

Geographical variation in bird song at different scales and its potential utility in monitoring the effects of habitat fragmentation

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by

Nafisa.A.Pathaw



Indian Institute of Science Education and Research Pune

Dr Homi Bhabha Road

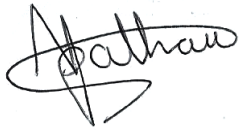
Pashan, Pune 411008

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Supervisor: Dr. Anand Krishnan,
Department of Biological Sciences
IISER Bhopal

Certificate

This is to certify that this dissertation entitled “Geographical variation in bird song at different scales and its potential utility in monitoring the effects of habitat fragmentation” 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 Nafisa. A. Pathaw at IISER Pune under the supervision of Dr. Anand Krishnan, Assistant Professor, Department of Biological Sciences, IISER Bhopal, during the year 2021-2022.



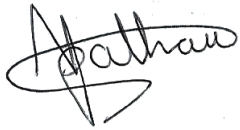
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Declaration

I hereby declare that the matter embodied in the report entitled “Geographical variation in bird song at different scales and its potential utility in monitoring the effects of habitat fragmentation” 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 Dr Anand Krishnan and the same has not been submitted elsewhere for any other degree.

A handwritten signature in blue ink, appearing to read 'A. Pathan', with a large, sweeping flourish underneath.

Signature of the Student

A handwritten signature in blue ink, appearing to read 'Dr. Anand Krishnan', with a long, sweeping flourish extending to the right.

Signature of the Supervisor

Abstract

The cultural transmission of birdsongs has been proven to be of significant importance. Several studies have found male birds interacting with neighbouring birds in the adjacent territories for purposes such as territorial defense and mate attraction. During such interactions, they have been found to share songs with their neighbours, which may involve them singing song types that are similar to that of their neighbours. Several studies have found that song-sharing between neighbouring birds may help males maintain territory tenure and reproductive success. A number of studies have also found song sharing to correlate with the geographical distance between birds' territories. Habitat fragmentation has been found to hamper this process of song-sharing between neighbouring birds. Thus we may use song sharing as an indicator of habitat fragmentation. I aimed at inquiring whether a correlation exists between song sharing and the geographical distances of the territories of the Ashy prinia (*Prinia socialis socialis*) at IISER Pune, Maharashtra and that of the Pale-chinned flycatcher (*Cyornis poliogenys*) in Nongkhyllam Wildlife Sanctuary, Meghalaya, respectively. No significant correlation was found as a result of this study. Habitat fragmentation or barriers may also lead to geographical variations in birdsong. I aimed to study this as well in this thesis by examining geographical variations between the respective three Ashy prinia (*Prinia socialis*) subspecies and three Grey-breasted prinia (*Prinia hodgsonii*) subspecies that are both found extensively in India. Data was collected from online song databases which had the acoustic data of the various subspecies taken in accordance with their respective habitat distributions. Analysis revealed that there was no significant evidence of this in the Grey-breasted prinia (*Prinia hodgsonii*) subspecies but statistical analysis showed a significant result for the Ashy prinia (*Prinia socialis*) subspecies, although this is preliminary on account of the low sample size. Nevertheless, studying song sharing in birdsong can still serve as a useful tool for conservation purposes, pending further study.

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1. INTRODUCTION

The twenty-first century has witnessed a large increase in urbanization and population density. The outcomes of this have often led to an increase in encroachment upon the habitats of many organisms such as forests, grasslands, and wetlands, to name a few. Consequently, this has resulted in the formation of habitat patches which have often had negative impacts on numerous populations of animals including birds in particular (Quesnelle et.al., 2013; Seress and Liker, 2015). As a result, many organisms' population size gets reduced due to predation, isolation of habitat patches, and parasitism caused by habitat fragmentation (Saunders et.al., 1991). The avifauna in particular are amongst the group of organisms affected by this. It has also led to a decrease in species richness and the overall biodiversity of the affected areas (Wilcox and Murphy, 1985). Therefore, understanding its ecological and behavioural effects on the avifauna is significant for conservation purposes.

Many animals communicate with their conspecifics using sound. This often involves a sender and receiver in which the signal or sound being transmitted may convey information to the latter (Bradbury and Vehrencamp, 1998). Birds are among such organisms that communicate in this manner. For instance, such signals can take the form of alarm calls often used by birds to warn their nestlings about predators that may be close by (T.N Suzuki, 2011; Marler and Evans, 1996). Another highly crucial signalling process of theirs is in the context of sexual selection and reproduction, requiring males to sing birdsongs in order to attract a mate. They may also sing to defend their territories against other competing males or to maintain group cohesion (Catchpole and Slater, 2003).

Songbirds learn songs directly from their parents or from neighbouring birds (Marler and Slabberkoorn, 2004; Peter J.B. Slater, 1986; Peter J.B. Slater, 1988). Each species has its characteristic set of songs composed of a varied number of syllables. These song types together collectively comprise their song repertoire (Catchpole and Slater, 2003; Marler and Slabbekoorn, 2004; Beecher 2005), which may be matched between neighbouring individuals. Several studies have been done on studying song sharing in

birds such as song sparrows (*Melospiza melodia*) (Hill et.al., 1999; Hughes et.al., 1998; Beecher et.al., 2000), white-crowned sparrows (*Zonotrichia leucophrys*) (Poesel et.al., 2012) and American redstarts (*Setophaga ruticilla*) (Lemon et.al., 1985). Studying the extent of song-sharing between neighbouring versus non-neighbouring birds in a habitat can also serve as a framework to measure the effects of habitat fragmentation on bird populations. Studies suggest that grassland fragmentation increases song-sharing between neighbours, leading to a reduction in overall song diversity in smaller patches (Laiolo and Tella, 2005). Such studies may therefore serve as early warning systems which may provide information on how fragmentation affects population viability. The first part of my thesis focuses on this. I aim to explore whether song sharing correlates with the geographical distance in the Ashy prinia (*Prinia socialis*) in Pune, Maharashtra and apply the same methods to the Pale-chinned flycatcher (*Cyornis poliogenys*) situated in Nongkhylllem Wildlife Sanctuary and Reserve Forest. The next section of my thesis focuses on studying geographical variation in the birdsongs of the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies, respectively. Through this project, I hope to gain a deeper understanding of the factors impacting bird behaviour, a phenomenon that remains poorly studied especially in India (Purushotham et.al., 2016). The next sections (1.1) and (1.2) of this chapter will give us a further detailed introduction to the study of song sharing and geographical variation in birdsong

1.1 Objective: Studying song sharing as a paradigm to monitor the effects of habitat fragmentation on birdsong

Null hypothesis 1: No correlation exists between song sharing and the geographical distance of the territories of the Pale-chinned flycatcher (*Cyornis poliogenys*)

Null hypothesis 2: No correlation exists between song sharing and the geographical distance of the territories of the Ashy prinia (*Prinia socialis*)

Social learning is an important process for birds. During the process of dispersal from their natal sites and reaching their new breeding sites, birds may spend a considerable

amount of time learning the songs of their neighbours (McGregor and Krebs, 1982; Beecher et.al., 1994). Song sharing can occur in two ways. First, birds can match the song types within their repertoires which have the closest similarity to that of their adjacent neighbours'. Second, they can match a song type which is not being sung at the moment by their neighbours but is still shared between them (Marler and Slabberkoorn, 2004). Birds may also seek out territories consisting of birds that sing similar songs (Catchpole and Slater, 2003). Song sharing plays an important role in many processes that affect birds' survival. It may influence the reproductive success of males by playing a part in their ability to acquire and retain territories. An example of this was found in a study on Song sparrows (*Melospiza melodia*) that showed that song sharing was a contributing factor to maintaining territories, as opposed to repertoire sizes (Beecher et.al., 2000). Females that grow up listening to male conspecifics singing local songs or dialects formed by song sharing amongst neighbouring local bird populations may also prefer choosing mates that do the same (O' Loghlen and Rothstein, 1995), thus influencing reproductive success. The Indigo Buntings (*Passerina cyanea*) serve as a good example of this, studies found that males that shared songs with their neighbours exhibited higher reproductive success (Payne, 1982). Song sharing has often been linked to aggression amongst males with adjacent territories, Song sparrows (*Melospiza melodia*) have been shown to exhibit this behaviour (Wilson & Vehrencamp 2001). Naturally, as the distance between individuals' territories increases, the songs shared between them may decrease as their interaction and influence over each other is hampered. This negative correlation of song sharing with distance has been found in male House Finches (*Carpodacus mexicanus*) (Bitterbaum and Baptista, 1979) within a distance of about 5kms. A similar case was found in the Song sparrow subspecies (*Melospiza melodia cooperi*) (Wilson et.al., 2000) and Great-tit (*Parus major*) where the latter study also found that increased habitat fragmentation had a negative correlation with dispersal tendencies (Rivera-Gutierrez et.al., 2010). A similar study which inspired this thesis was based on populations of the Dupont's lark (*Chersophilus duponti*) in Northern Africa and Spain (Laiolo and Tella, 2005). This research found an interesting correlation between variations in song sharing and ecological factors such as habitat fragmentation. As habitat fragmentation

increased, Dupont's Larks (*Chersophilus duponti*) were found to share more songs with neighbouring males compared to their non-neighbours, due to habitat barriers being formed. In contrast, as habitat fragmentation reduced, and the steppe land habitat became more connected, song sharing and song types between neighbours were lesser than that between non-neighbouring birds. This was found to occur because connected or continuous habitats provide more opportunities for individuals to disperse and interact with one another, instead of being restricted to just their local adjacent neighbours. Thus using song sharing to monitor the effects of habitat fragmentation on birdsong may serve as a useful tool to help us detect its negative effects at an earlier period; as compared to monitoring genetic changes. This section of the thesis takes two approaches towards exploring song-sharing in the species of interest. For the first approach, I test the null hypothesis stating that a correlation does not exist between song sharing and Euclidean distances in the principal component (PC) space of the Ashy prinia (*Prinia socialis*) song syllables' signal parameters. The Ashy prinia (*Prinia socialis*) is an understory insectivorous bird. It is a resident wren warbler at the study site, located at the IISER Pune (Indian Institute of Science Education and Research) campus in the state of Maharashtra. It is situated at the Deccan Plateau and has a rich biodiversity of avifauna. I recorded its breeding song which it sings from July to October (Chitnis et al., 2020). The purpose of this study was for me to practise and get accustomed to collecting and analysing acoustic data. The Ashy prinia (*Prinia socialis*) is found abundantly at the study site, is highly vocal during the breeding season and has a rather simple and stereotyped song. The methods applied for the Ashy prinia (*Prinia socialis*) study would eventually be tested in a forested habitat in Meghalaya, India. Next, I test the null hypothesis that states that there exists no correlation between song sharing and the geographical distances of the Pale-chinned flycatchers' (*Cyornis poliogenys*) territories, in the subtropical hill forest of Nongkhylllem Wildlife Sanctuary and Reserve Forest, Meghalaya. It is a small insectivorous oscine passerine bird that is found in Nepal, Bhutan and the Northeastern parts of India (Ali and Ripley 1983). It belongs to the Muscicapidae family, has a complex song and sings throughout the year. Not many studies have been done in India that explore the effects of ecological factors such as habitat fragmentation on song-sharing in insectivorous birds. There is even

lesser literature and research done on the song characteristics and behaviour of this particular bird.

1.2 Objective: To study the geographical variation in the songs of the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies

Null hypothesis 3: No geographical variation exists in the birdsongs of the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia socialis*) subspecies

We have discussed earlier that many oscine passerine birds tend to have characteristic vocalizations that help them communicate with each other and identify their conspecifics. We now dive deeper into addressing the topic of birdsong dialects. One cannot discuss geographical variation in birdsongs of the same species without bringing up song dialects. Before discussing this we must first discuss the process of song learning in birds. Studies conducted over the years by pioneers in ornithology such as Peter Marler, Konishi and W. H. Thorpe (Marler and Slabberkoorn, 2004; Marler and Tamura, 1964; Konishi 1985, 1965; Thorpe 1958, 1961) on the development of birdsong reveal that many oscine passerines learn songs by hearing a tutor bird such as their father sing, and proceed to imitate or copying them. Concerning the way birds learn songs, there are two types of learners, namely, close-ended and open-ended song learners. The former is only able to learn songs during the first year of their lives and cease to do so after the sensitive phase of song acquisition ends; whereas the latter can continue to add new changes or elements to their song repertoires, often during a particular time of the year. Examples of close-ended learners include the well-studied Zebra finches (*Taeniopygia guttata*), White-crowned sparrows (*Zonotrichia leucophrys*) and Swamp sparrows (*Melospiza georgiana*). Open-ended learners include the European starling (*Sturnus vulgaris*) and the canary (*Serinus canaria*). Several studies have been done exploring song dialects in birds. One of the earliest studies on birdsong found evidence that the resident White-crowned sparrow subspecies (*Zonotrichia leucophrys nutalli*) of California, USA (Marler and Tamura 1962, 1964; Baker 1974) possessed distinct geographical dialects. The same was found in the migratory

subspecies *Zonotrichia leucophrys pugentensis* by Luis Baptista (Baptista 1977).

Another well-studied bird where dialects have been found over large distances is the Great-tit (*Parus major*), (Hunter et.al, 1979). Song sharing amongst male birds holding territories within a particular location can lead to the formation of song types or repertoires that differ from that of birds found in a different location; despite them being the same species. Such songs occurring in distinctive regions are called dialects (Marler and Tamura 1962). The reason for the occurrence of such dialects can be many. One possible cause could be the generation of new syllables, leading to new combinations of song sequences within a song type arising from song sharing. It is a well-known phenomenon that many birds disperse from their natal territories and establish their breeding sites where they rear their hatchlings. It is at such breeding sites where they intend to set up their territories, that the establishment of song dialects is formed with their neighbours. The time frame of the occurrence of their dispersal as well as their ability to learn songs or modify them can be crucial for birds such as close-ended learners (Podos and Warren, 2007). The formation of dialects can occur on a microgeographic scale, occurring in populations with close territories, enabling them to interact or interbreed. Conversely, they can occur on a macrogeographic scale as well, where large distances between several territories hamper interactions between them (Catchpole and Slater, 2003).

The study of geographic variation in birdsongs of oscine passerines can serve an important purpose because we can obtain immense information about how songs have evolved with time and trace their history. As habitats continue to change in the highly urbanized world today, we can use this approach to monitor its effects on birdsong and song divergence amongst allopatric and sympatric populations; as well as study the cultural evolution of birdsongs. This section focuses on exploring whether geographical variations occur in the songs of the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies, respectively. *Prinia socialis socialis*, *Prinia socialis inglisi* and *Prinia socialis stewarti* are subspecies of the Ashy prinia (*Prinia socialis*) whereas *Prinia hodgsonii hodgsonii*, *Prinia hodgsonii rufula* and *Prinia hodgsonii albobularis* are the Grey-breasted prinia subspecies. The Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) are both small wren warbler species

belonging to the family *Cisticolidae* and the genus *Prinia*. Using data from online databases Xenocanto (<https://www.xeno-canto.org/>) and the Macaulay Library (<http://macaulaylibrary.org/>), I examined the subspecies' songs recorded from their respective habitat distributions within India.

2. MATERIALS AND METHODS:

This materials and methods section is divided into three sections. Section 2.1 focuses on materials and methods applied for studying the correlation of song sharing in the Ashy prinia (*Prinia socialis*) with their geographical distances at IISER Pune. The second (2.2) section addresses the materials and methods applied to study the correlation between song sharing and the geographical distances of territories occupied by Pale-chinned flycatchers (*Cyornis poliogenys*) at Nongkhylllem Wildlife Sanctuary and Reserve Forest, Meghalaya. The third section (2.3) addresses the materials and methods applied for studying geographical variation in the sympatric wren warblers' songs, the Grey-breasted prinia (*Prinia Hodgsonii*) and Ashy prinia (*Prinia socialis*) subspecies found in India.

