Individual-level discrimination in song characteristics of white-rumped shama, *Copsychus malabaricus*

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Passerines exhibit considerable variations in song characteristics, with crucial ecological, behavioural and conservation implications. We documented the individual variations and level of distinctness in the song characteristics of white-rumped shama, *Copsychus malabaricus*. Bioacoustic analysis revealed that songs were composed of repeated strophes (2–5 types per individual) with occasional whistles/trills or orphan syllables, made up of 3–13 elements, with dissimilar structures. Data analysis using 373 strophes from 22 males showed that all 8 variables (minimum, maximum, dominant and range of frequency, strophe duration, song rate, number of elements per strophe and type of element per strophe) differed significantly ($P < 0.001$). Song variations were higher among individuals compared to within individuals. The principal component analysis explained 56.87% variance. Discriminant function analysis exhibited 69.41% cumulative variance with 40.37% classification accuracy. The present study may be useful for further research on some other attributes of a song such as repertoire size, microstructure and seasonal/diurnal variations.

Keywords: Bioacoustic analysis, *Copsychus malabaricus*, discriminate function analysis, principal component analysis, song distinctness.

ASSESSMENT of individual variations in animals has crucial ecological, behavioural and conservation implications[1]. Songbirds are known to exhibit considerable variations in vocal characteristics both at geographical and individual levels[2,3]. Several avian species have been documented to possess individual distinctness in acoustic characters[4]. Such variations are known to play a significant role in individual recognition and are thus useful in a number of social interactions such as territory advertisement, neighbour–stranger discrimination, sexual selection and pair-bond maintenance[5–7]. Studies on vocal individuality also aid in our understanding of population dynamics, fecundity and survival[8,9]. Assessment of vocal distinctness at the individual and sub-population levels may help in understanding the mechanisms and driving forces behind the reproductive isolation leading to sub-population variations, ultimately resulting speciation[10].

For the assessment of individual variations and distinctness of songs, several analytical methods have been used by researchers[11]. Spectrographic cross-correlation (SPCC), discriminate function analysis (DFA) and coefficients of variance (CV) are the most frequently used methods[4], apart from hidden Markov models[12], artificial neural networks[13], mel-frequency cepstral coefficients[14] and repertoire-based individual acoustic monitoring[15]. DFA is widely used to access the variability and distinctness in song characteristics of birds[1,16–18]. Studies showed that researchers prefer to use multiple analytical techniques to achieve more accurate and robust results[18].

The white-rumped shama (*Copsychus malabaricus*, family Muscicapidae) is a small-sized (about 28–34 g average body weight), sexually dimorphic, territorial, shy passerine bird which looks like a thrush[19]. Males have a chestnut belly and glossy black above plumage, with a long graduated tail, white rump and outer tail feathers (Figure 1). Females, on the other hand, are greyish-brown in colour, and shorter than the males[20,21]. The bird is native to scrubs and secondary forests in South and Southeast Asia. It can be found in the cover of shady ravines and deep undergrowth, particularly

Figure 1. A male white-rumped shama, *Copsychus malabaricus* in its natural habitat.

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*For correspondence. (e-mail: anilsonta@gmail.com) in bamboo forests[21,22]. It primarily breeds in South Asia from April to June, and the breeding season is preceded by males performing song displays to attract females. The song is particularly rich and melodious, making it popular as a caged bird in South Asia. The white-rumped shama is known to imitate many different birds in captivity and also in the wild[19,21]. Information on the song characteristics of this species is almost negligible, while the singing behaviour and sociobiology of two other sympatric congeneric species (i.e. oriental magpie robin, *Copsychus saularis*, and Indian robin, *Copsychus fulicatus*) are well studied[23].

In the present study, we document the song characteristics and individual variations of this species. We conducted this study keeping the following hypotheses in view: (i) since the targeted species is an inhabitant of dense forests and strongly territorial, the individuals should possess distinctness in songs to maintain pair-bonds, recognize and deter rivals, and gain fitness benefits and (ii) spectral features of the song should act as a significant component for discrimination.

