SUPPLEMENTARY MATERIALS TO:

Unlearned adaptive responses to heterospecific referential alarm calls in two bird species from separate evolutionary lineages

Jungmoon Ha[1], Keesan Lee[1], Eunjeong Yang[1], Woojoo Kim[1], Hokyung Song[2], Injae Hwang[3], Larisa Lee-Cruz[4], Jinseok Park[1], Jihyeon Song[1], Chan-ryeol Park[5], Wooshin Lee[6], Piotr Jablonski[1,7]*, Sang-im Lee[8]*

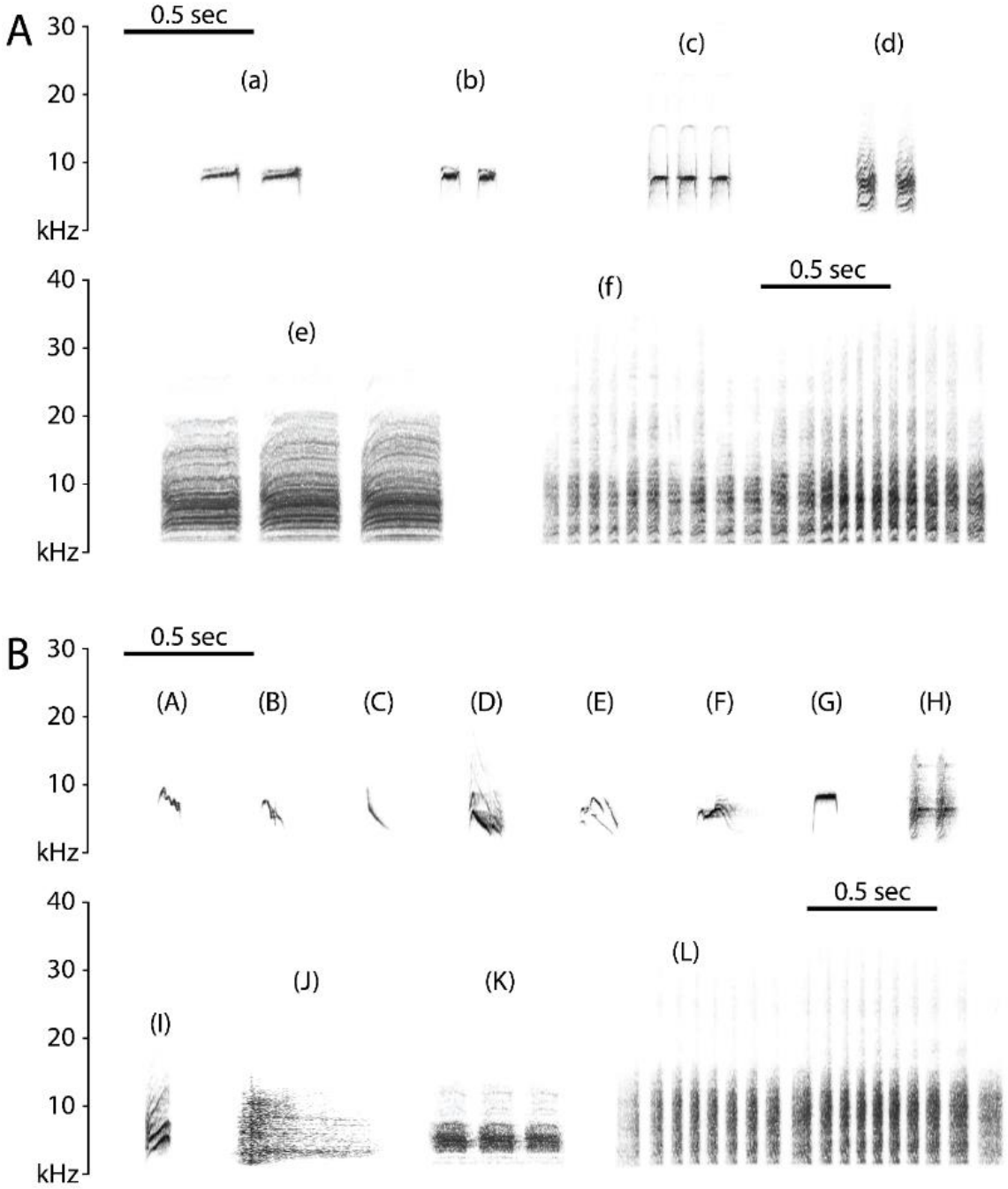
* Corresponding authors

Affiliations:

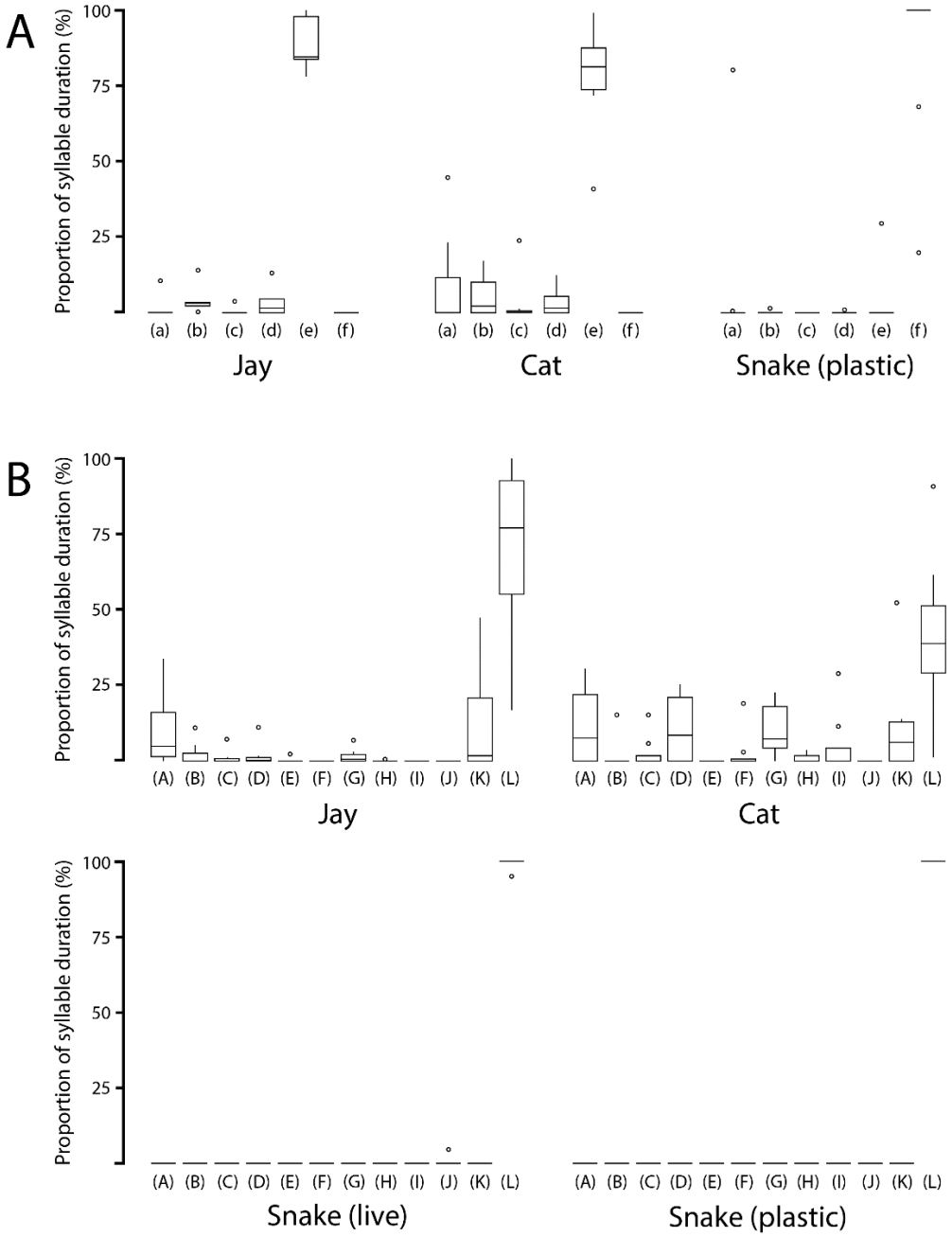
1. Laboratory of Behavioral Ecology and Evolution, School of Biological Science, Seoul National University, Seoul, Republic of Korea
2. Jeju National University, Jeju-si, Jeju-do, Republic of Korea
3. National Institute of Biological Resources, Seo-gu, Incheon, Republic of Korea
4. INRAE, UMR TETIS, Maison de la Télédétection, 500 Rue Jean François Breton, 34090 Montpellier, France
5. Urban Forests Division, National Institute of Forest Science (NIFoS), Dongdaemun-gu, Seoul, Republic of Korea
6. Department of Forest Sciences, CALS, Seoul National University, Seoul, Republic of Korea
7. Behavioral Ecology Group, Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, Poland
8. Laboratory of Integrative Animal Ecology, Department of New Biology, DGIST, Korea

