Sexual selection and speciation in field crickets

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Recent theoretical work has shown that sexual selection may cause speciation under a much wider range of conditions than previously supposed. There are, however, no empirical studies capable of simultaneously evaluating several key predictions that contrast this with other speciation models. We present data on male pulse rates and female phonotactic responses to pulse rates for the field cricket *Gryllus texensis*; pulse rate is the key feature distinguishing *G. texensis* from its cryptic sister species *G. rubens*. We show (*i*) genetic variation in male song and in female preference for song, (*ii*) a genetic correlation between the male trait and the female preference, and (*iii*) no character displacement in male song, female song recognition, female species-level song discrimination, or female song preference. Combined with previous work demonstrating a lack of hybrid inviability, these results suggest that divergent sexual selection may have caused speciation between these taxa.

The role that sexual selection plays in speciation is far from clear. Historically, courtship was assumed to have only a secondary or "reinforcing" role after the gradual divergence of taxa in allopatry (1). This is the classical view of reinforcement, which, although still controversial, has been recently gaining both theoretical and empirical support (for reviews, see refs. 2–7). Reproductive character displacement, which is the greater divergence of sexual signals and preferences in sympatry than in allopatry, is a predicted outcome of reinforcement. Reproductive character displacement has also received recent empirical support in taxa ranging from flies to fish, frogs, and birds (8–12), although studies not finding reproductive character displacement are at least as common (e.g., refs. 13–19). An important condition of the reinforcement hypothesis as originally formulated is that some degree of postmating genetic incompatibility has evolved in allopatry before reassociation in sympatry. That is, the reinforcement model depends on selection against male cues or female preferences that promote maladaptive crosstaxa pairings, where the pairings are maladaptive because of the divergence in traits, other than signals, that has already taken place.

In contrast with the reinforcement model, which proposes genetic divergence in allopatry sufficiently great to produce postmating incompatibility, some recent models suggest that premating incompatibility can evolve rapidly and with little genetic change (20–25). These models incorporate different assumptions about the underlying genetics, population size, the strength of natural selection against intermediate phenotypes, and the degree of assortative mating required for speciation. Some models conclude that speciation may be very rapid (e.g., ref. 26). Consistent with this, empirical evidence indicates that taxa likely to have undergone speciation involving sexual selection may have remarkably little genetic divergence—on the order of the genetic divergence normally found between populations (2, 27, 28). Rapid divergence will be greatly facilitated by positive assortative mating (26, 29) because of the development of a genetic correlation between female preferences and male traits (see also ref. 30). Such theoretical work strengthens the conclusions of many empiricists that sexual isolation may have been directly responsible for speciation in certain cases. In particular, the number of "cryptic" species—those species differing principally in mating signals and with often only a very limited degree of postzygotic isolation (31–33)—have suggested to some authors the possibility of sexual selection driving speciation even in the absence of any pronounced hybrid inferiority (see, e.g., refs. 28, 32–43).

