

Network analysis reveals context-dependent structural complexity of social calls in serrate-legged small treefrogs

Ke Deng^a, Qiao-Ling He^{a,b}, Tong-Liang Wang^c, Ji-Chao Wang^c, and Jian-Guo Cui^{a,*}

^aCAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

^bUniversity of Chinese Academy of Science, Beijing 100049, China

^cMinistry of Education Key Laboratory for Ecology of Tropical Islands, Key Laboratory of Tropical Animal and Plant Ecology of Hainan Province, College of Life Sciences, Hainan Normal University, Haikou 571158, China

*Address correspondence to Jian-Guo Cui. E-mail: cuijg@cib.ac.cn.

Handling editor: Zhi-Yun Jia

Abstract

Vocal communication plays an important role in survival, reproduction, and animal social association. Birds and mammals produce complex vocal sequence to convey context-dependent information. Vocalizations are conspicuous features of the behavior of most anuran species (frogs and toads), and males usually alter their calling strategies according to ecological context to improve the attractiveness/competitiveness. However, very few studies have focused on the variation of vocal sequence in anurans. In the present study, we used both conventional method and network analysis to investigate the context-dependent vocal repertoire, vocal sequence, and call network structure in serrate-legged small treefrogs *Kurixalus odontotarsus*. We found that male *K. odontotarsus* modified their vocal sequence by switching to different call types and increasing repertoire size in the presence of a competitive rival. Specifically, compared with before and after the playback of advertisement calls, males emitted fewer advertisement calls, but more aggressive calls, encounter calls, and compound calls during the playback period. Network analysis revealed that the mean degree, mean closeness, and mean betweenness of the call networks significantly decreased during the playback period, which resulted in lower connectivity. In addition, the increased proportion of one-way motifs and average path length also indicated that the connectivity of the call network decreased in competitive context. However, the vocal sequence of *K. odontotarsus* did not display a clear small-world network structure, regardless of context. Our study presents a paradigm to apply network analysis to vocal sequence in anurans and has important implications for understanding the evolution and function of sequence patterns.

Key words: call network, centrality, *Kurixalus odontotarsus*, vocal repertoire, vocal sequence.

Vocal communication has evolved in a broad range of taxa (Gerhardt and Huber 2002; Naguib et al. 2009; Chen and Wiens 2020), and it plays an important role in survival, reproduction, and animal social association. As the acoustic units of conveying information, different note types, call types and phrase types usually have distinct ecological implications (Toledo et al. 2015; Demartsev et al. 2019; Fan et al. 2022). Consequently, animals emit a variety of calls/songs in different contexts and even can use functionally referential calls to indicate specific danger (Casar and Zuberbuhler 2012; Suzuki 2018). In addition to the type, the sequence and combination of the acoustic units may also determine the information conveyed (Arnold and Zuberbuhler 2006; Suzuki et al. 2018). There has been a large body of studies on vocal sequence, however, most of them focus on vocal repertoire and syntactic patterns in primates and songbirds, which have relatively large repertoire sizes (Okanoya 2004; Gentner et al. 2006; Ouattara et al. 2009; Fishbein et al. 2020).

Vocalizations are conspicuous features of the behavior of most frogs and toads (Wells 2007). Males emit advertisement calls to attract females, and they usually alter their call efforts or call types according to ecological context to

improve the attractiveness/competitiveness (Köhler et al. 2017; Ryan et al. 2019). For example, a study with African clawed frogs *Xenopus laevis* showed that receptive calls from females increased the call duration of previously subordinate males (Xu et al. 2012). Similarly, a study with Emei music frogs *Nidirana daunchina* showed that female calls induced males to emit significantly more advertisement calls (Cui et al. 2010). In addition, Byrne and Keogh (2007) reported that male Australian terrestrial toadlets *Pseudophryne bibronii* significantly increased their call rate in response to conspecific odors (both from males and females). Furthermore, male toadlets emitted significantly fewer advertisement calls and more territorial calls in response to male odors (Byrne and Keogh 2007). However, as yet, very few studies have focused on vocal sequence in anurans (Bernal et al. 2009; Bhat et al. 2022).

Serrate-legged small treefrogs *Kurixalus odontotarsus* are suitable species to investigate whether the use and the sequence of acoustic units vary in different contexts. Male *K. odontotarsus* can emit 3 distinct types of notes (henceforth referred to as A notes, B notes, and C notes), and they emit different call types (a category of vocalizations emitted

Received 6 September 2022; accepted 20 December 2022

© The Author(s) 2022. Published by Oxford University Press on behalf of Editorial Office, Current Zoology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

in a particular social context with specific note type) with these 3 note types: 1. advertisement calls, which contain only A notes (Figure 1A); 2. aggressive calls, which contain only B notes (Figure 1B); 3. encounter calls, which contain only C notes (Figure 1C); 4. compound calls, which consist of at least 2 note types and can be further divided into 4 types according to the note types the calls contain (i.e., A and B, B and C, A and C, and A and B and C, Figure 1D–G). Generally, males vocalize on branches or in bushes and emit advertisement calls to attract females (Figure 1H). Females show a preference for longer calls (Deng et al. 2019, 2022). Previous studies also demonstrated that advertisement calls elicit a vocal response and induce rivals to emit more aggressive calls (Zhu et al. 2017b; Deng et al. 2020). In addition, males emit relatively more encounter calls and compound calls in competitive

context (field observations). Consequently, male *K. odontotarsus* can emit a variety of call phenotypes, which consist of notes of specific types and numbers in a particular order.