CONTENT:

SUPPLEMENTARY MATERIALS PART 1:
SYLLABLE TYPES AND THEIR USE IN VOCALIZATIONS

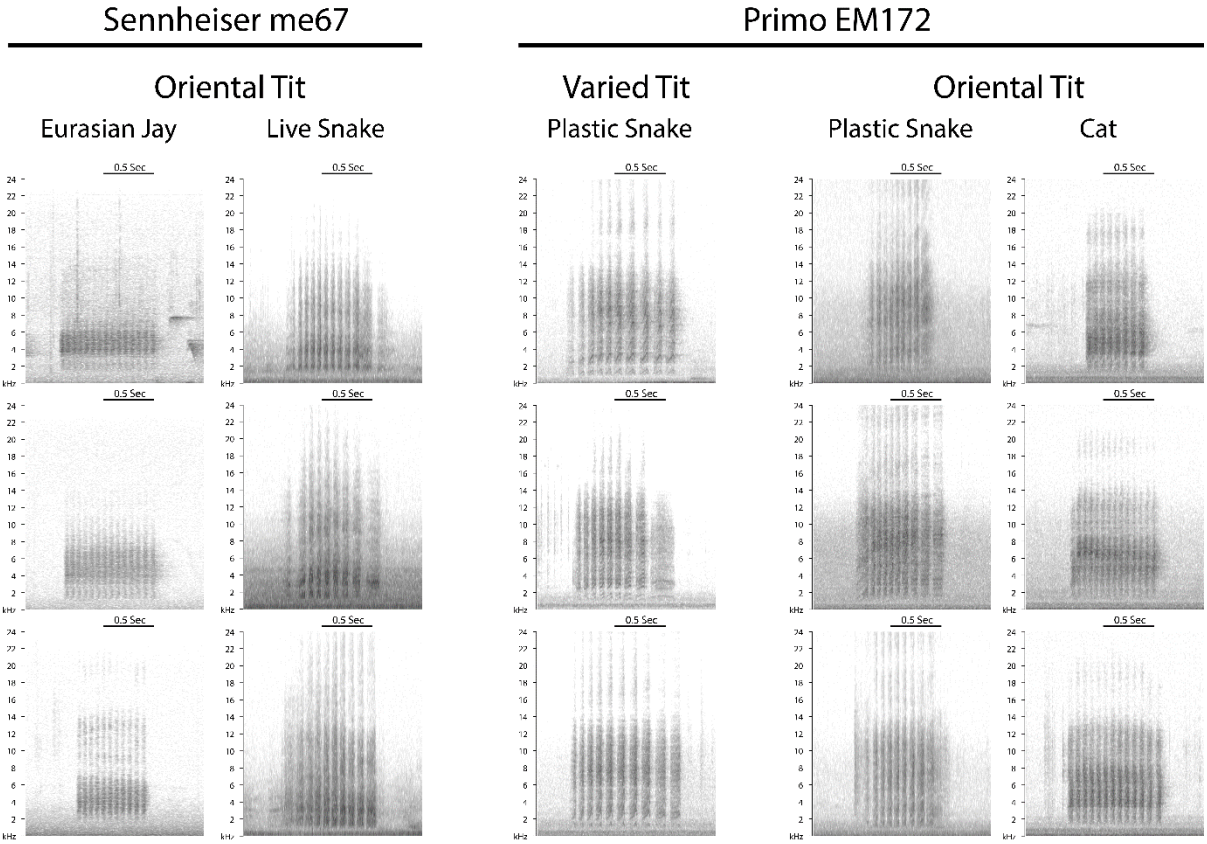


Supplementary Figure S1. Examples of typical sonograms of syllable types recorded in parental alarm calls in response to three predators: jays, cats, and snakes by the Varied Tit (A) and the Oriental Tit (B). Vocal responses were visually separated into several types based on their acoustic features in the sonogram, and each type was encoded by a different letter (lower case for the Varied Tit and upper case for the Oriental Tit).



Supplementary Figure S2. Syllable types and their use (proportion that each syllable type duration comprised in the total duration of all syllables uttered during a response) by the Varied Tit (A) toward jay, cat, and plastic snake presented near a nest, and by the Oriental Tit (B) toward jay, cat, live snake, and plastic snake presented near a nest. The duration of all syllables in each syllable type in a recording was aggregated to calculate their proportion in the total duration of all syllables in the same recording. Sample sizes are listed in Table 1 of the main text.

Vocal responses to snakes near nests were almost entirely composed of one syllable type repeated in phrases: syllable "f" and syllable "L" in the Varied Tit and the Oriental Tit respectively. Most syllables produced toward plastic snake by the Varied Tit were of the (f) syllable type, whereas the proportion of the (f) syllables was close to zero in the experiments with the other predators. This indicates that breeding Varied Tit used the (f) syllables almost exclusively as a response to snake tests. Similarly, the (L) syllables accounted for almost the total duration of all syllables in the recordings of the Oriental Tit toward live and plastic snakes. The proportion of (L) syllables was also high in the recordings from the jay and cat presentations, but those recordings also contained several other syllables (unlike recordings from snake presentation tests). As visual inspection of sonograms did not always allow for reliable distinction between various (L) type syllables, we run statistical analysis to determine if and how the (L) syllables differ among responses to the three different predators (see supplementary material part 2).



