

# Katydid synchronous chorusing is an evolutionarily stable outcome of female choice

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IN many animals that use rhythmic acoustic or bioluminescent sexual communication, neighbouring males precisely synchronize their signals<sup>1-4</sup>. This event has previously been interpreted as a development whereby cooperative individuals benefit from maintenance of species-specific signalling rates<sup>5,6</sup>, minimization of predation risks<sup>7,8</sup>, or maximization of peak signal amplitude of a local population<sup>2,9</sup>. Our recent findings on chorusing in the neotropical katydid *Neoconocephalus spiza* (Orthoptera: Tettigoniidae), however, refute for this species all three hypotheses that claim that synchrony is adaptive. Instead, we demonstrate that synchrony can be an epiphenomenon created by competitive interactions between males jamming each other's signals. The mechanism generating this interference is shown to be an evolutionarily stable strategy (ESS) maintained under sexual selection for exploiting a critical psychoacoustic feature: females orienting toward signalling males choose the leading call in a closely synchronized sequence.

We determined the nature of synchrony in male *N. spiza* by acoustic measurements and playback experiments in the field and laboratory. *N. spiza* produce loud (amplitude ~70 dB sound pressure level (SPL) at 3 m) advertisement 'chirps' by forewing-forewing stridulation. Chirps consist of 2-10 'pulses' and are 17-74 ms long ( $\bar{x}$  = 52 ms; s.e. = 0.87;  $n$  = 231 chirps from 31 insects); chirp periods ( $T$ ) are 278-550 ms ( $\bar{x}$  = 438 ms; s.e. = 6)<sup>10</sup>. Both intra- and inter-individual variation in  $T$  occur and are independent of ambient temperature (23-28 °C), but a soloing male usually maintains a regular chirp rhythm for several minutes. Males calling within distances of 10 m synchronize their chirps, except that a male typically drops out of the chorus for 1-2 chirp periods every 4-15 periods and then rejoins in phase<sup>11,12</sup>. Designating the onset time of male  $i$ 's chirp during period  $k$  as  $t_{i,k}$ , we gauge the separation of male  $i$ 's chirp rhythm from male  $j$ 's rhythm during that period by the phase angle  $\alpha_{ij,k}$  ( $=360^\circ \times (t_{i,k} - t_{j,k}) / \bar{T}_i$ ). Whenever 2 males call simultaneously, their chirp rhythms are invariably separated by an  $|\alpha| < 54^\circ$ . The leading role ( $\alpha$  slightly  $< 0^\circ$ ) within synchronizing pairs usually alternates between males during successive chirp periods.

Playback experiments on soloing males in the field in Panama defined the mechanism responsible for synchrony. When a single 55-ms playback stimulus, a genuine chirp of amplitude = 56-74 dB (at 3 m), was broadcast at a phase angle =  $\Theta$  ( $0^\circ < \Theta \leq 300^\circ$ ) after the focal insect's chirp, the insect delayed its next chirp by slightly  $< \Theta$  (Fig. 1a). Stimuli broadcast at the end of the insect's chirp period ( $-60^\circ < \Theta \leq 0^\circ$ ) failed to postpone the very next chirp, however, they advanced the second chirp by  $\sim |\Theta|$ . Presumably, the first chirp remained unaffected because it had already been 'triggered' by a central rhythm generator before the onset of the stimulus<sup>13</sup>. Lengthening playback stimuli by  $l$  (65-305 ms) induced commensurately longer delays  $\sim \Theta + (l/\bar{T}) \times 360^\circ$  in the next chirp. When series of 55-ms stimulus chirps with periods  $> \bar{T}$  ms were broadcast, males did not gradually synchronize with the stimuli but consistently called before each stimulus (Fig. 1b). The playback of stimulus chirp series with periods  $\ll \bar{T}$  ms, however, completely inhibited calling<sup>11</sup>.

These findings imply that a male is inhibited from calling by sound initiated  $> 70$  ms before its anticipated chirp and that inhibition continues until the sound ends. Immediately after

release from inhibition, the insect's first chirp is slightly advanced. This mechanism is accurately depicted by a modification of inhibitory resetting models describing firefly flash rhythms<sup>14,15</sup> (Fig. 2). Moreover, the findings indicate that synchrony does not result from amplitude-dependent, mutual entrainment of chirp rhythms, a reportedly general, phase-locking model<sup>16</sup>, but rather that synchrony is simply generated anew every chirp period. That is, when 2 males signal, each will not call until  $\sim \bar{T}$  ms (slightly  $< \bar{T}$  ms when a leader) after the other's chirps. If males sustain comparable  $T$ s, then runs of synchrony ensue by default. Otherwise, the faster male calls during most chirp periods, whereas the slower one usually remains silent