Traditionally, the sequential order of acoustic units has been illustrated by flow charts, and the transition probability between any 2 acoustic units has been calculated using Markov chain analyses (Bohn et al. 2009; Markowitz et al. 2013; Kershenbaum et al. 2014). Network analysis is a promising alternative to investigating the structure of call/song sequences, which has been used in studies with some mammals and birds. This analytical tool provides specific network metrics which allow researchers to quantify the connection and transition patterns between acoustic units and identify the syntactic features (Humphries and Gurney 2008; Deslandes et al. 2014; Weiss et al. 2014). Furthermore, since network

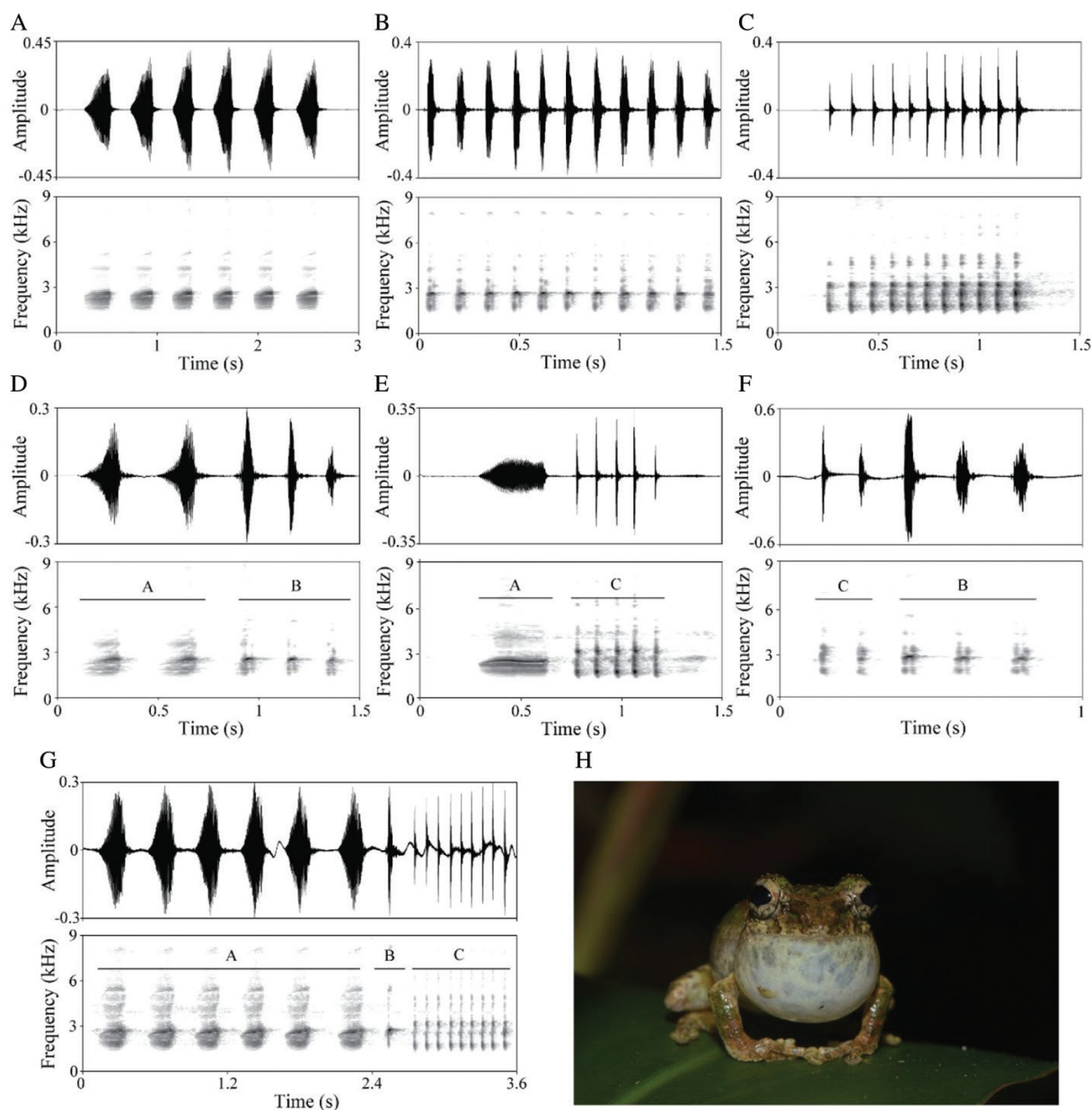


Figure 1 Illustrations of oscillogram (top) and spectrogram (bottom) for 7 call types (a–g) and a calling male *Kurixalus odontotarsus* (h, photo by K.D.). An advertisement call consisting of 6 A notes (A), an aggressive call consisting of 11 B notes (B), an encounter call consisting of 11 C notes (C), a compound call consisting of 2 A notes plus 3 B notes (D), a compound call consisting of 1 A note plus 5 C notes (E), a compound call consisting of 2 C notes plus 3 B notes (F), and a compound call consisting of 6 A notes, 1 B note plus 9 C notes (G).

analysis is based on graph theory, it allows researchers to visualize and evaluate the overall structure of the network (Sasahara et al. 2012; Cody et al. 2015; Allen et al. 2019).

Using the dataset from Deng et al. (2020), we used both conventional method and a network-based approach to examine the variation of vocal sequences of male *K. odontotarsus* in different contexts. We hypothesized that the presence of a competitive rival should influence the repertoire size, the sequence patterns produced, and consequently the call network structure. Since males increase their call rate and emit relatively more compound calls in competitive context, we predicted that the number of call phenotypes would increase in the presence of a rival's advertisement calls. We also predicted that specific call phenotypes might often co-occur in vocal sequence, and thus the connectivity at the network level would decrease in competitive context.

Materials and Methods

Data collection

All data of the present study were derived from the previous study (Deng et al. 2020), which was conducted from May to August 2019 at Diaoluo Mountain National Nature Reserve in Hainan, China (18.72°N, 109.87°E, elevation 933 m). Experiments were conducted in the field, which were far enough from the chorus to prevent the tested males from directly interacting with other males. Tested males were placed in enclosures constructed of wire mesh (42 × 32 × 90 cm), which were open to ambient air and sound. Soil and plants were provided, and male frogs could locomote freely in enclosures. We recorded males for 3 min before initiating a playback (i.e., spontaneous period), 3 min during the playback, and 3 min after the playback using a digital voice recorder (Sony PCM-D100). The acoustic stimuli were presented using Adobe Audition (version 3.0) and broadcasted using a speaker (amplified field speaker, Saul Mineroff Electronics, Inc.), which was 1 m away from the enclosure. We used advertisement calls with 5 notes (i.e., 5A) as the stimuli, which represented a competitive rival (Zhu et al. 2017b; Deng et al. 2019). Advertisement calls were broadcasted with 5-s interstimulus intervals (Zhu et al. 2017b), and the amplitude of the stimuli was 80 dB SPL (re 20 μPa), measuring at the central area of the enclosure using a sound pressure level meter (AWA 6291, Hangzhou Aihua Instruments Co., China). The experimental procedures are described in detail by Deng et al. (2020).

