



# Broad Selectivity for Courtship Song in the Cricket *Gryllus bimaculatus*

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## Abstract

Various characteristics of a long-distance acoustic signal have been shown to vary to different degrees. It has been suggested that female preferences based on stable song parameters are stabilising or weakly directional, and preferences based on variable parameters are strongly directional. We tested this hypothesis based on a short-distance signal (courtship song) produced by the field cricket, *Gryllus bimaculatus*. We studied the degree of variability of different courtship song parameters and the behavioural importance of several parameters using synthesised song models in playback experiments. We found that most of the courtship song elements of *G. bimaculatus* were quite variable (coefficient of variation, CV, in the range of 20–53%). The most variable parameter of the courtship song was the relative amplitude of two elements: high-amplitude ticks and low-amplitude pulses. Because songs containing only ticks (of rare occurrence) appeared to be more effective than songs with both ticks and pulses (of frequent occurrence), we consider female preferences to be directional. Alteration of less variable traits, such as the carrier frequency and duration of ticks (CV = 20–25%), had a different effect on female responsiveness. The synthesised songs with different carrier frequencies of ticks were as attractive to females as the positive control (courtship of muted males accompanied by playback of the recorded song). Altering the duration of ticks had a crucial effect on the female response rate, decreasing female responsiveness to the level observed in the negative control (courtship of muted males). Thus, we did not find a strong relationship between the variability of individual song parameters and their potential importance in song recognition and the evaluation of male quality. The partial inconsistency of our results with the data of other authors may be due to different patterns of past and current selection on long-distance and short-distance acoustic signals.

## Introduction

In many species of animals, acoustic signals play an important role in intraspecific communication and reproductive isolation. A long-distance acoustic signal (an advertisement signal or a calling song) shows a clearly species-specific structure, as this type of signal is often the only way to attract conspecific females. However, calling songs can also be used in the context of sexual selection, for females to discriminate among males on the basis of certain properties of calling

songs for mating. Various studies on insects and anurans show that the characteristics of calling song may vary to different degrees. It has been suggested that stable parameters are used for the process of recognition (essential recognition parameters sensu Popov & Shuvalov 1977 and static properties sensu Gerhardt 1991). The female preference functions associated with species recognition in insects and anurans are typically stabilising or 'unimodal' because females prefer males that exhibit intermediate values of calling song characters (Butlin et al. 1985; Ewing &

Miyan 1986; Ferreira & Ferguson 2002; Gerhardt & Huber 2002; Klappert & Reinhold 2003; Saarikettu et al. 2005). By contrast, preferences based on variable parameters are strongly directional or 'open ended' as long as females prefer males that present extreme values of calling song characters (Ritchie 1996; Shaw & Herlihy 2000; Gerhardt & Huber 2002). Variable parameters ('motivational' according to Popov & Shuvalov (1977), or 'dynamic' according to Gerhardt (1991)) have been suggested to be important for intraspecific competition.

Many cricket species display a repertoire that includes three structurally distinct signals, termed the calling, courtship and aggression songs (Alexander 1961). Studies on song recognition in crickets have primarily focused on calling songs. Behavioural studies on a number of species have shown that both the temporal pattern and carrier frequency are important cues for song recognition by females (e.g. Walker 1957; Popov & Shuvalov 1977; Pollack & Hoy 1979; Thorson et al. 1982; Doherty 1985; Stout & McGhee 1988; Hennig & Weber 1997; Pollack & Kim 2013). The most important temporal parameters of calling songs are suggested to be the pulse period and duration, the number of pulses in a chirp and the chirp period.

The courtship song, which is a close-range acoustic signal, is performed by males and aimed at a nearby female. In all of the cricket species of the genus *Gryllus* studied thus far, the courtship songs are more complex than the calling songs. In *Gryllus* species, courtship songs contain two elements that differ in both their temporal and frequency characteristics, whereas calling songs comprise only one element (Nocke 1972; Zhantiev & Dubrovin 1974; Rheinlaender et al. 1976; Ragge & Reynolds 1998; Wagner & Reiser 2000; Fitzpatrick & Gray 2001; Vedenina & Pollack 2012). Similar to calling songs, courtship songs exhibit a species-specific structure; however, it may be more variable than that of the calling song (Zuk et al. 2008; Vedenina & Pollack 2012). As a rule, the courtship song of *Gryllus* species consists of single high-amplitude, high-frequency pulses, alternating with numerous lower-amplitude, lower-frequency pulses.

