J. Agric. Sci., Tokyo Univ. Agric., 66 (1), 11–17 (2021)
東京農大農学集報，66（1），11–17（2021）

Call Classification of the Japanese Giant Flying Squirrel (*Petaurista leucogenys*) Using Principal Component Analysis

By

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(Received November 19, 2020/Accepted March 9, 2021)

Summary：The Japanese giant flying squirrel (*Petaurista leucogenys*) is a member of the arboreal Sciuridae. There are few studies conducted on their calls, even though this species produces loud calls. A previous study suggested that they have several call types, for example, the “Gururu” exchange call and the “Kyokyo” call used in alarm and mating contexts. “Gururu” calls were divided into five subtypes ; however, this classification was subjective and dependent on the researcher. We classified the calls of the Japanese giant flying squirrel at Mt. Takao in Tokyo and Daiyuzan in Kanagawa Prefecture, Japan using objective statistical methods such as principal component analysis (PCA) on six measurements : peak frequency, duration of the note, duration of the call bout, inter-note interval, number of pulses, and number of notes. PCA results indicated that the “Gururu” calls were not clearly distinguished between the natural calls and the response call, whereas the “Kyokyo” calls were distinguished with the first axis explained by the number of pulses and note duration, and the second axis explained by the number of notes and call duration. This suggests that the “Kyokyo” call can be classified into two subtypes, along with a sound character and context (alarm and mating).

Key words：call classification, principal component analysis, *Petaurista leucogenys*, playback experiments

1. Introduction

The Japanese giant flying squirrel (*Petaurista leucogenys*), a kind of rodent, is known for a large number of calls[1)]. They communicate with other individuals to make them aware of their location in the dark[2)]. Previous study conducted a spectrogram analysis to evaluate the calls of this animal and classified the calls into five types[1)] : (1) “Gururu”, which was the most common exchange call ; (2) “Kyokyo”, an alarm and mating call ; (3) “Zizizi” and (4) “Gua-o”, which are courtship behavioral calls between fighting males ; and (5) “Gugugu,” that is used between relatives, such as a mother and child. The research suggested that their calls are important for understanding their social behavior. In addition, it is important to classify calls accurately because we may identify the behavior of the Japanese giant flying squirrels through only listening to their calls. However, the classification in ANDO and KURAMOCHI (2008)[1)] was subjective and dependent on the researcher. For instance “Gururu” call types were divided into five subtypes ; however, these classifications were unclear and abstract because they were classified based on the contour of the calls. In terms of the “Kyokyo” call, the two opposite meanings, alarm and mating, indicates that the classification in ANDO and KURAMOCHI (2008)[1)] is probably inadequate.

Principal component analysis (PCA) is known to be effective for classifying unclear call types[3)]. Rodents are one of the most behaviourally diverse orders in mammals[4)], and they can be used as a comparative model for studying

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acoustic communication[5]. For example, the giant mole-rat (*Fukomys mechowii*) has the most varies vocal repertoire among all subterranean rodents, and that repertoire is also linked with high social interactions[6]. Bednárová *et al.* (2013)[6] showed the separation of calls using PCA. Alarm calls in Anatolian ground squirrel (*Spermophilus xanthoprymnus*), Taurus ground squirrel (*S. taurensis*) and European ground squirrel (*S. citellus*) were each distinguished into each three species using PCA, even though these calls have similar structures[7].

Playback experiments, where recorded calls are played back to animals to obtain a response, are known to be effective in investigating animals that emit calls frequently. Previous studies have suggested that the experiments are useful for research concerning the call features of primates[8–10]. In addition, Pallas's squirrels (*Callosciurus erythraeus*) were shown to respond acoustically to calls of conspecifics[11]. Therefore, we conducted playback experiments to classify call types objectively using PCA.