Supplementary Figure S3. Example sonograms of "L" syllable phrases to different predators for the Oriental Tit and “f” syllable phrases to snake presentations for the Varied Tit. Each sonogram was produced from a single phrase of a different pair. Note the visually perceptible higher similarity between snake alarm calls from the two species (i.e., between “L” and “f” syllables) and lower similarity between the “L” syllables to snakes and the two other predators (Eurasian Jay and cat).

SUPPLEMENTARY MATERIALS PART 2:
ACOUSTIC ANALYSES OF SYLLABLE TYPE "L" ["churr" type] IN THE ORIENTAL TIT AND RESULT DATASET OF PLAYBACK EXPERIMMENT

Supplementary Table S1. Summary of PCA (varimax-rotated principal components) and statistical analysis comparing (L) syllables between the live snake and the jay presentations to Oriental tits. Three principal components were extracted from 13 variables (7 time variables and 6 frequency variables; explanations of the acronyms are in Table S9). The columns of the principal components were arranged from largest to smallest proportion of variance explained by each component (% of variance explained shown in parentheses). Loadings higher than 0.5 are marked with grey background. This table corresponds to Fig. S4, S6, and Table S2.

Standardized loadings in varimax-rotated principal components			
Acoustic variables	**PC1: Frequency (32%)**	**PC2: Syllable (26%)** (*duration & rate*)	**PC3: Phrase (17%)** (*duration & syllable number*)
Time variables			
NS	0.13	-0.07	0.92
PD	0.17	0.39	0.81
ASD	0.05	0.91	0.03
SDSD	0.03	0.82	0.30
ACR	-0.18	-0.82	0.05
SDCR	0.05	0.59	0.58
AISI	-0.01	0.39	-0.39
Frequency variables			
ACFS	0.94	-0.17	-0.01
SDCFS	0.78	0.25	0.30
A1QFS	0.71	-0.42	-0.07
A3QFS	0.98	0.06	0.05
AIRFS	0.81	0.35	0.11
SDIRFS	0.64	0.33	0.20

Component	**Estimate (snake)**	**Standard error**	**p-value**	**Fitted distribution (link function)**
PC1: **Frequency**	5.425	0.031	<0.001	Power exponential (identity)
PC2: **Syllable**	4.207	0.087	<0.001	Johnson SU (identity)
PC3: **Phrase**	1.779	0.105	<0.001	Johnson SU (identity)

Biological meaning of the principal components:

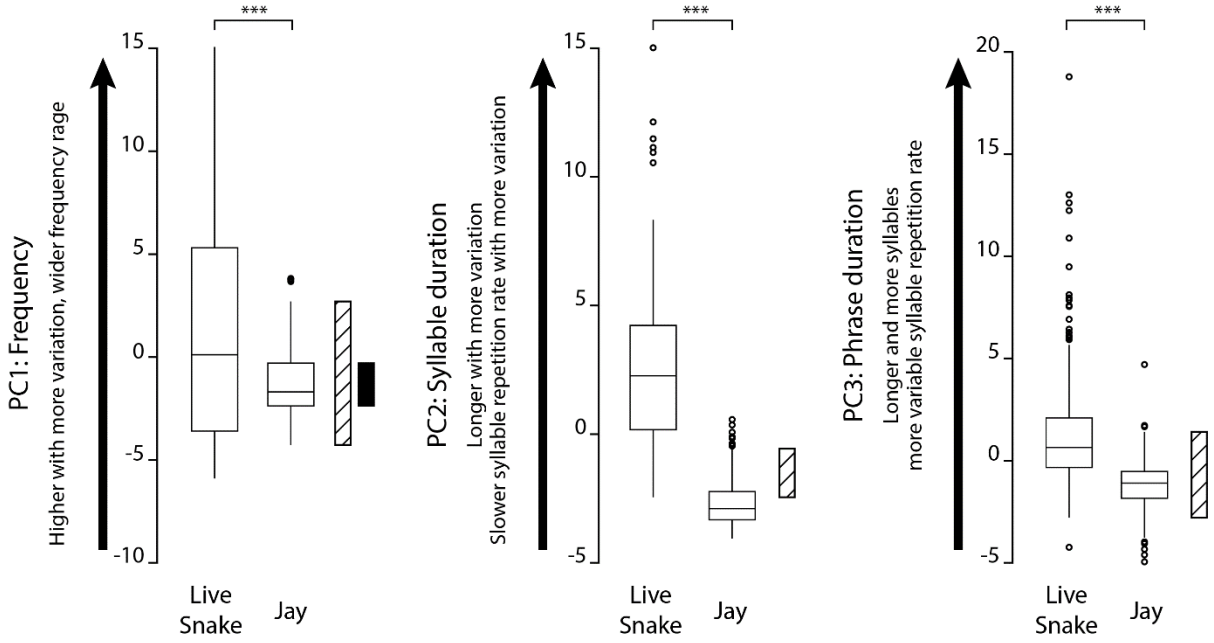
PC1: This is an index of how high the frequency of a syllable is, how variable the frequency of syllables within a phrase is, and how wide the frequency range of a syllable is. Higher values of PC1 indicate higher frequency of (L) syllables that vary more among the syllables within a phrase. It also indicates wider frequency range of a phrase composed of these syllables.

PC2: This is an index of how long and how variable is the duration of syllables in a phrase, and how fast syllables in a phrase were repeated. Higher values of PC2 indicate (L) syllables of longer duration that varies more among syllables within a phrase. Higher PC2 also indicate (L) syllables of slower repetition rate within a phrase that varies more among syllables within a phrase.

PC3: This is an index of how long is duration of a phrase, and how many syllables it contains. Higher values of PC3 indicate longer phrases of (L) syllables that have more syllables in the phrase. Higher PC3 also indicate (L) syllables of faster repetition within a phrase.

Supplementary Table S2. Comparison between the live snake and jay presentation experiments. Results of Variance component analysis (conducted using “VCA” function in R package "VCA") for the varimax-rotated principal components extracted from 13 characteristics of "L" phrases (PC1, PC2, PC3; Table S1). The error can be interpreted as a within-trial variation of the rotated component. This table corresponds to Fig. S4 and S6, and Table S1.

Rotated component (RC)	Variable level	df	Variance component	% to total	SD
PC1 (frequency properties)	total	17.79	19.23	100	4.38
	predator	1	0.97	5.04	0.98
	predator/nest	15	15.13	78.68	3.89
	error	446	3.12	16.26	1.76
PC2 (syllable properties)	total	1.91	18.99	100	4.35
	predator	1	13.38	70.48	3.65
	predator/nest	15	2.21	11.67	1.48
	error	446	3.38	17.84	1.84
PC3 (phrase properties and syllable variation)	total	6.75	7.83	100	2.79
	predator	1	2.76	35.26	1.66
	predator/nest	15	1.55	19.79	1.24
	error	446	3.52	44.93	1.87



Supplementary Figure S4. Boxplots of varimax-rotated principal components (PC1, PC2, PC3) for "L" phrases of the Oriental Tit for the comparisons between the live snake and the stuffed Eurasian Jay. Bars on the right side of each graph indicate the degree of overlap presented as either the overlap of the full ranges (from the minimum to the maximum value, excluding outliers) for the two predators (hatched bar) or the overlap of interquartile ranges (from the lower to the upper quartile) for the two predators (filled bar). Note that these differences in boxplots between predator type treatments do not consider the random effect of nest ID included in the statistical model. Supplementary Tables S1 & S2 contain the results of the statistical analysis, and Fig. S6 contains boxplots for the original variables.