We report here our attempts to clarify the role of sexual selection in speciation. We present results from the North American field cricket species *Gryllus texensis* (formerly *Gryllus integer*) and *Gryllus rubens*. These species are ideal candidates for study: they are cryptic sister species with extensive areas of both sympatry and allopatry, and prezygotic isolation appears to be virtually complete, whereas postzygotic isolation appears to be virtually absent. We review the evidence for each of these characteristics in slightly greater detail below. The two species occur throughout much of the south-central and southeastern United States: *G. texensis* ranges from west Texas east to extreme western Florida and Georgia; *G. rubens* ranges from eastern Texas east to Florida, Georgia, and North Carolina (http://csssrvr.entnem.ufl.edu/~walker/handbook/22gryll3.html). No known morphological differences separate males of these species; the only known difference is in the calling song used to attract sexually receptive females. *G. texensis* tend to produce trills with fewer pulses than *G. rubens*, but there can be considerable overlap (D.A.G., unpublished results); the only diagnostic song difference is pulse rate: *G. texensis* produce trills with a pulse rate of about 80 pulses per second (p/s), and *G. rubens* trill at about 56 p/s (both at 25°C; see Fig. 1 and ref. 44). When we initiated this study, there were no known morphological differences between females, but during the course of the study we discovered that female *G. texensis* tend to have slightly shorter ovipositors than *G. rubens* (45). Molecular phylogenetic evidence indicates that these are sister species (46). Laboratory crosses readily produce hybrid offspring that have fertility equal that of the parental species (47, 48); hybrids are intermediate in song (ref. 49; unpublished data) and female preference for song (unpublished data). Despite the ease of producing hybrids in the laboratory, two lines of evidence indicate that hybrids are either absent or rare in the field. First, the temperature-adjusted pulse rates of field-recorded males (or laboratory-recorded wild-caught males) are strongly bimodal, almost completely without overlap (ref. 44; this study). As hybrid song is known to be intermediate, this indicates that hybrids, if produced, may not survive to adulthood. Second, laboratory-reared sibships from field-caught field-inseminated females are all of one species or the other (ref. 44; this study). This indicates either that females do not mate with heterospecifics in the field or that there is a high degree of conspecific sperm precedence in dual-mated females (50, 51). On the basis of these two lines of evidence, we tentatively conclude that the two species do not hybridize or do

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Abbreviation: p/s, pulses per second.

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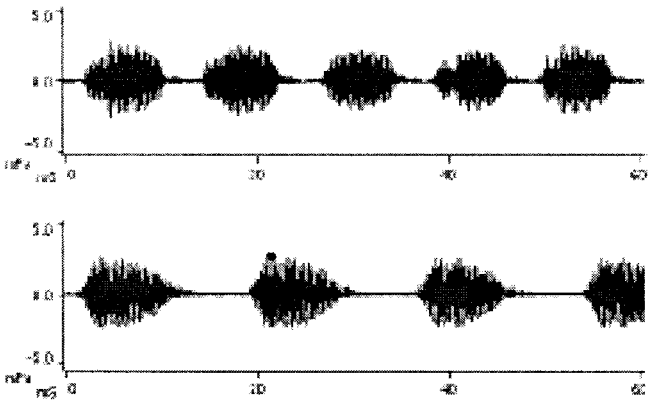

Fig. 1. Waveforms of the pulse rates of *G. texensis* (*Upper*) and *G. rubens* (*Lower*), both recorded at 25°C. The pulse rate difference remains the only diagnostic means of separating males of these species.

so only rarely. An important caveat: although based on over 100 recordings of field males (44) and over 150 sibships from field-caught females [ref. 44 plus this study (sympatric localities only)], the absence of obvious hybrids is negative evidence and therefore cannot be conclusive; molecular work is planned and may well indicate some past or present gene flow.

The relative evolutionary rates of pre- and postzygotic isolation are likely to vary depending on many factors. Nonetheless, we can make predictions regarding the average expectations under different speciation mechanisms (see, e.g., ref. 2). Had these taxa speciated entirely in allopatry, with no reinforcement, we would expect a degree of postzygotic isolation approximately equal the observed degree of prezygotic isolation. Such is not the case (reviewed above). Alternatively, if divergence was allopatric but with reinforcement completing the process in sympatry, we would expect (*i*) at least some degree of hybrid malfunction, and (*ii*) reproductive character displacement in male song, female preference for song, and/or female recognition of and/or discrimination against heterospecific song. If speciation occurred via sexual selection operating on prezygotic isolation, we would expect (*i*) trivial postzygotic isolation, (*ii*) near complete prezygotic isolation, (*iii*) a genetic correlation between male song and female preference, and (*iv*) no character displacement. Although we realize that no single study can definitively address each of these issues, we believe that our data come close and are more consistent with speciation via sexual selection than the alternatives.

