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## Divergence in female calling song discrimination between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae)

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**Abstract** Two species of closely related wood cricket, *Gryllus fultoni* (Orthoptera: Gryllidae) and *Gryllus vernalis*, occur together in some parts of the eastern United States and have a similar calling song structure, consisting of three-pulse chirps. A previous study revealed that chirp rate in *G. fultoni* was highest (greatest difference vis-à-vis chirp rate in *G. vernalis*) in sympatric populations, intermediate in near allopatric populations that were located close to the sympatric zone, and lowest in allopatric populations. A similar trend was observed in pulse rate, but the mean values of this trait showed much more convergence than chirp rate at the low end of the range of calling temperatures. In this study, we investigated the song discrimination of females from sympatric and allopatric populations of *G. fultoni* at about 23°C, which is near the middle of the normal range of calling temperatures. We used both single-stimulus and two-stimulus playback experiments to learn if geographical differences in song preferences paralleled those in calling songs. Stimuli presented were representative of calling songs in three classes of *G. fultoni* populations (sympatric, near allopatric, and far allopatric), a calling song of *G. vernalis*, and three calling songs with parameter values that were intermediate with respect to those of the songs of far allopatric *G. fultoni* and *G. vernalis*. In the single-stimulus playbacks, females of all *G. fultoni* populations responded poorly if at all to the heterospecific stimulus. Females of sympatric and near

allopatric populations responded poorly to all intermediate stimuli, but females of far allopatric populations frequently responded to these sounds. In the two-stimulus playbacks, females of sympatric and near allopatric populations generally discriminated against intermediate and heterospecific stimuli. However, females of far allopatric populations often did not discriminate against intermediate stimuli, whose characteristics resembled the calling songs of *G. vernalis*. The divergent pattern of female phonotactic discrimination between sympatric and far allopatric populations was thus generally congruent with the pattern of divergence in chirp and pulse rates and would be expected to significantly reduce heterospecific mating in sympatry. These geographical patterns of female song discrimination and male calling songs conform to a commonly used definition of reproductive character displacement.

**Keywords** Female song discrimination · *Gryllus fultoni* · *Gryllus vernalis* · Reproductive character displacement · Reproductive isolation

### Introduction

Reproductive character displacement (RCD) can be defined as a geographical pattern in which traits that reduce the chances of mating between two closely related species differ more in areas of sympatry than in allopatric areas (Brown and Wilson 1956; Grant 1972; Gerhardt and Huber 2002); this pattern of sympatric divergence may arise from selection against the adverse consequences of interspecific matings (Dobzhansky 1940; Howard 1993). Such selection has been termed reinforcement (sensu Blair 1955) and may contribute to speciation (Servedio and Noor 2003). RCD, once considered to be highly controversial, has recently been documented by numerous empirical studies (see Table 1 in Gabor and Ryan 2001; Gerhardt 1999; Marshall and Cooley 2000; Höbel and Gerhardt 2003), and several theoretical approaches have shown the feasibility of reinforcement (Liou and Price 1994; Kelly and Noor 1996; Servedio and Kirkpatrick 1997; Kirkpatrick and Servedio

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**Table 1** Ranges of population means ( $\pm$  SD) of sympatric ( $n=6$ ), near allopatric ( $n=3$ ), and far allopatric ( $n=4$ ) *G. fultoni* groups, as well as heterospecific group ( $n=6$ ) (see Jang and Gerhardt 2006)

	Chirp rate ( $s^{-1}$ )	Pulse rate ( $s^{-1}$ )	Pulse duration (ms)	Carrier frequency (kHz)
Sympatric	4.92 $\pm$ 0.62~5.53 $\pm$ 0.34	44.8 $\pm$ 3.7~51.1 $\pm$ 4.7	11.6 $\pm$ 1.5~13.2 $\pm$ 2.0	4.23 $\pm$ 0.20~4.42 $\pm$ 0.17
Near allopatric	4.35 $\pm$ 0.52~4.72 $\pm$ 0.48	44.1 $\pm$ 4.7~48.8 $\pm$ 3.1	10.2 $\pm$ 1.6~13.0 $\pm$ 3.4	4.07 $\pm$ 0.14~4.17 $\pm$ 0.24
Far allopatric	3.14 $\pm$ 0.56~3.81 $\pm$ 0.60	34.3 $\pm$ 2.1~42.8 $\pm$ 4.3	11.9 $\pm$ 2.5~15.3 $\pm$ 1.8	4.19 $\pm$ 0.26~4.27 $\pm$ 0.21
Heterospecific	2.55 $\pm$ 0.59~3.04 $\pm$ 0.30	29.8 $\pm$ 1.4~33.7 $\pm$ 1.4	13.0 $\pm$ 3.9~14.5 $\pm$ 3.7	4.22 $\pm$ 0.16~4.66 $\pm$ 0.27

Calling songs of *G. fultoni* and *G. vernalis* populations were recorded in the field (Jang and Gerhardt 2006), and the mean values of calling song characters were calculated using UNIANOVA with the temperature covariate set to 23°C for each population. The standard stimuli and the heterospecific stimulus were based on averages of the population means for each group (see Table 2)

1999; Kirkpatrick 2000). As pointed out by Servedio and Noor (2003), the controversy has been accentuated by the perspective that reinforcement must involve selection against hybrids, whereas a more “broad sense” definition asserts that any increase in premating isolation would qualify as reinforcement. By this latter definition, reinforcement is likely to contribute to the latter stages of many cases of sympatric speciation or merely to reduce gamete wastage when taxa complete speciation in allopatry and then reestablish geographical overlap in some parts of their ranges of distribution (e.g., Kirkpatrick and Ravigné 2002).

