

## RESEARCH ARTICLE

# Synchrony of complex signals in an acoustically communicating katydid

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## ABSTRACT

Investigating the ability to entrain to auditory stimuli has been a powerful approach to uncovering the comparative rhythm abilities of different animals. While synchrony to regular simple rhythms is well documented, synchrony to complex stimuli, with multiple components at unequal time intervals, is rarer. Several katydid species with simple calls have been shown to achieve synchrony as part of their natural calling interactions in multi-individual choruses. Yet no study so far has demonstrated synchrony in any insect with a complex call. Using natural calling behaviour and playback experiments, we investigated acoustic synchrony and the mechanisms underlying it in the katydid *Mecopoda* sp. ‘Two-part caller’. This katydid has a complex call consisting of a long trill followed by two or more chirps. We found that individual males synchronized trills and, to a lesser extent, chirps. Further investigation of trill synchrony showed that the timing of trills is modified by external trills but not chirps. Chirp synchrony is modified by external chirps, but also by trills. We suggest a qualitative two-oscillator model underlying synchrony in this species and discuss the implications for the evolution of acoustic synchrony.

**KEY WORDS:** Acoustic, Synchrony, Rhythm, Entrainment, *Mecopoda* spp., Bushcricket

## INTRODUCTION

Several animals have a striking ability to synchronize motor activity in response to external stimuli. The study of the comparative abilities of animals to do so has been an important field of research (Cook et al., 2013; Hasegawa et al., 2011; Jao Keehn et al., 2019; Kotz et al., 2018; Patel et al., 2009; Schachner et al., 2009; Wilson and Cook, 2016). Experiments investigating these abilities often involve playback of artificial rhythmic stimuli to animals and studying their response as evidence of their rhythmic abilities. Rhythmic responses have, however, also been demonstrated in studies of natural behaviour in fireflies, frogs and katydids (bushcrickets) (Greenfield, 2002; Henry et al., 2021). Males of several katydid species produce calls to attract mates over a long distance (Alexander, 1967). In many of these species, males call together in groups or assemblages called choruses (Greenfield, 1994) and interact with each other acoustically to produce striking temporal patterns such as synchrony and alternation (Greenfield and Roizen, 1993; Greenfield and Schul, 2008; Greenfield and Snedden, 2003; Hartbauer et al., 2005; Nityananda and Balakrishnan, 2007).

Their natural behaviour thus serves as an ideal method by which to investigate their rhythmic abilities. Studies of acoustic synchrony in katydids have therefore typically focussed on the responses of individuals to natural stimuli both in the context of playback and in multi-signaller choruses.

Acoustic synchrony in katydids is often imperfect and the calls of individual males lead or follow calls of other males by a few milliseconds (Greenfield and Roizen, 1993; Hartbauer et al., 2005; Nityananda and Balakrishnan, 2007). The mechanisms underlying this synchrony differ in different species and various models have been suggested to describe the mechanisms underlying both acoustic synchrony in katydids and the closely related phenomenon of synchronous flashing in fireflies (Buck et al., 1981a,b; Greenfield, 1994). In species of the katydid genus *Mecopoda*, these mechanisms have typically been investigated using phase response curves (PRCs), which represent the response of an internal oscillator when disturbed by single chirps at different phases of the calling cycle (Hartbauer et al., 2005; Nityananda and Balakrishnan, 2007; Sismondo, 1990). A typical PRC demonstrates how the animal increases or decreases the length of its calling period in response to external chirps. In the species *Mecopoda elongata*, the slope of the PRC seems to determine whether synchrony or alternation occurs (Sismondo, 1990). Simulations of duets based on PRCs also show that the male with the faster intrinsic chirp rate produces chirps that predominantly lead those of a male with a slower rate (Hartbauer et al., 2005). In the katydid *Mecopoda* sp. ‘Chirper’, a similar approach showed that synchrony is enabled in this species through a combination of chirp-chirp resetting and a change in intrinsic rate (Nityananda and Balakrishnan, 2007).

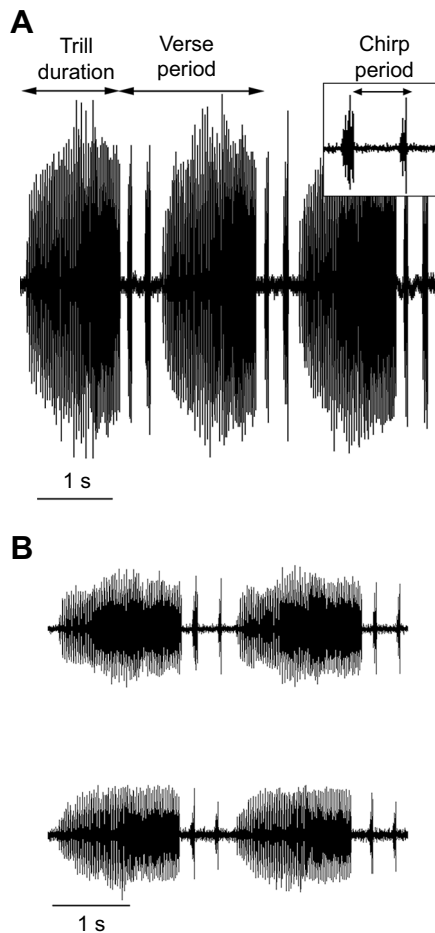
Different mechanisms thus enable synchrony in different species. All species that have been studied, however, have had simple calls consisting of a single chirp repeated at a regular rhythm. Studies investigating rhythm perception in other animals have also focussed on their ability to perceive these simpler (isochronous) rhythms (Celma-Mirallas and Toro, 2020). Evidence for entrainment to more complex rhythms has been shown for vocal learners such as parrots (Patel et al., 2009; Schachner et al., 2009), but also more recently from sea lions (Cook et al., 2013). In katydids, synchrony has thus far not been reported from species that have more complex calls and the mechanisms that might underlie such synchrony remain unknown. Many species of katydids and anurans have complex multi-component calls (Gerhardt, 1994; Gerhardt and Huber, 2002; Korsunovskaya, 2008; Ryan, 1980). Whether and how they synchronize their rhythms remains an open question.

We here demonstrate acoustic synchrony in *Mecopoda* sp. ‘Two-part caller’, a katydid with a complex call consisting of a trill, followed by two or more short chirps (Fig. 1). The frequency spectrum of the call is broadband, ranging from 2 to 70 kHz. This is one of five southern Indian *Mecopoda* spp. katydids that look identical but have dramatically different songs (Nityananda and Balakrishnan, 2006). This song type is found in the evergreen

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**Fig. 1. Oscillograms of the call of *Mecopoda* sp. 'Two-part caller'.**

(A) Oscillogram of male katydid showing verse structure. Each verse consists of a trill followed by chirps. Inset shows a magnified view of the chirps. (B) Oscillogram of two simultaneously calling males with synchronized trills.

forests of the state of Karnataka in South India and has a peak breeding season between the months of March and June. Using acoustic playback experiments, we investigated acoustic synchrony and the possible underlying mechanisms in this species.