The function of a courtship song is controversial. It has been shown that a courtship song is an important cue for eliciting mounting in females (Adamo & Hoy 1994; Libersat et al. 1994; Balakrishnan & Pollack 1996; Nelson & Nolen 1997; Vedenina & Pollack 2012). On the other hand, taking into account that a female has already chosen and approached a particular male based on his calling song, why does a courtship song show a species-specific structure? It has

been suggested that a more stereotyped calling song allows species-specific identification and ease of localisation, whereas a more variable courtship song is more likely to contain information about individual male quality (e.g. Fitzpatrick & Gray 2001; Gray 2005; Rebar et al. 2009). It has been shown that a courtship song may be used by females as a cue for choosing males with high immunocompetence (Rantala & Kortet 2003; Tregenza et al. 2006). The courtship song might play an additional role in species recognition when males use alternative mating strategies. When male crickets use satellite behaviour to locate females, they aggregate around calling males and intercept females that are attracted to the callers (Cade 1980). In such a situation, the intercepted females can only evaluate the courtship song of these satellite males.

Courtship song recognition has mainly been studied in three cricket species, *Teleogryllus oceanicus*, *Gryllus bimaculatus* and *Gryllus assimilis* (Burk 1983; Libersat et al. 1994; Balakrishnan & Pollack 1996; Rebar et al. 2009; Vedenina & Pollack 2012). Among these species, *T. oceanicus* appears to be the best studied. The courtship song of *T. oceanicus* consists of a short chirp, followed by a long trill. It has been shown that the chirp component alone is sufficient to evoke normal mounting, whereas the trill is only partially effective on its own; the high effectiveness of the chirp is due to its fundamental frequency and temporal pattern (Balakrishnan & Pollack 1996). Overall, females of *T. oceanicus* prefer males that exhibit longer courtship songs with a higher duty cycle (Rebar et al. 2009). Altering certain stable features of the courtship song of *G. assimilis* resulted in significant decreases in the mounting frequency, consistent with the notion that trait values that show little variability are constrained by stabilising selection exerted by females (Vedenina & Pollack 2012). However, alteration of a variable song trait had no effect on the female response rate, thus failing to support the hypothesis that variable traits provide a substrate for sexual selection.

As one of the most common cricket species used in behavioural and physiological studies, *G. bimaculatus* has been surprisingly poorly investigated with respect to courtship song recognition. The degree of variability in individual song parameters has not been studied in this species thus far. In one study, high-amplitude, high-frequency (11–16 kHz) pulses were shown to be a crucial component of a successful courtship song, whereas the role of less intense, low-frequency (4–5 kHz) pulses was not tested (Libersat et al. 1994). In another study, *G. bimaculatus* females preferred a courtship song with a high rate and long duration of high-frequency pulses, whereas the rate of

low-frequency pulses did not play any role in female preferences (Rantala & Kortet 2003). However, the aim of the latter study was to test whether the male song transmits information about male immunocompetence to females, rather than to assess the behavioural importance of various song parameters using synthesised song models.

In the present study, we investigate the degree of variability of different courtship song parameters, both within and between males of *G. bimaculatus*. We also study the behavioural importance of several courtship song parameters using synthesised song models in playback experiments. Similar to a previous study we performed in *G. assimilis* (Vedenina & Pollock 2012), we focus on the relationship between the variability of individual song parameters and their potential importance for song recognition and the evaluation of male quality. We tested the hypothesis that female preferences based on stable parameters are stabilising or weakly directional, while preferences based on variable parameters are strongly directional. We assumed that the shape of preferences for courtship song parameters would be different from the shape expected for the preferences for calling song characters.

## Materials and Methods

### Crickets

The crickets came from a laboratory stock obtained from the Moscow Zoo culture. This culture was originally obtained in 1985–1990 from the cricket farms and pet food stores in Germany and Great Britain. Since this period, the Moscow Zoo culture has been maintained at more than 1000 individuals at all times. The size of the laboratory stock varied from 30 to 200 individuals at different times; however, this stock was refreshed from the Zoo culture one–two times per year to reduce the potential effects of inbreeding. The crickets were reared in plastic containers (57 × 39 × 42 cm) at 22–27°C under a 12-h:12-h light/dark cycle. Food (dried gammarus and oat-flakes) and water were provided *ad libitum*. Males and females were separated before the imaginal moult. One to two days before the experiments, the crickets were individually isolated in small plastic containers (12 × 12 × 7 cm) with food and water. In all but one experiment, we used 3- to 8-d-old virgin females (Table 2). There was no difference between the responses of 3- to 5-d-old and 6- to 8-d-old females. As many behavioural studies conducted on *G. bimaculatus* have been performed on older females