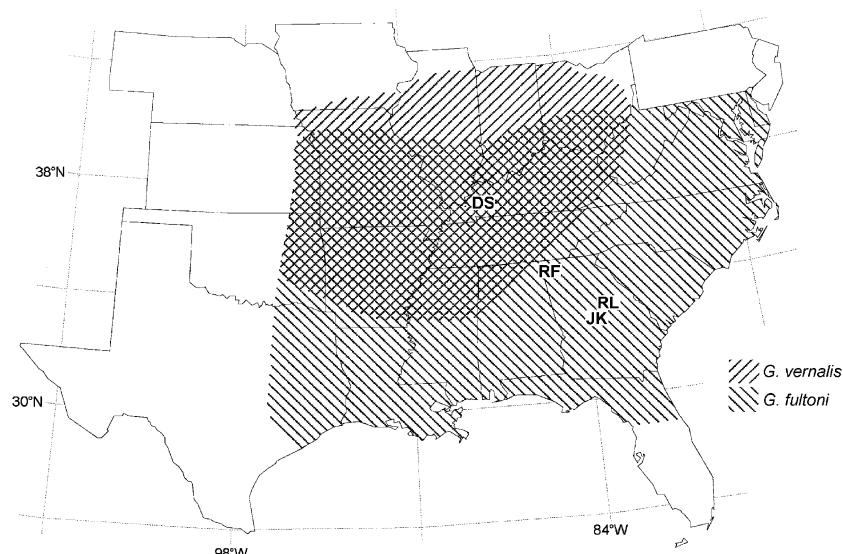
One limitation of most empirical studies of RCD is that they focus on either mating signals usually produced by the male or, more rarely, on the selectivity for these signals usually exhibited by the female (see Table 1 in Gabor and Ryan 2001). Thus, examination of only one component of the communication system may lead to a wrong conclusion about RCD. For example, a pattern of RCD may occur in male signals, but if there is no corresponding pattern in female preference, then there cannot be any enhancement of premating isolation. By contrast, if there are species differences in mating signals in both sympatric and allopatric areas, then selection can still act on receiver preferences, resulting in RCD of preferences but not signals (Waage 1979; review in Gerhardt and Huber 2002).

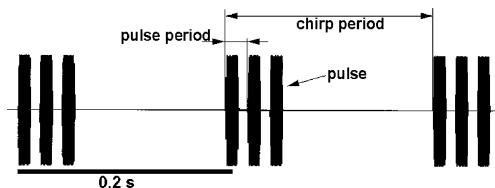
In the eastern United States, two species of wood cricket, *Gryllus fultoni* (Alexander) (Orthoptera: Gryllidae) and

*Gryllus vernalis* Blatchley, occur together in an area between eastern Kansas and northwest of the Appalachian Mountains (Fig. 1). The two species have a very similar calling song structure, consisting of three-pulse chirps (see Fig. 2). Alexander (1957) first noted qualitative differences in calling songs between sympatric and allopatric populations of *G. fultoni*. Recently, a formal analysis of geographic variation in calling songs of *G. fultoni*, corroborated by studies of the songs of laboratory-reared crickets, showed that values of chirp and pulse rates were highest in sympatric populations, lowest in far allopatric populations, and intermediate in the near allopatric populations (Jang and Gerhardt 2006). As a result, divergence in chirp and pulse rates between the two species was greater in areas of sympathy than in areas of allopatry. Thus, differences in chirp rate, pulse rate, or the combination of these two properties can potentially be used to promote reproductive isolation between *G. fultoni* and *G. vernalis* in sympathy. There was no statistical difference in calling song characters between sympatric and allopatric *G. vernalis* populations (unpublished data, Y. Jang).

In this study, we investigated whether the pattern of female song discrimination diverged in accordance with the pattern found in chirp or pulse rate between sympatric and allopatric *G. fultoni* populations. This investigation is necessary because the magnitude of divergence in chirp and pulse rate between *G. fultoni* and *G. vernalis* in sympathy

**Fig. 1** Geographic distributions of *G. fultoni* and *G. vernalis*. The right-hatched and left-hatched areas denote distributions of *G. fultoni* and *G. vernalis*, respectively. The cross-hatched area represents the sympatric zone. Playback experiments for this study were conducted on four *G. fultoni* populations. JK (Jackson, GA, USA) and RL (Rutledge, GA, USA) were the far allopatric populations, and DS (Dawson Springs, KY, USA) was the sympatric population. RF (Rising Fawn, GA, USA) was located close to the sympatric zone and was the near allopatric population





**Fig. 2** The stimulus representing calling songs of the *G. fultoni* sympatric populations. All other stimuli, including the heterospecific stimulus, had the same pulse shape and three pulses per chirp. The values of temporal and spectral parameters for all stimuli are shown in Table 2

decreased as temperature decreased (Jang and Gerhardt 2006). Thus, reproductive isolation based solely on divergence in calling song characters may not be reliable over the entire range of breeding temperatures. Playback experiments, employing both single-stimulus and two-stimulus designs, were used to examine the phonotactic selectivity of *G. fultoni* populations (two far allopatric, one near allopatric, and one sympatric). The single-stimulus design simulated a situation in which a female encounters an isolated calling song of any kind (conspecific, heterospecific, or intermediate). This test was critical because females of some species orient toward heterospecific signals in the absence of conspecific signals (review in Gerhardt and Huber 2002). The two-stimulus design simulated a situation in which a female encounters both conspecific and heterospecific calling songs at the same time. This design estimates the relative attractiveness of population stimuli compared to intermediate and heterospecific stimuli (for comparisons of two designs, see Doherty 1985; Wagner 1998).

## Materials and methods

### Biology of *G. fultoni* and *G. vernalis*

Both *G. fultoni* and *G. vernalis* are nymphal-diapause species (Alexander 1957) and are univoltine, except in north Florida where *G. fultoni* has a partial second generation with the adults occurring in August and September (Walker 1974). Both species are strictly micropterous in the field and have a narrower head than pronotum (Alexander 1957). Because they have similar life history and morphology, *G. fultoni* and *G. vernalis* are regarded as closely related species (D. Weissman, personal communication, 2003). The phylogenetic relationship based on mitochondrial DNA genes revealed that the *Gryllus* species in eastern North America may be divided into four clades characterized by a difference in life history traits (Huang et al. 2000). Unfortunately, *G. vernalis* was not incorporated into this phylogeny, and the phylogenetic relationship between *G. fultoni* and *G. vernalis* still needs to be determined.