2.1: Studying the correlation between song sharing and geographical distance in the Ashy prinia (*Prinia socialis*)

2.1.1 Study Site:

This study was conducted at the IISER Pune (Indian Institute of Science Education and Research) campus, Pune, Maharashtra situated in the Deccan plateau of India. The duration of the study was 27th July-13th October, 2021. This campus has a relatively large population of Ashy prinia (*Prinia socialis*) with their breeding season occurring during the months of July-October (Ali and Ripley, 1983; Chitnis, 2020).



Fig. 1. A Map of the sample sites at the IISER Pune Campus

Source: Map made in OpenStreetMap®

The black dots in figure 1 represent the Gps coordinates of each of the sampling points taken at IISER Pune, Maharashtra and represented on a map.

2.1.2 Sound Recording and sampling method:

Recordings were made using an ME62 Sennheiser (Wedemark, Germany) omnidirectional handheld microphone (frequency range 20 Hz-20,000 Hz) which was connected to a Zoom H6 recorder (Tokyo, Japan) via an XLR cable. The dawn chorus of the birds was recorded from 6:00 AM and would stop after 9 AM, as the study site is an urban area that tends to have high levels of noise from sources such as vehicles passing by. The duration of each recording was set to 1 minute, except in cases where the bird would take a longer time to begin singing. If the bird was present at a sampling point, it was recorded. I would then move ahead to the next sampling point by covering a distance that was greater than 50m, in order to avoid sampling the same individual

twice. This distance between the sampling points is maintained because understory insectivorous birds tend to hold territory sizes that are less than 50m (Hart et.al., 2018; Rivera-Gutierrez, 2010). Since it is not possible to sample the entire campus in one morning, I would sample areas in the IISER PUNE campus' Baner gate, Main building, Guesthouse, Lecture Hall Complex, undergraduate Labs and the dining areas in one day (named G1, \tilde{n} , Mendeleev and the Dining block). The following day, I recorded areas close to the vicinities of the campus' Pashan gate, Faculty housing areas, and Hostels 1, 2, and 3. In the next subsequent days, I made sure to sample the areas which I had missed out on the previous ones. 85 recordings were used in total for this analysis.

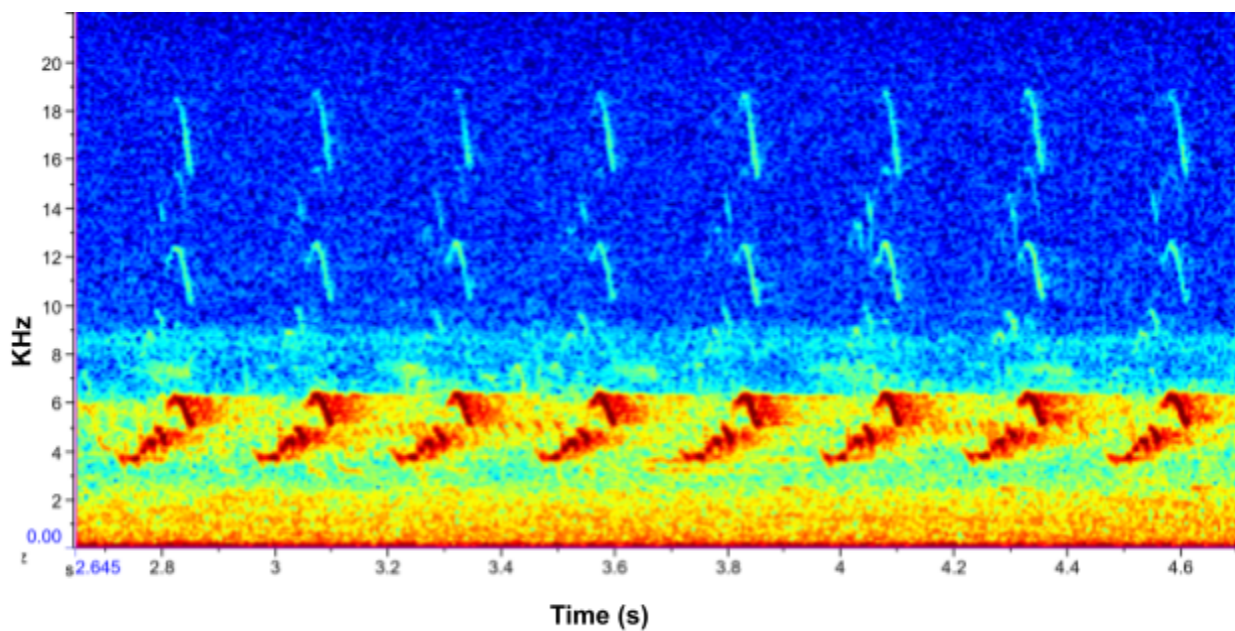


Fig. 2. A spectrogram depicting an Ashy prinia's (*Prinia socialis*) song syllables at the IISER Pune campus

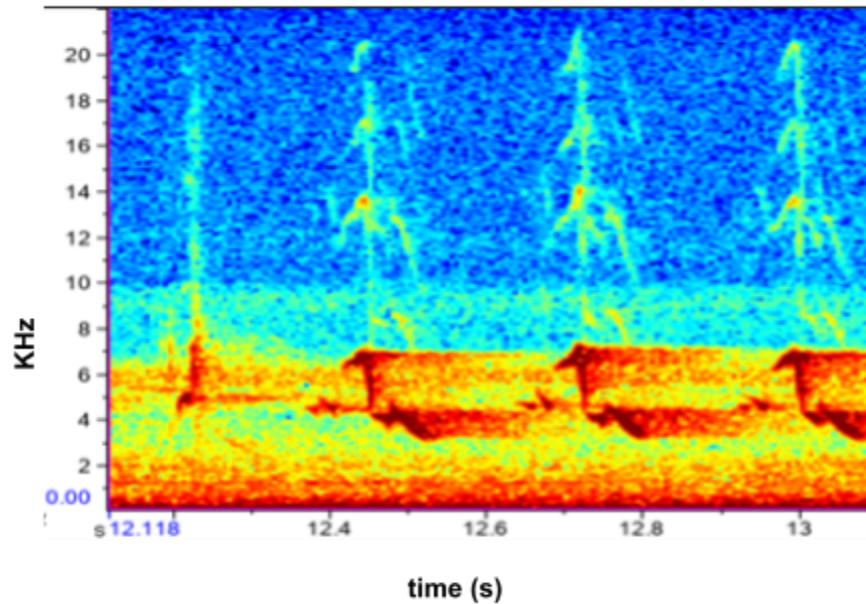


Fig. 3. A spectrogram depicting another syllable of the Ashy prinia's (*Prinia socialis*) syllable in the IISER Pune campus

2.1.3 Data Analysis:

I used the Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, NY) for the purpose of labelling each syllable of the different song types of the Ashy prinia. A note is represented as a continuous trace on a spectrogram and is the fundamental elementary component of a song. A set of two or more notes together in turn form a syllable in a song type. The following parameters for each syllable were calculated: low and high frequencies (Hz), the peak frequency contour at the start and end of the note (Hz), peak frequency contour maximum and minimum frequencies (Hz), 90% Bandwidth (Hz), centre frequency (Hz), Delta time (s), the Begin and End time (s), peak time relative, peak frequency average slope (Hz/ms) and Average Entropy (bits). Many of these parameters were selected based on the methods applied by Krishnan, 2019 and Chitnis et al., 2020 in their published work. For further analysis, the programming language Python 3.10 was used. I took the aid of my friend Damiki Laloo in order to be able to write the code for performing a principal component analysis on all the parameters except for the begin time, end time, low and high frequencies. A principal component analysis (PCA) helps one to visualize whether strong patterns or variations exist in the

data set and variables of interest. For data sets with a lot of parameters involved, it becomes difficult to uniformly perform various analytical methods to observe trends in the data. Hence, a PCA helps to reduce the data's dimensionality without compromising the data set's existing variations. Since each of the samples' parameter measurements was reduced to three principal components, the euclidean distances between their respective principal components (PC1, PC2 and PC3) were calculated.

In order to be able to check for a correlation in the extent of song sharing amongst neighbouring birds with geographical distance. I used the haversine formula in python 3.10 to measure the distance between the various sampling points/GPS coordinates in my study site. It computes the greatest circle distance amongst two points present on the surface of a sphere (the earth in this case), using their respective latitudes and longitudes as the input. The formula is given below as:

$$d = 2r\Phi \sin^{-1} \left(\sqrt{\sin^2 \left(\frac{\Phi_1 - \Phi_2}{2} \right) + \cos(\Phi_1) \cos(\Phi_2) \sin^2 \left(\frac{\Psi_2 - \Psi_1}{2} \right)} \right)$$

Where d represents the distance between two points on a great circle; r is the radius of the sphere (the earth in this case); Φ_1 and Φ_2 represent the latitudes of the two points (radians); Ψ_1 and Ψ_2 are the longitude inputs (radians). Upon obtaining the result, a scatter plot in python 3.10 was then made to check if a correlation existed between the euclidean distances of the samples' principal components and the actual geographical distances between them.

2.1.4 Statistical test:

For the statistical test, Spearman's correlation coefficient (ρ) was used. It is a non-parametric statistical test that helps us quantify the monotonic association (linearly correlated or not) existing between a pair of ranked variables i.e ordinal or continuous. It takes the ranked values of each variable into account for comparison. The values that it returns range from -1 (indicating a perfect negative correlation of ranks) to 1 (indicating a perfect positive correlation of ranks). A value of 0 indicates no association between the ranked variables. This was used on this part of my data as well using python 3.10,

to be able to execute this part of the analysis. The formula for Spearman's correlation coefficient is given below as:

$$\rho = 1 - \frac{6\sum d_i^2}{n(n^2 - 1)}$$

ρ represents the Spearman correlation coefficient; n is the number of observations and d_i is the difference between the two ranks of each observation.

2.2 Studying the correlation between song sharing and geographical distance in the Pale-chinned flycatcher (*Cyornis poliogenys*)

2.2.1 Study site:

The samples for this study were collected at Nongkhylllem Wildlife Sanctuary (29sq.km) and Reserve forest (96.91 sq. km) which are situated in Meghalaya's Ri Bhoi District. A major part of the sampling was done at the wildlife sanctuary, situated between 25°45'-26°00' N latitude and 91°45'-92°00' E longitude (Bhuyan, 2020). These areas are largely composed of tropical moist deciduous forests along with extensive areas of bamboo and tropical semi-evergreen forest (Dimos Khyntiam and Nibedita Sen, 2016). The Pale-chinned flycatcher, also known as Brook's flycatcher (*Cyornis poliogenys*) is an understory insectivorous bird that mostly forages on bamboos. The study was conducted from 2nd November 2021-4th December 2021. Although the study was not conducted during the breeding season, the bird was frequently found to sing even during the early winter season in the state.



Nongkhylllem Wildlife Sanctuary, Ri Bhoi District Meghalaya

2.2.2 Sound recording and sampling method:

Recordings were made using an ME62 Sennheiser (Wedemark, Germany) omnidirectional microphone (frequency range 20 Hz-20,000 Hz) which was connected to a Zoom H6 recorder (Tokyo, Japan) via an XLR cable. The recording was fairly challenging in the field and it was not easy to directly record a bird from the shrub or tree where it was singing. Hence using omnidirectional microphones was useful because they can capture sounds from all directions. Since the pale-chinned flycatcher's (*Cyornis poliogenys*) songs are loud, using this type of microphone setup

was not an issue. The dawn chorus was recorded on each day from about 5:00 AM and continued till 10:00 AM; on certain days it would go on till 11:00 AM. The duration of each recording was approximately 5 minutes. I would then move on to the next sampling point by walking a distance of roughly 100m, in order to avoid recording the same individual twice. Since Pale-chinned flycatchers (*Cyornis poliogenys*) stay still while singing, attempts were made at recording very close to their singing perches. As Nongkhylllem Wildlife Sanctuary and Reserve Forest have a large area, it was not possible to cover the entire area within a month. My sampling site consisted of five fixed routes/trails, on which I would sample on five continuous days of the week. For example, if I sampled along trail 1 on a Monday then I would move on to trail 2 for the next day (i.e a Tuesday) and so on. Owing to the vegetation of the sampling sites which mostly consisted of tropical moist deciduous forests and tropical mixed evergreen forest types which were dominated by mixed bamboo on which the bird was often found singing from, seeing the bird at all times both with and without binoculars was a challenge. Therefore to tackle this disadvantage, identification was made based on training my hearing abilities to correctly identify them by listening to their songs. On most sampling points when the bird was seen, the songs identified were the ones heard without seeing the bird. At the end of the 5 minutes recording, the location of each sampling point was noted via a Garmin Etrex 30x handheld GPS device and any additional info was noted verbally by speaking into the Sennheiser ME62 handheld microphone. Notes regarding the time of the recording and any other additional information were then transcribed into a notebook and google sheet. A total of 96 recordings were used in this analysis.

2.2.3 Data Analysis:

I used the software Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, NY) for the purpose of labelling each note of the different song types of the Pale-chinned flycatcher's (*Cyornis poliogenys*) repertoire. The following 14 parameters were measured for each syllable: low and high frequencies (Hz), the peak frequency contour at the start and end of the note (in Hz), peak frequency contour maximum and minimum frequencies (Hz), Bandwidth of 90% (Hz), centre frequency (Hz), Delta time (s), the

Begin and End time (s), peak time relative, Peak frequency average slope (Hz/ms) and Average Entropy (bits).

2.2.4 Classification of song sequences:

A good and well-accepted approach to be able to quantitatively measure and compare song sharing between different individuals in the study site is to classify different note types into their respective classes based on the acoustic properties of their songs Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, NY, USA). After classification song sequences were then made based on the order of occurrences of the respective notes/ syllables within a song type. The song types usually consisted of a series of notes which in turn formed syllables. Syllables in a particular order together then formed a song sequence. The following note/syllable class conditions were applied:

1. The first condition for classification was to look at the acoustic properties of the notes in their songs. Namely, peak frequency (kHz), peak frequency contour average slope (Hz/ms), 90% bandwidth, peak frequency contour minimum and maximum frequency were taken into account in order to classify them into separate note classes. This step was of the highest order of importance.
2. The second order of classification was to study how they looked visually on a spectrogram and assess their shapes: for example, an ascending note would usually have a sharp upsweep as compared to that of a descending note that has a sharp down sweep shape.
3. The next form of classification was to listen to how each note sounded. If the notes were too fast, the recording would be played at a slower playback speed.

The Pale-chinned flycatcher's (*Cyornis poliogenys*) notes in each song type were classified into the following categories:

1. Ascending notes (m,n,o,p)
2. Flat notes (v, a- l)
3. Descending notes (s, t, u)
4. Rattle/ trill syllables (r)

5. Click notes (w)
6. Notes that begin with an upsweep and end in a downsweep (y) or ascending+ descending notes.

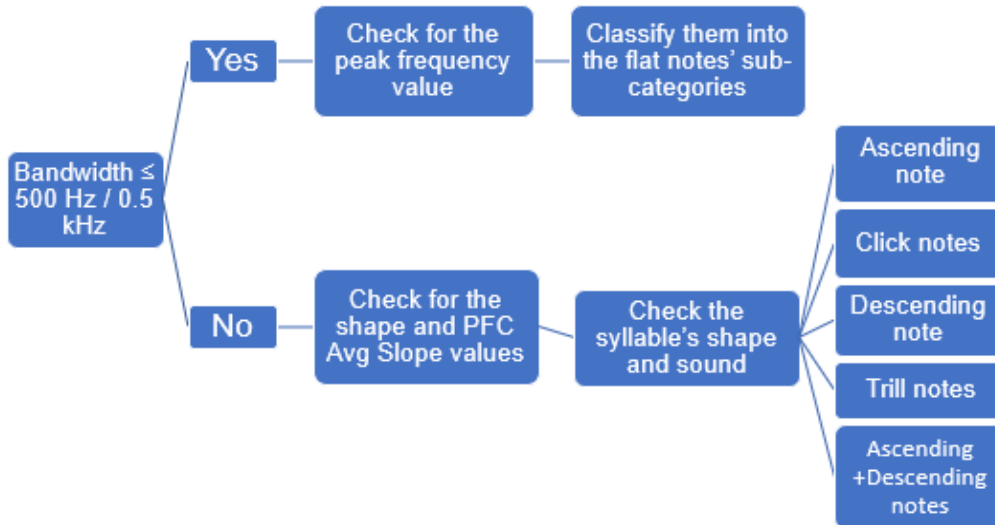


Fig. 4. This is a flow chart depicting a summary of the procedure of the notes/syllables' classification

A detailed explanation of the note/syllable classification forming their song sequences is given below as follows:

Flat notes/syllables

These are a series of brief notes, where the pitch remains fairly unchanged. They appear as clear traces in the spectrogram with unmodulated frequency ranges. All syllables where the difference between their minimum and maximum peak frequency contour ranges, or bandwidth measurements did not exceed 500 Hz or 0.5 kHz were grouped into this category. The Pale-chinned flycatcher's (*Cyornis poliogenys*) repertoire consists of a large number of such notes with peak frequencies ranging from as low as 2 kHz to as high as 9 kHz.