**Table 1:** The mean ( $\bar{x}$ ), standard deviation (SD) and coefficient of variation (CV) of courtship song parameters in *Gryllus bimaculatus*. Recordings and measurements were taken from the songs of 20 males, with each being measured at least 10 times

Song parameter	$\bar{x} \pm \text{SD}$	CV between males (%)	CV within males (%)
Tick period, s	0.282 ± 0.040	14	9
Tick duration, s	0.012 ± 0.002	20	17
Tick dominant frequency, Hz	11567 ± 2995	25	15
Tick amplitude	– <sup>a</sup>	–	16
Pulse amplitude	– <sup>a</sup>	–	24
Pulse/tick amplitude	0.067 ± 0.037	53	51
Pulse duration, s	0.013 ± 0.003	24	22
Pulse period, s	0.024 ± 0.006	25	24
Number of pulses between subsequent ticks	8.39 ± 3.02	36	30
Pulse dominant frequency, Hz	4811 ± 475	10	12

<sup>a</sup>Absolute measurements of tick and pulse amplitude could not be averaged because they were not calibrated in all recordings.

(10–14 d old; e.g. Adamo & Hoy 1994; Libersat et al. 1994; Rantala & Kortet 2003), we used 14- to 17-d-old virgin females in one series of experiments (when presenting stimulus 2) to test how female age affects female mating behaviour. The age of the males varied in the range of 1–2 wks, which corresponded to the most active courtship period of males. Recordings of courtship songs and behavioural experiments were conducted in the period from 2012 to 2014; animals from at least 10 laboratory generations were tested.

### Song Recording and Analysis

To record the courtship song, we introduced a male and a female into a cylindrical (15 × 15 cm) open-top arena, in which the floor was covered by a paper towel, and the walls were formed by a metallic grid. All recordings were performed under a red light at 24–28°C. A microphone (type 4191, 1/2 inch; Brüel & Kjær, Nærum, Denmark) was placed at a height of 8–10 cm from the top of the arena. The output of a conditioning amplifier (Brüel & Kjær 2690) was digitised (100 kHz sampling rate) using a custom-made A/D–D/A interface. The temporal parameters and power spectra of the songs were analysed with COLEDIT (Syntrillium, Seattle, WA) and TURBOLAB 4.0 (Bressner Technology, Gröbenzell, Germany). We measured six temporal and two frequency parameters (Table 1), with a minimum 10 instances of the relevant parameter being measured in the same song. Measurements were performed on songs of 20 males (each female placed with a male was used only once).

## Behavioural Experiments

The experiments were performed at 22–26°C in a cylindrical open-top arena. Illumination was provided only by a red light. We evaluated female preferences based on the readiness of the female to mount the male. During courtship, the male turns away from the female and presents his abdomen, while continuously stridulating. The female reacts by approaching from behind and touching his abdomen with her antennae and palps. This stimulates the male to spread his hind wings and flatten his abdomen, allowing the female to mount him. Mounting of the male by the female is a prerequisite for copulation (Alexander 1961; Adamo & Hoy 1994). A male was introduced into the arena, and after he stopped running and appeared calm (in 3–5 min), we introduced a female. If no antennal contact (when a cricket contacts a conspecific with at least one antenna, which is required for the initiation of courtship behaviour) occurred within 5 min, or if the male failed to produce a courtship song within 5 min after this contact, the trial was discarded. If the female did not make a choice within 5 min after the beginning of courtship, the trial was scored as ‘no mounting’. Two parameters of female preferences were measured: the percentage of females that mounted males and the latency from the onset of courtship song to the mounting response. We used each female only once, whereas each male might have been used multiple times (up to 10 times) because some individuals were more eager to court than others. After each trial, the paper towel was replaced to remove any olfactory cues that may have been left by the crickets.

