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ORIGINAL PAPER

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

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Abstract In several bushcricket species, individual males synchronise their chirps during acoustic interactions. Synchrony is imperfect with the chirps of one male leading or lagging the other by a few milliseconds. Imperfect synchrony is believed to have evolved in response to female preferences for leading chirps. We investigated the mechanism underlying synchrony in the bushcricket species *Mecopoda* 'Chirper' from Southern India using playback experiments and simulations of pairwise interactions. We also investigated whether intrinsic chirp period is a good predictor of leading probability during interactions between males. The mechanism underlying synchrony in this species differs from previously reported mechanisms in that it involves both a change in the oscillator's intrinsic rate and resetting on a chirp-by-chirp basis. The form of the phase response curve differs from those of previously reported firefly and bushcricket species including the closely related Malaysian species *Mecopoda elongata*. Simulations exploring oscillator properties showed that the outcome of pairwise interactions was independent of initial phase and alternation was not possible. Solo intrinsic chirp period was a relatively good predictor of leading probability. However, changing the intrinsic period during interactions could enable males with longer periods to lead during acoustic interactions.

Keywords *Mecopoda* · Synchrony · Phase response curve · Song oscillator · Bushcricket

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Abbreviations
PRC Phase response curve
SPL Sound pressure level

Introduction

Male crickets and bushcrickets call at night to attract potential mates over long distances (Alexander 1967). In certain species of bushcrickets, males call in groups often referred to as choruses (Greenfield 1994; Snedden and Greenfield 1998). Choruses consist of aggregations of simultaneously signalling conspecific individuals. This results in a high degree of competition for mates as well as close-range acoustic interactions between males (West-Eberhard 1984). The acoustic interactions can result in very precise timing relations between the chirps of individual males, such as synchrony (almost complete overlap of chirps) (Greenfield and Roizen 1993; Hartbauer et al. 2005; Walker 1969) or alternation (minimal overlap of chirps) (Greenfield et al. 1997; Minckley et al. 1995).

Typically, synchrony in choruses is imperfect, i.e., the chirps of one of the males lead the chirps of the other by a few milliseconds (Greenfield and Roizen 1993; Hartbauer et al. 2005). Phonotactic choice experiments in the bushcrickets *Neoconocephalus spiza* and *Mecopoda elongata* have shown that females exhibit a preference for leading chirps (Greenfield and Roizen 1993; Römer et al. 1997; Snedden and Greenfield 1998). Game theoretic models have shown that this preference for leading chirps could have driven the evolution of call synchrony and alternation (Greenfield and Roizen 1993; Greenfield et al. 1997).

Three major models have been suggested to explain the observed acoustic synchrony in bushcrickets and synchronous flashes in fireflies. These are the phase advance, phase delay and inhibitory resetting models (Buck 1988; Buck et al. 1981; Greenfield 1994; Hanson 1978). All three models assume an increasing level of excitation of a neural oscillator, which, on crossing a threshold level, fires and causes the production of a chirp. In the phase advance model, the oscillator is brought closer to threshold when an external stimulus is received and hence the next chirp is advanced (Buck 1988). Chirps can only be advanced and never delayed. In the phase delay model, the oscillator is reset to the basal level when an external stimulus is received but starts increasing immediately after being reset. In the inhibitory resetting model, the oscillator remains inhibited for the entire duration of the external stimulus (Greenfield 1994). The latter two models assume a delay between the firing of the oscillator and the production of a chirp (the effector delay). Both these models predict that if resetting happens during the effector delay, the timing of that chirp is not affected, but the chirp in the subsequent cycle is advanced.

Studies by Sismondo (1990) and Hartbauer et al. (2005), however, showed that the mechanism underlying the observed synchrony in chirps of the Malaysian bushcricket *M. elongata* (species 'S') was not explained by these models. Both studies examined the properties of the song oscillator using phase response curves (PRCs). Sismondo (1990) showed that the slopes of the PRCs determined whether synchrony or alternation would occur. Hartbauer et al. (2005) showed that one or the other partner in an acoustic interaction usually maintained lead in a large proportion of the chirps. They simulated duets using the PRCs and showed that the probability of a male's chirps leading the other male's chirps could be predicted by the relative intrinsic chirp rates of the two interacting males: the male with the faster intrinsic chirp rate was usually the leader in pairwise interactions. It is, however, not clear whether PRCs completely explain the acoustic interactions between males, especially given that previous studies have focussed on entrainment paradigms rather than on actual interactions between males.

We investigated pairwise acoustic interactions between individual males of the 'Chirper' song type of the genus '*Mecopoda*' from Southern India (Nityananda and Balakrishnan 2006). We used simulations to explore whether the PRCs were sufficient to reconstruct observed acoustic interactions between individuals. We investigated the mechanism underlying synchrony in this song type and compared it with the Malaysian *M. elongata* (Species 'S'). Both species are chirping species that synchronise. 'Chirper', however, produces chirps at four times the rate of *M. elongata* (Nityananda and Balakrishnan 2006; Römer et al. 1997). We compared the mechanisms underlying synchrony in 'Chirper' and *M. elongata* to examine whether closely related species with similar song structures shared similar oscillator properties. Finally, we investigated whether intrinsic chirp period was a good predictor of chirp leadership in this species.

Materials and methods

Song recording and analysis

All recordings were carried out in an anechoic chamber with the animals placed in acoustically transparent mesh cages. Recordings were made using tiepin microphones with custom-built amplifiers placed in front of the cages. The output of the microphones was digitised at a sampling rate of 16 kHz using a NI-DAQ AT-MIO-16E-2 card and the software Labview 6.0. During pairwise interactions and recordings for obtaining PRCs, the outputs of the two microphones were simultaneously acquired on to two separate channels. A customised MATLAB program (Chandra Sekhar, ECE, IISc) was used to obtain the time of onset and offset of the chirps. Customised MATLAB programs were then used to calculate the chirp periods and durations and the phase relationships between chirps. The ambient temperature during recordings was measured using a Testo 110 thermometer. The mean temperature across all song recordings was 24 (±0.77)°C. For solo recordings, males were placed in isolation in the anechoic chamber. For the pairwise interactions, two males were placed in acoustically transparent mesh cages kept 2 m apart from each other.

The time of the offsets was used to calculate periods for calling males during both solos and pairwise interactions. Chirp offsets were chosen because they were of larger amplitude and hence more easily recognised than onsets. The phase of each male's chirps relative to the chirps of the other calling male during the pairwise interactions was calculated using the formula

$$P = (t_{\mathrm{fm}} - t_{\mathrm{p1}})/(t_{\mathrm{p2}} - t_{\mathrm{p1}})$$

where P is the phase of the focal male, t_{fm} is the time of offset of the focal male's chirp, t_{p2} is the time of offset of the partner's chirp that directly follows the stimulus chirp and t_{p1} is the time of offset of the partner's chirp that directly precedes the stimulus chirp (Fig. 1a).

The phase values were converted to phase angles by multiplying them by 360°. Each phase angle was assumed to be a unit vector and the x and y components of each of these vectors were summed to obtain the x and y components of the mean vector. The length and angle of the mean vector was determined according to Batschelet (1981).

The length of the mean vector gave an index of the degree of spread around the mean vector. If there were no spread, the value of this index would be 1. The greater the spread, the closer the value would be to 0. A mean vector was also calculated using all the mean vectors in order to obtain a population level value. This 'mean vector of mean vectors' was calculated in the same way as each individual mean vector except that the x and y components of each mean vector were weighted by the length of each mean vector rather than assuming each one to be a unit vector.

A circular chi-square test (Batschelet 1981) was performed at a significance level of $\alpha = 0.01$ to test whether the distribution of mean vectors was different from uniform. A V-test (Batschelet 1981) was performed to examine whether the vectors were clustered around 0°.