In order to classify them in a more rigorous manner, they were further grouped into the following sub-categories:

2.5- 2.99 kHz	3-3. 49 kHz	3.5- 3.99 kHz	4-4. 49 kHz	4.5- 4.99 kHz	5-5. 49 kHz	5.5- 5.99 kHz	6-6. 49 kHz	6.5- 6.99 kHz	7-7. 49 kHz	7.5- 7.99 kHz	8-8. 49 kHz	8.5- 8.99 kHz
v	a	b	c	d	e	f	g	h	i	j	k	l

Table 1: Shows the various sub-categories of flat notes/syllables in the Pale-chinned flycatcher's (*Cyornis poliogenys*) song

This was based on their peak frequency contour (PFC) maximum and minimum measurements in Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, NY, USA) where each alphabet represents a group of flat notes with similar characteristics.

Descending notes

This category includes notes beginning at higher frequencies and ending at relatively lower ones. They undergo frequency modulations and appear as downward slopes or sweeps from the left to the right side in the spectrogram. Due to such frequency modulations, they have been further divided into subgroups based on their respective maximum and minimum peak frequency contour ranges (PFC minimum freq and PFC maximum freq) in Raven Pro 1.6. The average peak frequency contour slope measurement (Hz/ms) on Raven Pro 1.6 is usually a negative value for such notes. For example, a descending note with PFC minimum and maximum ranges being 4.52 kHz to 5.33 kHz would be classified as note class type "t" (see table 2). All of such note class types also have bandwidth ranges exceeding 0.5 kHz (or 500 Hz).

2-4kHz	4-6kHz	6-8kHz
s	t	u

Table 2: Show the various descending note class sub-categories in the Pale-chinned flycatcher (*Cyornis polio-genys*)' song based on their PFC minimum and maximum ranges.

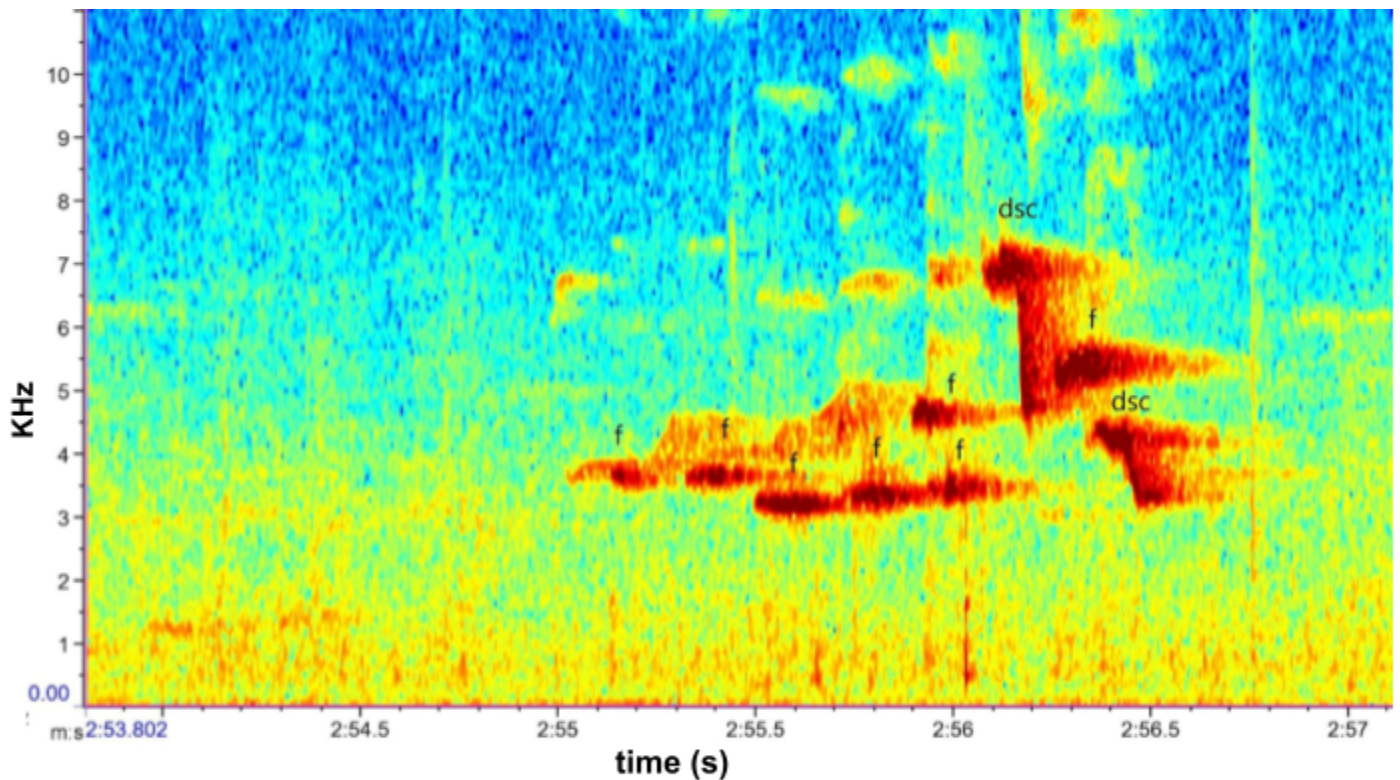


Fig. 5. A spectrogram (frequency vs time) displaying the pale-chinned flycatcher's (*Cyornis polio-genys*) descending notes (dsc) and flat notes (f) in a song type.

Ascending notes:

Ascending notes consist of those beginning at lower frequency ranges and ending at higher ones. Their frequencies are modulated as well and they appear as slopes slanting upwards from left to right in the spectrogram window. These have bandwidth measurements that exceed 500 Hz or 0.5 kHz and average peak frequency contour measurements (Hz/ms) that are positive values. They have been further classified into

sub-categories based on their maximum and minimum peak frequency contour values (in Raven Pro 1.6). For example, an ascending note with PFC minimum and maximum ranges being 6.66 kHz to 7.88 Khz, will fall under the note class category that is represented by the alphabet “o” (see table 3). The following table shows the various ascending notes class subcategories:

2-4 kHz	4-6 kHz	6-8 kHz	8-10 kHz
m	n	o	p

Table 3: Shows the various ascending note class sub-categories in the Pale-chinned flycatcher’s (*Cyornis poliogenys*) song based on their PFC minimum and maximum ranges.

Trill/ Rattle syllables: These consist of a series of fast duration notes with peak frequency ranges between 2-9 Khz. They often occur at the start or at the end of the song type. They are represented by the letter “r”.

Click notes:

A harsh-sounding note is produced consisting of a wide frequency range bandwidth from the lowest being about 1.4 kHz to the highest being approximately 3.5 kHz and peak frequency contour minimum and maximum ranges going from 1.2Hz to over 9 kHz. A brief stretch of white noise is heard when one hears the sound that we call a “click” (as defined by Catchpole and Slater, 2003). They are represented by the letter “w”.

A combination of ascending and descending notes (Asc + Dsc): There were very few of these notes, occurring about four times in three song types. These are notes that first begin with an upsweep and end with a down sweep with their minimum peak frequency contour being as low as 3.01 kHz to their maximum peak frequency contour value being 7.49 kHz. They are represented by the letter “y”

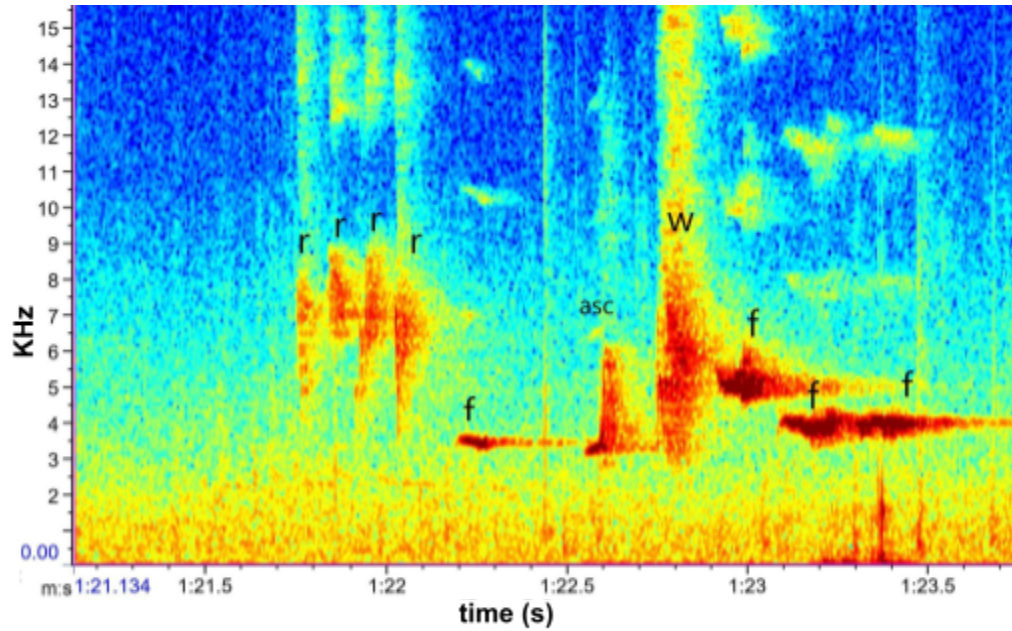


Fig. 6. A spectrogram (frequency vs time) displaying the Pale-chinned flycatcher's (*Cyornis poliogenys*) trill syllables (r), flat notes (f) and click notes (w) in a song type.

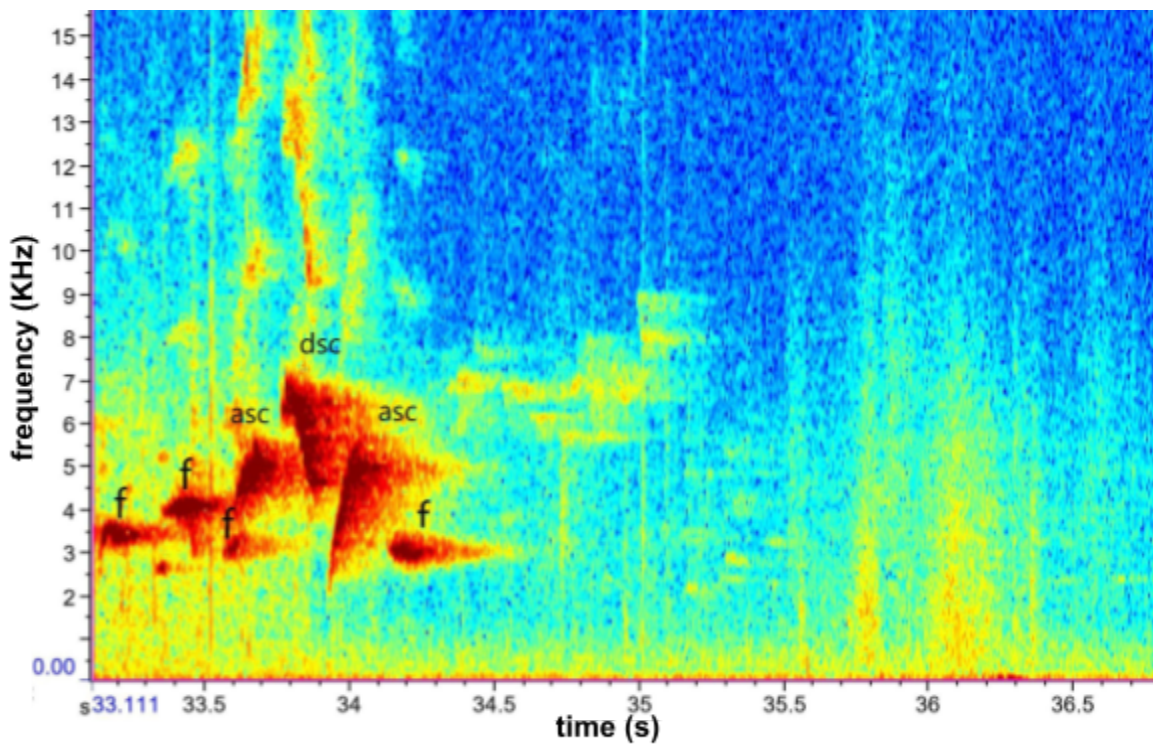


Fig. 7. A spectrogram (frequency vs time) displaying the Pale-chinned flycatcher's (*Cyornis poliogenys*) ascending (ASC), flat (f) and click (w) notes in a song type

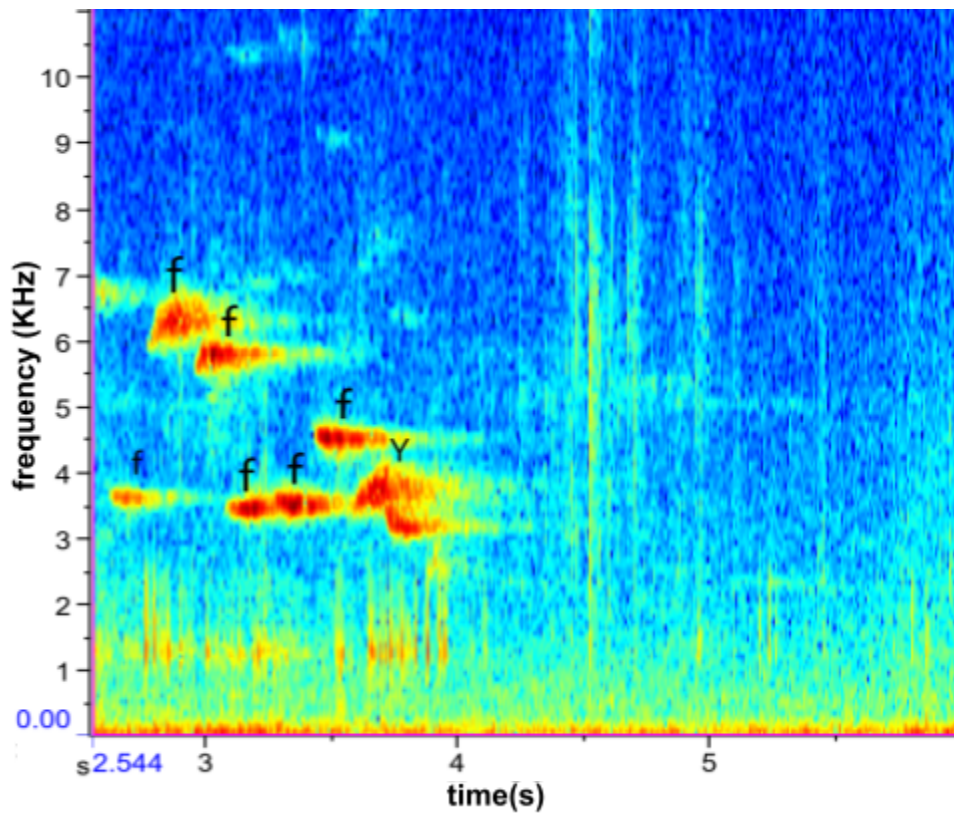


Fig. 8. Depicts a spectrogram (frequency vs time) displaying the Pale-chinned flycatcher's (*Cyornis poliogenys*) flat (f) notes and a note beginning with an upsweep and ending with a downsweep (Y) in a song type

2.2.7 Data Analysis

Levenshtein distance

This is also known as an “edit distance” metric that helps one quantify the similarity between song sequences. To quantify geographical distance I used the haversine formula here as well to compute the distance between the GPS coordinates of each sample in my study site using python 3.10. My friend Damiki helped me in being able to write the code for this part of the analysis.