Supplementary Table S3. Summary of varimax-rotated principal component analysis (PCA) and statistical analysis comparing (L) syllables between the snake and the cat presentations to Oriental tits. Four principal components were extracted from 13 variables (7 time variables and 6 frequency variables; explanations of the acronyms are in Table S9). The columns of the principal components were arranged from largest to smallest proportion of variance explained by each component (% of variance explained shown in parentheses). Loadings higher than 0.5 are marked with grey background. This table corresponds to Fig. S5, S6, and Table S4.

	Standardized loadings in varimax-rotated principal components			
Acoustic variable	**PC1: Frequency (21%)** (*duration & syllable number*)	**PC2: Phrase (17%)** (*duration & rate*)	**PC3: Syllable (24%)** (*range & variation*)	**PC4: Frequency: pitch (20%)** (pitch)
Time variable				
NS	-0.02	0.91	-0.23	0.08
PD	0.12	0.91	0.29	0.13
ASD	0.15	0.05	0.92	0.05
SDSD	0.44	0.36	0.57	0.09
ACR	-0.09	0.09	-0.90	-0.21
SDCR	0.53	0.52	0.15	0.30
AISI	0.09	0.05	0.83	0.14
Frequency variable				
ACFS	0.38	0.09	0.21	0.88
SDCFS	0.84	0.14	0.01	-0.05
A1QFS	-0.06	0.23	0.06	0.93
A3QFS	0.54	0.01	0.32	0.76
AIRFS	0.77	-0.17	0.37	0.25
SDIRFS	0.72	0.10	0.12	0.39

Component	**Estimate (snake)**	**Standard error**	**p-value**	**Fitted distribution (link function)**
PC1: **Frequency**	6.155	0.114	<0.001	Johnson SU (identity)
PC2: **Phrase**	1.103	0.130	<0.001	Johnson SU (identity)
PC3: **Syllable**	4.094	0.084	<0.001	Johnson SU (identity)
PC4: **Frequency: pitch**	5.724	0.097	<0.001	Johnson SU (identity)

Biological meaning of the principal components:

PC1: This is an index of how wide and how variable is the inter-quartile frequency range of syllables within a phrase, and how variable is the center frequency of syllables within a phrase. Higher values of PC1 indicate (L) syllables of higher inter-quartile frequency range that relatively varies more among syllables within a phrase. Higher PC1 values also indicates (L) syllables of more variable center frequency within a phrase.

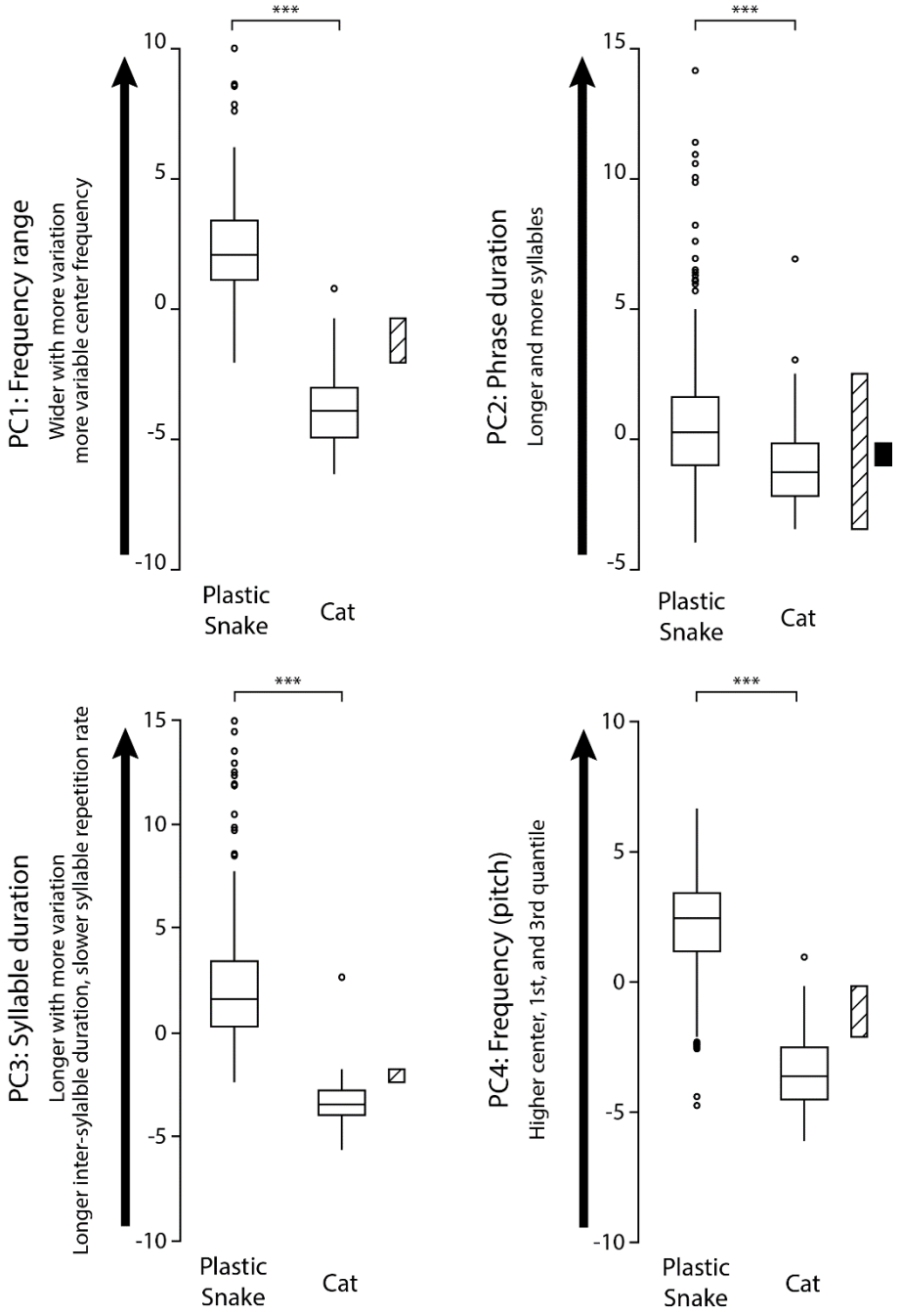
PC2: This is an index of how long is the duration of a phrase, and how many syllables there are in a phrase. Higher values of PC2 indicate longer phrases of (L) syllables that have more syllables in the phrase.