## Study populations

Adults of both cricket species were caught in the field in May 2002. The *G. fultoni* populations used for this study were from Jackson (JK, 35), GA, USA; Rutledge (RL, 23), GA, USA; Rising Fawn (RF, 27), GA, USA; and Dawson Springs (DS, 27), KY, USA (Fig. 1). The letters and numerals in parentheses represent the abbreviation and the number of field-caught females used for egg collection. JK and RL were designated as allopatric populations, and DS was a sympatric population. RF was located close to the sympatric zone and was designated as a near allopatric population. Immediately after capture, field-caught females were housed on wet sand in 1-gallon jars for egg collection, and the progeny of these crickets were reared from eggs to adults in the laboratory. All playback experiments were conducted on the first-generation crickets of the field-caught females. Cricket colonies were reared in plastic bins (33×50×29 cm) that had holes on the side for ventilation. Both juvenile and adult crickets were given shelter and were provided ad libitum with cricket chow (Fluker Farms, LA, USA) and lettuce. Adult crickets of both species were removed from colonies within 48 h of the final molt to ensure that all crickets used for this study were virgin (Solymar and Cade 1990). Newly emerged adults were individually housed in plastic containers (12×12×9 cm) until trials. All crickets were maintained at 23±1°C and with a 14:10 h, light to dark photoperiod during development. We tested the phonotactic selectivity of females over the same range of temperature.

### Stimulus design

The acoustic stimuli used for this study were synthesized using custom-designed software with 16-bit resolution and 44.1 kHz sampling rate (see Schul and Bush 2002). The pulse shape was constant for all stimuli, with 10% linear rise time, 80% flat time, and 10% fall time. The number of pulses per chirp was three for all stimuli. Each stimulus file was 10 min long and was written on a compact disc for playback.

Parameter values of stimuli were derived from the field recordings (Jang and Gerhardt 2006), and mean values of calling song characters used for test stimuli were adjusted to 23°C (Table 1). The seven acoustic stimuli ordered from high to low values of chirp and pulse rates were sympatric, near allopatric, far allopatric, and intermediate 1 (I1), intermediate 2 (I2), intermediate 3 (I3), and heterospecific stimuli (Table 2). The first three stimuli were designated as standard stimuli for their respective *G. fultoni* populations, and the characteristics of the standard stimuli were based on averages of calling songs of six sympatric, three near allopatric, and four far allopatric *G. fultoni* populations, respectively. Likewise, the characteristics of the heterospecific stimulus were based on averages derived from analyses of field recordings of six *G. vernalis* populations

**Table 2** Characteristics of stimuli used for female phonotaxis playback experiments

	CR ( $s^{-1}$ )	PR ( $s^{-1}$ )	PD (ms)	CF (Hz)
Sympatric	5.16	48.64	12.42	4,310
Near allopatric	4.56	46.05	11.48	4,123
Far allopatric	3.51	39.61	13.03	4,241
Intermediate 1	3.33	37.57	12.75	4,281
Intermediate 2	3.15	35.54	13.19	4,337
Intermediate 3	2.97	33.50	13.63	4,393
Heterospecific	2.79	31.47	14.07	4,449

CR chirp rate, PR pulse rate, PD pulse duration, CF carrier frequency are calling song characters. The top three stimuli were the standard stimuli for sympatric, near allopatric, and far allopatric *G. fultoni* populations. See the main text for how these parameter values of stimuli were derived

in sympatry. Intermediate stimuli had characteristics intermediate with respect to those of the far allopatric *G. fultoni* and sympatric *G. vernalis* populations. Values of chirp rate, pulse rate, pulse duration, and carrier frequency of adjacent intermediate stimuli were separated by  $0.18\ s^{-1}$ ,  $2.04\ s^{-1}$ ,  $0.44\ ms$ , and  $56\ Hz$ , respectively (Table 2).

Although only chirp and pulse rates showed the pattern expected of RCD in *G. fultoni* (Jang and Gerhardt 2006), we used stimuli representative of calling songs of *G. fultoni* and *G. vernalis* to examine the pattern of female song discrimination. We used this approach instead of holding all properties except chirp and pulse rates constant because the properties affecting female mate preferences are still unknown in both *G. fultoni* and *G. vernalis*. Moreover females of sympatric, near allopatric, and far allopatric *G. fultoni* populations may differ in their song discrimination and preference. In other words, preferences could be influenced by population-specific simultaneous variation in chirp rate and these other properties.

#### Single-stimulus experiment

All playback experiments were conducted in an octagonal arena that measured 50 cm from the center to the corner. The arena was built on a plywood base covered with a thin layer of carpeting. The boundary of the arena was a 12-cm high acoustically transparent screen. The stimulus was played back using a Sony CD player (Model No. D-SJ301, Japan), and the output from the CD player was sent to an attenuator-amplifier (TDL Technology, Model 439; Las Cruces, NM, USA; less than  $-0.1\ dB$  at 10 Hz at all gains and  $-0.5\ dB$  at 124 kHz at +20 dB). The signal from the attenuator-amplifier was sent to one of two tweeters (Dayton #275-100 Euro series textile tweeter; Springboro, OH, USA; flat frequency 2,000–20,000 Hz), which was placed at floor level outside of the arena wall (55 cm from the center of the arena). The angular separation of the two tweeters was  $120^\circ$ . Choice of a tweeter for playback trials was switched daily to prevent any phonotactic bias that might be present in the arena. Before a series of trials the amplitude level measured in the center of the arena was

calibrated to 75 dB SPL (0 dB=20  $\mu Pa$ ) using a Brüel and Kjær (Model 2209; Nærum, Denmark) sound-level meter with peak setting. During the trials, the anechoic chamber was lighted with three red 25-W incandescent bulbs.