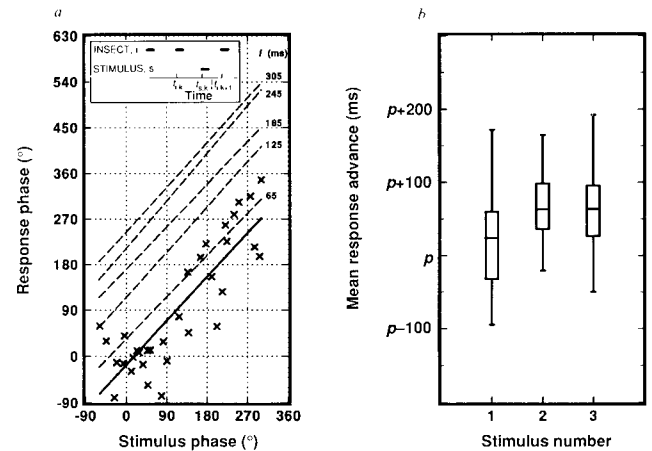


FIG. 1 a, The phase response function<sup>5,16</sup> for *N. spiza* males subject to a single playback stimulus at various stimulus phase angles  $\Theta$  ( $=360^\circ \times (t_{s,k} - t_{i,k}) / \bar{T}_i$ ;  $\bar{T}_i$  is insect  $i$ 's mean chirp period; see inset) during their chirp periods. Response phase angle ( $360^\circ \times ((t_{i,k+1} - t_{i,k}) - \bar{T}_i) / \bar{T}_i$ ) is the relative change in the length of the subsequent chirp period that ends at least 70 ms after the stimulus. Playback stimuli were identical, genuine chirps. We tested each male once with a 55-ms chirp (amplitude = 65 dB SPL; 0 dB re 20  $\mu$ Pa) presented at a random  $\Theta$  during the insect's period. A 2-channel tape recorder registered both the insect and stimulus, and their timing was analysed by simultaneously sampling both tape channels at 60 kHz with a computer. Solid line indicates least-squares regression (slope = 0.96, s.e. = 0.03; intercept = -16.64, s.e. = 2.87;  $r$  = 0.85;  $P$  = 0.01) obtained from data on playbacks to 33 randomly selected insects (crosses) in Panama. The predicted response phase of a male with  $\bar{T}$  = 440 ms, subject to a stimulus at  $\Theta$  = 180°, is 156°, a 190-ms increase in its subsequent chirp period. Effects of stimulus length were tested by playback of a randomized sequence of 5 chirps, each lengthened by a different amount ( $l$  = 65-305 ms) by a computer signal editing system that digitally copied and added pulses to the ending of the 55-ms stimulus. Successive chirps within a sequence were 7 s apart, and the sequence was re-randomized for each of 31 males. Dashed lines show the phase response functions obtained with chirps lengthened by given  $l$ . None of the 6 regression slopes shown differ significantly ( $F$ -test;  $P$  = 0.25); however, intercepts are shifted upward  $\sim (l/\bar{T}) \times 360^\circ$  ( $t$ -tests for comparing parallel regressions revealed that none of the lines except 245 and 305 ms are identical;  $P$  < 0.05). In an analogous playback test we broadcast a randomized sequence of three 55-ms chirps, each regulated to a different amplitude (=56, 65 and 74 dB). Comparison of the regression lines yielded by the 3 different chirps revealed no significant influences of amplitude on the phase response function. b, Responses of males subject to series of 3 identical 55-ms playback stimuli delivered with a 500-ms period.  $\bar{T}$  averaged 431 ms (s.e. = 6). Values given are response advances ( $=500 - (t_{i,k+1} - t_{s,k})$  ms) expressed relative to  $p$  ( $=500 - \bar{T}$  ms). These data were then averaged over repeated playbacks of the series to a given male, and ranges, 50% limits, and medians are shown for the means of 24 insects. The predicted response of a male with  $\bar{T}$  = 430 ms is a chirp  $\sim 90$  ms ( $=p + 20$  ms) before the second stimulus and  $\sim 130$  ms ( $=p + 60$  ms) before the subsequent ones. Response advances exceed  $p$  after the second and third stimuli in the series (Sign test;  $P$  = 0.02).