Synchrony was defined as overlap of chirps. The proportion of chirps that were in synchrony was calculated for each male in a pairwise interaction. The proportion of chirps that lagged or led the interacting partner's chirps was also calculated. The actual values in seconds of the lead or lag were also measured. A chirp was taken to be leading another if the chirps overlapped but the offset of the former preceded the offset of the latter. If the offset of the other chirp preceded the offset of a given chirp, the chirp was taken to be lagging the other chirp.

The mean periods of each calling male during solos and interactions were compared using unpaired t tests at a significance level of $\alpha = 0.05$ (Frank and Altheon 1994).

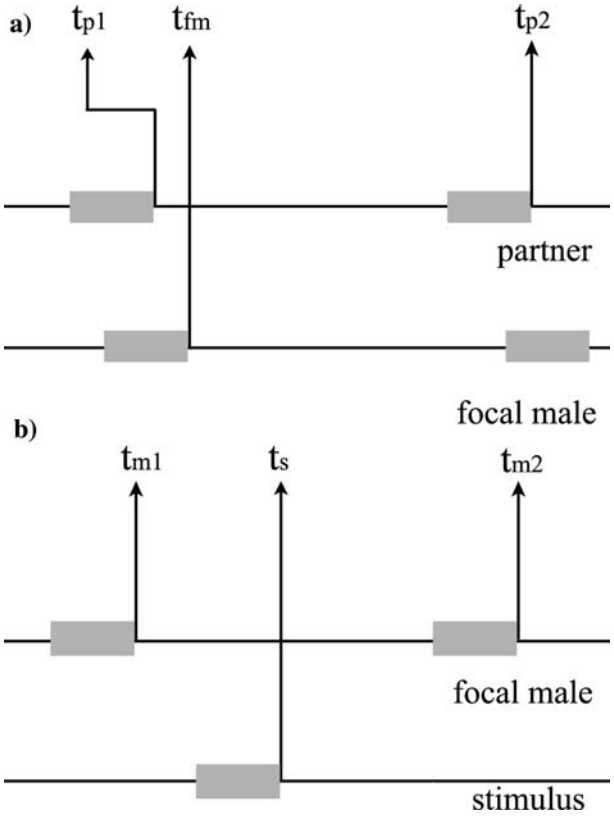

Fig. 1 **a** Diagram illustrating the calculation of the phase of one male's chirps with respect to the other male's chirps. **b** Diagram illustrating the calculation of stimulus phase and response phase. *Grey rectangles* represent chirps

Phase response curves

Each male was placed in an acoustically transparent mesh cage in an anechoic chamber. Once it started calling, a single pre-recorded conspecific chirp was played back to it at an output rate of 200 kHz using a NI-DAQ AT-MIO-16E-2 card through either a Tucker Davis Technology ES1 speaker (frequency range 2–110 kHz) using a Tucker Davis Technology ED1 electrostatic speaker driver or an Avisoft Ultrasonic Scanspeak speaker (frequency range 1–120 kHz) using an Avisoft amplifier. The chirp was played out to each male at two intensities: 68 and 78 dB SPL (*re* 2×10^{-5} N/m^2) as measured at the position of the calling male using a Bruel and Kjaer Sound Level Meter type 2231 with a ¼ in. 4939 microphone (frequency response 4 Hz–70 kHz). A customised Labview 6.0 program played out the chirp once every 9–13 periods of the calling male at a random phase during the period of the male. The chirp played out was taken from a previous recording of a solo calling male made using the Bruel and Kjaer Sound Level Meter and digitised at a sampling rate of 200 kHz using a NI-DAQ AT-MIO-16E-2 card and the software Labview 6.0.

Both the chirp played out and the chirps of the calling male were simultaneously recorded on separate channels as described. Onset and offset times of chirps were determined and stimulus phase calculated using the formula

$$S = (t_s - t_{m1})/T$$

where S is the stimulus phase, t_s is the time of offset of the stimulus (playback) chirp, t_{m1} is the time of offset of the calling male's chirp that directly preceded the

stimulus chirp and T is the undisturbed period of the calling male calculated as the mean of three calling periods before the stimulus chirp was played out (Fig. 1b).

Response phase was calculated using the formula

$$R = (t_{m2} - t_{m1})/T$$

where R is the response phase, t_{m2} is the time of offset of the calling male's chirp that directly follows the stimulus chirp, t_{m1} is the time of offset of the calling male's chirp that directly precedes the stimulus chirp and T is the undisturbed period of the calling male calculated as the mean of three calling periods before the stimulus chirp was played out (Fig. 1b).

Phase response curves were obtained by plotting the response phase R against the stimulus phase S. PRCs were obtained for ten animals at both intensities. PRCs were also obtained for the chirp following the response chirp to see if there was an effect of the stimulus chirp on the subsequent cycle of calling.

In addition to the PRC, analysis was carried out to examine the effect of the playback of the stimulus on the calling period of the animal. The mean of the four periods immediately preceding the stimulus and the mean of the four periods immediately following the disturbed period were calculated for every stimulus chirp played back to the animal. The distributions of these two means were compared. We also compared the means of four periods before one stimulus chirp with the means of four periods before the consecutive stimulus chirp to examine whether the change in period, if any, lasted until the next stimulus was played back (9–13 chirp periods later). Both comparisons were made using paired Student's t tests (Frank and Altheon 1994) at a significance level of $\alpha = 0.05$ for each of ten animals. For both of these tests, the average number of playback chirps in response to which the period change was examined was 36.8 (±7.3).

Simulations

The MATLAB tool Polyfit was used to fit cubic or quadratic equations separately to each arm of nine of the PRCs obtained at a sound pressure level (SPL) of 78 dB. This SPL was chosen because it was comparable to the level of the call of a male at a distance of 2 m in the anechoic chamber. A MATLAB program was developed to simulate pairwise interactions between two males using these equations.

The program assumed a phase counter for each male that began at zero and incremented until it reached one. At this point, the 'chirp' was produced and the phase was reset to zero. A 'chirp' in these simulations was modelled as a single point in time corresponding to either the onset or offset of a chirp. The increment for a male was the reciprocal of the intrinsic chirp period of the male. This means that the increments were shorter than the chirp period. In order to recreate the natural variability in an individual's calling period, this period was randomly chosen from a normal distribution with mean and standard deviation equal to that of a distribution of periods in a previously recorded solo calling bout of the male. If a 'chirp' was produced by one male before the phase counter of the other male reached one, the counter of the other male was reset so that the response would be as predicted by the PRC (using the fitted equations) for the given stimulus phase.

In order to incorporate the deviations of experimentally observed response phase from the equations fitted to the PRC, every time the fitted equations were used, an additional variable was added to the response phase predicted by the equation. This variable was drawn from a normal distribution with the same mean and standard deviation as the distribution of deviations from the equation fitted to the PRC arm. The times of production of 'chirps' in these simulated pairwise interactions were saved and used for further analysis.

Five sets of simulations were carried out using the PRCs:

1. The initial phase difference between the males was set at 0.5. Pairwise interactions were simulated between the PRCs of each of nine males with all the other eight males. This set of simulations was used to look at the influence of intrinsic chirp rate on leader probability at a population level.
2. Interactions were simulated between two identical PRCs. The initial phase difference between the males was set at 0.5. The mean intrinsic chirp period of one of the males in the simulation was set at the mean solo chirp period. In separate simulation runs, the mean intrinsic chirp period of the other male was changed from 50 ms less than the period of the other male to 50 ms more than the period of the other male in steps of 10 ms. These simulations were used to investigate the influence of intrinsic chirp rate on leader probability in pairwise interactions independent of the effect of differences between the PRCs of the partners.
3. Multiple simulations were carried out in which both the initial phase and the period difference between the males were varied. The mean chirp period of the male with constant chirp period (the focal male) was set at the mean solo intrinsic chirp

period for the population. The period of the other male was varied from 100 ms below the focal male's chirp period to 100 ms above in separate simulations. For every period difference, the simulation was run with 11 different initial phases. The initial phase was varied from 0 to 1 in steps of 0.1 with a different initial phase in each simulation. These simulations were used to examine the influence of initial phase on coupling phase across the range of rates seen in the population. Similar simulations were carried out using PRCs obtained at 68 dB SPL.

