Flash Activity in Two Synchronic Firefly Species (Coleoptera: Lampyridae)[1]

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Abstract Synchronic flashing in fireflies is a precisely timed behavior. This is a potentially useful tool to study sensory processing, the location and circuitry of the flash oscillator, and neuroeffector processing and coupling. Synchronic flashing, once thought to occur only in Southeast Asian fireflies, has recently been shown to be a prominent part of the behavior of a North American *Photinus* and *Photuris* species. To gain insights into the mechanisms of synchronic timing in fireflies, we compared spontaneous flashing and entrainment flashing in *Photuris frontalis* LeConte, a synchronic firefly found in Georgia's Coastal Plain, to analogous flashing in *Pteroptyx malaccae* Olivier, a synchronic firefly found in Malaysia. The timing of spontaneously produced flashes and entrainment flashes was recorded by photometry. Artificially produced, rhythmic stimulus flashes were used to induce a counterfeit synchrony (between subject fireflies and an LED), i.e., flash entrainment. We found that the spontaneously produced interflash intervals were repeated with a high degree of precision in *P. frontalis* and *P. malaccae*. However, the pattern of flashing was different during spontaneous flashing and flash entrainment. An isolated *P. frontalis* flashed intermittently during spontaneously flashing and entrainment flashing. Flash entrainment in *P. frontalis* started with an initial inhibition and then steady-state entrainment occurred with a fixed delay. In contrast, an isolated *P. malaccae* flashed continuously during spontaneous flashing and entrainment flashing. No initial inhibition occurred at the start of entrainment, and there was a gradual change in interflash interval until steady-state entrainment occurred at a fixed delay. We think that in-depth studies of the flash activities of different synchronic firefly species, including the locally available *P. frontalis,* could help our understanding of rhythmic temporal coordination of behavior by the nervous system.

Key Words Firefly, synchronous flashing, spontaneous flashing, flash entrainment

In firefly synchrony, groups of males flash rhythmically, repeatedly, and precisely in the species-specific pattern (Buck 1988). Such synchronic flashing, with its precise regular timing, is a potentially useful tool to study sensory processing, the location and circuitry of the flash oscillator, and neuroeffector processing and coupling. The rhythmic timing of Southeast Asian firefly synchrony was considered the most precise of any firefly flash behavior or of any rhythmically occurring animal behavior (Buck and Buck 1968, Buck 1988).

Until recently, only Southeast Asian fireflies were supposed to show synchrony as an obligatory part of their flash behavior (Buck 1988). In 1995, the North American

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firefly *Photinus carolinus* Green was shown to be synchronic (Copeland and Moiseff 1995) - and in 2000, the North American firefly *Photuris frontalis* LeConte was shown to be synchronic (Moiseff and Copeland 2000). This suggests that synchrony represents a generalizable solution to problems faced by fireflies and can, thus, provide insight into understanding the general issues of sensory processing, central pattern generation, and motor processing in fireflies. Toward this end, we decided to compare the spontaneous activity of a North American firefly *P. frontalis* to the well-studied Southeast Asian firefly *Pteroptyx malaccae* Olivier (Buck and Buck 1968; Buck 1988), and to compare as well a type of stimulus-driven synchronous flashing, called flash entrainment.

Materials and Methods

Pterptyx malaccae were collected in Pontian, Malaysia, and studied in Singapore in 1992. *Photuris frontalis* were collected and studied on Skidaway Island, GA, in 1997. Each firefly was placed in a 9-cm diameter Petri dish lined with wet filter paper. Apple shavings were changed every other day.

In all experiments, flashes were recorded from isolated 9-cm diameter Petri dish cages using a side-window RCA-9611 photomultiplier. Fireflies were tested during the night between 2000 and 100 h. Photomultiplier output was digitized (1000 samples/se) and processed off-line to determine flash timing, precision, and pattern.

In a flash entrainment experiment, customized software was used to provide a 1 min entrainment trial similar to that used by Hanson (1978) and others (Buck et al. 1981a, b). Entrainment experiments were conducted in 60-second trials. During the first 10 s of each trial, the subject's spontaneous flashing was recorded (i.e., no artificial stimuli were produced during this period). During the remaining 50 s, an entrainment stimulus was presented rhythmically at an interval just greater than the firefly's spontaneous interflash interval. The entrainment stimulus consisted of a green LED whose output was shaped to provide a counterfeit flash. The counterfeit flash was termed "normal-like" when the pattern and timing of the stimulus flash resembled the firefly's species-specific flash (a single pulse for *P. frontalis* and a double pulse flash for *P. malaccae*). When the counterfeit flash had an instantaneous onset and offset and a otherwise constant intensity for the duration of the pulse, the stimulus was called "rectangular".