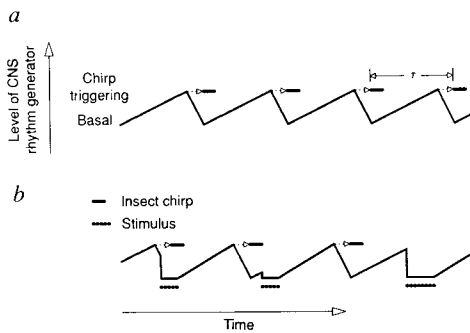


FIG. 2 a, Model depicting hypothetical chirp rhythm generator in the central nervous system (CNS) of an insect with a chirp period =  $T$ . b, Modification of the model in a, adapted from the scheme of Buck *et al.*<sup>15</sup> for firefly flash rhythms, to account for inhibition by acoustic stimuli and resetting of the subsequent chirp rhythm. Generator function is drawn steeper after inhibition to reflect the phase response lines in Fig. 1a.

because its central rhythm generator is repeatedly reset to the basal level before chirps are triggered.

Why do *N. spiza* males exercise inhibitory resetting and call in synchrony? This can be explained by the crucial way in which females orienting toward male songs discriminate synchronized calls. When series of shortened (i, 27.5 ms) and of normal (j, 55 ms) chirps, each with 400-ms  $T$ s and timed such that  $\alpha_{ij} = 180^\circ$ , were presented from 2 loudspeakers on opposite sides of an acoustically insulated laboratory arena, females tested in the arena oriented toward the longer chirps (Fig. 3). However, as  $\alpha_{ij}$  was adjusted such that short chirps began just 47.5 or 13.5 ms before long ones ( $\alpha_{ij} = -42.7^\circ$  or  $-12.4^\circ$ ), female preference abruptly shifted to the short (leading) chirps. To determine whether such phonotactic orientation toward leading chirps was due to stimulation by sounds intrinsic to chirp beginnings, which

remained unmasked in leading chirps, we then bisected the 55-ms chirp, equalized the power of its halves, and broadcast alternating series ( $T$ s = 400 ms;  $\alpha_{ij} = 180^\circ$ ) of the beginning (i) versus ending segments (j) from the 2 loudspeakers. Females showed no bias for beginning segments (44% preference; 2-tailed binomial test,  $n = 16$ ,  $P = 0.80$ ), indicating that selective phonotaxis resulted merely from the sudden onset of sound associated with leading chirps. In an experiment in which the 2 loudspeakers presented leading and following 55-ms chirps ( $T$ s = 400 ms;  $|\alpha| = 24.8^\circ$ ) in 3:1 and 1:3 ratios, respectively, females still favoured the predominantly leading source (80% preference; 2-tailed binomial test,  $n = 20$ ,  $P = 0.02$ ). Evidently, phonotaxis is influenced by a summation of stimulations over successive chirp periods.

Female orientation toward leading chirps is sufficiently potent to override the preference for length and would impose strong selection pressure on males to adopt a mechanism that improves the chance of calling slightly before a neighbour. Inhibitory resetting, whereby a male forgoes calling if its chirp is destined to follow that of a neighbour by  $>70$  ms but then shortens its next chirp period (Fig. 2b), meets this criterion. Computer simulation demonstrates this formally and also shows that synchrony results if 2 males with comparable  $T$ s use this mechanism (Box 1).

We tested the theoretical viability of inhibitory resetting (strategy R) by examining its evolutionary stability<sup>17,18</sup> against that of a hypothetical strategy (N) wherein a male's chirp rhythm is independent of its neighbour's calls (Box 1). A symmetrical game<sup>19</sup> is modelled in which simulated males adhering to either strategy are subject to the same energetic constraint yielding identical chirps with minimum mean periods =  $T^*$ . Males can

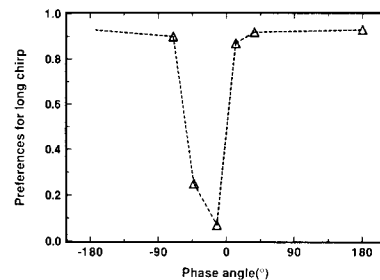


FIG. 3 Orientation of virgin female *N. spiza* to series of long (55 ms) versus short (27.5 ms) playback stimuli delivered with 400-ms periods and timed with various phase angles. The stimuli were presented through a 2-channel DAT recorder and two loudspeakers on opposite sides of an acoustically insulated laboratory arena (diameter = 4 m); amplitudes were adjusted to 60 dB SPL at the centre (female release point). Each playback stimulus was derived from the same digitized genuine chirp. This chirp was copied onto channel 1 of a computer signal editing system every 400 ms; the chirps on channel 1 were copied onto channel 2 and shortened by removal of ending halves. Channel 2 was then advanced or delayed to establish one of the 6 different phase angles tested, ranging from  $-69.8^\circ$  (short chirps begin 77.5 ms before long ones) to  $180^\circ$  (alternation). For each phase angle the signals on both channels were continuously 'looped' and transferred to a 10-min DAT tape segment. We tested females individually by playback of a tape segment (a given phase angle) during a 10-min trial. Individuals were repeatedly tested up to 6 times. In successive trials of the same female, we repositioned her at the arena centre, switched the loudspeakers broadcasting short and long chirps, and presented the next phase angle from a randomized sequence of the 6 angles; the presentation sequence was re-randomized for each female. Preference values at each phase angle are proportions of the females going to either loudspeaker ( $n = 14-20$ ) who moved toward and remained at the one broadcasting long chirps. All 6 values differ significantly from 0.5 (2-tailed binomial test adjusted by the Holm multiple test procedure;  $P < 0.05$ ), and significant reversals in preference occurred between  $-69.8^\circ$  and  $-42.7^\circ$  and between  $-12.4^\circ$  and  $12.4^\circ$  (McNemar test with Williams' correction;  $P < 0.05$ ).

