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# AN ANALYSIS OF ADVERTISEMENT SONGS OF *Hyalessa maculaticollis* (HEMIPTERA: CICADIDAE)

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**Abstract.** Advertisement songs play the critical role of a prezygotic reproductive isolation mechanism. Specifically, female cicadas rely on the fluctuation of spectral and temporal properties of males' songs for species recognition and pair formation. However, the structure of the advertisement song is often arbitrarily determined without proper delimitation criteria. Here, a new method for analyzing song structure was proposed which relied on fluctuation of spectral properties. First, time series of peak frequency and amplitude envelope were obtained from recorded songs. Then, a simple linear regression model based on those two time-series was tested for consistency of regression coefficients. This method was applied in analyzing advertisement songs of *Hyalessa maculaticollis*, a common cicada species whose advertisement songs contain multiple modulations in spectral properties in East Asia. Males of *H. maculaticollis* produced songs in separate phrases in which one phrase was equivalent to one complete song and they also changed their calling perches from tree to tree in between songs. One phrase of *H. maculaticollis* was divided into four main parts, in which the first two parts were repeated alternatively and the last two were produced once at the end. The proposed method suggests an effective objective way of analyzing acoustic characteristics of songs, especially for those animals with complex structures.

***Keywords***: cicada advertisement songs, *Hyalessa maculaticollis*, spectral fluctuation.

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# 1. Introduction

Cicadas belong to the group of insects well-recognized for their characteristic loud and complex species-specific advertisement songs for species recognition and pair formation [1]. Song production is solely observed in males by two main components: the tymbal apparatus, and the nervous system [2]. The first component consists of the bilateral coordination of a pair of tymbal muscles along with the buckles of tymbal ribs which generate the basic acoustic units of the song [3-4]. Afterward, the nervous system controls the overall operation of such tymbal apparatus to build up species-specific song patterns that are diverse in both frequency spectrum and amplitude modulation [2]. In turn, females recognize species-specific signals as their tympanal ridge vibrates at the precise peak frequency of conspecific male songs [5-7]. As a result, they depend on spectral properties and amplitude fluctuation to identify and select favorable mates.

To date, song structure identification has been done either by physiological experimenting on sound-producing apparatus [8-14] or subjective identification of song structure [15-18]. While experimental studies are time-consuming and require complicated simulation of sound producing apparatus, subjective identification is easily prone to oversimplification of fine-scale structures. Therefore, no method has been provided so far to solve such problems.

Therefore, a method was proposed in this study which employed a time series of peak frequency and amplitude envelope of a recorded song to detect changes in complex structures. We applied this method in analyzing advertisement songs of *Hyalessa maculaticollis*, a common cicada species in the Korean peninsula that produces songs containing multiple repeated fluctuation in the spectral domain.

# 2. Content

## 2.1. Methods

### 2.1.1. Sample collection

*Hyalessa maculaticollis* is one of the most abundant cicada species in the Korean Peninsula [19]. Adults of this species usually appear in summer and are easily recognized due to their loud and noisy advertisement songs. From July to August 2013, advertisement songs of this species were recorded in the vicinity of the Metropolitan Seoul, Republic of Korea. All songs were recorded during daytime, between 9 am and 5 pm, and no recordings were made on rainy days. During a recording session, the distance between the focal cicada and the recorder was kept at approximately 20-30 cm. Only those recordings containing more than one complete song were kept for data analysis.

### 2.1.2. Song recording and pre-processing

A digital recorder (PCM-D50; Sony Electronics Inc.; China) with a built-in microphone (-35.0 dB/Pa and 1 kHz sensitivity, maximum input level 120 dB SPL, and noise level 20.0 dB SPL) was used for song recording. A head-cover windshield was affixed to the recorder. Recordings were made at 44.1 kHz sampling rate and 16-bit resolution. Time series of peak frequency and amplitude envelop were extracted with wavelength of 512 samples and 0% overlap using package seewave version 2.2.1 and tuneR 1.4.4 in the program R (version 4.3.1; R Foundation for Statistical Computing; Vienna, Austria).

