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Individual Identification Based on the Songs of the Emei Shan Liocichla (*Liocichla omeiensis*)

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YF and CK designed the study. CK, YW and BC performed the experiments. CK, YW, SL, YF and SD carried out the analyses and wrote the manuscript. All authors read and approved the final manuscript.

Key words
The Emei Shan Liocichla, Songs, Song features, Individual identification, Southern Sichuan

ABSTRACT

In some oscine birds, individually distinctive vocalisations are thought to be more pronounced allowing individual recognition based on songs. In this study, we explore individual identification in the songs of male Emei Shan Liocichla, a vulnerable babbler that inhabits dense vegetation. We recorded territorial songs of male Emei Shan Liocichla from Laojunshan National Nature Reserve during the breeding season of 2016, in southern Sichuan, China. We used three methods to compare the acoustic difference among and within individuals. The results demonstrated that male Emei Shan Liocichlas had individually distinct songs and can be used to identify individuals. We also found that 4 to 5 songs were relatively certain of an individual identity. Our findings indicated that individual distinctiveness by vocalisation was reliable in the Emei Shan Liocichla. Monitoring song therefore provides a potential method to follow individuals in the field and thus facilitate ecological research of this secretive species.

INTRODUCTION

Many birds broadcast loud, easily detected sounds, especially during the breeding season. If these were individually distinctive, they could enable determination of the signalers' identity according to their vocalisations, allowing other individuals to discriminate between the vocalisations of breeding mates, familiar neighbors or intruders (Tibbetts and Dale, 2007; Wilson *et al*., 2010; Stoddard *et al*., 1991; Aubin and Jouventin, 1998). "Neighbor–stranger discrimination" is contingent on the ability of birds to distinguish from among individuals and vocalisations provide an accessible source of variation upon which to base these distinctions (Stoddard, 1996; Lovell and Lein, 2004; Budka and Osiejuk, 2013).

The prerequisite for individual recognition is that vocalisations have characteristics that are unique to each individual (Dale *et al*., 2001; Tibbetts and Dale, 2007). Fine structural features of vocalisations might be beneficial to individual distinctiveness. Individual distinctness of bird's vocalisations not only plays an important role in avian life history, but can also be used to monitor and manage bird populations. Identification of individuals is essential for research attempting to address a wide range of questions in bird biology. Traditional techniques such as radio-tracking and bird-banding involving artificial physical markers have been used to identify individuals (Grava *et al*., 2008; Arshad and Zakaria, 2011; Xia *et al*., 2012). However, capturing and marking birds may interfere with the behaviors of birds and compromise their welfare (Dixon, 2011; Rogers and Paton, 2005). In addition, many threatened species requiring detailed studies to assist with their conservation live in environments such as dense forests where detection and capture for tagging is difficult (Mennill and Vehrencamp, 2011) whilst others are mainly active at night, making direct observation difficult (Farnsworth and Russell, 2007; Odom and Mennill, 2010). Acoustic identification of individuals is a validated approach to solve these problems. Provided that a bird's vocalisations were individually distinctive, they can be used to identify individuals. Furthermore, it is non-invasive, thereby minimizing the influence on

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bird's behavior or movements (Mennill, 2011). Individual identification based on vocalisations has been used to estimate territory and home-range size (Betts *et al.*, 2005), explore dispersal patterns (Laiolo *et al.*, 2007) and forecast population size and dynamics (Hartwig, 2005).

The Emei Shan Liocichla (*Liocichla omeiensis*) is a small passerine and is endemic to a restricted range in southwestern China. The males sing characteristic songs (a loud and complex whistle) during the breeding season to maintain territories (Mackinnon *et al.*, 2000; Fu *et al.*, 2011; Fu *et al.*, 2013). This species is principally distributed in montane forest between 1,000 and 2,400 m in south-central Sichuan and northeast Yunnan and frequents thick bamboo and deep scrub, making individuals difficult to observe in the field (Fu *et al.*, 2011). Due to a declining population, narrow distribution range and severely fragmented habitat, the Emei Sahn Liocichla is regarded as a globally vulnerable species by the IUCN (IUCN, 2015). There are very no published studies documenting Emei Shan Liocichla vocalisations. Consequently, further investigation of the vocalisations of the Emei Shan Liocichla is imperative.

In this study, we aim to describe song variation in the Emei Shan Liocichla, and seek to document the potential for individual identity based on vocalisations.

