TEMPORALLY FLUCTUATING SELECTION OF SEX-LIMITED SIGNALING TRAITS IN THE TEXAS FIELD CRICKET, *GRYLLUS TEXENSIS*

SUSAN M. BERTRAM
Department of Biology, Arizona State University, Tempe, Arizona 85287–1501
E-mail: sbertram@asu.edu

Abstract.—*Gryllus texensis* males produce acoustic mating signals and display extensive heritable variation in when and how much time they spend signaling throughout the night. The goal of this research is to elucidate the potential mechanism responsible for maintaining this heritable variation. Mating signals attract female crickets. In low-density spring populations females select males that signal most often; in high-density fall populations mating appears random with respect to signaling time. Mating signals also inadvertently attract acoustically orienting parasitoid flies; parasitoids are prevalent during the first half of the evening in the fall mating season. I hypothesized that mating signals are influenced by sex-limited temporally fluctuating selection. I predicted how mating signals would respond to this pattern of cyclical selection a priori, and then measured the sexual characters over four successive generations. I provide correlative evidence that mating signals appear to respond to sex-limited temporally fluctuating selection. These results indicate that sex-limited temporally fluctuating selection may play a role in the maintenance of variation in these sexual characters.

Key words.—*Gryllus texensis*, mating, phenotypic evolution, temporally fluctuating sex-limited selection.

Received January 11, 2001.   Accepted June 26, 2002.

A primary goal of modern theoretical evolution is to understand the selective forces shaping variation in heritable traits. Fisher's fundamental theorem of natural selection indicates that at equilibrium selection should have depleted most genetic variance in fitness (Fisher 1958 [1930]). In fact, minimal heritable variance in either fitness or its components has become a long-held expectation, based on the notion that fitness is usually influenced by directional selection, and therefore a single best genotype should predominate (Falconer 1981; Charlesworth 1984; Charlesworth 1987). An extensive comparison of over 200 animal population studies indicates, however, that fitness-conferring traits exhibit higher levels of genetic variation than other traits (Houle 1992). This major inconsistency between data and theory reveals the importance of elucidating the mechanisms responsible for maintaining genetic variation in fitness conferring traits.

Sexual characters used by males to attract females have an inherent ability to influence fitness and should therefore display minimal amounts of heritable variation. A comprehensive literature review has, however, revealed that sexual characters exhibit even higher levels of additive genetic variation than other fitness-conferring traits in similar taxa (Pomiankowski and Møller 1995). Few investigations have directly examined how heritable variation is maintained in sexual characters (cf Pomiankowski and Møller 1995) making this question ripe for exploration.

The goal of my research is to investigate how heritable variation may be maintained in the sexual characters of male Texas field crickets (*Gryllus texensis*). Male Texas field crickets solicit potential mates by rubbing their raised forewings together to emit an acoustic mating signal known as ''calling song.'' Calling song is composed of repeated pulses of sound grouped into trills. Each pulse is produced by the closing stroke of the modified forewings. The ''scraper'' on the upper surface of the lower wing engages a ''file'' on the lower surface of the upper wing. Moving the scraper along the file causes the wings to vibrate a characteristic frequency, resulting in calling song (Bennett-Clark 1989).

Calling song is highly variable among males: males vary in peak frequency, number of pulses per trill, trill length, intertrill interval, amplitude, pulse rate, and percentage of missed pulses (Cade 1981; Cade and Wyatt 1984; Smith and Cade 1987; Souroukis et al. 1992; Gray and Cade 1999a). My research focuses on two characteristics of *G. texensis* calling song, time spent calling (TSC), and temporal calling pattern (TCP). TSC quantifies the amount of time a male calls throughout the night, is highly variable (Cade 1981; Cade and Cade 1992; Bertram and Johnson 1998; Bertram 1999, 2000), as well as highly heritable ($h_r^2 = 0.50$; Cade 1981). Some males never call, others call for a few minutes to a few hours a night, while others call for more than 10 hours in an evening (Bertram and Johnson 1998; Bertram 1999, 2000). TCP quantifies when a male calls throughout the night, is also highly variable among males, and includes a trio of parameters: start time, stop time, and mean time. ''Start time'' is the time that a male first calls and can be as early as dusk to as late as 0800 h. ''Stop time'' is the last time a male is observed to call in an evening. Some males stop calling by midnight whereas others do not stop until several hours after dawn. ''Mean time'' suggests how a male partitions his signals throughout the night and is indicative of peak calling time. Some males call most in the early evening, with mean times as early as 2100 h, whereas others concentrate their calls around dawn, with mean times as late as 0900 h.

Some variation in calling song is environmentally induced. Texas field crickets have two mating seasons each year, spring and fall, and are therefore exposed to a bimodal range of temperatures and photoperiods. Temperature affects the rate the wing opens in *G. texensis*, resulting in an affect on pulse length, interpulse length, peak frequency, trill length, intertrill interval, and pulse duty cycle (Martin et al. 2000). Even after temperature is controlled for, significant variation in peak frequency, trill length, and inter trill interval still exists, suggesting a genetic component (Martin et al. 2000).

Temperature at which males call does not appear to affect the number of pulses per trill, the proportion of pulses missed within a trill, or the trill duty cycle (Martin et al. 2000), which are all calling parameters that exhibit substantial variation. Further, the temperature at which *G. texensis* males are reared does not significantly influence the number of pulses per trill (Gray and Cade 1999b). Finally, photoperiod does not appear to influence *G. texensis* calling song. Walker (2000) reared *G. rubens* and *G. texensis* crickets in different temperatures (25°C and 32°C) and photoperiods (16:8 LD and 11:13 LD) and found that although rearing temperature affected pulse rate in *G. rubens*, rearing photoperiod did not significantly influence pulse rate in either species. Likewise, Bertram and Bellani (2002) reared *G. texensis* crickets in spring (14:10 LD) and fall (12:12 LD) photoperiods and found rearing photoperiod did not significantly influence TSC or TCP. Overall, environmental effects appear to only partially explain the variation observed in *G. texensis* mating signals.