Methods

From September through mid-October 1999, we collected female crickets from a number of localities across the southeastern United States. We caught females that had flown to lights at night and those discovered by searching likely places during the day. We placed females in individual containers with cat food and water and allowed them to oviposit in moist vermiculite both before and after bringing them into the laboratory. Offspring were reared in individual family containers at 28 ± 1°C with a 13:11 light/dark photoperiod and *ad libitum* access to cat food and water in cotton-plugged vials. Containers were checked weekly until last-instar nymphs were seen. Containers with last-instar nymphs were checked at minimum every 2 days, and newly emerged adults were separated and placed in individual containers with food and water. Females were tested at 11 ± 4 days of age; males were recorded at 10 ± 4 days (means ± SDs).

Males could unambiguously be identified to species on the basis of their temperature-corrected pulse rates (see Fig. 3 and *Results*); to our knowledge, this remains the only means of distinguishing males of these two species. Females were identified to species primarily on the basis of the songs of their brothers. In the few instances in which no sons were recorded for a family, females from sympatric sites (eight females from five families) were assigned to species on the basis of phonotactic response and ovipositor length, a character discovered during the course of this study to be fairly reliable although not definitive in all cases (45). No females from sympatric sites were assigned to species on the basis of phonotactic response alone.

Sufficient numbers of surviving offspring of *G. texensis* were obtained from the following localities (Fig. 2): Allopatric: Uvalde, TX, Kerrville, TX, San Antonio, TX, Lampasas, TX, Austin, TX, Round Rock, TX, and Port Aransas, TX; sympatric: Bastrop, TX, Sulfur, LA, Minden, LA, Alexandria, LA, Dumas, AR, Tallulah, LA, Greenwood, MS, Starkville, MS, Tuscaloosa, AL, Milton, FL, and Carrollton, GA. We collected too few *G. rubens* to allow meaningful comparison of their songs and preferences in relation to allopatry/sympatry with *G. texensis*. Nonetheless, we include summary data for *G. rubens* to help illustrate the differences between these species. The *G. rubens* were from Milton, FL (sympatric) and Marianna, FL (equivocal–allopatric). We list the sympatry/allopatry status of the Marianna site as "equivocal–allopatric" because it is probably outside of the normal geographic range of *G. texensis*, but a few individuals have been collected that far east.

We refer to the collection sites as "localities" rather than "populations," because both of these species have strong flight capability, and we have no data to indicate levels of gene flow among localities. We attempted to test a minimum of two males and two females per family. We calculated sibling heritability estimates following ref. 52 and the genetic correlation between males and females following ref. 53. Estimates are considered significant if greater than or equal to two standard errors from zero. Quantitative genetic parameters apply to the population sampled at the time of sampling. As we do not know the population substructuring of our collection localities, we calculated estimates for each locality separately, as well as a "metapopulation" estimate across all individuals irrespective of collection locality.

Male Song Recording and Analysis. We recorded approximately 30 seconds of calling song per male on a Sony (Tokyo) WM-D3 Professional Walkman by using an Archer electret microphone (no. 270090 PC) within a 14-cm diameter parabolic reflector. The recording temperature was noted to the nearest 1°C. Songs were then digitized at 22.05 kHz by using CANARY 1.2.4 (Cornell Laboratory of Ornithology, Ithaca, NY). We measured 50 pulse periods (the time from the start of one pulse to the start of the next pulse) for each male. Measurements were made by using the "measurement panel" and "data log" capabilities of the software. The measurement error was effectively one cursor width, so we scaled the on-screen song display to 3.94 ms/cm to give a measurement error approximately equivalent to measuring pulse rates to the nearest 1 p/s (at 22.05 kHz sampling, the error caused by the time lag between digitized samples is approximately equivalent to 0.15 to 0.3 p/s). The median value of the 50 pulse periods was used to calculate the unadjusted pulse rate as 1/median pulse period. The temperature-adjusted pulse rates were calculated as $\text{PULSE RATE}_{adj} = \text{PULSE RATE} + 3.5^*(25 - T_r)$, where T_r = the recording temperature. The slope of 3.5 pulses/°C is appropriate for *G. texensis* (54) but is slightly steeper than the slope for *G. rubens*. We corrected all songs to 25°C by using a slope of 3.5, then examined a histogram of the adjusted pulse rates and readjusted the pulse rates of males determined to be *G. rubens* by using a more appropriate slope of 2.8 p/°C (based on ref. 44). The recording temperature ranged from 24 to 28°C.

