Comparing Introductory Vocalisations in Estrildids

A Thesis

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by

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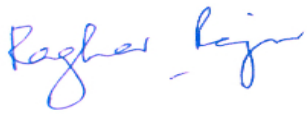
INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH PUNE

Certificate

This is to certify that this dissertation entitled Comparing Introductory Vocalisations in Estrildids 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 Nandu T S at Indian Institute of Science Education and Research under the supervision of Dr Raghav Rajan, Assistant Professor, Department of Biology, during the academic year 2022-2023.



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This thesis is dedicated to my parents

Declaration

I hereby declare that the matter embodied in the report entitled Comparing Introductory Vocalisations in Estrildids 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.

Nandu T S

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Abstract

Introductory notes (INs) are notes that occur at the onset of the vocalisation in several species, including songbirds. In Zebra finches, songs start with a variable number of IN repeats, followed by a fixed motif sequence of syllables that repeats across the song bout. To examine if INs also have a species specific pattern, in this study we aim to characterise INs across relatives of the Zebra finch, i.e. the Estrildidae family. We also looked at and quantified some general properties of the birdsong as it would allow us to compare and correlate properties of INs with properties of the song that follows. It was seen that all the Estrildid species had fairly stereotyped songs that consistently began with a few fixed syllables, despite the vast repertoire of unique syllables that the bird was capable of producing. While the majority of these species were seen to produce repeats of this starting syllable, a few of these species did not do so. Across the species, it was seen that the INs changed their acoustic properties as they repeated themselves, consistently getting longer and gaps between them getting shorter as they proceeded to song. Across all species, irrespective of syllable identity or IN properties, it was seen that the birds produced syllables faster and faster as they began their song. There was also a lot of similarity in song and IN properties among species of the same genus.

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Contributions

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Nandu T S, Vasudha Kulkarni, Dr Raghav Rajan	Validation
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Nandu T S	Visualisation
Dr Raghav Rajan	Supervision
Dr. Raghav Rajan	Project administration
Dr Raghav Rajan	Funding acquisition

This contributor syntax is based on the Journal of Cell Science CRediT Taxonomy[1].

[1] https://journals.biologists.com/jcs/pages/author-contributions

Introduction

Across the animal kingdom, a majority of males attract females through the use of various audio-visual displays, which have been studied to better understand communication among various animal species. In songbirds, the males attempt to attract females via their songs and other actions like plumage or reproductive displays (Mitoyen et al., 2019). Their songs, especially the songs of the zebra finch (*Taeniopygia guttata*) (Fee and Scharff, 2010) have been heavily studied to better elucidate the mechanisms behind learned complex motor sequences. Songs also provide an easily quantifiable instance of a self-initiated movement sequence.

But before we head further, it is important to adequately describe what songs are. A song is a complex sequence of vocalisations which consists of multiple notes and song is used for communication within a species, usually to facilitate mate selection (Catchpole and Slater, 2008). Every song is made up of several 'syllables', and each syllable is one continuous piece of sound that has a distinct acoustic structure. Songs are a unique behaviour in the sense that it has both innate and learned components. Even early on, people working on songbirds realised that a good singer needs a good tutor and that individuals could be bred to produce more elaborate or more simplified songs. Further, the discovery of the brain regions responsible for the control and production of songs managed to bring birdsong from a purely behavioural trait to something that lies at the confluence between behaviour and neurobiology. All these factors together contributed to making songs a highly pertinent field of study for neurobiologists (Konishi, 1989). The study of complex behaviour has had the hurdle of accurately describing the relevant behaviour objectively. Being able to represent complex vocalisations pictorially via spectrograms made birdsong an ideal model to learn more about the development of complex behaviour (Thorpe, 1954). The fact that it is quite simple to manipulate a bird's auditory input also helped greatly in unravelling the role of sensory guidance in development of this behaviour (Konishi,1989). These factors led to songs being favoured as a model to study complex behaviour in particular.

So how actually do birds produce these songs, or in fact any of their vocalisations? Song production in birds involves the transfer of dynamic fluid energy, i.e. airflow, into auditory energy, i.e. song. Sound is generated during expiratory pulses and the flow of air is driven by increasing expiratory pressure. The flow of air past the syrinx, the vocal organ, is created by the tightening of respiratory muscles while the syringeal muscles regulate the resistance in the airway via the motion of the laterally positioned labia in and out of the lumen of the bronchi (Suthers et al., 1999; Goller and Larsen, 2002). Lastly, as soundwaves exit through the bird's beak/bill, it behaves as an amplifier for the song as its soundwaves exit the bird (Demery et al. 2021). In a song, the length of each expiratory pulse can change greatly. The inspirations taken between the expiratory pulses are usually of a short length, and the inspiratory pressure here is greater than the pressure during quiet respiration, i.e. during non-song periods, to enable short

and deep inhalations (Hartley and Suthers, 1989). So, the pattern and potency of the respiratory patterns in birds are greatly changed for the production of song (Suthers and Goller, 1998). Additionally, the characteristics of the song produced are also influenced by muscle activity of the labial muscles and syringeal muscles. For instance, respirations were found to dictate the syllable length, sequence of syllables and the pattern of sound and silent intervals in time (Goller and Suthers, 1996a; Goller and Suthers, 1996b). Thus, song is produced by the bird via conversion of fluid air dynamic force into acoustic energy with the help of its syrinx and respiratory muscles, which then have an effect on the kind & properties of sound produced.

Coming to Zebra finches, these birds are small seed-eaters native to Australia. They show remarkable stereotypy in their songs and are easy to raise in captive conditions. This, in addition to them showing similarity to humans in vocal learning (Doupe and Kuhl, 1999; Brainard and Doupe, 2013; Prather et al., 2016), is why Zebra finches are an extremely popular model system for studying vocal learning. In Zebra finches, both the syllable types and their order in the motif are greatly stereotypical (Bruno and Ofer Tchernichovski, 2019).

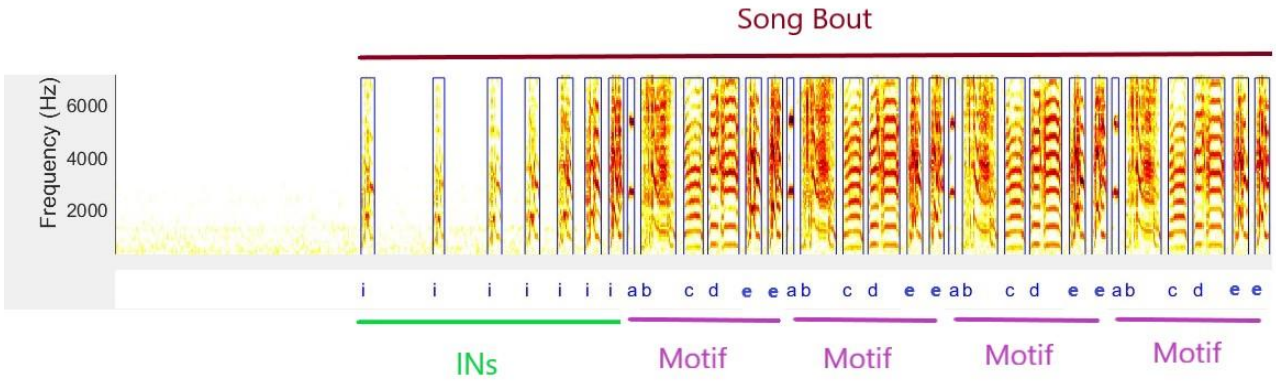

Fig 1. A spectrogram representation of a zebra finch song. The figure shows a single song bout of a Zebra finch, marked by the maroon line on top. The green line at the bottom shows the repeated intro note sequence in the bout while the purple line indicates the sequence of syllables (motif) that gets repeated across the bout. The alphabets indicate syllable types, with the 'i' syllables being the intro notes and syllables 'a' to 'e' representing motif syllables.

The male typically sings versions of its song motif in bouts that start with repetition of short duration vocalisations called introductory notes (aka an intro note or IN) which are preceding the first song-unit of a bout (Sossinka and Böhner, 1980), as shown in the figure above (Fig. 1). Thus, a stereotypical zebra finch song starts with a varying number of simple, identical INs, followed by a fixed motif syllable sequence that repeats across the song bout.

Like INs in zebra finches, similar introductory gestures have been observed and studied in other organisms and have various functions hypothesised about them. These include head bobbing in Anolis lizards (Fleishman, 1992), tail flicking in Jacky dragons (Peters, 2003), introductory notes

in advertisement and aggressive calls of treefrog (Wells and Schwartz, 1984), introductory grunts in Opsanus beta (vocalising fish) (Thorson and Fine, 2002), introductory whistle in golden-crowned sparrows and white-crowned sparrows (Marler and Tamura, 1962; Soha and Marler, 2000), introductory notes in *Geocrinia victoriana* (Eastern smooth frog) (Littlejohn and Harrison, 1985) and introductory notes in several songbird species (Shiovitz et al.,1975; Richards, 1981).

Now what makes these introductory gestures so important to study? This is because they have been shown to perform very vital functions in the communicative signals produced by animals. Head bobbing in Anolis lizards' territorial aggressive display is used to draw attention when the receiver is too far away (Fleishman, 1992) . In Jacky dragons visual display, introductory tail flicking of lower velocity & longer duration are used to maximise the signal efficacy (Peters, 2003). Long and short buzz-like introductory notes in treefrog (*Hyla ebraccata*) were used in close-range and short-range interactions respectively (Wells & Schwartz, 1984). In Opsanus beta, introductory grunts served to alert other fish that an advertisement call is imminent (Thorson & Fine, 2002). Introductory whistles in golden-crowned sparrows and white-crowned sparrows serve as recognition signals to the main song (Marler & Tamura, 1962; Soha and Marler, 2000). Introductory notes in *Geocrinia victoriana* are aimed at other males as a territorial call (Littlejohn and Harrison, 1985). A pattern of one or more INs followed by more brisk and elaborate notes is found in the songs of several passerine birds, like the rufous-collared sparrow Zonotrichia capensis, field sparrow (*Spizella pussila)*, American brown creeper (*Certhia familiaris*) and European Certhia, vesper sparrow (*Poocetes gramineus*) and song sparrow (*Melospiza melodia*) (Shiovitz et al.,1975; Richards, 1981). Several recent papers have linked zebra finch INs in particular to motor preparation (Rajan & Doupe, 2013; Rao et al. 2019), with the trend of INs going from a variable first IN to a more stereotypical last IN just before the start of song being similar to the sequence of preparatory neural activity seen in primates (Shenoy et al. 2011). Additionally, previous studies have also found preparative neural activity in brain regions responsible for song control long before the first IN of the bout (Hessler and Doupe, 1999; Kao et al., 2008; Rajan, 2018; Woolley et al., 2014). This suggests that INs represent only a portion of motor preparatory activity, rather than its entirety. So, INs in zebra finches are attributed crucial motor preparatory functions and could play a huge role in learning more about this highly relevant topic. Introductory whistles in golden-crowned sparrows are additionally known to act as an innately recognizable prompt that directs future learning of song in nestlings (Soha and Marler, 2000; Hudson and Shizuka, 2017). These introductory whistles are so innate in these birds that young male sparrows raised fully in isolation go on to sing simple songs consisting mostly of repetitive introductory whistling (Marler, 1970). Thus, while several hypotheses exist to explain these gestures, it is broadly agreed that these functions can be classified into functions that either enhance the communicative power of the display, act as recognition signals or have motor preparatory functions, all of which are vital for the animal. Learning more about these traits are very important in order to learn more about the behaviours of these animals and even the mechanisms underlying said behaviours.

Coming to introductory notes in zebra finches, several properties of these INs have been described. In these birds, INs are shown to have both innate and learnt components. How many times the IN gets repeated and the acoustic properties of the INs are two things juvenile birds learn socially from its tutor. However, they show an innate biological bias to create short-duration vocalisations as INs, about 3 times before initiation of their song, regardless of their tutor songs (Kalra et al., 2021). The number of INs are also dependent on the presence of calls (i.e. non-song vocalisations) prior to the start of the first IN. The acoustic state of the first IN produced before the song was also seen to correlate with the time taken to initiate the song (Rao et al., 2019). In zebra finches, as the bird goes from the first IN to the last IN before the motif, median inter-syllable interval becomes shorter and fluctuations in inter-syllable interval across renditions decrease. Looking at IN acoustic properties, it is seen that INs become louder, shorter, higher in mean frequency, and change in their entropy as the IN sequence progresses to the last IN before motif onset. Irrespective of the number of INs before a bout, it was seen that the syllable properties were similar for INs at a given position relative to the last IN. Thus, it would seem that in spite of differing initial conditions, the fluctuating numbers of INs always ensure that the song motifs start from a common premotif state. IN-related activity in the neurons of the premotor nucleus HVC was also seen to reach a unique common premotif state before the last IN (Rajan and Doupe, 2013). These findings taken together imply that INs represent preparation prior to the motif initiating, making sure that the bouts always begin from the same state, irrespective of the start conditions. It was also found that this progression of INs to a common premotif state is not dependent on any real-time sensory feedback, rather depending on internal neural processes. (Rao et al., 2019)

Zebra finches belong to the Estrildidae family, which are a group of small seed-eating songbirds naturally occurring across Africa, southern Asia and Australia. However, due to their popularity as cage birds, they have been introduced to various new habitats across the world. This family consists of birds with similar behaviour and body structure, but differ a lot in plumage and appearance. A defining character of the family is a pointed beak which is short yet thick. These birds vary in body size from 8 cm (in *Nesocharis shelleyi*, commonly called Shelley's oliveback) to 17 cm (in *Padda oryzivora,* commonly known as Java Finch) and vary in body mass from 6 g (in *Estrilda troglodytes,* commonly called black-rumped waxbill) to 25 g (in Java Finch). This family is thought to have diverged from the Viduidae family approximately 15.5 million years ago, and its most recent common ancestor is thought to have lived around 10.9 million years ago. This family, comprising of 40 genera, is further divided into 6 sub-families: Poephilinae (10 genera), Lonchurinae (6 genera), Erythrurinae (2 genera), Estrildinae (10 genera), Amandavinae (3 genera) and Lagonostictinae (9 genera) (Olsson & Alström, 2020).

While beginning any study into birdsongs, it is helpful to know what all factors will affect the song of a bird and its syllables, be they ecological, phylogenetic or physiological. Several studies have found the existence of ‘dialects’ in songbirds, which refers to how songs within a population of a species contain certain salient characteristics/patterns which differ from those

found in other populations of the same species. This is a geographic phenomenon which facilitates reproductive isolation and thus continued divergence among populations. Dialect evolution is attributed to limited or biassed dispersal of birds and to the fact that errors crop up while songs are learnt via vocal transmission (Podos and Warren, 2007). Coming to body size, it is a notable correlate of animal vocalisation in general. In birds specifically, larger bodies mean a larger syrinx, which translates into harsher constraints on tissue flexibility for abrupt frequency modulations. This means that birds with a larger body find it harder to produce higher fundamental frequencies (i.e. an inverse relation between body size and song frequency) (Wallschager, 1980) and more rapid frequency shifts in their songs. Bill size and shape also affect song syllables, with the latter in particular being able to filter the tonal elements produced from the syringeal pathway (Demery et al. 2021). Thus, bill size & shape, geographic distribution and body size are some of the important factors influencing bird song.

Of all the defining syllable properties studied for correlation with various traits, frequency is the trait we know most about. For instance, ambient noise has a lot of power in deciding which frequencies a bird might choose to ensure maximum propagation of its song (Brenowitz, 1982a and 1982b). Phylogenetic relationships between the birds must also be examined because the size-frequency correlation has been found to differ among certain groups of closely related species (Ryan, 1985b). Habitat nature, i.e. how heavily forested a habitat is, has also been found to be an important factor. Studies have shown the existence of certain frequency windows in low-forested habitats where sound attenuates very little, meaning birds there prefer to produce songs in these frequency windows (Morton, 1970 and 1975). It has also been found that birds attempt to create lower frequency sounds irrespective of body size or, to a certain extent, phylogeny in these low-forested habitats. This is because lower frequencies transmit the best in these habitats (Ryan & Brenowitz,1985). So, some of the factors affecting syllable frequency are ambient noise, phylogenetic relationships, body size and habitat nature.

Now, over the years, while there have been several studies that look at song structure across multiple species (Shiovitz et al.,1975; Richards, 1981), there have been no studies that compare INs in particular across species systematically. Since songs as a whole have been found to have species specific characteristics, it is also important to see if INs also have a species specific pattern. The presence of a ‘universal’ trend in INs across species would shed more light into the function & relevance of introductory notes. The absence of such a trend would imply a more species specific, innate, component for introductory note production.

So, for my thesis, I studied IN structure and features of different Estrildid birds to better understand IN function, and examine some of the phylogenetic, physiological & ecological constraints that have shaped them. I looked at Estrildid finches because so far we have been studying, and know most about, zebra finches and we wish to see how unique the characteristics of INs are to other relatives of zebra finches, i.e. birds that belong to the same family as zebra finches. While my focus is on INs, I have also looked at and quantified some general properties

of the whole birdsong of these species as it would allow me to compare and correlate properties of INs with properties of the song that follows. This would help provide a context to studies on motor preparatory function of INs in Zebra finches, letting us know to what extent we can generalise our findings to other birds of this family as well as providing an avenue of further research based on the extent of the universality observed.

Methods

Birds:

The birds chosen for the purposes of this study all belong to the Estrildidae family. I used good, clear audio recordings from birds belonging to each of the following species.

S. no.	Bird	Scientific name	Abbreviations used in text
1	Zebra Finch	*Taeniopygia castanotis*	ZF
2	Bengalese finch	*Lonchura striata domestica*	BF
3	Java finch	*Padda oryzivora*	JF
4	Spice finch	*Lonchura punctulata*	SF
5	African silverbill	*Euodice cantans*	ASB
6	Indian silverbill	*Euodice malabarica*	ISB
7	Gouldian finch	*Chloebia gouldiae*	GF
8	Blue-capped cordon-bleu	*Uraeginthus cyanocephalus*	BCC
9	Long-tailed finch	*Poephila acuticauda*	LF
10	Star finch	*Bathilda ruficauda*	StF
11	Red-billed firefinch	*Lagonosticta senegala*	RBF
12	Lavender waxbill	*Glaucestrilda caerulescens*	LW
13	Cherry finch	*Aidemosyne modesta*	CF
14	Double-barred Finch	*Stizoptera bichenovii*	DbF
15	Red-cheeked cordon-bleu	*Uraeginthus bengalus*	RCC
16	Diamond firetail	*Stagonopleura guttata*	DfT
17	Cut-throat finch	*Amadina fasciata*	CtF

Table1. List of Species, their scientific names and the abbreviations used for them in the text.

Of the 17 species listed here, only 3 species were available in the lab: Zebra, Bengalese and Java finches. Thus, for the other species, I relied on raw unlabelled audio recordings collected by

other songbird labs. At the start, the recordings were obtained from birds based on the following conditions:

1. Must have clear, legible isolated recordings (no group recordings from colonies)
2. Must have recordings from at least 5 different individuals per species.
3. Songs should be undirected songs.