Female crickets to be tested for their phonotactic selectivity were drawn randomly from the stock populations after eclosion. Only intact females that aged between 7 and 30 days were used. Each trial began by placing a female cricket in an opaque plastic cover located in the center of the arena. The plastic cover (250 ml) had holes for sound permeation. The cricket was placed in the arena for 1 min to acclimate without any sound and then was exposed to a stimulus broadcast from one of the tweeters for 1 min. Then the cricket was released by removing the cup, and 5 min was allowed for a response to the stimulus. We scored a positive phonotactic response if a female oriented toward a tweeter and touched the wall of the arena directly in front of the speaker broadcasting the stimulus or if a female made a “double reversal” movement in front of the tweeter. That is, crickets sometimes ran toward the arena screen after release without orientation and walked along the screen. A female cricket sometimes reversed its direction of walking in front of the tweeter broadcasting the test stimulus twice in a row within close proximity of the tweeter; each reversal of direction had to follow a crossing of the line between the tweeter and the female release point. The double reversal movement was a local search and occurred when an individual was very close to the object that it was searching (see Bell 1991). A score of “no response” was tabulated if the female left the cup but did not orient toward the test stimulus, did not make a double reversal movement, or failed to leave the cup within the 5-min trial.

In the single-stimulus playback experiment, each cricket was tested five times over a 2-day period. In a given day, a female cricket was tested two or three times. The order in which stimuli were presented was determined using a random number table at the beginning of the 2-day period. The total number of females used for the single-stimulus playback experiment was 30 for JK, 30 for RL, 31 for RF, and 30 for DS. Due to disappearance or death, the number of females used for five trials was not the same for each population, and the ranges of females used for each stimulus test were 29~30 for JK, 29~30 for RL, 29~31 for RF, and 28~30 for DS. The total number of phonotaxis trials for all populations was 589.

#### Two-stimulus experiment

The experimental setup and testing protocol of the two-stimulus choice experiment were similar to those of the single-stimulus experiment above. In the two-stimulus experiment, females of each population were given two stimuli simultaneously: the standard stimulus and an alternative stimulus with properties characteristic of intermediate or heterospecific calling song. In a pair of playback stimuli, one tweeter was designated for the standard stimulus and the other for the alternative stimulus. The tweeters assigned to these two kinds of stimuli were switched daily.

Each cricket was tested four times over a 2-day period. On a given day, a female cricket was tested twice. The order in which four sets of test stimuli were presented was determined using a random number table at the beginning of the 2-day testing period.

The total number of females used for the two-stimulus experiment was 30 for each population. Due to disappearance or death, the number of females used for four trials was not the same for each population, and the ranges of females used for each set of stimuli presentation were 29~30 for JK, 30 for RL, 29~30 for RF, and 28~30 for DS.

### Statistical analyses

Because female crickets were repeatedly subject to phonotactic trials, and because age might affect female selectivity (Prosser et al. 1997; Stout et al. 1989; Gray 1999), we used the generalized linear mixed models (GLIMMIX Macro) in the SAS (ver. 8.1; The SAS Institute; Cary, NC, USA) to determine whether three groups of the *G. fultoni* populations, sympatric (DS), near allopatric (RF), and far allopatric (JK and RL), differed in their phonotactic orientations. Predictor variables were “zone,” “test,” and “age”. In the single-stimulus experiment, the response variable was the female phonotactic orientation that was categorized as 1 (positive response) or 0 (all others). Zone indicated whether an individual cricket was sympatric, near allopatric, or far allopatric. Test was one of the five tests (standard, I1, I2, I3, and heterospecific). Age was the number of days between the final molting and the first phonotactic trial and was a random variable. Post hoc analyses using least square means were conducted with the Tukey–Kramer adjustment to determine which groups of *G. fultoni* populations differed.

The statistical procedures for testing whether the three groups of *G. fultoni* crickets differed in female phonotactic selectivity in the two-stimulus experiment were the same as above. The response variable was categorized as 1 (standard stimulus) or 0 (alternative stimulus). The total number of trials in the two-stimulus experiment across all populations was 480. Of these, 100 trials did not yield either 1 or 0 response, and they were treated as missing data.

## Results

### Single-stimulus experiment

The two allopatric populations did not differ in their phonotactic responses over the five single-stimulus tests (GLIMMIX, numerator  $df=1$ , denominator  $df=284$ ,  $F=0.96$ ,  $P=0.3279$ ) and were pooled for further GLIMMIX analyses. Results of the GLIMMIX analysis revealed that the female phonotactic orientation was significantly affected by zone and test, but not by age (Table 3). There was no significant interaction between zone and test for female phonotactic orientation. The post hoc analyses revealed that the far and near allopatric groups were significantly

**Table 3** Results of the generalized linear mixed (GLIMMIX) models for the single-stimulus (a) and two-stimulus (b) experiments

	Numerator $df$	Denominator $df$	$F$	$P$
(a) Single-stimulus experiment				
Age	1	573	1.17	0.2793
Zone	2	573	4.52	0.0113
Test	4	573	14.27	<0.0001
Zone × test	8	573	1.29	0.2462
(b) Two-stimulus experiment				
Age	1	367	0.37	0.5415
Zone	2	367	5.48	0.0045
Test	3	367	3.39	0.0182
Zone × test	6	367	0.39	0.8868

Predictor variables were “zone,” “test,” and “age”. See the text for more information

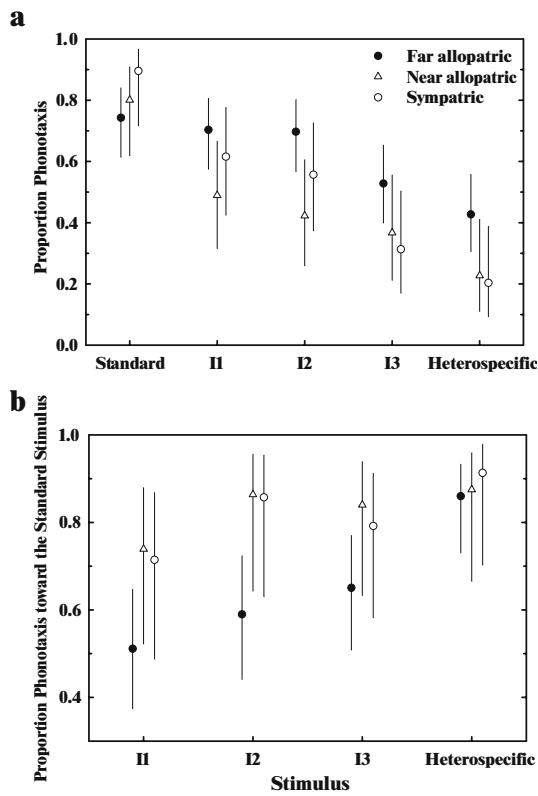
different in the overall female phonotactic orientations over five tests (Table 4 and Fig. 3a). The female phonotactic orientation was not significantly different between far allopatric and sympatric groups and between near allopatric and sympatric groups. However, the sympatric and far allopatric groups significantly differed in their orientations toward the heterospecific stimulus (Fig. 3a; GLIMMIX differences of least square means,  $df=573$ ,  $t=2.00$ ,  $P=0.0457$ ).