### 2.1.3. Spectral fluctuation analysis

First, a linear regression model of the spectral fluctuation against the amplitude fluctuation was tested:

$$y_i = x_i^T \beta_i + u_i \; (i = 1, \dots, n) \qquad (1)$$

where at the time index $i$, $y_i$ was Peak frequency (PF), $x_i$ was 2×1 vectors with the first component of 1 and the second component of Amplitude envelope (ENV) and $u_i$ was the remaining part of PF not explained by ENV. Then, the null hypothesis that all regression coefficients, $\beta_i$, were consistent

$$H_0: \beta_i = \beta_0 \; (i = 1, \dots, n)$$

was tested against the alternative hypothesis that at least one $\beta_i$ was different from others. If the null hypothesis was rejected, it could be assumed that there are $m$ breakpoints, where the regression coefficients differed from those of the former regressions. In other words, $m$ points could be located to separate the whole sequence into $m + 1$ segments of different structures, and Equation (1) can be rewritten as:

$$y_i = x_i^T \beta_i + u_i \; (i = i_{j-1} + 1, \dots, i_j; j = 1, \dots, m + 1) \qquad (2)$$

where $j$ was the segment index, $\{i_1,\dots,i_m\}$denoted the set of the breakpoints, and $i_0 = 0$ and $i_{m+1} = n$.

We used package strucchange 1.5-0 [20] in the program R to identify structural changes in the modulation of both the spectrum and amplitude of cicada songs. The package was robust in detecting multiple changes with unknown timing [21]. A general workflow of the overall analysis scheme is shown in Fig. 1. To estimate the numbers and locations of potential breakpoints, Zeileis et al. [21] mainly relied on peaks of the empirical fluctuation process. However, since numerous fluctuating and repeated patterns were expected, two criteria in estimating breakpoints were applied: peaks in the empirical fluctuation process and changes in fluctuation pattern. In other words, both the number of peaks and different fluctuation patterns observed in the empirical fluctuation process were investigated and then used to estimate the number and locations of potential structural changes.

In our workflow, the changes of fluctuation pattern were tested by using function efp (), in which we set type=”RE” to apply recursive ordinary least square estimates of regression coefficients [22] in Equation (2) to the test statistic. If there was evidence indicating potential structural changes, we dated the location of those changes using the function breakpoints (). In this function, the model to be tested was defined by a formula, e.g. PF~ENV, as in the symbolic description of Equation (1), and the data type used was a time-series dataset. For a given number of breakpoints, denoted as $m$, breakpoints () identified $m$ breakpoints within the data where inconsistencies in regression coefficients emerged in Equation (1) based on the theoretical work of Bai and Perron [23]. By employing the dynamic programming algorithm given by Bai and Perron [24], breakpoints () inspect all time indices to search for a time index in Equation (1) that could provide the minimal residual sum of squares when it was assumed as a breakpoint. When a breakpoint was identified, a new search for additional breakpoints was followed using the same method. However, the null hypothesis including the previously identified breakpoint was tested this time. This process continued until $m$ breakpoints were found or halted if no suitable additional breakpoints could be identified. The parameter $h$ was the minimal segment size to be analyzed, either as an integer number or as a fraction relative to the duration size of a time series. For each $h$, breakpoints () would calculate all possible segmentation of the data, each referring to a model of a certain value of $m$. Summaries of each model included a number of breaks, and locations of such breaks. We used earlier estimations of locations of breaks to decide $h$, then compared results yielded by breakpoints () and prior estimation of the number of breaks to select $m$.

