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Divergence between the Courtship Songs of the Field Crickets *Gryllus texensis* and *Gryllus rubens* (Orthoptera, Gryllidae)

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Abstract

Acoustic mating signals are often important as both interspecific prezygotic isolating mechanisms and as sexually selected traits in intraspecific mate choice. Here, we investigate the potential for cricket courtship song to act as an isolating mechanism by assessing divergence between the courtship songs of *Gryllus texensis* and *Gryllus rubens*, two broadly sympatric cryptic sister species of field crickets with strong prezygotic isolation via the calling song and little or no postzygotic isolation. We found significant species-level differences in the courtship song, but the song has not diverged to the same extent as the calling song, and considerable overlap remains between these two species. Only two related courtship song characters are sufficiently distinct to play a possible role in prezygotic species isolation.

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Introduction

Acoustic mating signals are often important determinants of the boundaries of gene flow both within and among species. Long-distance acoustic mating signals are species-specific, at least for a given locality and season. The taxonomy of several acoustically communicating groups of organisms often relies on behavioral differences exhibited in the long distance songs, usually termed 'calling songs' that males sing to attract females. Crickets (Orthoptera, Gryllidae) provide some of the best known examples (Fulton 1952; Alexander 1957; Walker 1962, 1963; Weissman et al. 1980; Otte 1994), but other acoustic groups such as cicadas

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(Marshall & Cooley 2000), lacewings (Wells & Henry 1998) and frogs (Gerhardt 1994; Márquez & Bosch 1997) provide additional examples. Calling song is an intuitive species-level criterion because male song and female song recognition together largely define the limits of gene flow. In crickets, several studies have shown that females prefer conspecific song (Walker 1957; Doherty & Callos 1991; Gray & Cade 2000) (for an exception, see Doherty & Howard 1996). However, calling song is not the only type of song that male crickets sing. In aggressive male–male interactions, males produce 'aggressive' song, and in close-range male–female encounters, usually following female phonotaxis to calling song, males produce 'courtship' song (Alexander 1961). Courtship song is typically quieter than calling song and by comparison has been much less studied. It appears, however, that courtship song is a requirement for normal inducement of female mounting and subsequent sperm transfer (mating requires female cooperation in crickets). The mating success of experimentally muted but paired males is only a small fraction of the mating success of males allowed to produce courtship song (Crankshaw 1979; Libersat et al. 1994; Balakrishnan & Pollack 1996).

In this paper, we quantitatively describe the courtship songs of two closely related North American field crickets, *Gryllus texensis* Cade & Otte and *Gryllus rubens* Scudder. These species both live throughout the south-central to south-eastern USAs, with an area of sympatry extending from eastern Texas to western Florida. Recent molecular phylogenetic work identifies these two species as closest relatives (Huang et al. 2000). Males are distinguishable by features of the calling song. *G. rubens* tends to produce trills with more pulses per trill than does *G. texensis* (D. A. Gray, pers. obs.), however, the best distinguishing feature is pulse rate: at 25°C, male *G. texensis* produce trills with an average pulse rate of about 80 pulses/s. At the same temperature, *G. rubens* trill at approximately 56 pulses/s (Walker 1998, 2000; Gray & Cade 2000; Martin et al. 2000). Provided that the temperature is above approximately 20°C, the temperature-corrected pulse rates of these two species are distinct, with virtually no overlap. The divergence in calling song is sufficient to allow females to behaviorally distinguish conspecifics with high certainty. Female *G. texensis* phonotactic response to conspecific calling song averages five times the response to heterospecific song (Gray & Cade 2000). Calling song differences remain the only known means of distinguishing males; many females can be distinguished with reasonable accuracy based on the length of the ovipositor relative to body size (Gray et al. 2001).