## MATERIALS AND METHODS

All experiments were carried out on male *Mecopoda* sp. 'Two-part caller'. The age of the individuals was unknown. The experiments comply with the legal principles of animal care and animal welfare of the Government of India. A minimum of 10 individuals was used for every experimental condition and 10 pairs for the duet condition. In the comparison of period changes this would yield a power of 0.8 if we assumed a difference similar to that observed in a previous study on *Mecopoda* sp. 'Chirper' (Nityananda and Balakrishnan, 2007). Individuals were not allocated to separate individual conditions and so the investigator did not need to be blinded to the condition.

In all experiments, individual males were placed in acoustically transparent nylon mesh cages and the calling of the males and any stimuli played back were recorded on separate channels in an anechoic chamber (2×2×3 m) using tiepin microphones and custom-built amplifiers placed directly in front of the cages. The output of the microphones was digitized at a sampling rate of 16 kHz using a NI-DAQ AT-MIO-16E-2 card and the software

Labview 6.0 (National Instruments Corporation). This sampling rate is sufficient to measure the temporal characteristics of the chirps and trills of the call, which fall in the range of 0.5–3 Hz.

All playback experiments used stimuli taken from a previous recording of an individual 'Two-part caller' male made using a Bruel and Kjaer sound level meter (Type 2231 with a ¼ inch microphone (4939: flat frequency response from 4 Hz to 70 kHz) and digitized at a sampling rate of 200 kHz using a NI-DAQ AT-MIO-16E-2 card and the software Labview 6.0 (National Instruments Corporation, USA). Stimuli were played out at a sampling rate of 200 kHz using a NI-DAQ AT-MIO-16E-2 card and an Avisoft Ultrasonic Scanspeak speaker (frequency range 1–120 kHz). The stimuli were played out at 91 dB SPL (peak) 30 cm from the speaker measured using the sound level meter described above. This corresponds to 74.5 dB SPL at 2 m at the position of the animals.

## Song recording and analysis

All recordings were made at night, during the natural calling time of the animals. Solo recordings were made with individuals isolated in the anechoic chamber and duets were recorded with the two cages placed 2 m apart in the anechoic chamber. During duets and the PRC experiments, outputs were simultaneously obtained on two separate channels from two microphones. Custom MATLAB (MathWorks, USA) programs were subsequently used to first obtain the time of call onsets and offsets and then to calculate the period, duration and phase relationships of the call elements. A Testo 110 thermometer (Testo Ltd., UK) was used to measure the ambient temperature during recordings. The temperature for all recordings was 22.23±0.30°C (mean±s.d.).

Phase relationships between trills were calculated according to the formula:  $P_{12} = (T_{11} - T_{21}) / (T_{22} - T_{21})$ , where  $P_{12}$  is the phase relationship between the trills,  $T_{11}$  is the time of offset of the trill,  $T_{21}$  is the time of offset of the preceding trill of the partner and  $T_{22}$  is the time of offset of the following trill of the partner.

Phase relationships of the chirps were calculated with the same formula using chirp offsets instead of trill offsets. Phase values thus obtained were multiplied by 360 deg to obtain the phase angles. The  $x$  and  $y$  components of each of these angles were determined assuming each angle was a vector of unit length. The components for each duet were then summed together to obtain the  $x$  and  $y$  components of the mean vector for each duet. The angle and length of the mean vectors were then calculated (Batschelet, 1981). A  $V$ -test (Batschelet, 1981) was performed to determine if the vectors were clustered around an angle of 0 deg which would indicate perfect synchrony. The mean trill and chirp periods during solos and duets were compared using unpaired  $t$ -tests at a significance level  $\alpha = 0.05$ .

## Phase response curves

To obtain the PRCs, a previously recorded stimulus was played out after the male initiated calling. The stimulus was played out at a random phase of the male's calling, approximately once every 7 verse periods. We obtained PRCs in response to playback of both trills (trill PRCs) and chirps (chirp PRCs). Trill PRCs were obtained from playback of 87.36±5.25 (mean±s.e.m.) trills per individual, while chirp PRCs were obtained from playback of 61.62±19.49 chirps per individual. In both trill and chirp playback experiments, we looked at the effect on both trills and chirps and obtained separate PRCs for each with calculations as described below. We thus had four types of PRCs demonstrating the effect of trill playback on trills, trill playback on chirps, chirp playback on trills

and chirp playback on chirps. For all four PRCs, the stimuli played out and the calls of the male were simultaneously recorded as described above. The times of offsets and onsets were obtained, and phases were calculated as detailed below.

### The effect of trill playback on trills

For this PRC, the stimulus phase was determined according to the formula:

$$S = (t_s - t_{fm1})/T, \quad (1)$$

where  $S$  is the stimulus phase,  $t_s$  is the time of offset of the stimulus trill,  $t_{fm1}$  is the time of offset of the preceding trill of the male and  $T$  is the undisturbed verse period of the calling male calculated as the mean of three verse periods of the male before the playback (Fig. 2A). Stimulus phases close to 0 represent stimuli played out soon after the end of preceding trill and phases close to 1 represent stimuli played out close to the next trill.

The response phase was determined according to the formula:

$$R = (t_{fm2} - t_{fm1})/T, \quad (2)$$

where  $R$  is the response phase,  $t_{fm1}$  is the time of offset of the trill preceding the stimulus,  $t_{fm2}$  the time of offset of the following trill of the male and  $T$  is the undisturbed verse period of the calling male calculated as the mean of three verse periods of the male before the playback (Fig. 2A). Response phases close to 1 represent no change in period in response to playback and phases greater than 1 represent increases in verse period in response to playback.

### The effect of trill playback on chirps

For this PRC, the calculations were made as in Eqns 1 and 2 except that  $t_{fm1}$  was the time of offset of the preceding chirp of the male,

$t_{fm2}$  was the time of offset of the following chirp of the male and  $T$  was the undisturbed chirp period of the calling male calculated as the mean of three chirp periods of the male before the playback. Note that when the period between two chirps included a trill, this chirp period was not used to calculate the undisturbed chirp period.

### The effect of chirp playback on trills

For this PRC, calculations were made using Eqns 1 and 2 except that  $t_s$  was the time of offset of the stimulus chirp.

### The effect of chirp playback on chirps

For this PRC, the calculations were made as for the effect of trill playback on chirps except that  $t_s$  was the time of offset of the stimulus chirp (Fig. 2B).

In all four cases, the PRC was obtained by plotting the response phase against the stimulus phase. The PRC gives us a picture of how the animal adjusts the timing of its next trill or chirp in response to stimulus playback at different phases in its calling cycle.

The means of three trill periods before and after the period disturbed by stimulus playback were calculated and compared using Student's  $t$ -tests with the significance level Bonferroni corrected for multiple comparisons (Cabin and Mitchell, 2010). The tests were used to determine if there was a significant difference in trill period caused by playback of the stimulus. A similar comparison of means was made between the means of three trill periods before one stimulus playback and the next one approximately 7 periods later. This enabled us to examine if the effects of stimulus playback on trill period were long lasting.