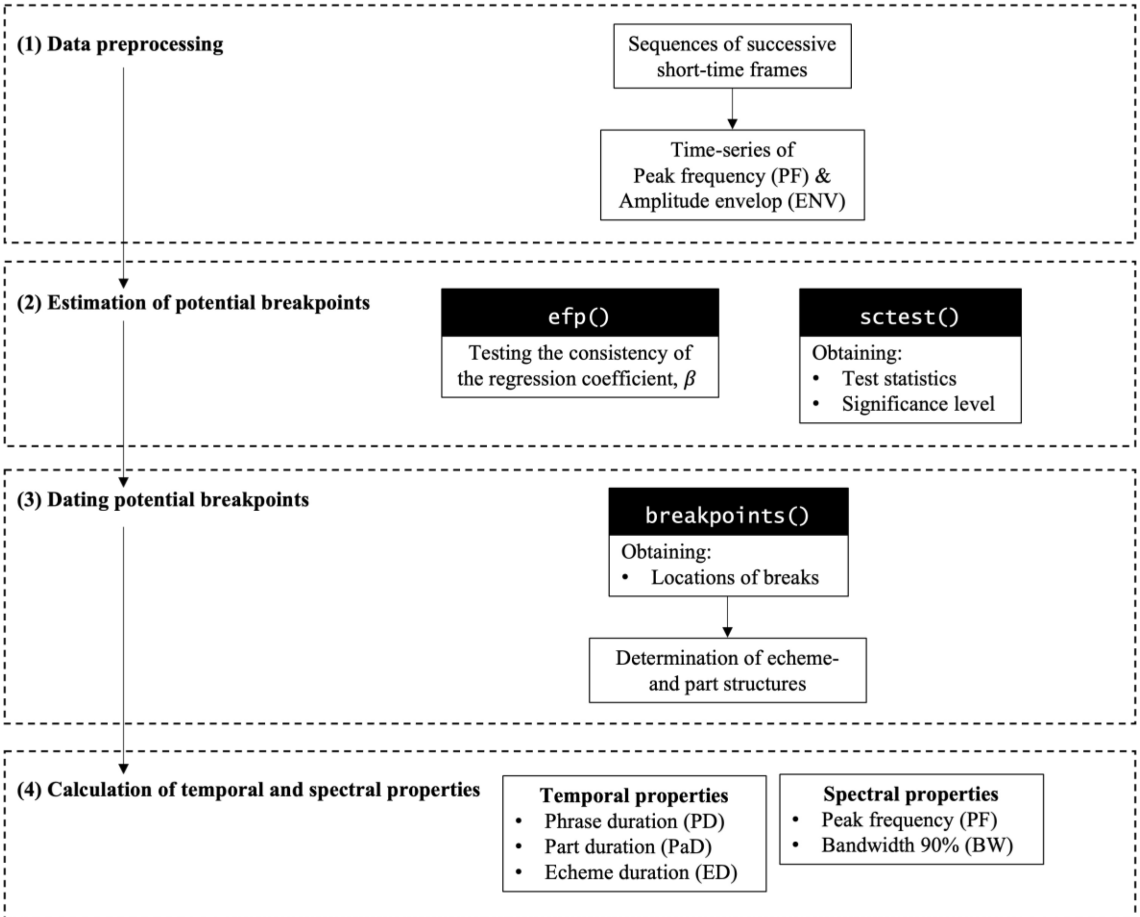


***Figure 1. A workflow of the overall spectral fluctuation analysis. Black headers indicate the relevant package functions used in each step***

### 2.1.4. Hierachical terminilogy of cicada song description

The hierarchical terminology employed to describe cicada songs in this study comprised of phrase, part and echeme. A phrase was one complete advertisement signal repeated in time [25], meanwhile an echeme was constituted by cycles of repeated tymbal muscle movement. In this study, we introduced part as a new structure that consisted of an aggregation of echemes so that one phrase is constructed by several main parts. After the determination of song structures, temporal and spectral measurements were conducted (Fig. 2). PD was the duration of a phrase, PaD was the duration of a part and ED was the duration of an echeme. No intervals between parts or between echemes were noted. With regards to spectral properties, two characteristics were measured: peak frequency (PF) and bandwidth 90% (BW90), in which PF was the frequency with the highest energy, whereas BW showed a 90% frequency range of a structure. Data was shown in mean ± standard deviation.