Comparison of sequences with respect to geographical distances:

Out of 26 days of sampling, the data from 20 days was deemed suitable for use in the analysis. This was done because the remaining 6 days of sampling consisted of either no data or data with a lot of noise levels in them. The GPS locations/sampling points recorded on each day were categorized into groups based on how far they were from the first sampling point of the data used. GPS locations that were 200m away from the starting sampling point were placed into one group (say group 1), GPS locations that were at a distance greater than 200m but less than 400m were placed into the next group (say group 2); group 3 consisted of those that were located at distances greater than 400m but less than 600m and so on. The grouping of locations was done for each of the 20 respective sampling days. The upper constraint distance of 10km (as the farthest distance traversed along a trail is 7km) from the starting GPS sampling point was set, this was done in order to keep a check on how many groups could be formed along each trail. Once the groups were made, the song sequences that corresponded to the GPS sampling points within each of these groups were compared with those of other groups by measuring the Levenshtein distance medians between them using Python 3.10.

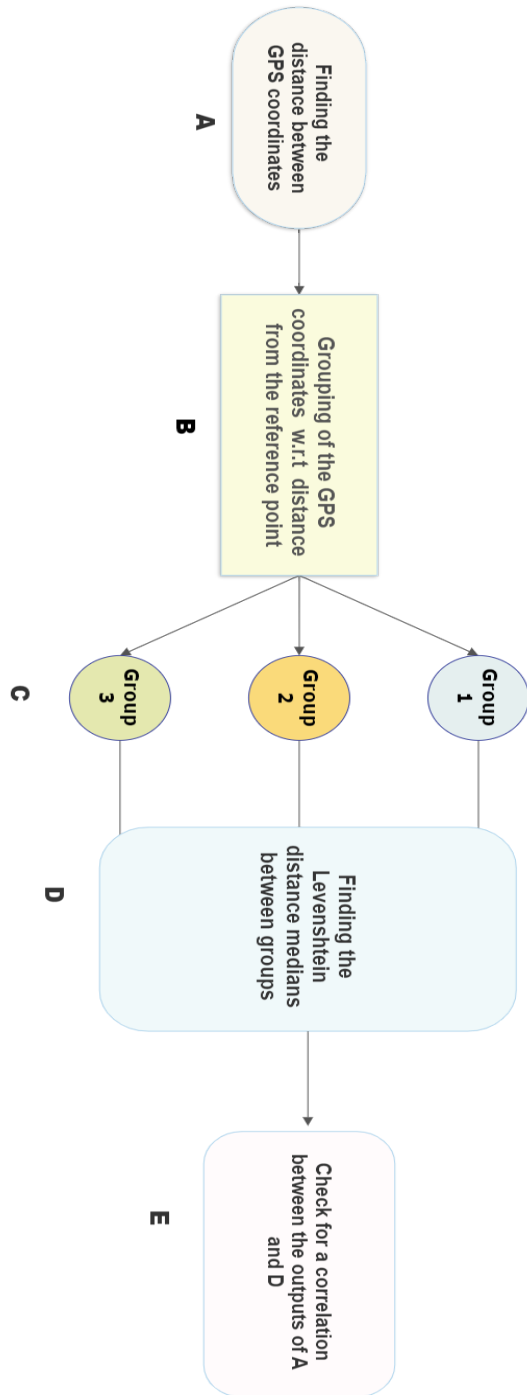


Fig. 9. A summary of the analysis for studying song sharing in the Pale-chinned flycatcher (*Cyornis polioagenys*)

2.2.8 Statical test:

A Spearman's correlation coefficient test was also performed for this part of the analysis to check if a correlation exists between song sharing and geographical distances.

2.3: Studying geographic variation in the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies

2.3.1 Data collection:

The song data of the Ashy prinia (*Prinia socialis*) subspecies and Grey-breasted prinia (*Prinia hodgsonii*) subspecies was curated from the online song database sources Xenocanto (<https://www.xeno-canto.org/>) and the Macaulay Library (<https://www.macaulaylibrary.org/>) (see Appendix). Their songs were then digitized in the Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, NY, USA) software. The same parameters as those mentioned earlier were calculated for each syllable i.e, 90% bandwidth, delta time, peak frequency, peak time relative, centre frequency, average entropy, peak frequency contour average slope, maximum and minimum peak frequency, and the peak frequency at the start and end of the note (calculated using the peak frequency contour feature in Raven Pro). There were 50 Ashy prinia (*Prinia socialis*) subspecies recordings used with 28 of them being *Prinia socialis socialis*, 18 being *Prinia socialis stewarti* and 4 being *Prinia socialis inglisi*, respectively. There were 53 Grey-breasted prinia (*Prinia hodgsonii*) recordings used for this analysis out of which 18 were *Prinia hodgsonii hodgsonii*, 17 were *Prinia hodgsonii rufula* and 18 were *Prinia hodgsonii albogularis*, respectively. From each recording, about 20 syllables were digitized. To obtain data for the geographic variation section, recordings which were taken from the subspecies' habitat distribution were required. The Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) recordings were taken from the online song databases mentioned earlier and were categorized into subspecies, based on where the recordings were taken from. The locations of the recordings are present in the online song database mentioned earlier. A strict criterion for categorizing them was based on referring to studies by Ali and Ripley, 1983 that defined the habitat distribution of the subspecies. Since the Grey-breasted prinia (*Prinia hodgsonii*) has a rather

complex song consisting of pre-rattle sequences (see fig.10) and successive rattle syllables, these two components of their song were digitized separately. On the other hand, the Ashy prinia (*Prinia socialis*) subspecies have comparatively simpler and more stereotyped songs consisting of repeating syllables (see fig.11).

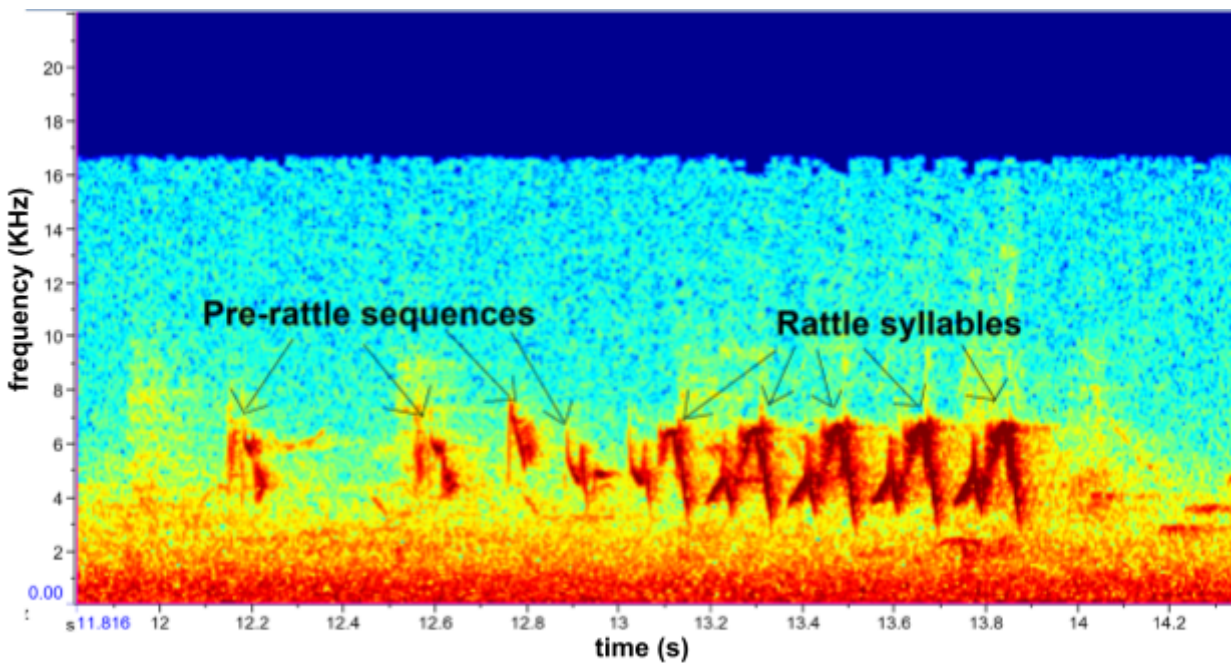


Fig. 10. Depicts a spectrogram showing pre-rattle sequences and rattle syllables of the song of a Grey-breasted prinia subspecies (*Prinia hodgsonii hodgsonii*)

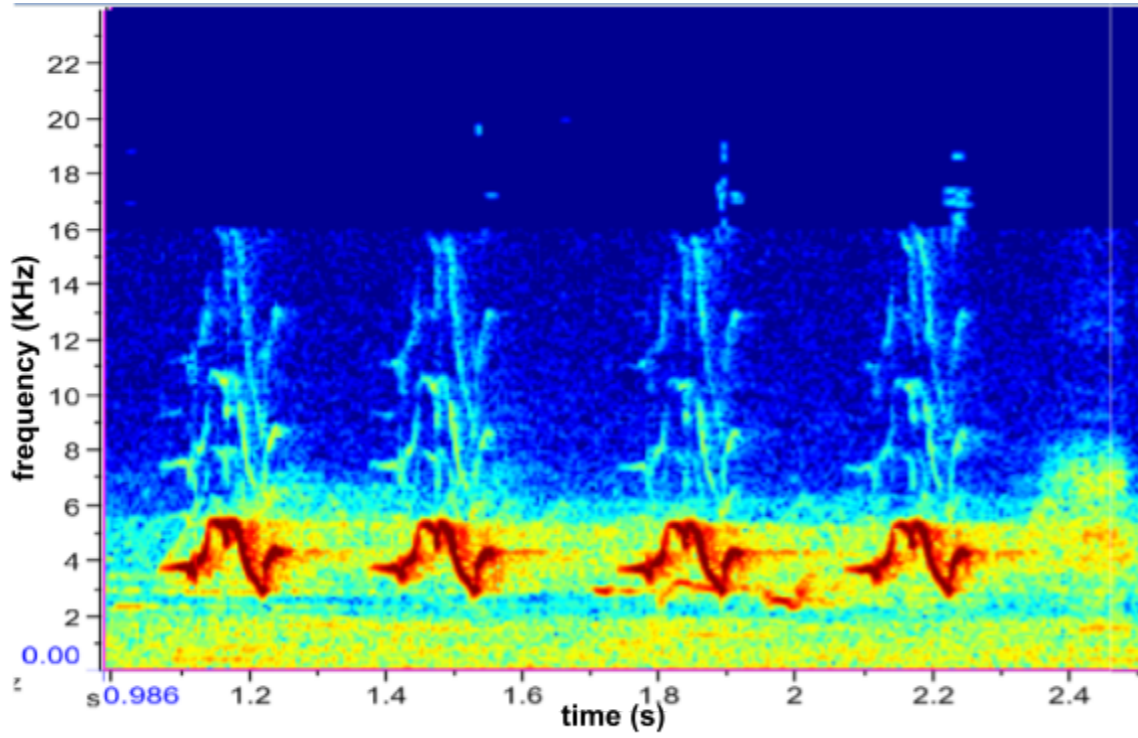


Fig. 11. Depicts a spectrogram showing syllables of the Ashy prinia subspecies' (*Prinia socialis socialis*) song

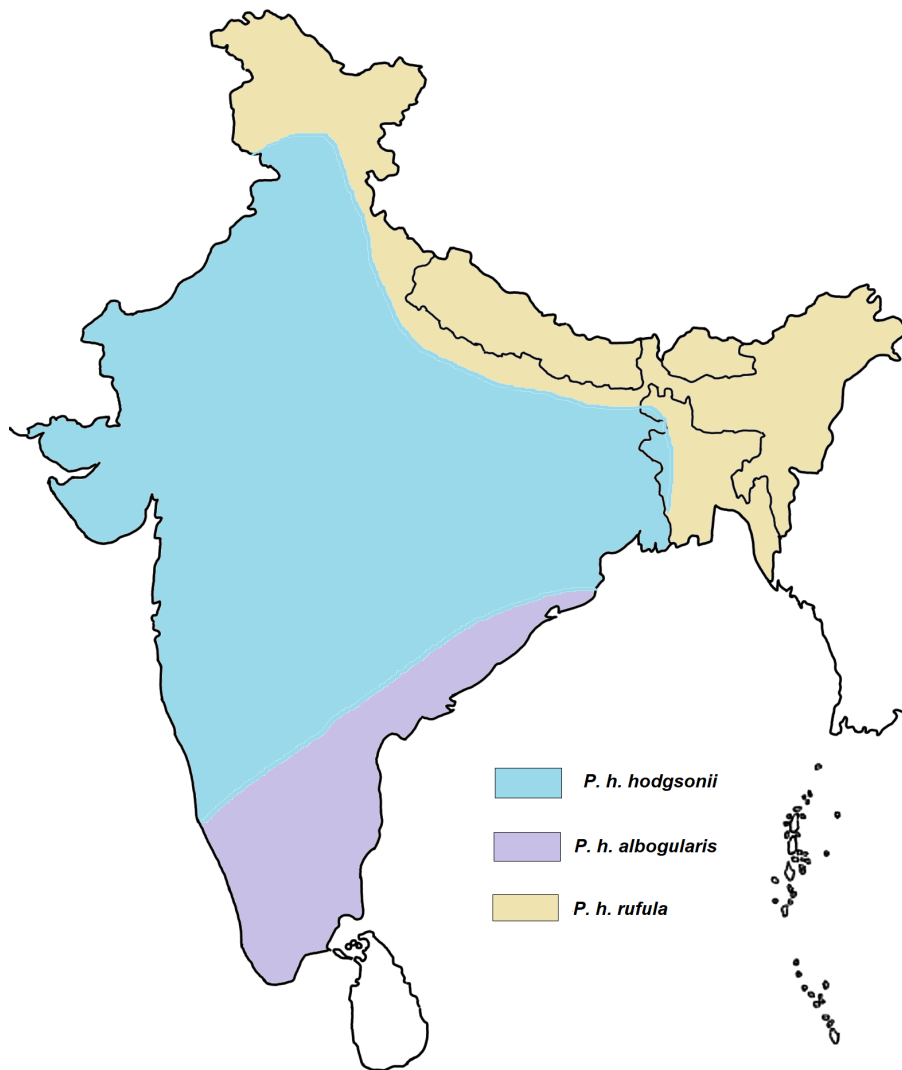


Fig. 12. Grey-breasted prinia (*Prinia hodgsonii*) subspecies distribution

Prinia hodgsonii hodgsonii (*P. h. hodgsonii*) resides in most parts of Central and North India (blue); *Prinia hodgsonii rufula*'s (*P. h. rufula*) habitat is restricted to the extreme parts of Northern India (beige) and the North East. Whereas, *Prinia hodgsonii albogularis* (*P. h. albogularis*) resides in most parts of South-eastern India (purple).

Note: The map was made with reference to the subspecies distribution in Ali and Ripley, 1983.