Several considerations motivated the present study. First, no detailed quantitative comparison of cricket courtship songs has ever been published (for qualitative comparative work, see Alexander 1961 and Leroy 1966; for quantitative non-comparative work, see Libersat et al. 1994; Balakrishnan & Pollack 1996; Wagner & Reiser 2000). Second, because each is the closest relative of the other, any differences in song represent divergence from a common ancestor. Examination of the divergence in courtship song relative to the divergence in calling song may provide insight into the relative roles of calling song and courtship song as pre-mating isolating mechanisms. Because most mating results from both female phonotaxis to calling song and subsequent acceptance of courtship song, we would a priori expect greater divergence in calling song. This is not a foregone conclusion, however. Both of these species can occur at such high abundance that some individuals undoubtedly encounter each other even in the absence of calling song (Cade 1979, 1981, 1989; Cade & Cade 1992; wherein *G. texensis* was studied under the name *G. integer*, see Cade & Otte 2000). Studies documenting species-level differences in potential isolating cues (Hoy et al. 1988; Ritchie & Gleason 1995) are an important first step in assessing pre-mating isolation. Finding significant divergence does not demonstrate that a trait functions as an isolating mechanism, but a finding of minimal divergence precludes a significant role for the trait as an effective isolating mechanism (Noor et al. 2000).

Methods

The crickets used in this study were from laboratory stock reared in plastic tubs (37 × 32 × 17 cm) supplied with cat food, cardboard shelter, and water in cotton-plugged vials. *G. texensis* stock were derived from field collections in Austin, Texas, in Sep. 1998; *G. rubens* stock were from field collections in Marianna, Florida, in Sep. 1999. Cultures were maintained at 28 ± 1°C on a 14-h/10 h light/dark cycle. Newly molted adults were removed daily and were housed in individual 500-ml plastic containers with the same provisions as above.

Individuals were transferred to a recording room at least 24 h prior to testing to allow for temperature acclimatization. Just prior to each trial, we removed the water bottle and cardboard shelter from the male's container, placed a conspecific female into the container and replaced the plastic lid with a screened lid. This container was then positioned in a recording chamber that was constructed by placing an approximately 20-l plastic pail that was lined with 2.54 cm thick corrugated foam within an 84-l plastic garbage can also lined with foam. The ambient temperature within the recording chamber was measured to the nearest 0.5°C using a Digi-Sense Type K Thermocouple Probe Thermometer (Cole-Parmer Instrument Corp., Vernon Hills, IL). The range of temperatures across recordings was 22–28°C ($\bar{x} = 23$, mode = 23). Song recordings were made using a LinearX M51 Precision Acoustic Measurement Microphone (10 Hz to 40 kHz ± 1 dB; LinearX Systems Inc., Tualatin, OR) connected to a TASCAM DA-P1 Digital Audio Tape Recorder (20 Hz to 20 kHz ± 1 dB; TASCAM America, Montebello, CA) set at mono, 48 kHz, 16-bit sampling, 20 kHz low-pass filter. The microphone was positioned 19 cm above the crickets.

We used Canary 1.2.4. bioacoustics software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) to analyse the courtship songs (44.1 kHz and 16-bit sampling). Before analysis, we digitally high-pass-filtered all recordings at 500 Hz to remove very low frequency noise not eliminated by the recording chamber. Filtering out sound below 500 Hz did not interfere with sound analysis because the minimum frequency of the courtship song is approximately 4.5 kHz (see Table 1). To calibrate amplitude measurements, a 5-kHz tone of known intensity (75 dB reference 20 μPa at 19 cm) was also recorded and analysed using the identical setup as used for this experiment. For analysis, a spectrogram of the

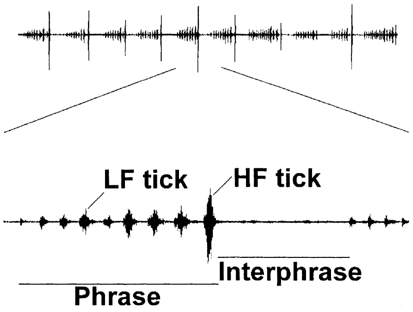

Fig. 1: Waveform representation of generalized *Gryllus* courtship song showing the prominent song features described in the text