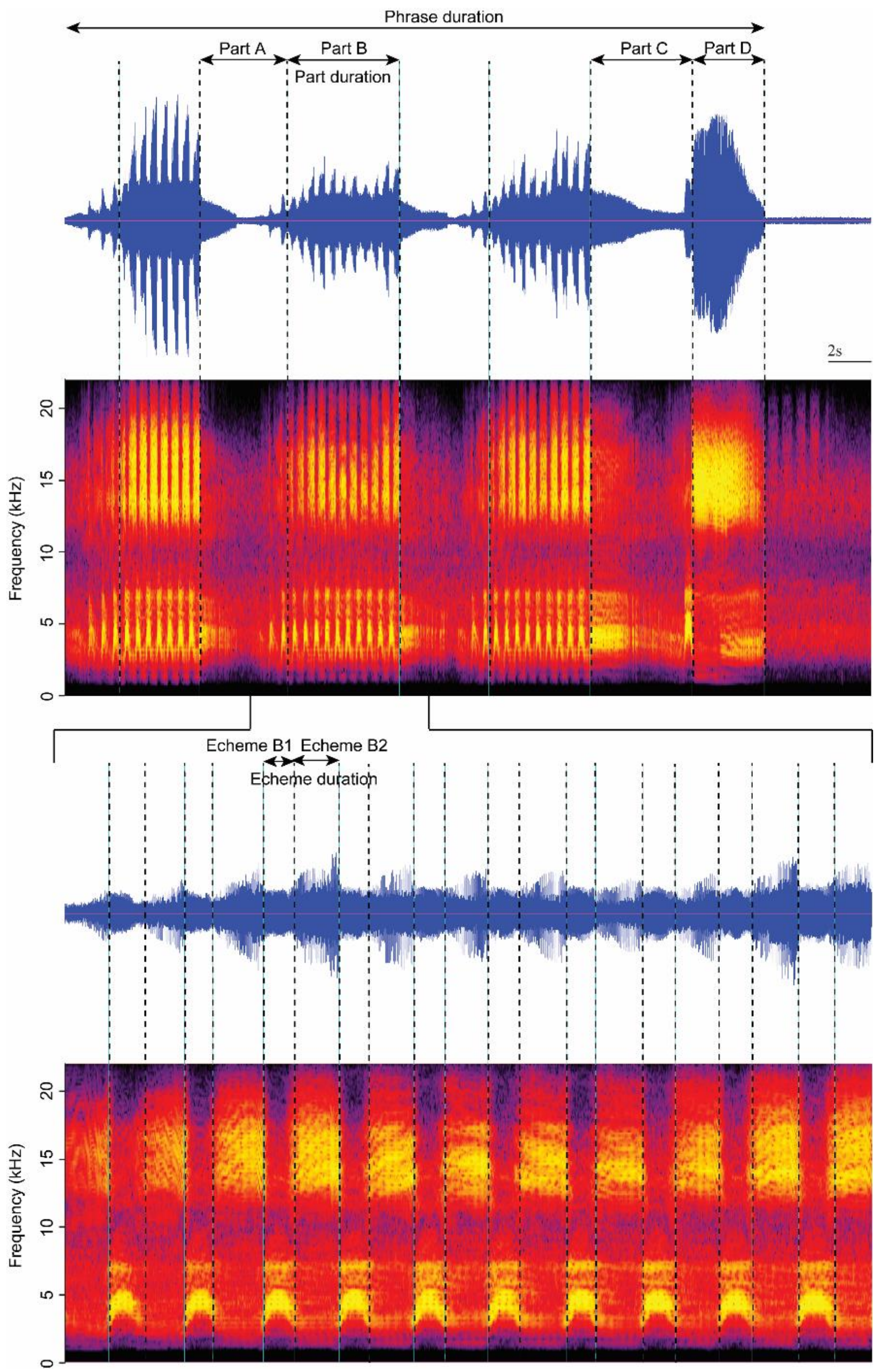


***Figure 2. Hierarchical terminology for cicada advertisement song description***

***(a) An advertisement song of H. maculaticollis consists of four parts; Phrase duration starts from the beginning to the end of one complete song, whereas part duration expands from the beginning to the end of one part; (b) One part B consists of two echemes B1 and B2 alternatively repeated, and echeme duration was the time between the beginning to the end of one echeme***

## 2.2. Results and discussion

### 2.2.1. Results

Advertisement songs of *H. maculaticollis* were easily recognizable owing to the alternative repetition of high- and low frequency structures within the song. The empirical fluctuation processes of 19 songs (Fig. 3) displayed such complexity and repetition, and since all of them crossed the significance boundary, more than one significant structural change was estimated. For this species, at least three different fluctuation structures were observed, and breakpoint estimation relied on both the peaks of the empirical fluctuation process and the number of different fluctuation patterns identified in the process.