Results

Spontaneous flashing by an isolated firefly. In an earlier study (Moiseff and Copeland 2000), individual *P. frontalis* flying males observed under free-flying field conditions were seen to flash intermittently. That is, though the firefly produced the species-specific flash at the species specific interflash interval, the pattern of flashes was punctuated by it stopping and then, after a pause, resuming. When two or more intermittently flashing *P. frontalis* assembled and flashed synchronically (Moiseff and Copeland 2000), the intermittent species specific flashing also was seen in the synchrony that was produced. The type of synchrony produced by two intermittent flashes was called intermittent synchrony (Copeland and Moiseff 2000). Both flying flashing males and males that had landed produced an intermittent synchrony.

To study synchrony with photometers at a high temporal resolution and to have stimulus control as well, field-captured individuals were placed in Petri dish cages.

The cages used were large enough to allow movement, and the fireflies flashed both spontaneously and synchronously when either walking or stationary.

Typical examples are shown in raster-like records (Fig. 1). The continuous 1-min records read from left-to-right and top-to-bottom. In the North American synchronizer *P. frontalis* (Fig. 1), the isolated spontaneously active firefly stopped (arrow) and started (circle) twice. When flashing began again, it occurred later than would have been predicted if the original interflash interval had been maintained during the cessation of flashing. (Arrows added to the record indicate the predicted occurrences of the missing flashes). If the stop-start indicated the gating of the control of the flash-producing organ (lantern), flashes would have resumed at the positions predicted by the arrows. However, when the flashes resumed, the flashes did not resume at the predicted position but, rather, at delayed times. This indicated that the stop-starts were not merely the result of on-off gating of the lantern control circuitry, but probably reflect a modulation of the oscillator circuitry itself.

The North American synchronizer *P. frontalis* showed stop-starts as part of its isolated spontaneous flashing (Fig. 1, Table 1) (Moiseff and Copeland 2000). To

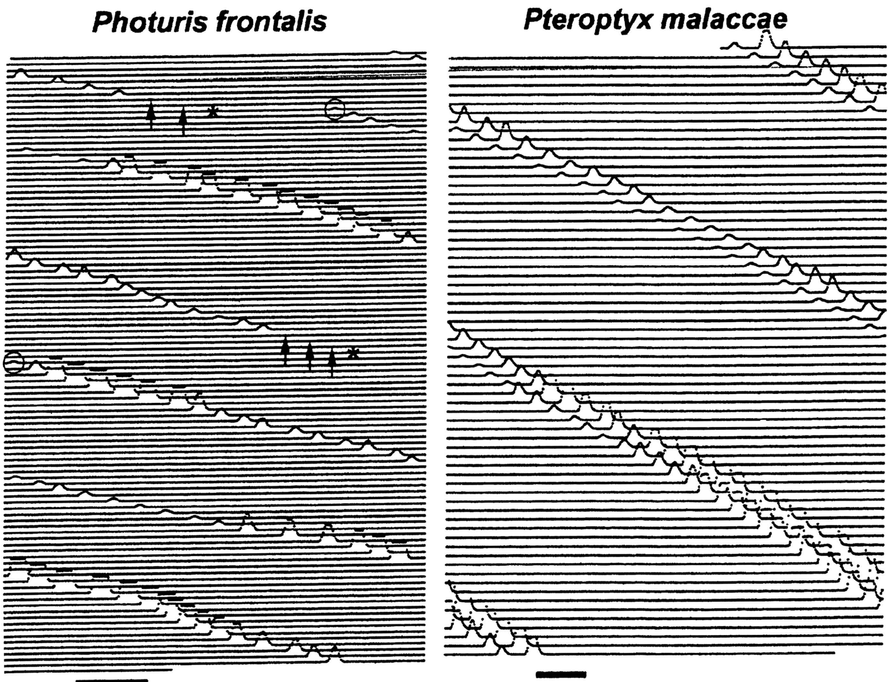

Fig. 1. Spontaneous flashing recorded by photometer from an isolated caged male. *P. frontalis* spontaneous flashing shows two stop-starts and *P. malaccae* shows continuous flashing with no stop-starts. Arrows indicate the expected position of the missing flashes that would have occurred if the flashing had continued. The asterisk (*) indicates where the expected resumed flash would have occurred, and the circle indicates the resumption of flashing. The black horizontal line at the bottom of each record indicates a 100 msec time base.