MATERIALS AND METHODS

Study area

Territorial songs of the Emei Shan Liocichla were recorded in Laojunshan National Nature Reserve (28° 39'–28° 43' N, 103° 57'–104° 04' E) (Fig. 1). From May to June 2016 we collected territorial songs from a total of 17 male Emei Shan Liocichlas. The study site was in southern Sichuan, China, with a subtropical climate, and annual average temperature from approximately 10 to 15°C and mean annual precipitation in excess of 1,500 mm annually. The vegetation at these sites is dominated by evergreen broad leaf forest and interspersed with some non-native coniferous forests and tea plantations (Fu *et al.*, 2011).

Field data collection

The songs were recorded using a SONY ICD-SX 1000 digital voice recorder (Sony, China) and RODE NTG-2 external microphone (Rode, Australia). We kept the distance between microphone and the singing male liocichlas within 30m to obtain clear recordings. In order to ensure that recordings were appropriately assigned to the right individuals, we only recorded those songs that we were certain of belonging to different individuals according to their territories. In total, we recorded 315 songs from 17 males, ranging from 8 to 33 songs for each male.

Fig. 1. Laojunshan National Nature Reserve, China.

Song classification and feature analysis

We used Goldwave 5.25 (Goldwave, Canada) to remove noise below 1 kHz, which is below the minimum frequency of the Emei Shan Liocichla vocalisations. We used Avisoft-SAS Lab Lite (Avisoft Bioacoustics, Germany) to analyze the songs and prepare sonograms. The sonogram parameters setting were indicated as follows: sampling frequency, 22.05 kHz; fast Fourier transform length, 256 points; hamming window: a frame size of 100% and an overlap of 50%, sample size, 16 bits, frequency resolution 86 Hz, and time resolution 5.8 msec (Lindholm and Lindén, 2007).

We classified song types according to their shapes on the spectrogram, mainly compared the number and sequence of different types of elements (Franco and Slabbekoorn, 2009). Song types that share most of the features (more than 75 percent) mentioned above were classified as the same song types (Baker *et al.*, 2000). Songs that varied only in the number of added or omitted or repeats elements but were otherwise similar in their shapes on sonogram, we also classified as the same song type (Sandoval *et al.*, 2014). We measured the repertoire size for the 17 males that we had recorded 20 or more songs (Sandoval *et al.*, 2014). Only 11 of 17 males met this criterion.

For each song, we obtained frequency and temporal measurements, and we measured 28 variables in total, the 28 measured variables showed in Table I (we used the codes to instead the variables in the following), and sound spectrograms of part of the variables depicted in Fig. 2.

Statistical analyses

Firstly, we randomly selected 10 sequential songs from each individual in order to avoid bias from those individuals where we recorded more songs. We only

Table I. The measured variables of the Emei Shan Liocichla songs.

Variables	Code
Highest frequency of the song	FH
Lowest frequency of the song	FL
Frequency at the start of the song	FS
Frequency at the end of the song	FE
Duration of the song	DT
Highest frequency of the1st note	F1H
Lowest frequency of the 1st note	F1L
Frequency at the start of the 1st note	F1S
Frequency at the end of 1st note	F1E
Duration of the 1st note	T1
Duration between 1st note and 2nd note	T1-2
Highest frequency of the2nd note	F2H
Lowest frequency of the 2ndnote	F2L
Frequency at the start of the 2nd note	F2S
Frequency at the end of 2nd note	F2E
Duration of the 2nd note	T2
Duration between 2nd note and 3rd note	T2-3
Highest frequency of the3rd note	F3H
Lowest frequency of the 3rd note	F3L
Frequency at the start of the 3rd note	F3S
Frequency at the end of 3rd note	F3E
Duration of the 3rd note	T3
Duration between 3rd note and 4th note	T3-4
Highest frequency of the 4th note	F4H
Lowest frequency of the 4th note	F4L
Frequency at the start of the 4th note	F4S
Frequency at the end of 4th note	F4E
Duration of the 4th note	T4
Total number of elements of the song	N

recorded 8 songs (<10 songs) for two males, so all songs were used into subsequent analyses. We calculated mean values of measured temporal and frequency variables of each individual. Then, we used the data to calculate the overall mean for each variable. For each individual, we used coefficients of variation (CV) to describe the extent of variability for each measured variables (Charrier *et al*., 2001; Xia *et al*., 2014). We calculated between-male coefficients of variation among individuals (CVa) and coefficients of variation within individuals (CVw). Subsequently, we calculated the potential of individual coding (PIC) for each variable (Charrier *et al*., 2001; Xia *et al*., 2014). PIC value is equal to CV_a /mean CV_w, and mean CV_w represented the average value of the CV_w of all individuals. For each variable, PIC value exceeds 1 indicating that this variable is available for individual recognition. We used Wilcoxon test to compare the variation coefficient between and within individuals as the data were not distributed normally.