4. The initial phases were set to the same values as in the real interactions between the males for whom the comparison was being made. The chirp periods were taken from solo periods obtained on the same day as the real interactions. This set of simulations was used to compare simulated interactions of individuals with real interactions of those individuals and to examine whether the characteristics of real interactions of particular individuals could be recreated using the PRCs of those individuals in simulated interactions.
5. The intrinsic chirp period used in the simulations was adjusted such that the resultant periods observed in the simulated interactions matched those seen in the real interactions. The initial phases were set to the same values as in the previous set of simulations. We examined whether the resultant leader probability for particular individuals in simulated interactions matched those in real interactions after adjustment of the chirp period.

Results

The song type 'Chirper' is found in Bangalore and Shimoga in Southern India (Nityananda and Balakrishnan 2006). It calls with an average intrinsic chirp period of 483 ms and mean chirp duration of 109 ms (Fig. 2a, Nityananda and Balakrishnan 2006). The frequency spectrum of this song type ranges from 2 to 70 kHz. The animals call in choruses from 6:30 to 9:30 p.m. Within a chorus, males synchronise their chirps with those of their neighbours (Fig. 2b).

Variation in the intrinsic chirp period

The mean intrinsic chirp period for 37 males was 490 ± 31 (SE) ms. The intrinsic period in this song type had on average a standard deviation of 16 ms (3% of the period) per male. At the population level, however, the variation was larger and the intrinsic chirp period ranged from 417 to 574 ms between individuals (Fig. 2c). Thus, animals might call with partners whose intrinsic chirp period could be as different as 157 ms.

Characterisation of pairwise interactions

During the pairwise interactions, a high degree of synchrony was observed between the chirps of the two males. The mean proportion of chirps in synchrony was 0.963 (±0.158) (Table 1). The angle of the mean vector was greater than 315.8° or less than 35° (Table 1, Fig. 3d). The length of the mean vector ranged from 0.73 to 0.98 showing that the spread of the phase angles was also low (Table 1). The distribution of the 20 mean

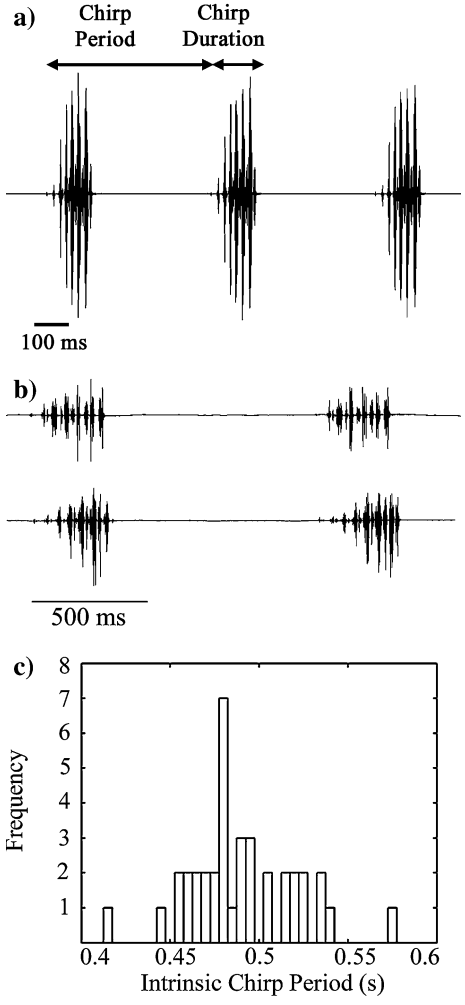

Fig. 2 **a** Oscillogram of the call of song type 'Chirper'. **b** Oscillograms of two males of the song type 'Chirper' with synchronous chirps. **c** Frequency histogram of the intrinsic chirp periods of the calls of 37 males recorded at 24 (±0.77)°C

Table 1 Pairwise acoustic interactions between males

Animal	Angle of mean vector[a] (degrees)	No. of phase angles	Length of mean vector	Proportion of leader chirps	Proportion of chirps in synchrony	Mean lead[b] (±SD) (s)	Mean lag[b] (±SD) (s)
camp12	0.72	910	0.92	0.50	0.99	0.020 (0.016)	0.023 (0.021)
camp14	7.67	617	0.98	0.22	1.00	0.013 (0.016)	0.016 (0.008)
camp17	6.39	499	0.95	0.33	1.00	0.019 (0.015)	0.022 (0.015)
camp19	35.04	506	0.96	0.01	1.00	0.002 (0.001)	0.046 (0.020)
camp21	338.72	751	0.97	0.92	1.00	0.031 (0.016)	0.010 (0.010)
camp23	17.51	517	0.96	0.20	0.99	0.020 (0.023)	0.026 (0.014)
camp25	16.35	511	0.97	0.09	1.00	0.009 (0.010)	0.026 (0.015)
camp26	4.17	597	0.95	0.40	0.99	0.013 (0.012)	0.017 (0.014)
camp32	337.37	391	0.73	0.75	0.89	0.032 (0.025)	0.032 (0.043)
camp30	8.97	586	0.89	0.31	0.97	0.017 (0.014)	0.025 (0.018)
camp13	359.38	913	0.91	0.50	1.00	0.023 (0.021)	0.020 (0.016)
camp15	352.40	619	0.98	0.78	1.00	0.016 (0.008)	0.013 (0.016)
camp18	353.77	500	0.94	0.66	1.00	0.022 (0.015)	0.019 (0.015)
camp20	325.08	506	0.97	0.99	1.00	0.046 (0.020)	0.002 (0.001)
camp22	21.36	759	0.96	0.07	1.00	0.010 (0.009)	0.031 (0.016)
camp24	342.97	514	0.96	0.80	1.00	0.026 (0.013)	0.020 (0.023)
camp28	343.77	511	0.97	0.90	1.00	0.026 (0.015)	0.009 (0.010)
camp27	356.30	594	0.95	0.59	1.00	0.017 (0.014)	0.013 (0.012)
camp33	22.18	367	0.79	0.19	1.00	0.032 (0.043)	0.032 (0.025)
camp31	5.18	715	0.86	0.68	0.98	0.025 (0.018)	0.017 (0.014)

[a] Mean vector values refer to the mean vector of all phase angles of one male's chirps with respect to the chirps of its partner in the pairwise interaction

[b] Lead and lag values refer to the time by which one male's chirps leads or lags the overlapping chirps of its partner in the pairwise interaction

vector angles (Fig. 3d) was significantly different from uniform ($P < 0.001$) and the V-test indicated that these angles were clustered around 0° ($P < 0.001$). Polar plots of representative examples of interactions and all the mean vectors are shown in Fig. 3a–d.

In six out of ten pairwise interactions, one of the partners led the other for more than 70% of the chirps (Table 1). However, there were cases where the lead was more evenly shared between the two males. The mean lead across 37 individuals was 25 ms (±14 SE) and the mean lag was 23 ms (±14 SE).