### Response to chirpless calls

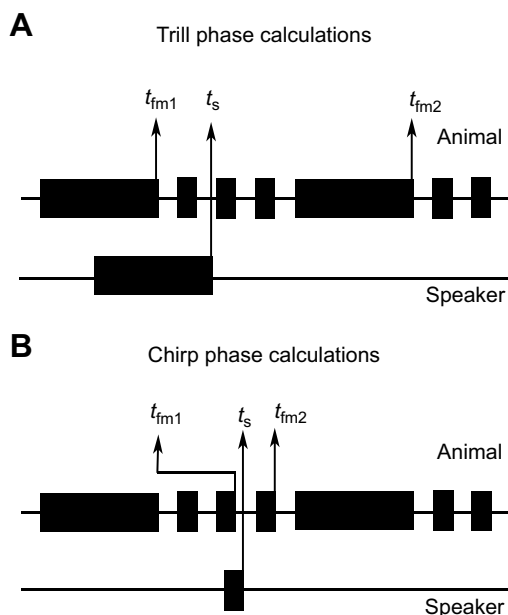
To investigate the influence of chirps on the synchronization of trills between males, we conducted a series of tests in which the response of males to calls without chirps was recorded. Two categories of experiments were conducted for each male: a positive control duet and a duet with chirpless calls. During the positive control duets, the recording played out to the male consisted of a standard 'Two-part caller' verse of duration  $1.89 \pm 0.11$  s (mean  $\pm$  s.d.) and a period of  $2.05 \pm 0.09$  s followed by two chirps. During the chirpless duets, the same call was used with the chirps removed and replaced by silence. The call onsets and offsets were obtained and analyzed as previously described.

## RESULTS

### Solos and duets

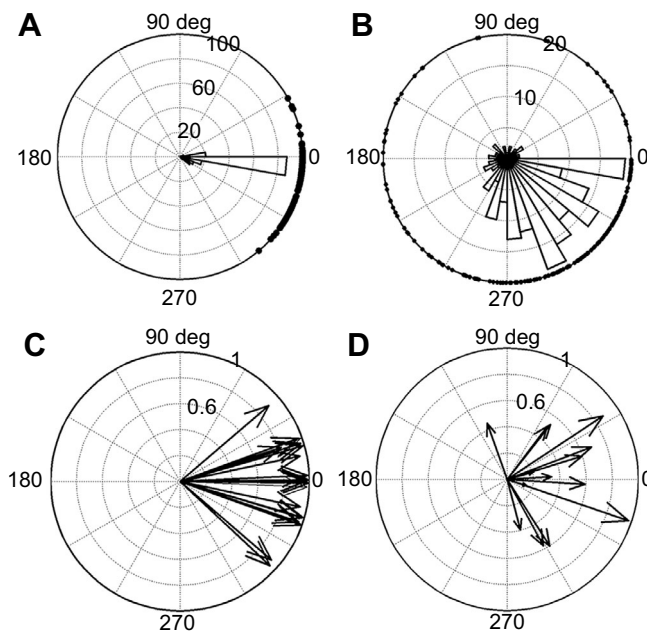
During solo calling, individuals ( $N=22$ ) produced verses with a mean period of  $1.92 \pm 0.10$  s (mean  $\pm$  s.e.m.;  $N=236.77 \pm 49.79$  verses per individual). These consisted of trills with a mean duration of  $1.31 \pm 0.07$  s followed by chirps of mean duration of  $67.8 \pm 0.36$  ms ( $N=272.09 \pm 82.99$  chirps per individual) (Fig. 1A). The mean number of chirps following the trills in the solo bouts was  $2.02 \pm 0.065$ .

In acoustic duets in the laboratory, individual males ( $N=18$  duets, 36 males) synchronized their trills ( $N=144.39 \pm 26.24$  trills per focal individual), with a mean vector angle of  $7.41$  deg, where an angle of  $0$  or  $360$  deg would represent perfect synchrony (Fig. 3A,C). The vector angles across all duets were within  $56$  deg of perfect synchrony ( $0/360$  deg) (Fig. 3C). The length of the vector across all duets was  $0.92 \pm 0.01$  (mean  $\pm$  s.e.m.) and ranged from  $0.76$  to  $0.99$  indicating a high degree of synchrony (a vector length of  $1$  represents perfect synchrony). The distribution of mean vector angles was significantly different from a uniform distribution and the  $V$ -test confirmed that the angles were centred at  $0$  deg ( $V$ -test,  $v=16.39$ ,  $P=2.35 \times 10^{-8}$ ).



**Fig. 2. Phase calculations for the phase response curves.** Long black rectangles represent trills and shorter rectangles represent chirps. (A) Trill PRC calculations show how playback affected the trills of a calling animal. Here, the stimulus played back from the speaker is depicted as a trill. For chirp playback, the phase calculations would be identical with a chirp stimulus replacing the speaker's trill. (B) Chirp PRC calculations show how playback affected the chirps of a calling animal. Here, the stimulus played back from the speaker is depicted as a chirp. For trill playback, the phase calculations would be identical with a trill replacing the speaker's chirp.





**Fig. 3. Synchrony in *Mecopoda* sp. 'Two-part caller'.** Representative polar plots of timing relationships between (A) trills and (B) chirps of two calling males. Points on the circumference represent phase values. Histograms represent frequency of phase values in bins of 10 deg. Mean phase vectors were centred at 0 deg for both (C) trills ( $N=18$  duets,  $V$ -test,  $v=16.39$ ,  $P=2.35 \times 10^{-8}$ ) and (D) chirps ( $N=18$  duets,  $V$ -test,  $v=11.52$ ,  $P=6.15 \times 10^{-5}$ ) across all interactions. Each vector represents the results from one male in a duet.

Males also synchronized their chirps ( $N=179.17 \pm 42.32$  chirps per individual; mean  $\pm$  s.e.m.); the distribution of chirp phase angles was also significantly different from a uniform distribution and centred around 0 deg ( $V$ -test,  $v=11.52$ ,  $P=6.15 \times 10^{-5}$ , Fig. 3B,D). The degree and precision of synchrony, however, was less than that of the trills (Fig. 3D) with an average vector length of  $0.64 \pm 0.07$  and spread over a larger range of angles with a maximum angle of  $121.99$  deg from perfect synchrony.

The verse period was significantly greater in duets than during solo calling for 9 out of 22 individuals (Fig. 4, Bonferroni corrected  $t$ -tests, all  $P < 0.001$ , all  $t < 6.02$  and  $> -238.14$ ) and significantly lower for 8 individuals (Fig. 4, Bonferroni corrected  $t$ -tests, all  $P < 0.001$ , all  $t < 16.91$  and  $> -4.21$ ). The mean absolute change in

verse period from solos to duets was  $276 \pm 60.17$  ms (mean  $\pm$  s.e.m.). The mean difference in verse period between pairs of duetting males was  $48.77 \pm 19.15$  ms. This was much less than the mean difference between the solo verse periods of each pair  $317.77 \pm 72.16$  ms as might be expected because of the synchrony during duets.