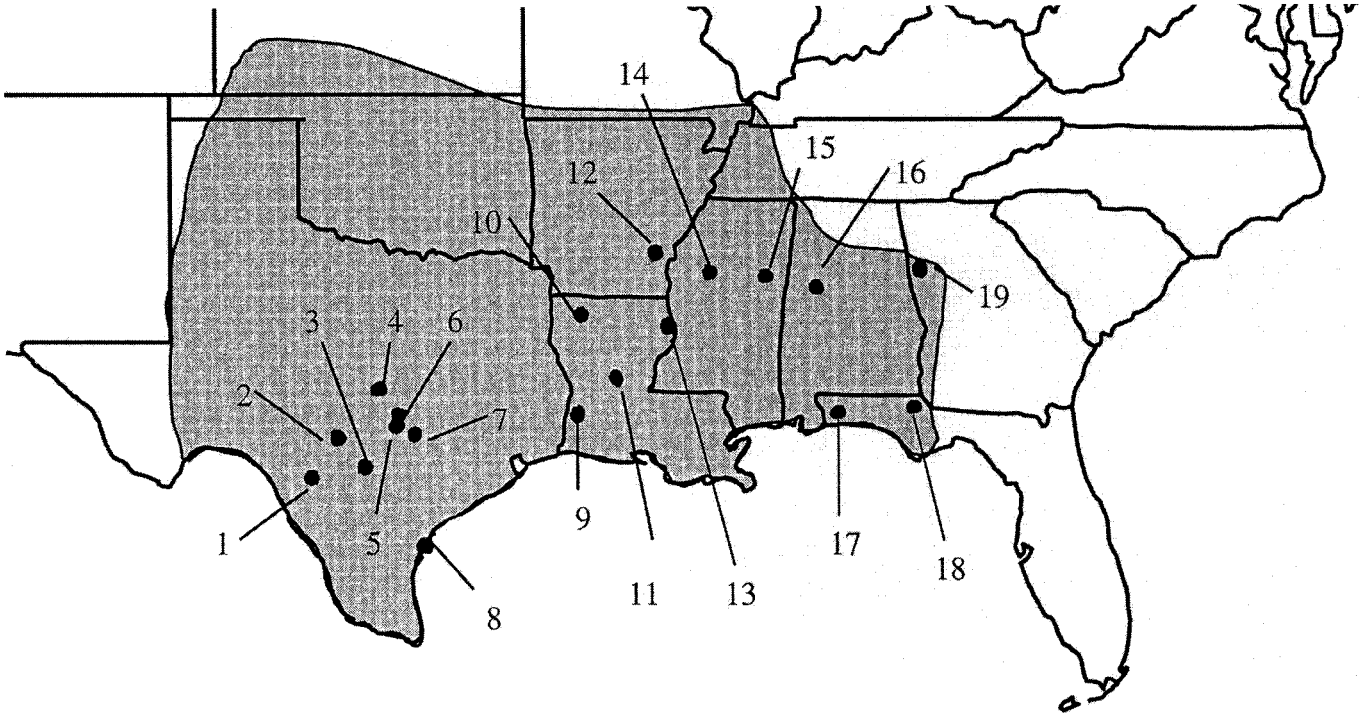

Fig. 2. Collection localities and geographic distribution of *G. texensis*. Numbered localities are: 1, Uvalde, TX; 2, Kerrville, TX; 3, San Antonio, TX; 4, Lampasas, TX; 5, Austin, TX; 6, Round Rock, TX; 7, Bastrop, TX; 8, Port Aransas, TX; 9, Sulfur, LA; 10, Minden, LA; 11, Alexandria, LA; 12, Dumas, AR; 13, Tallulah, LA; 14, Greenwood, MS; 15, Starkville, MS; 16, Tuscaloosa, AL; 17, Milton, FL; 18, Marianna, FL (*G. rubens*); and 19, Carrollton, GA.