Table 1: Statistical description of the courtship songs of *Gryllus rubens* and *Gryllus texensis*. Data were temperature corrected to 25°C. Comparison of songs via Mann–Whitney U-test. Amplitude measures in decibels (dB) are referenced to 20 μPa

	G. rubens (n = 23)		*G. texensis* (n = 36)				
Song character[1]	Mean	SD	Mean	SD	Urub	Utex	p
HF tick rate (ticks/s)	1.9	0.4	3.7	0.8	826	2	0.000
LF tick rate (ticks/s)	51.0	5.2	62.2	7.4	85	743	0.000
Number of LF ticks	24.7	4.9	14.3	3.0	812	16	0.000
Interphrase interval (ms)	39.4	11.5	51.1	15.1	214	614	0.002
HF tick duration (ms)	14.7	2.9	13.6	2.0	487	341	0.260
HF tick amplitude (dB)	79.8	3.9	79.8	4.4	397	431	0.798
LF tick amplitude (dB)	65.9	2.9	71.9	5.9	147	681	0.000
HF tick frequency (kHz)	12.52	4.91	9.80	4.60	553	275	0.031
LF tick frequency (kHz)	4.48	0.18	5.22	0.34	12	816	0.000

[1] HF = high frequency; LF = low frequency; see text.

song was created using the following specifications: filter bandwidth of 683.89 Hz, Fast Fourier Transform of 256 points, and a clipping level of −15 dB.

Field cricket courtship song is a temporally structured sequence of pulses arranged in groups (see Fig. 1). We call the basic grouping of pulses a ‘phrase’ of courtship song. Phrases are separated by a period of silence that we call the ‘interphrase interval’. A phrase of courtship song consists of two distinct types of sound, a series of lower frequency pulses of approximately 5 kHz that we call 'low frequency ticks' followed by a single high-frequency pulse that we call a 'high-frequency tick'. We measured nine characters of the courtship song (see Fig. 1). These were: the number of high-frequency (HF) ticks per second, hereafter 'HF tick rate' measured as the reciprocal of the HF tick period (the time from the start of one HF tick to the start of the next HF tick); the number of low-frequency (LF) ticks per phrase; HF tick duration; HF tick peak amplitude; interphrase interval; LF tick peak amplitude; HF tick peak frequency; LF tick peak frequency; and LF tick rate. Most measurements were made using the 'measurement panel' and 'data log' capabilities of the software but the number of LF ticks per phrase was counted manually. We attempted to make 15 measurements per song character per male, however, fewer measurements were used in the instances when 15 were impossible (i.e. short recordings). We succeeded in taking 15 song measurements per song character for 39 of the 59 males recorded (66%), but could only make between five and 14 measurements per song character for 16 males (27%); four males (7%) had very short recordings (less than five measurements per song character). The median value of each song character for each male was used in the analysis.

Prior to statistical comparison, we corrected all songs for differences in recording temperature to 25°C using the slopes from linear regressions of the song characters on temperature. Corrections were song character corrected to 25°C = song character + slope * (recording temperature −25). We examined interspecific differences for each of the song characters using the non-parametric Mann–Whitney U-test. Non-parametric analyses were used because several of the temperature-corrected song characters were not normally distributed (parametric tests, not shown, gave the same results). We attribute no biological significance to the non-normality, in particular the apparent bimodality of the interphrase interval in *G. texensis* (see Fig. 6, Results) is considered an anomaly of this sample. Two additional studies of the interphrase interval in *G. texensis*, each with sample sizes of over 100 individuals, both reveal unimodal normal distributions (Gray & Eckhardt, in press; D. A. Gray, K. Singh, & M. J. Fitzpatrick, unpubl. data).