However, in order to maximise the range of species that we could look at, we had to compromise on the number of individuals available from each species as well as the number of song bouts from each bird. Thus, the final list of birds we used in our analysis are as follows:

S. no.	Species	No. of individuals Studied	Avg. No. of Bouts Analysed per Individual bird
1	Zebra Finch	8	20
2	Bengalese finch	7	20
3	Java finch	6	20
4	Spice finch	6	20
5	African silverbill	6	20
6	Indian silverbill	5	20
7	Gouldian finch	6	10
8	Blue-capped cordon-bleu	8	20
9	Long-tailed finch	4	5
10	Star finch	6	20
11	Red-billed firefinch	2	3
12	Lavender waxbill	1	5
13	Cherry finch	6	20
14	Double-barred Finch	6	20
15	Red-cheeked cordon-bleu	2	5
16	Diamond firetail	1	7
17	Cut-throat finch	2	20

Table 2. List of number of individuals and number of bouts per individual bird used for analysis for each species.

Thus, of the 17 species, 6 species had songs from less than 5 individuals per species and 6 species had less than 20 song bouts per individual.For some birds, like the Diamond firetail & Cut-throat finch, the songs were recorded from a group setting rather than isolated recordings. We do not have any information about the relatedness of birds from each species.

To obtain the natural distribution data of each species, I used the eBird website (https://ebird.org/home), which is an online database with reliable information on bird observations in the wild, providing real-time data about bird distribution and abundance around the world.

Recording songs:

The following methods were used in the collection of songs from zebra finches, java finches and bengalese finches in the Rajan lab.

1. The bird being recorded was isolated for a minimum of 24 hours within a sound-proof recording box.
2. A microphone (model AKG C 417 PP) was attached to the roof of the bird's cage and was connected to a mixer that was only used as an amplifier.
3. Data from the microphone was saved on a computer by connecting the mixer output to the sound card. Data was recorded using a custom-written python-based program called PyCBS.
4. Alsamixer, a Linux-based graphical program was used to configure the sound settings and adjust the volume settings.
5. Audio files were saved in 'wav' format at 44100 Hz sampling rate.

Figure 2. Picture of the recording setup. The microphone is attached to the middle of the roof and the bird is provided with seed and water in the cage itself

Song Files obtained from collaborators:

As mentioned above, most of the songs we used in our analysis were obtained from our collaborators across the world. Their names and the species they gave are tabulated below.

S. No.	Collaborator	Birds
1	Dr. David Mets, Prof. Michael Brainard, Dr. William H Mehaffey (UCSF, San Francisco)	African Silverbill, Indian Silverbill, Spice Finch
2	Dr. Nao Ota (Max Planck Institute of Biological Intelligence, Seeweisen)	Blue-capped cordon-bleu
3	Dr. Henrik Brumm (Max Planck Institute of Biological Intelligence, Seeweisen)	Gouldian Finch
4	Prof. Kazuhiro Wada, Zhuocheng Hu and Feiyang Chen (Hokkaido University, Sapporo)	Star Finch, Spice Finch, Double barred Finch, Cherry Finch, African Silverbill
5	Prof. Sarah M N Woolley (Columbia University, New York)	Double barred Finch, Lavender Waxbill, Longtail Finch, Red billed Firefinch, Red cheeked Cordon-bleu, Star Finch
7	Dr. Sue Anne Zollinger, Alyssa Maxwell (Manchester Metropolitan University, Manchester)	Cut throat Finch, Diamond firetail, Double barred Finch, African Silverbill, Gouldian Finch

Table 3. List of collaborators and the species whose songs they gave

In most cases, the songs were provided such that each audio file contained only one song bout. However, in some instances, a single audio file contained multiple song bouts from an individual bird. In this case we used the presence of silence > 2s to separate vocalisations into bouts.The single audio file was split into multiple files containing one bout each using audio editing software like Audacity.

Analysis of Birdsongs:

All the raw audio files were screened for songs and labelled by custom Matlab based programs: *Screen-Song-File-Keyboard-Modified.m* and *Auto-Song-Segment-Label.m* (ASSL) (Created and developed by Raghav Rajan). *Screen-Song-File-Keyboard-Modified.m* (Screening) is used to

select proper audio files, whereas the ASSL (Labelling) labels individual syllables within the songs in the given files.

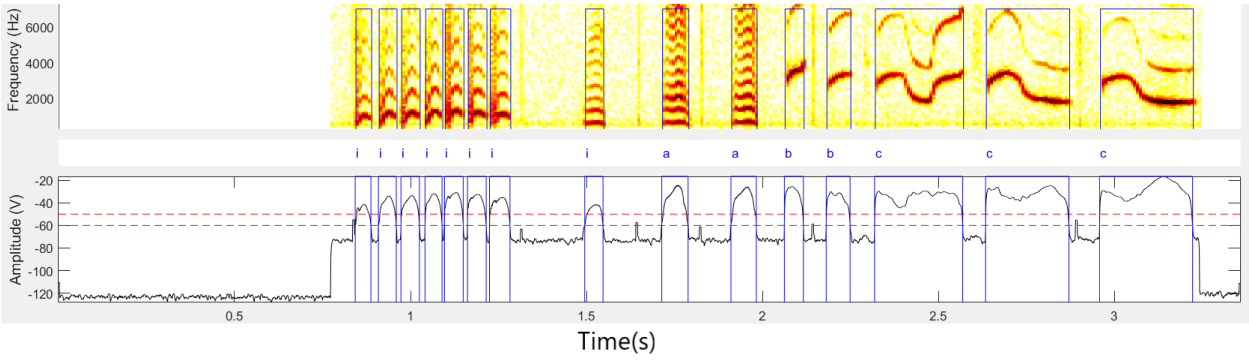

Figure 3. An example of a spectrogram as seen with ASSL (Labelling) on top and amplitude levels displayed below

The syllables were visualised using spectrogram representation, which is a visual representation of the various spectra of frequencies carried by a signal as it changes with time. The X-axis represents time and the Y-axis represents frequency. The amplitude of a given frequency at a given time is represented by the intensity or colour of each point in the image. The ASSL software used amplitude thresholds provided by the to segment the syllables. Syllables were then manually assigned labels, based on their visual appearance on the spectrogram. Similar looking syllables were given the same label. . While assigning labels to the syllables, calls and motif syllables were annotated differently for all the birds. Labelled song data with information such as syllable name, onset time, offset time, frequency, amplitude etc. was saved as an OnsetOffset text file.

Processing of Onset-Offset files:

After annotating the syllables, the output obtained as Onset Offset files were processed. Firstly, we inserted ‘Start’ and ‘End’ syllables at the
beginning and end of each song bout, with a silent gap of at least 2 seconds being used to determine the end of one song bout and the beginning of the next. The next step was to exclude bouts with very few unique syllables (less than 3 unique syllables). This was to ensure that we got proper, complete song bouts and not incomplete motifs or a collection of calls. These processed files were then used to calculate transition probability matrices, quantify different acoustic features, create plots and do statistical tests.

Calculations:

	Start	i	a	b	c	d	End	k	l
Start	0	0.94	0	0	0	0	0	0.06	0
i	0	0.27	0.73	0	0	0	0	0	0
a	0	0	0.24	0.76	0	0	0	0	0
b	0	0.09	0	0	0.75	0	0.12	0	0
c	0	0	0	0	0	0.78	0	0	0.14
d	0	0	0	1	0	0	0	0	0
End	0	0	0	0	0	0	0	0	0
k	0	0.75	0.12	0	0	0	0.12	0	0
l	0	0.07	0.4	0	0	0	0.05	0	0.48

Table 4. An example of a transition probability matrix. The alphabets a to i represent the song syllables while k & l represent calls of the bird. 'Start' and 'End' represent the beginning and end of the song respectively. The numbers represent the transition probability from one syllable to another.

The transition probability matrices, which looked at the probability of occurrence of every syllable transition possible, were calculated for each individual bird.

We calculated different quantifiers of song (Scharff et al., 1991; Hampton et al., 2009) to analyse their sequence stereotypy using the following formulae:

a) Sequence Linearity = No. of unique syllables / No. of unique transitions

Sequence Linearity is a measure of how many different ways the syllables are ordered in the song. It is calculated by dividing the total number of unique syllables in the bird's repertoire with the number of all possible syllable transitions for the bird. Sequence linearity values of a song vary from 0 to 1 where 0 corresponds to a highly nonlinear song, with very low stereotypy and a value of 1 would mean a perfectly linear, and a completely stereotyped, song.

b) Sequence Consistency = Sum of typical transition probabilities / Sum of all transition probabilities

Sequence Consistency is a measure of how often a particular sequence of syllables is produced within the song. It is calculated by dividing the sum of typical transition probabilities with the sum of all transition probabilities. Here, typical transition probability refers to the highest transition probability from a given syllable to any other syllable. Sequence consistency values of a song will be in the set (0,1], where a low consistency value would mean the absence of a

typical sequence of syllables in the song (i.e. very low stereotypy) and a value of 1 would mean the typical syllable sequence is reproduced perfectly (i.e. a completely stereotyped song).

c) Transition entropy = $\sum -P_i \log_2 P_i$

Transition entropy is a measure of the variability inherent in the song. Here Pi is the probability of any existing transition and the $-P_i \log_2 P_i$ term is summed over all possible transitions by the bird. Transition entropy values will always be in the set $(0,\infty)$, where a very low entropy value would mean the song is highly stereotyped and a very high value would mean the song is highly variable.

To find out how many times the bird repeated its syllables, the number of syllables per bout was divided by the number of unique syllables it possessed.
To look at the properties of initial syllables, we took the first five syllables that occurred at the beginning of each bout and then averaged the required acoustic properties (Mean frequency, log amplitude, syllable duration, Entropy variance and intersyllable interval) out for each bird. The acoustic property values were obtained from the onset offset files, which is calculated based on Sound Analysis Pro 2011 (http://soundanalysispro.com/). We then calculated the average acoustic property values for each species to use in our analysis.
To look at the properties of repeated start syllables, we took the start syllables of each bird which was getting repeated and then averaged out the required acoustic properties for each bird. Then we calculated the average acoustic property values for each species to use in our analysis.

Code:

The code used to calculate the various parameters were written in Python and can be found on the following Google drive folder, along with additional R scripts used in the study: https://drive.google.com/drive/folders/1IJRqmNb_SDiHBFpwZechCtJZ-olC5g3Q?usp=sharing .

GraphPad Prism 8.4.3 was used to plot most of the figures and to run statistical tests. The Shapiro–Wilk test was used to test for the normality of data. Based on whether the data came from a gaussian distribution or not, either a One-way ANOVA test or a Kruskal–Wallis test was used to test for the statistical significance of the data. We calculated Pearson correlation coefficient and Spearman's rank correlation coefficient to check for correlations between two data sets. To obtain fitting data like R squared values, adjusted R squared values and slopes, custom RStudio Desktop scripts were used.

Results

1. Geographic Distribution

Since geographic distribution is a trait of the birds that I was interested in, I decided to find out the natural distributions of the species whose song I collected. In the figure below (Fig 4) is mapped out the geographic ranges across which these birds are naturally found.

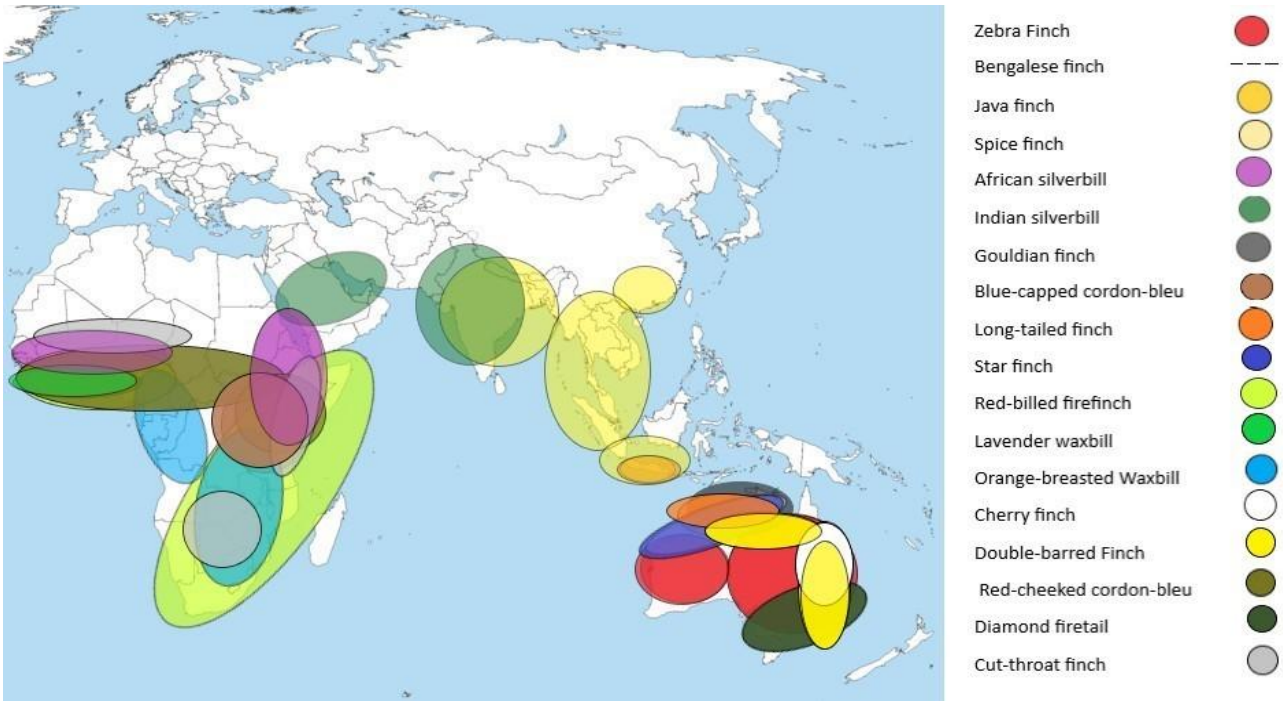

Figure 4: Natural Geographic Distribution of Estrildid Finches used in the study. Each translucent oval represents the entire range of a species. The species identity denoting each colour is explained in the legend described alongside the figure. The distribution map was created based on bird sightings as compiled by the eBird online database.

The distribution map was compiled based on information from the eBird website, which is an online database with reliable information on bird observations in the wild, providing real-time data about bird distribution and abundance around the world. The species are seen to be naturally distributed across sub-saharan Africa, South-East Asia and the whole of Australia. The

maximum number of species are concentrated in the East & West coasts of Africa and the North & East coasts of Australia. The Bengalese finch is a completely domesticated species and so does not have a natural geographic distribution.

2. Phylogeny

I mapped the different species onto a phylogenetic tree, adapted from Olsson & Alström (2020), the latest and most detailed study till date to look at the Estrildidae family. Also shown below is a table containing more detailed information about the genus and subfamilies that each of these species belong to.

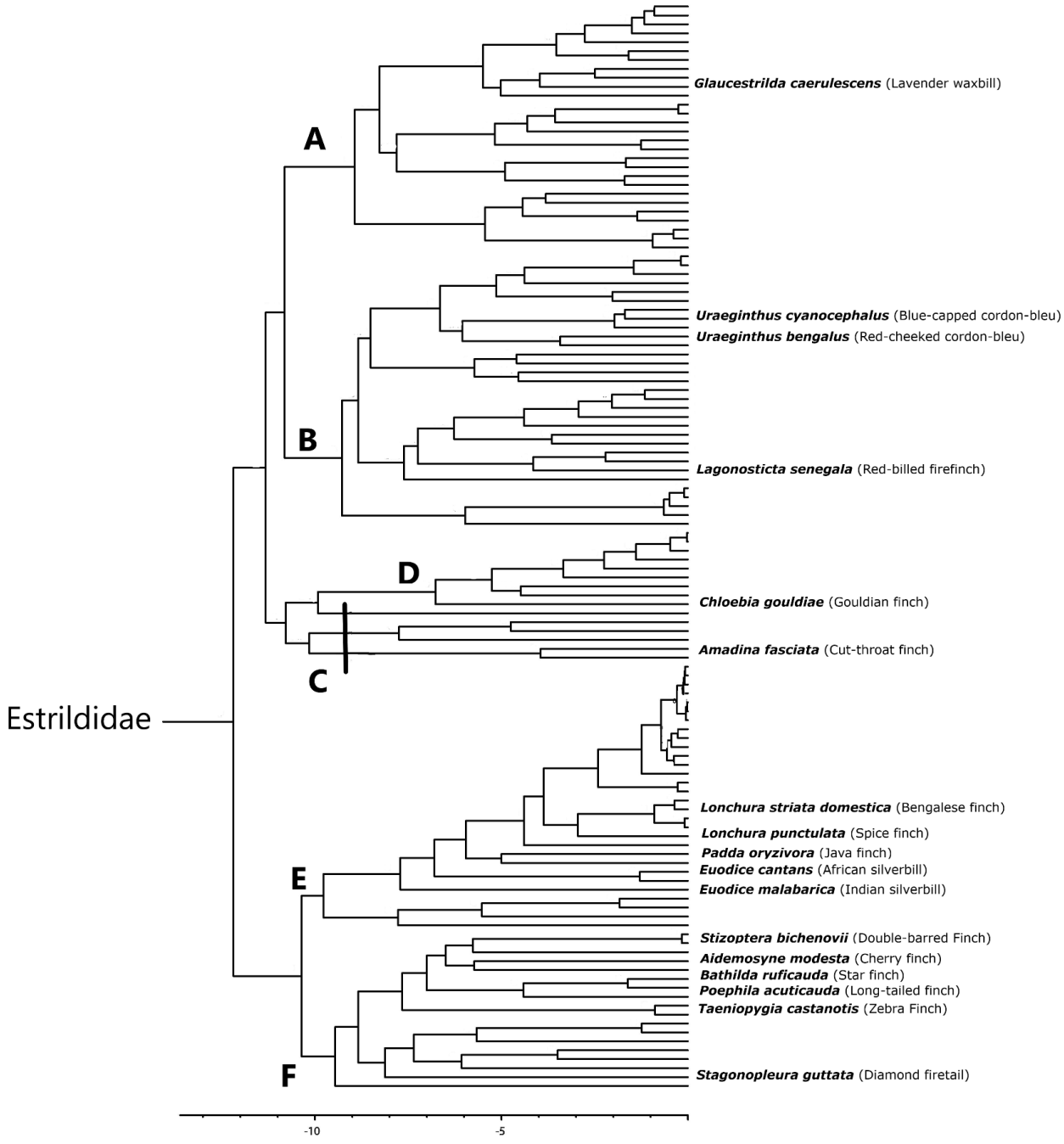

Figure 5: **Phylogeny tree.** Adapted from Olsson & Alström, 2020. The time axis at the bottom is at the scale of millions of years, beginning at the present day. From it we get a sense of when a clade we are interested in diverged from its closest sister clade. The alphabets A to F represent the 6 subfamilies.

Bird	Genus	Subfamily
Zebra Finch	Taeniopygia	Poephilinae
Bengalese finch	Lonchura	Lonchurinae
Java finch	Padda	Lonchurinae
Spice finch	Lonchura	Lonchurinae
African silverbill	Euodice	Lonchurinae
Indian silverbill	Euodice	Lonchurinae
Gouldian finch	Chloebia	Erythrurinae
Blue-capped cordon-bleu	Uraeginthus	Lagonostictinae
Long-tailed finch	Poephila	Poephilinae
Star finch	Bathilda	Poephilinae
Red-billed firefinch	Lagonosticta	Lagonostictinae
Lavender waxbill	Glaucestrilda	Estrildinae
Cherry finch	Aidemosyne	Poephilinae
Double-barred Finch	Stizoptera	Poephilinae
Red-cheeked cordon-bleu	Uraeginthus	Lagonostictinae
Diamond firetail	Stagonopleura	Poephilinae
Cut-throat finch	Amadina	Amandavinae

Table 5: Table containing more detailed phylogenetic details about the species.