Compared to responses toward their own standard stimuli, females of all *G. fultoni* groups responded poorly to the heterospecific stimulus (Fig. 3a; GLIMMIX differences of least square means,  $df=573$ ,  $t\geq 3.34$ ,  $P\leq 0.0009$ ) and to the I3 stimulus (GLIMMIX differences of least square means,  $df=573$ ,  $t\geq 2.35$ ,  $P\leq 0.0189$ ). Compared to responses toward their own standard stimuli, females of the sympatric (GLIMMIX differences of least square means,  $df=573$ ,  $t\geq 2.28$ ,  $P\leq 0.0228$ ) and near allopatric populations (GLIMMIX differences of least square means,  $df=573$ ,  $t\geq 2.42$ ,  $P\leq 0.0157$ ) responded poorly to all intermediate stimuli. Compared to responses toward their own standard stimuli,

**Table 4** Post hoc analyses testing group differences in the phonotactic responses in the single-stimulus (a) and two-stimulus (b) experiments

Zone 1	Zone 2	$df$	$t$	Adj $P$
(a) Single-stimulus experiment				
Far allopatric	Near allopatric	573	2.91	0.0106
Far allopatric	Sympatric	573	1.55	0.2682
Near allopatric	Sympatric	573	-1.01	0.5693
(b) Two-stimulus experiment				
Far allopatric	Near allopatric	367	-2.67	0.0216
Far allopatric	Sympatric	367	-2.55	0.0300
Near allopatric	Sympatric	367	0.03	0.9994

The generalized linear mixed models were used with the Tukey–Kramer adjustment



**Fig. 3** Results of female phonotaxis playback experiments. In the single-stimulus playback experiment (a), one of the following stimuli was presented to females of each *G. fultoni* population: standard, intermediate 1 (I1), intermediate 2 (I2), intermediate 3 (I3), and heterospecific stimulus (see Table 2). The standard (conspecific) stimulus had values representative of the calling songs of the female's population of origin. The Y-axis indicates the proportions of females responding to the standard, intermediate, or the heterospecific stimulus. In the two-stimulus playback experiment (b), we presented female crickets with a choice of two different stimuli broadcasting simultaneously. The standard stimulus was always played back with one of the alternative stimuli: I1, I2, I3, or heterospecific stimulus. Two far allopatric populations, JK and RL, were pooled to form the far allopatric *G. fultoni* group. The Y-axis indicates the proportions of females choosing the standard stimulus over alternatives with intermediate or heterospecific values. Vertical lines in both parts of the figure indicate upper and lower 95% confidence limits

females of the far allopatric populations responded well to the I1 and I2 stimuli.

#### Two-stimulus experiment

The two allopatric populations did not differ in their phonotactic responses over the four choice tests (GLIMMIX, numerator  $df=1$ , denominator  $df=188$ ,  $F=0.49$ ,  $P=0.4870$ ) and were pooled for further GLIMMIX analyses. Results of the GLIMMIX analysis revealed that the female phonotactic orientation was significantly affected by zone and test, but not by age (Table 3). There was no significant interaction between zone and test for female phonotactic orientation. The post hoc analyses revealed that the overall female phonotactic orientation was significantly different

between the far allopatric and sympatric groups and between the far and near allopatric groups (Table 4 and Fig. 3b). In the far allopatric populations, the proportion of the phonotactic orientation toward the standard stimulus was significantly higher than 0.5 in the heterospecific and I3 tests (Fig. 3b; GLIMMIX least square means,  $df=367$ ,  $t\geq 2.08$ ,  $P\leq 0.0385$ ) but was not in the I2 and I1 tests (Fig. 3b; GLIMMIX least square means,  $df=367$ ,  $t\leq 1.19$ ,  $P\geq 0.2349$ ). In contrast, females of the near allopatric or sympatric populations generally discriminated against intermediate stimuli. In the near allopatric population, the proportion of the phonotactic orientation toward the standard stimulus was significantly higher than 0.5 in all four tests (Fig. 3b; GLIMMIX least square means,  $df=367$ ,  $t\geq 2.15$ ,  $P\leq 0.0321$ ). When given a choice between the standard stimulus and I1, females of the sympatric population (DS) did not discriminate against the I1 stimulus (GLIMMIX least square means,  $df=367$ ,  $t=1.87$ ,  $P=0.0625$ ). However, DS females significantly discriminated against I2, I3, or the heterospecific stimuli (GLIMMIX least square means,  $df=367$ ,  $t\geq 2.81$ ,  $P\leq 0.0053$ ) in the two-stimulus playback experiment.

#### Discussion

This study demonstrated that the pattern of female song discrimination was different among sympatric, near allopatric, and far allopatric *G. fultoni* populations. Playback experiments using both single-stimulus and two-stimulus designs showed that females of sympatric and near allopatric *G. fultoni* females generally discriminated against intermediate stimuli whose characteristics were intermediate between calling songs of far allopatric *G. fultoni* and sympatric *G. vernalis* populations. By contrast, females of allopatric *G. fultoni* populations responded as well toward intermediate stimuli as they did toward their standard stimulus. Discrimination against intermediate stimuli would enhance premating reproductive isolation between *G. fultoni* and *G. vernalis* in sympatry because characteristics of intermediate stimuli, especially I2 and I3, were within the ranges of calling songs of sympatric *G. vernalis* populations (Jang and Gerhardt 2006). Therefore, the pattern of female song discrimination in *G. fultoni* was consistent with predictions of RCD.