2. Materials and methods

Study area

This study was conducted in two areas where the Japanese giant flying squirrel is distributed : Mt. Takao in Tokyo (35°37′N, 139°15′E) and Daiyuzan in Kanagawa Prefecture (35°18′N, 139°4′E), Japan. Situated at an elevation of 599 m and 433 m, respectively, these areas have a temple forest with many hollow trees, which can be used as dens by the Japanese giant flying squirrel. Mt. Takao is a tourist spot visited by approximately three million hikers per year. However, there are few tourists in Daiyuzan.

Recording and editing calls

We conducted a preliminary survey to record the calls of the Japanese giant flying squirrel in the two study areas. We recorded for a total of 40 days from March 27 to December 15, 2018 and the number of days for each area was as follows : 35 days on Mt. Takao and 5 days in Daiyuzan. The survey was conducted according to the activity time of this animal, 2 to 4 hours after dark[2]. For recording, we used a condenser microphone with frequency characteristics ranging from 40 Hz to 20 kHz (ECM-G5M ; SONY, Tokyo, Japan) and a recorder with a sampling frequency of 192 kHz (DR-701D, TASCAM, California, USA). We conducted a spectrogram analysis to distinguish call types. To edit and analyze the calls, we used SASLab Pro version 5.2.15 (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany). "Gururu" and "Kyokyo" recorded calls were selected as playback calls because these call types were produced frequently, and continued for approximately 10 s and 5 s, respectively (Fig. 1). These were recorded from the same female in October at Mt. Takao, which had the least noise in our recorded collection. These calls have been suggested to have a frequency range between 1 and 12 kHz[1]. However, this recording contained some noises, which were recorded as below 1 kHz. Therefore, in the present study, we removed all sounds below 1 kHz using SASLab Pro (Fig. 1). Spectrograms were generated with a Hamming window function, and a fast Fourier transform size of 1024 for the "Gururu" recorded call and 512 for the "Kyokyo" recorded calls, respectively.

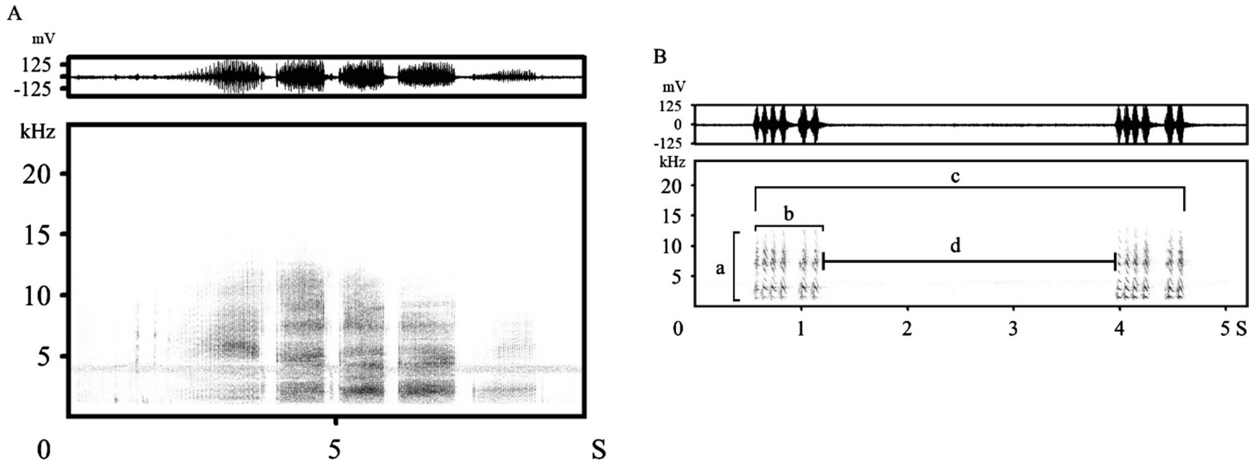

Fig. 1 Sound spectrograms of "Gururu" and "Kyokyo" recorded calls. A : "Gururu" recorded call for which the frequency range was between 1 kHz and 15 kHz. FFT size of 1024. B : "Kyokyo" recorded calls for which the frequency range was between 1 kHz and 12 kHz. FFT size of 512. The horizontal axis represents time (s) and the vertical axis represents the frequency (kHz). The call was recorded on Mt. Takao from a female individual. To reduce the background noise, we removed all sounds in the frequency range of 1 kHz. Call measurements are (a) peak frequency, (b) duration of the note, (c) duration of the call bout, and (d) inter-note interval.