#### BOX 1 Evolutionary stability of inhibitory resetting (R) versus independent (N) calling as determined by computer simulation of a pairwise game

Two simulated males are programmed to chirp according to the rules (see text and Fig. 2b) of 'strategies' R or N. They are assigned  $L$  and  $F$  'points', respectively, when in leading and following roles (synchrony with partial call overlap) during a chirp period and  $L + F$  and 0 points, respectively, when soloing and inhibited for a chirp period; they each earn  $(L + F)/2$  points if both call during the same chirp period but fail to overlap or if perfect synchrony (complete overlap) occurs. The averaged points that a male receives over many successive chirp periods reflect its success in attracting females and, ultimately, its fitness. Tabular entries represent  $E(i, j)$ , the (expected) fitness 'payoff' to male  $i$  calling in the presence of male  $j$ , as obtained from our simulations.

		Strategy, male $j$	
		R	N
Strategy, male $i$	R	$\sim(L + F)/2$	$u \times L + v \times F$
	N	$v \times L + u \times F$	$\approx(L + F)/2$

Assuming a long game (measured by number of chirp periods) and an interchirp interval  $\gg$  chirp length, we found that  $u > v$  (with  $u$  and  $v$  non-negative variables constrained by  $u + v = 1$ ) under a wide range of substitutions for the deterministic and stochastic parameters controlling interchirp interval in R and N. Furthermore, as game length and the number of games increased,  $E(R, R)$  and  $E(N, N)$  converged upon the tabular values. Therefore, if  $L > F > 0$ , then  $E(N, R) < E(R, R)$ , making R an evolutionarily stable strategy<sup>19</sup>; also,  $E(R, N) > E(N, N)$ , favouring the spread of R in a population. In simulations of 2 males adhering to R, synchrony always occurred during more than 78% of chirp periods. An extension of this model to an 'n-player scramble' and inclusion of various levels of inhibitory-resetting will be presented elsewhere.

momentarily speed up (to a period =  $T^* - d + \epsilon$ , where  $d$  is a constant increment, and  $\epsilon$  is a continuously distributed stochastic variable with mean = 0), but this is necessarily followed by compensatory slowing (to  $T^* + d + \epsilon$ ). If leading chirps are more attractive and the values of individual chirps are averaged across successive chirp periods to produce an overall index of a male's attractiveness to females, R is an ESS.

None of the hypotheses proposing adaptive, cooperative functions for synchrony in *N. spiza* are tenable. Females did not show an increased latency when orienting to alternated chirps in the arena trials (*t*-tests for paired comparisons;  $P > 0.20$ ), implying that synchrony did not result from a need to maintain a specific signalling rhythm within a neighbourhood. Unlike certain acoustic orthopterans<sup>20, 23</sup>, *N. spiza* has no known phonotactic natural enemies, and when we deployed loudspeakers broadcasting male calls in the field for several hours nightly during various seasons neither potential predators nor parasitoids were attracted. It is therefore doubtful that synchrony currently represents a strategy for evading natural enemies. Males are not distributed spatially in dense clusters which may then compete for females on an inter-group level by maximizing the group's peak signal amplitude by synchrony.

Female discrimination toward leading calls may have evolved due to various direct or indirect selection mechanisms<sup>24</sup>. The origin of the preference notwithstanding, males should compete to lead<sup>25, 26</sup>, particularly if their signals are somehow substandard. An evolutionarily stable means of such competition is inhibitory resetting, and a consequence of collective inhibitory resetting is synchrony. We do not claim that sequence discrimination and intermale signal jamming by inhibitory resetting underlie all synchronous phenomena. But we do establish empirically and theoretically that synchrony can be an incidental by-

product<sup>27</sup> of elementary interactions which did not evolve under selection pressure to generate the collective outcome. □

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