#### The effect of trill playback on trill phase response curves

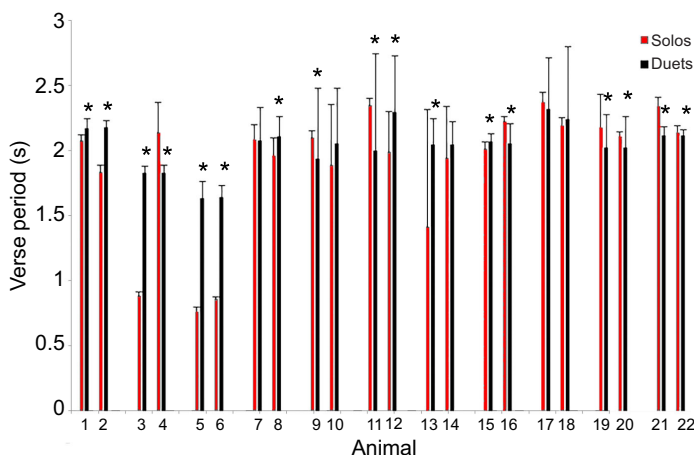
The PRCs of individuals rose for about half the stimulus phase after which they had a slight dip below 1 before returning to a value of 1 (Fig. 5A). This indicates a delay in response to early stimulus phases and an advance in response to the later ones. However, in all except three individuals (Fig. 5A, animals 2, 3 and 9), neither the delay nor the advance was pronounced. In a few individuals, there were some trills that were delayed by a large amount in response to trills in the middle stimulus phases (Fig. 5A, animals 2, 4, 5 and 13). There was no effect of stimulus phase on the timing of subsequent trills (Fig. 5B).

An analysis of verse periods before and after the playback of stimulus trills showed that in 7 out of 14 individuals, the average of three periods following the trill playback was significantly lower than the average of three periods preceding it (Bonferroni corrected  $t$ -tests, all  $P < 0.003$ , all  $t > 0.02$ ). The trend was the same for other animals, but the difference was not significant in these cases. The mean difference between the periods before and after the stimulus trills was  $64 \pm 16$  ms (mean  $\pm$  s.e.m.). There was, however, no significant difference between the averages of periods preceding a stimulus trill and periods preceding the subsequent stimulus trill ( $t$ -tests, all  $P > 0.68$ , all  $t > -0.13$  and  $< 0.42$ ) with the mean differences between these two sets being  $30 \pm 17$  ms.

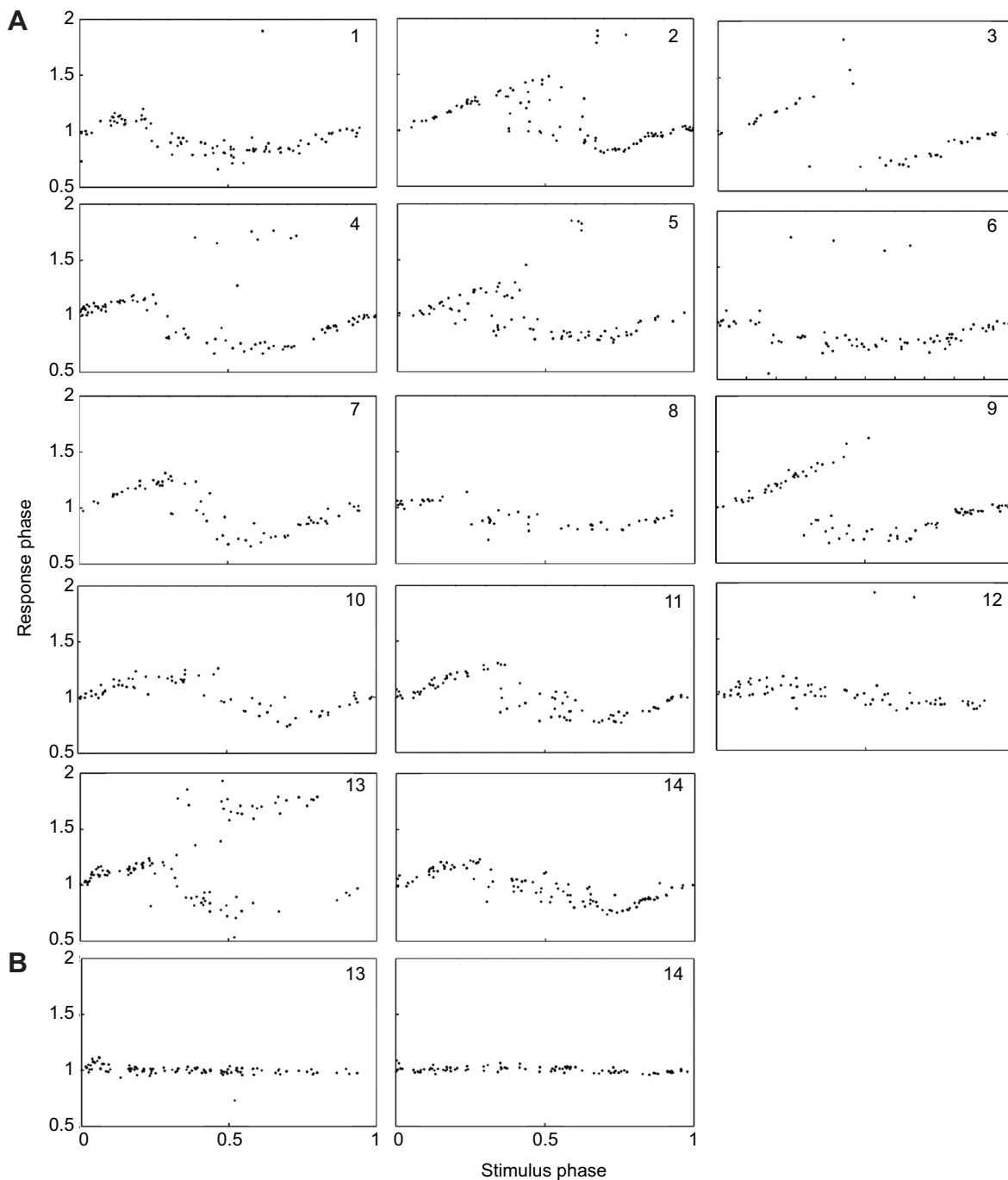
#### The effect of trill playback on chirp phase response curves

Since it was less likely that the onset of our playback trill would fall in the chirp section of the calling verse of the animal, we could not obtain individual PRCs for each animal. Instead, we combined data across all animals to generate a single PRC. This PRC indicates that the playback of trills increases the period of the chirps if ending in the first half of the calling period (stimulus phase less than 0.5) (Fig. 6A). There was, however, no systematic increase of response phase with increasing stimulus phase which is often a characteristic of PRCs in other species.