Methods

Study area and recording of songs

The study was carried out in the Himalayan foothills adjoining Paonta town in Sirmour district, Himachal Pradesh, near the Haryana border, located in northern India, between 30°24′21″–30°28′13″N lat. and 77°27′18″–77°31′26″E long. Col. Sher Jung National Park lies in close proximity to the study site. Dense sal wood trees with grassy glades make up the vegetation. The altitude ranges from 350 to 700 m. The habitat is comprised of mountainous heterogeneous mixed forests. The northeast and southeast-facing slopes feature gently sloping terrain and abundant vegetation. Steep carps (southwest, and northwest-facing slopes) have scant vegetation in comparison. There are rocky overhangs on several escarpments. The study area is dominated by sal (*Shorea robusta*), axlewood (*Anogeissus latifolia*), khair (*Acacia catechu*), salai (*Boswellia serrate*) and pine (*Pinus roxburghii*). It was classified as 3C/C2, and 5B/C2 vegetation types by Champion and Seth[24].

Songs of 22 unbanded male individuals were recorded during April and May 2021, in their native habitat. We recorded a total of 408 recordings (18.54 ± 1.78 recordings per individual). After editing (excluding the long silent temporal gaps, recordings having degraded/attenuated signals and acoustically overlapped signals), a total of 215 recordings with 373 strophes (derived from 13.27 ± 1.46 min duration recordings/individual; 17.0 ± 1.89 strophes/individual) were used for bioacoustic analysis. The recordings were made during peak singing time, i.e. between 0800 and 1100 h. Each bird was only recorded when it was visible, and each location was recorded only once. Due care was taken to ensure that individuals were not misidentified as neighbours. Most of the individuals were located about 100–400 m away from each other. To avoid the mixing of recordings of two neighbouring individuals prior to initiating song recording, the singing behaviour of focal males, song posts (a preferred branch of a tree or position used by the songbird to broadcast songs), and boundary of the territory of targeted males were identified during the first two weeks of fieldwork. Preliminary behavioural observations revealed the established territories (by guarding the territories, mostly using fixed song posts and chasing other birds having similar foraging requirements); usually, the owner did not leave his territory and did not allow other congeneric males into his territory. Occasionally, in case any rival male crossed the boundary, the owner chased it immediately, pushed back the rival, returned to the territory, and usually advertised his territory using a song. So, it was feasible to understand this behaviour to ascertain and identify the territory owner. Using this approach, we identified separate individuals for recordings. Moreover, the distribution of individuals was scattered (except for some individuals) owing to a thin population of the studied species, which further reduced the possibility of mixing recordings of different individuals. Mostly, one individual per day was recorded, and multiple recordings were made. For behavioural observations on territory boundaries and song posts, field binoculars (Nikon, 10X42) were used. All the songs were recorded from a distance of 2–10 m. A portable recorder (Tascam DR-100MKIII 192kHz/24-Bit Stereo Portable Recorder) and microphone (Sennheiser ME-66) were used to record the signals. The signals were recorded at a sampling frequency of 48 kHz. Behavioural correlates were also noted during field recordings.

Variable measurements and statistical analysis

After editing (sampling frequency conversion from 48 to 16 kHz for better spectrograms), high-quality cuts (excluding those of a few individuals due to poor quality) were analysed with the help of Windows-based sound analysis software (Avisoft SAS Lab Pro). Spectrograms were displayed on a computer monitor, and measurements of variables were made using frequency and time cursor (movable indicator to measure the values of variables in Avisoft). For the generation of spectrograms, the sampling frequency of the recordings was converted from 48 to 16 kHz at 16-bit resolution. A song consisted of discrete sections preceded and followed by temporal gaps, called strophes, and each strophe was made up of several smaller units known as elements. The dominant frequency (frequency of maximal amplitude) of a given strophe was analysed by generating a power spectrum. The frequency of the highest peak was recorded. The frequency bandwidth was calculated by subtracting the minimum frequency from the maximum frequency; the central frequency was the central value of the spectrum of the respective strophe on the *y*-axis in Avisoft. All spectrograms