Results

Representative courtship songs are shown in Figs 2 and 3. Statistical comparisons are summarized in Table 1. Seven of the nine characters measured were significantly different between the two cricket species. *G. texensis* typically produced HF tick rates that were approximately twice as fast as those of *G. rubens* (Fig. 4). This relationship was most likely due to *G. rubens* producing over 40% more LF ticks per phrase than did *G. texensis* (Fig. 5). *G. texensis* had longer interphrase intervals than *G. rubens* (Fig. 6). The peak amplitude of *G. texensis* LF ticks was significantly louder than that of *G. rubens*. *G. rubens* produced significantly higher HF tick peak frequencies, whereas *G. texensis* produced significantly higher LF tick peak frequencies. The HF ticks of the two species had similar durations and peak amplitudes.

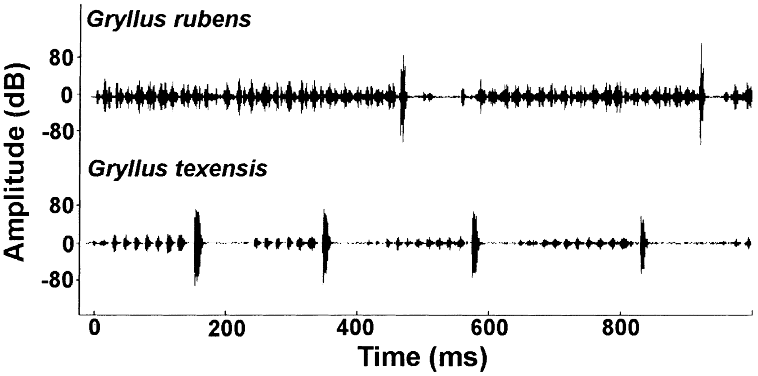

Fig. 2: Waveform representations of example *Gryllus rubens* and *Gryllus texensis* courtship song

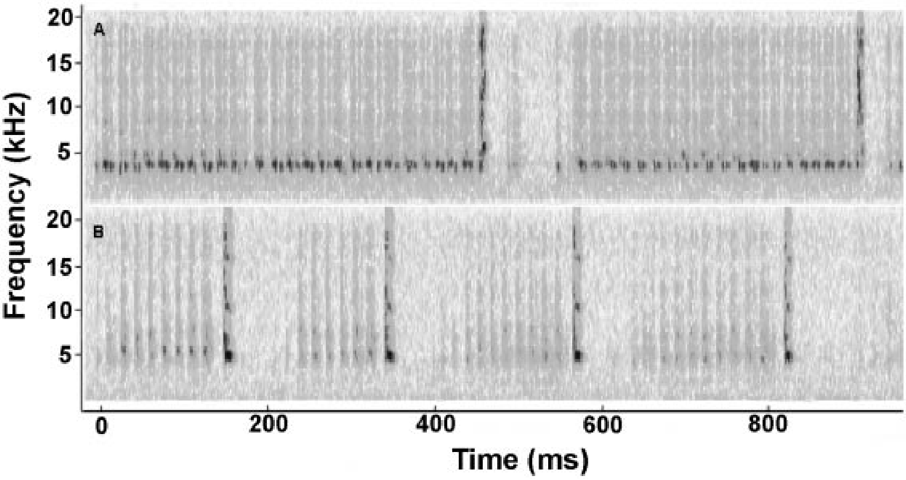

Fig. 3: Spectrogram representations of the songs in Fig. 2. Panel A is the *Gryllus rubens* song of Fig. 2, whereas panel B is the *Gryllus texensis* song

In the calling song, the pulse rate of *G. texensis* is significantly faster than that of *G. rubens* (80 vs. 56 pulses/s, respectively, at 25°C). Similarly, in the courtship song, *G. texensis* had significantly faster HF and LF tick rates than *G. rubens* (Fig. 7). The courtship song LF tick rates were not the same as the pulse rates of the calling songs (*G. texensis* one-sample t-test LF tick rate vs. 80 pulses, $t = -14.44$, $df = 35$, two-tailed $p < 0.000$; *G. rubens* one-sample t-test LF tick rate vs. 56 pulses, $t = -3.36$, $df = 22$, two-tailed $p < 0.003$).