Table 1. The number of times that intermittent flash activity (stop-starts) occurred during rhythmic spontaneous flashing in the North American synchronic firefly *P. frontalis* and the Southeast Asian synchronic firefly *P. malaccae*

P. frontalis				*P. malaccae*			
Individual	Total # of stop-starts	*N	Stop-starts/min	Individual	Total # of stop-starts	*N	Stop-starts/min
A	6	3	2.0	F	0	3	0
B	6	3	3.0	G	0	3	0
C	9	4	2.2	H	0	3	0
D	13	4	3.2	I	1	3	0.3
E	32	5	8.0	J	2	3	0.6

address the question of whether or not stop-starts represent an obligatory aspect of flash production or of the circuitry producing synchrony, we compared patterns produced by *P. frontalis* with data obtained from isolated spontaneously active *P. malaccae* tested under similar conditions and with identical instrumentation. Results from *P. malaccae* showed continuous flashing and did not show stop-starts as a regular part of its isolated spontaneous flashing (Fig. 1, Table 1). Stop-starts were a characteristic of the spontaneous flashing of isolated *P. frontalis* males and infrequently seen in *P. malaccae* males.

The precision (regularity) of the species-specific interflash interval of individual fireflies was first measured by Buck and Buck (1968). Because different firefly species can have average intervals differing by thousands of milliseconds, and because a larger average interflash interval can have a larger variability than a smaller average interflash interval, the coefficient of variation ($CV = s/X \times 100$) was used (Buck and Buck 1968, Lehner 1996). The differences between CV's were compared using the C-statistic (Lehner 1996).

A comparison of the average interflash intervals for five individuals of each species showed species-specific differences of several hundred milliseconds in average interflash interval (Table 2). However, a comparison of the precision of the interflash interflash of *P. frontalis* and *P. malaccae* indicated no difference (C statistic, $P > 0.05$). The CV values ranged from 1.74 to 3.13 in *P. frontalis* and 1.18 to 3.08 in *P. malaccae* (Table 2).

Flashing during Flash Entrainment. *In P. frontalis,* an initial inhibition occurred during an entrainment trial immediately after the LED began to flash (Fig. 2, Table 3). The initial inhibition continued for 5 to 22 s per trial (Table 3) and then flashing (flash entrainment) began, this time at a shorter interflash interval than prior to photic stimulation. The flash delay (the time measured from the beginning of the LED flash to the beginning of the next firefly flash) did not change appreciably once entrainment began (Fig. 2). Thus, in this species, once entrainment began, it was always a steady-state entrainment. Once entrainment flashing began, 4 to 7 stop-starts occurred during a 1-min trial (Fig. 2, Table 4).

Pteroptyx malaccae (Fig. 2, Table 3), on the other hand, showed no initial inhibition of spontaneous flashing at the beginning of an entrainment trial. It showed a gradual

Table 2. Precision of the flashes that make up the spontaneous interflash interval in the North American synchronic firefly *P. frontalis* and the Southeast Asian synchronic firefly *P. malaccae*

P. frontalis				*P. malaccae*			
Individual	Interflash interval X ± SD (msec)	Precision*	N**	Individual	Interflash interval X ± SD (msec)	Precision*	N**
A	629 ± 12	1.91	75	F	934 ± 11	1.18	47
B	634 ± 11	1.74	88	G	999 ± 22	2.20	58
C	660 ± 16	2.42	80	H	1138 ± 35	3.08	55
D	691 ± 12	1.74	79	I	1247 ± 16	1.28	54
E	704 ± 22	3.13	51	J	1310 ± 35	2.67	55

* Precision = (SD)/X · 100 = coefficient of variation.
** N = number of interflash intervals.