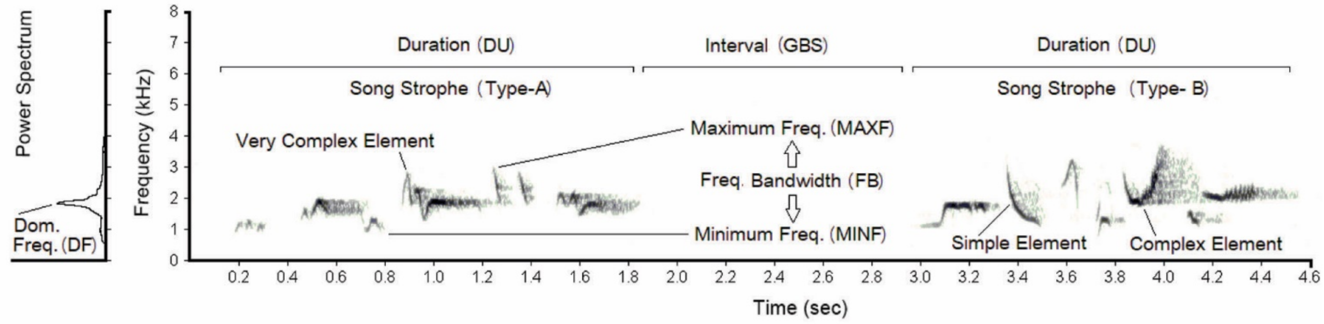

Figure 2. Spectrogram and power spectrum of two song strophes of a male *C. malabaricus,* and the measured acoustic variables. The interval between two strophes was reduced to bring both in the figure. On the basis of structure, the elements were classified into three categories, i.e. simple, complex and very complex. Simple elements comprised of simple structures without frequency inflections, i.e. slope of the frequency in a continuous sound on the spectrogram; complex elements had frequency-modulated elements with one change (peak or valley) in the slope of the frequency in a continuous sound on the spectrogram, while very complex elements had frequency-modulated elements with more than one change (peak or valley) in the slope of the frequency in a continuous sound on the spectrogram.

were generated using the following settings of SAS Lab: 512 FFT-length, 100% frame, Hann window and 75%-time window overlap. In the present study, minimum frequency (MINF), maximum frequency (MAXF), frequency bandwidth or range of frequency (FB), dominant frequency (DF), central frequency (CF), strophe duration (DU) and intervals/gap between strophes (GBS) in song strophes were measured (Figure 2). The number (NES), type (TES) of element per strophe and song rate (strophes/minute; SR) were estimated to define the acoustic features of songs. Since DF and CF were correlated, we excluded CF from the analysis. Variables were summarized as mean ± standard deviation, and Kruskal–Wallis ANOVA (non-parametric test) was used to test the differences in variables among individuals.

Statistical analyses were conducted using R (ref. 25). We examined a total of 373 song strophes at the spectrographic level. We used the coefficient of variance to compare differences within (CV_w), and among (CV_a) individuals and calculated the ratio of CV_a/CV_w to measure the potential individual coding (PIC). We used principal component analysis (PCA) to summarize the effect of song characteristics. Since two variables (range of frequency of strophes, and central frequency) had a high correlation with other variables ($r > 0.5$), we proceeded with further analysis using actual variables, after removing the said variables, rather than the principal components (PCs). We also scaled the data using scale() function for analysis. After removing multivariate outliers using the Mahalanobis distance test in the package rstatix[26], we tested differences in songs of individuals using multivariate analysis of variance (MANOVA). Additionally, we measured the effect size of MANOVA by calculating partial eta squared value (ηp^2) using the package effectsize[27]. We also performed DFA to classify the songs by individuals based on spectro-temporal variables using the package MASS[28]. Differences were considered significant at $P < 0.05$. In the analysis, each strophe was treated as an independent data point. To identify the 'alfa strophe' in an individual, all types of strophes were identified based on visual inspection of the spectrograms of songs of the respective males, and the most frequently uttered strophe was labelled as the alfa strophe of that particular individual.