The data included species from all 6 different subfamilies. The Poephilinae subfamily has the most number of representatives, followed by the Lonchurinae subfamily. We also have 3 pairs of species that belong to the same genus: Lonchura, Euodice and Uraeginthus.

3. Song Properties

I first looked at a few song properties across these birds with the aim of comparing IN properties to these song properties. I examined how these song properties varied phylogenetically and geographically.

3.1. Number of Unique syllables per bout

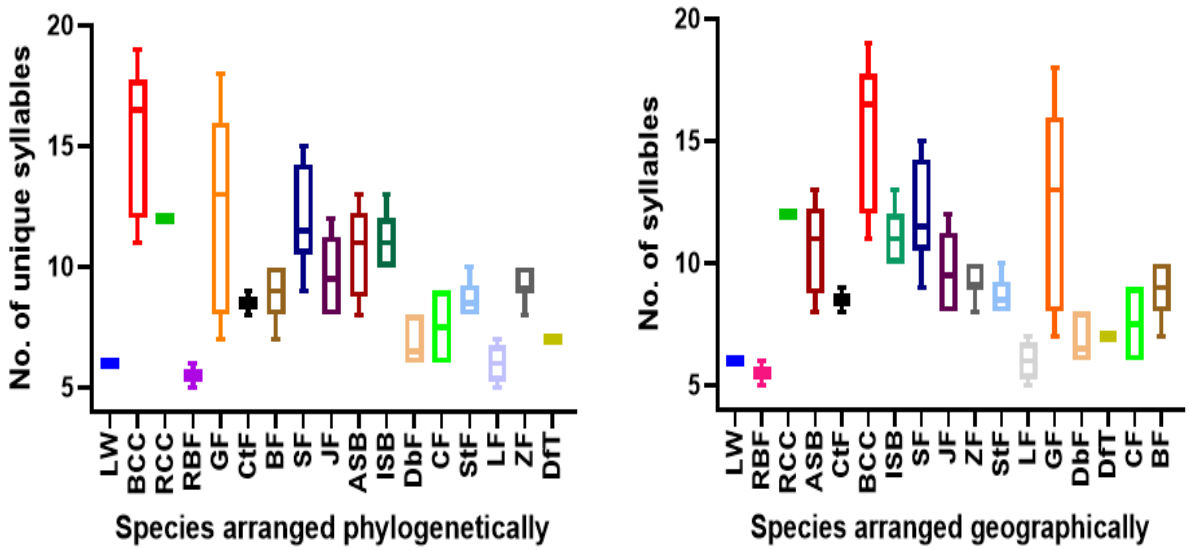

Figure 6: Number of unique syllables per species. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the number of syllables.

I calculated the total number of unique syllables that each species is capable of producing so as to see if a vast repertoire of syllables allows the bird to produce more kinds of IN syllables within its song. There does not seem to be any discernible trend across species either phylogenetically or geographically. Statistical tests were performed to determine if the number of syllables varied significantly across species. From the Kruskal-Wallis test it was found that it did vary significantly (P value <0.0001). From this result, we get an idea of how broad the syllable repertoire of each species is, which makes the idea that birds would still prefer to start their syllables with the same IN syllables all the more intriguing.

3.2. Average number of syllables per bout

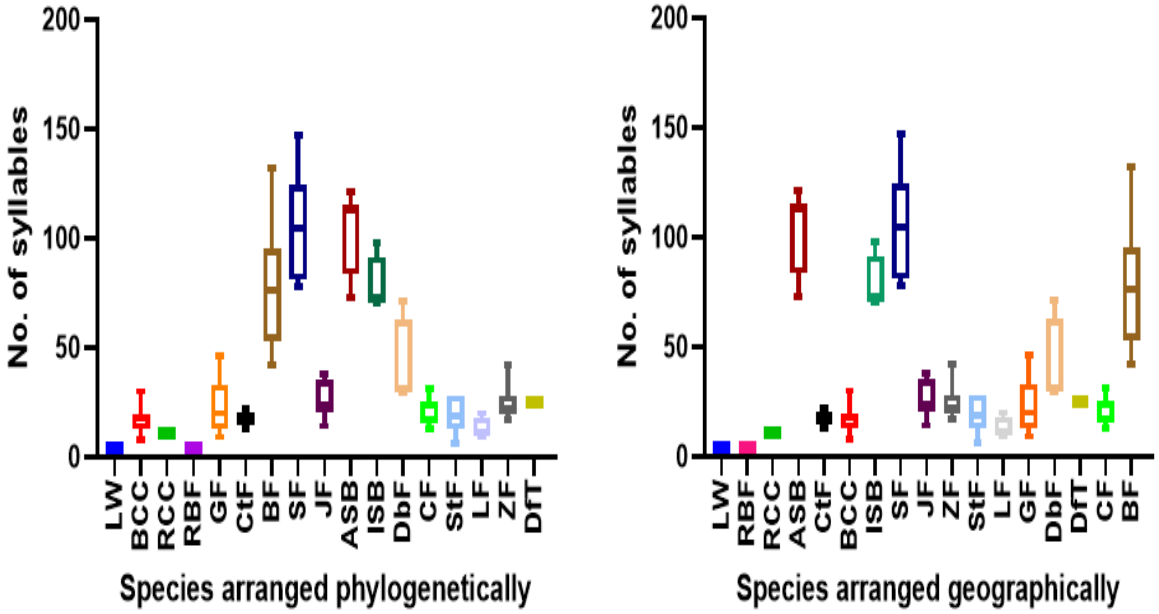

Figure 7: Average number of syllables per bout. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the number of syllables.

I calculated the average number of syllables each species can produce per bout to see how birds varied the lengths of their songs across species and to later on see if song length had a bearing on properties of INs. We can see a discernible trend in the average number of syllables produced per bout across species phylogenetically and geographically (Figure 7). In the phylogeny graph on the left, we see that species belonging to the Estrildinae, Lagonostictinae, Erythrurinae, Amandavinae and Poephilinae subfamilies tend to produce less number of syllables (less than 20) while the species of the Lonchurinae subfamily produce much higher numbers (more than 40). An outlier here are the Java Finches. In the geography graph on the right, we can see that both ISB and SF (which are found in the Indian subcontinent) do tend to produce a very high number of syllables when compared to their closest geographical neighbours. Statistical tests were performed to determine if the average number of syllables per bout varied significantly across species. From the Kruskal-Wallis test it was found that it did vary significantly (P value <0.0001). Here, we see that some birds produce much more syllables in their songs than the number of unique syllables they can produce.

3.3. Average repeats of a syllable

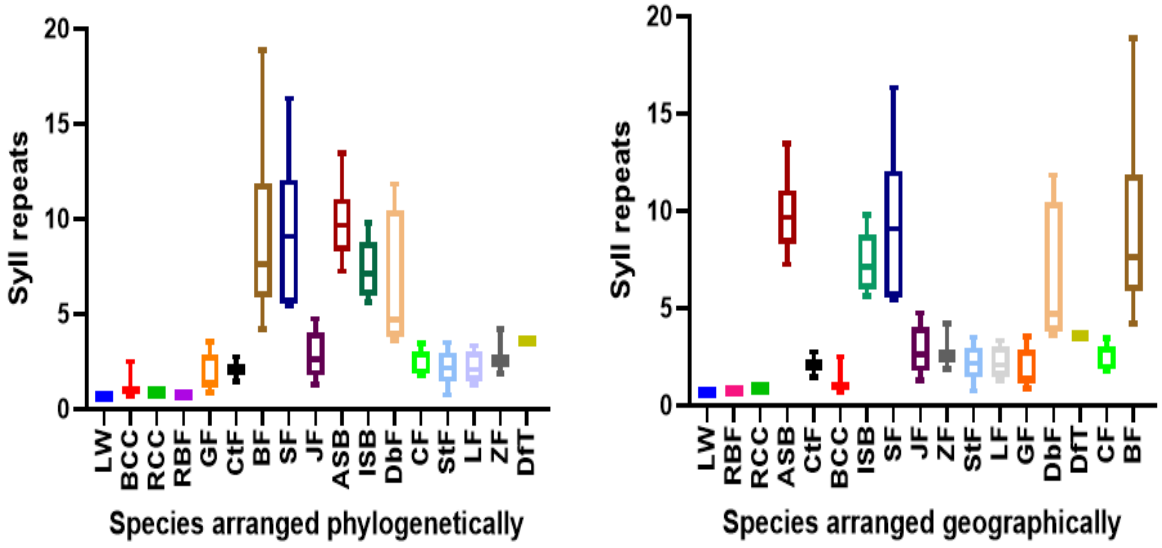

Figure 8: Average repeats of a syllable. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the number of times a syllable is repeated.

From the previous section (figure 7), I could see that some species produce syllables in their songs that were much more than the unique syllables in their repertoire. This is made possible by repeats of its syllables and so, I looked at how the number of times a syllable is repeated varies across species. Since a characteristic of INs is their repeatability, I wanted to see any links between the number of times a bird repeats syllables in its song and the number of times INs are repeated. In the figure, we can see a discernible trend across species phylogenetically and geographically. In the phylogeny graph on the left, we see a similar trend as the number of syllables per bout, i.e. species towards the ends tend to repeat syllables less (less than 3 times) while the species in the middle repeat syllables more (more than 5). An outlier here are the JFs, which despite being in the middle only repeats around 3 times. In the geography graph on the right, we can again see that both ISB and SF (which are found in the Indian subcontinent) do tend to produce more repeats than its geographic neighbours. So here again, we seem to see both a phylogenetic as well as geographic trend. But the geographic trend is observed only for 2 species and the phylogeny trend is seen for almost all the species barring one exception. Statistical tests were performed to determine if the number of times a syllable is repeated varied significantly across species. From the Kruskal-Wallis test it was found that it did vary significantly (P value <0.0001). Here, we can see that species like BF, SF, ASB, ISB and DbF repeated their syllables much more than the other species, which was expected considering that these species also tend to have many more syllables in a bout than the other species.

3.4. Song Stereotypy

The next step in our efforts was to study how consistent the song of each species was across multiple renditions. This was important because I wished to see if and how song stereotypy was related to similarities found in INs across the species. To see if IN trends across species still held true independent of song stereotypy. Thus I looked at the following three song consistency measures as described in the Methods section: sequence linearity, sequence consistency and transition entropy.

3.4.1. Sequence Linearity

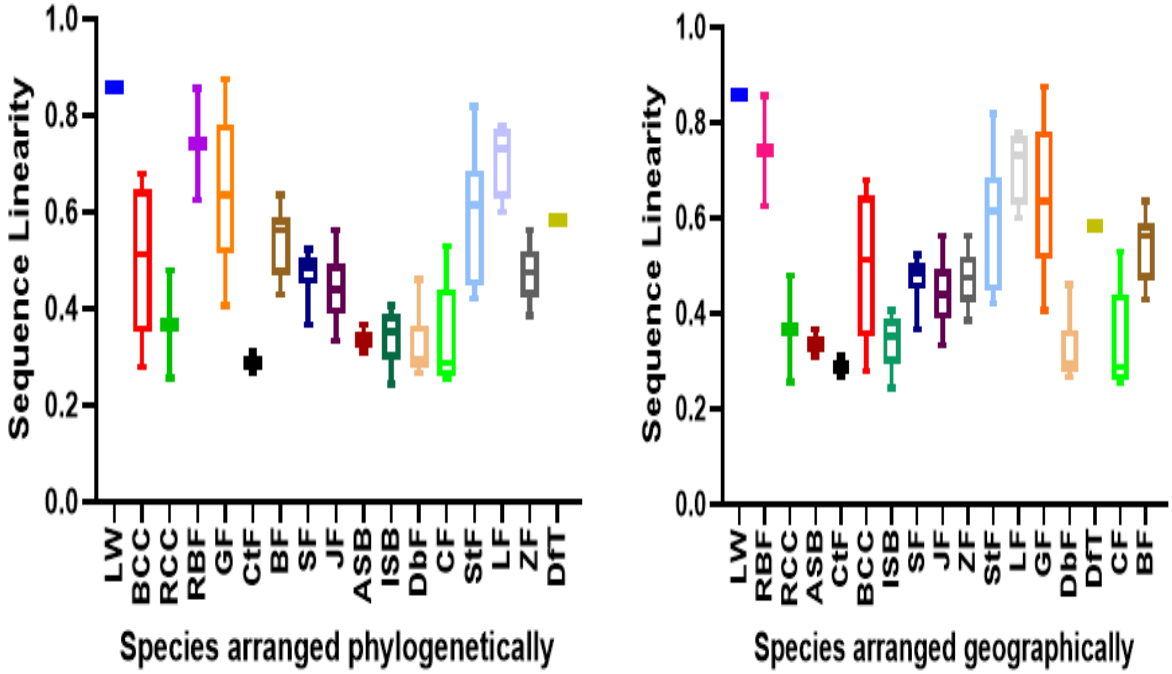

Figure 9: Sequence Linearity. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the sequence linearity.

Here, there does not appear to be any trends across species, either phylogenetically or geographically in how sequence linearity varies across species (Fig 9). We do see that BCC, GF, CF and StF species show a large range of linearity values, compared to species like ZF or JF which have similar sample size as them. Using ZF, which are known to have very stereotyped songs, as a benchmark, the results would indicate that 11 of these species (all except CtF, RCC,

ASB, ISB, DbF and CF) have very stereotyped songs (higher the linearity value, higher the stereotypy). Statistical tests were performed to determine if sequence linearity varied significantly across species. From the Kruskal-Wallis test it was found that it did vary significantly (P value <0.0001).

3.4.2. Sequence Consistency

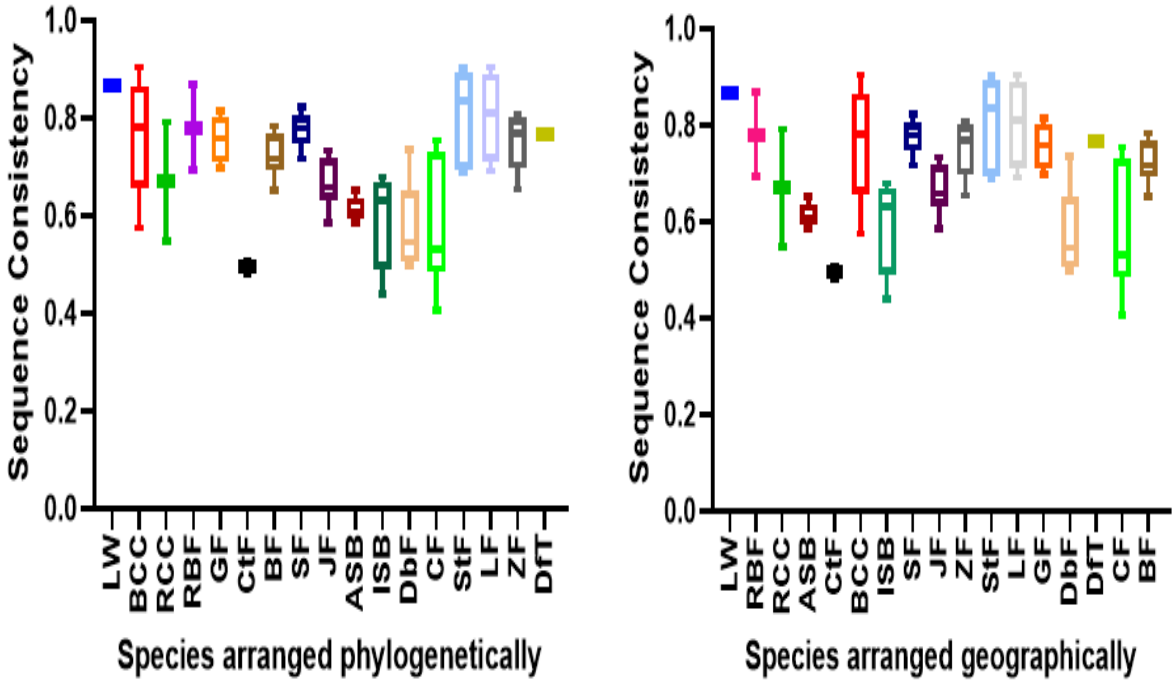

Figure 10: Sequence Consistency. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the sequence consistency.

Here, there does not appear to be any trends across species, either phylogenetically or geographically in how sequence consistency varies across species (Fig 10). We do see that the consistency values for all species are high (higher the consistency value, higher the stereotypy) and that the ranges of these values are also small, especially when compared to the previous sequence linearity values. Using ZF as a baseline, 10 of these species (all except CtF, JF, RCC, ASB, ISB, DbF and CF) have very stereotyped songs. Statistical tests were performed to determine if sequence consistency varied significantly across species. From the One-way ANOVA test it was found that it did vary significantly (P value <0.0001).

3.4.3. Transition Entropy

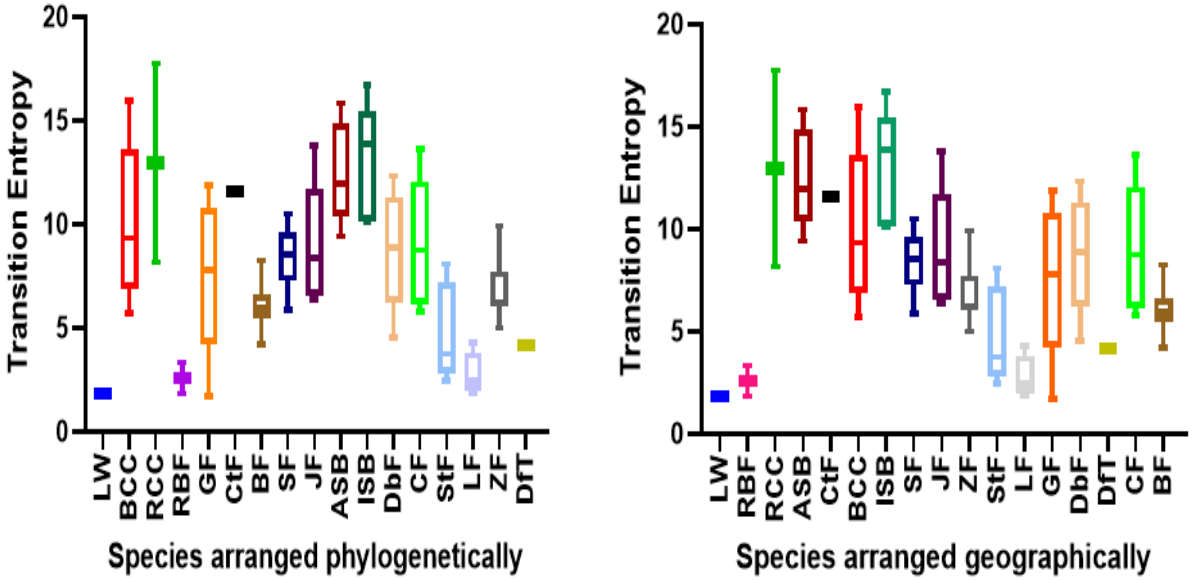

Figure 11: Transition Entropy. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the Transition Entropy.