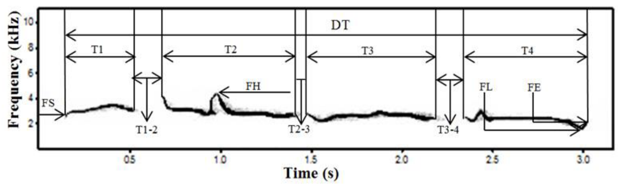

Fig. 2. Sonogram of the Emei Shan Liocichlas showing 12 measured frequency and temporal variables.

We complemented the potential of individual coding approach by utilizing discriminant function analysis (DFA), aimed at comparing differences in the measured temporal and frequency variables between males. We aimed to explore the fewest possible measured variables to explain the largest possible amount of differentiation between individuals. We established a model that included all measurements; we excluded from the discriminant analysis the variables with the lowest F-to-remove value until we obtained a model with the fewest variables that still provided the same or higher percentage of correct assignments relative to the original model that included all acoustic features. This analysis was conducted for each song type that was shared by more than 5 individuals and we had recorded at least 5 songs by each male; two song types satisfied these standards (as shown in Fig. 3). The results were conservative estimates and reported as percentages of classification accuracy based on the leave-one-out approach to cross-validation (Sandoval *et al*., 2014).

We also used discriminant function analyses to determine how much recording would have to record to be relatively certain of an individual identity. We mainly focused on the two dominant song types since we had recorded at least 5 songs for each individual. Firstly, we randomly selected 2 songs from each individual to conduct DFA. Then, we added the sample size of songs one by one until we obtained a stable high correct recognition rate. Our purpose was using the fewest sample size of songs while we were able to acquire stable high classification accuracy.

We used Kruskal-Wallis test to explore whether measured variables varied among different individuals since the data were not distributed normally. We only focused on the song types and variables that were detected by the discriminant function analysis as being favorable to individual identification.

All data are presented as means ± SE using two-tailed tests. Significant differences are determined at $P = 0.05$. Statistical analyses were performed with software SPSS 22.0 for Windows.

Table II. The features and coefficients of variation between and within individuals based on the measured variables of male Emei Shan Liocichla.

Variable	Mean ± SE	CVa (%)	Mean CVw (%)	PIC value
FH	3.86±0.37	9.59	6.55	1.47
FL	1.72±0.21	12.34	12.17	1.01
FS	2.89±0.44	15.14	10.31	1.47
FE	2.04±0.23	11.65	13.07	0.89
DT	2.18±0.25	11.41	14.86	0.77
F1H	3.75±0.37	10.09	6.82	1.48
F1L	2.43±0.17	6.96	9.90	0.70
F1S	2.81±0.43	15.18	13.07	1.16
F1E	2.90±0.18	6.27	7.54	0.83
T1	0.55±0.18	33.12	19.12	1.73
T1-2	0.14±0.03	18.88	19.52	0.97
F2H	3.56±0.52	14.50	8.06	1.80
F2L	2.16±0.34	15.68	12.09	1.30
F2S	3.06±0.58	19.06	25.29	0.75
F2E	2.60±0.40	15.49	10.96	1.41
T2	0.62±0.06	10.36	10.01	1.04
T2-3	0.12±0.03	25.73	23.84	1.08
F3H	3.18±0.20	6.15	5.83	1.06
F3L	1.96±0.34	17.42	13.12	1.33
F3S	2.70±0.41	15.15	12.95	1.17
F3E	2.40±0.44	18.39	17.05	1.08
T3	0.54±0.10	18.73	16.67	1.12
T3-4	0.09±0.04	39.90	24.66	1.62
F4H	3.02±0.22	7.35	3.85	1.91
F4L	1.80±0.925	16.19	7.50	2.16
F4S	2.39±0.29	12.37	9.43	1.31
F4E	1.99±0.30	14.73	7.52	1.96
T4	0.63±0.06	9.21	8.88	1.04

$P < 0.01$, Z=2.983, Wilcoxon test between CVa and mean CVw.