Results

Acoustic characteristics

Males sang frequently during the breeding season, mostly in the morning (0800–1100 h). Individuals were generally observed singing from exposed branches of trees and sometimes from the unexposed branches of small, dense trees. The song of white-rumped shama was composed of repeated strophes with occasional whistles (vocalizations phonetically rendered as whistles with almost no change in pitch), trills (comprised of a series of few similar elements with rapid utterance and at steady, inter-element, short gaps), and orphan syllables (comprised of one or two elements, and not matching with the elements used in strophes). The strophes were made up of 3–13 elements, with dissimilar structures, and occasional repetition of elements. The song repertoire was comprised of 2–5 types of strophes (average 3.13 ± 0.20 per individual; $N = 22$). Each male used a particular strophe (alfa strophe) most frequently (61.26% of total strophes). The structure of all alfa strophes was dissimilar in each individual (Figure 3). Based on visual inspection of the printed spectrograms of strophes, we did not find any strophe-sharing among individuals. The frequency of strophes ranged from 0.5 to 5.8 kHz. The dominant frequency of most strophes was 2.02 ± 0.56 kHz ($n = 373$). The central frequency of strophes was 1.96 ± 0.48 kHz ($n = 373$). The duration of strophes was 0.3 to 6.82 sec, preceded and followed by temporal intervals ranging from 0.2 to 40.14 sec. The white-rumped shama sang in moderate-sized song bouts ranging from 1 to 7 min duration.

Individual variations

Based on comparative analysis using MANOVA, the acoustic characteristics of 373 strophes from 22 males, it