One interesting aspect of trill playback was that the number of chirps produced by the animal was significantly greater when the trill playback overlapped with the offset of the animal's trill (paired



**Fig. 4. Change in verse period from solos to pairwise interactions.** Red bars represent mean values obtained during solos. Black bars represent mean values obtained during duets. Error bars depict s.d. Values obtained from 22 pairs of males that interacted with each other are grouped together and the numbers below identify individual animals. Asterisks indicate significant differences between the values obtained in solos and the values obtained in duets, after Bonferroni corrections ( $*P < 0.0023$ ).



**Fig. 5. Trill phase response curves obtained by plotting the response phase of trills in response to playback of stimulus trills.** (A) Individual trill PRCs for 14 animals. (B) Phase-dependent change of the period following the disturbed period in 2 individuals. Numbers refer to individual animals.

$t$ -test,  $t=4.83$ ,  $P=3.27\times 10^{-4}$ ,  $N=14$  males). The mean number of chirps produced was  $2.74\pm 0.5$  when the playback overlapped the animal's trill offset ( $N=14$  males,  $55.07\pm 15.37$  playback trills per individual) and  $2.10\pm 0.44$  when the playback did not overlap ( $N=14$  males,  $30.36\pm 8.35$  playback trills per individual).

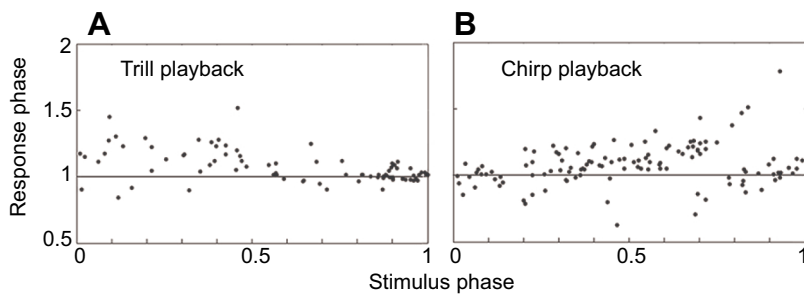
#### The effect of chirp playback on trill phase response curves

Chirps played to individual males at random phases during their calls had no effect on the timing of the trills of the males (Fig. S1). The PRCs obtained were flat and the response phase stayed at a value of approximately 1 for all stimulus phases. The average of three verse periods before and after chirp stimuli did not

significantly differ for 11 out of 12 individuals ( $t$ -tests, all  $P>0.12$ , all  $t>-0.27$  and  $<2.16$ ) and the mean difference between periods before and after the stimuli was  $32\pm 10$  ms.

#### The effect of chirp playback on chirp phase response curves

As discussed above, owing to the lack of cases when the playback chirps overlapped an animal's chirps, we were only able to obtain a combined PRC across all animals. This PRC described how each animal adjusted its chirp periods in response to playback of chirps (Fig. 6B). The response phase increased above 1 with increasing stimulus phase up to around a stimulus phase of 0.7. After a stimulus phase of around 0.7, the response phase was mostly below 1 and



**Fig. 6. Chirp phase response curves obtained by plotting the response phase of chirps in response to playback of stimulus trills and chirps.** (A) Response to stimulus trills ( $N=14$  animals). (B) Response to stimulus chirps ( $N=12$  animals). Both plots combine data from multiple animals.

reached 1 at a stimulus phase of 1. Thus, the chirp periods were increased in response to chirps played back early in the chirp period but were shortened in response to chirps played back later in the chirp period.

### Response to chirpless calls

Eleven out of 14 males synchronized their trills with those of the positive control stimulus played out to them (Fig. 7). The phase angles of the mean vectors obtained in these duets were different from a uniform distribution and were centred around 0 deg ( $V$ -test,  $v=9.36$ ,  $P=2.0044 \times 10^{-4}$ ). Three out of the 14 individuals, however, failed to synchronize their trills and instead had a phase angle closer to 90 deg.

Twelve out of 14 males also synchronized their trills with those of the stimulus (chirpless calls). The phase angles of the mean vectors obtained in these duets were not uniformly distributed and were centred around 0 deg ( $V$ -test,  $v=10.42$ ,  $P=4.11 \times 10^{-5}$ ). Similarly to the positive control duets, two out of 14 individuals failed to synchronize and had mean vector angles above 90 deg. These males were two of the three that also failed to synchronize with the positive control, as mentioned in the previous paragraph.

### DISCUSSION

Our results show that individual *Mecopoda* sp. ‘Two-part caller’, a katydid with a complex call, synchronize elements of their calls. The synchrony is governed by mechanisms similar to those in other synchronizing katydid species and gives us an insight into the oscillators underlying song production in this species.

### Phase response curve comparisons

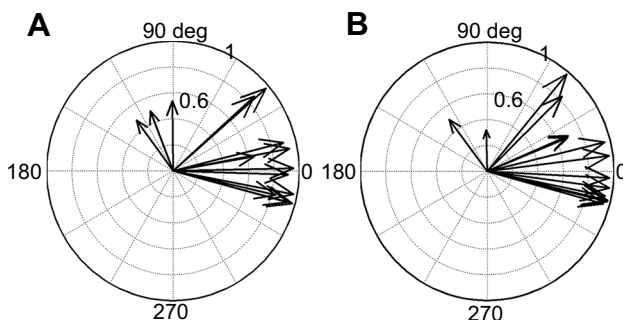
Like several synchronizing katydid species, the trill PRC obtained for ‘Two-part callers’ has both an ‘advance’ phase and a ‘delay phase’. Similarly to *M. elongata*, both phases affect the immediate

chirp and not the following chirp. This distinguishes the PRC from that of many other well-studied species such as the katydids *Neoconocephalus spiza* and the firefly *Pteroptyx cribellata* (Buck et al., 1981a; Greenfield and Roizen, 1993). In these species, the immediate chirp is only delayed, and the advance phase only affects the subsequent chirp. Previous models such as the phase advance and phase delay models that have been used to explain the mechanisms of synchrony are thus insufficient to explain trill synchrony in this species. Unlike the PRCs predicted by these models, the trill PRC obtained for most ‘Two-part callers’ is nonlinear and less pronounced. This PRC is perhaps more similar to that modelled for visual synchrony in other firefly species such as *Pteroptyx malacciae* (Ermentrout, 1991). Using one classification of firefly ‘pacemakers’ (Hanson, 1978), ‘Two-part caller’ has a variable intrinsic chirp period with a small amplitude PRC. In this respect it differs from both previously studied species of the genus. Both *M. elongata* and *Mecopoda* sp. ‘Chirper’ have high-amplitude PRCs with two distinct arms and a break point around 70% into the period (Hartbauer et al., 2005; Nityananda and Balakrishnan, 2007). In fact, the chirp PRC (Fig. 6B) we obtained for ‘Two-part caller’ fits with this pattern and resembles the PRC previously obtained for *M. elongata*. In contrast, the trill PRC for most individuals consists of a continuous curve without any break points. Thus, the mechanism for adjusting chirp periods resembles that of other chirping species but the mechanism for adjusting trill periods is different.