Here, there does not appear to be any trends across species, either phylogenetically or geographically in how transition entropy varies across species (Fig 11). We do see that the entropy values for most species show a wide range of values irrespective of sample size, except for ZF, SF and BF.
Again, using the highly stereotyped ZF as a baseline, the results indicate that 7 of these species (LW, RBF, BF, StF, LF, DfT and ZF) have highly stereotyped songs (higher the entropy value, lower the stereotypy). Statistical tests were performed to determine if transition entropy varied significantly across species. From the One-way ANOVA test it was found that it did vary significantly (P value <0.0001).

Thus, from the above three matrices of song stereotypy, we can say that at least 7 of these 17 species (i.e. LW, RBF, BF, StF, LF, DfT and ZF) have highly stereotyped songs as measured across these values.

4. Start Syllables

Now, we get into our main focus of Introductory Notes. However, a problem arises here. Till now, we have looked at song properties and parameters which have a preset definition that holds true across species. But now we come across INs which have not been studied across the Estrildidae family. So, I started by looking at syllables with which the song always begins, aka 'start syllables', and their characteristics across species.

4.1. Number of start syllables

The most defining property of INs is that they are the syllables with which the bird begins its song. Thus, I take a look at how many syllables each species uses to begin its song, i.e. how many 'start syllables' does each species have.

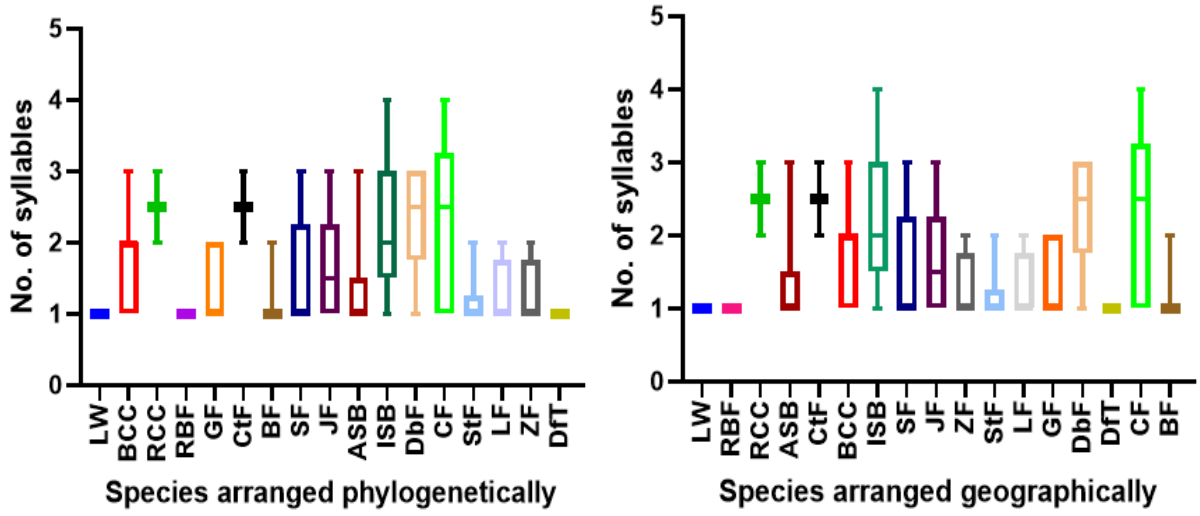

Figure 12: Number of Start syllables. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the number of syllables.

Here, there does not appear to be any trends across species, either phylogenetically or geographically in how the number of start syllables varies across species (Fig 12). We can see that all species on average possess between 1 and 3 start syllables, with the maximum number of start syllables that an individual bird possess being 4 (by a CF and ISB individual bird), which itself is quite low, considering that the species on average has a repertoire of 16 unique syllables. Across species, despite the vast repertoire of unique syllables possessed by each species, it is seen that they only begin their song with less than a handful of syllables. Statistical tests were

performed to determine if the number of start syllables varied significantly across species. From the Kruskal-Wallis test test it was found that it did not vary significantly (P value of 0.0911). Thus, this (along with the fact that all the previous song matrices did vary significantly from each other) reaffirms the notion that Estrildid finches can only begin their songs with a very limited subset of unique syllables, despite possessing a high repertoire of unique song syllables.

4.2. Average number of syllables with high start probability

Now, in figure 12 we saw that there were a handful of ways for these species to begin its song. However, most zebra finches have a single kind of INs, with some birds having two or three kinds (Zann, 1993). As such, I looked at the number of syllables across the species that had the probability of occurring at the start of the song, i.e. start transition probability, greater than 0.25. But why start with such a low probability threshold? After all, if the syllable always comes at the beginning of the song, should it not have a much higher start probability? The low threshold is because of the fact that INs are sometimes included as part of the motif, which is seen in several species (see spectrograms attached in the supplementary information). Thus to compensate for that, a much lower probability threshold was chosen.

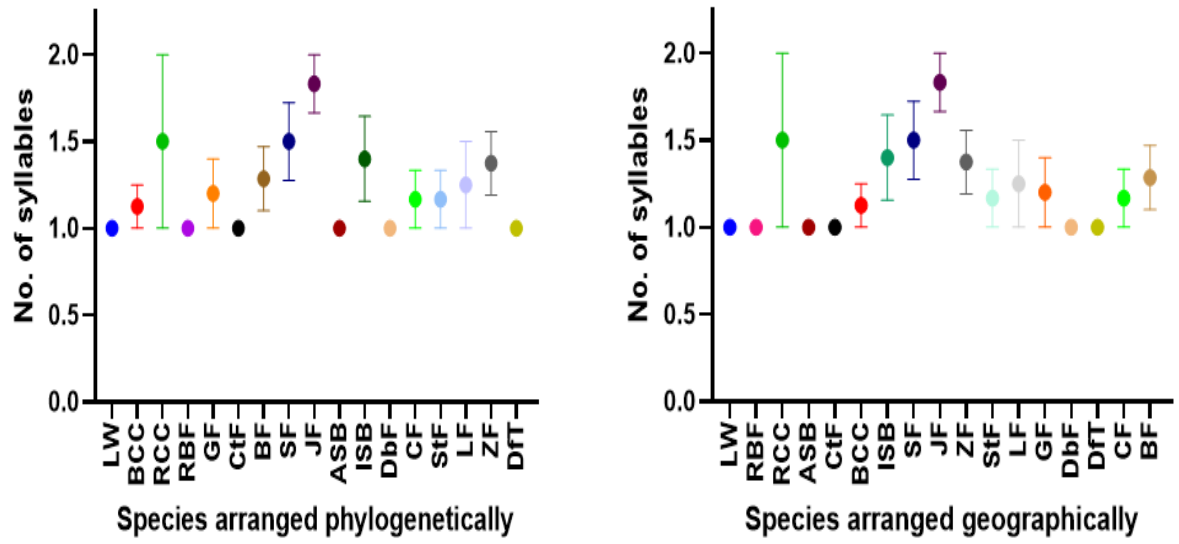

Figure 13: Number of syllables with start probability > 0.25. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the number of syllables. The dot represents the mean of the data while the error bars show the standard error of mean.

Figure 13 shows how the average number of syllables with start probability greater than 0.25 varies across species. While we don't see any trends phylogenetically, geographically we do see

one. While for most species towards the ends of the geography graph, the average number of syllables is one, for the species in the middle, i.e. whose natural habitat is South Asia, they show a peak of slowly rising averages that increase from 1 to 1.8 and then falls back to 1. The outlier to this trend is the RCC and CF, which shows an average of 1.5 and 1.2. BF doesn't count as an outlier because of its lack of a natural distribution. Trends aside, across species, it is seen that there are only one or two syllables with a high start probability, despite that wide array of unique syllables they possess. Statistical tests were performed to determine if the average number of syllables with start probability greater than 0.25 varied significantly across species. From the Kruskal-Wallis test test it was found that it did not vary significantly (P value of 0.1902). This, along with the fact that a geographic trend was observed, makes this an interesting result, in spite of the fact that the start probability threshold might lead to inaccurate results. As such, this result should be used carefully during interpretation.

4.3. Repeats of Start Syllable

Another characteristic property of INs is their repeatability. So in order to characterise that, here I looked at the start syllables and saw how many times on average they get repeated across species.

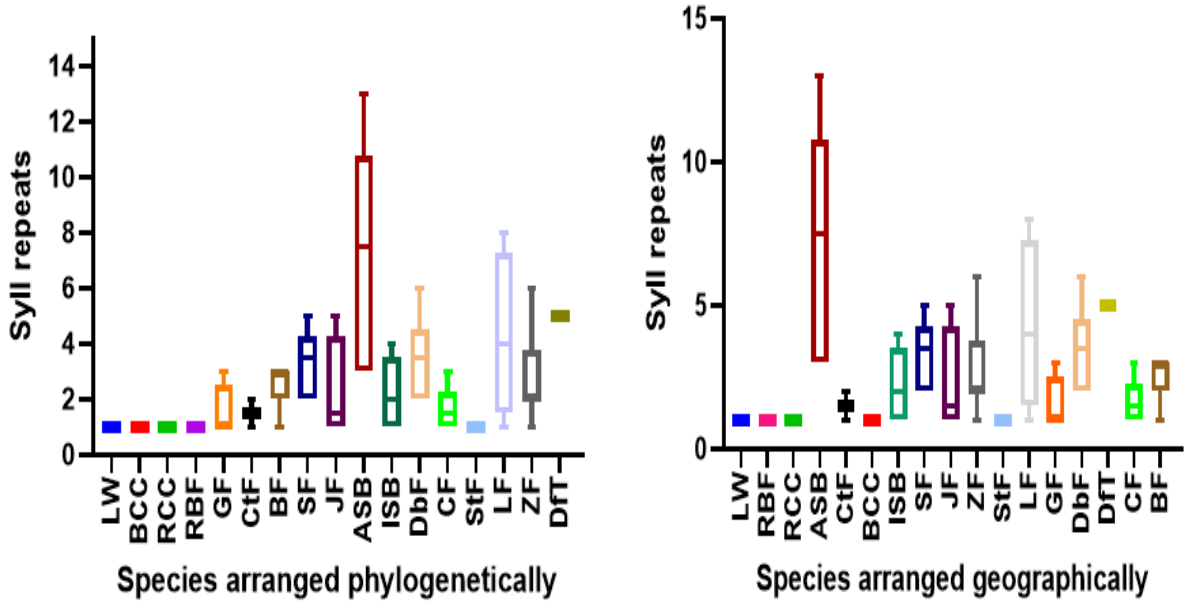

Figure 14: Average repeats of start syllable. The graph on the left has the species arranged on the X axis in the phylogenetic order while the graph on the right has them arranged geographically. The Y axis has the number of times the start syllable is produced.

Figure 14 shows how the number of times a syllable is repeated varies across species. Here, there does not appear to be any trends across species geographically. Phylogenetically, from the graph on the left, it would appear that birds with low repeats of the start syllable (i.e. species LW to JF with 1 to 4 repeats on average) mostly appear on the left hand side of the X axis. The exception to this is StF. One more interesting point is that ASB has a very wide range of average repeats, even when compared to other species with similar sample size. Statistical tests were performed to determine if the number of times a syllable is repeated varied significantly across species and with the Kruskal-Wallis test (P value <0.0001), it was found that it did vary significantly. These results indicate that while some species produce their start syllable only once, the majority of the species (11 of them) tend to repeat their start syllables.

4.4. Repeats per bout v/s Repeats of start syllable

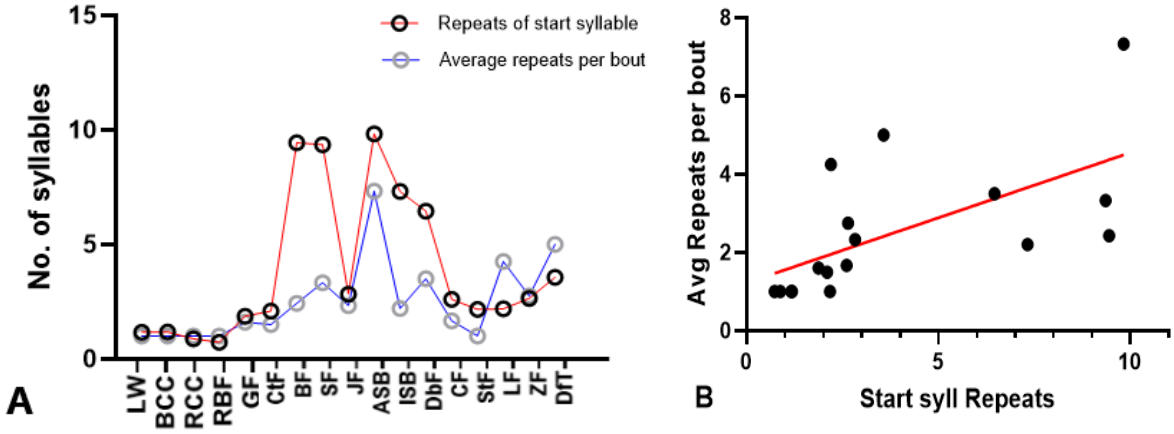

Figure 15 : Repeats per bout v/s Repeats of start syllable. (A) On the X axis are species and on the Y Axis are how many times the syllable is produced. The red line joins the average number of times a start syllable is produced while the blue line joins the average number of times the bird tends to repeat its syllables in the bout. **(B)** X axis has the number of times the start syllable is repeated and Y axis has the number of times the bird repeats syllables per bout. The red line is a simple linear regression.

Since we know that some species tend to repeat their syllables more than others, the above graph was made to see if the rate at which a bird repeats syllables on average is anyway linked to how many repeats of the start syllable it produces. From figure 15 A, we can see that for some species the repeats of the start syllable is similar to the number of times they repeat syllables in their bouts. So, to quantify this correlation, I performed a correlation test and a Spearman correlation coefficient (r) of 0.8115 was obtained (P value = 0.0002), indicating high correlation. Thus,

there is a high correlation between how much a bird repeats syllables and how many times it repeats the start syllable.

4.5. Start Probability v/s Repeat Probability of Start syllables

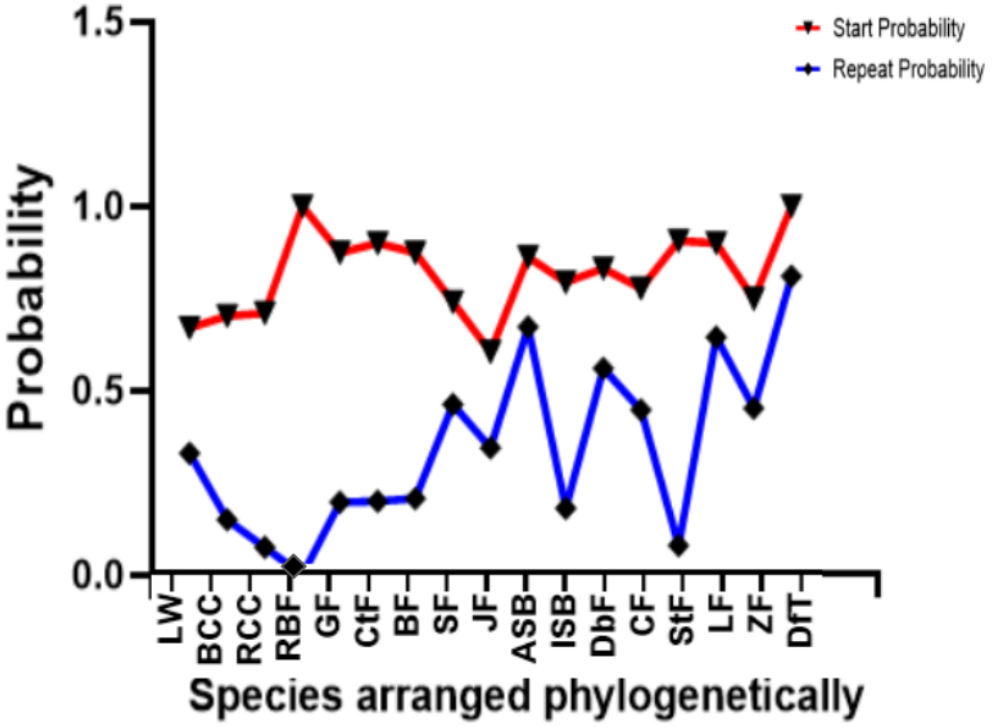

Figure 16: Start probability and repeat probability of start syllables. The Y axis represents probability and the X axis shows the species arranged phylogenetically. The red line joins the average start probabilities of the start syllable while the blue line joins the average repeat probabilities of said start syllable.

The syllable with the highest start probability for each bird was taken and the repeat probability (i.e. probability of a syllable to transition to itself) for this syllable was found. These values were then averaged across each species and the results are shown in figure 16. As expected, the start probabilities of these syllables are all very high across species, ranging from 0.6 to even 1. However, the repeat probabilities show a wide range, from 0 to 0.8. Statistical tests were performed to determine if the highest start probability varied significantly across species and with the Kruskal-Wallis test (P value = 0.1133), it was found that it did not vary significantly. However, it was seen that repeat probability of said start syllable did vary significantly across species (Kruskal-Wallis test P value = 0.0013). Our aim was to see if there existed syllables which began the song and repeated itself consistently across species. However these results

indicate that while some syllables do consistently start the song, they do not equally repeat themselves across species.

5. Syllable Properties

Now that we have looked at the general properties of the start syllables, it's time to look at the acoustic syllable properties of these repeating start syllables. For this, the repeating start syllables were taken and their acoustic properties were measured out as they repeated themselves. The aim was to see how their properties changed from the first start syllable repeat to the last. Since I was looking at the change in the start syllables, I had to exclude birds from my analysis that produced their start syllables only once. As such, the sample sizes of certain species and even some species entirely had to be excluded. So, the list of birds used to get these results are as follows.

S. no.	Species	No. of individuals Studied
1	Zebra Finch	7
2	Bengalese finch	6
3	Java finch	3
4	Spice finch	6
5	African silverbill	6
6	Indian silverbill	3
7	Gouldian finch	2
8	Long-tailed finch	3
9	Cherry finch	3
10	Double-barred Finch	6
11	Diamond firetail	1
12	Cut-throat finch	1

Table 6. List of number of individuals for each species and the species used for calculating acoustic properties of start syllables.

As such, the number of species studied has been reduced to 12 and the number of individual birds per species has reduced as shown in the table.