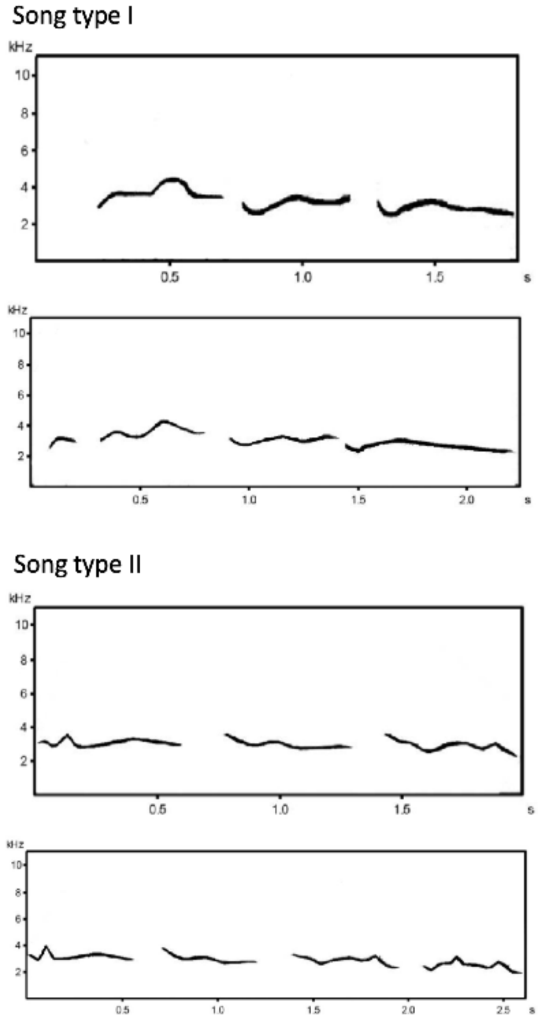

Fig. 3. Spectrogram of two dominant song types and their song type variations.

RESULTS

Song features

We found 9 unique song types of 17 male Emei Shan Liocichlas from Laojunshan National Nature Reserve, and two song types were dominant in this population, which were respectively shared by 10 and 13 males. The repertoire sizes varied from 2 to 4, mean 2.55±0.69 for the 11 males with more than 20 songs. A complete song usually consists of two to four elements, mean 3.07±0.61 for the 17 males, more detail of the measured song variables see Table II. We also detected that the Emei Shan Liocichla prefer to generate various kinds of song type variants, mainly through the following method: omit, add or repeat an element of a whole song.

Table III. Results of discriminant function analysis used to evaluate individual identification in the Emei Shan Liocichla songs.

Song type	N	Percent correct classification (%)	Wilks'λ	F_{df}	Variables retained in backwards DFA
Type I	10	97.4	<0.001	49_{116}	F1H, F2H, T1, T1-2, T2-3, FS
Type II	13	96.3	<0.001	84_{314}	DT, F1L, F1E, F2L, F2E, F3H, F3S, T1-2, T3, FH, FL, FE

Sample size (N) shows the total number of males that sang each song type. The Wilks'λ and F values show the results of backwards DFA. The features retained in the backwards DFA correspond to the measured temporal and frequency variables.

Coefficients of variation

PIC values were greater than 1.0 for most of the measured variables (22 of 28 variables exceeded 1).There were significant variant between CVa and mean CVw by Wilcoxon test (P < 0.01, Z=2.983, as shown in Table II), suggesting that variation between individuals was greater than within individuals.

Discriminant analyses

Discriminant function analyses on the basis of the two dominant song types allocated songs to the correct male and produced a high percentage of correct classification, ranging from 96.3% to 97.4%, indicating a high rate of individual identification. This high rate of discrimination was acquired with a segment of measured variables retained in backwards DFA, six typical variables for song type I, and twelve typical variables for song type II, as shown in Table III.