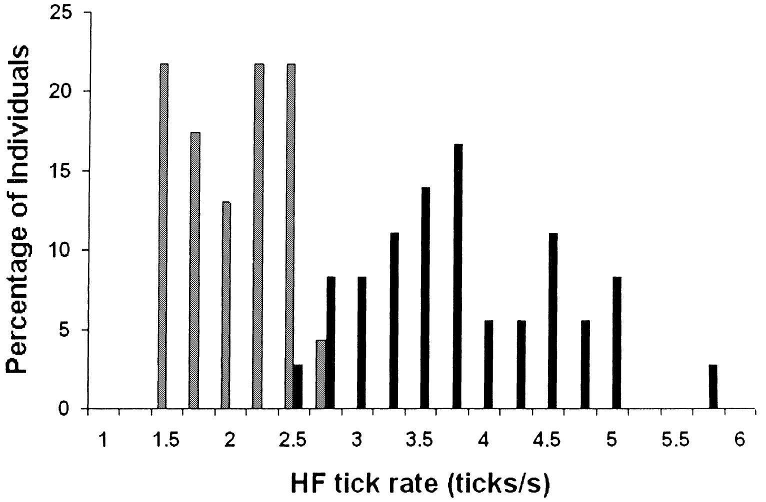

Fig. 4: Distribution of the high-frequency (HF) tick rate showing minimal overlap between *Gryllus rubens* (light bars) and *Gryllus texensis* (dark bars)

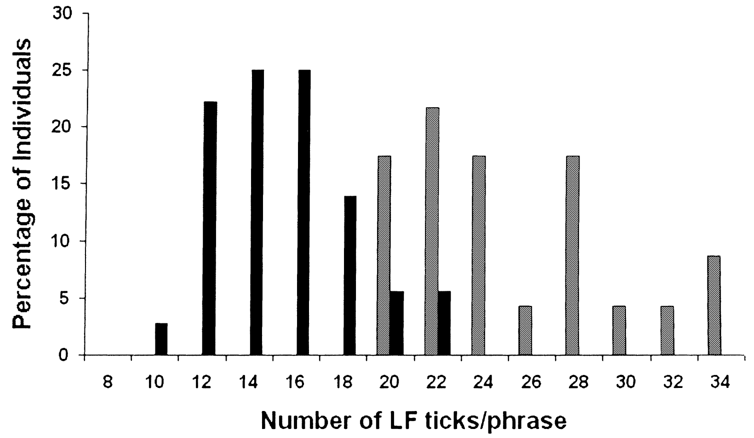

Fig. 5: Distribution of the number of low-frequency (LF) ticks per phrase showing minimal overlap between *Gryllus rubens* (light bars) and *Gryllus texensis* (dark bars)

Discussion

We have shown clear differences between the courtship songs of these two species. Most of the song parameters are significantly different between the species, and in general, *G. rubens* courtship song is slower than that of *G. texensis*. This difference is in the same direction as the difference in the calling song pulse