The number of calls, the number of each call type, the number of each call phenotype, and the order of call phenotypes during each period (before, during, and after the playback of advertisement calls with 5 notes) were recorded in the present study. In total, 58 male *K. odontotarsus* were tested, 8 of them did not emit any call after the playback period.

Network construction and visualization

All analyses were performed using the software R 4.1.0 (<http://cran.r-project.org>). According to the order of call phenotypes in the vocal sequence, call networks before, during and after the playback period were constructed using the time-ordered package (Blonder and Dornhaus 2011). A connection was defined as any 2 call phenotypes occurring next to each other in the vocal sequence (undirected), and a transition was defined as one call phenotype followed by another call phenotype (directed). For the depiction of vocal

sequences in networks, the visualizations were constructed using the Fruchterman–Reingold algorithm in iGraph package (Fruchterman and Reingold 1991; Csardi and Nepusz 2006), which essentially pulls nodes that are highly connected closer together. Nodes represent call phenotypes in the visualized call networks, and edges represent the connections between call phenotypes. Thickness of the edges represents the frequency of connections and the arrows represent transitions between call phenotypes.

Network metrics

All network metrics were calculated using iGraph package (Csardi and Nepusz 2006). Three centrality metrics were calculated for each call phenotype in each network, and the mean values of these metrics were used to evaluate group-level changes in network structure. Degree: describes the sum of all connections to the focal call phenotype. Closeness: describes how well connected a call phenotype is to all others in the network. Betweenness: describes how important a call phenotype is for connections and stability of the call network. The removal of the high-betweenness call phenotype will likely fragment network connectivity. These 3 metrics were calculated based on directed networks, and were normalized to facilitate comparisons across networks of different sizes (Maldonado-Chaparro et al. 2015). The normalized degree was the raw degree divided by $n - 1$, and normalization for closeness was performed by multiplying the raw closeness by $n - 1$ (the maximum number of possible connections), where n was the number of nodes in the network (Freeman 1979). The normalized betweenness was calculated from the following formula: $B_{\text{normalized}} = \frac{2B}{n^2 - 3n + 2}$, where B is the raw betweenness and n is the number of nodes in the network (Freeman 1979).

To investigate the transition motifs (patterns) of call phenotypes, in-degree and out-degree were calculated for each call phenotype in each network. In-degree was based on the number of different call phenotypes that immediately preceded focal call phenotype, and out-degree was based on the number of different call phenotypes that immediately followed focal call phenotype. The proportion of both degrees was used to estimate the transition motifs based on the definitions in Sasahara et al. (2012) 1) One-way: for a given call phenotype, a less than an average number of call phenotypes both precedes and follows; 2) Bottleneck: for a given call phenotype, a greater than an average number of call phenotypes precedes and a less than an average number of call phenotypes follows; 3) Hourglass: for a given call phenotype, a greater than an average number of call phenotypes both precede and follow; 4) Branch: for a given call phenotype, a less than an average number of call phenotypes precedes and a greater than an average number of call phenotypes follows. Deterministic motifs (one-way and bottleneck) have fewer than average call phenotypes following any specific call phenotype. Non-deterministic motifs (hourglass and branching) have greater than average call phenotypes following any specific call phenotype (Sasahara et al. 2012).

To describe the call network structure before, during, and after the playback period, 2 network-level metrics were calculated based on undirected networks. Average path length (L): the average of all path lengths between all pairs of nodes in the network (Wey et al. 2008). The larger L is, the more steps are required for any call phenotype to reach another, and vice versa. Clustering coefficient (C): describes how densely the

network is clustered around the focal node (Wey et al. 2008). It represents the overall tendency of different call phenotypes to form groups that are highly likely to co-occur in a vocal sequence. Furthermore, we used average path length and clustering coefficient in combination to calculate the small-world coefficient (S): $S = (C/C_{\text{rand}})/(L/L_{\text{rand}})$ (Humphries and Gurney 2008). C and L were calculated on observed networks, and C_{rand} and L_{rand} were calculated on randomly permuted Erdős-Renyi networks with the same number of nodes and edges. We performed 1,000 permutations and then calculated small-world coefficient using average C_{rand} and L_{rand} . Small-world networks were characterized by a small-world coefficient greater than 1 (Humphries and Gurney 2008) and clusters of call phenotypes above a certain degree of modularity (Q) (Newman 2006). Consequently, we used the Girvan-Newman algorithm (Girvan and Newman 2002) to define network communities (NC) in a network and find the most fitted number of communities (Newman and Girvan 2004). Generally, the larger the value of Q , the more accurate the partition is into communities (Newman and Girvan 2004).

Statistical analysis

We evaluated the variations in call rate and the number of call phenotypes among periods using a Friedman test followed by pairwise comparisons (Wilcoxon signed rank test), because not all data indicate a normal distribution (Shapiro-Wilk test: $P < 0.05$). To examine group-level changes in network structure between the playback periods, we conducted a linear mixed model with each network metric (degree, closeness, and betweenness centrality) as the dependent variable. The playback period (before, during, and after) was used as the independent variable. We included individual ID as a random effect. We used Spearman's rank correlation test to examine whether centrality of call phenotypes during the playback period correlates with their frequency of occurrence during that period. $P < 0.05$ was considered statistically significant.

Results

The Friedman test showed that call rate of tested male *K. odontotarsus* varied significantly across 3 periods ($\chi^2 = 22.59$, $df = 2$, $P < 0.001$, $N = 50$, Figure 2A). Specifically, males emitted significantly more calls during the playback period than before (Wilcoxon signed rank test: $V = 166$, $P < 0.001$) and after (Wilcoxon signed rank test: $V = 1098$, $P < 0.001$) the

playback period (Figure 2A). Similarly, there were significant differences in the number of call phenotypes among periods (Friedman test: $\chi^2 = 74.72$, $df = 2$, $P < 0.001$, $N = 50$, Figure 2B). Specifically, the number of call phenotypes during the playback period was significantly greater than that before (Wilcoxon signed rank test: $V = 16$, $P < 0.001$) and after (Wilcoxon signed rank test: $V = 1271$, $P < 0.001$) the playback period (Figure 2B). In addition, males emitted relatively more calls containing only A notes before and after the playback period (Figure 2C). During the playback period, the proportion of calls containing only B notes or C notes, and the proportion of calls consisting of different note types increased (Figure 2C).