Specifically, a phrase of *H. maculaticollis* lasted 54.27 ± 21.36 s and it could be determined by four main parts, in which the first two parts A and B were repeated alternatively six times in the beginning, parts C and D were produced only once at the end of the song (Fig. 4). Echeme structures were analyzed in all parts (Fig. 5). Part A was further divided into three types of echemes from A1 to A3, in which echeme A1 was considered to play to the role of warming up for the repetitive modulation of frequency in the middle of the song. Part B consisted of two types of echemes B1 and B2 and they were alternatively produced approximately seven times in the song. Not only the peak frequency of echeme B1 was remarkably lower than echeme B2 but also the bandwidth of 90% of the former was narrower compared to the latter. This attribute made up the signature song pattern of *H. maculaticollis*. Part C was comprised of three echemes C1 to C3, which was similar to the structure of part A. Subsequently, two echemes in part D were also generated one time in all songs analyzed. Detailed descriptive statistics of temporal and spectral properties are provided in Table 1.

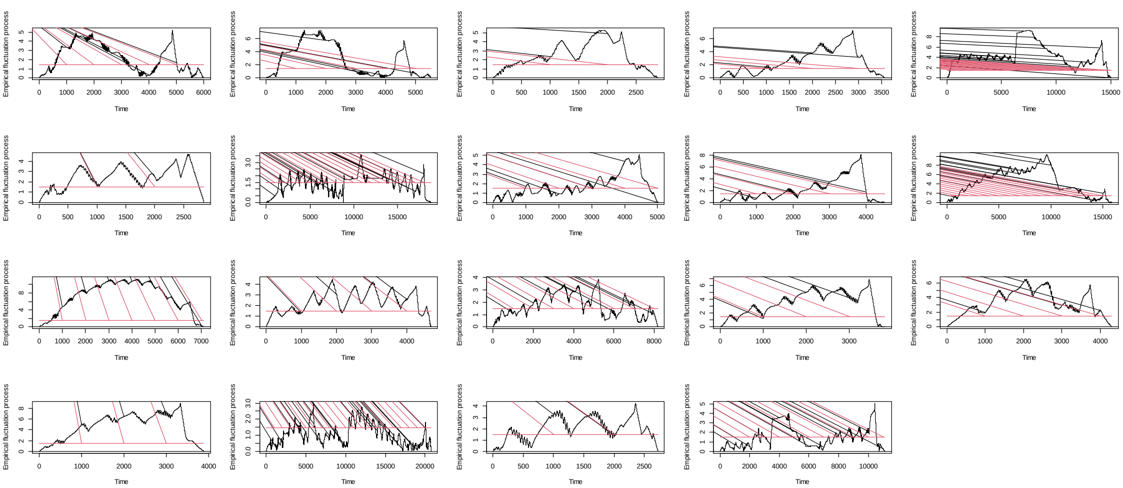

***Figure 3. Empirical fluctuation processes of 19 H. maculaticollis' advertisement songs analyzed***

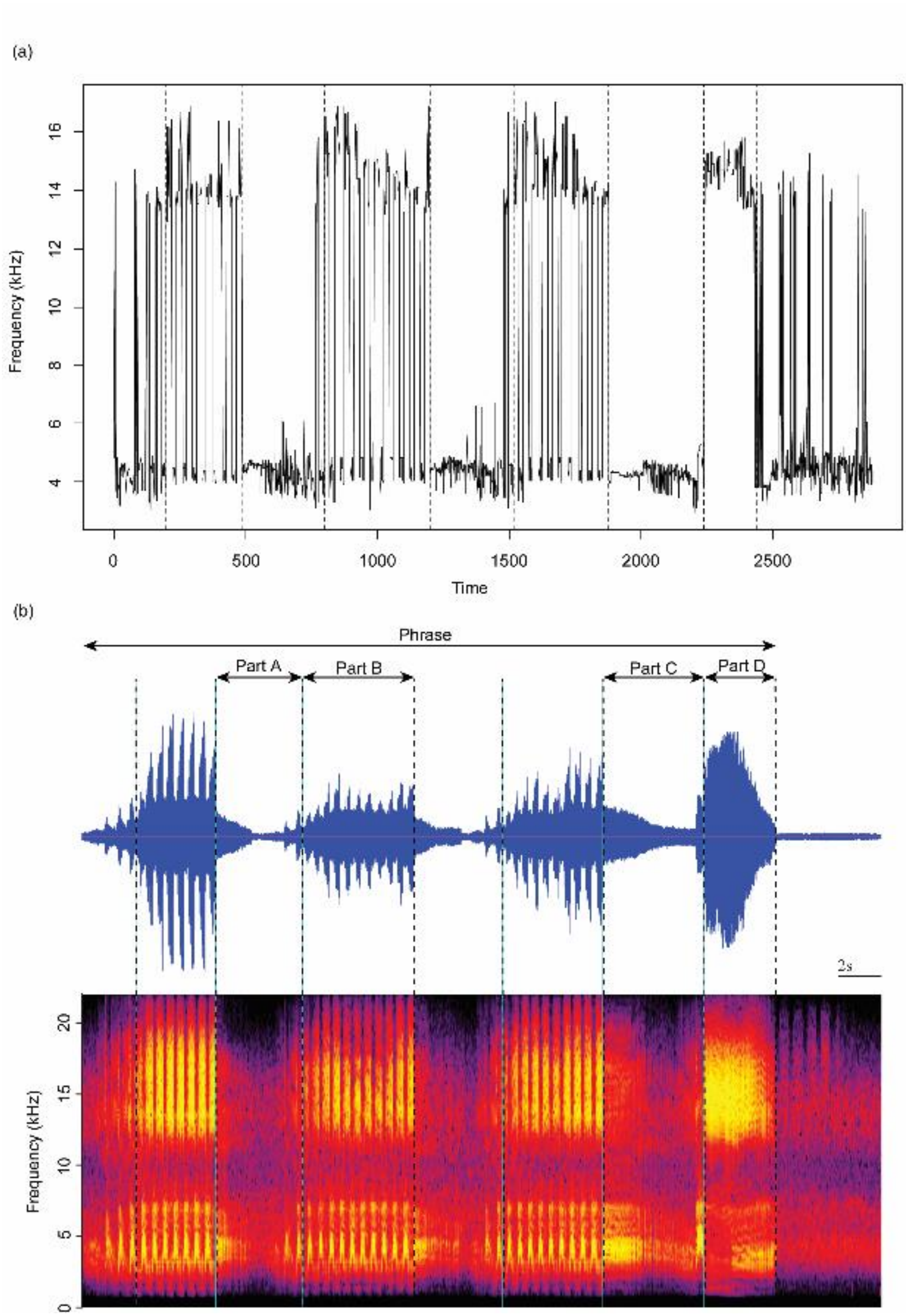


***Figure 4. An advertisement song of H. maculaticollis in which one phrase consisted of four main parts A to D***

***(a) Plot of peak frequency with determined part structures (dashed lines represented located breakpoints); (b) Song shown in oscillogram (above) and spectrogram (below)***