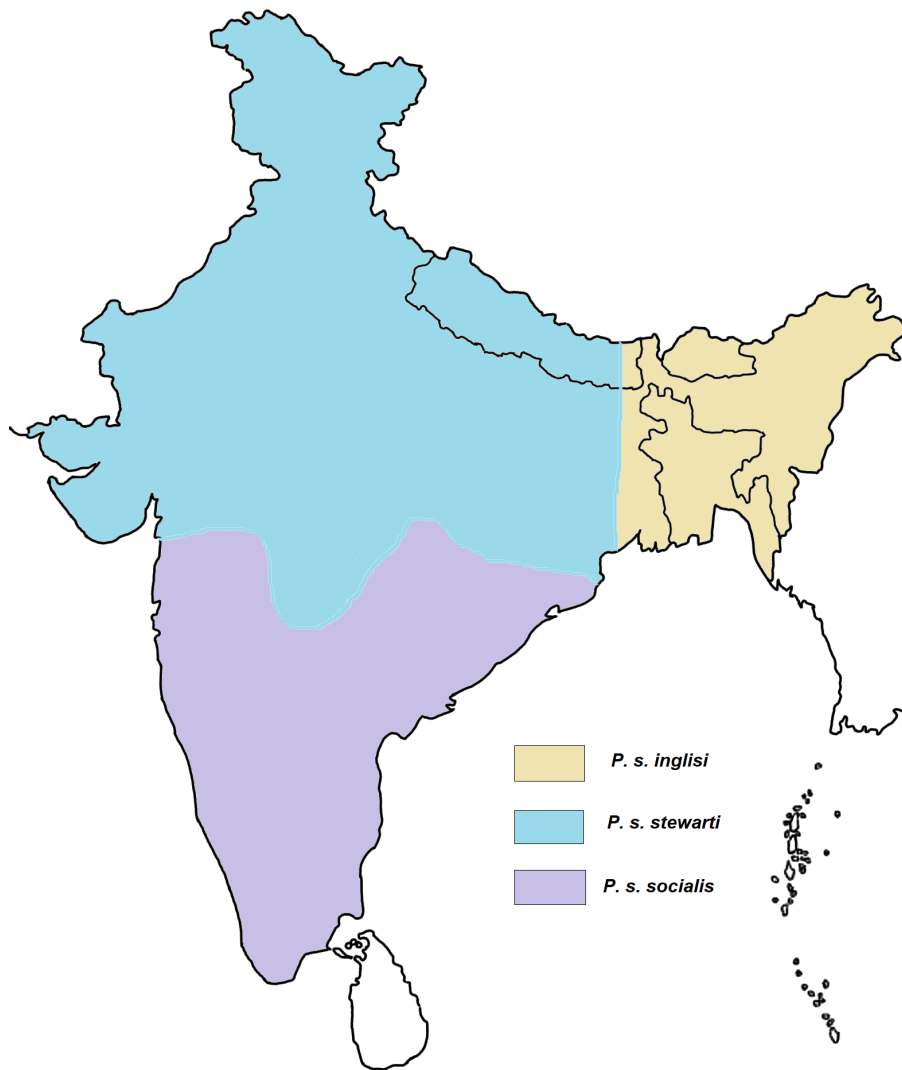


Fig. 13. A map depicting the geographical ranges of the Grey-breasted prinia (*Prinia hodgsonii*) subspecies' habitats.

Prinia socialis socialis (*P. s. socialis*) occupies most parts of peninsular India (purple). *Prinia socialis stewarti's* (*P. s. stewarti*) habitat distribution lies mostly in the Northern, Western parts of India and Nepal (blue). *Prinia socialis inglisi* (*P. s. inglisi*) (*beige*) resides in most parts of Northeast India, Bhutan, Northeast Bangladesh and parts of West Bengal.

Note: The map was made with reference to the subspecies distribution studied by Ali and Ripley, 1983.

2.3.2 Data Analysis:

I used the software Raven Pro 1.6 (Cornell Laboratory of Ornithology, Ithaca, NY) for the purpose of labelling the syllables and sequences present in the song data. The following 15 parameters were measured: low and high frequencies (Hz), the peak frequency contour at the start and end of the note (in Hz), peak frequency contour maximum and minimum frequencies (Hz), peak frequency (Hz), bandwidth of 90% (Hz), centre frequency (Hz), delta time (s), the begin and end time (s), peak time relative, peak frequency average slope (Hz/ms) and average entropy (bits). A principal components analysis (PCA) was done using MATLAB (Mathworks Inc., Natick, MA, USA) on the correlation matrix of eleven parameters (excluding begin and end time, low and high-frequency measurements). The principal component (PC) scores obtained were then used to make a three-dimensional scatter plot of the song parameters, representing their acoustic signal space (Krishnan 2019; Chitnis et al., 2020; Lahiri et al., 2021). For the Grey-breasted prinia (*Prinia hodgsonii*), three-dimensional scatter plots of the PC scores of their rattle and pre-rattle syllables, were made separately.

Linear Discriminant Analysis (LDA): A linear discriminant analysis (LDA) was done on the syllable parameters of the songs using the Classification Learner app in MATLAB (Mathworks Inc., Natick, MA, USA). Just like the PCA, LDA is a dimension reduction analysis that helps in the classification and visualisation of the data. Thus to execute this, Matlab's Classification Learner app helps us train a linear discriminant model and checks its accuracy at classifying notes/syllables of the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies respectively, based on the acoustic signal measurements of their songs' syllables; using a 5-fold cross-validation.

95% confidence ellipse plots: The 95% confidence ellipse for the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) were also made showing where the centroids of each subspecies' data are present.

2.3.3 Statistical test:

The principal component (PC) scores obtained in the earlier section (2.3.2) by performing a PCA using MATLAB (Mathworks Inc., Natick, MA, USA) on the acoustic signal parameters, were then subjected to a one-way multivariate variate analysis of variance (MANOVA) in Python 3.10. The python package statsmodels (Seabold et.al, 2010) was used and the MANOVA gets subjected to various test statistics such as Wilk's lambda, Pillai's trace, Hotelling's Lawley trace and Roy's greatest root using the python function "fit.mv test ()" which automatically computes these. These help us to identify differences between group means and to check if they are significantly different based on the acoustic signal properties of their songs.

3. RESULTS

This chapter is divided into three sections. The first two sections (3.1 and 3.2) consist of the results of the work done on exploring the correlation between song sharing and geographical distance in the Ashy prinia (*Prinia socialis*) at the IISER Pune campus and Pale-chinned flycatcher (*Cyornis poliogenys*), at Nongkhylllem Wildlife Sanctuary and Reserve Forest, respectively. The second consists of results from studying geographic variation in the Grey-breasted prinia (*Prinia hodgsonii*) and Ashy prinia (*Prinia socialis*) subspecies found in India (3.3)

3.1 Results of studying song sharing in the Ashy prinia (*Prinia socialis*) subspecies at the IISER Pune campus, Maharashtra

3.1.2 Principal Component Analysis:

Principal component analysis (PCA) done in Python 3.10 gives us the principal components 1, 2 and 3 as results. PC 1 accounts for 26.71% of the variance, PC 2 for 20.26% and PC 3 for 16.27% .Overall the three PCs account for 63.24% of the variance amongst the different individuals. Plotting the euclidean distance between the respective principal component scores of the samples vs their geographical distance gives us the following graph (fig.14)

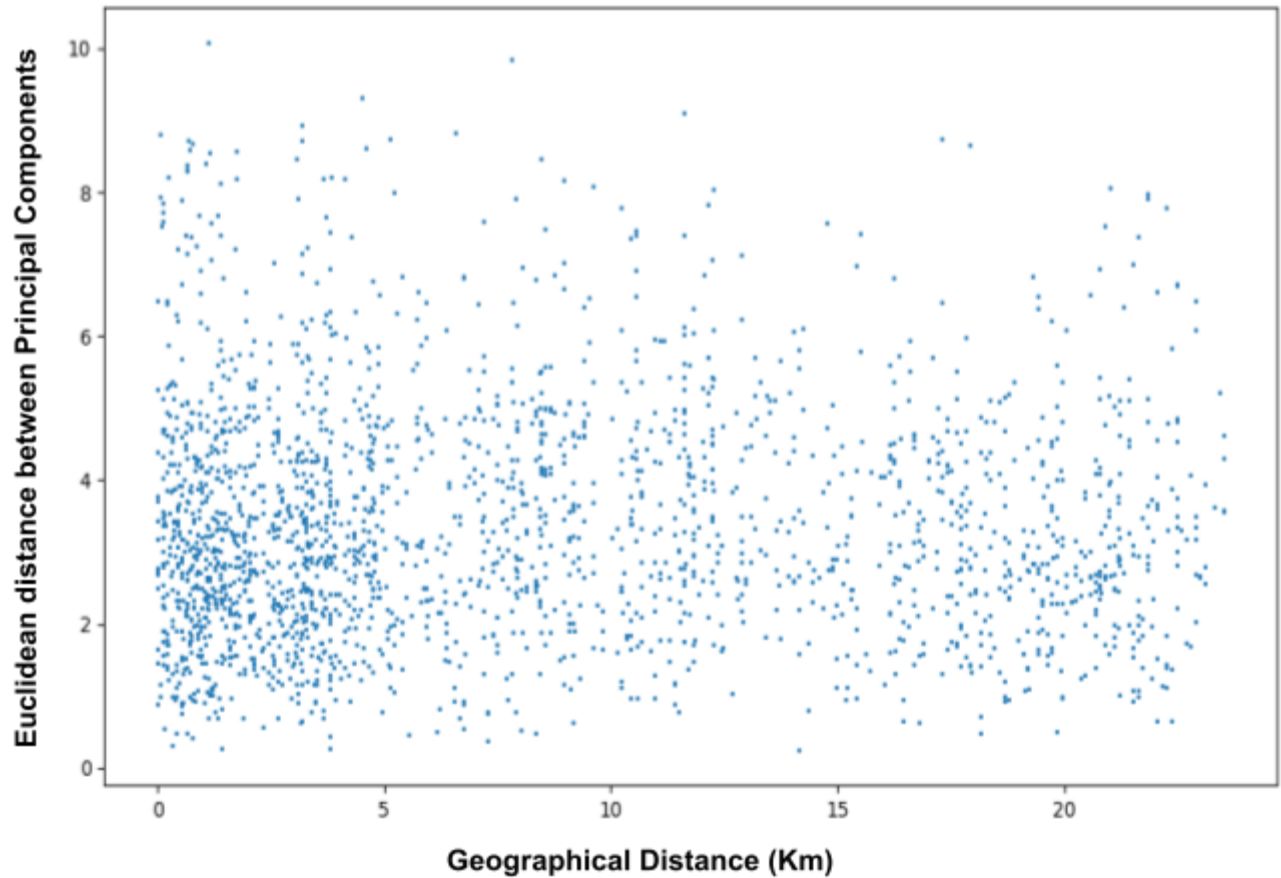


Fig. 14. Depicts the graph of the Euclidean distances between the principal components of each (PC 1, 2 and 3) respective sample pairs vs the geographical distances between them.

In the figure, the blue data points represent the result of the Euclidean distances between the Principal components (1, 2 and 3) of each respective sample pairs plotted with respect to the geographical distances between them. For the null hypothesis to be rejected, a trend is supposed to be observed in the figure. In the case of a positive linear correlation, the Euclidean distances between the PCs in the PC signal space would increase along with the geographical distances between their territory sizes. A negative linear correlation between the two variables would result in the euclidean distances decreasing as the geographical distances between their territory sizes increase. In this case, no trend is observed as the figure depicts. But a statistical test is required to confirm this and make a conclusion.

3.1.3 Statistical test result:

As mentioned in the methods section, a statistical test called Spearman's rho correlation analysis was done in python 3.10. The resulting overall Spearman's rho correlation coefficient is 0.037 with an overall p-value of 0.067.

3.2 Results on exploring the correlation between song sharing and geographical distance in the Pale-chinned flycatcher's (*Cyornis poliogenys*) song.

3.2.1 Levenshtein distance vs geographical distance: The number of song sequences present amounted to 174. A lot of the song sequences/ song types shared similar note class types but the order in which they occurred differed which led to such a high number of song sequences. Using Python 3.10 the Levenshtein distance medians between them were plotted against the geographical distance between their respective territories (fig.15)

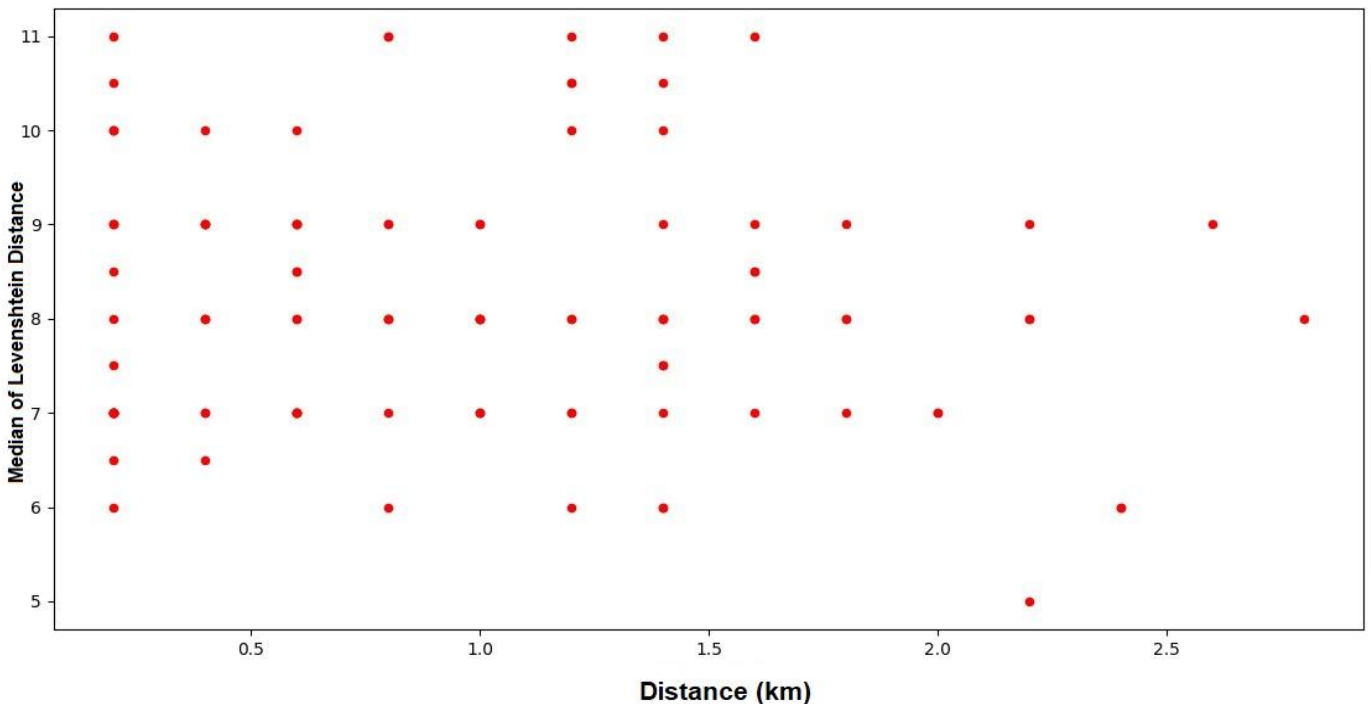


Fig. 15. Depicts the scatter plot of the Levenshtein distance medians (red) between the song sequences of the samples versus that of the geographical distances between them.

To reject the null hypothesis, there needs to either be a positive or negative linear correlation between the two variables. However, the graph obtained does not seem to indicate either of the two. Thus, here as well Spearman's rho correlation coefficient analysis is done to help us interpret the results better.

3.2.2 Statistical test result: Spearman's rho correlation coefficient for this data is -0.0545 with a p-value of 0.579. This is not a significant result and hence, we fail to reject the null hypothesis in this case as well.

3.3 Results of studying geographical variation in the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies' songs

3.3.1 Principal Component Analysis for the Ashy prinia (*Prinia socialis*) subspecies' song data:

The first three principal components out of eleven parameters (see fig.16) explained more than 75.59% of the variation present in the acoustic signals of the three Ashy prinia subspecies (*Prinia socialis*). Visually, the plot shows that some amount of overlapping in their acoustic signals occurs. But this is only a preliminary analysis and we cannot make concrete conclusions from this.