Figure 3 gives a visualized presentation of vocal sequences. Compared with before and after the playback period, more multi-note calls (Figure 3A) and more compound calls (Figure 3B,C) occurred during the playback period. Calls containing only B notes occupied more central positions in the call networks than other call types, and they had a relatively higher degree (represent as bigger size in the graphs, Figure 3). Linear mixed model revealed that the average degree ($t = 4.500$, $P < 0.001$), closeness ($t = 60.996$, $P < 0.001$), and betweenness ($t = 2.703$, $P = 0.008$) varied significantly across 3 periods (Figure 4). Call phenotypes had significant higher centralities either before (degree: $t = 5.204$, $P < 0.001$; closeness: $t = 2.714$, $P = 0.010$; betweenness: $t = 2.668$, $P = 0.011$) or after (degree: $t = 2.345$, $P = 0.024$; closeness: $t = 4.316$, $P < 0.001$; betweenness: $t = 2.061$, $P = 0.046$) than during the playback period (Table 1, Figure 4). The degree ($S = 4043.9$, $P < 0.001$), closeness ($S = 54394$, $P < 0.001$), and betweenness ($S = 33696$, $P < 0.001$) of call phenotypes during the playback period significantly correlated with their frequency of occurrence during that period (Spearman's rank correlation test).

Deterministic motifs were more common than non-deterministic motifs for each period (average of deterministic motifs = 67.3%, Table 2), most of which were one-way motifs (Table 2). Hourglass motifs were the second most common motif (average = 29.3%), while bottleneck motifs and branch motifs were far less common for each period (less than 10%, Table 2). Compared with before and after the playback period, the proportion of one-way motifs increased, and the proportion of hourglass motifs decreased during the playback period (Table 2).

Compared with before and after the playback period, the average path length increased, and the clustering coefficient decreased during the playback period (Table 3). Therefore,

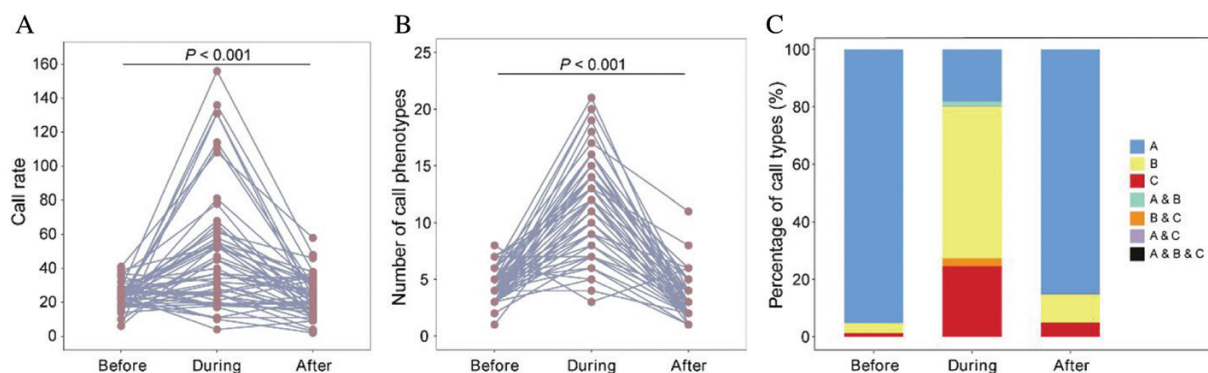


Figure 2 The variations in (A) call rate ($N = 50$), (B) the number of emitted call phenotypes ($N = 50$), and (C) percentage of call types ($N = 58$ for before and during, and $N = 50$ for after the playback period) among periods.