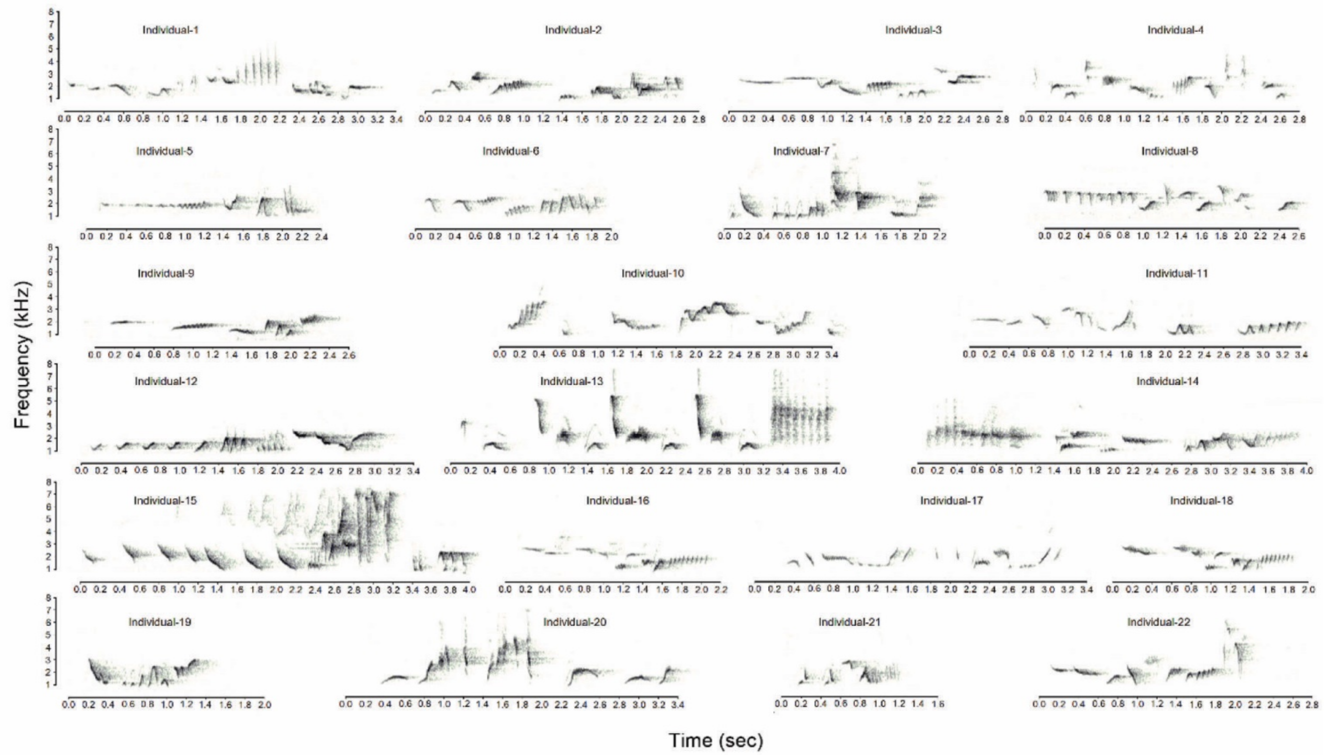

Figure 3. Spectrograms of alfa strophes of all 22 males shows that all individuals used their own strophes without any sharing.

Table 1. Song characteristics of white-rumped shama, *Copsychus malabaricus* (male individuals) show that song variations among individuals are more than those within individuals

Song variables	Mean ± SD (n = 373, N = 22)	CV_a	CV_w	Potential individual coding
Minimum frequency (kHz)	0.90 ± 0.26	28.89	18.10	1.60
Maximum frequency (kHz)	3.02 ± 0.88	29.14	17.30	1.68
Dominant frequency (kHz)	2.02 ± 0.56	27.72	22.09	1.25
Frequency bandwidth (kHz)	2.13 ± 0.88	41.31	25.23	1.64
Strophe duration (s)	2.06 ± 1.16	56.31	36.43	1.55
Song rate (strophes/min)	6.13 ± 2.59	42.25	18.56	2.28
Number of element/strophe	3.53 ± 1.88	53.26	28.49	1.87
Type of element/strophe	2.10 ± 0.64	30.48	19.03	1.60

CV_a, Coefficient of variance among individuals; CV_w, Coefficient of variance within individuals.

was found that all 8 spectro-temporal variables differed significantly ($P < 0.001$). The PIC values for all parameters were >1.00, indicating that song variations among individuals were greater than those within individuals (Table 1). It can be inferred from this analysis that the selected variables can be used for the recognition of individuals. PCA on eight variables yielded two components (eigenvalue >1.00) and explained 56.87% variance (Table 2). PC1 (39.44% variance) exhibited greater loadings for the variables maximum frequency, range of frequency, strophe duration and number of elements per strophe, while PC2 (17.43% variance) showed greater loadings for minimum frequency, dominant frequency, song rate and type of element per strophe (Table 2). MANOVA using PCs revealed that the variations in song characteristics among individuals were significant (Pillai's trace = 2.14, $F = 7.13$, $P < 0.001$) with a strong effect size ($\eta^2 = 0.31$). DFA exhibited 84.38% cumulative variations with 40.37% classification accuracy. LD1 explained 51.36% of the variance, while LD2 and LD3 showed 18.05%, and 14.97% variance respectively (Table 3). DFA showed that all 22 individuals were distinct from each other (Figure 4). However, some of them clustered closely, while a few localized distinctly.

Discussion

The present study reveals that white-rumped shama uses complex songs comprised of a limited number of strophes made up of simple, complex and very complex elements

(Figures 2 and 3), which differ in structure among the neighbours. The dominant frequency or peak song frequency (i.e. 2.02 kHz) was comparatively lower than that of the congeneric species *C. fulicatus* (4.21 kHz)[23]. The central frequency (1.96 kHz) was considerably lower than expected (3.2–3.5 kHz), according to the correlation of central frequency and body weight in passerines by Wallschlager[29], for the body weight of white-rumped shama (i.e. 28–34 g)[20]. Similar patterns have been reported in some other passerines[30]. Several studies showed that the dominant frequency of passerines is significantly associated with habitat[31]. The species in forest habitats produce relatively low-frequency vocalizations compared to those in open habitats[32,33]. This seems true for the white-rumped shama as it is an inhabitant of dense forests in our study area, while the Indian robin prefers open scrublands[23]. However, other factors such as sexual selection, body size, phylogeny, morphology and physiology may also lead to low song frequencies, as reported in previous studies[30,31,34,35].