Female Phonotaxis Trials. Females were tested in response to 14 synthetic songs of differing pulse rates. Seven of the songs were typical of *G. rubens*, and seven were typical of *G. texensis*. For each, the seven songs consisted of one song with the average pulse rate for the temperature plus six songs with pulse rates typical of plus and minus 3°C in 1°C increments. For example, at 25°C, the stimulus set had the *G. rubens* typical song of 56 p/s plus 6 songs typical of *G. rubens* at 22, 23, 24, and 26, 27, and 28°C (with corresponding pulse rates of 47.6, 50.4, 53.2, and 58.8, 61.6, and 64.4 p/s) plus the *G. texensis* typical song of 80 p/s and variants representing plus and minus 3°C (i.e., pulse rates of 69.5, 73.0, 76.5, and 83.5, 87.0, and 90.5). Pulse rates were based on previous work with these species (44, 54). Songs were constructed of repeated single artificial pulses created by using COOL EDIT '96 (Syntrillium Software Corporation, Phoenix, AZ). Pulses were sine waves sweeping from 5.25 to 4.75 kHz with 500 Hz modulation and a 3-kHz modulation frequency. Pulse length was 10 ms for the 17°C song and decreased by 0.05 ms for each 2°C increase in temperature (54). Pulses were shaped by using an amplitude envelope with symmetrical rise-and-fall times of 30% of the pulse length and were then bandpass filtered from 3.5 to 6.5 kHz (Fast Fourier Transform size = 6,400, Blackman windowing). Each song had 45 ± 6 pulses per trill and intertrill intervals of 175 ± 50 ms. The within-song variation was the same for each song and was introduced to mimic natural variation and reduce habituation.

We tested female responses to the broadcast songs by using a noncompensating treadmill called a "kugel." The kugel has been described in detail elsewhere, so our treatment here is brief. We refer interested readers to previous work (55–57). Briefly, the kugel consists of a 16.2-cm-diameter sphere that floats on a column of air. A test female was tethered on the sphere such that when she walked toward a broadcast song, the sphere rotated beneath her. Rollers connected to a personal computer measured the speed and direction of sphere movement relative to an active speaker once per second. Female movement was converted to a net vector phonotaxis score as the cosine of the angle of movement (relative to the active speaker, designated as 0°) multiplied by the speed of movement, summed for each second of the trial. Thus the kugel acts similarly to an oversized upside-down computer "mouse" that measures directed female phonotaxis toward male calling song. Songs were broadcast at 84 dB sound pressure level (20 μPa) measured at the female tether point. The order of song presentation as well as which speaker played which song was randomized for each female.

Because female responses to male cues may be viewed as representing a continuum from sexual selection to species recognition (58), we analyzed female response data at three levels. First, we tested female "preferences" as the stimulus level that elicits the greatest positive response. Female preferences were standardized to 25°C by using a linear female temperature response (e.g., a female that preferred the 23°C song when tested at 24°C was assigned a 24°C preference at 25°C); linear temperature responses are appropriate because it is known that both male songs and female preferences are temperature coupled and show identical linear responses (59–61). Second, we tested female "recognition" of heterospecific stimuli in terms of whether females showed average positive phonotaxis when presented with heterospecific song. A third level of female response may be that females do recognize both conspecific and heterospecific signals but show "discrimination" between them. Thus we also tested the magnitude of female response to heterospecific relative to conspecific song.

Results

Genetic Effects. Summary data, including sample sizes, are presented in Table 1. The within-locality quantitative genetic estimates for *G. texensis* were highly variable and had high standard errors, precluding conclusions regarding any particular locality. The within-locality sibling heritability estimates for male pulse

Table 1. For each locality, the mean and SD of pulse rate in male song are given as well as the mean and SD of the female preference for pulse rate