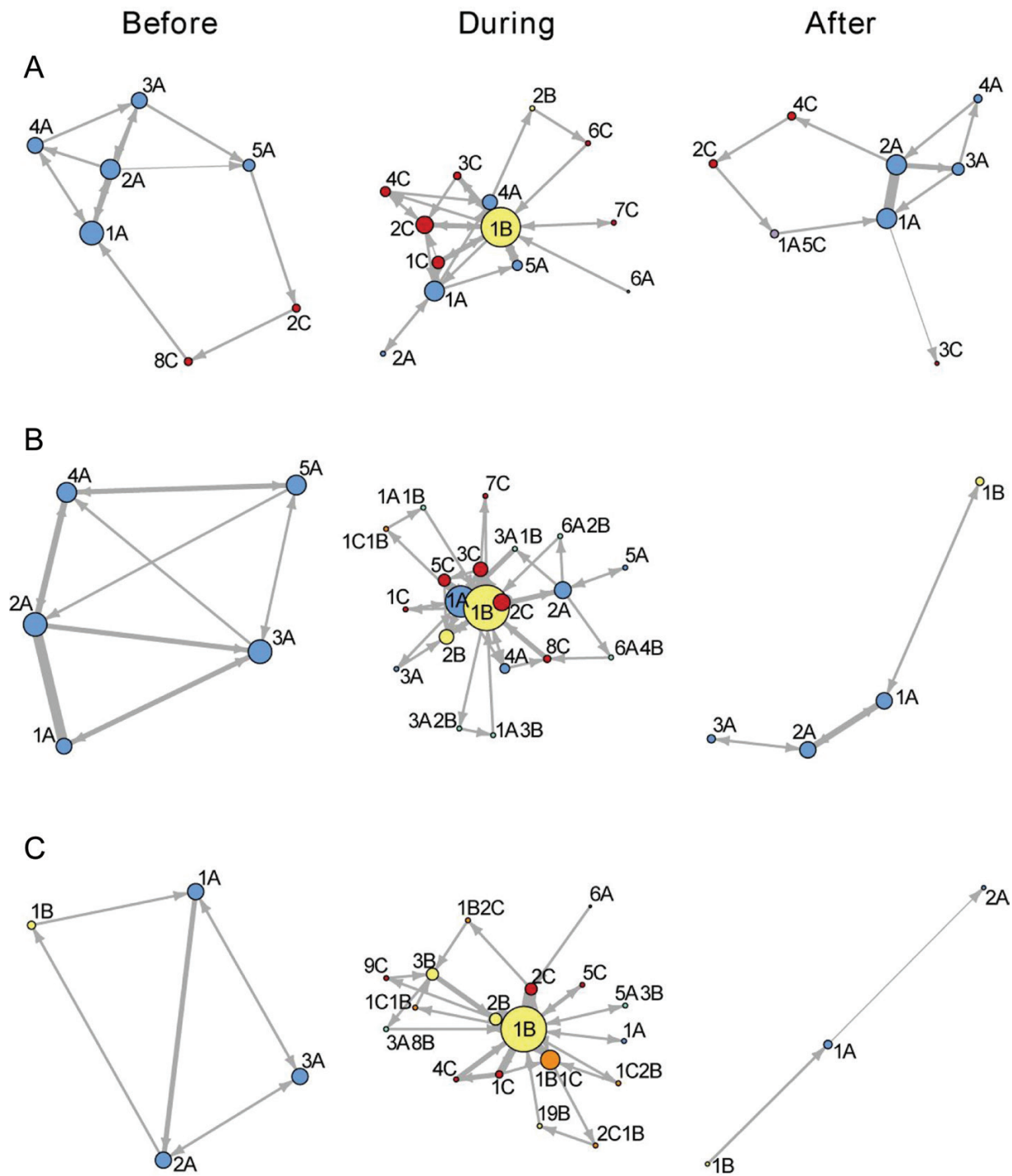


Figure 3 Call networks of 3 male *Kurixalus odontotarsus* before, during, and after the playback of advertisement calls (A–C). Nodes represent call phenotypes, and size of nodes is proportional to degree centrality. Colors represent call types: calls containing only A notes (blue), calls containing only B notes (yellow), calls containing only C notes (red), calls consisting of A and B notes (green), calls consisting of A and C notes (purple), and calls consisting of B and C notes (orange). Thickness of the edges represents the frequency of connections and the arrows represent transitions between call phenotypes.

the small-world coefficient during the playback period was the highest ($C = 2.90$, Table 3), indicating that call phenotypes within vocal sequences clustered into highly connected groups with short distances between them. Accordingly, the call network during the playback period had 6 network communities (Table 3). Nevertheless, the Q value was too small for each period (≤ 0.18 , Table 3),

suggesting that neither of them had a clear small-world network structure (Figure 5).

Discussion

We used both conventional methods and network analysis to investigate the vocal sequences of male *K. odontotarsus*.

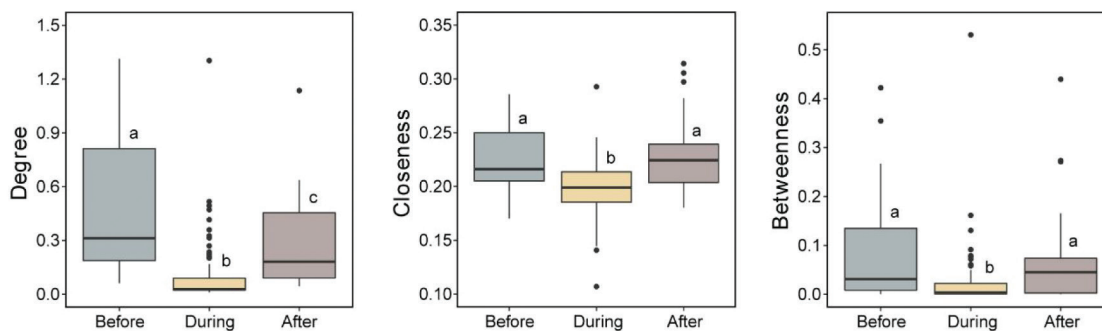


Figure 4 Comparison of degree, closeness, and betweenness centrality among periods ($N = 58$ for before and during, and $N = 50$ for after the playback of advertisement calls). Different superscript letters indicate significant differences ($P < 0.05$) as determined by linear mixed model.

Table 1 Linear mixed model testing the variation of each network metric across different periods ($N = 58$ for before and during, and $N = 50$ for after the playback of advertisement calls). Factor reference category is “period | during the playback period”

Network metric	Factor	Coefficient	SE	t	P
Degree	Period before	0.260	0.050	5.204	<0.001
	Period after	0.104	0.044	2.345	0.024
Closeness	Period before	0.020	0.007	2.714	0.010
	Period after	0.029	0.006	4.316	<0.001
Betweenness	Period before	0.051	0.019	2.668	0.011
	Period after	0.035	0.017	2.061	0.046

Table 2 Total number and percentage of transition motifs for each period ($N = 58$ for before and during, and $N = 50$ for after the playback of advertisement calls)

Period	Deterministic		Non-deterministic	
	One-way	Bottleneck	Hourglass	Branch
Before	10 (59%)	1 (6%)	6 (35%)	0 (–)
During	67 (75%)	1 (1%)	21 (23%)	1 (1%)
After	13 (57%)	1 (4%)	7 (30%)	2 (9%)

Table 3 The average path length (L), clustering coefficient (C), the small-world coefficient (S), number of network communities (NC), and modularity (Q) for each period ($N = 58$ for before and during, and $N = 50$ for after the playback of advertisement calls)

Period	L	C	S	NC	Q
Before	1.75	0.53	0.96	3	0.05
During	2.14	0.29	2.90	6	0.15
After	2.00	0.36	1.13	3	0.18

Consistent with previous study (Zhu et al. 2017b), we found that males significantly increased their call rate when presented with rival’s advertisement calls. Males also changed the call types they used. Specifically, males emitted relatively fewer advertisement calls, but more aggressive calls and encountered calls in competitive context, which demonstrates an agonistic function for sequences consisting of B notes and C notes. Similar result has been found in a study of Amboli bush frogs *Pseudophilautus amboli*, which reported that males mainly

emitted certain types of notes either vocalizing alone or with a neighbor, whereas they switched to a different group of notes in a territorial dispute (Bhat et al. 2022). We also found that male *K. odontotarsus* emitted significantly more compound calls during the playback period (as shown in Figures 3 and 5), which resulted in a larger repertoire size. This result suggests that males also modify their vocal sequences in the presence of a competitive rival by incorporating various combinations of different note types. Many anuran species emit calls consisting of several notes of different types (Nali and Prado 2014; Narins and Meenderink 2014; Furtado et al. 2016). Generally, males tend to emit notes of a certain type when they call alone but add a series of other types of notes during the vocal competition (Bevier et al. 2004; Reichert 2009; Bhat et al. 2022), resulting in a more elaborate vocal sequence.