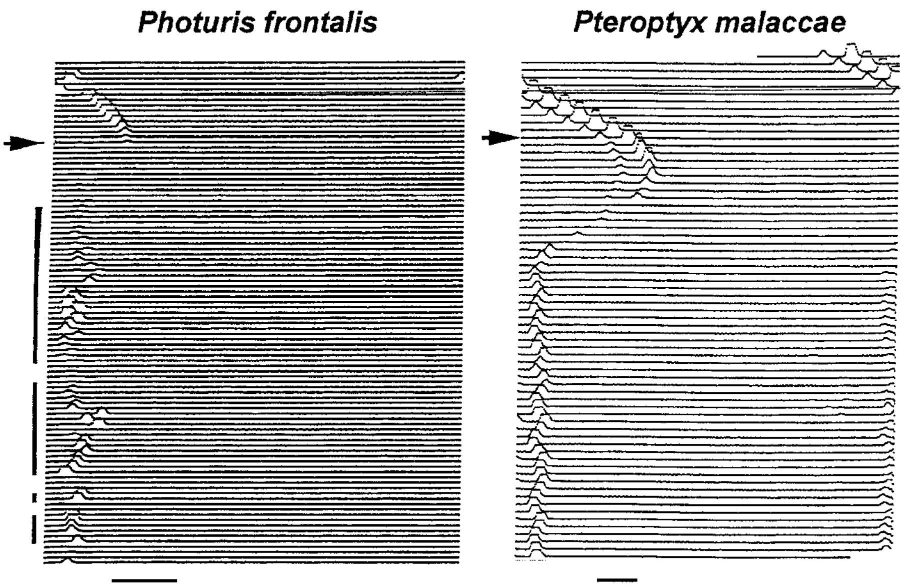

Fig. 2. Flash entrainment recorded by photometer from an isolated caged male. The arrow indicates the start of LED flashing. Both species show steady state entrainment. In *P. frontalis,* initial inhibition and stop-starts occurred. Initial inhibition began at the arrow and ended at the black line (where entrainment flashing began). Three stop-starts (breaks in the black line) occurred in this example. To be considered a stop-start, a pause had to extend beyond two interflash intervals. Flash entrainment is gradual in *P. malaccae* and shows no stop-starts. The black horizontal line at the bottom of each record indicates a 100 msec time base.

Table 3. Duration of the initial inhibition that occurred during flash entrainment in the North American synchronic firefly *P. frontalis* and the Southeast Asian synchronic firefly *P. malaccae*

P. frontalis			*P. malaccae*		
Individual	Duration of initial inhibition X ± SD (sec)	N*	Individual	Duration of initial inhibition X ± SD (sec)	N*
Synthesized Species Specific Stimulus					
A	5.8 ± 4.8	5	F	0	1
B	7.4 ± 6.8	3	G	0	2
C	10.1 ± 5.1	6	H	0	2
D	18.3 ± 14.7	8	I	9.0 ± 8.9	7
E	22.5 ± 12.7	2			
Synthesized Rectangular Stimulus					
J	13.6 ± 11.3	5	L	0	1
K	50	1	M	0	3
			N	0	5
			O	0	
			P	2.1 ± 1.9	7

* N = number of 1 minute samples examined.

lengthening and shortening of the interflash interval (Fig. 2) until a steady state entrainment occurred. Flash entrainment in *P. malaccae,* like spontaneous flashing, was continuous and showed no stop-starts, making it different from *P. frontalis.*

Discussion

We compared spontaneous flashing and entrainment flashing in the well-studied Southeast Asian synchronizer *P. malaccae* and the recently discovered North American synchronizer *P. frontalis* to determine whether *P. frontalis* might serve as a useful preparation for neurobehavioral studies of synchrony and the precise control of rhythmic behaviors in insects.

P. frontalis and *P. malaccae* show similarities in their flash behavior. In both, spontaneous (Fig. 1) and synchronic flashing occurs in caged (unpubl. data) and free-flying animals (Moiseff and Copeland 2000). Spontaneous and synchronic flashing is not unusual for caged male *Pteroptyx* fireflies (Buck and Buck 1968, Ballentyne 1987, Ballentyne and McLean 1970), but male *Photuris* fireflies rarely show spontaneous species-specific flashing while caged (Barber 1951, Carlson et al. 1982, Carlson and Copeland 1985). In both firefly species, synchronic flashing can be produced by flash entrainment (Fig. 2), the flash entrainment involves a change in interflash interval, and stable entrainment occurs at a fixed delay. In *P. cribellata,* another well studied Southeast Asian synchronizer (Hanson 1978, Buck et al. 1981a, b), the interflash interval does not change during flash entrainment.

