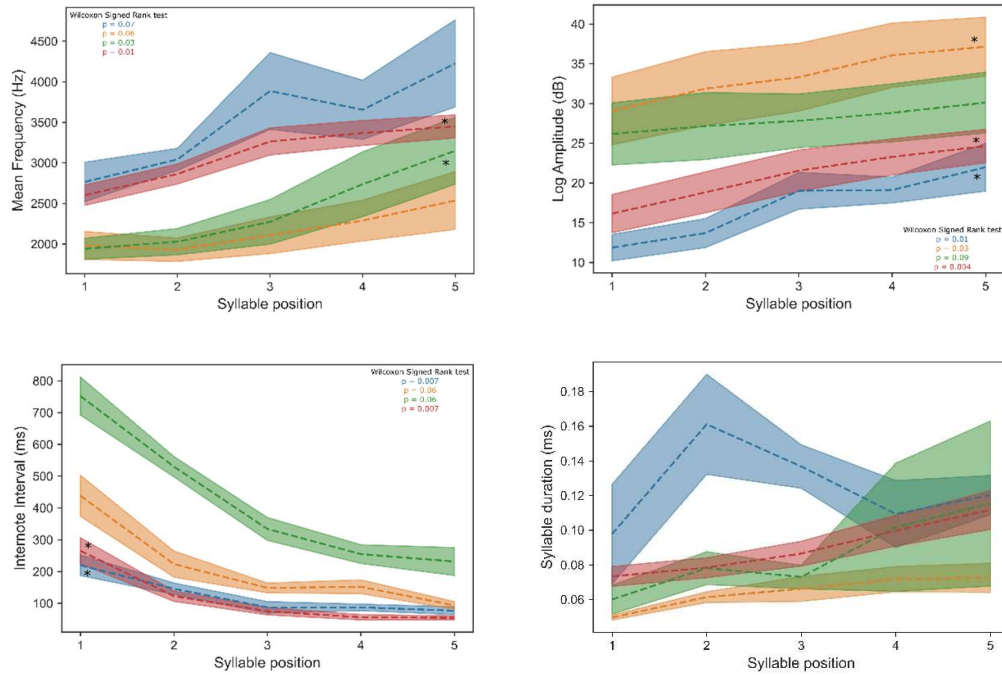

Figure 8. a) Mean log amplitude, b) Mean frequency c) Mean internote interval and d) Syllable duration across first 5 syllable positions

Species	Amplitude ('greater')	Frequency ('greater')	Syllable duration ('two-sided')	Internote interval ('less')
BCC	**0.01171875**	0.07421875	0.25	**0.0078125**
BF	**0.03125**	0.0625	0.0625	0.0625
JF	0.09375	**0.03125**	0.8125	0.0625
ZF	**0.00390625**	**0.01171875**	**0.0078125**	**0.0078125**

Table 3. Result summary of statistical analyses of Wilcoxon signed rank test for comparison of acoustic features of 1st and 5th syllable of a song. The word in the bracket indicates the alternative hypothesis used to test the data

We also looked at how acoustic features such as amplitude, frequency, internote interval and syllable duration varies across initial five syllables of the song. As seen in Fig. 8, there is a clear increasing trend in amplitude and frequency, and a decreasing trend can be seen in internote interval across first 5 syllable positions. Syllable duration shows no clear trend, except in Zebra finches, where the syllable duration increases and this could be because of short duration of INs as compared to that of motif syllables (Fig. 8d). To test the increase or decrease, I did a Wilcoxon signed rank test for average values of first and fifth syllable, the results of which are provided in Table 3.
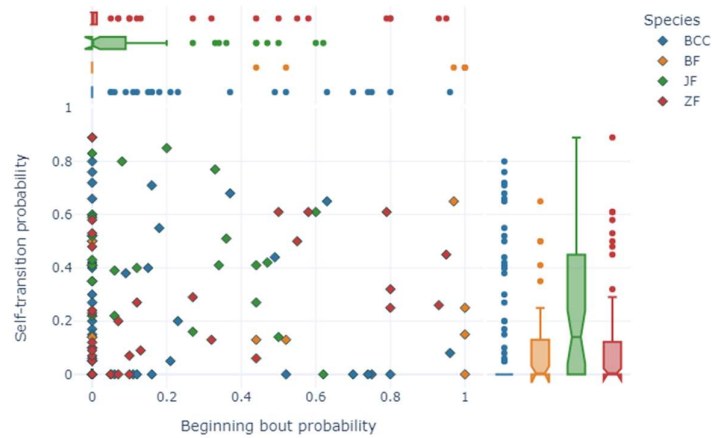
Figure 9. Self-transition and beginning bout probability distribution of all unique syllables of all species, with marginal distributions represented as boxplots