Can the phonotactic discrimination by females of sympatric *G. fultoni* populations lead to reproductive isolation between *G. fultoni* and *G. vernalis* in sympatry? In both single-stimulus and two-stimulus experiments, these females discriminated against the I2, I3, and heterospecific stimuli. Thus, the magnitude of differences sufficient for phonotactic discrimination at 23°C was 2.01 s<sup>-1</sup> in chirp rate and 13.1 s<sup>-1</sup> in pulse rate (see Table 1). The minimum temperatures at which the mean differences were greater than 2.01 s<sup>-1</sup> in chirp rate and 13.1 s<sup>-1</sup> in pulse rate were 19.4 and 18.9°C, respectively (Jang and Gerhardt 2006). Thus, reproductive isolation based on average song differences may be possible only above 18.9°C. Reproductive isolation is more likely at higher temperatures because the

differences in chirp and pulse rates increase at higher temperatures (Jang and Gerhardt 2006). However, reproductive isolation solely based on song differences and female song discrimination between *G. fultoni* and *G. vernalis* in sympatry may not be possible at the lower end of the temperature range. Even at higher temperatures, reproductive isolation based on differences in chirp and pulse rates may not always be reliable due to variability of these properties.

The conclusion stated above about reproductive isolation between *G. fultoni* and *G. vernalis* in sympatry is tentative for two additional reasons. First, male song characters and female selectivity are temperature-coupled in many acoustic insects and frogs (Gerhardt 1978). That is, the change in calling song characters due to temperature change may be matched by a change in female selectivity. We have not tested whether temperature coupling occurs in these two species of crickets. Second, there may be an interaction between rearing environment and expression of calling song characters in *G. fultoni* (see Walker 2000). The values of chirp and pulse rates were generally lower in the laboratory populations than those in the field populations (Jang and Gerhardt 2006). In the laboratory data, the mean difference in chirp rate between *G. fultoni* and *G. vernalis* in sympatry was less than  $2.01\text{ s}^{-1}$  throughout the tested temperature range (between 18 and  $28^\circ\text{C}$ ), whereas that in pulse rate was greater than  $13.1\text{ s}^{-1}$  throughout this range. In other words, reproductive isolation may be possible based only on pulse rate in the laboratory data. The difference between field and laboratory data may reflect differences in developmental temperatures between field and laboratory populations. Therefore, to properly determine reproductive isolation between *G. fultoni* and *G. vernalis* in sympatry, we need information about how calling song characters and female selectivity are expressed under various temperature conditions.

The pattern of female song discrimination toward synthesized stimuli was largely parallel to the pattern of divergence in chirp and pulse rates between sympatric and far allopatric *G. fultoni* populations. Chirp and pulse rates of the sympatric *G. fultoni* populations were significantly higher than those of the far allopatric *G. fultoni* populations (Jang and Gerhardt 2006). Thus, the southern wood cricket, *G. fultoni*, is one of the rare cases where both male signals and female song discrimination have been examined and have shown the pattern of RCD. Another case of RCD for both male signaling and female song discrimination is a North American species of periodical cicada, *Magicicada neotredecim* (Marshall and Cooley 2000). *M. neotredecim* is a 13-year species and overlaps a sibling 13-year *Magicicada tredecim* in the central United States. In sympatric areas, the dominant male call pitch of *M. neotredecim* increases from approximately 1.4 to 1.7 kHz, whereas that of *M. tredecim* remains comparatively stable. The average preferences of female *M. neotredecim* for call pitch show a similar geographic pattern, changing with the call pitch of conspecific males.

Although our playback experiments found a pattern of RCD in female song discrimination in *G. fultoni*, our design did not allow us to specify what calling song characters or combinations were promoted in female song discrimination. In other cricket species, chirp rate (*Acheta domesticus*, Stout and McGhee 1988; *Gryllus lineaticeps*, Wagner 1996), pulse rate (*Gryllus campestris*, Thorson et al. 1982; *Gryllus bimaculatus*, Doherty 1985; *Gryllus firmus*, Doherty and Storz 1992), and carrier frequency (*Teleogryllus oceanicus*, Hennig and Weber 1997) are used for discrimination of male calling songs. In *G. fultoni*, all four calling song characters had sufficient between-individual variation, which is a necessary condition for female song discrimination to affect the evolution of male traits (Jang and Gerhardt 2006). There were also significant differences in chirp rate and pulse rate between sympatric *G. fultoni* and *G. vernalis* populations, which could potentially be used for premating reproductive isolation between the two species. Thus, chirp rate and pulse rate could be used for female mate choice, as well as reproductive isolation between *G. fultoni* and *G. vernalis* in sympatry. However, pulse duration and carrier frequency alone are unlikely to be used for reproductive isolation between *G. fultoni* and *G. vernalis* in sympatry because ranges of these two characters overlap extensively between sympatric *G. fultoni* and *G. vernalis* populations. Alternatively, females of *G. fultoni* may simultaneously evaluate differences in several calling song characters to select among potential mates (see Brooks et al. 2005). More playback experiments using stimuli, in which values of one parameter vary at one time while other parameter values are held constant, are needed to see what properties of calling songs females of *G. fultoni* use for song discrimination.

To conclude definitively that the divergent patterns of the male calling song and female song discrimination between the sympatric and far allopatric *G. fultoni* populations were driven by the reinforcement process, we need more information about postzygotic reproductive isolation between *G. fultoni* and *G. vernalis*. Alexander (1968) indicated that *G. fultoni* and *G. vernalis* produced hybrids in some mating attempts. However, in an earlier study, no offspring were obtained from four *G. fultoni* ♂ × *G. vernalis* ♀ pairs and four *G. fultoni* ♀ × *G. vernalis* ♂ pairs, whereas all intraspecific matings produced offspring (Alexander 1957). We also failed to produce interspecific hybrids (none of the crosses of 17 *G. fultoni* ♂ × *G. vernalis* ♀ pairs and 14 *G. fultoni* ♀ × *G. vernalis* ♂ pairs produced instars) in laboratory studies (Y. Jang, unpublished data). These results support the conclusion that postzygotic isolation between these two taxa is almost complete.