Like ‘Chirper’, however, ‘Two-part caller’ also has a variable intrinsic chirp period. In both species, the chirp period before external acoustic stimulation differs from that after, but the difference is lost if periodic stimulation is missing. This resembles a model for firefly synchrony (Ermentrout, 1991), which assumes an adaptable free-running period that enables synchrony. Our results could also be modelled using phase oscillator differential models (Kuramoto, 1984) that have been used to describe synchrony in Japanese tree frogs (Ota et al., 2020). Here, although each oscillator has a preferred period, this period can vary during interactions with other coupled oscillators. Oscillators in both models have a period that adapts to the period of the external stimulus from another oscillator until the two are in synchrony. This means that whether the period of the male in duets increases or decreases will also ultimately depend on the rate changes that occur in the duetting partner, as can be seen in our results from actual duets (Fig. 4). If the period of the external stimulus or duetting partner is out of the range of adaptability, the first model also predicts phase-locking with a small phase difference instead of synchrony. This might be more like the results obtained in the positive control playback experiments of our study where all males lagged the stimulus by a specific amount, and some had poor synchrony.

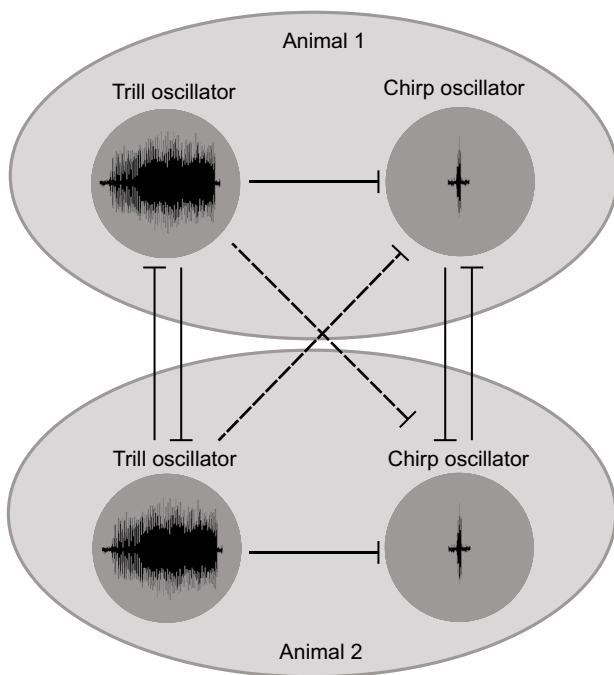
### A two-oscillator model of synchrony

In contrast to other species from which synchrony has been reported, ‘Two-part caller’ has a call with more than one call



**Fig. 7. Mean phase vectors of trills during interactions with a positive control and a chirpless playback stimulus.** Mean phase vectors of trills were significantly centred around 0 deg during interactions with (A) a positive control playback stimulus ( $N=14$  animals,  $V$ -test,  $v=9.36$ ,  $P=2.0044 \times 10^{-4}$ ) and (B) a chirpless playback stimulus ( $N=14$  animals,  $V$ -test,  $v=10.42$ ,  $P=4.11 \times 10^{-5}$ ).

component: a trill and a series of chirps. Models of oscillators in chirping species such as *Necoconocephalus spiza* assume a single oscillator that increases in level and is reset when an external chirp is heard (Greenfield and Roizen, 1993). In ‘Two-part caller’, each calling component could, however, differentially affect the production of calls. In separate playback experiments, we played back both trills and chirps to calling males and examined how they affected calling. The results of our experiments indicate that while trill playback affects the timing and synchrony of trills, chirp playback does not seem to affect them. This shows that trill production is governed by an oscillator that is unaffected by the chirps. Playback of trills does affect the adjustment of chirp periods, as does playback of chirps. We therefore propose a dual-oscillator model in which the production of trills and chirps are governed by two different oscillators (Fig. 8). During solo calling, the trill oscillator suppresses the production of chirps for the duration of the trill, after which the chirp oscillator produces the chirps. Interestingly, the verse period of ‘Two-part caller’ is very similar to that of the chirp period of *M. elongata* (Nityananda and Balakrishnan, 2006), which suggests that perhaps both are modifications of the same oscillator to produce different calls. In ‘Two-part caller’, while interacting with another calling male, the trills heard reset the trill oscillator as seen in the trill PRC. The chirps, however, do not reset the trill oscillator but affect the production of chirps by the chirp oscillator if heard in between trills, thus enabling the level of chirp synchrony seen. Such a model would also predict that when a male’s trills are suppressed by external trills, the suppression of the focal male’s chirps should be released, and we would expect more chirps to be produced in this case. Our results seem to bear this out, since more chirps are produced when external trills extend beyond a male’s trills, and thus presumably suppress the trill oscillator.



**Fig. 8. Two oscillator model for synchrony in *Mecopoda* sp. ‘Two-part caller’.** Circles represent oscillators governing the production of trills and chirps. Larger ellipses represent each of two animals acoustically interacting with each other. Lines depict inhibitory processes. Inhibition between the trill oscillator and chirp oscillator of the other male is indicated as a dotted line to suggest a possibly different process.

### Advantages of leading trills and implications for the evolution of synchrony

In other synchronizing species of katydids, females have been shown to prefer chirps of males that lead the chirps of neighbouring males by a short window of time. In the species *N. spiza*, this preference could over-ride other preferences for louder or longer chirps (Greenfield and Roizen, 1993; Snedden and Greenfield, 1998). The mechanisms enabling chirp synchrony could have evolved as an evolutionarily stable strategy (ESS) in response to such a female preference (Greenfield and Roizen, 1993; Greenfield et al., 1997). A female preference for leading chirps was also demonstrated in the species *M. elongata* (Fertschai et al., 2007). Such a preference may be due to the fact that leading chirps are preferentially represented in the auditory system of the female (Römer et al., 2002). The representation of the following chirp is inhibited for a specific time window following the leading chirp owing to a process of contralateral inhibition. These results have important implications for the synchrony of trills in ‘Two-part caller’. Given that longer calls with a higher duty cycle have greater inhibitory effects on the auditory system in katydids (Römer and Krusch, 2000), we would expect the long trills to have a large inhibitory effect on the auditory system of the female. If contralateral inhibition mechanisms similar to *M. elongata* operate in this species, a male with leading trills could therefore gain a huge advantage: the call of the following male would be inhibited for a long duration during which only the male with the leading trill would be heard. This could mean that females strongly prefer males with leading trills. Such a preference would create a strong selective pressure on the males to produce the same and could have driven the evolution of synchrony in this species in a process like that seen in *N. spiza*. An alternative evolutionary scenario could be that females prefer calls with chirps, and that the males need to synchronize the trills so that the chirps can be heard in the gaps. There might then be resemblances to the calls of the frog, *Physalaemus pustulosus*, which consists of a whine followed by ‘chucks’ where females prefer calls with chucks compared with calls without (Ryan, 1980).