Our findings reveal that acoustic characteristics play a significant role in song discrimination among individuals, although the outcome may vary with different analytical techniques. Therefore, an integrated approach (using more than one method) has been adopted in most of the previous studies[18]. Keeping this in view, we also adopted the same approach and achieved consolidated and reliable differentiation. The variability was higher among individuals compared to within individuals. Several studies conducted in the past on other species such as brownish-flanked bush warbler (*Cettia fortipes*)[11], buff-breasted flycatcher (*Empidonax fulvifrons*)[36], ortolan bunting (*Emberiza hortulana*)[37] and common cuckoo (*Cuculus canorus*)[17] also showed similar trends of variability. The PIC values revealed that both spectral and temporal variables play a crucial role in individual distinctness. It has already been established that bird songs are sexually selected and mediated through mate attraction or territorial defence. Thus, they are expected to be highly plastic (enough varied to possess individual distinctness) among individuals for mate recognition to maintain pair-bonds, and to deter neighbours and guard territory[3]. Since the white-rumped shama inhabits forest habitats, individual identity may be crucial to push away rivals and maintain cohesiveness with the paired female.

Table 2. Factor loadings of eight song variables used for principal component analysis with eigenvalues >1.00 in 22 individuals of *C. malabaricus*. Significant loadings are shown in bold

Song variables	Principal components PC 1	 PC 2
Eigenvalues	3.16	1.39
Variance explained (%)	39.44	17.43
Minimum frequency (kHz)	0.03	**0.60**
Maximum frequency (kHz)	**–0.48**	0.31
Dominant frequency (kHz)	–0.28	**0.45**
Frequency bandwidth (kHz)	**–0.49**	0.13
Strophe duration (s)	**–0.45**	–0.20
Song rate (strophes/min)	0.21	**0.42**
Number of element/strophe	**–0.39**	–0.22
Type of element/strophe	–0.23	**–0.25**

Table 3. Variable coefficients of linear discriminant function analysis performed on song characteristics in *C. malabaricus* (22 male individuals)

Song variables	LD 1	LD 2	LD 3
Minimum frequency (kHz)	–0.04	0.13	0.10
Maximum frequency (kHz)	0.10	–1.18	0.07
Dominant frequency (kHz)	0.05	0.02	–0.09
Strophe duration (s)	–0.02	–0.14	–0.33
Song rate (strophes/min)	–1.57	–0.48	0.19
Number of element/strophe	0.43	–0.21	0.44
Type of element/strophe	–0.09	0.40	1.18
Variance explained (%)	51.36	18.05	14.97

In the present study, each individual used his strophe repertoire. Hence, it appears that the structure of these strophes carries individual signatures, and may play a significant role in individual discrimination. We observed about 40% discrimination in the songs of 22 individuals, with the use of only some basic spectro-temporal parameters. The distinctness may increase further after adding more variables mainly based on song complexity such as frequency modulation and microstructure (fine-scale acoustic characteristics of vocal elements of strophes). Since the population size of white-rumped shama in the study area was low, each male individual had few neighbours (roughly 1–4). We argue that the total number of *C. malabaricus* individuals (i.e. 22) in the present study is much higher than the expected number of neighbours (1–4 in the present study) experienced by the territory owner. So, it can be inferred that such variation (40%) in song features may enable a territory owner to recognize his neighbour correctly as the number of neighbours is low. Similar findings have been reported in Eastern Phoebes (*Sayornis phoebe*)[38]. In some studies, playback experiments have demonstrated that the distinctness of vocalizations can be used to discriminate among conspecifics[39,40].