Genetic effects have been found to influence calling song in a variety of cricket species (Hedrick 1988; Olvido and Mousseau 1995; Mousseau and Howard 1998; Roff et al. 1999), and several *G. texensis* calling song parameters exhibit a strong heritable component. Time spent calling is heritable, based on realized heritability estimates resulting from selection on high and low lines over four generations (h_r^2 high = 0.50; h_r^2 low = 0.53; TSC in *G. texensis* from Austin, TX; Cade 1981). Heritability of number of pulses per trill has been estimated at $h^2 = 0.37 \pm 0.13$ (F$_1$ full-sib design), $h^2 = 0.40 \pm 0.14$ (F$_2$ full-sib design), and $h^2 = 0.39 \pm 0.19$ (father-son regression; *G. texensis* from Austin, TX; Gray and Cade 1999b). Further, in *G. integer*, a closely related species, heritability of the percentage of time spent in short calling bouts has been estimated at $h^2 = 0.75 \pm 0.25$ (father-son regression) and $h^2 = 0.69 \pm 0.24$ (F$_1$ full-sib design; Hedrick 1988).

Provided a portion of the variation is heritable, two general forces can potentially contribute to higher heritable variance in sexual characteristics: weaker selective loss and higher mutational input (Pomiankowski and Møller 1995). In an effort to determine the process by which the heritable variation may be maintained in male Texas field cricket's calling song, I created an a priori hypothesis about the pattern of selection influencing male sexual characters. I then predicted how calling song should respond to this hypothesized pattern of selection and measured male calling song over four successive generations.

I hypothesized that the pattern of selection influencing the mating signals of the Texas field cricket is (1) sex-limited, (2) countervailing, and (3) temporally fluctuating in nature. First, I hypothesized that selection is sex-limited because only males produce mating signals. Second, I hypothesized that sexual and natural selection are countervailing forces because calling song positively influences mate choice and concomitantly increases the likelihood of a calling male cricket being parasitized by acoustically orienting parasitoid flies (Diptera: Tachinidae, *Ormia ochracea*) (Cade 1975; Gray and Cade 1999b). Reported parasitism rates vary for *G. texensis* from 12% (Adamo et al. 1995a) to 64% (Walker and Wineriter 1991), with flies primarily parasitizing male

crickets. Larvae burrow into the cricket host to feed and grow (Adamo et al. 1995a). Parasitism results in cricket death in approximately seven to 10 days (Adamo et al. 1995b).

Last, I hypothesized that selection is temporally fluctuating. Selection fluctuates in a cyclical manner between spring and fall mating seasons. In spring there are few parasitoids and the density of crickets is low. In low, spring-like densities, field experiments indicate females mate with males that call most often ($r = 0.32$, $P < 0.001$), and there is significant direct selection (β') in favor of calling time ($\beta' \pm SE = 0.26 \pm 0.09$, $t = 2.8$, df = 107, $P = 0.005$; Cade and Cade 1992). Cricket and parasitoid densities are much higher in fall. In high, fall-like densities, field experiments indicate mating is random with respect to calling time ($r = 0.05$, $P > 0.05$), and there is no significant direct selection (by female crickets) on calling time ($\beta' \pm SE = 0.02 \pm 0.1$, $t = 0.2$, df = 89, $P = 0.8$; Cade and Cade 1992). As parasitoid flies acoustically orient to calling male crickets (Cade 1975), fall selection should confer a selective disadvantage to males with high TSC and a selective advantage to males with low TSC.

Selection also appears to fluctuate within a night. At what time during the night a male cricket calls appears to influence his likelihood of being parasitized and his likelihood of mating for the following three reasons: (1) parasitoid flies orient to signaling male crickets early in the evening, with fly prevalence dropping dramatically by 0200 h (Cade et al. 1996); (2) female crickets orient toward calling male crickets throughout the night (Cade 1979); and (3) most mating does not occur until the dawn hours (French and Cade 1987). This suggests that in fall, when parasitoids are prevalent and female crickets do not discriminate between potential mate's TSC, early morning callers should avoid parasitism and attract mates, whereas males calling in the early evening should have a higher probability of being parasitized. In spring, when parasitism rates are very low and female crickets select mates dependent on their TSC, males that call throughout the night should confer the highest selective advantage.

Based on the hypothesis that the pattern of selection is sex-limited and temporally fluctuating, my field research focused on the following hypotheses and predictions. (1) Because parasitism is not prevalent in spring, and females in low, spring-like densities select males that call often, I hypothesized that spring selection should confer an advantage to high TSC males that call throughout the night. I predicted that the subsequent fall population would exhibit higher overall TSC phenotypes than the previous spring population and that they would partition their calls evenly throughout the night. (2) Because parasitoids acoustically orient to calling males, parasitoids are most common in early evenings in fall, and female crickets in high fall-like densities select mates at random with respect to their TSC phenotypes, I hypothesized that fall selection should confer an advantage to males that call less often and partition most of their calls around the predawn and dawn hours. Therefore, I predicted that the subsequent spring population would display lower TSC phenotypes shifted towards dawn calling, in comparison with the previous fall population's phenotypes. I tested these predictions by monitoring male calling behavior in the field over four successive generations.

My goal is to determine how heritable variation may be maintained in *G. texensis* mating signals. High heritable variation can be maintained by either weaker selective loss or higher mutational input (Pomiankowski and Møller 1995), and I have hypothesized that the pattern of selection resulting in weak selective loss is sex-limited temporally fluctuating selection. Complete support for this hypothesis requires (1) that *G. texensis* respond across generations in a manner consistent with the hypothesized pattern of selection; (2) that differences between generations are genetically based and not due to phenotypic plasticity; and (3) the performance of a selection experiment to show that sex-limited temporally fluctuating selection is capable of maintaining heritable variation. This research focuses on the first of these three criteria. I will show that *G. texensis* mating signals respond in a manner consistent with the hypothesis of sex-limited temporally fluctuating selection. I will also provide support for the second criterion; I will show preliminary evidence suggesting variation in *G. texensis* mating signals is genetically based, and not due to phenotypic plasticity.