To the best of our knowledge, this is the first report of acoustic synchrony of complex calls in an insect. This suggests that the ability to rhythmically respond to complex stimuli could be more widespread amongst animals than has previously been thought. However, it is important to clarify that the mechanisms underlying such abilities could be very different in different species. Our two-oscillator model suggests that the mechanisms of synchrony effectively reduce the differing rhythms in the call to two separate patterns of trills and chirps and the oscillators respond separately to each of these. This converts what might appear to be a complex pattern into a combination of two simpler mechanisms, which might not underlie rhythm perception in vertebrates, for example. It is possible that other species of katydids and anurans with complex calls could also similarly use multiple oscillators to synchronize different components of their calls. However, even amongst the closely related species of the katydid genus *Mecopoda*, it seems as if differing neural mechanisms could govern call synchrony. How these mechanisms have evolved and the selective pressures on them makes for a fascinating future area of research. Further studies, for example, are needed to shed light on female preferences for leading trills and the neural representation of these in the nervous system of ‘Two-part caller’. It would also be important to investigate if, despite the similarities in synchronizing behaviour, the processes leading to the evolution of the behaviour in this species could differ, as has



been suggested for *Mecopoda* sp. ‘Chirper’ (Nityananda and Balakrishnan, 2009).

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: V.N., R.B.; Methodology: V.N., R.B.; Formal analysis: V.N.; Investigation: V.N.; Resources: R.B.; Data curation: V.N.; Writing - original draft: V.N.; Writing - review & editing: V.N., R.B.; Visualization: V.N.; Supervision: R.B.; Project administration: R.B.; Funding acquisition: R.B.

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#### Data availability

All data supporting this paper are available on Figshare: <https://doi.org/10.6084/m9.figshare.13293089.v1>

#### References

- Alexander, R. D. (1967). Acoustical communication in arthropods. *Annu. Rev. Entomol.* **12**, 495-526. doi:10.1146/annurev.en.12.010167.002431
- Batschelet, E. (1981). *Circular Statistics in Biology*. New York: Academic Press.
- Buck, J., Buck, E., Case, J. F. and Hanson, F. E. (1981a). Control of flashing in fireflies V. Pacemaker synchronization in pteroptyx cribellata. *J. Comp. Physiol. A* **144**, 287-298. doi:10.1007/BF00612560
- Buck, J., Buck, E., Hanson, F. E., Case, J. F., Mets, L. and Atta, G. J. (1981b). Control of flashing in fireflies IV. Free run pacemaking in a synchronic pteroptyx. *J. Comp. Physiol. A* **144**, 277-286. doi:10.1007/BF00612559
- Cabin, R. J. and Mitchell, R. J. (2010). To bonferroni or not to bonferroni: when and how are the questions. *Bull. Ecol. Soc. Am.* **81**, 246-248.
- Celma-Miralles, A. and Toro, J. M. (2020). Discrimination of temporal regularity in rats (*Rattus norvegicus*) and humans (*Homo sapiens*). *J. Comp. Psychol.* **134**, 3-10. doi:10.1037/com0000202.
- Cook, P., Rouse, A., Wilson, M. and Reichmuth, C. (2013). A California sea lion (*Zalophus californianus*) can keep the beat: Motor entrainment to rhythmic auditory stimuli in a non vocal mimic. *J. Comp. Psychol.* **127**, 412-427. doi:10.1037/a0032345
- Ermentrout, B. (1991). An adaptive model for synchrony in the firefly *Pteroptyx malaccas*. *J. Math. Biol.* **29**, 571-585. doi:10.1007/BF00164052
- Fertschai, I., Stradner, J. and Römer, H. (2007). Neuroethology of female preference in the synchronously singing bushcricket *Mecopoda elongata* (Tettigoniidae; Orthoptera): why do followers call at all? *J. Exp. Biol.* **210**, 465-476. doi:10.1242/jeb.02655
- Gerhardt, H. C. (1994). The evolution of vocalization in frogs and toads. *Annu. Rev. Ecol. Syst.* **25**, 293-324. doi:10.1146/annurev.es.25.110194.001453
- Gerhardt, H. C. and Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: University of Chicago Press.
- Greenfield, M. D. (1994). Synchronous and alternating choruses in insects and anurans: common mechanisms and diverse functions. *Am. Zool.* **34**, 605-615. doi:10.1093/icb/34.6.605
- Greenfield, M. D. (2002). *Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication*. Oxford: Oxford University Press.
- Greenfield, M. D. and Roizen, I. (1993). Katydid synchronous chorusing is an evolutionary stable outcome of female choice. *Nature* **364**, 618-620. doi:10.1038/364618a0
- Greenfield, M. D. and Schul, J. (2008). Mechanisms and evolution of synchronous chorusing: emergent properties and adaptive functions in *Neoconocephalus* katydids (Orthoptera: Tettigoniidae). *J. Comp. Psychol.* **122**, 289-297. doi:10.1037/0735-7036.122.3.289
- Greenfield, M. D. and Snedden, W. A. (2003). Selective attention and the spatio-temporal structure of Orthopteran choruses. *Behaviour* **140**, 1-26. doi:10.1163/156853903763999863
- Greenfield, M. D., Tourtellot, M. K. and Snedden, W. A. (1997). Precedence effects and the evolution of chorusing. *Proc. R. Soc. B Biol. Sci.* **264**, 1355-1361. doi:10.1098/rspb.1997.0188
- Hanson, F. (1978). Comparative studies of firefly pacemakers. *Fed. Proc.* **37**, 2158-2164.
- Hartbauer, M., Kratzer, S., Steiner, K. and Römer, H. (2005). Mechanisms of synchrony and alternation in song interactions of the bushcricket *Mecopoda elongata* (Tettigoniidae: Orthoptera). *J. Comp. Physiol. A* **191**, 175-188. doi:10.1007/s00359-004-0586-4
- Hasegawa, A., Okanoya, K., Hasegawa, T. and Seki, Y. (2011). Rhythmic synchronization tapping to an audio-visual metronome in budgerigars. *Sci. Rep.* **1**, 120. doi:10.1038/srep00120
- Henry, M. J., Cook, P. F., De Reus, K., Nityananda, V. and Kotz, S. A. (2021). An ecological approach to measuring synchronization abilities across the animal kingdom. *Phil. Trans. R. Soc. Lond. B.* (In press).
- Jaoo Keehn, R. J., Iversen, J. R., Schulz, I. and Patel, A. D. (2019). Spontaneity and diversity of movement to music are not uniquely human. *Curr. Biol.* **29**, R621-R622. doi:10.1016/j.cub.2019.05.035
- Korsunovskaya, O. S. (2008). Acoustic signals in katydids (Orthoptera, Tettigoniidae). *Communication I. Entomol. Rev.* **88**, 1032-1050. doi:10.1134/S0013873808090029
- Kotz, S. A., Ravignani, A. and Fitch, W. T. (2018). The Evolution of Rhythm Processing. *Trends Cogn. Sci.* **22**, 896-910. doi:10.1016/j.tics.2018.08.002
- Kuramoto, Y. (1984). *Chemical Oscillations, Waves, and Turbulence*. Berlin, Germany: Springer.
- Nityananda, V. and Balakrishnan, R. (2006). A diversity of songs among morphologically indistinguishable katydids of the genus *Mecopoda* (Orthoptera: Tettigoniidae) from Southern India. *Bioacoustics* **15**, 223-250. doi:10.1080/09524622.2006.9753552
- Nityananda, V. and Balakrishnan, R. (2007). Synchrony during acoustic interactions in the bushcricket *Mecopoda* “Chirper” (Tettigoniidae:Orthoptera) is generated by a combination of chirp-by-chirp resetting and change in intrinsic chirp rate. *J. Comp. Physiol. A* **193**, 51-65. doi:10.1007/s00359-006-0170-1
- Nityananda, V. and Balakrishnan, R. (2009). Modeling the role of competition and cooperation in the evolution of katydid acoustic synchrony. *Behav. Ecol.* **20**, 484-489. doi:10.1093/behecol/arp022
- Ota, K., Aihara, I. and Aoyagi, T. (2020). Interaction mechanisms quantified from dynamical features of frog choruses. *R. Soc. Open Sci.* **7**, 191693. doi:10.1098/rsos.191693
- Patel, A. D., Iversen, J. R., Bregman, M. R. and Schulz, I. (2009). Experimental evidence for synchronization to a musical beat in a nonhuman animal. *Curr. Biol.* **19**, 827-830. doi:10.1016/j.cub.2009.03.038
- Römer, H. and Krusch, M. (2000). A gain-control mechanism for processing of chorus sounds in the afferent auditory pathway of the bushcricket *Tettigonia viridissima* (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **186**, 181-191. doi:10.1007/s003590050018
- Römer, H., Hedwig, B. and Ott, S. R. (2002). Contralateral inhibition as a sensory bias: the neural basis for a female preference in a synchronously calling bushcricket, *Mecopoda elongata*. *Eur. J. Neurosci.* **15**, 1655-1662. doi:10.1046/j.1460-9568.2002.02003.x
- Ryan, M. J. (1980). Female mate choice in a neotropical frog. *Science* **209**, 523-525. doi:10.1126/science.209.4455.523
- Schachner, A., Brady, T. F., Pepperberg, I. M. and Hauser, M. D. (2009). Spontaneous Motor Entrainment to Music in Multiple Vocal Mimicking Species. *Curr. Biol.* **19**, 831-836. doi:10.1016/j.cub.2009.03.061
- Sismondo, E. (1990). Synchronous, alternating, and phase-locked stridulation by a tropical katydid. *Science* **249**, 55-58. doi:10.1126/science.249.4964.55
- Snedden, W. A. and Greenfield, M. D. (1998). Females prefer leading males: relative call timing and sexual selection in katydid choruses. *Anim. Behav.* **56**, 1091-1098. doi:10.1006/anbe.1998.0871
- Wilson, M. and Cook, P. F. (2016). Rhythmic entrainment: Why humans want to, fireflies can't help it, pet birds try, and sea lions have to be bribed. *Psychon. Bull. Rev.* **23**, 1647-1659. doi:10.3758/s13423-016-1013-x