PCA exhibited greater loadings for variables, namely maximum frequency, range of frequency, strophe duration and the number of elements per strophe in PC1, while PC2 showed greater loadings for minimum frequency, dominant frequency, song rate and type of element per strophe. Contrary to our assumptions, both spectral and temporal variables contributed to song discrimination. Thus, discrimination in song characteristics of white-rumped shama could be the outcome of both spectral and temporal features shaped by multiple ecological and social factors. Previous studies have revealed that different factors ranging from habitat structure to genetic and cultural drifts are accountable for the variation in song characteristics[41,42]. In the white-rumped shama, further studies are required to address this aspect in-depth.

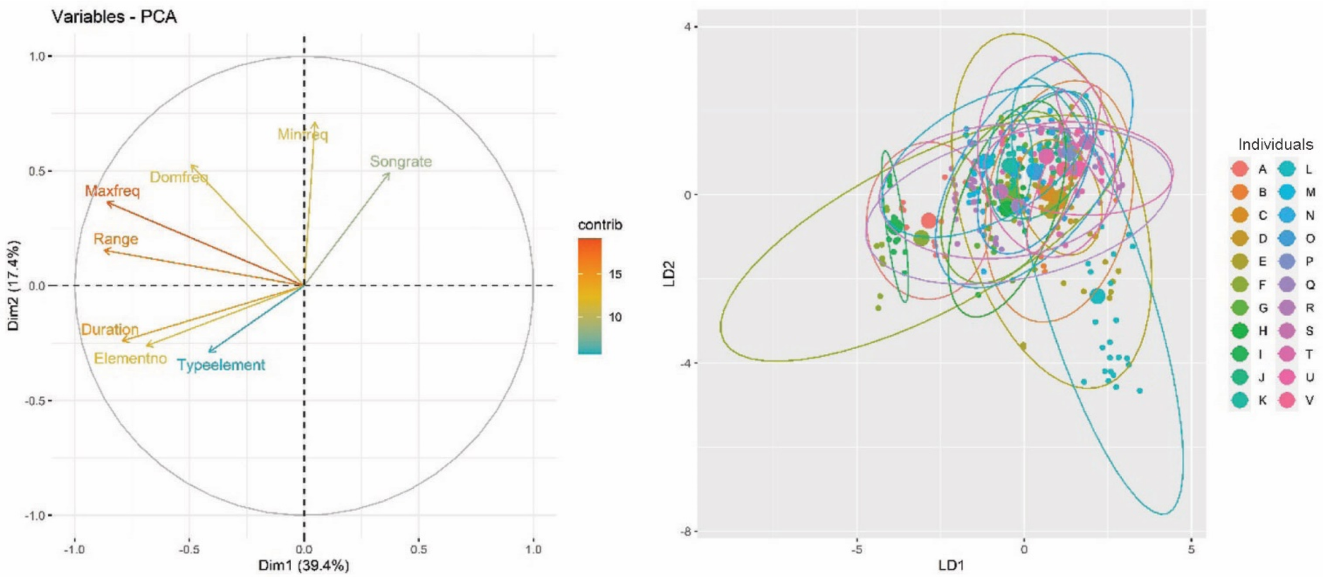

Figure 4. Principal component analysis with a projection of the variables and scatterplots of discriminate function analysis (DFA) (LD1 and LD2) showing the discrimination of song characteristics in *C. malabaricus* among 22 individuals (A to V). Song variables are plotted against the first two scores (functions), obtained from canonical DFA.

Conclusion

The present study demonstrates that song characteristics play a significant role in the individual identification. Vocalizations are vital for mate recognition (essential for pair-bonding) and successful territory guarding, which may have evolved to gain fitness benefits. Spectral features of songs have a major role in distinctness and may carry information about male quality. From a data collection standpoint, this study exhibits individual variations in a species of thick forest habitat that are difficult to spot or observe for population surveys. It has been reported that the identification of individuals in song birds is useful for research pertaining to behavioural ecology, population estimation and conservation studies[4]. In recent years, several portable devices have been developed for species identification based on acoustic signal characteristics. We have studied the individual song variations in white-rumped shama. This can act as a base for elaborate studies on other attributes such as song repertoire, song complexity, microstructure and seasonal/diurnal variations in the acoustic characteristics of a song.

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