PC3: This is an index of how long and how variable is the duration of syllables within a phrase, and how fast the syllables in a phrase were repeated. It is also an indicator of the inter-syllable duration. Higher values of PC3 indicate (L) syllables of longer duration that relatively varies more among syllables within a phrase. Higher PC3 values also indicates (L) syllables of slower repetition rate in a phrase that relatively has longer inter-syllable duration within a phrase.

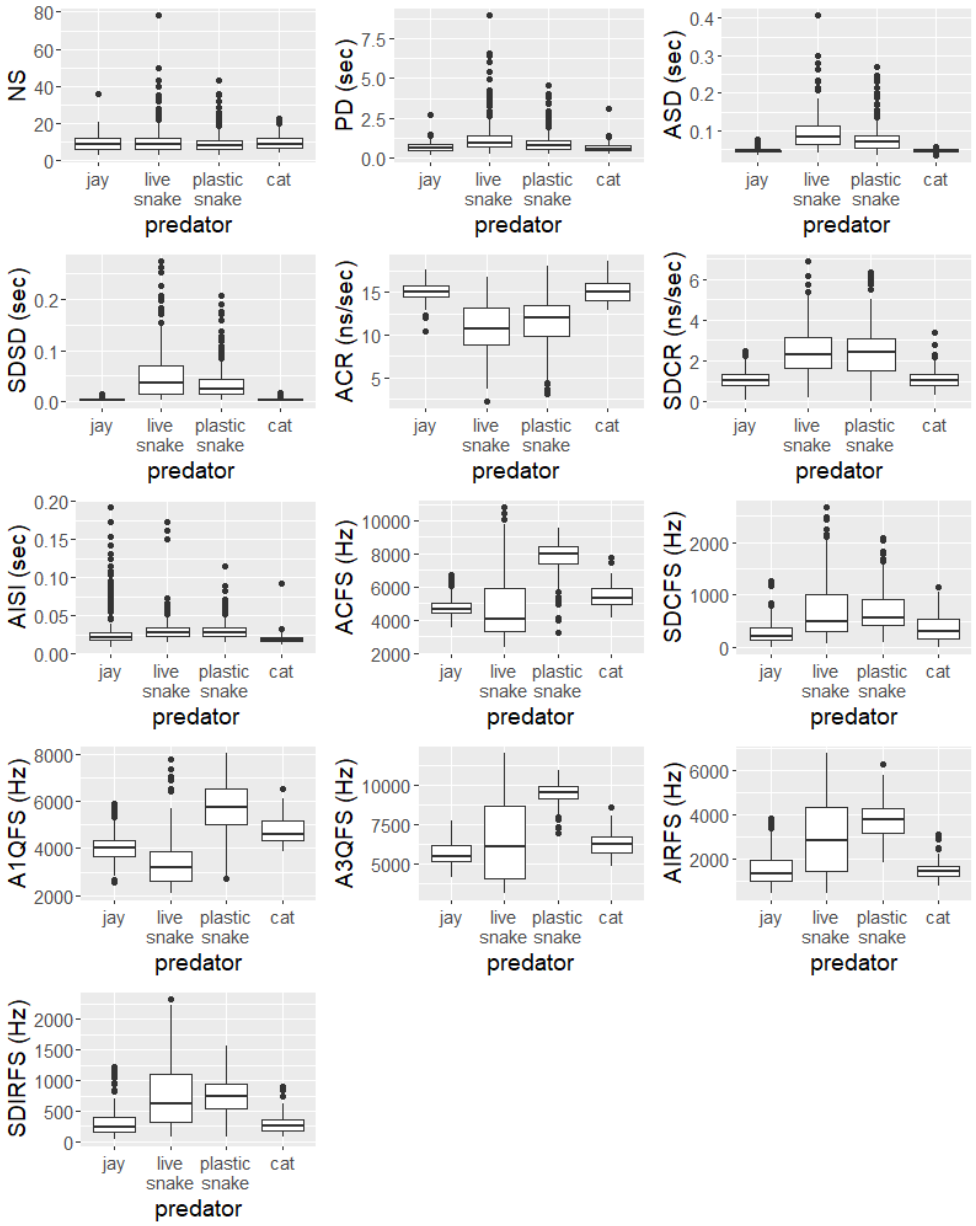
PC4: This is an index of how high is the general frequency (pitch) of a syllable. Higher values of PC4 indicate (L) syllables of higher center, first quartile, and third quartile frequency.

Supplementary Table S4. Comparison between the plastic snake and toy cat presentation experiments. Results of Variance component analysis (conducted using “VCA” function in R package "VCA") for the varimax-rotated principal components extracted from 13 characteristics of "L" phrases (PC1, PC2, PC3, PC4; Table S3). The error can be interpreted as a within-trial variation of the rotated component. This table corresponds to results presented in Fig. S5, S6, and Table S3.

Rotated component (RC)	Variable level	df	Variance component	% to total	SD
PC1 (frequency properties)	total	1.27	21.76	100	4.66
	predator	1	19.18	88.12	4.37
	predator/nest	16	0.53	2.43	0.72
	error	500	2.05	9.43	1.43
PC2 (phrase properties)	total	17.12	6.49	100	2.54
	predator	1	1.34	20.62	1.15
	predator/nest	16	1.11	17.22	1.05
	error	500	4.03	62.14	2.00
PC3 (syllable properties)	total	1.84	20.79	100	4.56
	predator	1	15.14	72.83	3.89
	predator/nest	16	0.99	4.76	0.99
	error	500	4.65	22.39	2.15
PC4 (frequency pitch properties)	total	1.36	18.78	100	4.33
	predator	1	15.85	84.37	3.98
	predator/nest	16	1.36	7.24	1.16
	error	500	1.57	8.38	1.25



Supplementary Figure S5. Boxplots of varimax-rotated principal components (PC1, PC2, PC3, PC4) for "L" phrases of the Oriental Tit for the comparisons between the plastic snake and the furry toy cat. Bars on the right side of each graph indicate the degree of overlap presented as either the overlap of full ranges (from the minimum to the maximum value, excluding outliers) for the two predators (hatched bar) or the overlap of interquartile ranges (from the lower to the upper quartile) for the two predators (filled bar). Note that these boxplots do not consider the random effect of nest ID included in the statistical model. Supplementary Table S3 & S4 contain the results of the statistical analysis, and Fig. S6 contains boxplots for the original variables.



Supplementary Figure S6. Boxplots for acoustic variables describing the syllable type "L" phrases of the Oriental Tit for the four predator treatments: jay, live snake, plastic snake, and furry toy cat. Please see Table S9 for the full names, definitions, and calculation methods of the variables.

Supplementary Table S5. Summary of PCA (varimax-rotated principal components) and statistical model analysis comparing (L) syllables of the Oriental Tit and (f) syllables of the Varied Tit in response to the plastic snake (here we used "LF/churr" syllable to referring both (L) and (f) syllable). Four principal components were extracted from 13 variables (7 time variables and 6 frequency variables; explanations of the acronyms are in Table S9). The columns of the principal components were arranged from largest to smallest proportion of variance explained by each component (% of variance explained shown in parentheses). Loadings higher than 0.5 are marked with grey background. This table corresponds to Fig. S7, S8, and Table S6.