Figure S1

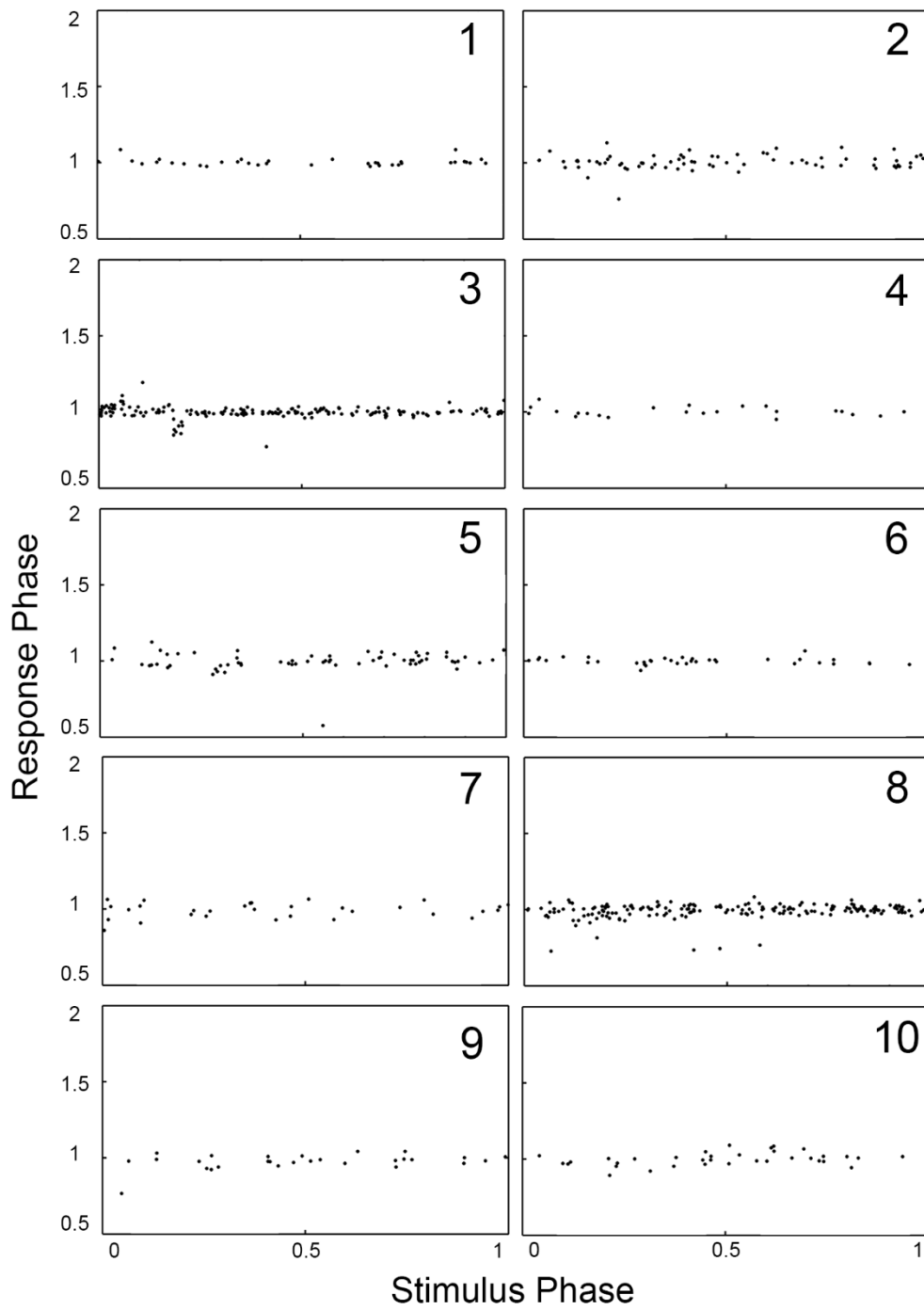


Fig. S1. Trill Phase Response Curves obtained by plotting the response phase of trills in response to playback of stimulus chirps. The last row depicts the phase response in the period following the disturbed period. Numbers refer to individual animals.