Photuris frontalis and *P. malaccae* show differences in their flash behavior as well. Spontaneous activity (Fig. 1, Tables 1 and 2), synchronic activity (Moiseff and Copeland 2000 unpubl. data), and flash entrainment (Fig. 2, Tables 3 and 4) are all

Table 4. The number of times that intermittent flash activity (stop-starts) occurred during flash entrainment in the North American synchronic firefly *P. frontalis* and the Southeast Asian synchronic firefly *P. malaccae*

P. frontalis			*P. malaccae*		
Individual	Number of stop-starts X ± SD (msec)	*N	Individual	Number of stop-starts X ± SD (msec)	*N
Synthesized Species Specific Stimulus					
one pulse (40 msec)			double pulse (80 msec)		
A	4.5 ± 2.3	8	F	0	1
B	5.8 ± 4.8	5	G	0.25 ± 0.1	2
C	6.8 ± 1.4	6	H	0.25 ± 0.2	2
D	7.0 ± 1.0	3	I	2.3 ± 8.9	7
E	7.5 ± 0.7	2			
Synthesized Rectangular Stimulus					
J	0 ± 5	5	L	0	1
K	7	1	M	0	3
			N	0	5
			O	0	5
			P	0	7

intermittent in *P. frontalis* and continuous in *P. malaccae* (Figs. 1 and 2) (Buck and Buck 1968). When flash entrainment occurs in *P. frontalis* (Fig. 2), it happens following an immediate initial inhibition; whereas, an initial inhibition is not characteristic of flash entrainment in *P. malaccae,* and the change in interflash interval is gradual.

Little is known about the neural mechanisms of synchrony at this time, but we speculate if the circuit were to consist of eye—to central flash oscillator—to lantern control circuitry, and if the central flash oscillator were spontaneously active and produced the spontaneous species -specific flash, flash differences between *P. frontalis* and *P. malaccae* might be explained by differences in the dynamics of the flash oscillator. The oscillator of *P. frontalis* could spontaneously reset itself and a stop-start (pause) might be produced. This would manifest itself as a resetting at the correct rhythm (interflash interval), but at a new phase from the previous rhythm (Fig. 1). If flash entrainment comes about when rhythmic photic input modulates the central flash oscillator via a flash entrainment network, *P. frontalis* and *P. malaccae* flash entrainment might be produced similarly if the lantern control circuitry (motor output) were inhibited while the central flash oscillator gradually changed its interflash interval. This could explain the appearance of a seemingly instantaneous entrainment in *P. frontalis* following the initial inhibition (Fig. 2).

Spontaneous interflash interval flash precision (CV) is similar in both species (Table 1). The precision for *P. frontalis* is similar to the precision for *P. malaccae* (as measured here) and for Southeast Asian fireflies (Buck and Buck 1968). Overall, there have been few data collected to allow for a comparison of CV in North American *Photuris* and *Photinus* rover (non-synchronic) fireflies. However, where data have been collected with high temporal resolution instrumentation (Buck and Buck 1968, 1972), a larger CV (less precision) has been reported in other North American fireflies than has been found for *P. frontalis* and *P. malaccae.* Because Southeast Asian firefly synchrony has been considered to be the most precise rhythmic behavior in animals (Buck and Buck 1968), we were surprised at the similarity of interflash interval precision in *P. frontalis* and *P. malaccae.*

Our goals were to gain insights into the mechanisms of synchronic flashing in fireflies and to see if the more accessible Georgia coastal plain firefly *P. frontalis* was sufficiently similar to the well-studied Southeast Asian synchronizer *P. malaccae* to recommend its further usage in neurobehavioral studies of biological timing. We have found that the species are similar in their spontaneous activity precision, but they have notable differences in the pattern of spontaneous activity (stop-starts) and their entrainment flashing (initial inhibition and stop-starts). These differences might reflect physiological differences in the neural machinery and have no behavioral meaning. Alternatively, these differences are clues about the enigmatic behavioral function of synchrony (Buck 1988).

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