Playback experiments and data analysis

The playback experiments were conducted at the sites where we found squirrels in the preliminary survey or where we found them without playing back the calls in the two study areas. The recorded calls were played back from a speaker that was 2 to 3 m high (SLINKMICROORG ; BOSE, Massachusetts, USA) and the volume was raised so that it was audible from a distance of at least 50 m. We conducted playback experiments for a total of 32 days from January 6 to November 19, 2019 as follows: 15 days on Mt. Takao and 17 days in Daiyuzan. The experiments were conducted 2 to 4 h after dark at the same time as in the preliminary survey. According to Koli and Bhatnagar (2014)[12], we defined call bout when it was separated by intervals of more than 2 min until the next call emitted in other types or from other individuals. In this study, exchange calls were defined as when flying squirrels responded within 2 min.

We performed principal component analysis (PCA) to classify the call type objectively between the two settings, which were as follows : the "artificial setting" where the Japanese giant flying squirrels responded to the playback call, and the "natural setting" where they emitted calls naturally. In this analysis, we used six call measurements peak frequency, duration of the note, duration of the call bout, inter-note interval, number of pulses, and number of notes (Fig. 1). We used normalized variate data transformed from the raw data, using the following equation :

$$Z_i = \frac{Xi - \bar{X}}{S},$$

where Z_i is the z-transform, X_i is each measurement (X_1, $X_2 \cdots X_i$), $\bar{X}$ is the average of all measurements, and S is the variance. Once their calls were classified, these measurements were then compared using the Wilcoxon signed-rank test to determine differences between the artificial and natural settings under the row data.

We set two clusters when using PCA in order to know whether calls in the two settings are different call types or not. However, we had better verify how many clusters were the optimal numbers. Therefore, the number of clusters in call types were reconsidered by *k*-means and using the silhouette analysis. The average silhouette width was calculated from 2 to 10 to identify the most appropriate number of clusters[13].

To compare the differences in the response ratio between "Gururu" and "Kyokyo," we performed a chi-square test. All statistical analyses were performed using R version 3.5.3[13].

3. Results

The natural and response calls of "Gururu" were recorded in all seasons. On the other hand, the natural calls

Table 1 Results of playback experiments in Mt. Takao and Daiyuzan.

Playback call type	Number of playback calls	Response call			
		"Gururu"	"Kyoyo"	Total	Ratio (%)
Gururu	453	65	14	79	17.4
Kyokyo	267	20	7	27	10.1

Table 2 Factor loadings for the first three principal components which account for ca. 80% of total variation from the PCA.

Measurements	"Gururu" call			"Kyokyo" call		
	PC1	PC2	PC3	PC1	PC2	PC3
Peak frequency	0.28	-0.22	0.84	-0.37	0.44	-0.31
Number of notes	-0.33	**-0.76**	0.00	0.28	**0.62**	0.07
Number of pulses	-	-	-	**-0.61**	0.09	0.17
Duration note	**0.62**	0.24	0.09	-0.59	0.20	0.14
Duration call	0.48	-0.53	-0.12	0.25	0.58	0.37
Inter note interval	0.44	-0.19	-0.53	-0.05	-0.19	0.85
SD	1.40	1.11	0.98	1.53	1.39	1.10
Proportion (%)	0.39	0.25	0.19	0.39	0.32	0.20
Cumulative (%)	0.39	0.64	0.83	0.39	0.71	0.91

The measurement, number of the pulse in "Gururu" call was removed because number of pulses was not clear.
Bold: the first largest factor loading in each vector.

of “Kyokyo” were recorded in June, December, January which months are their breeding season. They responded acoustically to recorded call using “Kyokyo” on January, March, May and November.