5.1. Frequency of start syllables

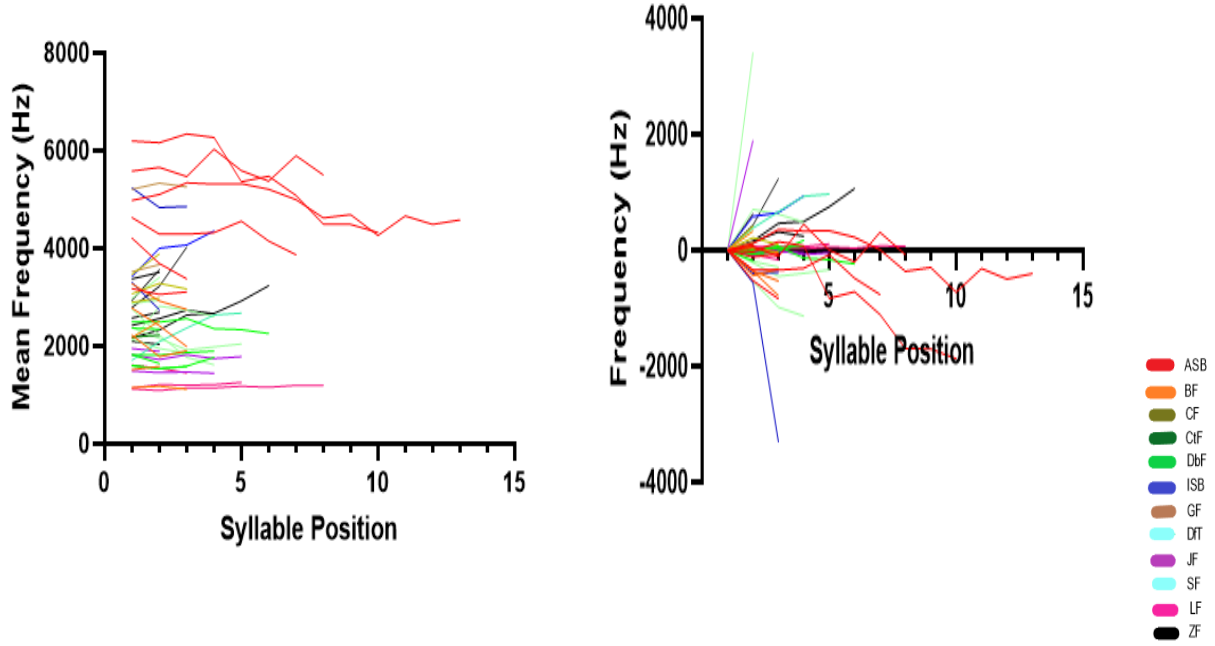

Figure 17: Frequency of start syllables. The X axis represents syllable position while the Y axis represents mean frequency in Hz. Each line in these graphs represents the syllable progression of a single bird.The species that each bird belongs to is indicated by the legend given besides the graphs. The graph on the left shows the frequency values of the syllable progression plotted as is, while the graph on the right shows the frequency values normalised to start from the same point (so as to allow for easier interpretation of the results).

From the Normalised mean frequency graph, it is hard to see a discernible trend across species in how the frequency changes as the start syllables progress to song. To make it more simpler to analyse, these curves were fitted to a simple linear model so as to give their slopes. Their slopes were averaged across each species and were tabulated, along with fitting information like the R^2 values as given below.

Species	**Slope**	**R^2**
ASB	-142.508	0.548007
BF	-72.2834	0.796562
CF	183.0253	0.732828

CtF	39.403	1
DbF	-22.8947	0.701996
DfT	248.8263	0.943602
GF	84.19075	0.567937
ISB	-153.213	0.866235
JF	-26.3236	0.587345
LF	-14.9716	0.862758
SF	1.771333	0.798532
ZF	246.2401	0.950812

Table 7 : Averaged mean frequency slope values across species, along with R^2 values.

All species show a good R^2 value (R^2>0.5). This indicates that a linear regression is a good fit for mean frequency values and for extracting slope information. From the slopes, we can see that half of the species show a decrease in mean frequency as the syllable sequence progresses while the other half show an increase in mean frequency, implying no uniform trend in how mean frequency changes across the species.

5.2. Amplitude of start syllables

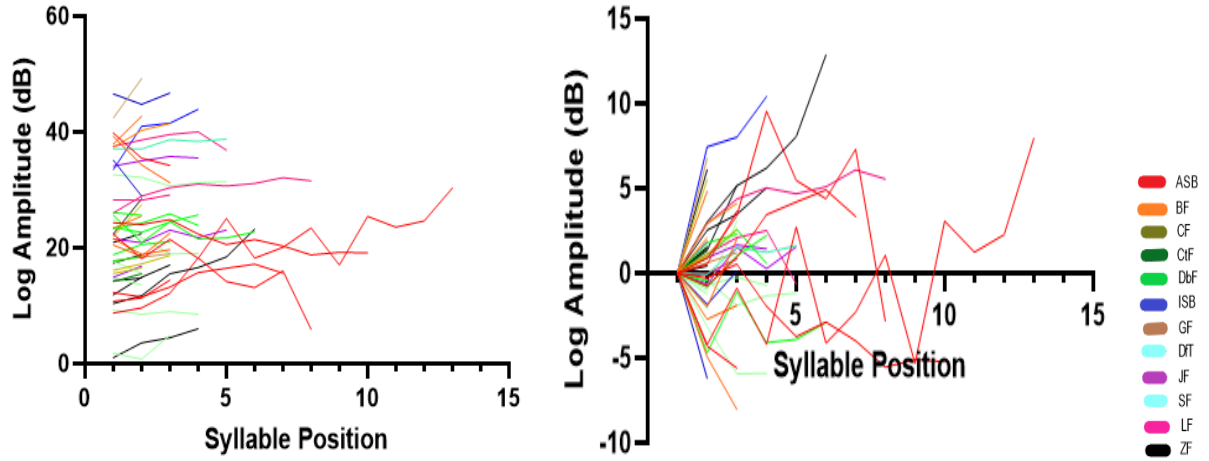

Figure 18: Amplitude of start syllables. The X axis represents syllable position while the Y axis represents Log Amplitude in dB. Each line in these graphs represents the syllable progression of a single bird.The species that each bird belongs to is indicated by the legend given

besides the graphs. The graph on the left shows the amplitude values of the syllable progression plotted as is, while the graph on the right shows the values normalised to start from the same point (so as to allow for easier interpretation of the results).

From the Normalised log amplitude graph, it is hard to see a discernible trend across species in how the amplitude changes as the start syllables progress to song. To make it more simpler to analyse, these curves were fitted to a simple linear model so as to give their slopes. Their slopes were averaged across each species and were tabulated, along with fitting information like the R^2 values as given below.

Species	**Slope**	**R^2**
ASB	-0.01905	0.616248
BF	0.832983	0.774733
CF	2.587667	0.998167
CtF	1.338	1
DbF	0.38845	0.6579
DfT	0.4539	0.7382
GF	3.684	0.99985
ISB	-0.98419	0.611439
JF	0.923467	0.736733
LF	0.371317	0.480668
SF	-0.72003	0.709533
ZF	2.304586	0.9875

Table 8 : Averaged amplitude slope values across species, along with R^2 values.

All species, except one, show a good R^2 value ($R^2>0.5$). This indicates that a linear regression is a good fit for log amplitude values and for extracting slope information. From the slopes, we can see that 3 of the species show a decrease in log amplitude as the syllable sequence progresses while the rest show an increase in log amplitude, implying that most of the species increase their amplitude as the sequence progresses.

5.3. Duration of start syllables

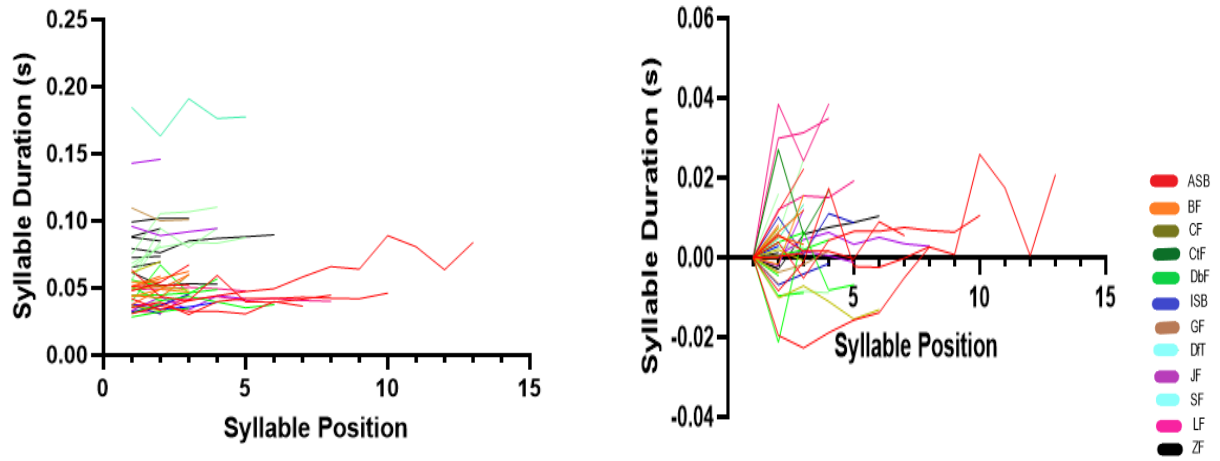

Figure 19: Duration of start syllables. The X axis represents syllable position while the Y axis represents syllable duration in seconds. Each line in these graphs represents the syllable progression of a single bird.The species that each bird belongs to is indicated by the legend given besides the graphs. The graph on the left shows the duration values of the syllable progression plotted as is, while the graph on the right shows the values normalised to start from the same point (so as to allow for easier interpretation of the results).

From the Normalised syllable duration graph, while we can estimate that most of the birds are increasing syllable duration as the start syllables progress to song, it is still hard to discern cross species trends in how it changes. To make it more simpler to analyse, these curves were fitted to a simple linear model so as to give their slopes. Their slopes were averaged across each species and were tabulated, along with fitting information like the R^2 values as given below.

Species	**Slope**	**R^2**
ASB	0.002591	0.547132
BF	0.005636	0.839383
CF	0.001285	0.694987
CtF	0.1281	1
DbF	0.001305	0.784497
DfT	-7.70E-05	0.000137
GF	0.284885	0.84255

ISB	0.000269	0.709233
JF	0.001521	0.433522
LF	0.002052	0.3169
SF	0.013361	0.824033
ZF	0.001314	0.868671

Table 9 : Averaged syllable duration slope values across species, along with R^2 values.

Nine out of the twelve species show a good R^2 value (R^2>0.5), indicating that for syllable duration, a linear regression is a good fit for extracting slope information. From the slopes, we can see that one of the species show a very slight decrease in duration as the syllable sequence progresses while the rest all show an increase in duration, implying that most of the species increase their syllable duration as the sequence progresses.

5.4. Entropy Variance of start syllables

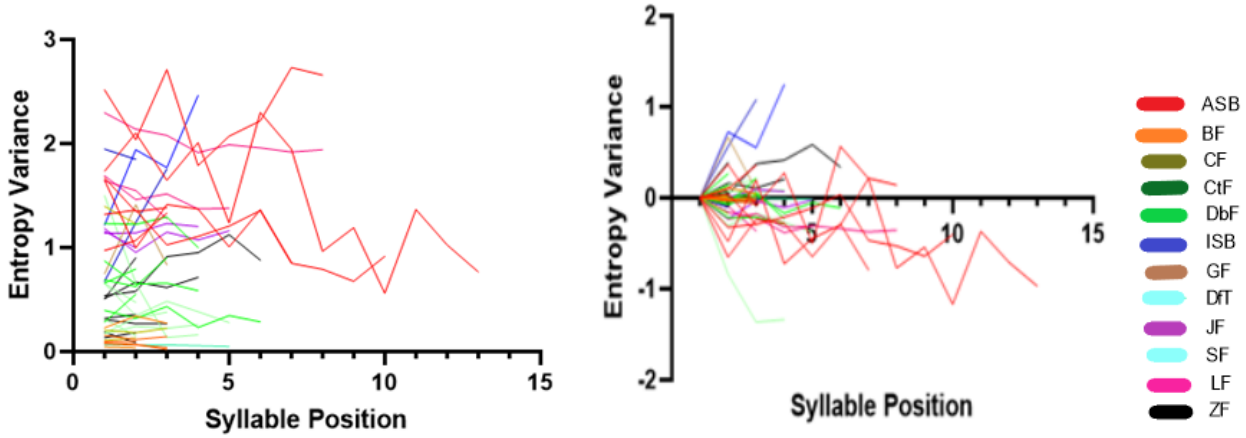

Figure 20: Entropy variance of start syllables. The X axis represents syllable position while the Y axis represents entropy variance. Each line in these graphs represents the syllable progression of a single bird.The species that each bird belongs to is indicated by the legend given besides the graphs. The graph on the left shows the duration values of the entropy variance plotted as is, while the graph on the right shows the values normalised to start from the same point (so as to allow for easier interpretation of the results).

From the Normalised entropy variance graph, it is hard to see a discernible trend across species in how the entropy variance changes as the start syllables progress to song. To make it more simpler to analyse, these curves were fitted to a simple linear model so as to give their slopes.

Their slopes were averaged across each species and were tabulated, along with fitting information like the R^2 values as given below.

Species	**Slope**	**R^2**
ASB	-0.02662	0.505463
BF	-0.00753	0.684448
CF	-0.04647	0.719967
CtF	0.07427	1
DbF	0.056997	0.659183
DfT	-0.00404	0.8423
GF	0.012305	0.50734
ISB	0.265517	0.9341
JF	-0.04015	0.557663
LF	-0.09919	0.793233
SF	-0.12389	0.847367
ZF	0.073116	0.861643

Table 10 : Averaged entropy variance slope values across species, along with R^2 values.

All species show a good R^2 value ($R^2>0.5$), meaning a linear regression is a good fit to extract slope information. From the slopes, we can see that 5 of the species show an increase in entropy variance as the syllable sequence progresses while the rest all show an decrease in entropy variance, implying that the majority of the species decrease their entropy variance as the sequence progresses.

5.5. Inter-syllable Interval of start syllables

Since Inter-syllable interval trends need at least 3 repeats of the start syllable, some more of the birds had to be removed from the analysis for inter syllable interval trends. As such, the species and number of birds used for this result are given in the table below.

S. no.	Species	No. of individuals Studied
1	Zebra Finch	3
2	Bengalese finch	4
3	Java finch	2
4	Spice finch	4
5	African silverbill	6
6	Indian silverbill	2
7	Gouldian finch	1
8	Long-tailed finch	3
9	Cherry finch	1
10	Double-barred Finch	4
11	Diamond firetail	1

Table 11. List of number of individuals for each species and the species used for calculating intersyllable intervals of start syllables.

Thus, the species under analysis for this particular result has decreased to 11 and the number of individuals per species has also decreased.

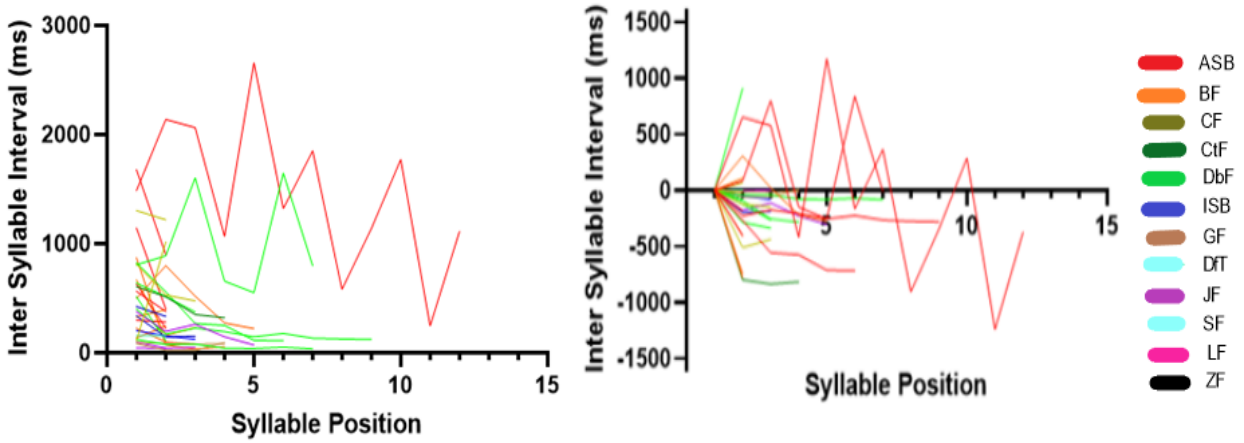

Figure 21: Inter-syllable Interval of start syllables. The X axis represents syllable position while the Y axis represents Inter-syllable Interval. Each line in these graphs represents the syllable progression of a single bird.The species that each bird belongs to is indicated by the legend given besides the graphs. The graph on the left shows the Inter-syllable Intervals plotted as is, while the graph on the right shows the values normalised to start from the same point (so as to allow for easier interpretation of the results).

From the Normalised Inter-syllable interval graph, while we can estimate that most of the birds are decreasing inter-syllable duration as the start syllables progress to song, it is still hard to discern cross species trends in how it changes. To make it more simpler to analyse, these curves were fitted to a simple linear model so as to give their slopes. Their slopes were averaged across each species and were tabulated, along with fitting information like the R^2 values as given below.

Species	**Slope**	**R^2**
ASB	-139.282	0.62387
BF	-487.143	1
CF	108.4	1
DbF	-83.715	0.760225
DfT	-249	0.6169
GF	909	1
ISB	-127.5	0.93105
JF	-116.85	0.9721
LF	-2.45667	0.585435
SF	-57.364	0.859275
ZF	-38.2897	0.7078

Table 12 : Averaged inter-syllable interval slope values across species, along with R^2 values.

All species show a good R^2 value ($R^2>0.5$), indicating that a linear regression model is a good fit to extract slope information. From the slopes, we can see that 2 of the species show an increase in inter-syllable duration as the syllable sequence progresses while the rest all show an decrease in inter-syllable duration, implying that most of the species decrease their inter-syllable interval as the sequence progresses.

6. Properties of Initial syllables

In the previous section, I managed to characterise the acoustic properties of the repeating start syllables. However, the fact that I relied upon each bird needing to repeat its start syllable meant that I had to exclude several birds and in some cases entire species from our results, which would

reduce the scope of our study. As such, in an effort to avoid this, I came up with the idea of examining the first five syllables of a bird's song, i.e. its initial syllables, and studying the changes in their acoustic properties. This would not only allow me to include the birds and species that I removed from our earlier analysis, it would also tell me how acoustic properties vary during the initial phase of the bird song, across syllable identities. As to why only the first five syllables? This was because the bout length of most of our species, especially during the beginning of our study, was around 20 syllables. As such it made sense to take the first quarter of the syllables, i.e. the first five syllables, as the initial syllables. As I incorporated more species like LW and RBF, their very short bout lengths ensured that I would have to exclude them if we considered more than 5 syllables. An additional fact is that most birds repeated their start syllables less than 5 times, meaning I could include the repeats of the start syllable of most of the birds if I looked at the first 5 syllables.

In order to allow for easier interpretations of the results, the properties were averaged across the species and were fitted to a simple linear model so as to give their slopes. Their slopes are tabulated below, along with fitting information like the R^2 values.

6.1. Frequency of initial syllables

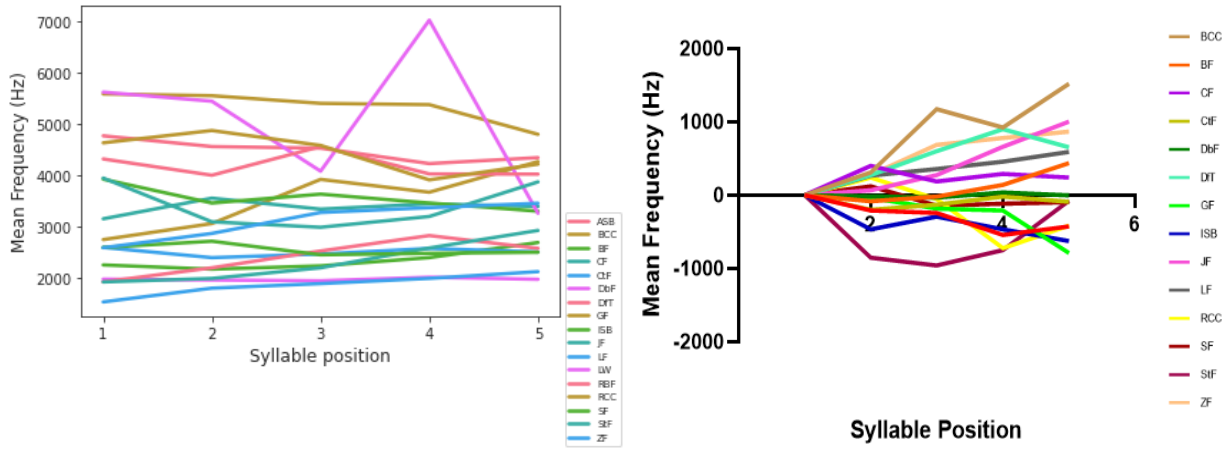

Figure 22: Frequency of initial syllables. The X axis represents syllable position while the Y axis represents mean frequency in Hz. Each line in these graphs represents the average syllable progression of a single species.The species that each line belongs to is indicated by the legend given besides the graphs. The graph on the left shows the frequency values of the syllable progression plotted as is, while the graph on the right shows the frequency values normalised to start from the same point (so as to allow for easier interpretation of the results).