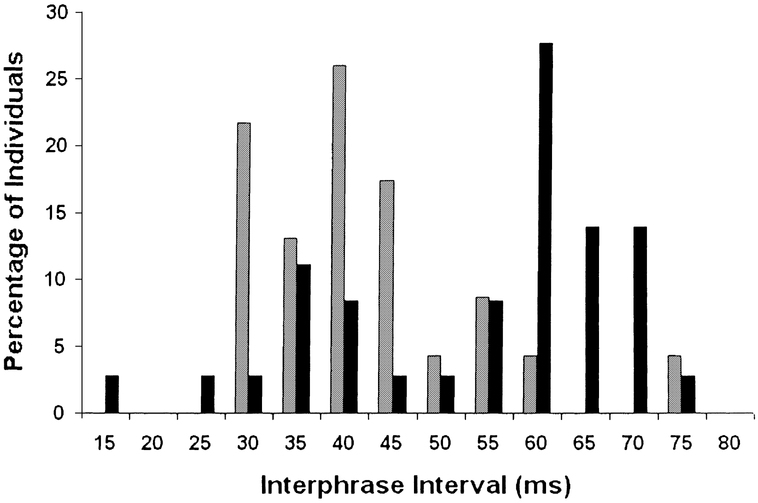

Fig. 6: Distribution of interphrase intervals showing substantial overlap between *Gryllus rubens* (light bars) and *Gryllus texensis* (dark bars)

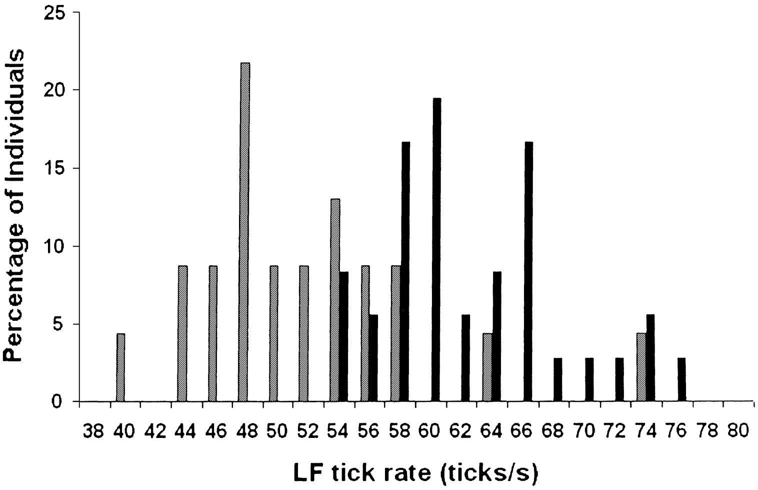

Fig. 7: Distribution of the low-frequency (LF) tick rates showing moderate overlap between *Gryllus rubens* (light bars) and *Gryllus texensis* (dark bars)

rate but is not of the same magnitude. For most song characters there remains considerable overlap between the species. The most strongly separated courtship song characters are the number of LF ticks per phrase and the HF tick rate. For a given interphrase interval and LF tick rate, these song characters are necessarily strongly correlated.

In our study, the source populations for both species were allopatric with respect to the other species. The use of allopatric sources of crickets was beneficial in that it decreases the chance that any observed song similarity is due to past hybridization. Hybrids of these two species can easily be produced in the laboratory (Smith & Cade 1987; Cade & Tyshenko 1990; Gray & Cade 2000), but have not been observed in the field (Walker 1998, 2000; Gray & Cade 2000). The disadvantage of using crickets from allopatric populations is that it is possible that crickets from sympatry may show greater differences in courtship song. This remains to be tested, however, we note that for *G. texensis* there is no difference in calling song with respect to sympatry or allopatry with *G. rubens* (Gray & Cade 2000).

We conclude that significant courtship song differences exist between *G. texensis* and *G. rubens*. The courtship song characters that best separate the species are the HF tick rate and the number of LF ticks per phrase. It is interesting to note that *G. rubens* has more LF ticks per phrase in the courtship song and, typically, has more pulses per trill in the calling song than does *G. texensis*. We do not know if quantitative genetic variation underlies the number of LF ticks per phrase in the courtship song, but we note that for *G. texensis*, the number of pulses per trill in the calling song does have a significant additive genetic component (Gray & Cade 1999). Overall, the divergence in courtship song does not generally match the divergence in the calling song pulse rates. This may be expected because species-level differences in courtship song will typically, although not always, come into play only after female phonotaxis to calling song. Documenting the degree of interspecific variation is the first step in assessing the potential importance of traits as isolating mechanisms; that this variation influences female mating decisions remains to be demonstrated.