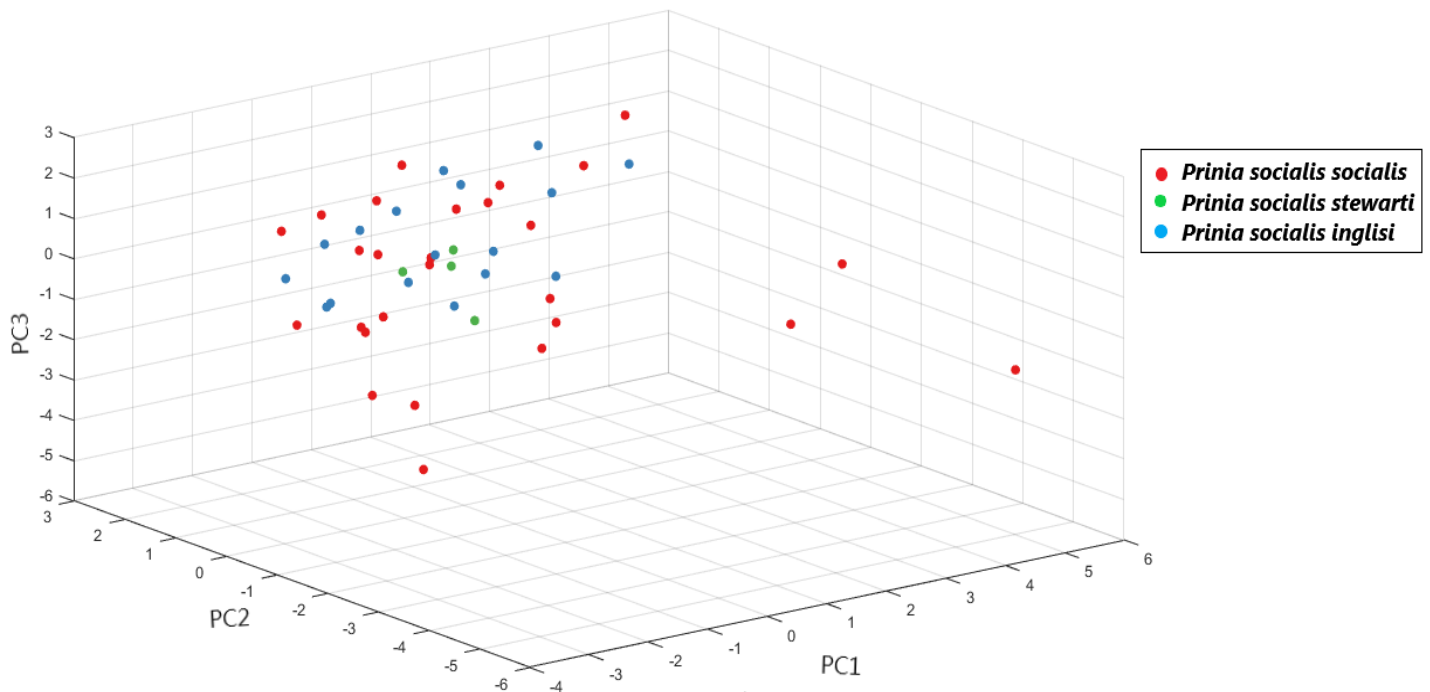


Fig. 16. The figure above indicates a three-dimensional scatter plot of PC1, 2 and 3 of the song parameters of the Ashy Prinia subspecies: *Prinia socialis socialis* (red), *Prinia socialis stewarti* (blue) and *Prinia socialis inglisi* (green).

3.3.2 Statistical test: The MANOVA done through Python 3.10 on the three PC scores gives us a table consisting of results when the MANOVA is subject to various statistical (fig.17) tests such as Wilk's lambda, Pillai's trace, and Hotelling's Lawley trace and Roy's greatest root. We can see in the extreme right section of fig.17, that all the p values are less than 0.05 which indicates that a significance does exist between their acoustic signal parameters. This tells us that geographic variation does exist between the songs of the three Ashy prinia subspecies *Prinia socialis socialis* (*P. s. socialis*), *Prinia socialis stewarti* (*P. s. stewarti*) and *Prinia socialis inglisi* (*P. s. inglisi*). However, it should be noted that a very small sample size of *P. s. inglisi* is available and hence, more data is necessary to be able to make stronger inferences.

```

-----
species      Value  Num DF  Den DF  F Value  Pr > F
-----
Wilks' lambda 0.7864 3.0000 46.0000 4.1642 0.0108
Pillai's trace 0.2136 3.0000 46.0000 4.1642 0.0108
Hotelling-Lawley trace 0.2716 3.0000 46.0000 4.1642 0.0108
Roy's greatest root 0.2716 3.0000 46.0000 4.1642 0.0108
=====

```

Fig. 17. Depicts the MANOVA results on the three PCs of the Ashy prinia (*Prinia socialis*) subspecies' song parameters via the programming language python 3.10 when it is subject to various test statistics with their p values displayed towards the extreme right column (Pr > F)

3.3.3 Linear Discriminant Analysis:

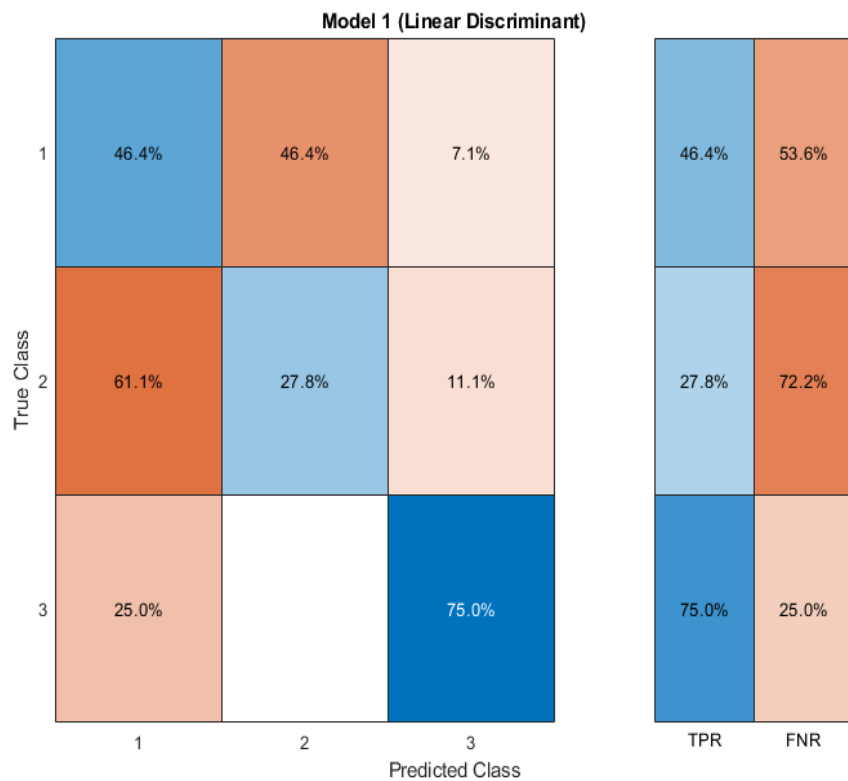


Fig.18: Depicts the LDA done on the Ashy prinia (*Prinia socialis*) subspecies' signal parameters
 1- Labels: 1-*Prinia socialis socialis*; 2-*Prinia socialis stewarti*; 3-*Prinia socialis inglisi*

Fig.18 above depicts a linear discriminant analysis (LDA) on the syllable parameters data of the Ashy prinia (*Prinia socialis*) subspecies' breeding songs, by using the Classification Learner app in MATLAB (Mathworks, Inc., Natick, MA).

We may infer from the LDA, that *P. s. socialis* and *P. s. stewarti* often gets misclassified from each other by this model. *P. s. socialis* gets classified as itself 46.4% of the time, as *P. s. stewarti* 46.4% of the time and as *P. s. inglisi* 7.1% of the time. *P.s. stewarti* on the other hand gets classified as itself 27.8% of the time, as *P. s. socialis* 61.1% of the time and 11.1% of the time as *P. s. inglisi*. Lastly, *P. s. inglisi* gets classified as *P. s. socialis* 25% of the time and itself 75% of the time but the sample size is also too small to make any strong claims.

3.3.4 95% confidence ellipse:

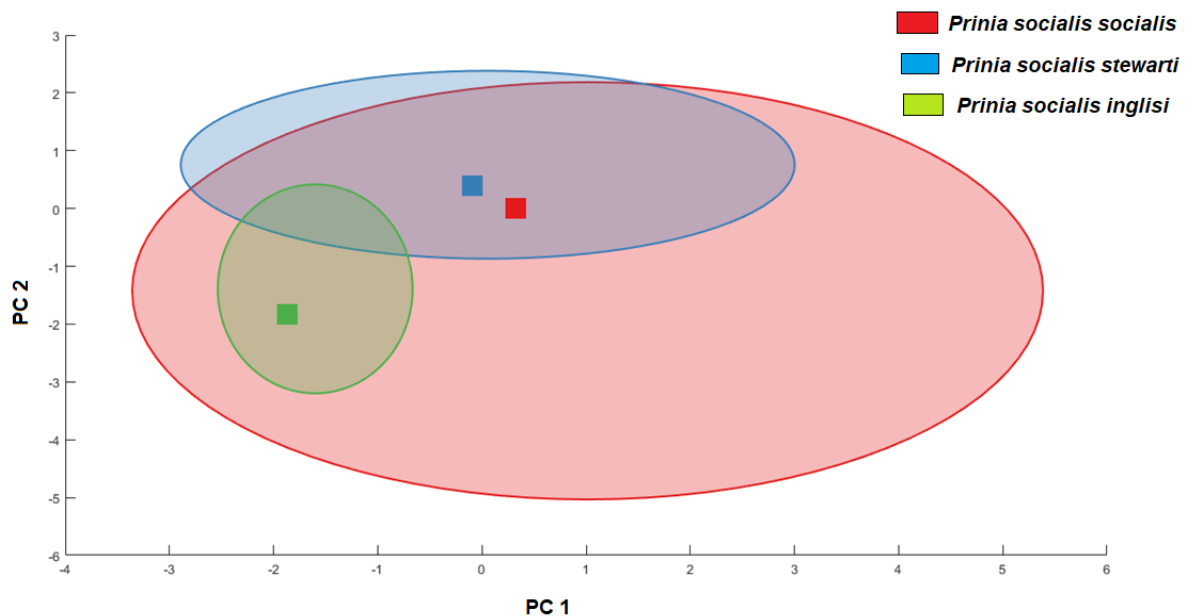


Figure 19: Depicts the 95% confidence ellipse of the Ashy prinia subspecies' (*Prinia socialis*) data. Labels: red- *Prinia socialis socialis*; blue- *Prinia socialis stewarti*; green- *Prinia socialis inglisi*

The 95% confidence ellipse in fig.19 shows where most of the three subspecies' sample means will be present. We observe that the sample means of *P. s. socialis* and *P. s. stewarti* are close unlike that of *P. s. inglisi*.

3.3.5 Principal Component Analysis for the Grey-breasted prinia (*Prinia hodgsonii*) subspecies' pre-rattle sequences:

The first three principal components of eleven parameters (see figure). PC1, PC2 and PC3 account for 75.75% of the variation present in the acoustic signals of the three Grey-breasted Prinia (*Prinia hodgsonii*) subspecies.

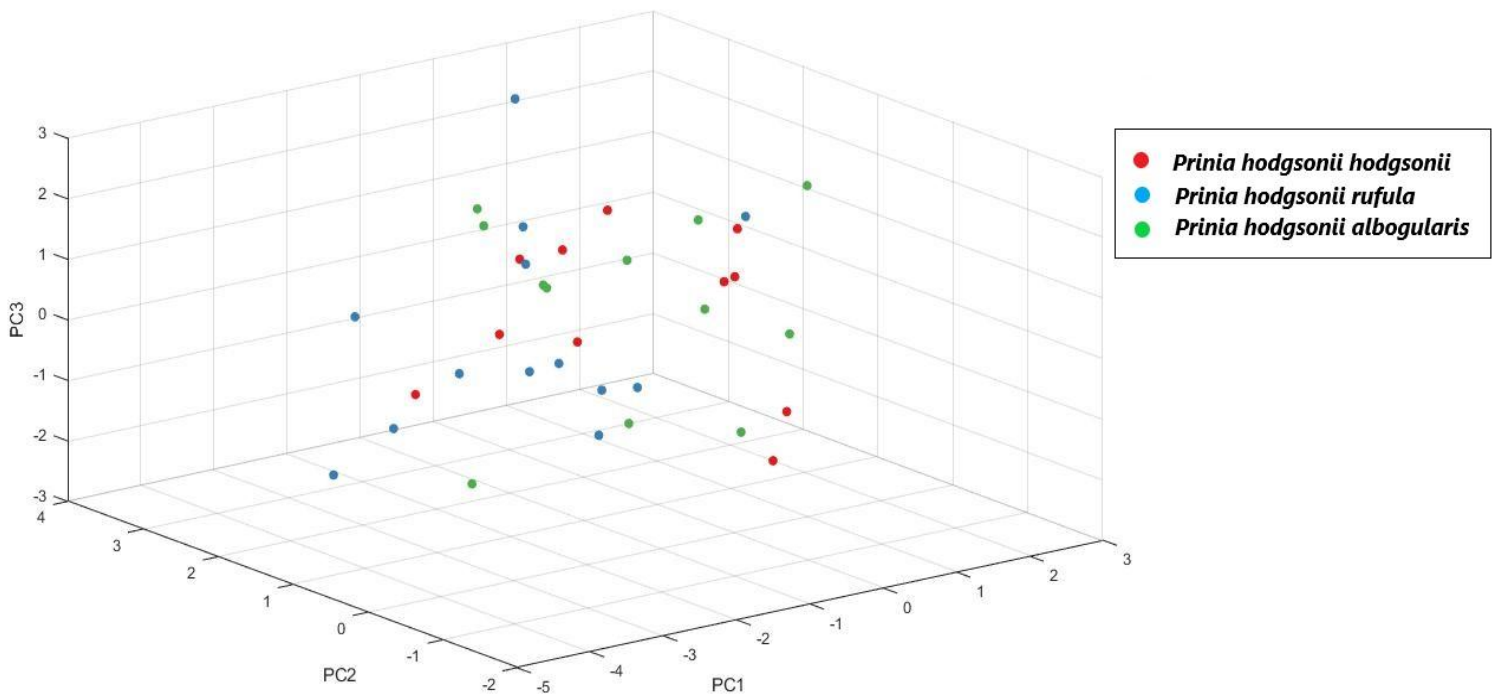


Figure 20: Depicts the 3D signal space comprised of PC1, 2 and 3 of the Grey-breasted Prinia (*Prinia hodgsonii*) subspecies' pre-rattle sequences: *Prinia hodgsonii hodgsonii* (red), *Prinia hodgsonii rufula* (blue) and *Prinia hodgsonii albogularis* (green)

3.3.6 Linear Discriminant Analysis:

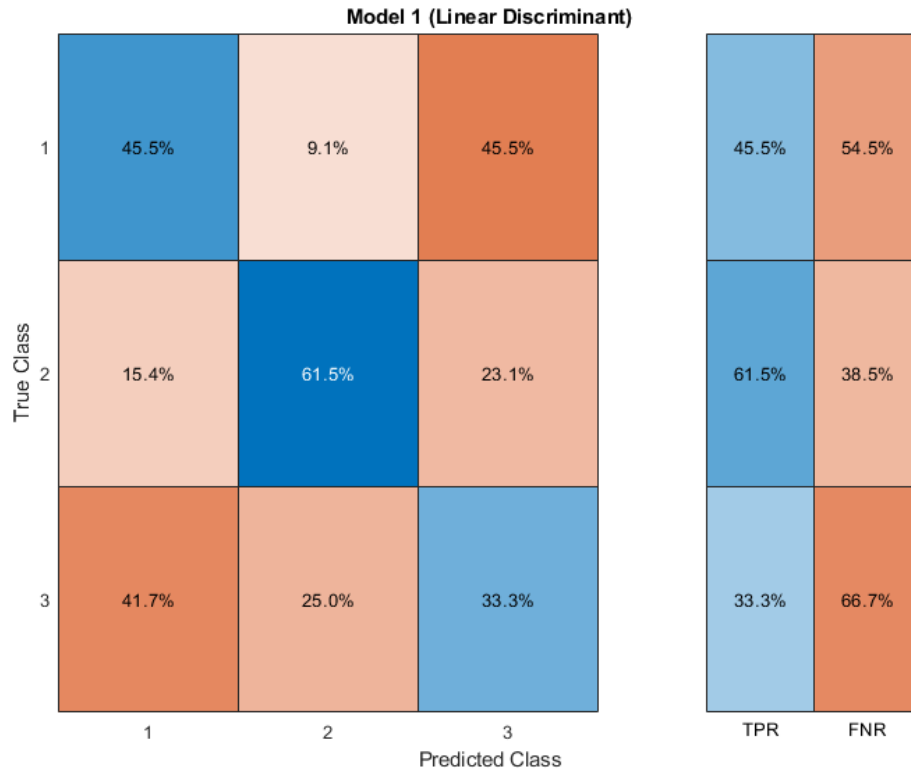


Fig. 21. Depicts the LDA on the pre-rattle sequences' signal parameter data present in the Grey-Breasted Prinia (*Prinia hodgsonii*) subspecies' breeding song using the Classification Learner app in MATLAB.