	Standardized loadings in varimax-rotated principal components			
Acoustic variable	**PC1: Frequency: pitch (24%)** (*pitch*)	**PC2: Phrase (18%)** (*duration & syllable number*)	**PC3: Syllable (17%)** (*duration & rate*)	**PC4: Frequency (14%)** (*range & variation*)
Timing variables				
NS	-0.07	0.89	-0.12	-0.13
PD	-0.09	0.92	0.15	-0.10
ASD	0.15	-0.06	0.93	0.07
SDSD	0.31	0.27	0.59	0.38
ACR	0.11	0.16	-0.81	0.25
SDCR	0.31	0.56	-0.01	0.42
AISI	-0.42	0.09	0.53	-0.08
Frequency variables				
ACFS	0.97	0.00	0.00	0.03
SDCFS	-0.15	0.17	-0.08	0.79
A1QFS	0.91	0.16	0.01	-0.28
A3QFS	0.91	-0.10	0.03	0.24
AIRFS	0.10	-0.36	0.03	0.73
SDIRFS	0.09	0.39	-0.06	0.30

Component	**Estimate (VT)**	**Standard error**	**p-value**	**Fitted distribution (link function)**
PC1: **Frequency: pitch**	-3.895	0.096	<0.001	Johnson SU (identity)
PC2: **Phrase**	0.513	0.144	<0.001	Johnson SU (identity)
PC3: **Syllable**	0.334	0.089	<0.001	Johnson SU (identity)
PC4: **Frequency**	-1.776	0.108	<0.001	Johnson SU (identity)

Biological meaning of the principal components:

PC1: This is an index of how high is the general frequency (pitch) of a syllable. Higher values of PC1 indicate "LF/churr" syllables of higher center, first quartile, and third quartile frequency.

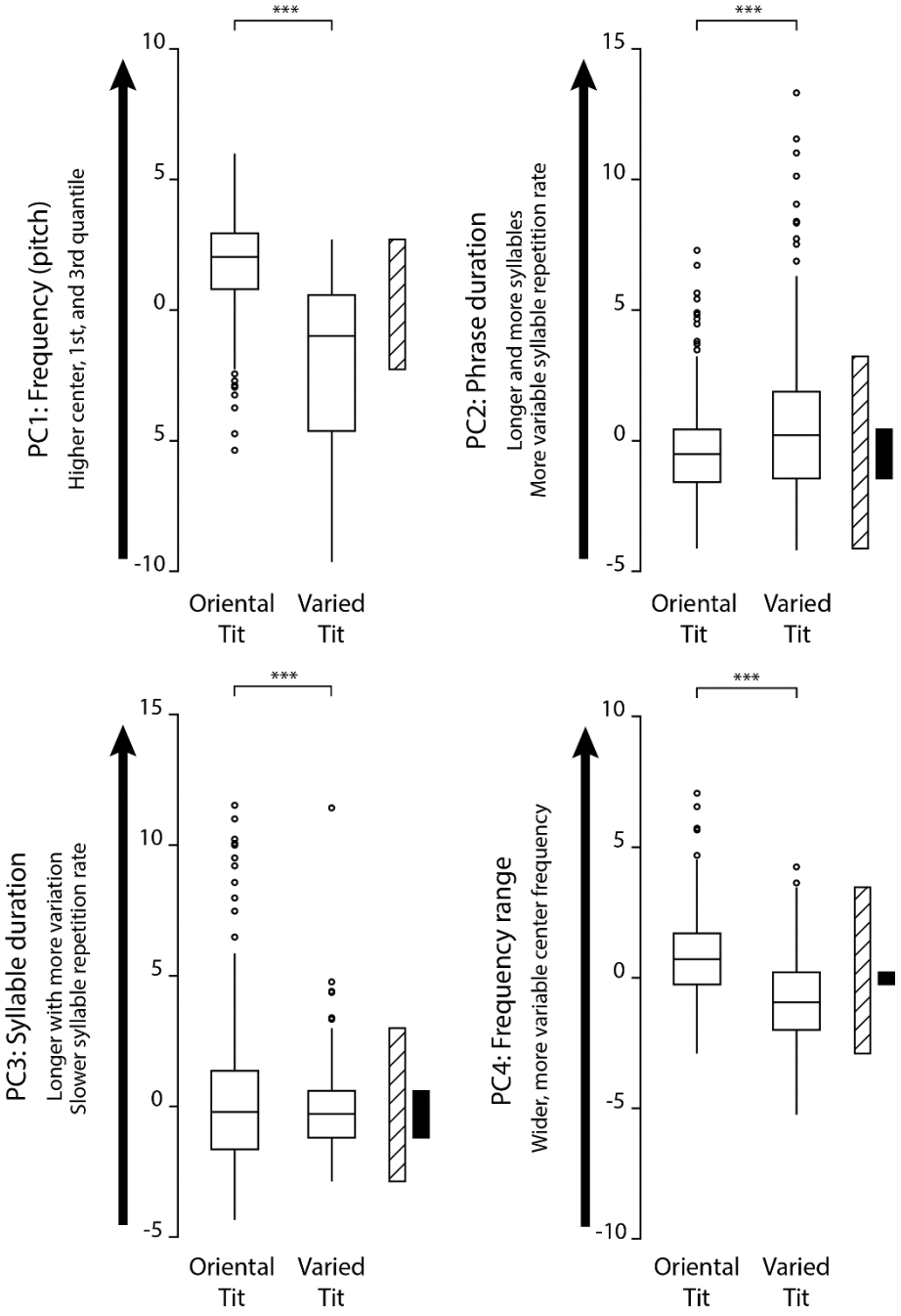
PC2: This is an index of how long is the duration of a phrase, and how many syllables there are in a phrase. Higher values of PC2 indicate longer phrases that have more " LF/churr " syllables. It also indicates higher variation of repetition rate of syllables within a phrase.

PC3: This is an index of how long and how variable is the duration of syllables within a phrase, and how fast the syllables in a phrase were repeated. Higher values of PC3 indicate " LF/churr " syllables of longer duration that relatively varies more among syllables within a phrase. Higher values of PC3 also indicate " LF/churr " syllables of slower repetition rate in a phrase with relatively longer inter-syllable duration.