MATERIALS AND METHODS

To test my predictions that male crickets in fall call throughout the night with higher TSC phenotypes, whereas male crickets in spring shift their calls to the second half of the night with lower TSC phenotypes, I collected several hundred adult *G. texensis* males during the spring (May) and fall (September) breeding seasons of 1998 and 1999 and monitored their calling behavior in a natural field setting. I monitored a total of 174 males in spring and 192 males in fall of 1998. In 1999, I monitored 179 males in spring and 96 males in fall. Individuals were monitored for up to five continuous nights, however, due to raccoon predation, many were only monitored for one or two evenings. Results are averaged for each individual monitored for more than one evening.

Male crickets were collected from aggregations at bright lights of a driving range in the northern outskirts of Austin, Texas. These male crickets were housed together in clear plastic containers with food and water in natural light conditions for the first 18–42 h in the Brackenridge Field Laboratory (BFL) in Austin, Texas. Each male was then transferred to its own individual one-liter plastic container. Each plastic container had four side panels cut from it and wire screens glued in place to allow adequate air ventilation. The containers were each placed in a shallow Styrofoam container filled with a water ''moat'' to ensure that imported fire-ant pests (*Solenopsis invicta*) could not attack the crickets. During the day, males were kept inside a BFL laboratory to ensure adequate temperature control and unlimited access to food and water. Males were moved to a BFL field in the late afternoon and their containers were provisioned with water and shelter; food was not provided in the field to reduce the attraction of predators.

Thirty-two males were monitored simultaneously. Males were separated in a gridlike fashion, using a 6×6 design with the last row holding only two individuals. A distance of 2 m separated all males, greater than the minimum 1.2 m observed between calling males in nature (Cade and Wyatt 1984).

Male mating calls were monitored electronically from 1800 h until 1000 h the next day. Two microphones were hung in each container, attached to a central circuit board and computer. These microphones monitored the sound decibel level in the male's container. The central circuit board compared the sound decibel level in the container to a preset level; each time the microphone was monitored and the cricket was silent a zero was scored on the computer's hard drive; a one was scored when the cricket was calling. Each male's calling/noncalling behavior was monitored and recorded six times per second throughout the night, ensuring continuous courtship information (Bertram and Johnson 1998; Bertram 2000). Each male's raw data consisted of two parallel columns (one from each microphone) of ones and zeros, identifying when the male was calling and silent throughout the night. Use of two microphones ensured accurate and conservative data collection for each male. When both microphones scored a one the animal was recorded as calling. When only one microphone scored a one, or when both microphones scored a zero, the animal was recorded as not calling.

Male TSC was quantified by determining the number of hours a male called per night. TCP was described by quantifying start time, which is the time the male is first observed to signal relative to sunset (sunset = 0, positive numbers represent hours after sunset, negative numbers are hours before sunset); stop time, which is the last time the male was observed to call relative to sunrise (sunrise = 0, positive numbers represent hours after sunrise, negative numbers are hours before sunrise); and mean time, which is the normalized mean of the indicator function of time relative to sunrise. The indicator function takes the values of zero or one, depending on whether the male is calling. For example, three males (A, B, and C) that signal first at 2200 h, last at 1000 h, and call for a total of three hours could have very different mean times. If male A called for one hour from 2200–2300 h and then for two hours from 0800–1000 h, his mean time would be 0530 h (-1.0 with a 0630 h sunrise). If male B called for two hours from 2200–2400 h and then for one hour from 0900–1000 h, his mean time would be 0230 h (-4.0). Whereas, if male C called for one hour from 2200–2300 h, for another hour from 0330–0430 h, and then again from 0900–1000 h, his mean time would be 0400 h (-2.5). For further details on the electronic apparatus see (Bertram and Johnson 1998), for further details on calling parameter calculations see (Bertram 2000).

$$\text{mean time} = \frac{\int_{1800\,h}^{1000\,h} I(t)t \, dt}{\int_{1800\,h}^{1000\,h} I(t) \, dt}$$

I tested results for normality using the Shapiro-Wilk statistic. Because TSC, start time, mean time and stop time were not normally distributed, I used the Kolmogrov-Smirnov (KS) goodness of fit test to determine significant differences between seasons and years. To test my prediction that calling parameters change in a predictable manner from one mating season to the next, I conducted pairwise seasonal comparisons: spring 1998 versus fall 1998; fall 1998 versus spring

TABLE 1. Differences in time spent calling (TSC) and temporal calling pattern (TCP) in the field cricket, *Gryllus texensis,* over four mating seasons

| | 1998 | | 1999 | |
	Spring	Fall	Spring	Fall
No. Calling/No. Monitored (% Calling)	54/174 (31%)	67/192 (35%)	69/179 (39%)	31/96 (32%)
Calling Males TSC Average (SE)***	1.42 (0.18)	**1.35 (0.17)**	**1.00 (0.16)**	**1.19 (0.22)**
Start Time Minimum	−0.50	1.10	−1.26	1.41
Start Time Average (SE)***	6.5 (0.53)	6.2 (0.51)	**6.4 (0.57)**	**5.8 (0.78)**
Start Time Maximum	12.19	13.78	12.69	14.79
Mean Time Minimum	−9.56	−9.92	−9.94	−9.94
Mean Time Average (SE)***	**−0.5 (0.42)**	**−2.3 (0.46)**	**−0.6 (0.43)**	**−2.1 (0.65)**
Mean Time Maximum	2.72	2.66	2.69	3.19
Stop Time Minimum	−6.88	−9.48	−9.94	−9.81
Stop Time Average (SE)***	1.7 (0.30)	**1.3 (0.34)**	**1.7 (0.39)**	1.9 (0.45)
Stop Time Maximum	3.70	3.50	4.46	3.63

*** Underlined bolded number pairs signify statistically significant seasonal differences at $P < 0.05$.

1999; and spring 1999 versus fall 1999. This resulted in twelve different analyses, necessitating Bonferroni corrections to $\alpha^1 < 0.0042$. To determine whether seasonal differences in calling song correlated with any obvious seasonal differences in environment, I used a regression to determine if there was a relationship between calling behavior and air temperature, rainfall, or scotophase. This resulted in twenty different analyses, which I Bonferroni adjusted to $\alpha^1 < 0.0025$.