We used a sample size of 18–20 females in each series of experiments (Table 2). Males were muted the day before trials by cutting off the right forewing proximal to the stridulatory file. As a negative control, we employed muted males in the absence of any song playback. As a positive control, the courtship of muted males was accompanied by playback of the courtship song shown in Fig. 1e. We chose this song as positive control because the values of the song parameters were very close to the mean values shown in Table 1. In the playback experiments, each time the muted male attempted to sing (as indicated by movement of the remaining forewing), a touch of the keyboard commanded the computer to play the test song from a loudspeaker mounted above the arena at a height of 20 cm. All trials were video recorded (Sony DCR-TRV 355E), and the video signals were transferred to a PC for the analysis of courtship. The courtship trials were analysed with the ADOBE PREMIERE and VIRTUAL-DUB programs.

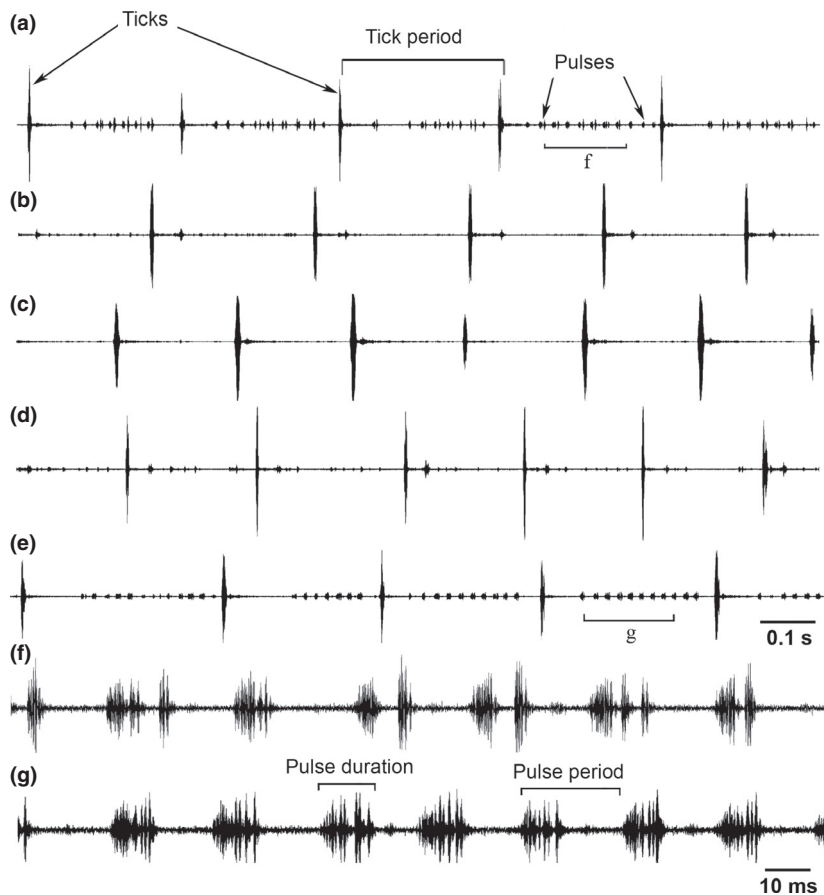
## Acoustic Stimuli

The structures of the test songs were based on the recorded courtship songs of *G. bimaculatus* (Fig. 1, Table 1). To distinguish between two song elements (high-amplitude and low-amplitude pulses), we referred to the high-amplitude pulses as ‘ticks’. We tested female responsiveness to 11 synthesised songs (Table 2), in which we altered the carrier frequency, the duration of ticks and the number of song elements (songs with either both ticks and pulses, or only ticks). In five song treatments (stimuli 1 and 3–6), the carrier frequency of ticks was manipulated; in one song

**Table 2:** Details of the stimuli, sample size and age of the females used in behavioural experiments

Stimulus	Treatment	Sample size	Female age (d)	Tick frequency (kHz)	Tick duration (ms)	Pulses between subsequent ticks
Positive control	Muted male + courtship song	19	3–8	11.831 ± 0.097a	12.5 ± 0.9a	Present
Negative control	Muted male + no playback	20	3–6	–	–	–
1	Tick frequency	20	4–7	5	12	Present
2	Female age	19	14–17	5	12	Present
3	Tick frequency	20	3–5	8	12	Present
4	Tick frequency	20	4–7	11	12	Present
5	Tick frequency	20	3–6	14	12	Present
6	Tick frequency	20	5–8	17	12	Present
7	Absence of pulses	20	4–8	17	12	Absent
8	Absence of pulses	20	4–7	11	12	Absent
9	Tick duration	20	5–7	11	24	Absent
10	Tick duration	18	3–8	11	30	Absent
11	Tick duration	20	3–7	11	36	Absent

<sup>a</sup>The  $\bar{x} \pm$  standard deviation.



