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Short Communication

Influence of photoperiod on Temporal Mate Signaling Patterns in the Texas Field Cricket, *Gryllus texensis*

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INTRODUCTION

Crickets appear to be entrained to the circadian mechanism in which they are reared (Wiedenmann, 1988; Wiedenmann *et al*., 1988), and photoperiod influences a variety of cricket behaviors including egg laying (Olvido *et al*., 1998), induction of embryonic diapause (Shiga and Numata, 1996), egg hatching (Olvido *et al*., 1998; Itoh and Sumi, 2000), nymphal growth rate (Carriere *et al*., 1996), and adult wing from (Masaki and Shimizu, 1995). Photoperiod is also known to influence mate signaling in a variety of insect species including calling onset and duration in gypsy moths, *Lymantria disar* (Webster and Yin, 1997), and calling onset and temporal pattern in Australian common armyworms, *Mythimna convecta* (Del Socorro, 1997). In crickets, however, the evidence that photoperiod affects acoustic mate signaling is suggestive but not conclusive.

Photoperiod influences time spent calling in the house cricket, *Acheta domesticus*. In an experiment designed to test the effect of a strong electromagnetic field on the circadian singing activity, Shaw *et al*. (1995) found that control males (unexposed to the electromagnetic field) monitored in total darkness (0L:24D cycle) called significantly more often than males monitored in a more natural light cycle (12L:12D cycle). This research,

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while suggestive of the potential influence of photoperiod on calling behavior, is based on such extreme scotophase shifts that interpretations cannot be easily extrapolated to more environmentally natural conditions. Conversely, in an experiment designed to determine if developmental conditions and parental generation affect the pulse rate of calling field crickets, Walker (2000) reared sibships of *Gryllus rubens* and *G. texensis* crickets under two environmentally natural temperatures and photoperiods and found that while rearing temperature influenced pulse rate, photoperiod did not significantly affect it. Lastly, Olvido and Mousseau (1995) reared striped ground crickets (*Allonemobius fasciatus*) in two environments differing in photoperiod and temperature (31°C, 15L:9D and 24°C, 11L:13D) and then monitored mating signals at three temperatures. Statistically significant interactions were found between monitoring temperature and rearing environment for several calling parameters (including chirp rate, chirp duration, interchirp interval, pulse number, and carrier frequency), signifying that the environment in which nymphs develop determines how the adult environment influences calling song. While this research suggests that environmentally realistic shifts in photoperiod may influence several parameters of calling song, the experimental design precludes teasing apart the influences of photoperiod from the influences of temperature. Thus it is necessary to determine how cricket calling behavior is influenced by natural shifts in photoperiod.

The Texas field cricket, *Gryllus texensis*, is a useful species for investigating whether photoperiod directly influences calling behavior. Bivoltine populations occur in the southeastern United States, where first-generation adults mature in spring under a 14L:10D photoperiod. They mate and produce primarily nondiapausing second-generation progeny that develop through summer. Second-generation progeny mature in fall under a 12L:12D photoperiod. Fall adults mate and produce diapausing progeny who become the first-generation (spring) adults the following year.

Previous field research provides indirect correlative evidence suggesting that photoperiod influences mate-signaling behavior. Although there is extensive variation among individuals (Cade, 1991), spring and fall populations differed in their temporal calling components when captured as adults and monitored in the field over four successive mating seasons. Spring populations called most in the early morning hours, whereas fall populations distributed their calls more evenly throughout the night (Bertram, 1999, 2002b). Further, fall populations spent more time calling than spring populations (Bertram, 1999, 2002b). Our goal is to determine whether the 2-h difference in scotophase between the spring and fall mating seasons influences the temporal calling components of male crickets, thereby explaining the seasonal differences observed in the field.

METHODOLOGY

Juvenile crickets were reared to maturity and monitored under spring (sunset 7:30 PM; sunrise 5:30 AM; 14L:10D) and fall (sunset 6:30 PM; sunrise 6:30 AM; 12L:12D) photoperiods to determine the influence of photoperiod on acoustic mate signaling. All crickets were first-generation offspring of several hundred adults captured from light aggregations in Austin TX in September 2000 and then maintained in the laboratory under a fall photoperiod regime at ~26°C. First to third instars were randomly placed in spring and fall treatments and reared through maturity. Crickets experienced an abrupt transition between light and dark at sunrise and sunset and were reared at ~26°C with unlimited food and water. Once males reached maturity, they were housed individually in half-liter containers to minimize social effects. Individual housing also allowed us to monitor each male's acoustic mating signals.