We played back “Gururu” and “Kyokyo” recorded calls in the two study areas from January to November 2019. Seventy-nine of the 453 “Gururu” call playbacks (17.4%) were responded to acoustically over the 32 days. In addition, 27 of the 267 “Kyokyo” call playbacks (10.1%) were responded to acoustically over the 32 days (Table 1). The “Gururu” calls were responded to more frequently than “Kyokyo” calls ($x^2 = 6.61$, $P = 0.01$). The “Gururu” and “Kyokyo” recorded calls were responded to with not only the matching call but also the other calls (Table 1).

In the PCA, more than 80% of the total variation was summarized by the first three axes (Table 2). The measurement of the number of pulses of the “Gururu” call was removed because the number of pulses was not clear in a call bout, and the “Gururu” calls were not distinguished clearly and divided into subtypes (Fig. 2A). In contrast, the “Kyokyo” calls were distinguished by their setting (Fig. 2B). As shown in Fig. 2B, the first axis (PC1) is explained by the number of pulses and duration notes, whereas PC2 is explained by the number of notes and duration calls. Significant differences in the PC scores were only assessed for the first two PC axes, which explained 71.4% of the total variation (Fig. 2B, Table 2). As shown in Fig. 3, there were significant differences between the settings regarding peak frequency ($W = 27$, $P < 0.01$), duration of the note ($W = 36$, $P < 0.01$), duration of the call bout ($W = 185$, $P < 0.01$), the number of pulses ($W = 12.5$, $P < 0.001$), and the number of notes ($W = 191.5$, $P < 0.01$).

The number of clustering in “Gururu” and “Kyokyo” calls were reconsidered by *k*-means and using the silhouette analysis. The optimal number of clusters was identified three and five clusters from the average silhouette width in “Kyokyo” calls and “Gururu” calls, respectively. In three clusters in “Kyokyo” calls, all response calls (except one call) were classified into one cluster and all-natural calls were classified into the other two clusters. On the other hand, there were both natural and response calls in every five clusters in “Gururu” calls.

4. Discussion

The Japanese giant flying squirrel responded acoustically to recorded calls at both Mt. Takao and Daiyuzan. This suggests that they recognized the recorded calls as conspecific calls because the acoustic response ratio was similar to that in a natural setting (13% ; Ando and Kuramochi 2008[1]). We considered that the experiments were effective in obtaining acoustical responses from the Japanese giant flying squirrel.

The “Gururu” recorded calls were responded to more frequently than the “Kyokyo” recorded calls, consistent with the results reported by Ando and Kuramochi (2008)[1] (Table 1). However, the “Gururu” and “Kyokyo” recorded calls were responded to with both “Gururu” and “Kyokyo” calls from them (Table 1). Ando and Kuramochi (2008)[1] did not observe the Japanese giant flying squirrel respond to the “Gururu” call with a “Kyokyo” call, nor a “Kyokyo” recorded call with a “Gururu” call. Our results indicate