The relative intrinsic chirp period was not a very good predictor of the exact proportion of leading chirps (Fig. 4a, R^2 value = 0.1952). In seven out of nine cases, however, males whose intrinsic chirp periods were shorter than their partner's had a proportion of leading chirps greater than 0.5. Two of the pairwise interactions formed an exception to this rule (Fig. 4a): the animal with the higher intrinsic period had a greater proportion of leading chirps. A linear relationship between the proportion of leading chirps and the relative intrinsic chirp period was observed if the difference between the intrinsic periods of the partners was within 20 ms (Fig. 4a).

The mean chirp period was significantly less during pairwise interactions than when calling in isolation in 16 out of 20 animals (Fig. 5). Animals with different intrinsic chirp periods adjusted their periods to match those of their partners, usually at a shorter chirp period than those of either partner (Fig. 5).

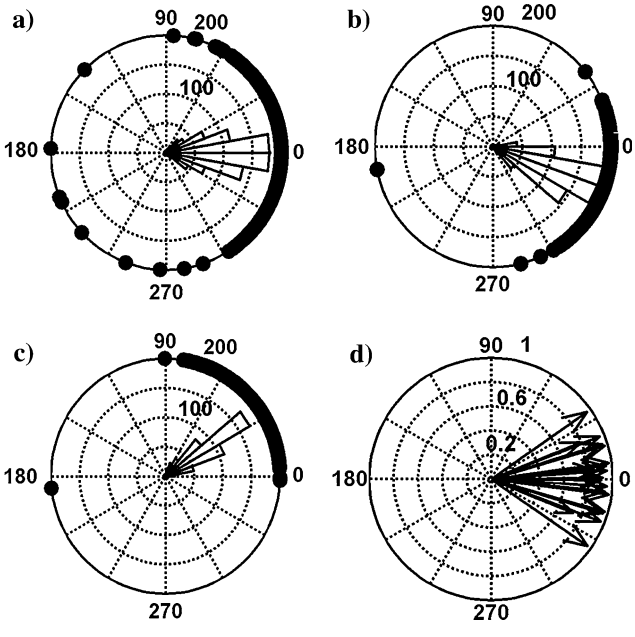

Fig. 3 **a–c** Representative polar phase plots for pairwise interactions between males. Points on the circumference represent phase values. The histogram represents frequency of the phase values in bins of 10°. **d** Mean vectors for all the interactions. See text for further details

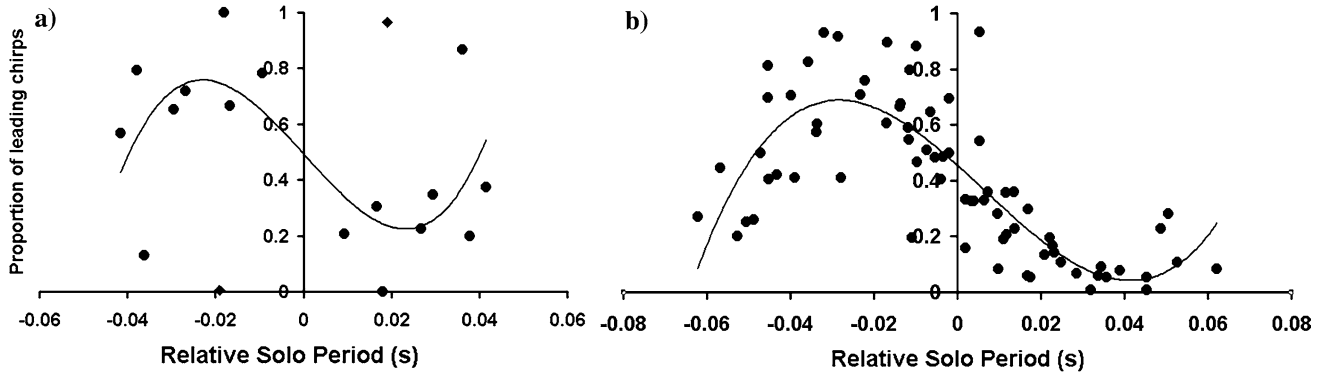

Fig. 4 Relative solo intrinsic chirp period as a predictor of the proportion of leading chirps in **a** real pairwise interactions and **b** simulated pairwise interactions. The fitted curve in **a** excluded the values for the points marked as *diamonds* and had an R^2 value of 0.1952. The curve in **b** had an R^2 value of 0.6456

Phase response curves

The PRC represents the response of the oscillator underlying song to external stimuli. The response to external chirps was dependent on the SPL of the stimulus chirp (Fig. 6). Chirps played out at lower levels (68 dB SPL) did not elicit much response. However, chirps played out at the higher SPL (78 dB) elicited a response. During the cycle in which the stimulus was applied, the curves obtained at 78 dB SPL showed an increasing delay for about 70% of the calling period. Beyond this point, there was still a delay in the production of the chirp. However, for later phases this delay was less. This resulted in the PRC having two arms: the left one increasing with an increase in phase and the right one decreasing with an increase in phase. There was usually no gap between the two arms of the PRC. Furthermore, there was no advance of chirps for any phase. The stimulus chirp did not affect the chirp produced in the subsequent cycle after the response chirp in any of the individuals tested (examples for two individuals shown in Fig. 6, bottom row).

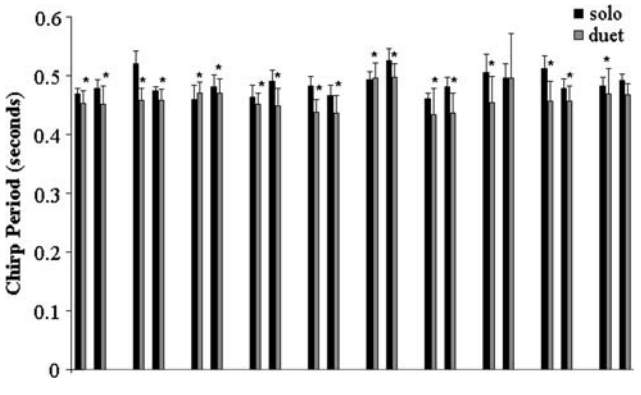

Fig. 5 Change in the chirp period from solo to pairwise interaction. *Black bars* represent values obtained from solo calling bouts. *Grey bars* represent values obtained from pairwise interactions. Each set of *one black and one grey bar* represents the value for one animal. Partners in the pairwise interactions are grouped together. *Asterisks* indicate significant differences between the solo and interaction values ($P < 0.05$). Average number of chirps analysed for solos = 734 (±284). Average number of chirps analysed for interactions = 598 (±217)

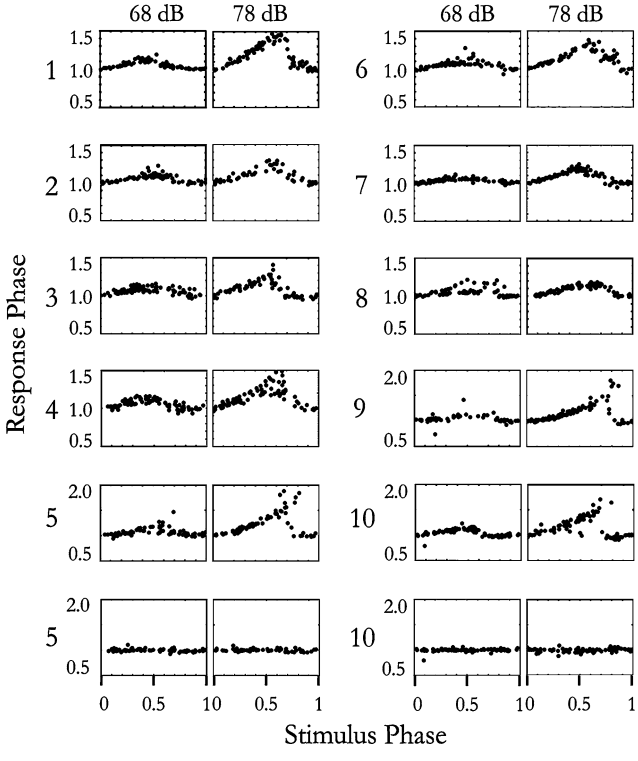

Fig. 6 Phase response curves obtained by playback of individual chirps at 68 and 78 dB SPL for ten animals. The *bottom row* shows PRC plots for the cycle subsequent to the disturbed cycle for the animals 5 and 10. Numbers 1–10 on the left of each plot refer to the individual animal for which the PRC was obtained

In 7 out of 11 animals, the calling period after the playback of the stimulus was significantly lower than the calling period before the playback. The reduction in period for these seven animals was on average 10.7 (±5.2 SE) ms (Table 2). This was lower than the reduction in period seen in pairwise interactions between animals where the periods on average reduced by 22.9 (±17.7 SE) ms. The calling periods before consecutive stimulus chirps, however, did not differ significantly, indicating that the periods returned to previous values within a maximum of 9–13 chirps (Table 2).