Network analysis revealed that the mean degree, mean closeness, and mean betweenness of the call networks significantly decreased during the playback period, suggesting that both direct and indirect connections among call phenotypes at group level were lowest in competitive context. In addition, there were significant correlation between centralities of call phenotypes and their frequency of occurrence during the playback period. Therefore, the changes in network structure might result from an increased difference in the frequency of occurrence of call phenotypes. That is, some certain call phenotypes were repeated frequently in the vocal sequence, whereas others occurred only a few times. Male frogs generally enhance their competitiveness by emitting calls containing more notes (Bernal et al. 2009; Zhu et al. 2017a). However, long calls and compound calls also carry costs such as increasing energetic costs or risks of predation and parasitism (Ryan et al. 1982; Bernal et al. 2006; Wells 2007), so frogs do not produce these calls at all times. For example, although females are preferentially attracted to whines with chucks over whines alone, and males also increase their call rate in response to calls containing chucks (Ryan et al. 2019), nearly 70% of the calls emitted by male túngara frogs *Physalaemus pustulosus* in the chorus are simple whine-only calls (Bernal et al. 2007). According to the definition, nodes that have more connections with others (more active or more co-occurrence) usually have higher centralities (occupy more central positions in the network). In the present study, 1B (aggressive calls with one note) had the highest degree centrality during the playback period (as shown in Figure 3), probably resulting from its high frequency of occurrence in vocal sequence. This call type (1B) is effective at suppressing rivals (Zhu et al. 2017b) and has low production costs, making this an effective strategy for dealing with rivals.

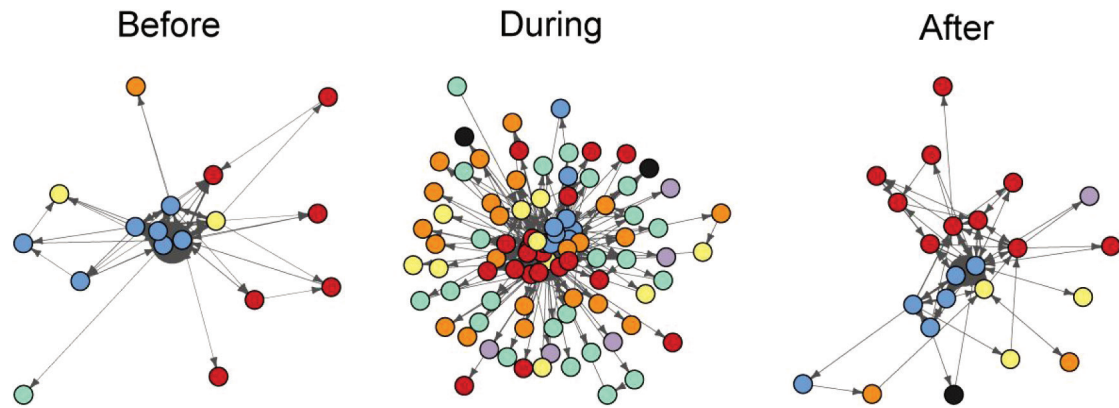


Figure 5 Call networks of male *Kurixalus odontotarsus* before ($N = 58$), during ($N = 58$), and after ($N = 50$) the playback of advertisement calls. Nodes represent call phenotypes, and colors represent call types: calls containing only A notes (blue), calls containing only B notes (yellow), calls containing only C notes (red), calls consisting of A and B notes (green), calls consisting of A and C notes (purple), calls consisting of B and C notes (orange), and calls consisting of A, B, and C notes (black). Thickness of the edges represents the frequency of connections and the arrows represent transitions between call phenotypes.

On the other hand, the changes in network structure revealed that the difference in the number of connections of call phenotypes increased during the playback period. Although a variety of compound calls occurred in the presence of advertisement calls, most of which were typically found on the network's periphery because they connected with few call phenotypes (as shown in Figure 5). Only a few simple calls (consisting of one note type) of high connection occupied the key positions, which acted as hubs in the call networks. Because compound calls consist of several notes of different types, they may convey separate messages simultaneously (Nali and Prado 2014). For example, male *Eleutherodactylus coqui* emit a two-note “co-qui” call, where the “co” is directed towards males whereas the “qui” is directed towards females (Narins and Capranica 1978). In *K. odontotarsus*, A notes are necessary and sufficient to attract females, and B notes can suppress rival's vocalization (Zhu et al. 2017b; Deng et al. 2019). However, compound calls consisting of A and B notes do not have both functions, but only functions similar to advertisement calls (consisting of only A notes, Zhu et al. 2017b; Deng et al. 2020). The functions of these compound calls are still not well understood, especially calls consisting of B and C notes, which need a further investigation.

The increased proportion of one-way motifs and average path length also indicates that the connectivity of the call network decreased in competitive context. However, the vocal sequence of *K. odontotarsus* did not display a clear small-world network structure. Small-world structure has been found in human language, bird song, and humpback whale song (Cancho and Solé 2001; Cody et al. 2015; Allen et al. 2019), where certain acoustic units shape closely-knit subgraphs with high rates of internal transitions. One possible reason for the lack of small-world structure is that male frogs flexibly modify their calls, any number of identical notes or different types of notes might be added or subtracted (Chuang et al. 2016; Furtado et al. 2016; Liu et al. 2018), which might generate variable vocal sequences. Bernal et al. (2009) reported a gradual increase in call complexity in túngara frogs, and males never transitioned from 3 to 1 or 0 chunks in either direction. However, certain acoustic units occur periodically as relatively cohesive groups in vocal sequence have not been identified in anurans.