Labels: 1- *Prinia hodgsonii hodgsonii*; 2- *Prinia hodgsonii rufula* and 3- *Prinia hodgsonii albogularis*

The LDA results show that *P. h. hodgsonii* gets classified as itself by 45.5%, as *P. h. rufula* and *P. h. albogularis* by 9.1% and 45.5%, respectively. *P. h. rufula* gets classified as itself by 61.5% and as *P. h. hodgsonii* by 15.4%; as *P. h. albogularis* by 23.1%. Lastly, *P. h. albogularis* gets classified as itself by 33.3%; as *P. hodgsonii rufula* by 25% and *P. h. hodgsonii* by 41.7%.

In summary, this analysis classifies *P. h. hodgsonii* as itself by 45.5%. It correctly classifies *P. hodgsonii rufula* and *Prinia hodgsonii albogularis* by 61.5% and 33.3%, respectively.

3.3.7 95% Confidence Ellipse of the first three PCs of the Grey-breasted Prinia's (*Prinia hodgsonii*) subspecies' pre-rattle sequence song parameters:

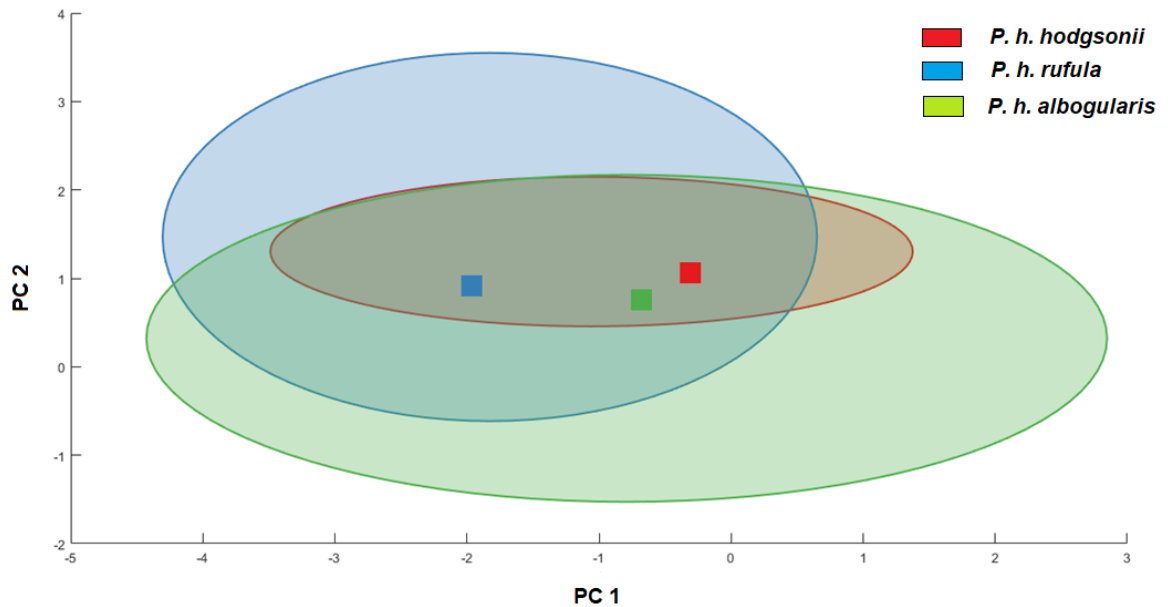


Fig. 22. Depicts the 95% confidence ellipse based on the PC scores of the song parameters of the pre-rattle sequences present in the Grey-breasted prinia (*Prinia hodgsonii*) subspecies' song. Labels: red - *Prinia hodgsonii hodgsonii*; blue- *Prinia hodgsonii rufula*; green- *Prinia hodgsonii albogularis*

The 95% confidence ellipse here shows where most of the three subspecies' sample means will be present. Visually, we can see that the three subspecies' sample means lie rather close to each other in the distribution.

3.3.8 Results of the MANOVA done on the first three principal components of the Grey-breasted prinia (*Prinia hodgsonii*) subspecies' pre-rattle sequence signal parameters:

```
-----  
species          Value  Num DF  Den DF  F Value  Pr > F  
-----  
Wilks' lambda   0.9288  3.0000  32.0000  0.8172  0.4939  
Pillai's trace  0.0712  3.0000  32.0000  0.8172  0.4939  
Hotelling-Lawley trace 0.0766  3.0000  32.0000  0.8172  0.4939  
Roy's greatest root 0.0766  3.0000  32.0000  0.8172  0.4939  
=====
```

Fig. 23. Depicts the MANOVA results for the pre-rattle syllables when it is subject to various test statistics with their p values shown towards the extreme right column in the figure above

We can see from fig. 23 that the p values (Pr > F column) of Wilk's lambda, Pillai's trace, Hotelling-Lawley trace and Roy's greatest root are all greater than 0.05. Therefore, we fail to reject the null hypothesis in this case. This indicates that no such significant difference between their acoustic signal parameters occurs and hence, the possibility of geographic variation occurring amongst the three different Grey-breasted prinia (*Prinia hodgsonii*) subspecies' pre-rattle sequences is ruled out

3.3.9 Principal Component Analysis for the Grey-breasted Prinia (*Prinia hodgsonii*) subspecies' rattle syllables:

Out of eleven parameters (see fig 24), the first three principal components PC1, PC2 and PC3 account for 75.75% of the variation present in the acoustic signals of the three Grey-breasted Prinia (*Prinia hodgsonii*) subspecies.

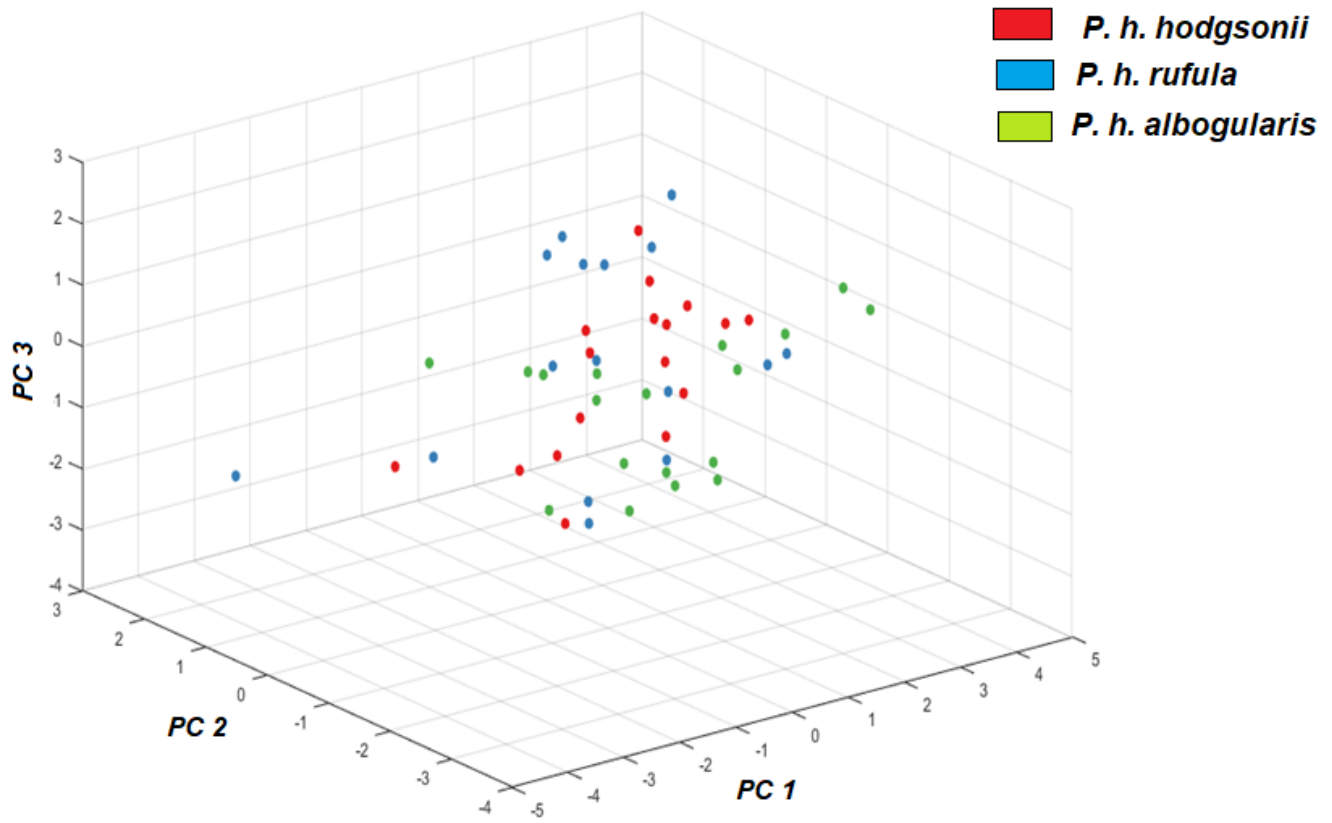


Fig.24. Depicts the three-dimensional signal space comprised of PC1, 2 and 3 of the Grey-breasted prinia (*Prinia hodgsonii*) subspecies' rattle syllables present in their songs. Labels: *Prinia hodgsonii hodgsonii* (red), *Prinia hodgsonii rufula* (blue) and *Prinia hodgsonii albogularis* (green).

3.3.10 Linear discriminant Analysis on the song parameters of the rattle syllables of the Grey-Breasted prinia subspecies (*Prinia hodgsonii*)

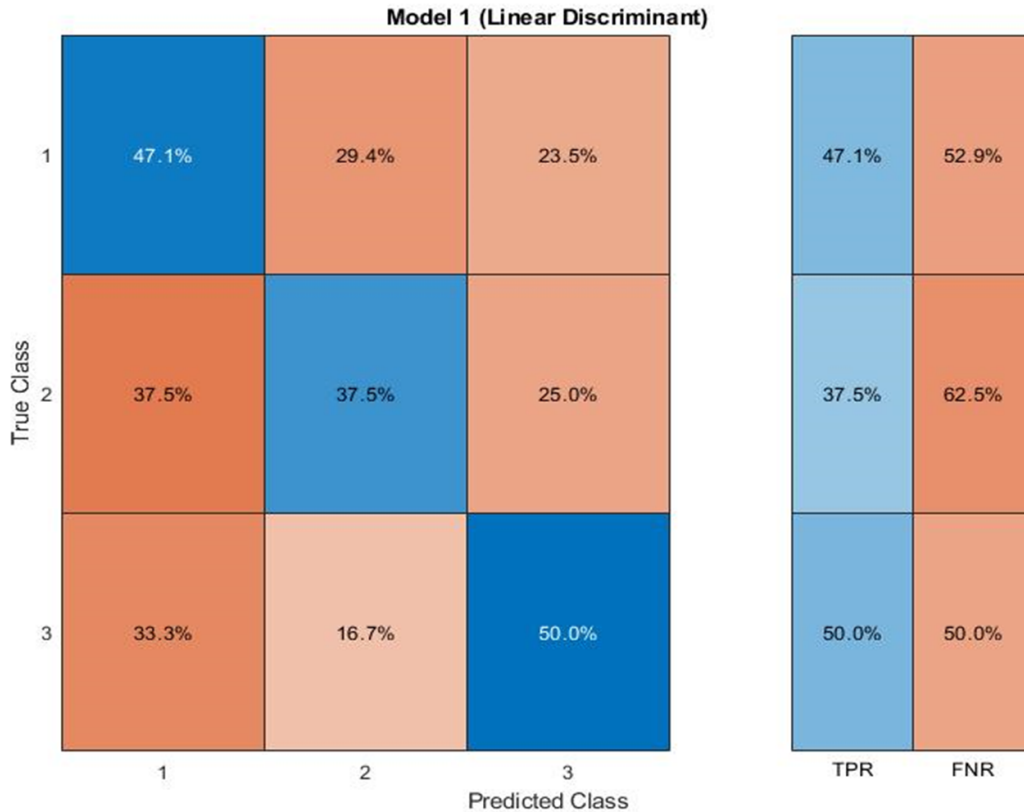


Fig. 25. Depicts the result of the LDA done on the song parameters of the rattle syllables of the Grey-Breasted Prinia subspecies (*Prinia hodgsonii*) using the Classification Learner app in MATLAB (Mathworks, Inc. Natick, MA) .

Labels: 1- *Prinia hodgsonii hodgsonii*; 2- *Prinia hodgsonii rufula*; 3- *Prinia hodgsonii albogularis*

The LDA results show that *P. h. hodgsonii* gets classified as itself 47.1% of the time, as *P. h. rufula* by 29.4% and as *P. hodgsonii albogularis* by 23.5%. *P. h. rufula* gets classified as itself and as *P. h. hodgsonii* by 37.5%; as *P. h. albogularis* by 25%. Lastly, *P. h. albogularis* gets classified as itself by 50%, as *P. h. rufula* by 16.7 % and *P. h. hodgsonii* by 33.3%.

In summary, this analysis classifies *P. h. hodgsonii* as itself by 47.1% of the time. It correctly classifies *P. hodgsonii rufula* and *Prinia hodgsonii albogularis* by 37.5% and 50%, respectively.