**Fig. 1:** Courtship songs recorded in five males (a–e) of *Gryllus bimaculatus*, illustrating their variability. The analysed temporal parameters are shown in (a) and (g). Fragments of the songs shown in (a) and (e) are shown in (f) and (g) at an expanded scale.

treatment (stimulus 2), the effect of female age on mounting response and latency was tested; and in three song treatments (stimuli 9–11), the duration of ticks was manipulated. The low-amplitude pulses were present in stimuli 1–6 and absent in stimuli 7–11. To study the importance of low-amplitude pulses, we compared the female responses to stimulus 4 with the responses to stimulus 8 (tick frequency 11 kHz) and compared the responses to stimulus 6 with the responses to stimulus 7 (tick frequency 17 kHz). Test songs were synthesised in SOUND FORGE software. The peak intensities of the ticks were 85–90 dB SPL; the sound pressure levels were calibrated using a Brüel & Kjær (type 2235) sound level meter.

#### Statistical Analyses

We employed the coefficient of variation (CV) for courtship song characters to measure the variability between and within males. For each male, CV was calculated as the ratio of the standard deviation to the mean. We examined differences in the mounting response between different series of experiments using Fisher's exact test. Differences in mounting

latency between the various treatments were measured using the nonparametric Kruskal–Wallis test and Mann–Whitney *U*-test. Nonparametric analyses were employed because some of the samples were not normally distributed.

## Results

### Variation of the Courtship Song Traits

In most of the specimens of *G. bimaculatus*, the courtship song consisted of two distinct elements: high-amplitude ticks and low-amplitude pulses (Table 1, Fig. 1). In some specimens, however, pulses were almost absent, as shown in Fig. 1c. According to the statistical analysis of the song parameters presented in Table 1, the relative amplitude of ticks and pulses was the most variable character (CV = 53% between males, and CV = 51% within males). Additionally, the absolute amplitude of pulses varied more (CV = 24%) than the amplitude of ticks (CV = 16%). The least variable traits were the tick period (CV = 14% between males; CV = 9% within males) and pulse dominant frequency (CV = 10% between

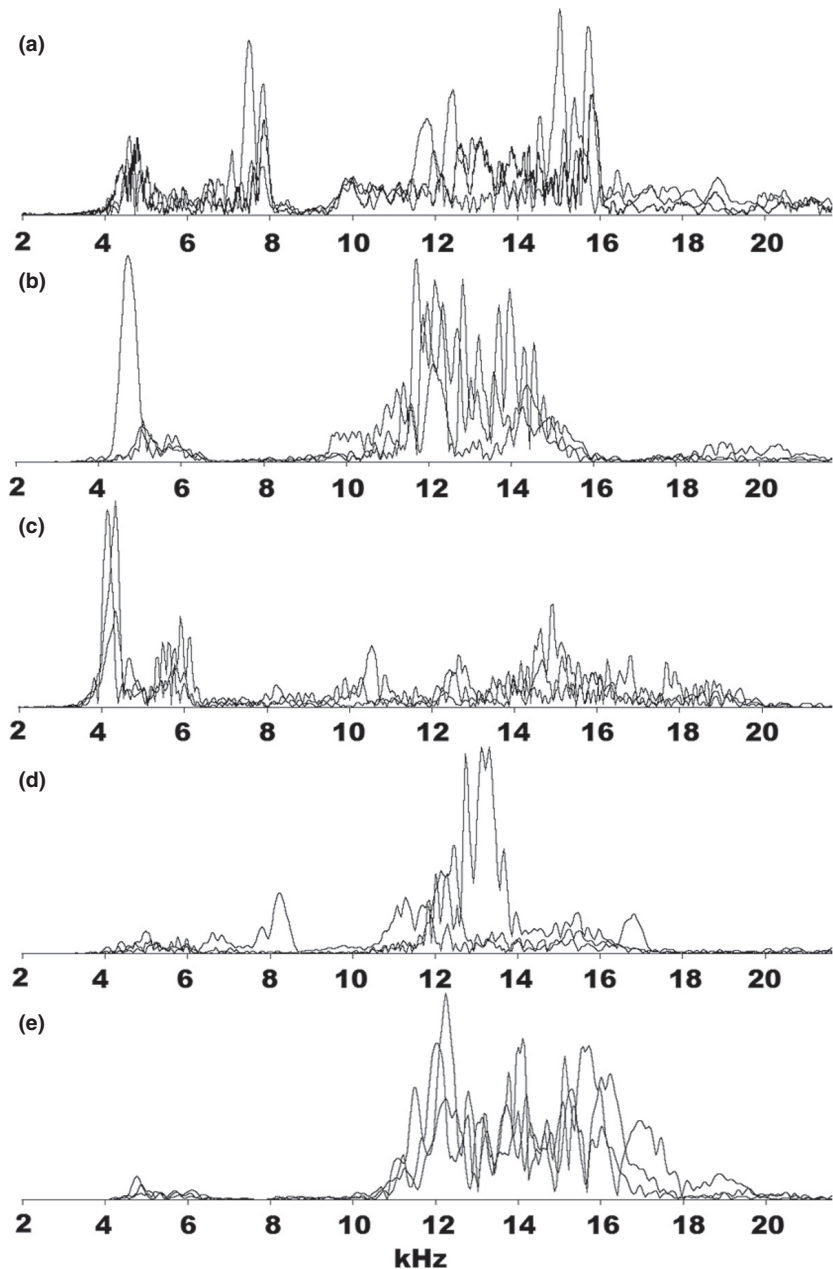
males; CV = 12% within males). Other traits studied varied in the range of 20–36% between males and 15–30% within males.