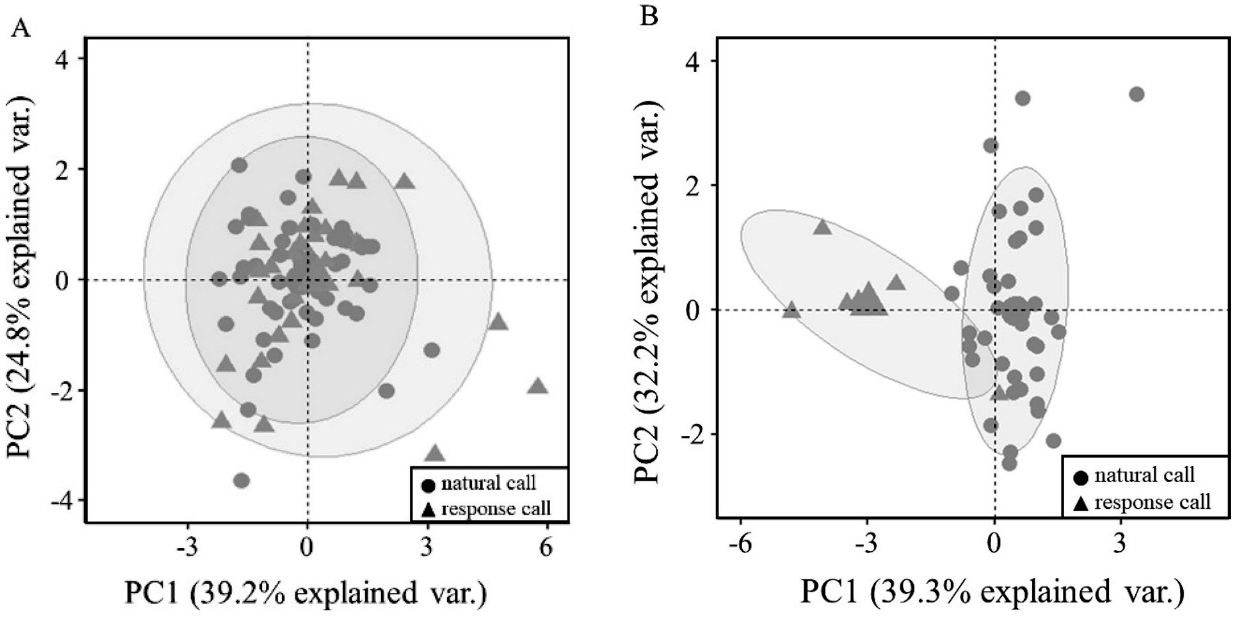

Fig. 2 Two-dimensional plots of PC1 vs. PC2 in natural and artificial settings. A : “Gururu” calls in the two different settings. B : “Kyokyo” calls in the two different settings. PC = principal component.