3.3.11 Results of the 95% Confidence Ellipse for the first three PCs of the Grey-Breasted prinia (*Prinia hodgsonii*) subspecies' song parameters

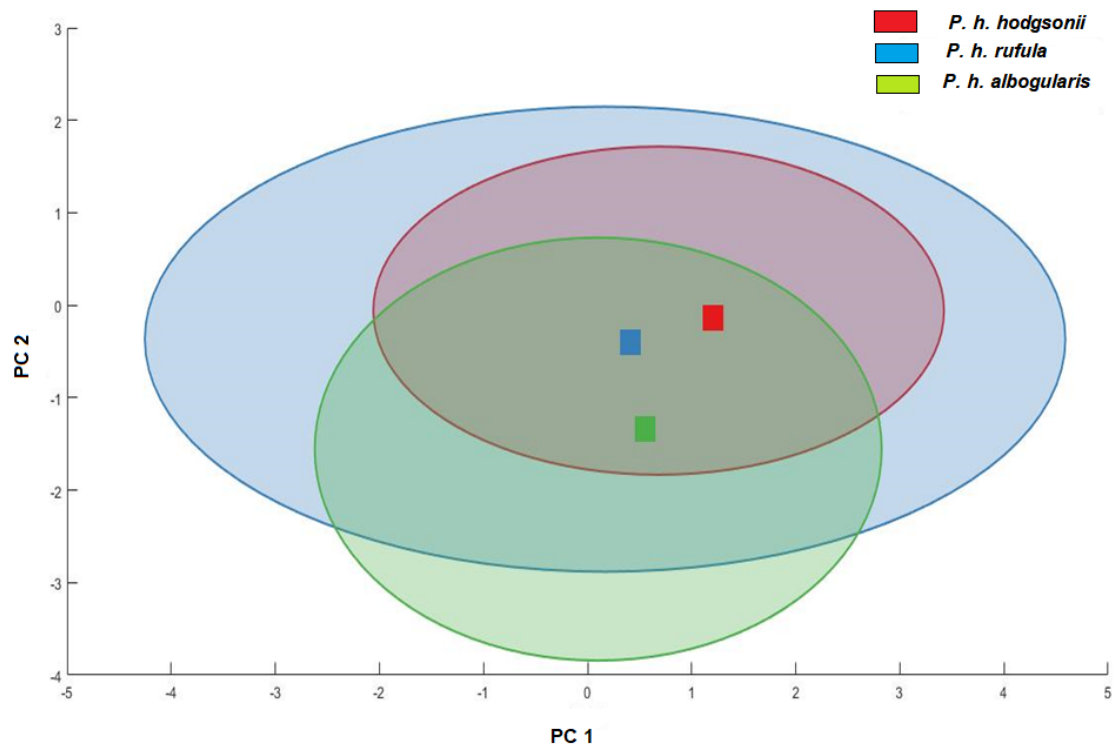


Fig. 26. Depicts the 95% confidence ellipse based on the PCs (1, 2 and 3) of the signal parameters of the rattle syllables present in the Grey-breasted prinia (*Prinia hodgsonii*) subspecies' song.

The ellipse shows where most of the three subspecies' sample means will be present. We observe that just like in the case with the pre-rattle syllables', the three subspecies' sample means here lie rather close to each other as well in the distribution.

3.3.12 Results of the MANOVA done on the first three principal component of the Grey-breasted prinia (*Prinia hodgsonii*) subspecies' s rattle syllables signal parameters

```
-----  
species          Value  Num DF  Den DF  F Value  Pr > F  
-----  
Wilks' lambda   0.8719  3.0000  47.0000  2.3025  0.0892  
Pillai's trace  0.1281  3.0000  47.0000  2.3025  0.0892  
Hotelling-Lawley trace 0.1470  3.0000  47.0000  2.3025  0.0892  
Roy's greatest root 0.1470  3.0000  47.0000  2.3025  0.0892  
=====
```

Fig. 27. Depicts the MANOVA results for the PC scores (1,2,3) of the Grey-Breasted prinia (*Prinia hodgsonii*) subspecies' rattle syllables when it is subject to various test statistics with their p-values shown towards the extreme right column.

We see from the figure that the p values (Pr > F column) of Wilk's lambda, Pillai's trace, Hotelling-Lawley trace and Roy's greatest root are all greater than 0.05. Therefore, we fail to reject the null hypothesis in this case. This also indicates that no such significant differences exist between their acoustic signal parameters and hence, the possibility of geographic variation occurring amongst the three different Grey-breasted prinia (*Prinia hodgsonii*) subspecies' rattle syllables is ruled out.

4. DISCUSSION

The discussion as well will be divided into three sections. The first two sections (4.1 and 4.2) focus on discussing the results found for exploring if correlations exist between song sharing versus geographical distance in the Ashy prinia (*Prinia socialis*) and the pale-chinned flycatcher (*Cyornis poliogenys*), respectively. The second section focuses on discussing the results found for studying geographic variation between the birdsongs of the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies found in India.

4.1: Exploring the correlation between song sharing and geographical distance between territories of the Ashy prinia (*Prinia socialis*) at IISER Pune

Null hypothesis: No correlation exists between song sharing and the geographical distance between territories of the Ashy Prinia (*Prinia socialis socialis*)

No correlation between song sharing and the Euclidean distances between their principal components (PCs) was observed. The introduction to this thesis has earlier mentioned that the extent to which a bird is able to learn songs or its learning period and its the timing of dispersal from its natal territory can play a significant role in how it shares songs with its neighbours in its breeding site (Slater 1989). In several studies, it was found that resident populations of birds shared more song with their neighbours than the migratory birds of the same species did (Hill et.al., 1999; Ewert 1994). Since the Ashy Prinia (*Prinia socialis*) population is a resident breeder in the IISER Pune campus. A possible explanation for the results could be that it shares songs with its neighbours to a great extent and hence as the distance between these territories increases, song sharing remains unaffected and shows no correlation as the geographical distance between territories changes. Anecdotally, the male birds were often seen and heard singing and responding to each other's songs. If one bird for example would begin singing, another male that hears it would also begin singing. Hence, there was no isolation or lack of interaction observed amongst the species studied. However, no

study so far has explored in detail the process of song acquisition by the Ashy prinia (*Prinia socialis socialis*) or inferred if it is a close-ended or open-ended learner. If we get more insight or information regarding this we may be able to better understand their song-sharing processes with their neighbours. In order to get better results towards this approach of studying song-sharing, one can in the future try banding the birds. A marked population makes it easier to compare levels of song sharing between two individuals or neighbours. It also helps us avoid double sampling by recording the same individual twice.

4.2: Exploring the correlation between song-sharing and geographical distance between territories of Pale-chinned flycatcher (*Cyornis poliogenys*)

Null hypothesis: No correlation exists between song-sharing and the geographical distance between territories of the Pale-chinned flycatcher (*Cyornis poliogenys*)

In the case of the pale-chinned flycatcher (*Cyornis poliogenys*) which has a much larger song repertoire and different habitat, we again see no significant results of a correlation occurring between song sharing and geographical distance. A possible reason could be that these species do not, in reality, share songs with each other even in close proximity. A more improved and controlled approach to this can be applied such as banding individuals to be able to recognise them better and avoid double sampling errors; quantifying home range sizes may help us identify birds better. Many studies have found evidence of a decrease in song sharing as the distance between the species' territory sizes increases (Laiolo et al., 2005; Rivera-Gutierrez et.al; Mcgregor and Krebs, 1982) they either banded birds in order to be able to identify individuals, or had bird populations that had been well marked and studied prior to the start of their experiments, which can be of significant help. This was the first attempt at trying to apply such methods to this study area. In this case, I compared the song sequences or song types shared amongst the individuals in order to access how similar they were and tried to correlate it with the distance between their territories. There could be several hidden outliers that may exist in my field methods and data analysis. Firstly, field data is

subject to many variables and one cannot control for all of these on a non-uniform terrain. The field site was a subtropical hill forest that had very mixed vegetation. This made it hard to strictly maintain the same distance between each sampling point as the study site was not just a smooth straight trail, albeit, I tried to minimize this irregularity as much as possible. Further improvements can be made to the sampling methods. Playback experiments as well as the banding of birds would require permits that I could not obtain before the duration of this brief field study came to an end. However, my study serves as a trial of field methods and a feasibility analysis for undertaking such a study on poorly-known bird species.

4.3: Studying geographic variation in the Ashy (*Prinia socialis*) and Grey-breasted (*Prinia hodgsonii*) prinia subspecies.

Null hypothesis: There is no geographical variation between the songs of the Ashy prinia (*Prinia socialis*) and Grey-breasted prinia (*Prinia hodgsonii*) subspecies found in India

From the geographical variation study, we can see that the Ashy prinia subspecies' data do in fact show some geographic variation. *P. s. inglisi* gets correctly classified with 75% accuracy. The Ashy prinia have a fairly small repertoire size with stereotyped songs (Chitnis, 2020). However, more sampling points for *P. s. inglisi* are needed to strengthen these claims, as it is very poorly represented in recording databases. In the case of the Grey-breasted prinia (*Prinia hodgsonii*) subspecies, the null hypothesis could not be rejected and the corresponding LDA on their song parameters also gave us results that supported this. This suggests that their complex and variable songs do not show geographical dialects, but further study is needed on interactions between territorial males.

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Appendix

Table 1:

This table depicts the catalogue names of the Ashy prinia (*Prinia socialis*) subspecies recordings downloaded from the online song database Xenocanto (<https://www.xeno-canto.org/>) and the Macaulay library (<http://macaulaylibrary.org/>). In the subspecies column 1 represents *Prinia socialis socialis*, 2 represents *Prinia socialis stewarti* recordings, and 3 represents *Prinia socialis inglisi*.

Catalogue	Subspecies
XC658723 - Ashy Prinia - song	2
XC578479 - Ashy Prinia - song	1
XC183296 - Ashy Prinia - song	1
XC182174 - Ashy Prinia -song	1
XC657869 - Ashy Prinia - song	1
XC464269 - Ashy Prinia.song	1
XC195760 - Ashy Prinia - song	1
XC568902-Ashy Prinia song	1
XC569653 - Ashy Prinia - song.	1
XC507722 - Ashy Prinia -song.	2
XC369474 - Ashy Prinia - song	1
XC369472 - Ashy Prinia - song	1
XC472990 - Ashy Prinia -song	2
XC472989 - Ashy Prinia song	2
XC426835 - Ashy Prinia - song	3
XC426834 - Ashy Prinia-Song.	3
XC426836 - Ashy Prinia - song.	3
XC369475 - Ashy Prinia - song	1
XC369473 - Ashy Prinia - song	1
XC369471 - Ashy Prinia -song	1
XC472991 - Ashy Prinia - song	2
XC309696 - Ashy Prinia song	1
XC149798 - Ashy Prinia song	2
XC424246 - Ashy Prinia - <i>Prinia socialis</i>	1

XC73018 - Ashy Prinia - song	2
XC73015 - Ashy Prinia -song	2
XC73016 - Ashy Prinia - song	2
XC73014 - Ashy Prinia - song	2
XC73017 - Ashy Prinia - song	2
XC201047 - Ashy Prinia -song.	3
XC98663 - Ashy Prinia -song	2
XC547601 - Ashy Prinia song	1
XC487515 - Ashy Prinia - song	1
XC441384 - Ashy Prinia - song	1
XC214126 - Ashy Prinia - song	1
XC322644 - Ashy Prinia - song.	1
XC551770 - Ashy Prinia - song.	1
XC424246 - Ashy Prinia - song	1
XC322644 - Ashy Prinia - song	1
XC598048-Ashy prinia song	1
ML213256-ashy prinia song	1
ML567.ashy prinia song	1
ML174642.Ashy prinia song	2
XC568704 - Ashy Prinia - song.	1
XC530822 - Ashy Prinia -song	1
ML2039134110-Ashy prinia-song	2
ML348595771-ashy prinia song	2
ML203911261-Ashy prinia song	2
ML277252941-ashy prinia song	2
ML354136061-Ashy prinia	2

Table 2:

This table depicts the catalogue names of the Grey-breasted prinia (*Prinia hodgsonii*) pre-rattle sequence recordings downloaded from the online song database Xenocanto (<https://www.xeno-canto.org/>) and the Macaulay library (<http://macaulaylibrary.org/>). In the subspecies column, 1 represents *Prinia hodgsonii hodgsonii*, 2 represents *Prinia hodgsonii rufula* recordings, and 3 represents *Prinia hodgsonii albogularis*.

Catalogue	Subspecies
XC641510 - Grey-breasted Prinia	1
XC641519 - Grey-breasted Prinia	1
XC423740 - Grey-breasted Prinia	3
XC487517 - Grey-breasted Prinia	1
XC409924 - Grey-breasted Prinia	1
XC157915 - Grey-breasted Prinia	2
XC195496 - Grey-breasted Prinia	2
XC157914 - Grey-breasted Prinia	2
XC255541 - Grey-breasted Prinia	1
XC153013 - Grey-breasted Prinia	1
XC189334 - Grey-breasted Prinia	1
XC472981 - Grey-breasted Prinia	2
XC426833 - Grey-breasted Prinia	2
XC467427 - Grey-breasted Prinia	2
XC467426 - Grey-breasted Prinia	2
XC74151 - Grey-breasted Prinia	2
XC326854 - Grey-breasted Prinia	1
XC276855 - Grey-breasted Prinia	3
XC21131 - Grey-breasted Prinia	1
XC487517 - Grey-breasted Prinia	1
XC362110 - Grey-breasted Prinia	3
XC111004 - Grey-breasted Prinia	3
ML169612.grey-breasted prinia	2
ML182938.Grey-breasted prinia	2
ML216168.Grey breasted prinia	2
XC334898 - Grey-breasted Prinia	3
XC409925 - Grey-breasted Prinia	1
ML216171-grey-breasted prinia	2
ML364066941-Grey-breasted prinia	3
ML357935921 Grey-breasted prinia	3
ML249756991-Grey-breasted prinia	3

ML285996901-Grey-breasted prinia	3
ML360351751- Grey-breasted prinia	3
ML269203111-Grey-breasted prinia	3
ML152243411-Grey-breasted prinia	3
XC472983 - Grey-breasted Prinia	2

Table 3:

This table depicts the catalogue names of the Grey-breasted prinia (*Prinia hodgsonii*) rattle syllable recordings downloaded from the online song database Xenocanto (<https://www.xeno-canto.org/>) and the Macaulay library (<http://macaulaylibrary.org/>). In the subspecies column 1 represents *Prinia hodgsonii hodgsonii*, 2 represents *Prinia hodgsonii rufula* recordings, and 3 represents *Prinia hodgsonii albogularis*.

Catalogue	Subspecies
XC641522 - Grey-breasted Prinia	1
XC641510 - Grey-breasted Prinia	1
XC641519 - Grey-breasted Prinia	1
XC487517 - Grey-breasted Prinia	1
XC409924 - Grey-breasted Prinia	1
XC157915 - Grey-breasted Prinia	2
XC195496 - Grey-breasted Prinia	2
XC157914 - Grey-breasted Prinia	2
XC153013 - Grey-breasted Prinia	1
XC468290 - Grey-breasted Prinia	2
XC189334 - Grey-breasted Prinia	1
XC472981 - Grey-breasted Prinia	2
XC472980 - Grey-breasted Prinia	2
XC369477 - Grey-breasted Prinia	3
XC472983 - Grey-breasted Prinia	2
XC426833 - Grey-breasted Prinia	2
XC472984 - Grey-breasted Prinia	2
XC467427 - Grey-breasted Prinia	2
XC467426 - Grey-breasted Prinia	2

XC423740 - Grey-breasted Prinia	3
XC184999 - Grey-breasted Prinia	1
XC73265 - Grey-breasted Prinia	2
XC74151 - Grey-breasted Prinia	2
XC326853 - Grey-breasted Prinia	1
XC326854 - Grey-breasted Prinia	1
XC276855 - Grey-breasted Prinia	3
XC21131 - Grey-breasted Prinia	1
XC487517 - Grey-breasted Prinia	1
XC488788 - Grey-breasted Prinia	1
XC362110 - Grey-breasted Prinia	3
XC111004 - Grey-breasted Prinia	3
XC597948 - Grey-breasted Prinia	3
ML169612.grey breasted prinia	2
ML182938-Grey breasted prinia	2
ML216168.Grey breasted song.	2
XC334898 - Grey-breasted Prinia	3
XC183297 - Grey-breasted Prinia	1
XC409925 - Grey-breasted Prinia	1
XC319053 - Grey-breasted Prinia	1
XC634264 - Grey-breasted Prinia	3
ML24770.Grey breasted prinia	1
ML364066941-Grey breasted prinia	3
ML357935921-Grey breasted prinia	3
ML249756991-grey breasted prinia	3
ML285996901-Grey breasted prinia	3
ML360351751-Grey breasted prinia.	3
ML269203111-grey breasted prinia	3
ML283389-Grey breasted prinia	3
ML178532041-grey breasted prinia	3
ML152243411-grey breasted prinia	3
ML30750261-Grey breasted prinia	3