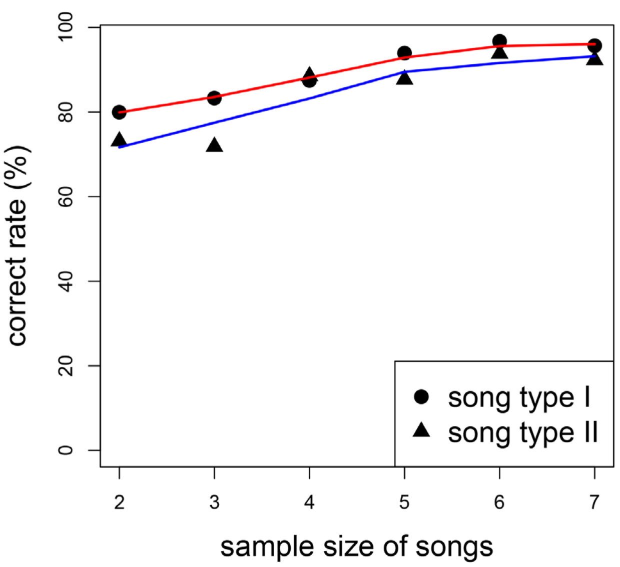

Fig. 4. The relationship between the correct rate based on DFA and sample size of songs, which are based on only two dominant song types.

The result of DFA also showed that the classification accuracy rose with the increasing sample size of songs for the two dominant song types. To get a reliable identity on an individual bird (i.e. the correct rates were nearly 90%), 4 to 5 songs are needed at least (Fig. 4).

Table IV. Results of Kruskal-Wallis test used to compare typical variables among different individuals.

Song type	Typical variables	χ2	df	P Value
Type I	F1H	68.084	9	<0.01
	F2H	70.218	9	<0.01
	T1	71.259	9	<0.01
	T1-2	41.091	9	<0.01
	T2-3	46.854	9	<0.01
	FS	49.061	9	<0.01
Type II	DT	69.958	12	<0.01
	F1L	75.676	12	<0.01
	F1E	54.245	12	<0.01
	F2L	91.221	12	<0.01
	F2E	88.742	12	<0.01
	F3H	69.636	12	<0.01
	F3S	84.504	12	<0.01
	T1-2	47.925	12	<0.01
	T3	77.925	12	<0.01
	FH	87.468	12	<0.01
	FL	81.384	12	<0.01
	FE	70.212	12	<0.01

Typical variables were retained in backwards DFA from two dominant song types.

Kruskal-Wallis test

We analyzed the variance among individuals of typical variables of two dominant song types that were detected by the discriminant function analysis. The results of Kruskal-Wallis test indicated that the typical variables for the two dominant song types that we analyzed were significant variant among different individuals (song type

I: df=9, P<0.01; song type II: df=12, P<0.01; as shown in Table IV).

DISCUSSION

This study demonstrated that male Emei Shan Liocichlas had individually distinct songs. The potential of individual coding (PIC) revealed that the variation of measured song variables among individuals were greater than within individuals. DFA based on the two dominant song types yielded a high level of accuracy. And Kruskal-Wallis test indicated that the typical variables were significant variant among different individuals. may among, Our findings showed that the individual distinctiveness by vocalisation was reliable in the Emei Shan Liocichla, which provides a potential method to follow individuals in the field and thus facilitate ecological research (such as population density monitoring and breeding behavior studies) of this secretive species.

Discriminant function analyses also acquired high correct recognition rates only based on 4 to 5 songs for each individual. This would be a useful analysis as it would inform ecologists working on this species that only 4 to 5 recordings they would have to collect to be relatively certain of an individual identity.

Steere's liocichla (*Liocichla steerii*), a close relative of Emei Shan Liocichla, exhibits a characteristic duetting behavior, which is thought to function as a joint-territory defense (Yuan *et al*., 2005). The leading male song in duets of Steere's liocichlas was highly individualistic (Weng *et al*., 2012; Mays *et al*., 2006). That would create selection for individuality so that females may answer the song of their mates (Mays *et al*., 2006). However, we didn't find this behavior in Emei Shan Liocichla, the female Emei Shan Liocichlas are incapable to sing a song (Kong, C.P., unpublished data). How this difference evolved between the two species is a problem needs further study.

In bird species, individually distinctive vocalisations have been reported not only in oscine birds (Nelson and Poesel, 2007; Ellis, 2008; Benedict and McEntee, 2009), but also in a number of sub-oscine and non-oscine bird species (Fitzsimmons *et al*., 2008; Sandoval and Escalante, 2011; Garcia *et al*., 2012). Consequently, we speculate that avian individual identification by vocalisations might be a common phenomenon in nature. Further work is needed to collect more examples in wider bird species and ascertain its evolutionary mechanism. In addition, we suggest that more attention should be paid to whether the song features of birds are consistent over time as it is imperative to identify individuals steadily.

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Statement of conflict of interest

The authors have declared that no competing interests.

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