Species	Slope	R^2
ASB	-118.2833	0.802
BCC	365.3222	0.8599
BF	109.927	0.6995
CF	37.2104	0.15659
CtF	0.3079	3.58E-05
DbF	4.7818	0.087978
DfT	193.598	0.7546
GF	-174.83	0.757739
ISB	-124.708	0.68962
JF	260.24	0.941322
LF	137.176	0.9508
LW	-314.441	0.115969497
RBF	34.237	0.04704705
RCC	-179.32	0.55944
SF	-42.333	0.384867
StF	-4.49449	0.000245
ZF	223.6868	0.91756

Table 13 : Averaged mean frequency slope values of initial syllables across species, along with R^2 values.

Talking about R^2 values, 10 of the 17 species show a good fit ($R^2>0.5$). This indicates that while a linear regression is a good fit for the majority of species, for the other 7 species another regression model will yield more accurate results. From the slopes, we can see that 7 species show a decrease in mean frequency as the song progresses to the fifth syllable, while the rest of the species show an increase in the mean frequency. This points against a unifying trend across the species on how frequency varies as the song begins.

6.2. Amplitude of initial syllables

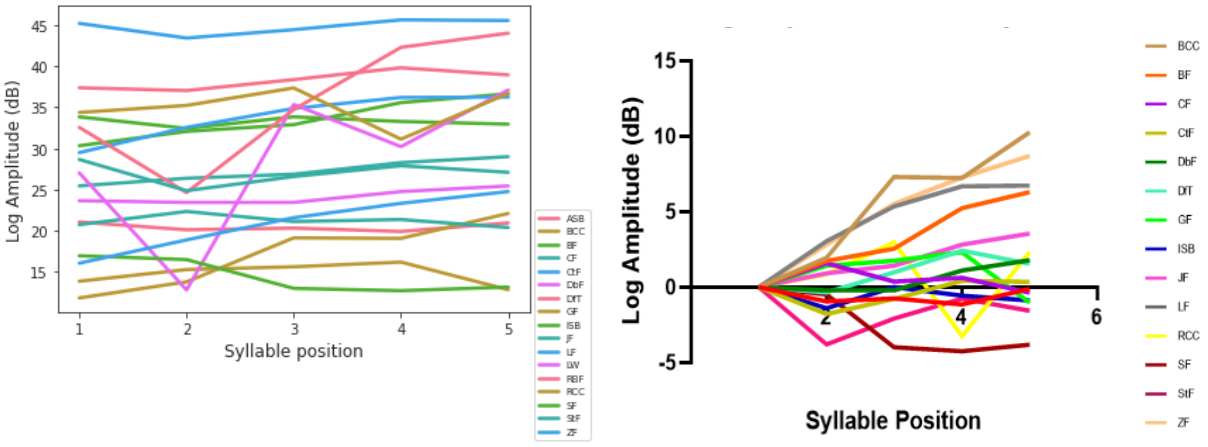

Figure 23: Amplitude of initial syllables. The X axis represents syllable position while the Y axis represents Log Amplitude in dB. Each line in these graphs represents the average syllable progression of a single species.The species that each line belongs to is indicated by the legend given besides the graphs. The graph on the left shows the amplitude values of the syllable progression plotted as is, while the graph on the right shows the amplitude values normalised to start from the same point (so as to allow for easier interpretation of the results).

Species	**Slope**	**R^2**
ASB	-0.0411	0.016228
BCC	2.582	0.92963
BF	1.61224	0.97686
CF	-0.1684	0.128884
CtF	0.29094	0.246333
DbF	0.489656	0.729617
DfT	0.589648	0.67774
GF	-0.115	0.017497
ISB	-0.09203	0.058837
JF	0.90029	0.982158
LW	3.75545	0.378985259

LF	1.713721	0.893055
RBF	4.12369	0.7279145
RCC	0.053377	0.001197
SF	-1.13917	0.750282
StF	-0.00812	7.95E-05
ZF	2.18597	0.978656

Table 14 : Averaged log amplitude slope values of initial syllables across species, along with R^2 values.

Talking about R^2 values, 9 of the species show a good fit (R^2>0.5). This indicates that while a linear regression is a good fit for the majority of species, for the other 8 species another regression model will yield more accurate results. From the slopes, we can see that 6 species show a decrease in log amplitude as the song progresses to the fifth syllable, while the rest of the species show an increase in the amplitude. This points against any unifying trends across the species on how amplitude varies as the song begins.

6.3. Duration of initial syllables

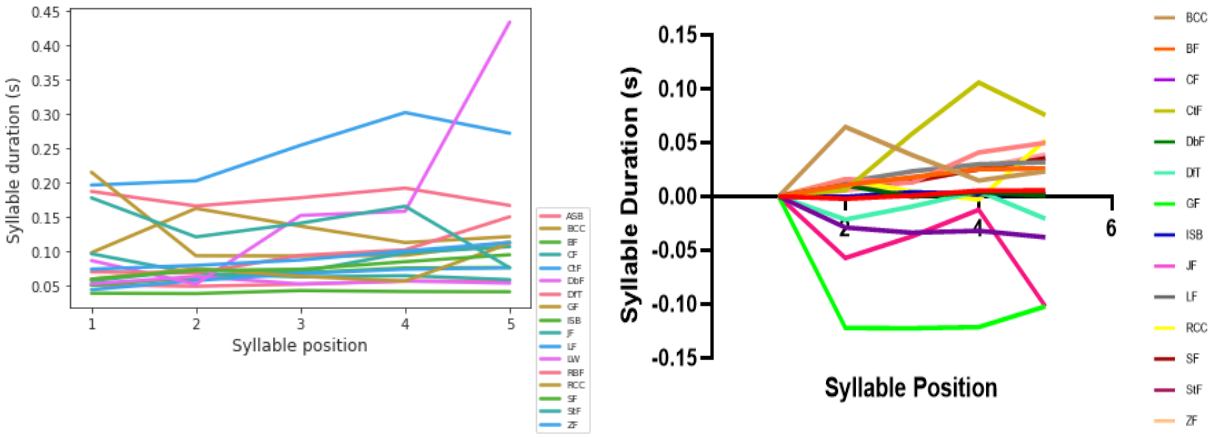

Figure 24: Duration of initial syllables. The X axis represents syllable position while the Y axis represents syllable duration in seconds. Each line in these graphs represents the average syllable progression of a single species.The species that each line belongs to is indicated by the legend given besides the graphs. The graph on the left shows the duration values of the syllable progression plotted as is, while the graph on the right shows the duration values normalised to start from the same point (so as to allow for easier interpretation of the results).

Species	Slope	R^2
ASB	0.001854	0.755951
BCC	-0.00029	0.000349
BF	0.006667	0.933907
CF	-0.00786	0.672793
CtF	0.02511	0.7624
DbF	-0.00047	0.02741
DfT	-0.0015	0.040427
GF	-0.02037	0.368716
ISB	0.000717	0.38966
JF	0.01246	0.901505
LW	0.08029047	0.7066482
LF	0.00805	0.92964
RBF	0.01423013	0.8168806
RCC	0.008633	0.364871
SF	0.008384	0.967934
StF	-0.01596	0.390614
ZF	0.009867	0.972236

Table 15 : Averaged syllable duration slope values of initial syllables across species, along with R^2 values.

Coming to R^2 values, 9 of the species show a good fit ($R^2>0.5$), indicating that for the majority of the species, the linear regression will give reliable slope information. From the slopes, we can see that 6 species show a decrease in duration as the song progresses to the fifth syllable, while the rest of the species show an increase in the syllable duration. This points against a unifying trend across the species on how duration varies as the song begins.

6.4. Entropy Variance of initial syllables

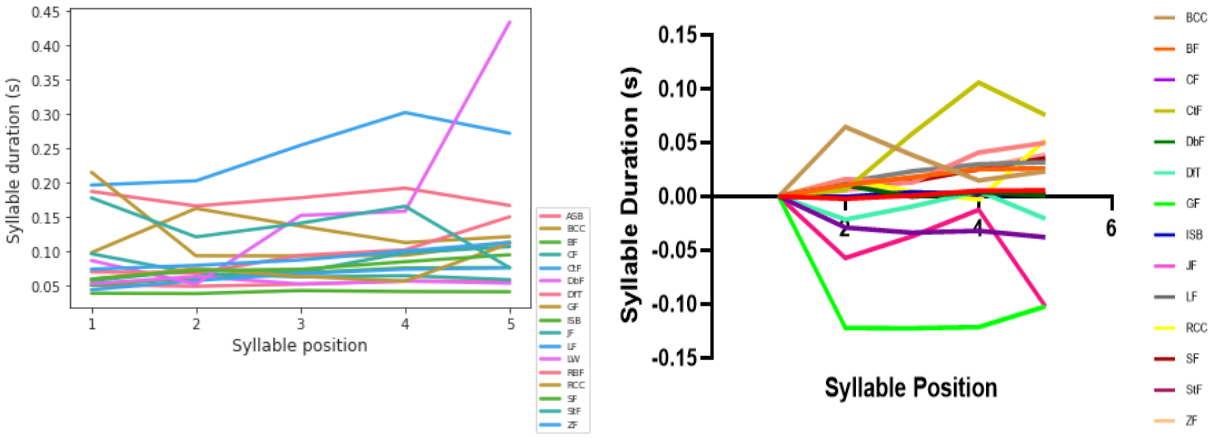

Figure 25: Entropy variance of initial syllables. The X axis represents syllable position while the Y axis represents entropy variance. Each line in these graphs represents the average syllable progression of a single species.The species that each line belongs to is indicated by the legend given besides the graphs. The graph on the left shows the entropy variance values of the syllable progression plotted as is, while the graph on the right shows the values normalised to start from the same point (so as to allow for easier interpretation of the results).

Species	**Slope**	**R^2**
ASB	0.003646	0.01176
BCC	0.1152	0.9364
BF	0.07925	0.9424
CF	0.06604	0.9854
CtF	-0.05834	0.5682
DbF	0.04116	0.9817
DfT	0.000291	0.00063
GF	0.01229	0.4664
ISB	0.03316	0.2597
JF	0.03636	0.6485
LW	-0.60463	0.453243

LF	0.04036	0.8323
RBF	-0.29009	0.636725
RCC	-0.1498	0.5879
SF	-0.00455	0.03773
StF	-0.06824	0.3682
ZF	-0.01251	0.184

Table 16 : Averaged entropy variance slope values of initial syllables across species, along with R^2 values.

Talking about R^2 values, 10 of the species show a good fit ($R^2>0.5$), suggesting that for the majority of the species a linear regression is a good fit to get slope information, but for 7 of the species, it is not so. From the slopes, we can see that 7 species show a decrease in entropy variance as the song progresses to the fifth syllable, while the rest of the species show an increase in the entropy variance. This points against a unifying trend across the species on how entropy variance varies as the song begins.

6.5. Inter-syllable interval of initial syllables

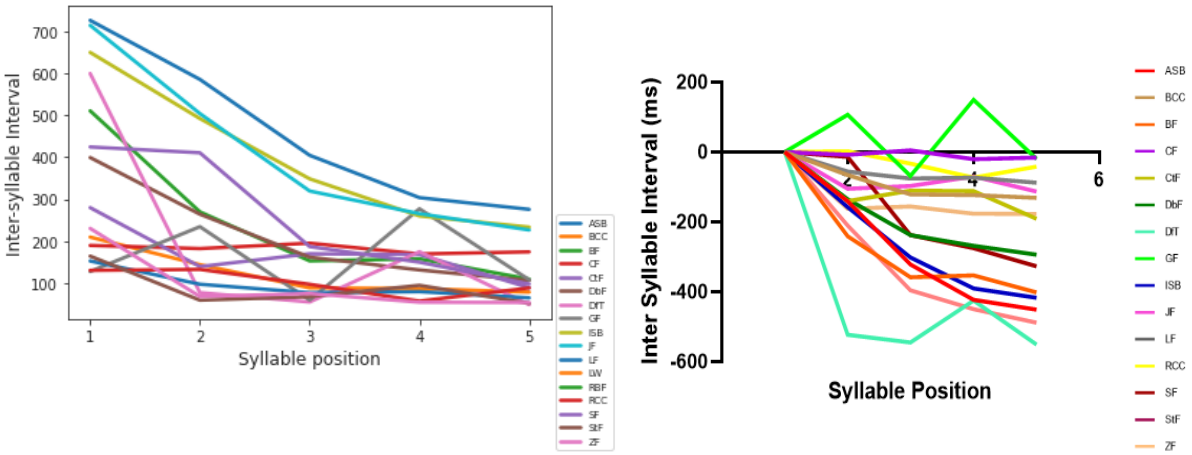

Figure 26: Inter-syllable interval of initial syllables. The X axis represents syllable position while the Y axis represents inter-syllable interval. Each line in these graphs represents the average syllable progression of a single species.The species that each line belongs to is indicated by the legend given besides the graphs. The graph on the left shows the inter-syllable interval values of the syllable progression plotted as is, while the graph on the right shows the values normalised to start from the same point (so as to allow for easier interpretation of the results).

Species	Slope	R^2
ASB	-118.376	0.944695
BCC	-31.9362	0.82202
BF	-91.4951	0.792229
CF	-4.35335	0.42341
CtF	-35.3111	0.636457
DbF	-71.9717	0.891066
DfT	-100.476	0.460504
GF	0.31447	2.98E-05
ISB	-106.584	0.93838
JF	-121.526	0.898049
LW	-186.685	0.366398
LF	-19.3471	0.778626
RBF	-5.4043	0.049492828
RCC	-15.9334	0.640871
SF	-91.4738	0.895363
StF	-18.9898	0.427242
ZF	-36.7255	0.590947

Table 17 : Averaged inter-syllable interval slope values of initial syllables across species, along with R^2 values.

Talking about R^2 values, 11 of the species show a good fit ($R^2>0.5$), suggesting that for the majority of the species a linear regression is a good fit to get slope information, but for 6 of the species, it is not so. From the slopes, we can see that except for one species (that too with a very small slope), all the other species show a decrease in inter-syllable interval as the song progresses to the fifth syllable. This points towards a unifying trend across the species on how inter-syllable intervals reduce as the song progresses.

7. Correlation to body size

Now, it is known that in birds, body size is linked to frequency and frequency modulation of songs. As such, I obtained body size information on each of these species to check for correlation against the slopes of the various initial syllables as well as the repeated start syllables. This was an effort to see whether size correlates with the rate of change of acoustic features of these syllables as the song progresses.

Species	**Body Size (cm)**
Zebra Finch	10-11
Bengalese finch	10
Java finch	14-17
Spice finch	10–12·5
African silverbill	11
Indian silverbill	11
Gouldian finch	15
Blue-capped cordon-bleu	13–13·5
Long-tailed finch	15·5
Star finch	11·5
Red-billed firefinch	9–10
Lavender waxbill	10
Cherry finch	11
Double-barred Finch	10-11
Red-cheeked cordon-bleu	12-13
Diamond firetail	12
Cut-throat finch	11-12

Table 18: Body sizes of the species used in the study

7.1. Correlation to acoustic features of repeated start syllables

Acoustic Property	**Normal Distribution?**	**Correlation coefficient (r)**	**Correlation?**
Mean frequency	Yes	-0.00457	No correlation
Amplitude	Yes	0.2437	Negligible correlation
Syllable duration	No	0.1838	Negligible correlation
Entropy Variance	Yes	-0.3412	Moderate negative correlation
Inter-syllable interval	No	0.3318	Moderate correlation

Table 19: Normality and Correlation coefficient of slopes of acoustic properties of repeated start syllables to bird size.

From the results, it can be seen that the properties are differently correlated to body size, with entropy variance and inter-syllable interval showing moderate correlation. However, surprisingly enough, mean frequency showed no correlation whatsoever. This is unexpected seeing as how frequency has been shown to correlate with size (Wallschager, 1980). As such, it was decided to check correlation of the magnitude of the slopes, to see if and how the correlation changes.

Property	**Normal Distribution?**	**Correlation coefficient (r)**	**Correlation?**
Mean frequency	Yes	-0.3630	Moderate negative correlation
Amplitude	Yes	0.1700	Negligible correlation
Syllable duration	No	0.1838	Negligible correlation
Entropy Variance	No	0.02120	No correlation
Inter-syllable interval	No	-0.05070	No correlation

Table 20: Normality and Correlation coefficient of magnitude of slopes of acoustic properties of repeated start syllables to bird size.

From the results, it can be seen that the magnitude of slopes of properties show very little correlation to body size, with only mean frequency showing a moderate negative correlation. The correlation of all the properties changed when correlation to size was checked against the magnitude of the slope.

7.2. Correlation to acoustic features of initial syllables

Property	Normal Distribution?	Correlation coefficient (r)	Correlation?
Mean frequency	Yes	-0.1796	Negligible negative correlation
Amplitude	Yes	-0.2135	Negligible negative correlation
Syllable duration	No	-0.2537	Negligible negative correlation
Entropy Variance	No	0.2352	Negligible correlation
Inter-syllable interval	Yes	0.2224	Negligible correlation

Table 21: Normality and Correlation coefficient of slopes of acoustic properties of initial syllables to bird size.

From the results, it can be seen that the properties all show negligible correlation to body size.

Property	Normal Distribution?	Correlation coefficient (r)	Correlation?
Mean frequency	Yes	0.6481	Highly correlated

Amplitude	No	-0.2771	Negligible negative correlation
Syllable duration	No	0	No Correlation
Entropy Variance	No	-0.2993	Negligible negative correlation
Inter-syllable interval	Yes	-0.2214	Negligible negative correlation

Table 22: Normality and Correlation coefficient of the magnitude of slopes of acoustic properties of initial syllables to bird size.

From the results, it can be seen that the properties are differently correlated to body size, with mean frequency showing highly positive correlation with body size. For the initial syllables, the correlation of the properties did not show a huge change when correlation was checked against the magnitude of the slope, except for mean frequency which was highly correlated.