RESULTS

I hypothesized that in spring there would be sexual selection via female choice for high TSC males, coupled with virtually no parasitism. From this I predicted that male crickets in fall would call throughout the night with elevated TSC. I further hypothesized fall selection would include natural selection via acoustically orienting parasitoids during the first half of the evening, coupled with female crickets selecting mates randomly (relative to their TSC). From this I also predicted that the subsequent spring population would have males that call most at dawn with reduced TSC. My predictions were generally supported.

Two of three seasonal comparisons support the prediction that TSC is higher following spring selection and lower following fall selection (see Table 1). The spring and fall populations were not significantly different in 1998 (KS: $P = 0.495$), but following fall selection in 1998 the spring 1999 population had a significantly reduced TSC (KS: $P = 0.002$). Following spring selection in 1999 the fall population had a higher TSC (KS: $P = 0.015$), but this trend was not statistically significantly because acceptable significance was Bonferroni corrected to $\alpha^1 < 0.0042$.

Predictions about how male crickets apportioned their calls throughout the night (TCP) were also supported via four different sets of measurements described in the following four paragraphs: (1) overall depiction of how calls were distributed throughout the night; (2) seasonal shifts in start times; (3) seasonal shifts in mean times; and (4) seasonal shifts in stop times.

Male crickets in spring shifted their calls towards dawn; males crickets in fall called more evenly throughout the night. I divided the nightly monitoring period into four time intervals (1800–2200 h; 2200–0200 h; 0200–0600 h; 0600–1000 h), and determined the number of minutes males called during each interval, for each season and year. I divided the night into four discrete time periods because four divisions provided enough statistical power, while enabling me to investigate calling behavior before and after 0200 h, the time when 90–95% of parasitoid flies are no longer prevalent (Cade et al. 1996). I then ran two regressions, one for each season (both years included in the analyses), using the data on the number of minutes males called during each 4-h-time interval. The slope of the regression of the fall data was not significantly different from zero, indicating that male crickets in fall call evenly throughout the night (see Fig. 1; $P = 0.244$, $t = 1.219$; df = 6). However, the slope of the regression of the spring data was positive and significantly different from zero, indicating that male crickets in spring focus their calls around the second half of the night (see Fig. 1; $P = 0.003$, $t = 4.877$; df = 6).

I predicted that following spring selection, males in fall would start calling earlier in the evening, and following fall selection, males in spring would start calling later in the night. The average seasonal start times were slightly earlier in fall than in spring (see Table 1). Although these differences were small and not statistically significant, note that start time data are presented relative to hours after sunset and that sunset arrives an hour earlier in fall compared to spring. In 1998, male crickets in fall started to call, on average, 18 min earlier than males in spring (KS: $P = 0.570$). Male crickets in spring 1999 called 6 min later than male crickets the previous fall (KS: $P = 0.102$). In 1999, male crickets started to call 36 min earlier than males in spring (KS: $P = 0.034$). When I combined the data from the two springs and compared them with the two fall mating seasons, there was a trend for spring males to start calling later than fall males (KS: $P = 0.048$).

Following spring selection, I predicted that male crickets
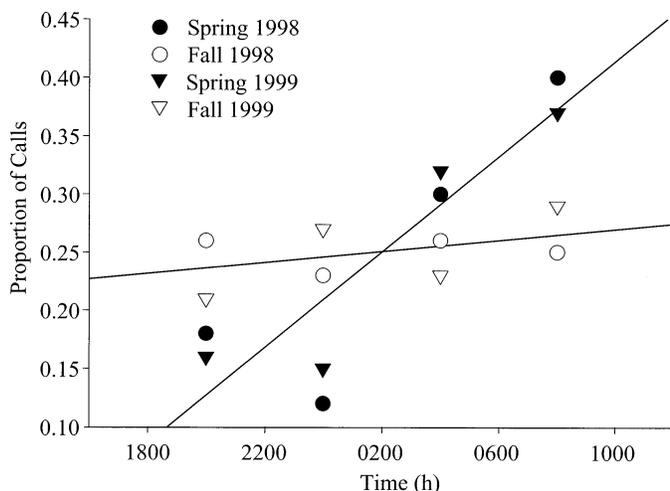
FIG. 1. Comparison of how males apportioned their acoustic mating signals in spring versus fall in 1998 and 1999. The 16-h nightly monitoring period was divided into four different time intervals, each four hours in duration. The proportion of calls produced in each four-hour interval of time was calculated for each mating season. The slope of the line of best fit through the fall data did not differ significantly from zero, suggesting that males in fall typically call throughout the night. The slope of the line of best fit through the spring data was positive and significantly different from zero, suggesting that males in spring call more in the early morning hours than in later hours.

in fall would have earlier mean times and following fall selection, I predicted that male crickets in spring would have later mean times. These predictions were supported. Mean time differed significantly between two of the three seasonal comparisons, and the third showed a nonsignificant trend (see Fig. 2). As with the start time data, note that the mean time data are presented relative to hours after sunset; sunset arrives an hour later in spring than in fall. Males in spring had later mean times than males in fall in 1998 (KS: $P = 0.031$), significantly so in 1999 (KS: $P = 0.002$), and males in fall 1998 had significantly earlier mean times than males in spring 1999 (KS: $P = 0.001$). In addition, when I combined the two springs and compared them to the two falls, spring males had significantly later mean times than fall males (KS: $P = 0.001$).

I predicted that male crickets in spring would have later stop times than males in fall. This prediction was supported in one of three seasonal comparisons (see Table 1). Male crickets in spring 1999 had significantly later stop times than the male crickets in fall 1998 (KS: $P = 0.001$). The other seasonal comparisons did not differ significantly (KS: 1998 $P = 0.450$; 1999 $P = 0.148$). When I combined the two springs and compared them to the two falls, males in spring did not differ significantly in their stop times from males in fall (KS: $P = 0.137$).