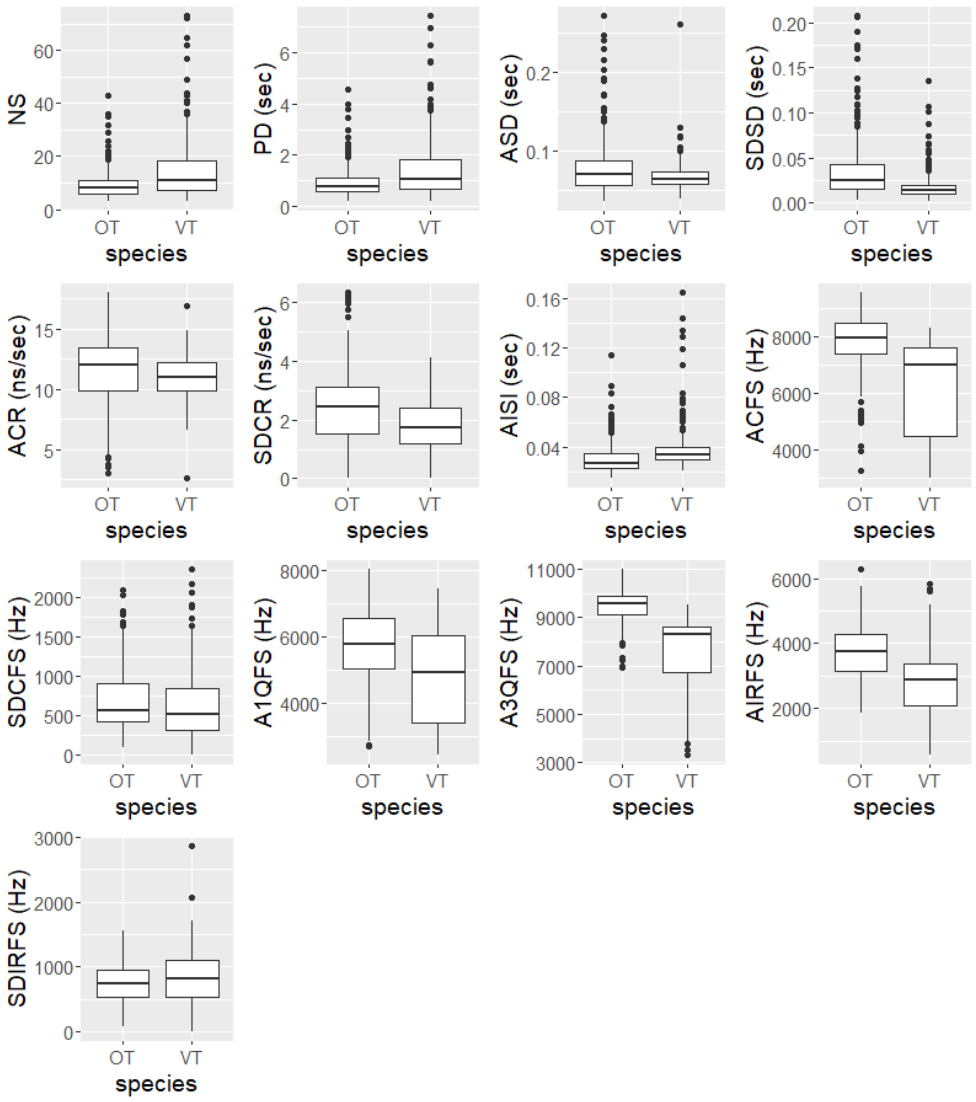
PC4: This is an index of how wide is the inter-quartile frequency range of a syllable, and how variable is the center frequency of syllables in a phrase. Higher values of PC4 indicate " LF/churr " syllables of wider inter-quartile frequency range and more variation of center frequency within a phrase.

Supplementary Table S6. Comparison between (L) syllables of the Oriental Tit and (f) syllables of the Varied Tit in response to the plastic snake. Results of Variance component analysis (conducted using "VCA" function in R package "VCA") for the varimax-rotated principal components extracted from 13 characteristics of "L" phrases of the Oriental Tit and (f) syllables of the Varied Tit (PC1, PC2, PC3, PC4; Table S5). The error can be interpreted as a within-trial variation of rotated component. This table corresponds to results presented in Fig. S7 and Table S5.

Rotated component (RC)	Variable level	df	Variance component	% to total	SD
PC1 (frequency pitch properties)	total	3.54	12.84	100	3.58
	species	1	6.30	49.08	2.51
	species/nest	19	4.05	31.60	2.01
	error	589	2.47	19.30	1.57
PC2 (phrase properties)	total	127.49	5.96	100	2.44
	species	1	0.30	5.15	0.55
	species/nest	19	1.00	16.79	1.00
	error	589	4.65	78.05	2.15
PC3 (syllable properties)	total	254.54	4.95	100	2.22
	species	1	0	0	0
	species/nest	19	1.02	20.63	1.01
	error	589	3.93	79.36	1.98
PC4 (frequency properties)	total	9.25	4.00	100	2.00
	species	1	1.19	29.85	1.09
	species/nest	19	0.88	22.01	0.93
	error	589	1.92	48.13	1.38



Supplementary Figure S7. Boxplots of varimax-rotated components (PC1, PC2, PC3, PC4) for the comparisons between "L" phrases of the Oriental Tit and "f" phrases of the Varied Tit in response to the plastic snake. Bars on the right side of each graph indicate the degree of overlap presented as either the overlap of full ranges (from the minimum to the maximum value, excluding outliers) for the two species (hatched bar) or the overlap of interquartile ranges (from the lower to the upper quartile) for the two species (filled bar). Note that these boxplots do not consider the random effect of nest ID included in the statistical model. Supplementary Table S5 & S6 contain the results of statistical analysis.



Supplementary Figure S8. Boxplots for acoustic variables of "L" phrases of the Oriental Tit (OT) in response to the plastic snake and "f" phrases of the Varied Tit (VT) in response to the plastic snake. Please see Table S9 for the full names, definitions, and calculation methods of the variables.

Supplementary Table S7. Results of playback experiments using two playback treatments toward nestlings of the Oriental Tit and the Varied Tit.

	Nestling Species					
	Oriental Tit			**Varied Tit**		
Playback Species	Fledged	Not Fledged	Total	Fledged	Not Fledged	Total
Oriental Tit	13	2	15	10	0	10
Varied Tit	10	5	15	10	0	10
Total	23	7	30	20	0	20

SUPPLEMENTARY MATERIALS PART 3:

LIST OF SUPPLEMENTARY MEDIA FILES WITH EXPLANATIONS

Supplementary Audio S1. One of the playback samples for the snake alarm calls of the Oriental Tit.