Signal monitoring commenced 7–10 days after final molt. Males were monitored from 5:00 PM to 10:00 AM for 3–7 days in their rearing photoperiod. We monitored 109 males in the spring photoperiod and 155 males in the fall photoperiod. Two temporal characteristics of *G. texensis* mating song were monitored: time spent calling (TSC) per 24-h period, and temporal calling pattern (TCP) a family of parameters (start time, mean time, and stop time) depicting how males partition calls throughout the night (Bertram and Johnson, 1998). TSC was quantified using the number of hours called per night. TCP was described using: (1) start-time: the first time the male was observed to call within the night, (2) stop-time: the last time the male was observed to call within a night, and (3) mean time: a general index of when a male calls through the night, and measured as the normalized mean of the indicator function of time, $I(t)$ (Bertram, 2000).

$$\text{Mean time} = \frac{\int_{6:00\ \text{PM}}^{10:00\ \text{AM}} I(t)t\,dt}{\int_{6:00\ \text{PM}}^{10:00\ \text{AM}} I(t)\,dt}$$

TSC and TCP were monitored using an electronic device that recorded each male's signaling/nonsignaling behavior, six times per second (Bertram and Johnson, 1998). To ensure microphone accuracy, two microphones were placed in each container. When the result of both microphones were positive, the animal was recorded as calling. When only one microphone was positive, or both microphones were negative, the animal was recorded as not calling.

The data presented are averages for each male. We tested the results for normality using the Shapiro-Wilk statistic. Because the temporal calling

components were not normally distributed, we used the nonparametric Kolmogorov-Smirnov goodness-of-fit tests to determine if calling song differed significantly between the two laboratory populations reared under different photoperiods. We also used a Kolmogorov-Smirnov goodness-of-fit test to determine if the two laboratory populations differed from crickets captured and monitored in the field. Field populations were collected from bright streetlight aggregations at a driving range, surrounded by large grassy fields, in the outskirts of Austin, Texas, in the spring and fall of 1998 and 1999 (see Bertram, 2002b, for details). These analyses resulted in 12 different tests, necessitating Bonferroni corrections to $P < 0.0042$. We provide exact probabilities throughout.

RESULTS

Males differed dramatically in when and how much they called throughout the night (Table I; Figure 1). Approximately 33% of males called, some for only a few minutes, others for an hour or two, and one male called for almost 6 h a night. Average TSC of calling males was around 40 min a night. Some males started calling as early as an hour before dark, while others did not call until morning. Average start time was just after 11:00 PM. Some males stopped calling very early in the night, others did not stop calling until several hours after sunrise. Average stop time was around 3:00 AM.

Table I. Time Spent Calling (TSC) and Temporal Calling Pattern (TCP: Start Time, Mean Time, and Stop Time) Summaries (Mean ± SE) and Statistical Comparisons Between Populations Reared and Monitored in Spring and Fall Photoperiod[a]

	Spring	Fall	*P*
Sunset	7:30 PM	6:30 PM	
Sunrise	5:30 AM	6:30 AM	
Scotophase	10 hr	12 hr	
Monitored (*N*)	109	155	
Calling [*N* (%)]	36 (33)	49 (32)	
TSC (callers)	48 ± 15 min	36 ± 6 min	0.012
Start time min.	5:12 PM	5:06 PM	
Start time average	11:06 PM ± 46 min	11:30 PM ± 46 min	0.101
Start time max.	9:30 AM	9:48 AM	
Mean time min.	5:24 PM	5:12 PM	
Mean time average	12:54 AM ± 39 min	1:06 AM ± 41 min	0.716
Mean time max.	9:30 AM	9:48 AM	
Stop time min.	5:24 PM	5:18 PM	
Stop time average	2:54 AM ± 44 min	3:18 AM ± 42 min	0.790
Stop time max.	9:30 AM	9:54 AM	

[a] Because 12 Kolmogorov-Smirnov tests were run, the typical significance value of $P < 0.05$ was Bonferroni adjusted to $P < 0.0042$.