Simulated interactions using individual phase response curves

In the interactions simulated using the PRCs, the mean proportion of chirps in synchrony was 0.805 (±0.183 SE) (Table 3), which was less than the proportion of chirps in synchrony observed in real pairwise interactions. The angle of the mean vector across all 72 simulated interactions was less than 103.2° and greater than 250.4° (Fig. 7d). Individual mean vector angles were less than 60° and greater than 322° (Table 3). The average length of the mean vector was 0.81 (±0.18 SE) and ranged from 0.11 to 0.92 across 72 interactions. Thus, the degree of synchrony observed in the simulated pairwise interactions was high but less than that observed in real pairwise interactions. The distribution of the 72 mean vector angles was significantly different from uniform and was clustered around 0° ($P < 0.001$ in both cases). Polar plots of representative examples of interactions and all the mean vectors are shown in Fig. 7.

One of the partners led the other for 70% of the chirps in only 25 out of 72 pairwise interactions. There were many more cases where the partners had a comparable proportion of leading chirps. The mean lead across all 72 pairwise interactions was 46 (±17 SE) ms and the mean lag was 63 (±56 SE) ms (Table 3). The relative intrinsic chirp period was a relatively good predictor of the proportion of leading chirps (Fig. 4b, R^2 value = 0.6456) in the simulated interactions. A linear relationship was observed if the difference between the intrinsic periods of the partners was around 30 ms. Within this range, the animal with the lower intrinsic period had the greater proportion of leading chirps. If the difference between the intrinsic chirp periods (relative solo rates) was greater than 40 ms, the proportion of leading chirps went down for the male with the lower intrinsic chirp period. Thus, the simulations indicate that there is a strong tendency for males whose intrinsic chirp periods are shorter than their partners' by up to 40 ms to have a greater proportion of leading chirps. In almost all cases there was a small but significant increase in chirp period from the intrinsic chirp rate to the simulated interaction (Fig. 8).

The effect of chirp period on the proportion of leading chirps

This set of simulations was used to investigate the influence of intrinsic chirp rate independent of the effect of the difference between the PRCs of the partners. If the intrinsic chirp period of a male was lower than its partner's by 20 ms or less, it had a greater proportion of leading chirps (Fig. 9a). This advantage was, however, reduced if the difference between their periods was greater than 20 ms. If the difference was about 50 ms, they had approximately equal proportions of leading chirps.

The effect of initial phase on the coupling phase during interactions

In the third set of simulations, simulated pairwise interactions were used to examine the effect of initial

Table 2 Change in period in response to an individual playback stimulus chirp

Animal	Mean of four periods before the *i*th stimulus chirp (±SD) (s)	Mean of four periods after the period disturbed by the *i*th stimulus chirp (±SD) (s)	Mean of four periods before the *i* + 1th stimulus chirp (±SD) (s)
camp21	0.446 (0.007)	0.444 (0.011)	0.446 (0.007)
camp23	0.537 (0.018)	0.526[a] (0.013)	0.538 (0.017)
camp24	0.508 (0.006)	0.505 (0.009)	0.508 (0.006)
camp25	0.44 (0.009)	0.435[a] (0.008)	0.44 (0.009)
camp26	0.488 (0.015)	0.476[a] (0.015)	0.489 (0.015)
camp27	0.526 (0.021)	0.509[a] (0.010)	0.527 (0.021)
camp28	0.491 (0.009)	0.485[a] (0.013)	0.491 (0.009)
camp30	0.472 (0.013)	0.454[a] (0.005)	0.472 (0.013)
camp31	0.479 (0.005)	0.477 (0.008)	0.479 (0.005)
camp32	0.497 (0.004)	0.497 (0.004)	0.497 (0.004)
camp33	0.398 (0.008)	0.391[a] (0.008)	0.398 (0.008)

[a] Value is significantly different from the value in the first column

Table 3 Simulated pairwise acoustic interactions

Animal	Angle of mean vector[a] (degrees)	No. of phase angles	Length of mean vector	Proportion of leader chirps	Proportion of chirps in synchrony	Mean lead[b] (±SD) (s)	Mean lag[b] (±SD) (s)
camp21	337.20	8	0.60	0.61	0.88	0.052 (0.036)	0.071 (0.059)
camp23	60.30	8	0.46	0.17	0.61	0.052 (0.040)	0.065 (0.038)
camp24	35.11	8	0.75	0.17	0.90	0.034 (0.031)	0.054 (0.029)
camp25	5.16	8	0.55	0.35	0.85	0.048 (0.041)	0.059 (0.049)
camp28	22.09	8	0.74	0.27	0.91	0.032 (0.031)	0.047 (0.041)
camp30	339.40	8	0.44	0.49	0.75	0.038 (0.028)	0.058 (0.056)
camp31	347.25	8	0.55	0.48	0.83	0.043 (0.033)	0.068 (0.048)
camp32	337.11	8	0.43	0.46	0.74	0.056 (0.042)	0.077 (0.044)
camp33	322.16	8	0.56	0.57	0.77	0.059 (0.041)	0.065 (0.053)

[a] Values for one animal are the means across values obtained in its simulated interactions with all other eight animals. Mean vector values for one animal are values of the mean vector of all mean vectors obtained in each of its simulated interactions with all other eight animals

[b] Lead and lag values refer to the time by which one male's chirps leads or lags the overlapping chirps of its partner in the pairwise interaction

phase on the outcome of a simulated interaction between partners. The phase during pairwise interactions was plotted in a phase-rate plot (Fig. 9b, c). This set of simulations was carried out using PRCs obtained at both 78 and 68 dB SPL (PRCs of the first animal in Fig. 6).

There was very little effect of initial phase on the outcome of the interactions between partners. Synchrony between the partners increased with decreasing difference between their periods (Fig. 9b). Interestingly, synchrony was seen in almost all cases except when the partner had a period slower than the focal male by 70 ms or more. At no point was the angle of the mean vector of an interaction close to 180°. This indicates that alternation is not possible in this song type for any range of initial phase or intrinsic period differences between PRCs at 78 dB SPL (Fig. 9b). Even at 68 dB SPL, alternation was not seen (Fig. 9c). There was, however, a slightly greater influence of initial phase at this level as is indicated by the greater spread of coupling phases for a given difference in period between the two partners.