The degree of premating and postmating reproductive isolation between *G. fultoni* and *G. vernalis* contrasts markedly with that between *Gryllus texensis* (formerly *Gryllus integer*, see Cade and Otte 2000) and *Gryllus rubens*. The latter two species occur together in the southeastern United States and produce trilled calling songs. They are closely related sister taxa (Huang et al. 2000) and are morphologically very similar. Such features have generated interest in what processes may have caused the

divergence of these two species. In the laboratory, they produce hybrid offspring that are as viable as within-taxon mating (Smith and Cade 1987; Cade and Tyshenko 1990), whereas in the field, the hybrids are seldom found (Walker 1998; Gray and Cade 2000). However, there is a strong premating isolation that is primarily based on the difference in pulse rates between these two species (Walker 1998, 2000; Gray and Cade 2000). The lack of postzygotic reproductive isolation between *G. texensis* and *G. rubens* relative to premating isolation suggests processes other than reinforcement (Gray and Cade 2000). Instead, divergent sexual selection was strongly implicated for speciation between these two taxa (Gray and Cade 2000).

Within the scenario of reinforcement, we suggest that populations of *G. fultoni* developed some degree of song discrimination against *G. vernalis* before secondary contact. That is, the strength of assortative mating was sufficiently high when the initial contact occurred (see Kirkpatrick and Ravigné 2002). All four *G. fultoni* populations in this study discriminated against the stimulus representative of *G. vernalis* calling songs. However, the song discrimination between *G. vernalis* and allopatric *G. fultoni* populations was incomplete because females of far allopatric *G. fultoni* populations oriented toward stimuli with character values that were intermediate between *G. fultoni* and *G. vernalis*. Furthermore, one may assume that females of *G. fultoni* have mate preferences for conspecific calling songs. That is, linkage disequilibrium is not zero (see Kirkpatrick and Ravigné 2002). The traditional view of reinforcement envisions low hybrid fitness after the secondary contact. Thus, viability selection against hybrids would be a main driving force to complete reproductive isolation between *G. fultoni* and *G. vernalis* after secondary contact. The effects of this reinforcement process may be divergence, both in male calling song characters and female mating preferences between allopatric and sympatric populations of *G. fultoni*.

Because the phylogenetic relationship between *G. fultoni* and *G. vernalis* is still unresolved, the possibility of reproductive interaction with other cricket species should be considered in terms of divergence in calling songs and female selectivity between sympatric and allopatric populations of *G. fultoni*. Besides *G. vernalis*, there are at least three other *Gryllus* species that might reproductively interact with *G. fultoni*. In the field localities where we collected *G. fultoni* or *G. vernalis*, *G. rubens* was the most frequently encountered and heard. This species has a trilled calling song, consisting of a continuous train of sound pulses. Females of *G. rubens* prefer the conspecific trilled calling song over the chirped calling song that has the characteristics of *G. fultoni* (Doherty and Callos 1991). However, the geographic distribution of *G. rubens* (Walker 2001) in relation to that of *G. fultoni* does not explain the marked difference in chirp and pulse rates between *G. fultoni* populations across the Appalachian Mountains. Two other candidates for reproductive interaction are *G. texensis* and *Gryllus veletis*. *G. texensis* has a trilled calling song with pulse rate faster than that of *G. rubens*. *G. veletis* has a chirped calling song with longer pulse durations, slower

chirp rates, and higher pulse numbers than those of *G. fultoni*. Currently, the female preferences of *G. fultoni* in tests of calling songs of these two species are unknown. Again, the geographic distributions of *G. texensis* and *G. veletis* (Walker 2001) are inconsistent with the divergent pattern of *G. fultoni* calling songs. Finally, *Gryllus pennsylvanicus*, *G. firmus*, and *Gryllus ovisopis* occur in the eastern US. However, the adult phase of these three species occurs primarily in the fall; hence, the probability of reproductive interaction with *G. fultoni* is slim. Given the similarity of calling songs, geographic distributions, breeding seasons, and habitats, *G. vernalis* is thus the most likely species to interact reproductively with *G. fultoni*, thereby driving the divergence in calling songs and female selectivity between sympatric and allopatric populations.

As Lemmon et al. (2004) emphasized, RCD is just one of the possible outcomes of reinforcement and can be generated without the process of reinforcement. Other less likely explanations for the divergence in both male calling song and female song discrimination in sympatric populations of *G. fultoni* include ecological character displacement, clinal variation, interference in the communication system between *G. fultoni* and *G. vernalis* (see Howard 1993; Noor 1999), and interspecific aggression (Tynkkynen et al. 2004). To address our interpretation and these alternatives, more information is needed about past and present gene flow, frequency and fitness of hybrids, genetics of male calling song and female preference, and heterospecific mating attempts in the field. In addition, it is imperative to recognize that studies of RCD must accompany identification of selective forces responsible for the pattern of divergence in characters between allopatric and sympatric populations (Jang and Gerhardt 2006).