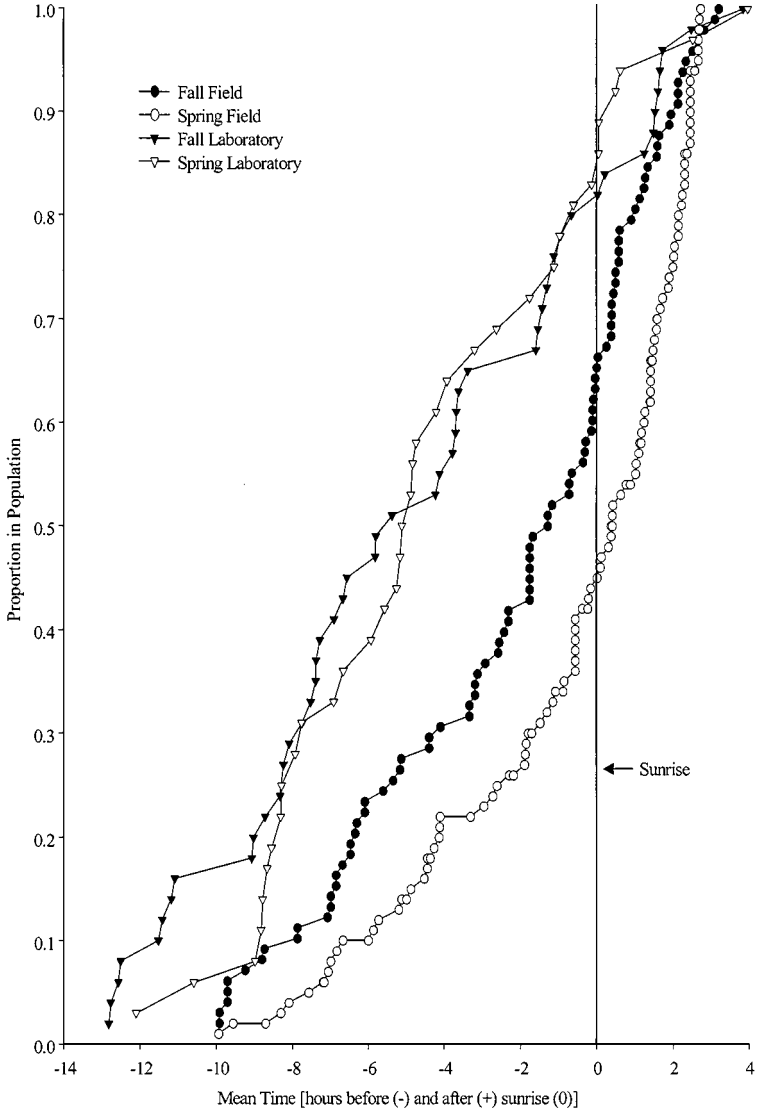

Fig. 1. Mean time, depicting when males focus their calls throughout the night, of individuals laboratory-reared and monitored in fall and spring photoperiod treatments (triangles) along with data of individuals captured as adults in the field and monitored in spring and fall during the 1998 and 1999 breeding seasons (Bertram, 2002b) (circles). Mean time is presented with respect to hours before (−) or after (+) sunrise. There was no significant difference in calling behavior between the two laboratory-reared treatments ($P = 0.716$). However, laboratory-reared males called earlier in the evening than males captured and monitored in the field during the spring ($P < 0.001$) and fall ($P = 0.002$) mating seasons.

Mean time, a general index of when a male calls through the night, was also highly variable. Some males called most in the early evening, others called throughout the night, while others called most around dawn. Average mean time was around 1:00 AM.