Our results with these two particular crickets may be of more general interest given the large numbers of acoustically communicating species distributed across several taxonomic groups. Long-range acoustic cues, although often showing high levels of intraspecific variation in some components (dynamic traits sensu Gerhardt 1991), are likely to be subject to the constraints of providing species-specific identification and ease of localization, which may often result in at least some song traits being relatively stereotyped (static traits sensu Gerhardt 1991). In systems such as those of crickets, where close range courtship (acoustic or otherwise) precedes mating but temporally follows long-range taxis to species-specific cues, we might expect courtship to play an important role in intraspecific mate choice and a lesser role in interspecific discrimination. This generalization is likely to hold across both invertebrate and vertebrate taxa.

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Literature Cited

Alexander, R. D. 1957: The taxonomy of the field crickets of the eastern United States (Orthoptera: Gryllidae: *Acheta*). Ann. Entomol. Soc. Am. **50,** 584—602.

Alexander, R. D. 1961: Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). Behaviour **17,** 130—223.

Balakrishnan, R. & Pollack, G. S. 1996: Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. Anim. Behav. **51,** 353—366.

Cade, W. H. 1979: The evolution of alternative male reproductive strategies in field crickets. In: Sexual Selection and Reproductive Competition in Insects (Blum, M. S. & Blum, N. A., eds). Academic Press, New York. pp. 343—380.

Cade, W. H. 1981: Field cricket spacing and the phonotaxis of crickets and parasitoid flies to clumped and isolated cricket songs. Z. Tierpsychol. **55,** 365—375.

Cade, W. H. 1989: Nightly and hourly rates of attraction of flying field crickets, *Gryllus integer*, to conspecific song. Can. J. Zool. **67,** 2540—2542.

Cade, W. H. & Cade, E. S. 1992: Male mating success, calling and searching behaviour at high and low densities in the field cricket, *Gryllus integer*. Anim. Behav. **43,** 49—56.

Cade, W. H. & Otte, D. 2000: *Gryllus texensis* n. sp. A widely studied field cricket (Orthoptera; Gryllidae) from the southern United States. Trans. Am. Entomol. Soc. **126,** 117—123.

Cade, W. H. & Tyshenko, M. G. 1990: Geographic variation in hybrid fertility in the field crickets *Gryllus integer*, *G. rubens*, and *Gryllus* sp. Can. J. Zool. **68,** 2697—2700.

Crankshaw, O. S. 1979: Female choice in relation to calling and courtship songs in *Acheta domesticus*. Anim. Behav. **27,** 1274—1275.

Doherty, J. A. & Callos, D. 1991: Acoustic communication in the trilling field cricket, *Gryllus rubens* (Orthoptera: Gryllidae). J. Insect. Behav. **4,** 67—82.

Doherty, J. A. & Howard, D. J. 1996: Lack of preference for conspecific calling songs in female crickets. Anim. Behav. **51,** 981—989.

Fulton, B. B. 1952: Speciation in the field cricket. Evolution **6,** 283—295.

Gerhardt, H. C. 1991: Female choice in treefrogs: static and dynamic acoustic criteria. Anim. Behav. **42,** 615—636.

Gerhardt, H. C. 1994: Selective responsiveness to long-range acoustic signals in insects and anurans. Am. Zool. **34,** 706—714.

Gray, D. A. & Cade, W. H. 1999: Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. Evolution **53,** 848—854.

Gray, D. A. & Cade, W. H. 2000: Sexual selection and speciation in field crickets. Proc. Natl. Acad. Sci. USA. **97,** 14449—14454.

Gray, D. A. & Eckhardt, G. E. In press: Is cricket courtship song condition dependent? Anim. Behav., in press.