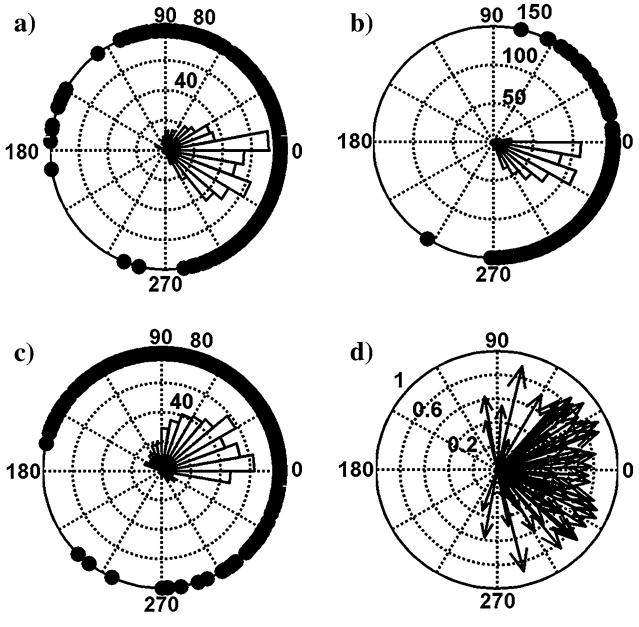

Fig. 7 a–c Representative polar phase plots for simulated pairwise interactions between males. Points on the circumference represent phase values. The histogram represents frequency of the phase values in bins of 10°. **d** Mean vectors for all the simulated interactions

Comparison between real and simulated interactions

The difference between the results obtained using the PRCs as a model of the oscillator and the actual interactions led us to examine how closely the PRCs predicted the characteristics of the interactions not only at population level but also for specific individuals. Towards this objective, we simulated pairwise interactions using PRCs of partners in real pairwise interactions in a fourth set of simulations. For these simulations, we used the intrinsic chirp periods obtained for the interacting partners on the same day as their pairwise interaction. We then compared the simulated and real interactions in three aspects: the degree of synchrony, intrinsic chirp rate as a predictor of the proportion of leading chirps and the change in rate from solo to pairwise interaction. The idea was to see how well the PRC of an individual recreated that individual's interactions when used in a simulation.

The proportion of chirps in synchrony between real and simulated interactions was comparable in

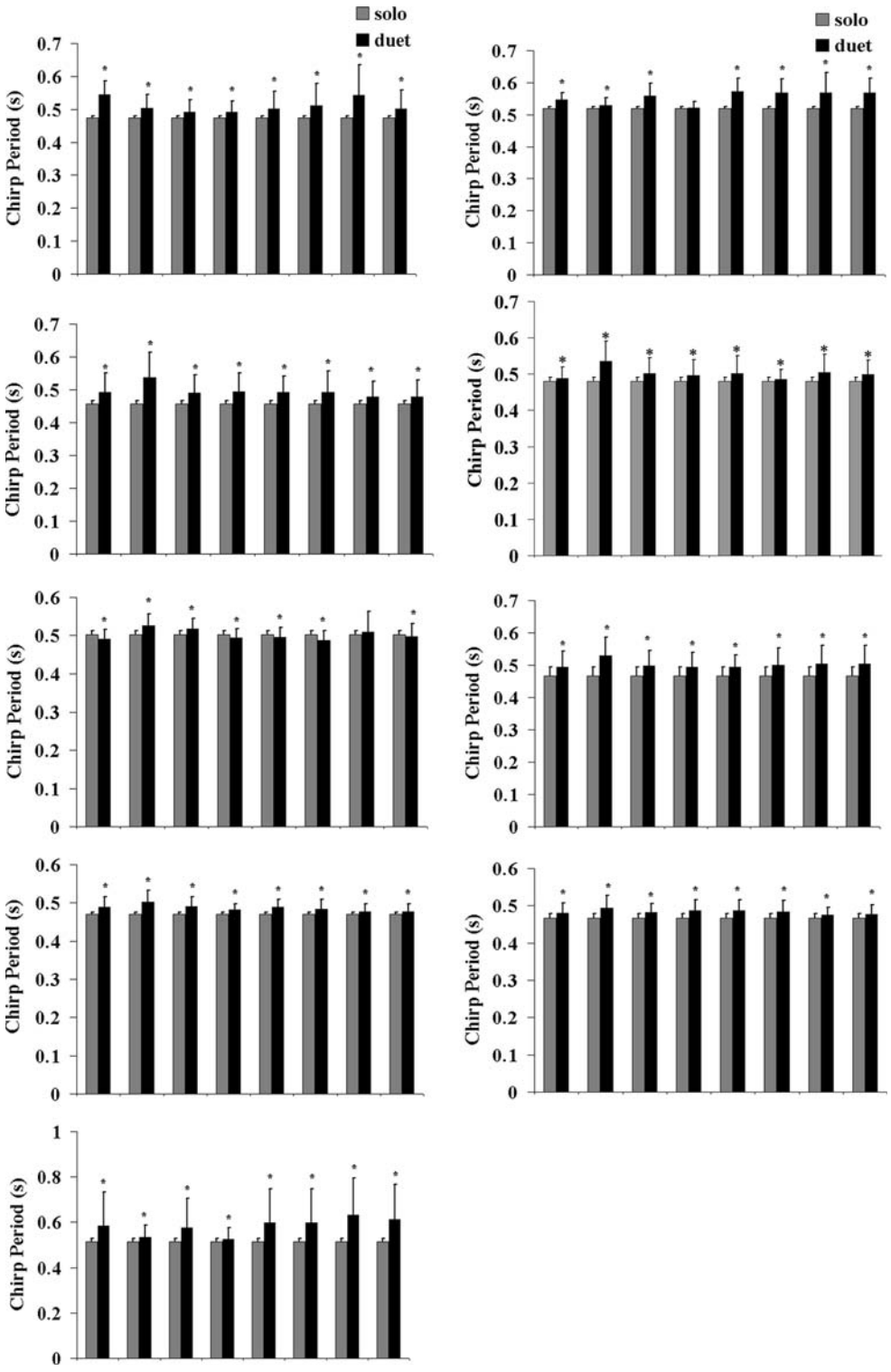

Fig. 8 Change in chirp period from solo calling to pairwise interaction in the simulated interactions for nine males. Each graph represents the interactions of one male with the other eight males. *Grey bars* represent values obtained from solo calling bouts. *Black bars* represent values obtained from the simulated interactions. *Asterisks* indicate significant difference between the solo and interaction values ($P < 0.05$). Average number of chirps analysed for interactions = 581 (±48)

four out of ten cases (Fig. 10a). However, in the other six animals the proportion of chirps in synchrony was less in the simulated interactions. The average proportion of chirps in synchrony was 0.809 in the simulated interactions as compared to 0.983 in the real interactions (Table 4). The lower degree of synchrony is also reflected in the average length of the mean vector. In the real interactions, the average length was 0.947 and the length ranged from 0.91 to 0.98, while in the simulated interactions the length ranged from 0.38 to 0.97 and the average length was 0.709.