The dominant frequency of ticks was more variable between males (CV = 25%) than within males (CV = 15%). In the courtship songs of some males, the spectral maxima of ticks occurred at 4–5 kHz (fundamental frequency), whereas higher harmonics were less intense. In other males, the energy of ticks was concentrated around approx. 11–16 kHz (Fig. 2). On average, the high-frequency component of the tick spectra was only 5 dB higher than the low-frequency

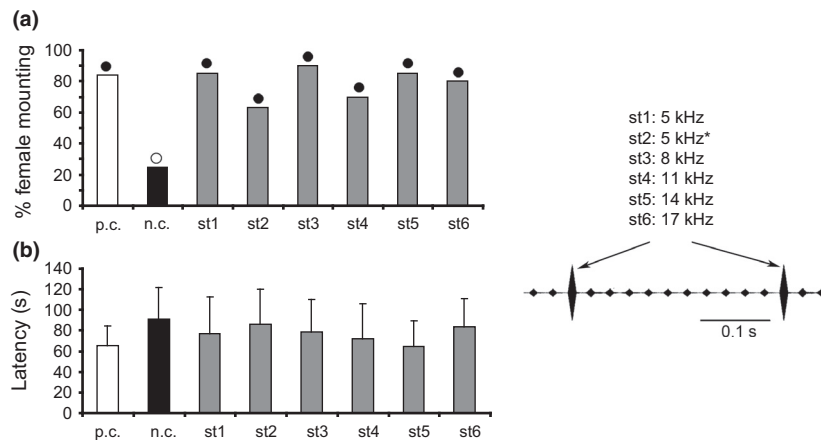
component, and the difference was insignificant (*U*-test:  $U = 22$ ,  $p = 0.29$ ).

#### Female Preference in Positive and Negative Controls

In the positive controls (experiments with muted males + playback of the courtship song), 84% of females exhibited a mounting response, whereas only 25% of females mounted courting males in the negative control (trials with muted males with no playback) (Fig. 3a). This difference was highly significant (Fisher's exact test, two-tailed:  $p = 0.0003$ ). However,



**Fig. 2:** Power spectra of courtship ticks recorded in five males (a–e) of *Gryllus bimaculatus*, illustrating their variability. The analysed ticks were obtained from the corresponding songs shown in Fig. 1. For each male, the power spectra of three ticks were superimposed.



**Fig. 3:** The role of the carrier frequency of courtship ticks in female behaviour in *Gryllus bimaculatus*. (a) Percentage of females that mounted males. The percentages are presented for courtship by muted males accompanied by the playback of a natural song (positive control, p. c.), or by muted males with no playback (negative control, n. c.), or by muted males accompanied by the playback of stimuli 1–6 (st1–st6), shown at the right. The asterisk near stimulus 2 indicates playback of the song containing 5 kHz ticks to the older females. Black circles above the bars indicate significant differences from the negative control; an open circle indicates a significant difference from the positive control ( $p < 0.05$ ). (b) Latency (the  $\bar{x} \pm SD$ ) from the onset of the courtship song to the mounting response.

the mounting latency observed in the positive control (the  $\bar{x} \pm SD$ :  $65 \pm 44$  s) did not differ significantly ( $U$ -test:  $U = 31$ ,  $p = 0.46$ ) from the latency in the negative control ( $91 \pm 69$  s) (Fig. 3b). There was no difference between the responses of 3- to 5-d-old and 6- to 8-d-old females in either the mounting response (Fisher's exact test, two-tailed:  $0.58 < p < 1$ ) or mounting latency ( $U$ -test:  $7 < U < 29$ ,  $0.09 < p < 0.75$ ).