We hypothesized that the population reared in the spring photoperiod treatment would call most in the early morning hours around dawn, whereas the population reared in the fall photoperiod treatment would call more often than the spring-reared population and distribute its calls evenly throughout the night. Our hypothesis was not supported. Spring and fall populations did not differ in when they called throughout the night (Table I; Figure 1). Further, while there was a trend for males reared in the fall photoperiod treatment to call for less time than males reared in the spring photoperiod treatment, this trend was not statistically significant (acceptable significance Bonferroni corrected to $P < 0.0042$) and in a direction that was contrary to our hypothesis. While there was no significant difference in calling behavior between the two laboratory-reared treatments, these males did call earlier in the evening than the males monitored in the field during the spring and fall mating seasons (Figure 1; Kolmogorov-Smirnov goodness-of-fit tests: Start time springs $P < 0.001$, falls $P = 0.031$; mean time springs $P < 0.001$, falls $P = 0.002$; stop time springs $P < 0.001$, falls $P < 0.001$).

DISCUSSION

Contrary to our hypothesis, spring/fall photoperiod differences did not lead to the spring-reared population calling less often than the fall-reared population. The two populations did not differ in their TSC behavior. Furthermore, photoperiod differences did not lead to the spring-reared population calling most around dawn. Both spring and fall-reared populations distributed their calls evenly throughout the night. Overall, these results suggest that a 2-h increase in darkness coupled with a 2-h decrease in light does not influence when and how much crickets signal through the night.

The 2-h difference in scotophase between spring and fall breeding seasons mimics scotophase shifts observed in *G. texensis*'s natural environment. Photoperiod regulation and response is a complex series of events, including photoreception, scotophase measurement, integration, and storage of the cryptic effects of scotophase measurements to some internal threshold, and the regulation of the appropriate responses when the scotophase measures surpass the threshold (Nunes and Saunders, 1999). Because photoperiod regulation and response is so complex, it is possible that the 2-h scotophase shift used here was less than the threshold necessary to cause a behavioral response.

The photoperiods examined were based on the assumption that the scotophase for *G. texensis* is the time between sunset and sunrise and the photophase is the time between sunrise and sunset. Because insects often count evening and morning twilights (approximately 30 min each at 30 degrees latitude) as part of the photophase, the photoperiods may have had scotophases about an hour shorter than the natural photoperiods. However, since both fall and spring laboratory populations did not have an evening or morning twilight effect, we doubt that including one would result in the seasonal differences observed in the field populations.

To date, much research on photoperiod and insect calling behavior has concentrated on scotophase shifts greater than 2 h. For example, in the Australian common armyworm, *Mythimna convecta*, peak temporal calling pattern (similar to mean time) did not differ between individuals reared under 16L:8D and 14L:10D environments, but shifted an hour earlier in the 12L:12D treatment (Del Socorro, 1997). Manipulating the photoperiod in more extreme ways may result in a calling behavior response in *G. texensis*.

Overall, our results suggest that photoperiod differences between spring and fall breeding seasons do not explain the field observations that spring populations call less often than fall populations and that spring populations call most around dawn while fall populations disperse their calls more evenly throughout the night. Seasonal differences in calling song may be rooted in differences in the selective regimes with which the populations were exposed (Bertram, 2002a,b). It is also possible that temperature differences, or a combination of temperature and photoperiod differences, influence calling song, as thermoperiods co-occur with photoperiods in the field. Supporting this argument, the temperature at which calling song is produced affects the rate at which *G. texensis* opens its wings, resulting in an affect on pulse length, interpulse length, peak frequency, trill length, intertrill interval, and pulse duty cycle (Martin *et al*., 2000). However, the temperature at which *G. texensis* are reared does not influence the number of pulses per trill (Gray and Cade, 1999) or the pulse rate in *G. texensis*, although Walker (2000) attributes this negative result to a small sample size after finding a significant affect in *G. rubens*. Further evidence against this argument comes from the fact that in the four successive generations in which seasonal differences in calling song were observed, neither TSC nor TCP components significantly correlated with seasonal temperature differences (Bertram, 2002b).

Attention should be drawn to the fact that there are calling song differences between the laboratory-reared populations and the field-monitored populations. It is unlikely that these differences are due to the monitoring environment, as Bertram (2002a) has shown that males captured in the field and monitored in the laboratory produce similar songs to those of males captured and monitored in the field. Laboratory-reared males likely call earlier in the

evening than field-monitored males because there is either something fundamentally abnormal about laboratory rearing conditions or crickets reared in the field are exposed to different selective regimes than those reared in the laboratory. Bertram (2002a) deals directly with the question of why calling songs may differ between laboratory and field populations.

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