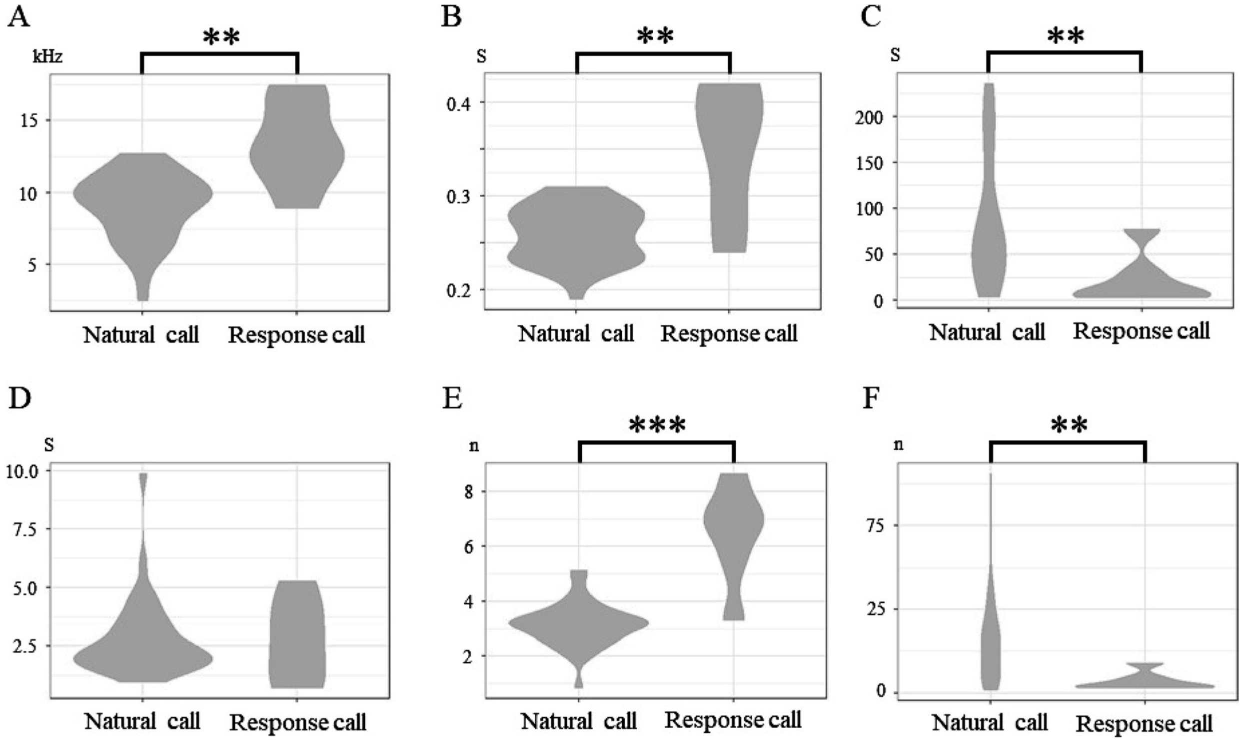

Fig. 3 The results of differences between situations for each measurement : A : peak frequency, B : duration of the note, C : duration of call bout, D : inter-note interval, E : number of pulses, and F : number of notes (Wilcoxon signed-rank test, $^{**}P<0.01$, $^{***}P<0.001$).

that they communicate with conspecifics with various call types and complexity. They do not appear to respond to one call type with only a single call type.

The "Gururu" call did not differ among settings according to the limited data available, whereas the "Kyokyo" call was distinct between settings (Fig. 2B). "Gururu" calls may have five subtypes as mentioned by Ando and Kuramochi (2008)1) from the results that the optimal number of clusters were five in *k*-means and silhouette analysis. However, "Gururu" calls are not distinguished into natural and response calls in this study. The data of the "Gururu" calls were limited, and more data should be recorded to investigate differences between settings, areas, or sexes. Ando and Kuramochi (2008)1) reported that "Kyokyo" calls were emitted both within an alarm context and on the day of estrus. In the present study, there was a difference in the acoustic characteristics between the natural and playback settings. This suggests that the "Kyokyo" call can be classified into two subtypes, along with a sound character and context (alarm and mating). This suggestion is supported by the result that the natural call (mating call) was produced only in the breeding season. The results that natural and response calls were classified into different clusters by *k*-means analysis also support this suggestion. "Kyokyo" calls may have two subtypes in natural calls from the results that the clusters were divided into two clusters in *k*-means and silhouette analysis. However, the number of data of the "Kyokyo" calls were not much. Therefore, more information (sample size, sex, season) is needed to reveal the subtype in the response call. This should be confirmed by acquiring more data from different individuals, sexes, and areas.

To our knowledge, this is the first study to conduct playback experiments with Japanese giant flying squirrels. "Gururu" calls were not divided into subtypes ; in contrast, "Kyokyo" calls were observed to be divided into an alarm context and a mating context as indicated through objective analysis using PCA. We conducted playback experiments in which only two types of calls were recorded from a single individual on Mt. Takao. Further study is required using more call types from various individuals in different areas.

Acknowledgments

We would like to offer special thanks dedicated to the memory of the late Dr. Motokazu Ando for his encouragement, which helped us complete our research. We are grateful to K. Shimizu (current affiliation : curator in Toyama Science Museum) and H. Moriya, the staff members of Tsukuiko Shiroyama Park. We would also like to thank S. Yoshida and M. Shigeta, members of the MACHIMUSA Research Group, and T. Morisaka, associate professor at Mie University. We also thank the members of the Laboratory of Wild Animals at Tokyo University of Agriculture for supporting the fieldwork.

References

1) Ando M, Kuramochi Y (2008) Vocal Communication of the Japanese giant flying squirrel *Petaurista leucogenys*. *J. Agri. Sci.* **53** : 176–183 (In Japanese).

2) Ando M, Imaizumi Y (1982) Habitat utilization of the white-cheeked giant flying squirrel *Petaurista leucogenys* in a small shrine grove. *J. Mamm. Soc. Japan.* **9** : 70–81 (In Japanese).

3) Sørensen PM, Wisniewska DM, Jensen FH, Johnson M, Teilmann J, Madsen PT (2018) Click communication in wild harbour porpoises (*Phocoena phocoena*). *Sci. Rep.* **8** : 1–11.