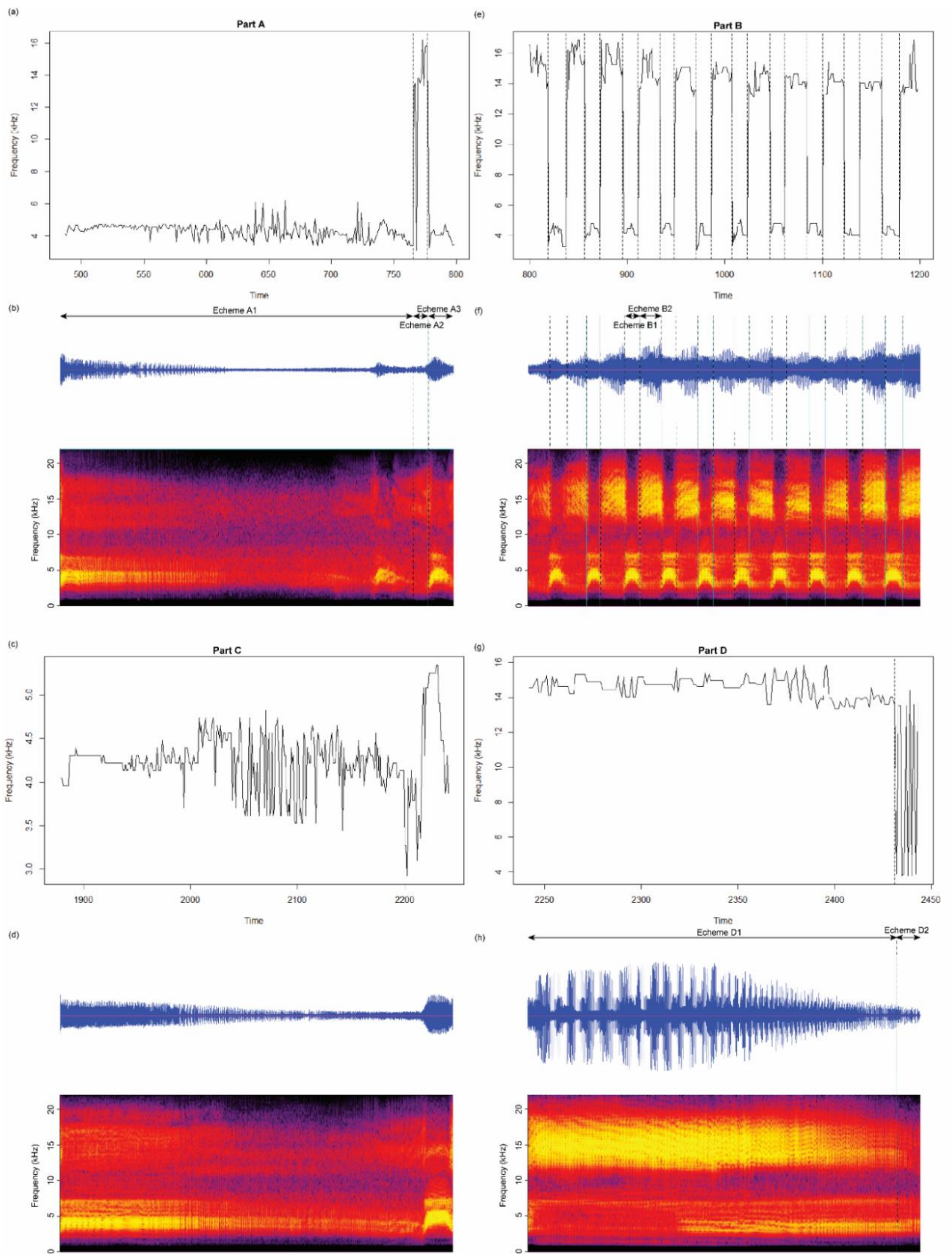


***Figure 5. Echeme structures in each part of H. maculaticollis' advertisement songs***

***Plots (a), (c), (e) and (g) display peak frequency with determined echeme structures of each part; Plots (b), (d), (f) and (h) are oscillograms (above) and spectrograms (below) of each structure shown in each part. Dashed lines represented located breakpoints.***

***Table 1.*** ***Acoustic properties of H. maculaticollis' advertisement songs (19 songs)***

| | n | PaD (s) | ED (s) | PF (kHz) | BW (kHz) |
|---|---|---|---|---|---|
| Part A | 6 ± 2.4 | 4.33 ± 1.26 | | | |
| Part B | 6 ± 2.4 | 3.28 ± 0.60 | | | |
| Part C | 1.06 ± 0.25 | 4.01 ± 0.78 | | | 6.21 ± 1.70 |
| Part D | 1.06 ± 0.25 | 2.53 ± 0.59 | | | |
| Echeme A1 | 1.01 ± 0.04 | | 3.63 ± 0.96 | 4.49 ± 0.29 | 9.33 ± 1.62 |
| Echeme A2 | 1.56 ± 0.74 | | 0.14 ± 0.03 | 11.69 ± 1.44 | 13.46 ± 0.86 |
| Echeme A3 | 1.56 ± 0.76 | | 0.52 ± 0.40 | 4.22 ± 0.25 | 8.09 ± 0.56 |
| Echeme B1 | 6.99 ± 1.26 | | 0.19 ± 0.03 | 4.23 ± 0.14 | 6.55 ± 0.75 |
| Echeme B2 | 7.95 ± 1.32 | | 0.23 ± 0.03 | 14.15 ± 0.75 | 10.67 ± 1.86 |
| Echeme C1 | 1.13 ± 0.34 | | 3.29 ± 1.05 | 4.32 ± 0.17 | 8.09 ± 0.56 |
| Echeme C2 | 1.50 ± 0.58 | | 0.14 ± 0.03 | 12.76 ± 1.98 | 13.98 ± 2.83 |
| Echeme C3 | 1.67 ± 0.58 | | 1.94 ± 0.50 | 4.30 ± 0.07 | 6.65 ± 0.37 |
| Echeme D1 | 1.25 ± 0.77 | | 2.34 ± 0.42 | 14.62 ± 1.02 | 13.19 ± 1.08 |
| Echeme D2 | 1.25 ± 0.50 | | 0.69 ± 0.96 | 8.74 ± 2.44 | 11.99 ± 2.37 |