To determine if the seasonal differences in calling song might be explained by environmental differences, I tested to see if there was a relationship between calling behavior and scotophase, temperature, or rainfall. Seasonal differences in male calling behavior were not significantly correlated with seasonal rainfall or temperature differences (see Table 2). There was a non-significant trend for mean time and stop
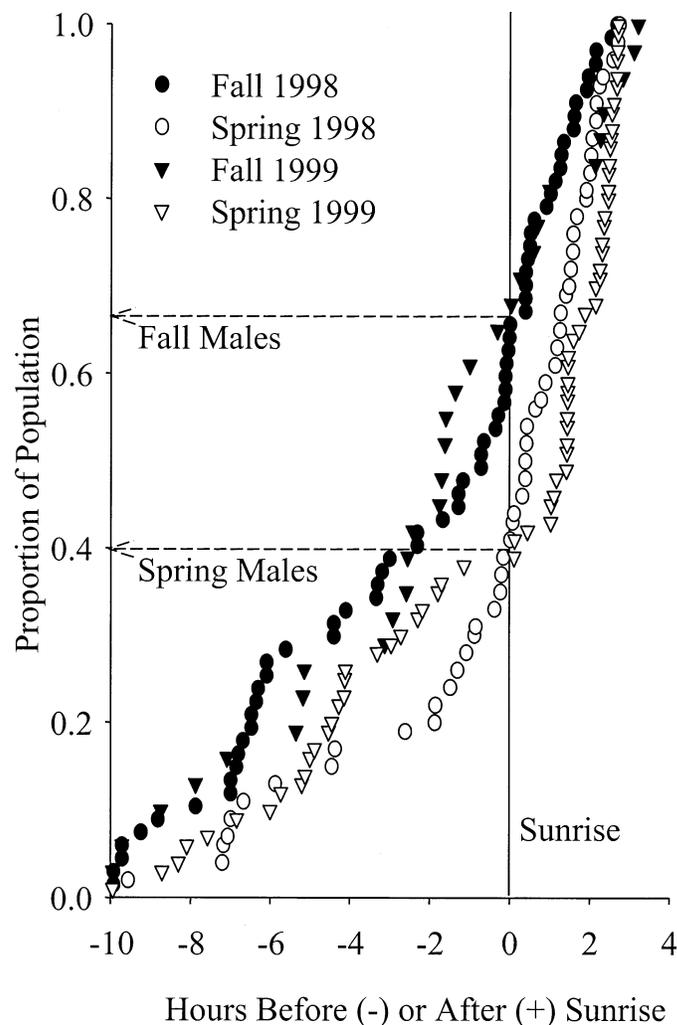

FIG. 2. Cumulative probability plot of male mean times in both the spring and fall mating seasons of 1998 and 1999. The males that called in fall had significantly earlier mean times compared to the males that called in spring. Further, 67% of the males in fall had mean times before sunrise, as compared to only 40% of the males in spring. All mean time data was calculated using sunrise as zero time. Negative values indicate hours before sunrise, positive values indicate hours after sunrise.

time to negatively correlate with scotophase, the dark portion of the photoperiod (Linear regression: mean time $t = -14.78$, $P = 0.005$; stop time $t = -14.758$, $P = 0.005$; acceptable significance Bonferroni corrected to $\alpha^1 < 0.0025$). Male crickets in spring had later mean and stop times, even though they experienced a scotophase that was two hours shorter than the scotophase experienced by fall males. Spring mating season occurs in May with a 14-hour light to 10-hour dark photoperiod (14:10 LD, LD = light:dark; 1930 h sunset, 0530 h sunrise). The fall mating season occurs in September with a 12:12 LD photoperiod (1820 h sunset, 0620 h sunrise).

DISCUSSION

I monitored male Texas field cricket time spent calling, start time, mean time, and stop time over four consecutive generations and found seasonal differences in 58% of the

TABLE 2. Environmental influences on time spent calling (TSC) and temporal calling pattern (TCP) in the field cricket, *Gryllus texensis,* over four mating seasons.

| | 1998 | | 1999 | | Regression results | | | | |
	Spring	Fall	Spring	Fall	TSC (All)	TSC (Callers)	Start time	Mean time	Stop time
Sunset	1930 h	1820 h	1930 h	1820 h	—	—	—	—	—
Sunrise	0530 h	0620 h	0530 h	0620 h	—	—	—	—	—
Scotophase	10 h	12 h	10 h	12 h	$t = 0.470$ $P = 0.684$	$t = 0.267$ $P = 0.814$	$t = -2.183$ $P = 0.161$	$t = -14.780$ $P = 0.005$	$t = -14.758$ $P = 0.005$
Avg. Precipitation	0.076	0.559	0.127	0.076	$t = 1.441$ $P = 0.286$	$t = 0.464$ $P = 0.688$	$t = -0.020$ $P = 0.986$	$t = -1.076$ $P = 0.394$	$t = -1.076$ $P = 0.394$
Avg. Low Temp. (°C)	19	22	20	20	$t = 0.769$ $P = 0.552$	$t = 0.080$ $P = 0.943$	$t = -0.409$ $P = 0.722$	$t = -1.625$ $P = 0.246$	$t = -1.625$ $P = 0.246$
Avg. High Temp. (°C)	32	32	31	34	$t = -0.610$ $P = 0.957$	$t = 0.287$ $P = 0.801$	$t = -2.583$ $P = 0.123$	$t = 1.222$ $P = 0.384$	$t = -1.106$ $P = 0.384$

paired consecutive comparisons (7 of 12 were significantly different at $\alpha^1 < 0.05$); further, 33% of those comparisons (4 of 12) showed significant differences at the Bonferroni corrected value of $\alpha^1 < 0.0033$. Male crickets in spring called most in the second half of the night and called less frequently than males in fall. By contrast, males in fall distributed their calls evenly throughout the night and had earlier start, mean, and stop times. Two nonmutually exclusive hypotheses potentially explain these observed seasonal differences in male acoustic mating behavior: (1) environmentally induced phenotypic plasticity, and (2) evolutionary response to seasonal selection differences.