4) Kay EH, Hoestra HE (2008) Rodents. *Curr. Biol.* **18** : 406–410.

5) Briggs JR, Kalcounis-Rueppell MC (2011) Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Anim. Behav.* **82** : 1263–1273.

6) Bednářová R, Hrouzková-Knotková E, Burda H, Sedláček F, Šumbera R (2013) Vocalizations of the giant mole-rat (*Fukomys mechowii*), a subterranean rodent with the richest vocal repertoire. *Bioacoustics.* **22** : 87–107.

7) Schneiderová I, Policht R (2012) Acoustic analysis of the alarm call of the Anatolian ground squirrel *Spermophilus xanthoprymnus* : a description and comparison with alarm calls of the Taurus *S. taurensis* and European *S. citellus* ground squirrels. *Sci. Nat.* **99** : 55–64.

8) Sugiura H (1998) Matching of acoustic features during the vocal exchange of coo calls by Japanese macaques. *Anim. Behav.* **55** : 673–687.

9) Kajikawa S, Hasegawa T (2000) Acoustic variation of pant hoot calls by male chimpanzees : a playback experiment. *J. Ethol.* **18** : 133–139.

10) Fichtel C (2004) Reciprocal recognition of sifaka (*Propithecus verreauxi verreauxi*) and redfronted lemur (*Eulemur fulvus rufus*) alarm calls. *Anim. Cogn.* **7** : 45–52.

11) Tamura N, Kasahi T, Kaneda M, Mitarai N, Shigeta M, Shigeta Y, Yamasaki F, Morisaki M, Tsuda T, Ono S, Hasegawa N, Waguri M (2013) Sound playback surveys to reveal the distribution of invasive alien Pallas's squirrels, *Callosciurus erythraeus*. *Mammal Study*. **38** : 97–103.

12) Koli KV, Bhatnagar C (2014) Calling activity of Indian giant flying (*Petaurista philippensis* Elliot, 1839) in the tropical deciduous forests, India. *Wildl. Biol. Pract.* **10** : 102–110.

13) Kaufman L, Rousseeuw PJ (2008) Finding groups in data : an introduction to cluster analysis. Wiley, Hoboken. pp. 126–163.

14) R Core Team (2020) R Core Team (2018) R : A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

主成分分析を用いたムササビ（*Petaurista leucogenys*）の音声タイプの分類

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（令和2年11月19日受付/令和3年3月9日受理）

要約：ムササビ *Petaurista leucogenys* は樹上性のリス科の動物である。本種は大きな声で鳴く動物であるが，音声についての研究はあまり行われていない。先行研究では，鳴き交わしに使われる「グルル」や繁殖や警戒時に発される「キョキョ」等のいくつかの音声タイプを発することが明らかになっている。一方で，「グルル」にはサブタイプが確認されているが，その分類方法は主観的であるため，より客観的な方法での分類が求められる。そのため本研究では，東京都高尾山と神奈川県大雄山にて，プレイバック実験によってムササビの音声を効率的に録音し，鳴き交わしに使われる「グルル」と繁殖や警戒の意味を持つ「キョキョ」の2タイプについて，6つの要素（ピーク周波数，ノートの持続時間，コールバウトの持続時間，ノート間隔，パルス数，ノート数）について主成分分析を用いて客観的に音声分類を行った。プレイバックの鳴き声に対する反応と，自然に発される音声を比較したところ，「グルル」は音声に変化がなかったのに対し，「キョキョ」は音声に違いが見られ，第一主成分はパルス数とノートの持続時間，第二主成分はノートの数とコールバウトの持続時間の寄与率が高かった。「キョキョ」はプレイバックに対する鳴き返しの時と，繁殖行動時に自発的に発せられた時で分かれたことから，「キョキョ」にはサブタイプがあり，繁殖の時と警戒の時で異なる音声を発している可能性が示唆された。

キーワード：鳴き声分類，主成分分析，*Petaurista leucogenys*，プレイバック実験

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