#### Female Preference in Experiments with Playback of Test Songs

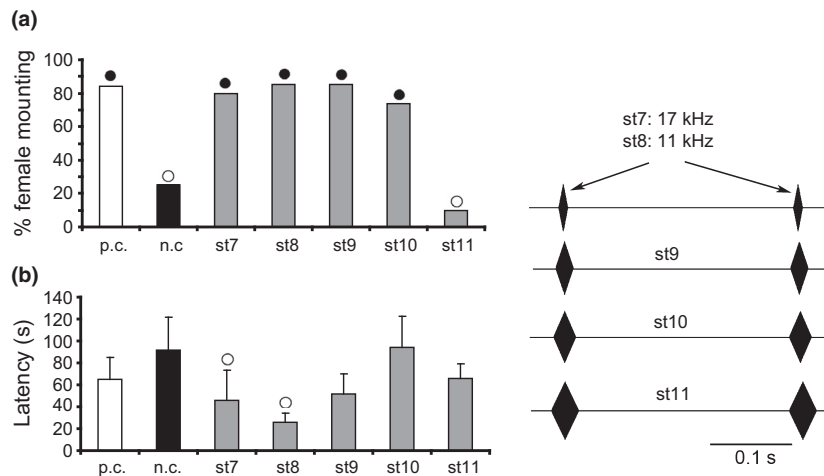
We tested the importance of various song elements for female responsiveness in playback experiments using computer-generated song models. The eleven song models were divided into two groups, depending on whether it was mainly frequency or temporal song characters that were manipulated. For ease of comparison, the data for the positive and negative controls shown in Fig. 3 are replicated in Fig. 4.

Changing the carrier frequency of ticks (5, 8, 11, 14, 17 kHz) did not result in a significant change in female responsiveness (in either the mounting response or latency). The mounting response level varied between 70% and 90%, which was comparable to that in the positive control (Fisher's exact test:  $p > 0.45$ ; Fig. 3a). Playback of the song containing 5 kHz ticks was less effective among the older females (stimulus 2) than among the younger females (stimulus 1) (63% vs. 85%); however, this difference was insignificant (Fisher's exact test:  $p = 0.15$ ). The

recorded mounting latencies did not differ among all of the stimuli presented (Kruskal–Wallis test:  $H = 1.77$ ,  $p = 0.97$ ; Fig. 3b).

Considering the great variability of the relative amplitude of pulses and ticks in the recorded courtship songs, we tested the importance of low-frequency pulses for females. Pulses were completely absent in stimuli 7 and 8, in which the frequency of ticks was 17 or 11 kHz, respectively. The efficiency of the two stimuli was comparable with that in the positive control (80% and 85%, respectively; Fig. 4a). However, the mounting latencies for the responses to both stimuli were significantly decreased in comparison with those in the positive control (Fig. 4b). In the experiments involving playback of stimulus 7, the mean mounting latency ( $\pm SD$ ) was  $46 \pm 27$  s ( $U$ -test:  $U = 70$ ,  $p = 0.03$ ). In the experiments with playback of stimulus 8, the latency was even lower, at  $26 \pm 8$  s ( $U = 42.5$ ,  $p = 0.0004$ ). Thus, the songs that only contain ticks are more effective than the songs with both ticks and pulses.