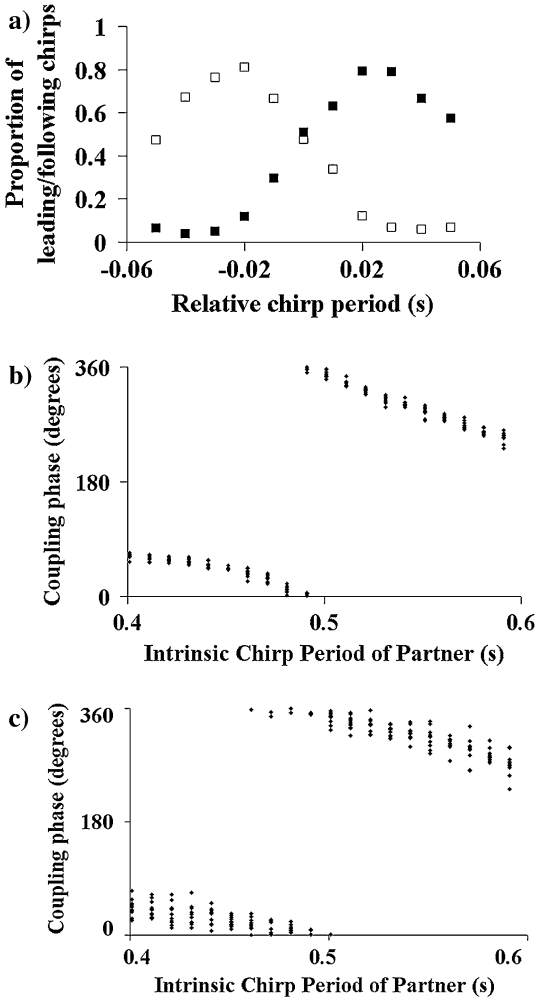

Fig. 9 **a** The influence of relative intrinsic chirp period on the proportion of leading and following chirps in an interaction. The initial phase for all these simulated interactions was 0.5. White squares = proportion of leading chirps; black squares = proportion of following chirps. **b** Phase-rate plot obtained from simulated interactions using PRCs obtained at 78 dB SPL. **c** Phase-rate plot obtained from simulated interactions using PRCs obtained at 68 dB SPL. In **b** and **c** each point represents the mean vector of the phase angles obtained in the interaction, given a particular phase and period difference between the partners. All points for a given intrinsic chirp period of the partner correspond to mean vectors from interactions with the same chirp period difference but with different initial phases between the two partners. The intrinsic chirp period of the focal male was kept constant at 490 ms in all cases

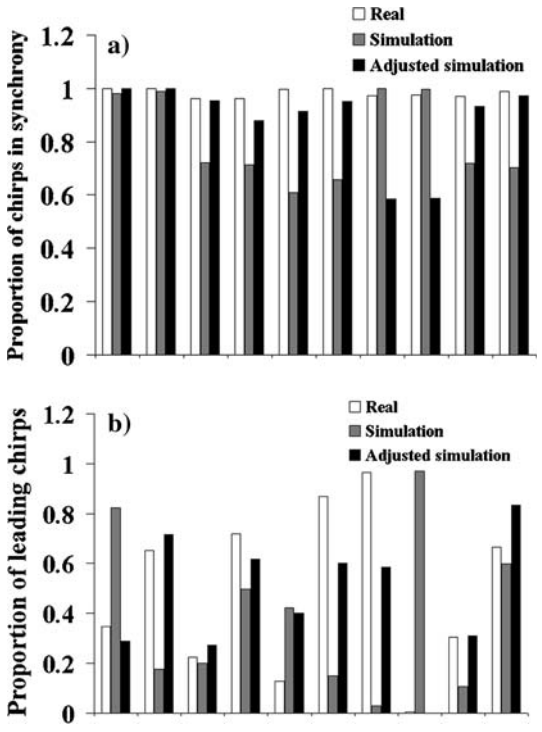

Fig. 10 Proportions of chirps **a** in synchrony and **b** leading during pairwise interactions. *White bars* represent values from real pairwise interactions. *Grey bars* represent values from simulated pairwise interactions using the intrinsic chirp periods from solos of males. *Black bars* represent values from simulated pairwise interactions in which the intrinsic chirp period values have been adjusted so that the resultant periods in the simulated pairwise interactions matched the periods obtained in real pairwise interactions

The leader–follower relationship predicted by the simulation did not match those in real interactions (Table 4, Fig. 10b). In six out of ten animals, there was not even a qualitative match of which animal emerged with a greater proportion of leading chirps in the interactions.

The simulated interactions did not appear to capture the characteristics of the real interactions, especially the prediction of which animal would be the leader in an interaction. We hypothesised that this was because the intrinsic chirp period used for the simulations (the solo intrinsic chirp period) might not reflect the actual chirp period of the animal during the real pairwise interactions. If the actual period of the neural song oscillator changed during interactions, then the period obtained during solo recordings might not reflect the period of the oscillator during interactions. To investigate this further, we performed a fifth set of simulations in which we adjusted the chirp periods used during the simulations so that the resultant mean chirp periods during interactions matched those of the mean chirp periods observed during actual interactions. We then examined whether the leader–follower relations between animals in these simulated interactions matched those observed in the real interactions.

In this set of simulations the average proportion of chirps in synchrony was 0.88, which was higher than the proportion observed in simulated interactions without adjusted intrinsic chirp periods (Fig. 10a). There was also a better match between the proportion of leading

Table 4 Comparison between real and simulated pairwise interactions of specific individuals

Animal	Angle of mean vector[a] (degrees)	No. of phase angles	Length of mean vector	Proportion of leader chirps	Proportion of chirps in synchrony	Mean lead[b] (±SD) (s)	Mean lag[b] (±SD) (s)
Real interactions							
camp34	2.81	736	0.98	0.35	1.00	0.014 (0.014)	0.011 (0.010)
camp35	357.17	732	0.98	0.65	1.00	0.011 (0.010)	0.014 (0.014)
camp36	11.18	435	0.91	0.23	0.96	0.017 (0.017)	0.027 (0.017)
camp37	349.23	438	0.91	0.72	0.96	0.027 (0.017)	0.017 (0.017)
camp38	19.08	711	0.95	0.13	1.00	0.016 (0.017)	0.019 (0.014)
camp39	340.78	708	0.95	0.87	1.00	0.019 (0.014)	0.016 (0.017)
camp40	315.77	419	0.96	0.96	0.97	0.075 (0.018)	NA
camp41	44.22	420	0.96	0.00	0.98	NA	0.075 (0.018)
Vbw	7.13	167	0.92	0.31	0.97	0.018 (0.015)	0.024 (0.017)
Vidot	353.12	162	0.95	0.67	0.99	0.024 (0.017)	0.018 (0.015)
Simulated interactions							
camp34	338.04	616	0.90	0.82	0.98	0.038 (0.032)	0.019 (0.009)
camp35	18.23	620	0.90	0.18	0.99	0.017 (0.009)	0.034 (0.030)
camp36	30.75	548	0.78	0.20	0.72	0.031 (0.015)	0.046 (0.023)
camp37	329.12	602	0.59	0.50	0.71	0.045 (0.025)	0.035 (0.017)
camp38	294.13	620	0.38	0.42	0.61	0.057 (0.042)	0.030 (0.030)
camp39	68.33	574	0.52	0.15	0.66	0.028 (0.030)	0.063 (0.042)
camp40	37.62	587	0.97	0.03	1.00	0.014 (0.013)	0.055 (0.017)
camp41	322.34	588	0.97	0.97	1.00	0.055 (0.017)	0.012 (0.010)
Vbw	47.62	534	0.54	0.11	0.72	0.062 (0.039)	0.054 (0.042)
Vidot	311.75	554	0.54	0.60	0.70	0.055 (0.041)	0.054 (0.038)

[a] Mean vector values refer to the mean vector of all phase angles of one male's chirps with respect to the chirps of its partner in the pairwise interaction

[b] Lead and lag values refer to the time by which one male's chirps leads or lags the overlapping chirps of its partner in the pairwise interaction

chirps observed in real interactions and this set of simulated interactions (Fig. 10b).

Discussion

The song type 'Chirper' differs from the Malaysian *M. elongata* (Species 'S') and other synchronising or alternating animals in the mechanism underlying synchrony.