We also looked at the Beginning bout probability and Self transition probability distribution of all unique syllables of all species (Fig. 9). Broadly, most syllables occur in the motif and hence have very low beginning bout probability and very low self transition probability, and this distribution is true for all species, except for Java finches, which have a higher number of syllables with greater self-transition probability (through pairwise Kolmogorov-Smirnov tests, but without any corrections).

D. Discussion

Different species have different song durations and BCC have significantly higher number of unique syllables, but there no differences in sequence linearity and consistency across species, which indicates that all birds have fairly stereotypical songs, given that they are adult birds with crystalized songs. Although different species differ in their song structure and number of unique syllables, they can begin their song with a maximum of 4 unique syllables, which means they are constrained in the number of ways they can initiate a song sequence.

Through this project we also wanted to identify INs in other species, so we looked at features of starting syllables - the first syllable of BCC rarely repeats, whereas that of other species repeats a similar number of times. Moreover, the starting syllable in BF also occurs several times in the middle of the song bout. This throws up a snag in the definition of introductory notes, defined by their beginning bout probability and repeatability in Zebra finches. Perhaps we should consider a broader definition of INs for other species.

The results of acoustic feature trends across initial syllables of the song is similar to the trend of INs in Zebra finches (Rajan and Doupe 2013). Amplitude and Frequency systematically increase and Internote interval consistently decreases along the first five syllables of the song (Fig 8). This could imply a similar motor preparation trend in other species, without similarly repeating initial syllables.

The overall probability distribution of all syllables of various species for their propensity to repeat and to occur at the beginning of a bout is largely similar. This, along with similar sequence linearity and consistency, tells us that song structure across these 4 species can be

considered to be the same – with a few introductory notes/starting syllables, long motifs and a few call notes.

An interesting observation is that the spread of data points for BCC in most measures seems more variable than that of other species. This could be because of differences in learning mechanisms as juveniles because both males and females of this species sing songs, although adult females have less elaborate songs. It could also be because these birds have a tapping display as part of their courtship behaviour (Ota et al., 2015), and the presence of this extra modality of conspecific recognition might have decreased the selection pressure on the song to remain very consistent through the species.

Thus, different species differ in the number of syllables and song duration but seem to have similar song structures and begin their song in similar ways.

E. Future directions

The future plans for this project involve collecting data for other Estrildid birds, such as African silverbills, Indian silverbills, Red-cheeked cordonbleus, Gouldian finches etc., quantifying different measures and mapping these differences to their phylogeny. Simultaneously, we could also study their initial syllables more closely to find similarities and differences across the clade.

In Zebra finches, INs are defined as short vocalizations that repeat and occur at the beginning of the song. But, as we have seen, first syllable of Blue-capped cordonbleu rarely repeats (Fig. 7b) and first syllable of Bengalese finches often repeats in the later parts of song bouts as well (personal observation). It would be interesting to check if the first syllable of Bengalese finches (that also occurs in the bout) always occurs at the beginning of the motif. It might also be interesting to expand the definition of INs to include first syllable of the song bout, without the expectation of repetition.

F. Acknowledgements

G. References

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