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## References

- Alexander RD (1957) The taxonomy of the field crickets of the eastern United States (Orthoptera: Gryllidae: Acheta). Ann Entomol Soc Am 50:584–602
- Alexander RD (1968) Life cycle origins, speciation, and related phenomena in crickets. Q Rev Biol 43:1–41
- Bell WJ (1991) Searching behavior. Chapman & Hall, London, UK
- Blair WF (1955) Mating call and stage of speciation in the *Microhylla oliveacea*–*M. carolinensis* complex. Evolution 9:469–480
- Brooks R, Hunt J, Blows MW, Smith MJ, Bussiere LF, Jennions MD (2005) Experimental evidence for multivariate stabilizing sexual selection. Evolution 59:871–880
- Brown WL, Wilson EO (1956) Character displacement. Syst Zool 5:49–64
- Cade WH, 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 WH, Tyshenko MG (1990) Geographic variation in hybrid fertility in the field crickets *Gryllus integer*, *Gryllus rubens*, and *Gryllus* sp. Can J Zool 68:2697–2700
- Doherty JA (1985) Phonotaxis in the cricket, *Gryllus bimaculatus*: Comparisons of choice and no-choice paradigms. J Comp Physiol A 157:279–290
- Doherty JA, Callos JD (1991) Acoustic communication in the trilling field cricket, *Gryllus rubens* (Orthoptera: Gryllidae). J Insect Behav 4:67–81
- Doherty JA, Storz MM (1992) Calling song and selective phonotaxis in the field crickets, *Gryllus firmus* and *G. pennsylvanicus* (Orthoptera: Gryllidae). J Insect Behav 5:555–569
- Dobzhansky T (1940) Speciation as a stage in evolutionary divergence. Am Nat 74:312–321
- Gabor CR, Ryan MJ (2001) Geographical variation in reproductive character displacement in mate choice by male sailfin mollys. Proc R Soc Lond B 268:1063–1070
- Gerhardt HC (1978) Temperature coupling in the vocal communication system of the gray tree frog *Hyla versicolor*. Science 199:992–994
- Gerhardt HC (1999) Reproductive character displacement and other sources of environmental selection on acoustic communications systems. In: Hauser M, Konishi M (eds) The design of animal communication. MIT Press, Cambridge, MA, pp 515–534
- Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans. Univ. of Chicago Press, Chicago, IL
- Grant PR (1972) Convergent and divergent character displacement. Biol J Linn Soc 4:39–68
- Gray DA (1999) Intrinsic factors affecting female choice in house crickets: time cost, female age, nutritional condition, body size, and size-relative reproductive investment. J Insect Behav 12:691–700
- Gray DA, Cade WH (2000) Sexual selection and speciation in field crickets. Proc Natl Acad Sci U S A 97:14449–14454
- Hennig RM, Weber T (1997) Filtering of temporal parameters of the calling song by cricket females of two closely related species: a behavioral analysis. J Comp Physiol A 180:621–630
- Howard DS (1993) Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis. In: Harrison RG (ed) Hybrid zones and the evolutionary process. Oxford Univ Press, Oxford, UK, pp 46–69
- Höbel G, Gerhardt HC (2003) Reproductive character displacement in the acoustic communication system of green treefrogs (*Hyla cinerea*). Evolution 57:894–904
- Huang Y, Ortí G, Sutherlin M, Duhachek A, Zera A (2000) Phylogenetic relationship of North American field crickets inferred from mitochondrial DNA data. Mol Phylogenet Evol 17:48–57
- Jang Y, Gerhardt HC (2006) Divergence in the calling songs between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae). J Evol Biol (in press)
- Kelly JK, Noor MAF (1996) Speciation by reinforcement: a model derived from studies of *Drosophila*. Genetics 143:1485–1497
- Kirkpatrick M (2000) Reinforcement and divergence under assortative mating. Proc R Soc Lond B 267:649–1655
- Kirkpatrick M, Ravigné V (2002) Speciation by natural and sexual selection: models and experiments. Am Nat 159:S22–S35
- Kirkpatrick M, Servedio MR (1999) The reinforcement of mating preferences on an island. Genetics 151:865–884
- Lemmon AR, Smadja C, Kirkpatrick M (2004) Reproductive character displacement is not the only possible outcome of reinforcement. J Evol Biol 17:177–183
- Liou LW, Price TD (1994) Speciation by reinforcement of premating isolation. Evolution 48:1451–1459
- Marshall DC, Cooley JR (2000) Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. Evolution 54:1313–1325
- Noor MAF (1999) Reinforcement and other consequences of sympatry. Heredity 83:503–508
- Prosser M, Murray AM, Cade WH (1997) The influence of female age on phonotaxis during single and multiple song presentations in the field cricket, *Gryllus integer* (Orthoptera: Gryllidae). J Insect Behav 10:437–449
- Schul J, Bush SL (2002) Non-parallel coevolution of sender and receiver in the acoustic communication system of treefrogs. Proc R Soc Lond B 269:1847–1852
- Servedio MR, Kirkpatrick M (1997) The effects of gene flow on reinforcement. Evolution 51:1764–1772
- Servedio MR, Noor MAF (2003) The role of reinforcement in speciation: theory and data. Annu Rev Ecol Syst 34:339–364
- Smith CJ, Cade WH (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
- Solymar BD, Cade WH (1990) Heritable variation for female mating frequency in field cricket, *Gryllus integer*. Behav Ecol Sociobiol 26:73–76
- Stout JF, McGhee R (1988) Attractiveness of the male *Acheta domesticus* calling song to females. J Comp Physiol A 164:277–287
- Stout J, Walikonis R, Atkins G (1989) The influence of age and JHIII levels on the phonotactic response of female crickets (*Acheta domesticus*). In: Erber J, Menzel R, Pflüger HJD, Todt D (eds) Neural mechanisms of behavior. Thieme Medical, New York, NY, p 75
- Thorson J, Weber T, Huber F (1982) Auditory behaviour of the cricket. II. Simplicity of calling-song recognition in *Gryllus* and anomalous phonotaxis at abnormal carrier frequencies. J Comp Physiol 146:361–378
- Tynkkynen K, Rantala MJ, Suhonen J (2004) Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. J Evol Biol 17:759–767
- Waage JK (1979) Reproductive character displacement in *Calopteryx* (Odonata: Calopterygidae). Evolution 33:104–116
- Wagner WE Jr (1996) Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. Behav Ecol 7:279–285
- Wagner WE Jr (1998) Measuring female mating preferences. Anim Behav 55:1029–1042
- Walker TJ (1974) Character displacement and acoustic insects. Am Zool 14:1137–1150
- Walker TJ (1998) Trilling field crickets in a zone of overlap (Orthoptera: Gryllidae: Gryllus). Ann Entomol Soc Am 91:175–184
- Walker TJ (2000) Pulse rates in the songs of trilling field crickets (Orthoptera: Gryllidae: Gryllus). Ann Entomol Soc Am 93:565–572
- Walker TJ (2001) Singing insects of North America. <http://buzz.ifas.ufl.edu>