Locality	Male pulse rate (*N* males, *N* families)	Female preferred pulse rate (*N* females, *N* families)
Uvalde, TX	78 ± 3 (25, 12)	77 ± 6 (24, 12)
Kerrville, TX	80 ± 4 (16, 9)	79 ± 4 (15, 9)
San Antonio, TX	77 ± 5 (19, 10)	81 ± 5 (14, 8)
Lampasas, TX	80 ± 4 (21, 12)	79 ± 5 (21, 11)
Austin, TX	79 ± 5 (20, 11)	79 ± 5 (20, 11)
Round Rock, TX	81 ± 4 (20, 11)	78 ± 4 (18, 9)
Port Aransas, TX	79 ± 2 (32, 17)	81 ± 4 (32, 17)
Bastrop, TX	79 ± 5 (17, 9)	82 ± 4 (13, 8)
Sulphur, LA	78 ± 1 (5, 3)	83 ± 4 (3, 2)
Minden, LA	82 ± 4 (18, 10)	80 ± 4 (17, 10)
Alexandria, LA	79 ± 5 (12, 7)	79 ± 4 (17, 10)
Dumas, AR	79 ± 7 (12, 7)	80 ± 6 (13, 7)
Tallulah, LA	82 ± 4 (30, 17)	79 ± 5 (33, 16)
Greenwood, MS	80 ± 4 (15, 8)	80 ± 3 (15, 8)
Starkville, MS	79 ± 3 (23, 12)	82 ± 5 (26, 14)
Tuscaloosa, AL	75 ± 7 (5, 3)	79 ± 5 (8, 4)
Milton, FL	77 ± 4 (11, 6)	80 ± 4 (9, 5)
Carrollton, GA	77 ± 4 (7, 3)	77 ± 9 (6, 3)
All *G. texensis*	79 ± 4 (308, 167)	80 ± 5 (294, 158)

Sample sizes are given in parentheses.

rate averaged 0.27 (SE = 0.12, $n = 18$ localities). The "metapopulation" estimate across all *G. texensis* families was also significant ($F_{166, 141} = 1.34$, $P < 0.0352$) and moderately higher [$(h^2) = 0.40 \pm 0.16$]. Results were similar for female preference for pulse rate [mean ± SE of within-locality estimates, 0.33 ± 0.18, $n = 18$ localities; "metapopulation" estimate: $h^2 = 0.38 \pm 0.17$, $F_{157, 136} = 1.43$, $P < 0.0164$]. The coefficients of additive genetic variation [CV_A (62)] were fairly low (CV_A males 3.20%, females 3.85%).

Because of negative estimates for some variance components in the heritability analysis, the within-locality genetic correlation could be estimated only in 10 localities. Across these 10, the genetic correlation averaged 0.78 ± 0.25 (SE) but is strongly biased upwards by inclusion of three localities with estimated correlations >1. Setting the correlation in each of these localities to 1 gives a mean ± SE across the 10 localities of 0.15 ± 0.05. The "metapopulation" genetic correlation was significant [$F_{175, 149} = 1.30$, $P < 0.0499$, where F = mean square $(MS)_{Family}/MS_{Family \times Sex}$ (55)] with an estimated genetic correlation of 0.49 ± 0.23. There was no significant covariation of male and female locality means (Pearson correlation $r = 0.04$, $n = 18$, $P = 0.8712$).

Character Displacement: Male Song. Fig. 3 shows the distribution of pulse rates in male song corrected to 25°C. We tested for character displacement by using family means because siblings within families were not independent data points (see above). We used nested ANOVA (localities nested within type of locality) to test the hypothesis of reproductive character displacement; nested ANOVA is the appropriate model for character displacement studies because it uses the geographic variation unrelated to sympatry/allopatry (i.e., variation because of clines, etc.) as the error term. There was no character displacement in *G. texensis* pulse rates (Tables 1 and 2); moreover, there was little variation among localities.

Character Displacement: Female Phonotaxis. *Female "preference."* Female preferences were, on average, strongly coincident with average male song ($t = 1.05$, df = 600, $P = 0.2948$; see Table 1; also compare Figs. 3 and 4). Thus female preference currently exerts stabilizing selection on male song. There was no evidence of reproductive character displacement and little variation among localities (Tables 1 and 2).