Both physical and social environments change between the spring and fall mating seasons. However, observed seasonal differences in calling behavior did not correlate with seasonal differences in temperature or rainfall. There was a trend (nonsignificant) for components of male TCP to correlate with seasonal scotophase differences. Nevertheless, a laboratory experiment indicates that males reared in spring photoperiod conditions produce calling songs with TSC and TCP components that are not significantly different from males reared in fall photoperiod conditions (Bertram and Bellani 2002). These results suggest that seasonal changes in the physical environment are unlikely to explain the observed seasonal differences in calling song.

Observed seasonal shifts in acoustic mate signaling behavior are unlikely to result from shifts in the social environment. Preliminary experiments suggest that a male's calling song may slightly influence the mate signaling behavior of his nearest neighbors. S. X. Orozco and S. Bertram (pers. com.) measured signaling behavior of 64 *G. texensis* males arranged in an 8 × 8 grid over four nights. Cross-correlation analyses indicate nearest neighbors may have higher correlations between their respective mate signaling patterns than more distant neighbors. However, the average correlation between even closest neighbors is low (average Pearson's correlation coefficient ± SE, based on 7574 paired correlations between calling males is 0.132 ± 0.002), suggesting that only a small fraction of variation between individuals is explained by the calling behavior of nearest neighbors. It is therefore unlikely that the observed seasonal differences are due to a small number of males shifting their calling behavior and influencing other males to do the same.

Both cricket and parasitoid densities increase dramatically from the spring to the fall mating season, and density influences calling behavior in the Texas field cricket; in low-density populations males call more and search less than in high-density populations (Cade and Cade 1992). I made every attempt to control for social environment in my experimental design. I used the same location and kept the same distance between monitored males each season. However, because the experiment was conducted in the natural environment, overall cricket density (the combined density of both monitored crickets and free ranging crickets) was likely substantially higher in the fall mating seasons than it was in the spring seasons. If density were key to explaining the observed seasonal patterns, TSC should have been lower in the fall than in the spring mating seasons. However, I found the opposite result, suggesting that seasonal density differences do not explain the observed seasonal differences in calling song.

Overall, preliminary evidence suggests minimal support for the hypothesis that environmentally induced phenotypic plasticity explains the seasonal differences in acoustic mating behavior. However, determination as to whether my interpretation is correct awaits direct experimentation to determine that the observed seasonal differences are in fact genetically based, and not due to phenotypic plasticity.

Evidence suggests that the second hypothesis, evolutionary response to selection, explains some of the observed seasonal differences in male acoustic mating behavior. Half of the variation in TSC is thought to be genetically based. Using the same population of Texas field crickets as this study, Cade (1981) performed selection experiments for high and low TSC on multiple lines over four generations. His estimates of heritability are $h_r^2 = 0.50$ for high lines and $h_r^2 = 0.53$ for low lines. Unfortunately, we do not yet have estimates of the genetic components of variance for TCP (start, mean, or stop time). Given that half the variation in TSC has a genetic basis, observed differences in acoustic mating signals between consecutive mating seasons are likely to be partially explained by the second hypothesis, evolutionary response to sex-limited temporally fluctuating selection.

Endler (1986) defined ten classes of methods used for detecting selection in natural populations. These methods vary in their ability to directly demonstrate natural selection; some test for trait heritabilities and relationships between the traits

and mating success and/or survivorship, whereas others test the predicted outcome of selection. I used the method that involved predicting the outcome of selection (Endler 1986). I predicted how traits would change each generation using known trait properties and the predicted relationships between traits and both mating success and survival. Because spring selection should confer an advantage to high TSC males, I predicted that the subsequent fall population would exhibit higher TSC phenotypes with calls distributed evenly throughout the night. Further, because fall selection should confer an advantage to males that call less often and partition their calls around dawn, I predicted that the subsequent spring population would display lower TSC phenotypes shifted towards dawn calling. My data supported my predictions in a number of the seasonal comparisons, suggesting that the observed seasonal mating behavior patterns may result from evolutionary responses to sex-limited temporally fluctuating selection.

Endler (1986) strongly recommended combining a subset of the 10 methods used for detecting selection to circumvent ''adaptationist program'' (Gould and Lewontin 1979) criticisms. Endler (1986), along with Lande and Arnold (1983), also strongly encouraged the use of quantitative measures of selection and derived retrospective measures based on observed changes within a generation. Because my methodology does not allow the use of this selection measure, I instead use Cade and Cade's (1992) selection measures taken from the same population.

Cade and Cade (1992) studied the mating behavior of male *G. texensis* in a large outdoor enclosure at BFL. They measured TSC, searching behavior, weight, and mating frequency over 113 nights for 93 males at high density (falllike conditions) and 111 males at low density (springlike conditions). They quantified the standardized selection differential (s') using a covariance analysis and estimated direct selection on each trait using the partial regression coefficients (β') of mating frequency on the standardized traits (Lande and Arnold 1983). There was a positive correlation between mating frequency and TSC at low density ($r = 0.32$, $P < 0.001$), but no relationship at high density ($r = 0.05$, $P > 0.05$). At low density there was significant total selection for TSC ($s' = 0.31$) and against searching ($s' = -0.17$) and significant direct selection in favor of elevated TSC ($\beta' \pm SE = 0.26 \pm 0.09$, $t = 2.8$, df $= 107$, $P = 0.005$). At high density total selection did not significantly influence TSC ($s' = 0.02$), searching behavior ($s' = 0.02$), or body weight ($s' = -0.05$). At high density there was also no significant direct selection on TSC ($\beta' \pm SE = 0.02 \pm 0.1$, $t = 0.2$, df $= 89$, $P = 0.8$; Cade and Cade 1992). Cade and Cade's (1992) study did not measure selection on TSC that resulted from acoustically orienting parasitoid flies, nor did their study measure selection on TCP that resulted from either mating success or parasitism. However, when combined with my results, their study provides further support for the hypothesis that the observed seasonal acoustic mating behavior patterns result from evolutionary responses to temporally fluctuating sex-limited selection.