Discussion

The aim of this study was to characterise the properties of Introductory Notes (INs) in the birds of the Estrildidae family. For this, we collected songs from 17 different species from this family and characterised various features of their song. It was found that birds from this family can produce anywhere from 5 to 18 unique syllables in their repertoire and that they produced anywhere from 5 to 120 syllables per bout. Looking at how stereotyped the songs of these birds are, we compared their stereotypy values to zebra finches as they are known to have very stereotyped songs (Bruno and Ofer Tchernichovski, 2019). It was seen that of these 17 species, at least 7 of them have highly stereotyped songs. The stereotypy values of the other birds did not deviate greatly from that of Zebra finches. Looking at how these species began their song, on average they have between 1 and 3 unique syllables with which they begin their song (aka start syllables). 11 of these 17 species show a tendency to repeat their start syllables while the other 6 show no repeats of this syllable. It was also found that a correlation exists between the number of times a bird repeats syllables in its bout and the number of times it repeats its start syllables. By looking at syllables with the highest probability of being the first syllable and the tendency of these syllables to repeat themselves, it was seen that while the starting probabilities are all consistently high across species (ranging from 0.6 to 1), the repeat probabilities show a wide range of values (from 0 to 0.8). Looking at the acoustic properties of start syllable repeats, it was found that amplitude and syllable duration of most species are seen to increase as the start syllable sequence progresses while the inter-syllable interval of most species seem to decrease. Next, the acoustic properties of the first 5 syllables of the bird's song (aka initial syllables), irrespective of syllable identity, were calculated and it would seem that for inter-syllable intervals, for almost all species, seem to decrease as the song begins. Statistical tests showed that none of the slopes of the repeated start syllable properties were significantly different from the slopes of the initial syllable properties. Looking at whether acoustic properties of the syllables correlated with the size of the bird, it was found that while the slopes themselves showed no correlation, the magnitudes of the mean frequency slopes, i.e. the rate at which the mean frequency changes, did. The start syllable repeats showed a moderate negative correlation while the initial syllables showed a high positive correlation to body size.

Song Properties

Coming to the general properties of songs themselves, the species showed a wide range of unique syllables. They were seen to have a huge variance in the number of syllables produced in a bout, with members of the Lonchurinae subfamily (with the exception of Java Finches) tending to produce very long songs, with more than 40 syllables per bout. This trait of producing longer songs by the Lonchurinae species could be attributed to their low plumage colour, as songs have

been known to be influenced by the plumage of the bird across species (Badyaev et al., 2002). But then why are Java finches an exception? Literature states that Java finches tend to incorporate several types of trills (series of repetitive syllables which are more demanding on the birds physically) in their songs, which its relatives do not (Kagawa and Soma, 2013). Considering that trills are costly to sustain, they might have opted for a smaller number of syllables in their bouts to sustain these trills, setting them apart from its closest relatives. The geographic trend in the two birds of the Indian subcontinent is undercut by the phylogenetic relationship between the two species (they both belong to the Lonchurinae subfamily), indicating that the geographic trend might just be the consequence of their genetic relatedness. A potentially significant geographic factor I did not quantify was habitat overlap amongst the species, as done by others (Payne, 1978), to check if that influenced the song in Estrildid finches. The phylogenetic and geographic trends were also repeated for the mean number of times the birds repeated syllables in their bouts, which makes sense, since increasing the number of repeats is the only way a bird with a fixed repertoire of unique syllables can increase the number of syllables per bout. Apart from the number of syllables per bout and number of repeats per bout, none of the other song features showed a distinct trend geographically or phylogenetically. Overall the results do not suggest any geographic trends for songs, while phylogeny seems to be an influential factor.

Introductory Notes

Now, coming to the meat of the matter, do these species actually have INs? In Zebra finches, it is known that song bouts start with repetition of INs (Sossinka and Böhner, 1980), meaning they always start the song, repeat themselves and are sometimes even included in the motif. It was seen that all the species in our study began their song consistently with a small number of syllables, in spite of the large repertoire of unique syllables they had access to. Upon performing statistical tests, it was seen that the number of start syllables did not vary significantly from each other, despite the number of unique syllables and the length of the song varying across species. Most of the species (11 of them) show a tendency to repeat their start syllables while 6 of them showed no repeats. Statistics showed that the number of repeats varied significantly across species. So, it can be seen that the majority of the species in our analysis tend to repeat their INs, suggesting that the trend in the family is to repeat their INs. However, there is a problem here of under-representation. As mentioned, except 1, all the species that showed no repeats of the syllable belong to the Estrildinae, Lagonostictinae and Erythrurinae subfamilies. There are only one species each to represent Estrildinae and Erythrurinae and the number of songs from these species are also less. So, it is hard to conclude whether all the birds in these two subfamilies show no IN repeats. Now, what does this tendency to repeat INs mean? As mentioned before, introductory gestures as a whole have 3 broad functions attributed to them: enhance communicability of the signal (Fleishman, 1992; Thorson & Fine, 2002; Peters, 2003), aid in conspecific identification (Marler & Tamura, 1962; Soha and Marler, 2000) and motor

preparation (Rajan & Doupe, 2013; Rao et al. 2019). So the tendency to repeat INs by itself would not tell us more about the function INs perform in these 17 species. But a lack of IN repeats would argue against a motor preparatory hypothesis, since there is no trend of INs going from a variable first IN repeat to a more stereotyped last IN to compare to the progression of neural preparatory activity. Thus, we can conclude that all of the species used in our analysis use INs to begin their song, yet the number of times said INs are repeated vary significantly across species. The number of times a bird repeats its INs was also seen to correlate with how much a bird repeats syllables in its bout, indicating that even if INs and motif are two distinct parts of a song, the underlying mechanisms that determine their properties are the same across these species.

Introductory Note Acoustic Properties

Looking at syllable properties of Zebra finch INs, it is seen that they become shorter, louder, higher in mean frequency, and change their entropy as the IN sequence progresses to the last IN before motif onset (Rajan and Doupe, 2013). The INs of the 17 Estrildid species were seen to get louder, longer and get produced faster as the repeat sequence progresses to song, with the exception of two or three species. The other acoustic properties did not display a clear trend as this, with a significant number of species (5-6) displaying an opposite trend in how acoustic properties change. This could be because the acoustic properties that don't show similar trends, i.e. mean frequency and entropy variance, are what the bird uses to distinguish between different INs, allowing discrimination between conspecific and foreign song like in white crowned sparrows (Marler & Tamura, 1962; Soha and Marler, 2000). A similar observation was made in Jacky dragons, where it was found that the duration of their introductory tail wagging was the most important characteristic, contributing more to the signal efficacy, rather than other components of its introductory gestures (Peters, 2003). Shiovitz (1975) and Richards (1981) both reported observations that INs in several songbirds, not just Estrildid finches, had narrow frequency ranges and were widely spaced from each other, but claimed that there were no other "pronounced species specific characteristics". However, my results indicate that while there are some intro note characteristics which hold true across species, there are still marked differences between INs of different species, from their acoustic syllable properties to their tendency to repeat themselves.

Looking at the properties of syllables as the birds of these species begin their song, it was seen that for almost all the species, except one, the inter-syllable intervals decrease as the song begins. So, across species, irrespective of whether their INs repeat, all these Estrildid finches produce syllables faster and faster as their songs begin. This implies that the rate at which syllables are produced is an important feature in the strength of songs as a communicative signal. Shiovitz (1975) and Richards (1981) both reported observations that INs in several songbirds were widely spaced, indicating that the first few syllables produced as part of song all have a large

inter-syllable interval across all birds. Statistical tests showed that none of the slopes of the repeated start syllable properties were significantly different from the slopes of the initial syllable properties, implying that irrespective of whether the bird used IN repeats to start their song or not, the trends of how the syllable properties changed at the beginning of the song remained consistent across these species. It is also interesting to note that Gouldian finches, the only representative from its subfamily, did not follow the majority trend for most of the properties measured for both the INs and initial syllables. A unique feature about Gouldian finches that might explain this deviation is that they have the most brightly and vividly coloured plumage of all the birds used in our study, which has been known to influence song structure and complexity (Badyaev et al., 2002), but we would need songs from other brightly plumed Estrildids to verify this. We would also need more representatives from this subfamily to definitively state whether it is only this species that shows a deviation or whether other members of the Erythrurinae subfamily also behave similarly wrt syllable properties of its INs and initial syllables.

Now, it is known that the frequency of bird songs are negatively correlated with body size (Wallschager, 1980). Thus, in order to see if and how the frequency of the repeated start syllable progression was related to body size, we tried a correlation test on the slopes obtained. We saw that while the slopes themselves showed no correlation, the magnitudes of the mean frequency slopes, i.e. the rate at which the mean frequency of the bird changes, showed a moderate negative correlation to bird size, which is in line with what we expect from literature, which says that frequency shows an inverse correlation to size of the bird (Wallschager, 1980). Similarly for the initial syllables, again the slopes themselves showed a small correlation and the magnitudes of the mean frequency slopes showed a high positive correlation to body size. This result from the initial syllables went against what is known about frequency size correlations (Wallschager, 1980) and can be attributed to either us using inaccurate fits (slopes were calculated from simple regression curves with low R squared values for 7 of the 17 species) or the initial syllables have an altogether different relationship with body size than the whole song. To clarify whether this is due to poor fits, fitting other kinds of regression curves with better R squared values would help. To check if initial syllables do show different trends from the whole song, other physical traits like bill size and shape (Demery et al. 2021), can be checked to see if they correlate differently with mean frequency. None of the other syllable properties showed a correlation to the magnitudes of the slopes.

Properties of Closely related Species

As mentioned, there are three pairs of closely related species in our samples: Blue-capped cordon-bleu (*Uraeginthus cyanocephalus*) and Red-cheeked cordon-bleu (*Uraeginthus bengalus*); Bengalese finch (*Lonchura striata domestica*) and Spice finch (*Lonchura punctulata*) and African silverbill (*Euodice cantans*) and Indian silverbill (*Euodice malabarica*). Since these birds are closely related, I wanted to see how similar their songs and in particular INs were:

1) Both the cordon-bleus belong to the Uraeginthus genus, which is made up of three species in total. Coming to song structure, both these birds show similarities in almost all the properties, but showed a considerable difference in the number of syllables in a bout. There were similarities in the number of start syllables. They don't repeat their start syllables and so their properties weren't measured. Coming to how they start their songs, their initial syllables showed a consistent increase in amplitude and decrease in inter syllable interval. On the other syllable properties, the trends differed. It was also seen that while fitting their acoustic properties to a linear curve, the Blue-capped cordon-bleu showed good fits (R^2 above 0.65) for almost all the properties while the Red-cheeked cordon-bleus showed moderate to poor fits ($R^2 \leqq 0.5$) for the same properties. On the whole, these two birds showed a lot of similarity in their song and most IN properties but differed significantly in how well these properties fit to a linear curve.
2) Coming to Bengalese and Spice finches, they belong to the Lonchura genus, which has 27 species. Coming to song properties, they show similarities in song stereotypy and number of repeats used in song, but show different number of syllables per bout and number of unique syllables. There were similarities in the number of start syllables and the number of INs produced per bout. They had the same trends for syllable duration, entropy variance and inter-syllable interval of INs while showing opposite trends for mean frequency and amplitude. Coming to how they begin their song, they showed the same trends for syllable duration and inter-syllable interval of their initial syllables while for the other properties they showed opposite curves. Looking at their fits across acoustic properties, the two birds behaved similarly in how well their properties fitted to a linear curve. For almost all the properties, they showed a good fit (R^2 above 0.75) to a linear line. On the whole, these birds showed a lot of similarity in their song and most IN properties. They also were similar in how well these properties fit to a linear curve.
3) Now coming to the silverbills, they both belong to the Euodice genus, which contains only these two species. Coming to song properties, they show similarities in song stereotypy and number of unique syllables, but show different number of syllables per bout and number of repeats in bout.There were significant differences in the number of start syllables and the number of INs per bout. They had the same trends for all properties of the INs except entropy variance. Coming to how they begin their song, they showed the same trends for all the syllable properties. Looking at their fits across acoustic properties, both the silverbills behaved similarly in how well their properties fitted to a linear curve. For almost all the properties, they showed a good fit (R^2 above 0.6) to a linear line. On the whole, both silverbills showed a lot of similarity in their song and most IN properties. They also were similar in how well these properties fit to a linear curve.

So, it is seen that most of the song and IN properties were similar among species belonging to the same genus. While it was known that certain song properties (like frequency) were influenced by genetic relatedness among species (Ryan, 1985b), these results indicate that a large

number of song and IN properties like structure, repeats and acoustic syllable properties were similar for birds belonging to the same genus.

Conclusion

So, it is seen that these Estrildid species begin their songs with a small number of start syllables, indicating that introductory notes do occur in all Estrildid finches. However, said INs do not repeat consistency across the family like in Zebra finches, especially with the species belonging to the Estrildinae, Lagonostictinae and Erythrurinae subfamilies. The INs that do repeat show changes to their mean frequency, amplitude, entropy, duration and inter-syllable interval as they repeat themselves. Across species, the consistent trend of the IN acoustic properties (barring 2 or 3 species) is that they get louder, longer and the gaps between them get shorter as the INs repeat themselves. The magnitude at which the mean frequency of INs change shows moderate negative correlation to the size of the bird. Across all species, irrespective of syllable identity or whether INs repeat, it was seen that the bird produces syllables faster and faster as the song starts. Lastly, it was seen that most of the song and IN properties were similar among species belonging to the same genus, as seen from the three pairs of species that belong to the same genus.

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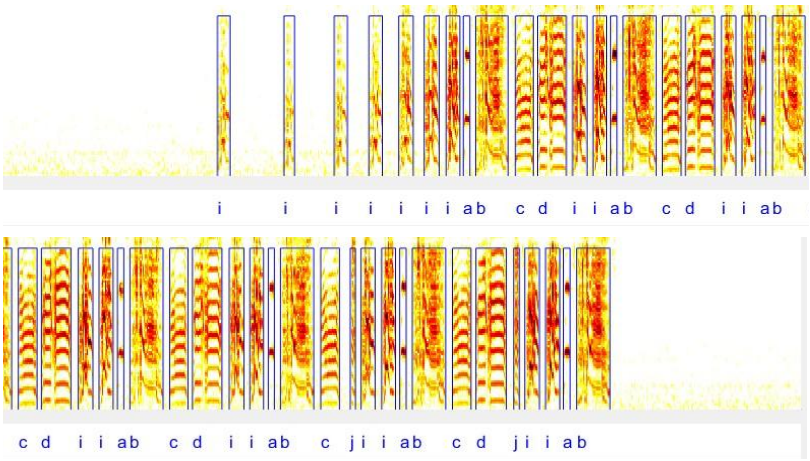
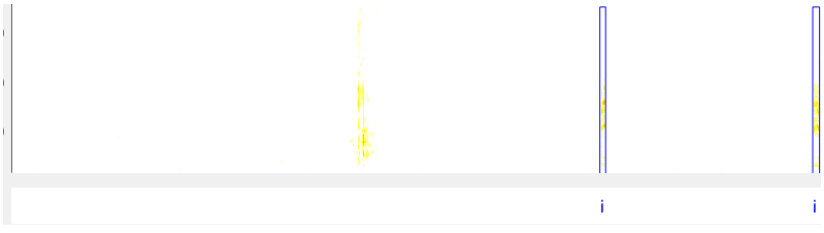
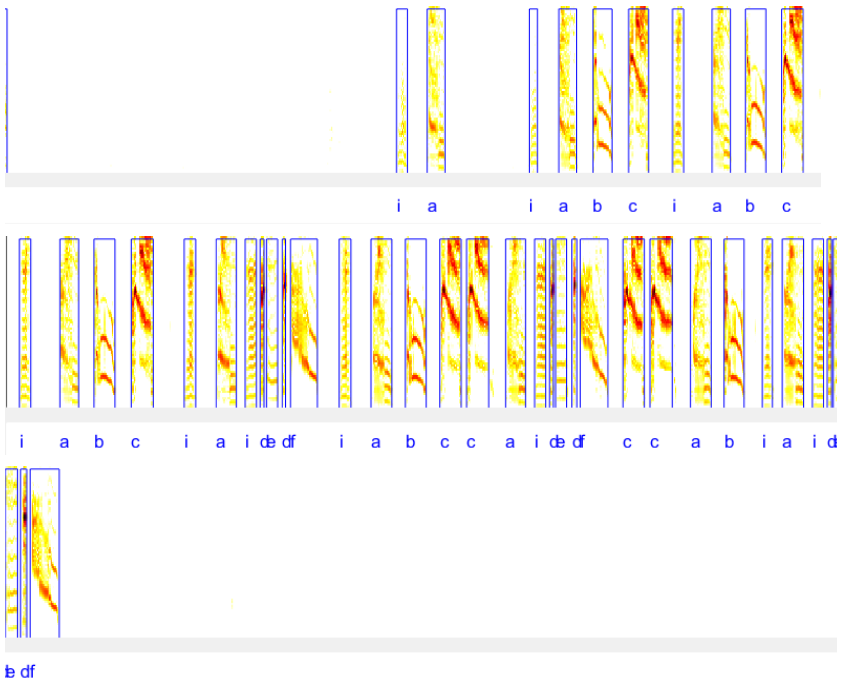
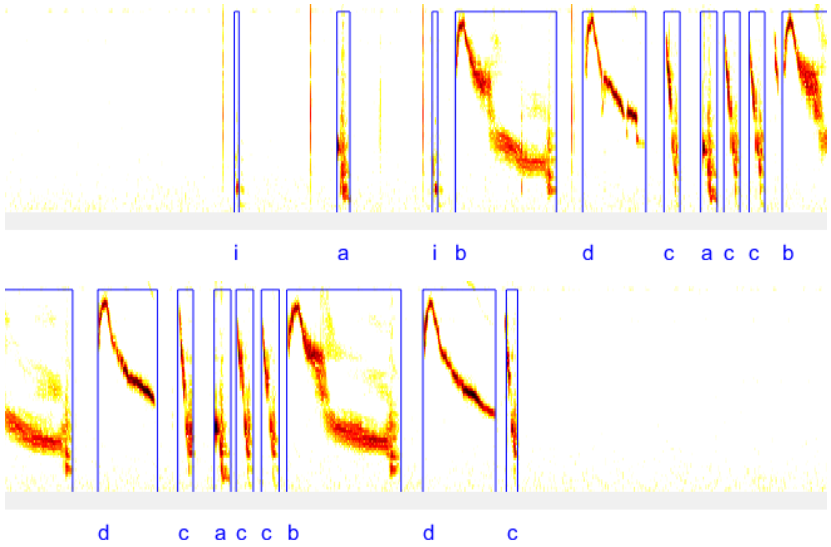
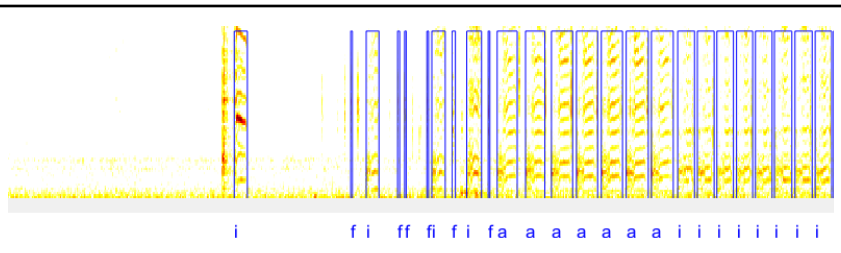
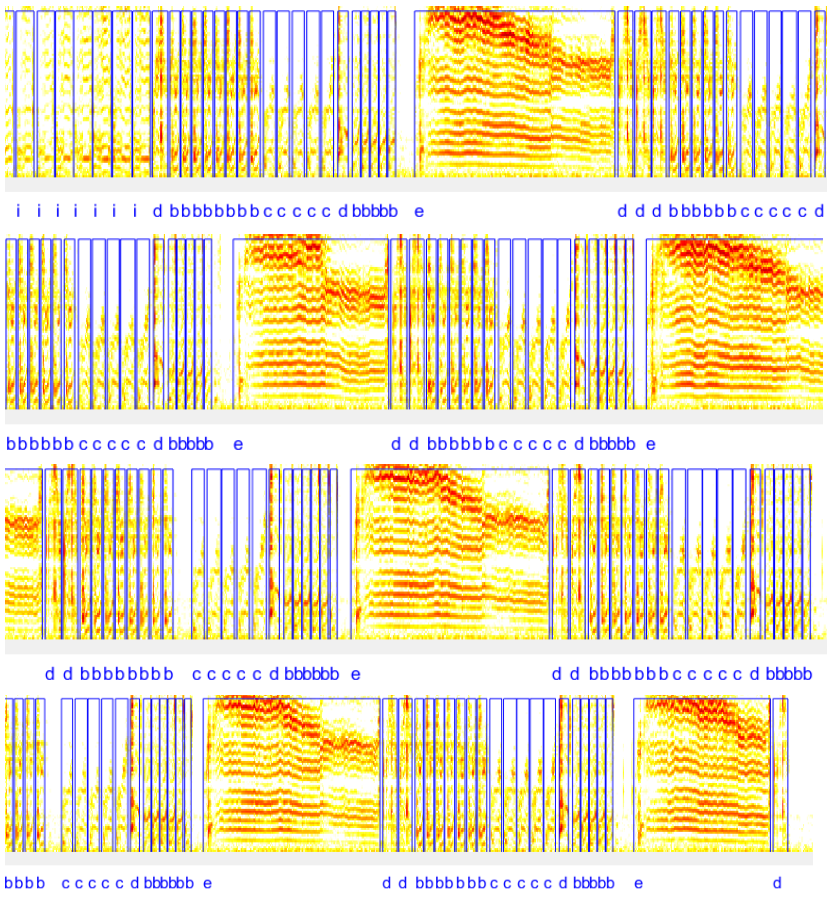
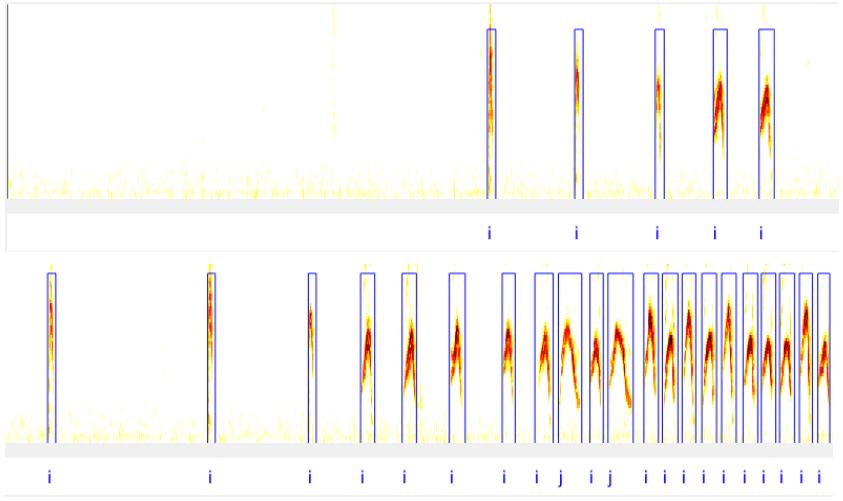
Supplementary Information