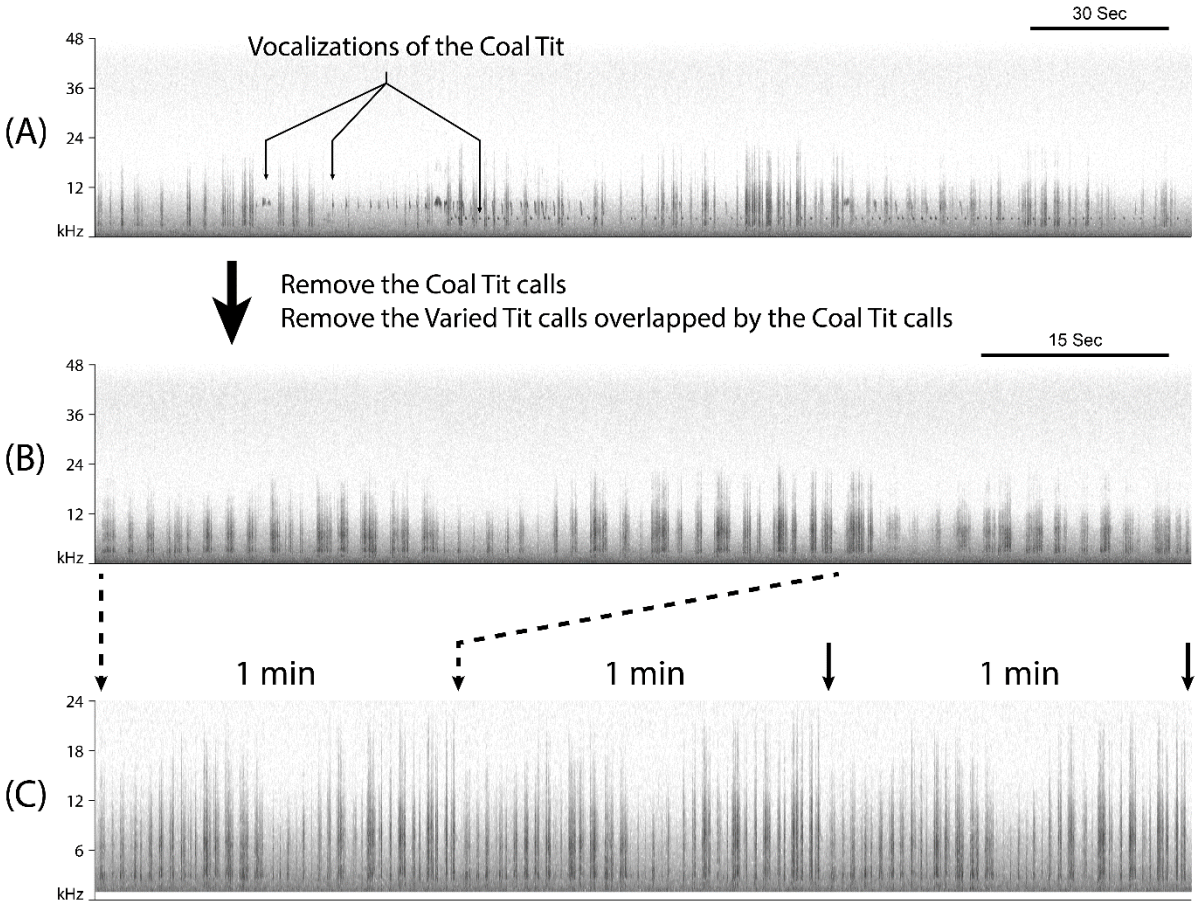
Supplementary Audio S2. One of the playback samples for the snake alarm calls of the Varied Tit.

Supplementary Video V1. Examples of alarm calls of the Oriental Tit and the Varied Tit toward a toy cat.

Supplementary Video V2. Examples of alarm calls of the Oriental Tit and the Varied Tit toward a stuffed jay.

Supplementary Video V3. Examples of alarm calls of the Oriental Tit and the Varied Tit toward a snake.

SUPPLEMENTARY MATERIALS PART 4:
ADDITIONAL INFORMATIONS ABOUT METHODS



Supplementary Figure S9. Examples of sonograms showing a schema of the preparation process of playback samples. (A) The full 4-minutes recording of alarm calls from the Varied Tit responded to the plastic snake model. One Coal Tit was attracted to the alarm calls of the Varied Tit and vocalized their own calls. (B) The same recording after deleting the alarm calls of the Coal Tit and alarm calls of the Varied Tit overlapped by the Coal Tit calls. We also fixed the maximum inter-phrase duration to 1 second based on the timely characteristics of natural vocal responses in the recordings (interquartile range of inter-phrase duration is from 0.4 to 1.7 seconds) to control the intensity of the alarm calls at high level by removing sections with long silences. (C) The final 3-minutes playback sample. The first 1-minute of the (B) was filtered by a 1 kHz high-pass filter after it was converted to a 48 kHz sampling rate. Then the 1-minute section was repeated three times to produce the final 3-minutes playback sample.

Supplementary Table S8. Symbols of syllable types of the Oriental Tit in the current study matched with the syllable names previously used in the literature. The table shows the names of syllable types for the Great Tit and the Oriental Tit previously used in the literature and the corresponding syllable types distinguished in our study (see sonograms of different syllable types in our study presented in the Supplementary Figure S1).

Previous studies	Study species of the research	Expression of syllable types in the literature	Syllable type symbols of the Oriental Tit in the current study
Hinde 1952	Parus major	"pit" "dit" "chat" "twink"	"A" "B" "C" "D" "E" "F" "G"
		"dur"	"K"
		"churr" "chich"	"L"
Hailman 1989	Parus major	"HF-notes"	"A" "B" "C" "D" "E" "F" "G"
		"IF-notes"	"H" "I"
		"LF-notes"	"J" "K"
		"LF-notes" "churr"	"L"
Carlson et al. 2018	Parus major	"tonal"	"A" "B" "C" "D" "E" "F" "G"
		"chirp"	"H"
		"jar/rattle/D"	"L"
Suzuki 2011,2014,2015	Parus minor	"chicka"	"A" "B" "C" "D" "E" "F" "G"
		"jar"	"L"
Ha et al. 2018	Parus minor	"jar"	"L"
Ha et al. 2020	Parus minor	"twink/chicka"	"A" "B" "C" "D" "E" "F" "G"
		"LF/churr/rattle/jar"	"L"

Supplementary Table S9. Abbreviation of acoustic variables used in the statistical analysis and explanation for each of them.

Variable acronyms	Descriptions
Time variables	
NS	Number of syllables in a phrase
PD	Phrase duration (sec)
ASD	Average from all syllable durations within a phrase (sec)
SDSD	Standard deviation of syllable duration in a phrase (sec)
ACR	Average "instantaneous" calling rate (number of syllables/secs in a phrase) calculated as an average from values determined for each syllable in a phrase according to the formula: 1/(preceding inter-syllable interval + syllable duration).
SDCR	Standard deviation of the "instantaneous" calling rate (number of syllables/secs in a phrase)
AISI	Average from all inter-syllable intervals in a phrase (sec)
Frequency variables	
ACFS	Average center frequency of syllables in a phrase (Hz) calculated from all center frequencies determined for each syllable in a phrase.
SDCFS	Standard deviation of syllable's center frequency in a phrase (Hz)
A1QFS	Average 1st quartile frequency of syllables in a phrase (Hz)
A3QFS	Average 3rd quartile frequency of syllables in a phrase (Hz)
AIRFS	Average interquartile range frequency of syllables in a phrase (Hz)
SDIRFS	Standard deviation of interquartile range frequency of syllables in a phrase (Hz)

Supplementary Table S10. Basic data structure for the analyses of detailed acoustic properties of phrases made of Oriental Tits' "L" and Varied Tits' "f" syllables: number of recordings and number of phrases recorded per recording in each of the different predator and species group.

			unit: number of "L" phrases / recording			
Paridae species	**Predator group**	**Number of recordings**	**Mean**	**Standard deviation**	**Minimum**	**Maximum**
Oriental Tit	Live snake	9	26	10.6	7	45
	Plastic snake	10	32.2	12.2	8	49
	Stuffed jay	8	28.6	10.0	10	43
	Toy cat	8	24.5	20.0	1	56
			unit: number of "f" phrases / recording			
Varied Tit	Plastic snake	11	26.1	11.2	5	44
	Stuffed jay	5	0	0	0	0
	Toy cat	7	0	0	0	0