Gray, D. A., Walker, T. J., Conley, B. E. & Cade, W. H. 2001: A morphological means of distinguishing females of the cryptic field cricket species, *Gryllus rubens* and *G. texensis* (Orthoptera: Gryllidae). Fla. Entomol. **84,** 314—315.

Hoy, R. R., Hoikkala, A. & Kaneshiro, K. 1988: Hawaiian courtship songs: evolutionary innovation in communication signals of *Drosophila*. Science **240,** 217—219.

Huang, Y., Ortí, G., Sutherlin, M., Duhachek, A. & Zera, A. 2000: Phylogenetic relationships of North American field crickets inferred from mitochondrial DNA data. Molec. Phylog. Evol. **17,** 48—57.

Leroy, Y. 1966: Signaux acoustiques, comportement et systématique de quelque espécies de Gryllides (Orthoptera, Ensiferes). Bull. Biol. Fr. Belg. **10,** 1—134.

Libersat, F., Murray, J. A. & Hoy, R. R. 1994: Frequency as a releaser in the courtship song of two crickets, *Gryllus bimaculatus* (deGeer) and *Teleogryllus oceanicus*: a neuroethological analysis. J. Comp. Physiol. A **174,** 485—494.

Márquez, R. & Bosch, J. 1997: Male advertisement call and female preference in sympatric and allopatric midwife toads. Anim. Behav. **54,** 1333—1345.

Marshall, D. C. & Cooley, J. R. 2000: Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. Evolution **54,** 1313—1325.

Martin, S. D., Gray, D. A. & Cade, W. H. 2000: Fine-scale temperature effects on cricket calling song. Can. J. Zool. **78,** 706—712.

Noor, M. A. F., Williams, M. A., Alvarez, D. & Ruiz-Garcia, M. 2000: Lack of evolutionary divergence in courtship songs of the *Drosophila pseudoobscura* subspecies. J. Insect. Behav. **13,** 255—262.

Otte, D. 1994: The Crickets of Hawaii. The Orthopterists' Soc., Philadelphia.

Ritchie, M. G. & Gleason, J. M. 1995: Rapid evolution of courtship song pattern in *Drosophila willistoni* sibling species. J. Evol. Biol. **8,** 463—479.

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

Wagner, W. E. Jr & Reiser, M. G. 2000: The importance of calling song and courtship song in female mate choice in the variable field cricket. Anim. Behav. **59,** 1219—1226.

Walker, T. J. 1957: Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. Ann. Entomol. Soc. Am. **50,** 626—636.

Walker, T. J. 1962: The taxonomy and calling songs of United States tree crickets (Orthoptera: Gryllidae: Oecanthinae). I. The genus *Neoxabea* and the *niveus* and *varicornis* groups of the genus *Oecanthus*. Ann. Entomol. Soc. Am. **55,** 303—322.

Walker, T. J. 1963: The taxonomy and calling songs of United States tree crickets (Orthoptera: Gryllidae: Oecanthinae). II. The nigricornis group of the genus. *Oecanthus*. Ann. Entomol. Soc. Am. **56,** 772—789.

Walker, T. J. 1998: Trilling field crickets in a zone of overlap (Orthoptera: Gryllidae: *Gryllus*). Ann. Entomol. Soc. Am. **91,** 175—184.

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

Weissman, D. B., Rentz, D. C. F., Alexander, R. D. & Loher, W. 1980: Field crickets (*Gryllus* and *Acheta*) of California and Baja California, Mexico (Orthoptera: Gryllidae: Gryllinae). Trans. Am. Entomol. Soc. **106,** 327—356.

Wells, M. M. & Henry, C. S. 1998: Songs, reproductive isolation, and speciation in cryptic species of insects. In: Endless Forms: Species and Speciation (Howard, D. J. & Berlocher, S. H., eds). Oxford Univ. Press, New York. pp. 217—233.

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