Phase response curves of 'Chirper' and other species

The song type 'Chirper' differed from other bushcrickets and fireflies in the form of its PRC (Buck et al. 1981; Greenfield and Roizen 1993; Hartbauer et al. 2005; Walker 1969). Typically, PRCs that have been studied to date show a delay in the production of a chirp whenever a stimulus is played back before a certain transition phase, which usually occurs around 70% of the intrinsic chirp period. After this transition phase, in most cases, there is no delay of the chirp. The stimulus chirp, however, usually advances the timing of the chirp in the subsequent cycle. This type of PRC has been reported in the synchronising bushcricket species *N. spiza* (Greenfield and Roizen 1993), the tree cricket *Oecanthus fultoni* (Walker 1969) and in the firefly *Pteroptyx cribellata* (Buck et al. 1981). Hartbauer et al. (2005) reported a different form of PRC for *M. elongata* in which the immediate chirp and not the next one is advanced in the later stimulus phases. Like *M. elongata*, our song type also lacked any advance of the chirp in the cycle subsequent to the disturbed cycle. However, it differed from *M. elongata* in that there was no advance for any stimulus phase. In this, it was similar to *Pholidoptera griseoaptera*, a largely alternating bushcricket species (Jones 1974). However, while *P. griseoaptera* has an increasing response phase with increase in stimulus phase up to a phase of 1, 'Chirper' had a decreasing response phase with increase in stimulus phase after about 0.7. In *P. griseoaptera*, chirps heard very late in the phase would delay the chirp of the male such that it was produced between the next two chirps of the partners (alternation). In 'Chirper', however, chirps heard very late in the phase, would have close to no effect and so the next chirp of the focal male would be produced at around the same time as the partner's next chirp (synchrony).

The difference in the form of the PRCs reflects differing mechanisms that bring about synchrony. In particular, as in *M. elongata* (species 'S'), the inhibitory

resetting or phase delay models do not adequately describe the mechanism operating in this song type. The models evoke the idea of an effector delay in order to explain the advance of the chirp in the cycle subsequent to the disturbed cycle and the lack of delay in the disturbed cycle in the later phases. In the song type 'Chirper', however, we saw no change in the subsequent cycle and a delay in the disturbed cycle for later phases. This cannot be explained by the effector delay. It is, however, possible that the mechanism producing synchrony in 'Chirper' may in fact be similar to that of *M. elongata* (Species 'S') and may simply reflect the outcome of the same mechanism at four times the rate.

Synchrony and alternation

Simulations have shown that the mechanism leading to synchrony in *M. elongata* (Species 'S') also leads to alternation for some values of initial phase and intrinsic period differences between the partners (Hartbauer et al. 2005). Sismondo (1990) showed in *M. elongata* that alternation could occur if the slopes of the PRCs were not steep, as happens when the stimulus intensity is low. He also indicated that when males of *M. elongata* were separated by distances greater than 4–5 m, they hear each other at reduced intensities and so call in alternation. In contrast to *M. elongata*, we found that within the range of periods occurring in the population, alternation was not seen for any initial phase in simulations even at lower sound intensities. The lack of alternation in the song type 'Chirper' could be due to its faster chirp rate in comparison to *M. elongata*. Greenfield (1994) observes that alternation is usually seen in species with lower chirp rates whereas in species that call at rates faster than one chirp per second, synchrony is more common. This is probably because there is a physiological constraint on how fast the oscillator can reach threshold after being reset. In species that call faster, there would not be time for the oscillator to reach threshold fast enough for a chirp to be produced without overlapping with the chirp of the next partner.

Phase response curves and rate change

Regardless of the actual mechanism that generates the observed PRC, the PRC alone could not have brought about the change in rate observed in real interactions. This is especially so in this song type, because the PRC indicates only a delay in chirp production that would lead only to slower rates during pairwise interactions, while in the real interactions we observed faster rates in the pairwise interactions than in solo bouts. Forrest et al. (1998) similarly found that their model based on the PRC was unable to recreate the entrainment seen in *O. fultoni*. They suggested that this was because the model assumed that the period of the oscillator remained constant after stimulation. In the case of 'Chirper,' also, the rate change could be due to a change in the intrinsic chirp period of the song oscillator.

While trying to understand the underlying mechanism that brings about synchrony, it is useful to consider the types of firefly entrainment mechanisms discussed by Hanson (1978). These were (1) a constant intrinsic chirp period with large amplitude PRC, (2) constant intrinsic chirp period with small amplitude PRC and (3) variable intrinsic chirp period with small amplitude PRC. This classification indicates that while the actual resetting could occur based on the shape of the PRC, the change in rate could involve a separate mechanism and need not be caused by the PRC itself. Our studies seem to indicate that the mechanism operating in this song type is a fourth kind, namely a variable intrinsic chirp period with large amplitude PRC.

The variable intrinsic chirp period suggests that this mechanism is similar to those described in the context of coupled oscillators (Ermentrout 1991; Mirollo and Strogatz 1990; Strogatz and Stewart 1993). Models of coupled oscillators may also be particularly applicable to the mechanism underlying synchrony in crickets, since the neuronal circuits underlying song production behave as oscillators (Bentley 1969). The model of Mirollo and Strogatz (1990), however, is applicable only for a phase advance mechanism. The Ermentrout (1991) model for the firefly *P. malaccae* is the one that best captures the features of the mechanism we have observed. In this model, the frequency (rate) of the oscillator is adaptable, i.e., changes in response to external stimuli. This enables the oscillator to entrain to and synchronise with stimuli produced at frequencies different from its own natural frequency (up to 15% in the firefly *P. malaccae*), which is not possible in the non-adaptable frequency model (constant intrinsic period). One would expect to see this mechanism in synchronising species with a larger variation in intrinsic rate in the population, as this would then enable individual animals to maintain synchrony with other individuals even if they had greatly differing rates.

Interestingly, the comparisons of mean periods before and after single chirp playback stimuli indicate that a single chirp can significantly decrease the period of the calling male but this change is soon lost. Given that this decrease in period is smaller than the overall change in chirp period seen during duets, it appears that the mechanism for rate change involves the cumulative effect of multiple external chirps.

Intrinsic chirp rate as a predictor of leadership in interactions

Our studies on male acoustic interactions show that relative intrinsic chirp period is not always a good predictor for leader probability in this song type. However, similar to *M. elongata*, males with lower intrinsic chirp periods tend to lead their partners in pairwise interactions. Results from the simulated interactions also suggest that males with lower intrinsic chirp periods tend to lead but only within a limited range of relative chirp periods (0–40 ms). The range in which leader probability and relative intrinsic chirp period had a linear relationship was smaller in real interactions than in simulated interactions (Fig. 2). The mismatch between these results and also the poor reproduction of individual male chirp rates and leader probabilities by simulations using the PRCs indicate that there might be other mechanisms that work in concert with the PRC during pairwise interactions. As described above, one such mechanism could be a change in the rhythm of the oscillator during interactions. This would imply that the intrinsic periods of two interacting males could differ from their solo intrinsic periods. Indeed, when the intrinsic periods were changed so that the resultant periods matched those observed in real interactions, the leader probability predicted by the PRC was much more similar to the observed probability than it was without incorporating the rate change.

The implication of this is that having a slower solo intrinsic chirp rate is not necessarily a disadvantage to a male during interactions as the male might be able to change the intrinsic rate of its oscillator during the interaction. This would enable it to increase the proportion of leading chirps and so the degree of attractiveness to a female. Males who can adjust their intrinsic periods enough might thus be able to gain an advantage. Another interesting aspect of the influence of intrinsic chirp period on leader probability is that the advantage conveyed by having a shorter intrinsic chirp period is only over a small range (a maximum of 40 ms using the results of the simulations). Since the range of periods seen in the population is more than double this range, males might frequently encounter other males whose periods are greatly different from theirs. In order to gain an advantage during interactions, males in the field would therefore either have to choose neighbours whose intrinsic periods were close to theirs or be able to change their period sufficiently and match those of their neighbours.

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