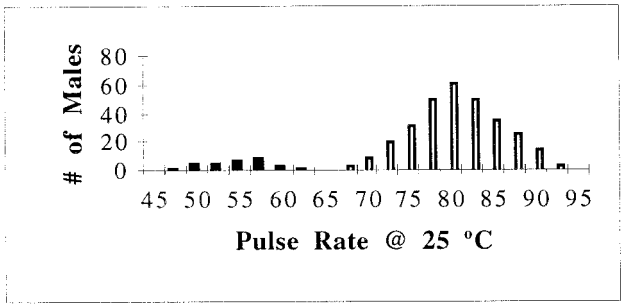

Fig. 3. Distributions of pulse rates in male songs of *G. rubens* (dark bars) and *G. texensis* (light bars) corrected to 25°C.

Heterospecific song "recognition." Most female *G. texensis* showed average positive phonotaxis when presented with *G. rubens* song: 241 of 294 females (82%) were positively phonotactic. This was significantly higher than the 50% expected by chance [continuity-corrected logarithm (log)-likelihood ratio: $G_{adj,1} = 131.67$, $P < 0.0001$]. Furthermore, 33% of the 241 positively phonotactic females had average phonotaxis significantly greater than 0 at the $P < 0.05$ level. This difference was also significant (33% of 241 vs. a null expectation of 5%; continuity-corrected log-likelihood ratio: $G_{adj,1} = 187.25$, $P < 0.0001$). Females from allopatric and sympatric localities did not differ in their probability of phonotaxis toward *G. rubens* song (allopatric 119 of 144 (83%), sympatric 122 of 150 (81%), $\chi^2 = 0.08$, $P = 0.77$); the conditional probability of significantly positive phonotaxis, given average positive phonotaxis, also did not differ in relation to sympatry/allopatry [allopatric 39 of 119 (33%), sympatric 41 of 122 (34%), $\chi^2 = 0.02$, $P = 0.89$].

Heterospecific "discrimination." Despite apparently "recognizing" heterospecific song, female *G. texensis* showed fairly strong discrimination in favor of conspecific song. We used two different measures of species-level discrimination. The first was the ratio of heterospecific response to conspecific response calculated for each female. On average, females' responses to conspecific song were almost five times greater than responses to heterospecific song: the heterospecific/conspecific ratio averaged 0.21 ± 0.64 (mean ± SD). We also performed one-tailed *t* tests for each female of the hypothesis that conspecific response exceeded heterospecific response. The mean ± SD one-tailed *P* value for the 294 females was 0.07 ± 0.11. Females from

Table 2. Nested ANOVA table showing no effect of sympatry on either male pulse rate or female preference for pulse rate in *G. texensis* from localities either sympatric or allopatric with *G. rubens*

Source	df	MS	*F*	*P*
Males (*N* = 167 families, 18 localities)				
Type of locality	1	2.11	0.08	0.7808
Locality (type)	16	26.30	1.61	0.0735
Error	149	16.37		
Females (*N* = 158 families, 18 localities)				
Type of locality	1	14.37	0.75	0.4016
Locality (type)	16	19.35	0.84	0.6430
Error	140	23.14		

Localities are nested within types of locality (sympatric/allopatric). MS, mean square.

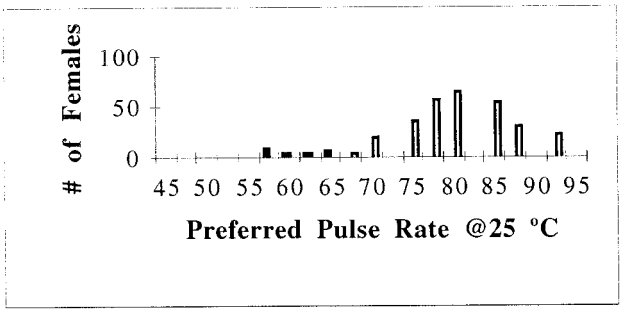

Fig. 4. Distributions of pulse rates most preferred by females of *G. rubens* (dark bars) and *G. texensis* (light bars). The horizontal axis is scaled as in Fig. 3 to facilitate comparison of female preferences and male songs.

allopatric and sympatric localities did not differ in their degree of species discrimination by using either measure (Wilcoxon two-sample normal approximation with continuity correction of 0.5, heterospecific/conspecific ratio $Z = 0.82$, $P = 0.41$; one-tailed P values from t tests $Z = 1.39$, $P = 0.17$).