In summary, our results demonstrate that male *K. odontotarsus* modify their vocal sequence and call network structure according to context. Anuran vocalizations consist of various acoustic units, such as different note types, different call types, and various combinations of different note types and call types (Toledo et al. 2015; Köhler et al. 2017). Studies that focus on the structure and the function of vocal sequences in anurans are invaluable for understanding the origin and evolution of syntactical rules.

Acknowledgments

We thank Rong-Ping Bu, Chen-Xu Wang, and Xing-Yu Tao for assistance with animal collection. We also thank Bi-Cheng Zhu for providing acoustic stimuli.

Funding

This work was supported by the National Natural Science Foundation of China (31772464, 32000313), Youth Innovation Promotion Association CAS (2012274), Sichuan Science and Technology Program (2022JDTD0026), Natural Science Foundation of Sichuan Province (2022NSFSC1736), Open Research Program in Ministry of Education Key Laboratory for Ecology of Tropical Islands (HNSF-OP-202002).

Conflict of Interest Statement

The authors declare no conflict of interest.

Authors' Contributions

K.D. and J.G.C. conceived and designed the study. K.D., T.L.W., and J.C.W. arranged the technical equipment. K.D. and Q.L.H. performed the experiments. K.D. analyzed the data and drafted the manuscript. All authors revised the paper critically and gave final approval for publication.

Data Accessibility Statement

The raw data are available as supplementary material.

References

- Allen JA, Garland EC, Dunlop RA, Noad MJ, 2019. Network analysis reveals underlying syntactic features in a vocally learnt mammalian display, humpback whale song. *Proc Biol Sci B* 286:20192014.
- Arnold K, Zuberbuhler K, 2006. Language evolution: semantic combinations in primate calls. *Nature* 441:303.
- Bernal XE, Akre KL, Baugh AT, Rand AS, Ryan MJ, 2009. Female and male behavioral response to advertisement calls of graded complexity in túngara frogs *Physalaemus pustulosus*. *Behav Ecol Sociobiol* 63:1269–1279.
- Bernal XE, Page RA, Rand AS, Ryan MJ, 2007. Cues for eavesdroppers: Do frog calls indicate prey density and quality? *Am Nat* 169:409–415.
- Bernal XE, Rand AS, Ryan MJ, 2006. Acoustic preferences and localization performance of blood-sucking flies (*Corethrella* Coquillett) to túngara frog calls. *Behav Ecol* 17:709–715.
- Bevier CR, Larson K, Reilly K, Tat S, 2004. Vocal repertoire and calling activity of the mink frog *Rana septentrionalis*. *Amphib-Reptilia* 25:255–264.
- Bhat AS, Sane VA, Seshadri KS, Krishnan A, 2022. Behavioural context shapes vocal sequences in two anuran species with different repertoire sizes. *Anim Behav* 184:111–129.
- Blonder B, Dornhaus A, 2011. Time-ordered networks reveal limitations to information flow in ant colonies. *PLoS ONE* 6:e20298.
- Bohn KM, Schmidt-French B, Schwartz C, Smotherman M, Pollak GD, 2009. Versatility and stereotypy of free-tailed bat songs. *PLoS ONE* 4:e6746.
- Byrne PG, Keogh JS, 2007. Terrestrial toadlets use chemosignals to recognize conspecifics, locate mates and strategically adjust calling behaviour. *Anim Behav* 74:1155–1162.
- Cancho RFI, Solé RV, 2001. The small world of human language. *Proc Biol Sci B* 268:2261–2265.
- Casar C, Zuberbuhler K, 2012. Referential alarm calling behaviour in New World primates. *Curr Zool* 58:680–697.
- Chen Z, Wiens JJ, 2020. The origins of acoustic communication in vertebrates. *Nat Commun* 11:369.
- Chuang MF, Kam YC, Bee MA, 2016. Quantitative description of the vocal repertoire of the territorial olive frog *Babina adenopleura* from Taiwan. *Bioacoustics* 25:1–18.
- Cody ML, Stabler E, Sánchez Castellanos HM, Taylor CE, 2015. Structure, syntax and “small-world” organization in the complex songs of California thrashers *Toxostoma redivivum*. *Bioacoustics* 25:41–54.
- Csardi G, Nepusz T, 2006. The IGRAPH software package for complex network research. *Interj Complex Syst* 1695:1–9.
- Cui JG, Wang YS, Brauth S, Tang YZ, 2010. A novel female call incites male-female interaction and male-male competition in the Emei music frog *Babina daunchina*. *Anim Behav* 80:181–187.
- Demartsev V, Gordon N, Barocas A, Bar-Ziv E, Ilany T et al., 2019. The “Law of Brevity” in animal communication: sex-specific signaling optimization is determined by call amplitude rather than duration. *Evol Lett* 3:623–634.
- Deng K, He QL, Zhou Y, Zhu BC, Wang TL et al., 2020. Male serrate-legged treefrogs adjust competition strategies according to visual or chemical cues from females. *J Exp Biol* 223:jeb229245.
- Deng K, Zhou Y, Zhang HD, He QL, Zhu BC et al., 2022. Conspecific disturbance odors act as alarm cues to affect female mate choice in a treefrog. *Behav Ecol Sociobiol* 76:58.
- Deng K, Zhu BC, Zhou Y, Chen QH, Wang TL et al., 2019. Mate choice decisions of female serrate-legged small treefrogs are affected by ambient light under natural, but not enhanced artificial nocturnal light conditions. *Behav Process* 169:103997.
- Deslandes V, Faria LR, Borges ME, Pie MR, 2014. The structure of an avian syllable syntax network. *Behav Process* 106:53–59.
- Fan PL, Li JX, Yang LT, Sun T, Wu SJ et al., 2022. Vocal repertoire of the critically endangered white-headed langur *Trachypithecus leucocephalus*: Call types, acoustic structures, and related social-ecological contexts. *Zool Res* 43:875–879.
- Fishbein AR, Idsardi WJ, Ball GF, Dooling RJ, 2020. Sound sequences in birdsong: how much do birds really care? *Philos Trans R Soc Lond B* 375:20190044.
- Freeman LC, 1979. Centrality in social networks I: Conceptual clarification. *Soc Netw* 1:215–239.
- Fruchterman TMJ, Reingold EM, 1991. Graph drawing by force-directed placement. *Softw Pract Exp* 21:1129–1164.
- Furtado R, Santos SP, Dias TM, Bastos RP, Nomura F, 2016. Vocal repertoire during reproductive and aggressive contexts of three Brazilian tree frogs: *Bokermannohyla sapiranga*, *Hypsiboas albopunctatus* and *H. goianus* (Anura: Hylidae). *S Am J Herpetol* 11:136–147.
- Gentner TQ, Fenn KM, Margoliash D, Nusbaum HC, 2006. Recursive syntactic pattern learning by songbirds. *Nature* 440:1204–1207.
- Gerhardt HC, Huber F, 2002. *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: University of Chicago Press.
- Girvan M, Newman MEJ, 2002. Community structure in social and biological networks. *Proc Natl Acad Sci USA* 99:7821–7826.
- Humphries MD, Gurney K, 2008. Network “small-world-ness”: A quantitative method for determining canonical network equivalence. *PLoS ONE* 3:e0002051.
- Kershenbaum A, Bowles AE, Freeberg TM, Jin DZ, Lameira AR et al., 2014. Animal vocal sequences: not the Markov chains we thought they were. *Proc Biol Sci B* 281:20141370.
- Köhler J, Jansen M, Rodriguez A, Kok PJR, Toledo LF et al., 2017. The use of bioacoustics in anuran taxonomy: Theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251:1–124.
- Liu QC, Wang TL, Zhai XF, Wang JC, 2018. Call characteristics of two sympatric and morphologically similar tree frogs species, *Polypedates megacephalus* and *Polypedates mutus* (Anura: Rhacophoridae), from Hainan, China. *Asian Herpetol Res* 9:240–249.
- Maldonado-Chaparro AA, Hubbard L, Blumstein DT, 2015. Group size affects social relationships in yellow-bellied marmots *Marmota flaviventris*. *Behav Ecol* 26:909–915.
- Markowitz JE, Ivie E, Kligler L, Gardner TJ, 2013. Long-range order in canary song. *PLoS Comput Biol* 9:e1003052.
- Naguib M, Janik VM, Clayton NS, Zuberbuhler K, 2009. *Vocal Communication in Birds and Mammals*. Amsterdam: Elsevier.
- Nali RC, Prado CPA, 2014. Complex call with different messages in *Bokermannohyla ibitiguara* (Anura, Hylidae), a gladiator frog of the Brazilian Cerrado. *J Herpetol* 48:407–414.
- Narins PM, Capranica RR, 1978. Communicative significance of the two-note call of the treefrog *Eleutherodactylus coqui*. *J Comp Physiol* 127:1–9.
- Narins PM, Meenderink SW, 2014. Climate change and frog calls: Long-term correlations along a tropical altitudinal gradient. *Proc Biol Sci B* 281:20140401.
- Newman ME, Girvan M, 2004. Finding and evaluating community structure in networks. *Phys Rev E* 69:026113.
- Newman MEJ, 2006. Modularity and community structure in networks. *Proc Natl Acad Sci USA* 103:8577–8582.
- Okanoya K, 2004. Song syntax in Bengalese finches: proximate and ultimate analyses. In: Slater P, Rosenblatt J, Snowdon C, Roper T, Brockmann HJ eds. *Advances in the Study of Behavior*. San Diego: Academic Press, 297–346.
- Ouattara K, Lemasson A, Zuberbuhler K, 2009. Campbell’s monkeys concatenate vocalizations into context-specific call sequences. *Proc Natl Acad Sci USA* 106:22026–22031.
- Reichert MS, 2009. Aggressive thresholds in *Dendropsophus ebraccatus*: Habituation and sensitization to different call types. *Behav Ecol Sociobiol* 64:529–539.
- Ryan MJ, Page RA, Hunter KL, Taylor RC, 2019. “Crazy love”: Nonlinearity and irrationality in mate choice. *Anim Behav* 147:189–198.
- Ryan MJ, Tuttle MD, Rand AS, 1982. Bat predation and sexual advertisement in a Neotropical anuran. *Am Nat* 119:136–139.
- Sasahara K, Cody ML, Cohen D, Taylor CE, 2012. Structural design principles of complex bird songs: a network-based approach. *PLoS One* 7:e44436.