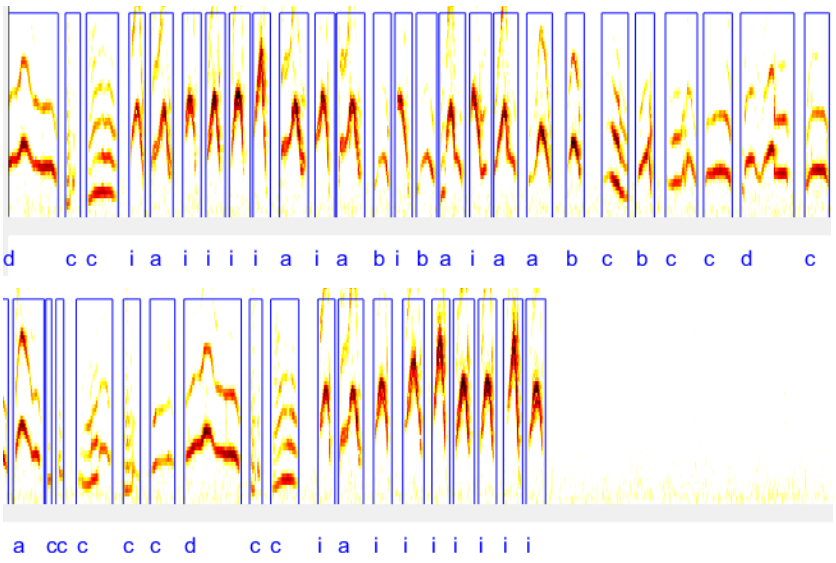
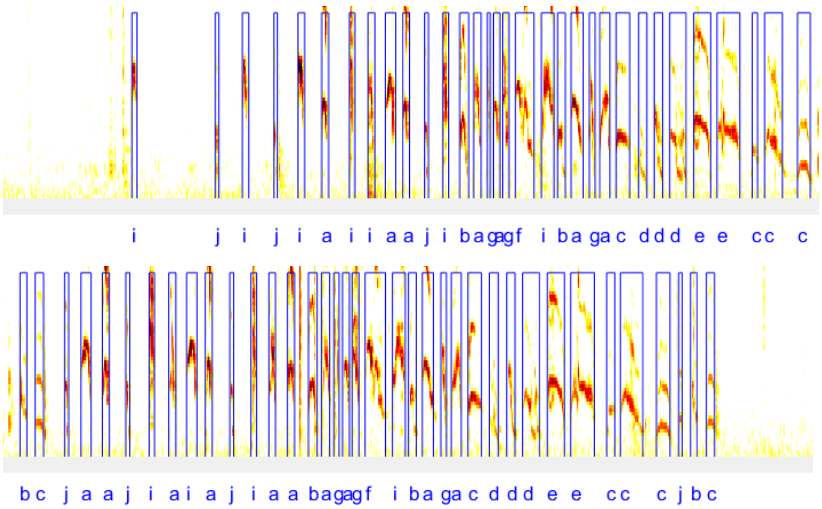
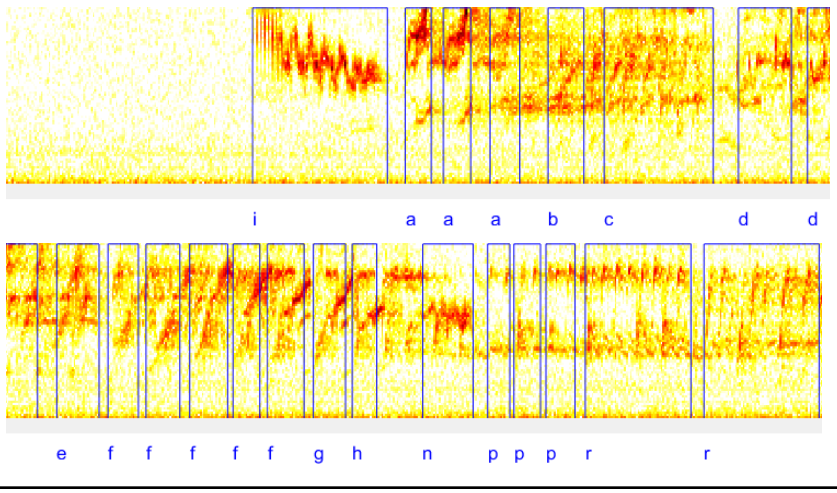
Song Spectrograms

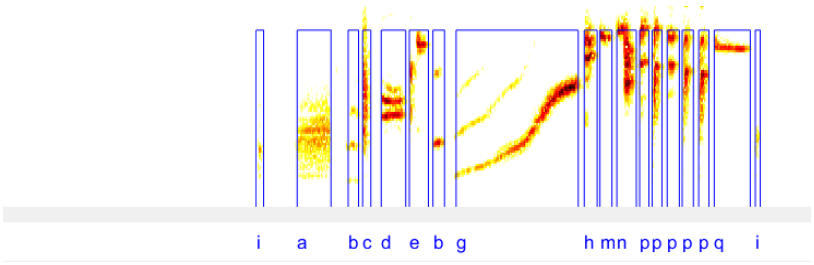
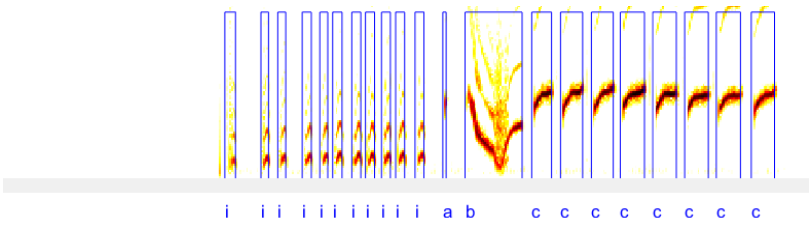
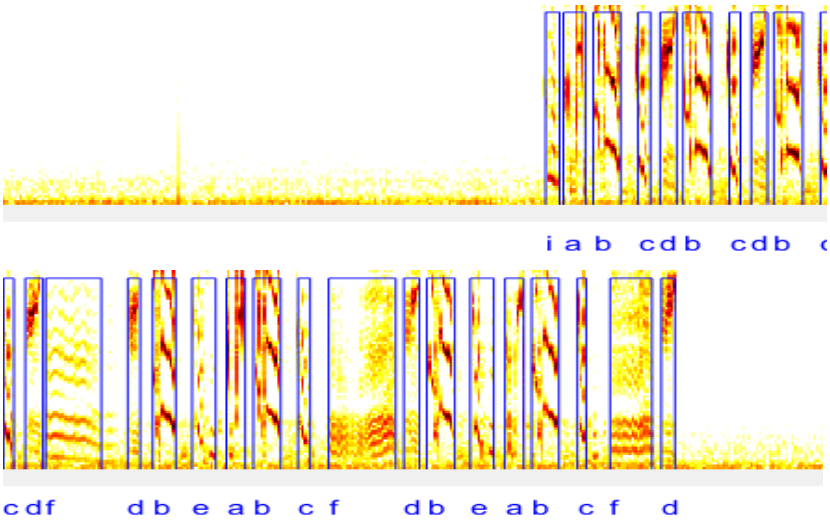
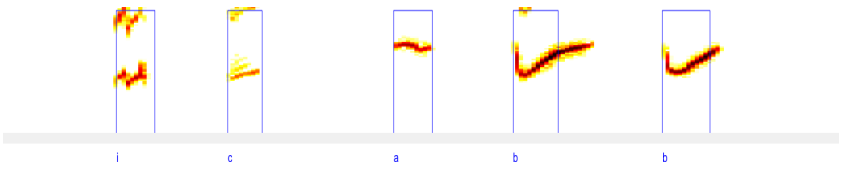
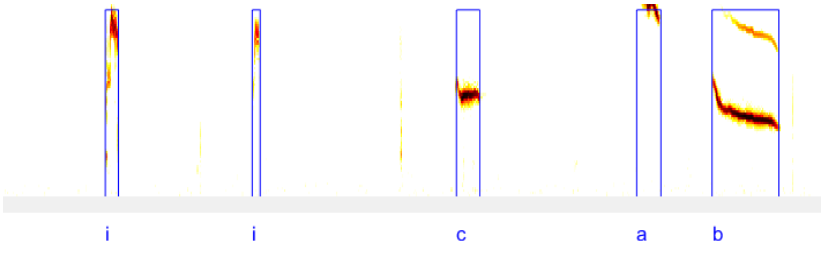
As mentioned in the introduction, the best way to visually represent songs is via spectrograms. A spectrogram is defined as a visual representation of the various spectra of frequencies carried by a signal as it changes with time. The X-axis represents time and the Y-axis represents frequency. The amplitude of a given frequency at a given time is represented by the intensity or colour of each point in the image. On frequency spectrograms, the syllables can be observed and can be identified & distinguished via their different rigid structures, which makes it easy for us to visualise the songs and song structures of different species.
Spectrogram samples one individual per species from each species is shown below:

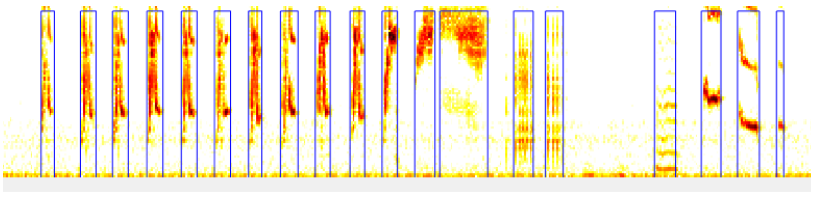
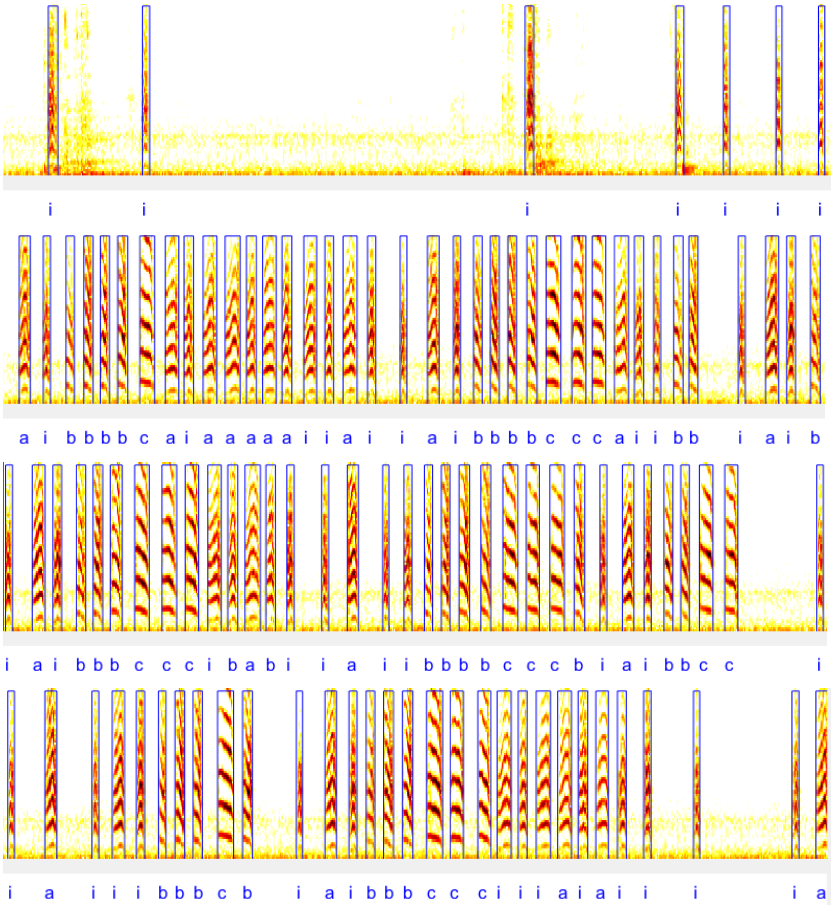
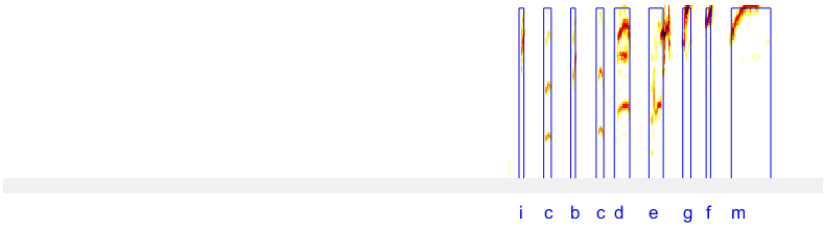
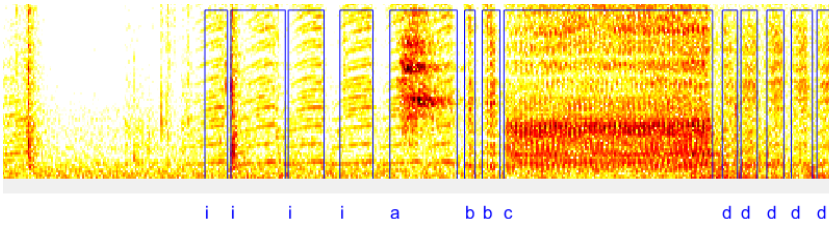
S. No.	Species	Spectrogram
1	Zebra Finch	
2	Bengalese finch	

		
3	Java finch	
4	Spice finch	

		
5	African silverbill	

		
6	Indian silverbill	
7	Gouldian finch	

8	Blue-capped cordon-bleu	
9	Long-tailed finch	
10	Star finch	
11	Red-billed firefinch*	
12	Lavender waxbill	

13	Cherry finch	
14	Double-barred Finch	
15	Red-cheeked cordon-bleu	
16	Diamond firetail	

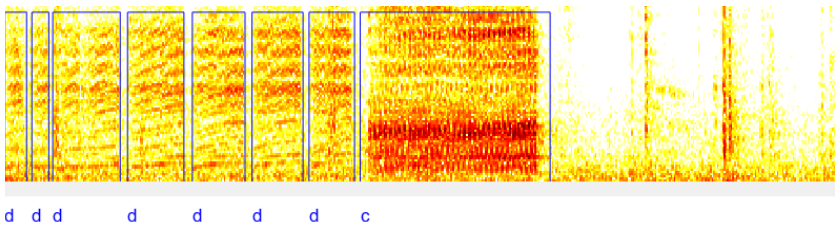
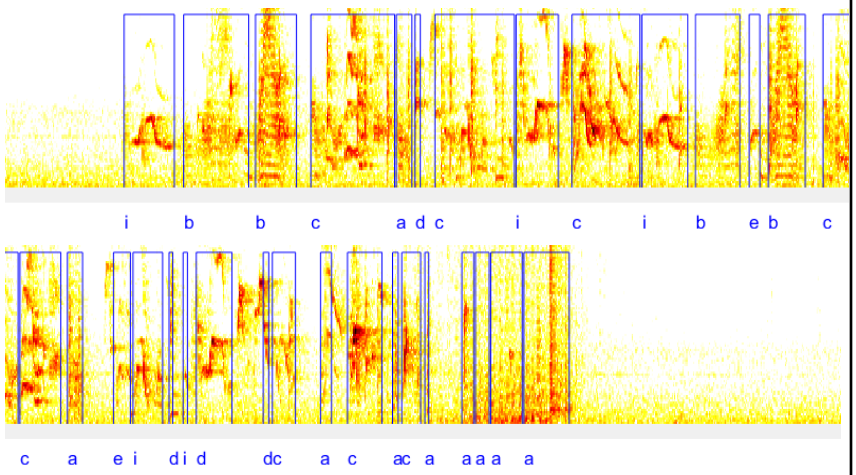
		
17	Cut-throat finch	

Table 23. Spectrogram samples from each of the 17 species. Each species has a single song bout of the bird shown beside it. Almost all the spectrograms are 5 seconds long, except for the species marked with (*), which is 2 seconds long (Red Billed Firefinch). The alphabets indicate syllable types, with the 'i' & 'j' syllables being the intro notes and the rest of the syllables representing motif syllables.