Discussion

Definitive evidence about past evolutionary events is almost impossible to find. Nonetheless, we view these results, combined with the results of previous studies, as substantially more consistent with speciation caused by sexual selection than they are with the alternatives. First, this study and previous work indicate that prezygotic isolation is probably the primary mechanism separating these species: male pulse rates are almost unambiguously species specific (ref. 44; this study); female phonotaxis to heterospecific song is only 20% of the response to conspecific song (this study); laboratory hybrids are easily produced (refs. 46–48; unpublished data), fertile (47, 48), and intermediate in male song and female preference (ref. 49; unpublished data) and yet are apparently absent in the field (ref. 44, sibships from sympatry; this study). The ease of producing viable hybrids in the laboratory does not in any way preclude low-hybrid viability in the field, but because sibships from field-caught field-inseminated females are of one species or the other, the difference between life in the laboratory and life in the field may be moot. We reiterate, however, that molecular genetic work remains to be done and may well indicate some past and/or present gene flow. Even if there is some gene flow, it remains very likely that prezygotic mechanisms (including potential conspecific sperm precedence; refs. 50, 51) are far more important in maintaining species integrity than are postzygotic ones. The precondition of some degree of hybrid malfunction invoked by reinforcement models of speciation is either nonexistent or is hard to demonstrate in this case.

To this background we have added the following results: no character displacement (predicted by reinforcement models) and genetic variation and correlation (predicted by sexual selection models). Our results are noteworthy for several reasons. First, we have demonstrated the linkage between divergence in reproductive characters and the sexual isolation often assumed to result from that divergence. Second, the degree of species discrimination shown by females in this study is likely to be only a minimum estimate because of our sequential presentation of songs; species discrimination is usually greater in simultaneous stimulus presentation "choice" designs (see ref. 63). Third, in terms of both numbers of individuals and numbers of sample localities, this is one of the larger studies of reproductive character displacement to have been conducted, and yet there is no evidence suggesting displacement. Ours is also one of only a few studies to address character displacement in female responses as well as male signals. Furthermore, we address female responses at three levels ranging from "recognition" to "discrimination" to "preference." At none of these levels is there any suggestion of character displacement. There is, instead, little geographic variation in the male signal or the female responses. Furthermore, both male pulse rate and female preference for pulse rate show significant levels of genetic variation and are significantly genetically correlated. The genetic correlation across all individuals is not simply because of covariation of means across localities.

Thus, of the two major predictions of reinforcement models, (*i*) postzygotic isolation equal to or exceeding prezygotic isolation in allopatry and (*ii*) prezygotic isolation mechanisms enhanced in sympatry, neither is supported by the currently available data. Of the predictions of speciation by sexual selection models (*i*) preeminence of prezygotic mechanisms in both allopatry and sympatry, (*ii*) no character displacement in male signals or female responses, and (*iii*) a positive genetic correlation between male signals and female responses, all are supported by present knowledge. We note that our finding of a positive genetic correlation means that runaway sexual selection could have occurred but does not demonstrate that it did occur; moreover, the reinforcement model in no way precludes a genetic correlation, and not all models of speciation by sexual selection require runaway. Nonetheless, our study is among the most complete in providing empirical evidence favoring the sexual selection model (see also refs. 28, 33, 42). The evidence presented here should be taken as encouragement of research examining speciation by sexual selection. Our results and discussion are not intended to be in any way critical of the widely accepted allopatric model with no involvement of sexual selection (i.e., vicariance) nor of the allopatric model invoking reinforcement. Instead, we are willing to suppose that because there are many millions of animal species on earth, there may have been more than one mechanism of speciation.

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