Gryllus texensis displays significantly greater variation in mating signals than other closely related species that are not exposed to the acoustically orienting parasites parasitoids (Cade 1991). The same pattern of variation has been observed in populations of the field cricket *Teleogryllus oceanicus*; populations exposed to the parasitoid *O. ochracea* express significantly greater song variance than populations not exposed to parasitoids (Rotenberry et al. 1996; Zuk et al. 2001). Provided traits are heritable, variation is maintained by a balance between the generation of fresh variability through mutation and introgression and the loss of that variation by selection (Bulmer 1980). Therefore, *G. texensis* and the subset of *T. oceanicus* populations exposed to acoustically orienting parasitoids must experience either weaker selective loss and/or higher input from mutation and/or introgression. *Gryllus texensis* and *T. oceanicus* are unlikely to display higher rates of mutation or introgression than closely related species (populations in the case of *T. oceanicus*). Therefore, I focused on identifying the pattern of selection influencing *G. texensis*'s mating signals, predicting how the mating signals would respond to this hypothesized selection pattern, then measuring the intergenerational changes in a natural field setting. My data, coupled with Cade and Cade's (1992) selection measures, suggest that the pattern of selection influencing male acoustic mating behavior is sex-limited temporally fluctuating selection.

I propose that sex-limited temporally fluctuating selection may act to reduce the selective loss of heritable variation in the acoustic mating signals observed in *G. texensis*. The consensus on present evolutionary theory, however, indicates temporally fluctuating selection without overdominance is too restrictive to maintain heritable variation in a population (Hedrick 1986), unless generations overlap to provide a ''storage effect'' for less advantageous genotypes to survive until the next favorable selective event occurs (Ellner and Hairston 1994; Sasaki and Ellner 1995; Ellner 1996; Ellner and Sasaki 1996; Sasaki and Ellner 1997; Ellner et al. 1999; Sasaki and de Jong 1999). The conundrum is that *G. texensis* has discrete generations, and thus overlapping generations cannot account for the necessary prerequisite of a traditional ''storage effect.'' Sasaki and Ellner (1997) speculate that variation will be maintained in populations with discrete generations provided the temporally fluctuating selection is sex limited in nature.

Following Sasaki and Ellner's (1997) reasoning, I hypothesize that when temporally fluctuating selection is combined with sex-limited selection it results in a storage effect and contributes significantly to the maintenance of heritable variation in sexual characters. Because males are the only sex to produce acoustic mating signals, selection on their sex-limited traits can only affect male reproductive success and survival. Genes coding for the traits carried by females would be unexpressed and therefore hidden from selection within a single generation. Some proportion of the alleles coding for sex-limited traits would therefore be shielded from selection. Through mating and genetic segregation, subsets of less advantageous genes could be transferred from mothers into offspring (Bertram 1999; Reinhold 1999).

Partial support for the hypothesis that sex-limited temporally fluctuating selection can maintain variation in heritable characters is provided by a one locus, two-allele analytical model, and a multilocus simulation model (Reinhold 2000). Because most behavioral traits are quantitative, future

work should focus on building a quantitative genetic model to determine whether sex-limited temporally fluctuating selection can maintain variation in a population, and can do so within a large fraction of parameter space.

To the best of my knowledge this is the first field demonstration of how a population without overlapping generations may respond to predictable temporal fluctuations in sex-limited selection. This work confirmed my a priori predictions that (1) males in the fall call more and partition their calls evenly throughout the night, and (2) males in the spring call less and shift their calling towards dawn. Demonstrating the expected effects in the field is a fundamentally important component of testing the hypothesis that genetic variation can be maintained by sex-limited temporally fluctuating selection. A preponderance of support additionally requires (1) experimentation to directly test my claim that seasonal differences in calling song are genetically based and not due to phenotypic plasticity, and (2) the performance of a selection experiment to show that sex-limited temporally fluctuating selection is capable of maintaining heritable variation at the levels observed in nature. Future research should therefore concentrate on testing these two criteria.