*n = number of units produced in a phrase, PaD: part duration, ED: echeme duration, PF: peak frequency, BW: bandwidth 90%.*

### 2.2.2. Discussion

During the course of spectral fluctuation analysis, there was a tradeoff between the analysis time and the resolution of fine-scale structure. As the window size of spectrogram configuration increases, better resolution of structures could be observed and investigated; however, this substantially increases the computational time of breakpoint dating, which may be a nuisance to the operating system. On the other hand, the dating of potential breakpoints was also affected by the minimal segment size to be analyzed. Depending on the precision of the analytical scheme, an examination of different fragmentation sizes should be conducted for the selection of the most suitable parameter. It is suggested that the determination of general structures of all advertisement songs of a species was first done before exploring any intraindividual variation in song structures. Therefore, the integration of the researchers' knowledge and advanced mathematical methods is critical for the well-defined delimitation of acoustic structures. This was also one of the limitations of the current work which required the optimization of the fragmentation sizes for each species.

To validate our result of the song structure, addressing the way to select the optimal number of breakpoints that identify the echeme structure in a song is important. Function breakpoints() in strucchange provided an automatic decision in the optimal breakpoints using model selection criteria, like the Bayesian Information Criteria (BIC). However, before accepting these criteria, we evaluated our results with the nature of the error and regressor in Equation (1) that Bai and Perron [23] assumed to test the hypotheses regarding breakpoints. Bai and Perron [23] concerned two cases for their work; one considers no lagged dependent regressor and accepted autocorrelation and heteroscedasticity of residuals, and the other focuses on the accepted lagged dependent regressors and the uncorrelated error term, $u_i$ in Equation (1) and (2). By inspecting the

ENV time-series dataset, there was a strong correlation between ENV and its lag (Supplementary Fig. 1), but the autocorrelation and heteroscedasticity in residuals by investigating residual plots for the fitted model of Equation (2) (Supplementary Fig. 1).

This may likely lead to an inaccurate assessment of the breakpoints. Besides, BIC tends to choose a higher number of breakpoints than that should be when there is the presence of autocorrelation of residuals [24]. Therefore, we manually chose the optimal number of breakpoints by inspecting the ENV plot for each song. To relieve the issues in estimated residuals, we evaluated the linearity assumption in Equation (1) and (2) works well in our data (Supplementary Fig. 3). Since many data points seem to deviate from the fitted model of Equation (1) and (2), the linearity assumption might not fit well in our data. The discrepancy between the model and the data possibly contributed to the heteroscedasticity of the residuals. Thus, further study is needed to test for suitable transformation for each variable or a method that assumes a non-linear relationship when identifying the breakpoints.

## 3. Conclusions

Song analysis has mainly relied on subjective delimitation between different structures within songs. For those animals that produce songs of complicated structures such as cicadas, birds, primates, etc., it is necessary to use an objective method of segregating different structures within songs. Spectral fluctuation analysis could serve as an effective tool for such studies which mainly rely on identifying significant changes in the peak frequency and amplitude envelope of the advertisement songs produced by male cicadas. Afterward, locations of such structural changes would be determined for subsequent measurement of spectral and temporal properties. The proposed method has been successfully applied to analyze the advertisement songs of *H. maculaticollis*, a cicada species that produced songs of complex structure.

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