- Suzuki TN, 2018. Alarm calls evoke a visual search image of a predator in birds. *Proc Natl Acad Sci USA* **115**:1541–1545.
- Suzuki TN, Wheatcroft D, Griesser M, 2018. Call combinations in birds and the evolution of compositional syntax. *PLoS Biol* **16**:e2006532.
- Toledo LF, Martins IA, Bruschi DP, Passos MA, Alexandre C et al., 2015. The anuran calling repertoire in the light of social context. *Acta Ethol* **18**:87–99.
- Weiss M, Hultsch H, Adam I, Scharff C, Kipper S, 2014. The use of network analysis to study complex animal communication systems: A study on nightingale song. *Proc Biol Sci B* **281**:20140460.
- Wells KD, 2007. *The Ecology and Behavior of Amphibians*. Chicago and London: The University of Chicago Press.
- Wey T, Blumstein DT, Shen W, Jordán F, 2008. Social network analysis of animal behaviour: A promising tool for the study of sociality. *Anim Behav* **75**:333–344.
- Xu F, Cui JG, Song J, Brauth SE, Tang YZ, 2012. Male competition strategies change when information concerning female receptivity is available. *Behav Ecol* **23**:307–312.
- Zhu B, Wang J, Sun Z, Yang Y, Wang T et al., 2017a. Competitive pressures affect sexual signal complexity in *Kurixalus odontotarsus*: insights into the evolution of compound calls. *Biol Open* **6**:1913–1918.
- Zhu B, Wang J, Zhao L, Chen Q, Sun Z et al., 2017b. Male–male competition and female choice are differentially affected by male call acoustics in the serrate-legged small treefrog, *Kurixalus odontotarsus*. *PeerJ* **5**:e3980.