LITERATURE CITED

Adamo, S. A., D. Robert, J. Perez, and R. R. Hoy. 1995a. The response of an insect parasitoid, *Ormia ochracea* (Tachinidae), to the uncertainty of larval success during infestation. Behav. Ecol. Sociobiol. 36:111–118.
Adamo, S. A., D. Robert, and R. R. Hoy. 1995b. Effects of a tachinid parasitoid, *Ormia ochracea*, on the behavior and reproduction of its male and female field cricket hosts (*Gryllus* spp). J. Ins. Physiol. 41:269–277.
Bennett-Clark, H. 1989. Songs and the physics of sound production. Pp. 227–261 *in* F. Huber, T. Moore, and W. Loher, eds. Cricket behavior and neurobiology. Cornell Univ. Press, Ithaca, NY.
Bertram, S. 1999. Understanding intrapopulation variation in the mating behavior of a field cricket. Ph.D. diss. Department of Biology, Arizona State University, Tempe, AZ.
———. 2000. The influence of age and size on temporal mate signaling behaviour. Anim. Behav. 60:333–339.
Bertram, S., and R. Bellani. 2002. The influence of photoperiod on temporal mate signaling patterns in the Texas field cricket, *Gryllus texensis*. J. Ins. Behav. 15:607–615.
Bertram, S., and L. Johnson. 1998. An electronic technique for monitoring the temporal aspects of acoustic signals of captive organisms. BioAcous. 9:107–118.
Bulmer, M. G. 1980. Effects of selection on genetic variability. Heredity 45:141–141.
Cade, W. H. 1975. Acoustically orienting parasitoids: fly phonotaxis to cricket song. Science 190:1312–1313.
———. 1979. The evolution of alternative male reproductive strategies in field crickets. Pp. 343–378 *in* M. S. Blum and N. A. Blum, eds. Sexual selection and reproductive competition in insects. Academic Press, New York.
———. 1981. Alternative male strategies: genetic differences in crickets. Science 212:563–565.
———. 1991. Interspecific and intraspecific variation in nightly calling duration in field crickets, *Gryllus integer* and *G. rubens* (Orthoptera, Gryllidae). J. Ins. Behav. 4:185–194.
Cade, W. H., and E. S. Cade. 1992. Male mating success, calling and searching behavior at high and low-densities in the field cricket, *Gryllus integer*. Anim. Behav. 43:49–56.
Cade, W. H., and D. R. Wyatt. 1984. Factors affecting calling behavior in field crickets, *Teleogryllus* and *Gryllus* (age, weight, density, and parasites). Behaviour 88:61–75.
Cade, W. H., M. Ciceran, and A. M. Murray. 1996. Temporal patterns of parasitoid fly (*Ormia ochracea*) attraction to field cricket song (*Gryllus integer*). Can. J. Zool. 74:393–395.
Charlesworth, B. 1984. The evolutionary genetics of life histories. Pp. 117–133 *in* B. Shorrocks, ed. Evolutionary ecology. Blackwell, Oxford, U.K.
———. 1987. The heritability of fitness. Pp. 21–40 *in* J. W. Bradbury and M. B. Andersson, eds. Sexual selection: testing the alternatives. Wiley, Chichester, U.K.
Ellner, S. 1996. Environmental fluctuations and the maintenance of genetic diversity in age or stage-structured populations. Bull. Math. Biol. 58:103–127.
Ellner, S., and N. G. Hairston. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. Am. Nat. 143:403–417.
Ellner, S., and A. Sasaki. 1996. Patterns of genetic polymorphism maintained by fluctuating selection with overlapping generations. Theor. Popul. Biol. 50:31–65.
Ellner, S. P., N. G. Hairston, C. M. Kearns, and D. Babai. 1999. The roles of fluctuating selection and long-term diapause in microevolution of diapause timing in a freshwater copepod. Evolution 53:111–122.
Endler, J. A. 1986. Natural selection in the wild. Princeton Univ. Press, Princeton, NJ.
Falconer, D. S. 1981. Introduction to quantitative genetics. Ronald Press, London.
Fisher, R. A. 1958. The genetical theory of natural selection. 2d ed. Dover, New York.
French, B. W., and W. H. Cade. 1987. The timing of calling, movement, and mating in the field crickets *Gryllus veletis*, *Gryllus pennsylvanicus*, and *Gryllus integer*. Behav. Ecol. Sociobiol. 21:157–162.
Gould, S. J., and R. C. Lewontin. 1979. The spandrels of San Marco and the panglossian paradigm: a critique of the adaptationist programme. Proc. R. Soc. Lond. B 205:581–598.
Gray, D. A., and W. H. Cade. 1999a. Sex, death and genetic variation: natural and sexual selection on cricket song. Proc. R. Soc. London B 266:707–709.
———. 1999b. Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. Evolution 53:848–854.
Hedrick, A. 1988. Female choice and the heritability of attractive male traits: an empirical study. Am. Nat. 132:267–276.
Hedrick, P. W. 1986. Genetic polymorphism in heterogeneous environments: a decade later. Annu. Rev. Ecol. Syst. 17:535–566.
Houle, D. 1992. Comparing evolvability and variability of quantitative traits. Genetics 130:195–204.
Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.
Martin, S. D., D. A. Gray, and W. H. Cade. 2000. Fine-scale temperature effects on cricket calling song. Can. J. Zool. 78:706–712.
Mousseau, T. A., and D. J. Howard. 1998. Genetic variation in cricket calling song across a hybrid zone between two sibling species. Evolution 52:1104–1110.

Olvido, A. E., and T. A. Mousseau. 1995. Effect of rearing environment on calling-song plasticity in the striped ground cricket. Evolution 49:1271–1277.

Pomiankowski, A., and A. P. Møller. 1995. A resolution of the lek paradox. Proc. R. Soc. Lond. B 260:21–29.

Reinhold, K. 1999. Evolutionary genetics of sex limited traits under fluctuating selection. J. Evol. Biol. 12:897–902.

———. 2000. Maintenance of a genetic polymorphism by fluctuating selection on sex-limited traits. J. Evol. Biol. 13:1009–1014.

Roff, D. A., T. A. Mousseau, and D. J. Howard. 1999. Variation in genetic architecture of calling song among populations of *Allonemobius socius*, *A. fasciatus*, and a hybrid population: drift or selection? Evolution 53:216–224.

Rotenberry, J. T., M. Zuk, L. W. Simmons, and C. Hayes. 1996. Phonotactic parasitoids and cricket song structure: an evaluation of alternative hypotheses. Evol. Ecol. 10:233–243.

Sasaki, A., and G. de Jong. 1999. Density dependence and unpredictable selection in a heterogeneous environment: compromise and polymorphism in the ESS reaction norm. Evolution 53: 1329–1342.

Sasaki, A., and S. Ellner. 1995. The evolutionarily stable phenotype distribution in a random environment. Evolution 49:337–350.

———. 1997. Quantitative genetic variance maintained by fluctuating selection with overlapping generations: Variance components and covariances. Evolution 51:682–696.

Smith, C. J., and W. H. Cade. 1987. Relative fertility in hybridization experiments using 3 song types of the field crickets *Gryllus integer* and *Gryllus rubens*. Can. J. Zool. 65:2390–2394.

Souroukis, K., W. H. Cade, and G. Rowell. 1992. Factors that possibly influence variation in the calling song of field crickets: temperature, time, and male size, age, and wing morphology. Can. J. Zool. 70:950–955.

Walker, T. J. 2000. Pulse rates in the songs of trilling field crickets (Orthoptera: Gryllidae: Gryllus). Ann. Entomol. Soc. Am. 93: 565–572.

Walker, T. J., and S. A. Wineriter. 1991. Hosts of a phonotactic parasitoid and levels of parasitism (Diptera, Tachinidae *Ormia ochracea*). Fl. Entomol. 74:554–559.

Zuk, M., J. T. Rotenberry, and L. W. Simmons. 2001. Geographical variation in calling song of the field cricket *Teleogryllus oceanicus*: the importance of spatial scale. J. Evol. Biol. 14:731–741.