In stimuli 9–11, we changed the duration of ticks. In all of these stimuli, only ticks with a frequency of 11 kHz were present, whereas pulses were absent, similar to stimulus 8 (Table 2, Fig. 4). Stimulus 9 involved ticks with a doubled duration compared with that in stimulus 8 (24 ms), while stimulus 10 involved ticks that were 2.5 times longer (30 ms), and stimulus 11 involved ticks that were three times longer (36 ms). The mounting frequencies associated with stimuli 9 and 10 were relatively high (85% and 74%, respectively) and were comparable to those for



**Fig. 4:** The role of temporal parameters of the courtship song in female behaviour in *Gryllus bimaculatus*. (a) Percentage of females that mounted males. The data for the positive and negative controls are replotted from Fig. 3. The test songs are shown at the right. In all of the stimuli (st7–st11), only ticks were present, while pulses were absent. Stimulus 7 contained ticks with a frequency of 17 kHz, whereas stimuli 8–11 contained ticks with a frequency of 11 kHz. In stimuli 7 and 8, the tick duration was 12 ms (as in the positive control and in stimuli 1–6), whereas in stimuli 9–11, the tick duration varied (st9 – 24 ms, st10 – 10 ms, st11 – 36 ms). The symbols are the same as in Fig. 3. (b) Latency (the  $\bar{x} \pm \text{SD}$ ) from the onset of the courtship song to the mounting response.

stimulus 8 and the positive control (Fig. 4a). However, the mounting latency for the response to stimulus 9 (the  $\bar{x} \pm \text{SD}$ :  $51 \pm 19$  s) was increased in comparison with the latency for stimulus 8 ( $U = 65.5$ ,  $p = 0.006$ ; Fig. 4b). In turn, the latency for the response to stimulus 10 ( $94 \pm 28$  s) was increased in relation to that for stimulus 9; however, this difference was barely significant ( $U = 70$ ,  $p = 0.05$ ). Stimulus 11 (in which the longest ticks were presented; 36 ms) was inefficient for females: the number of positive mounting responses to stimulus 11 was comparable to that in the negative control (10% and 25%, respectively) and was significantly different from that in the positive control and for all other test songs (Fisher's exact test:  $p < 0.001$ ). Thus, altering this parameter is crucial for female responsiveness.

## Discussion

We found that most of the courtship song elements observed in *G. bimaculatus* are quite variable. Six of the eight studied song parameters showed between-male CV values of  $>20\%$  (Table. 1). Even greater variability of courtship traits was reported in *G. assimilis* (Vedenina & Pollack 2012). The relative amplitude of pulses and ticks was shown to be the most variable song character in both species. At the same time, the dominant frequency of ticks was quite consistent in *G. assimilis* and variable in *G. bimaculatus*. For the dominant frequency of pulses, the situation was reversed. In *Gryllus texensis* and *Gryllus rubens*, the

courtship song traits were shown to be as variable as in *G. bimaculatus* (Fitzpatrick & Gray 2001). In particular, we observed similarity between the courtship songs of *G. bimaculatus*, *G. texensis* and *G. rubens* regarding the high variability of the dominant frequency of ticks. The high variability of this parameter in *G. bimaculatus* was not previously documented. The high-amplitude ticks in the courtship song of this species have generally been suggested to vary in the range of 11–16 kHz (Zhantiev & Dubrovin 1974; Rheinlaender et al. 1976; Libersat et al. 1994). However, our analysis of the spectra of ticks showed the high-frequency component (11–16 kHz) to be only 5 dB higher than the low-frequency component (4–5 kHz); moreover, this difference was insignificant.

Changing the carrier frequency of the ticks from 5 to 17 kHz did not result in a significant change in female responsiveness. This result is not consistent with the data obtained by Libersat et al. (1994), who found that a courtship song containing a low-frequency component (4.5 kHz) resulted in a poor mating success rate, whereas accompaniment by a 13.5 kHz synthetic song restored the female responses to normal rates. The inconsistency of the results might be explained by the different methods applied. First, the females used by Libersat et al. (1994) were relatively old (2–3 wk old); second, the synthesised songs were presented to non-virgin, experienced females; and third, each male courted only one female. To check the first assumption, we presented a synthesised song containing 5 kHz ticks (stimulus 2) to older



females (14–17 d old). The older females responded more poorly than the younger females, but this difference was insignificant. Thus, female age is an unlikely reason for the inconsistent results. The use of non-virgin, experienced females by Libersat et al. (1994) may be a more feasible explanation for the different results. Virgin females are known to be more eager to mate and less selective than mated females. However, because many other behavioural studies on cricket song recognition have been conducted on virgin females (Popov & Shuvalov 1977; Pollack et al. 1984; Adamo & Hoy 1994; Balakrishnan & Pollack 1996; Nelson & Nolen 1997; Wagner & Reiser 2000; Rantala & Kortet 2003; Gray 2005; Tregenza et al. 2006), we used the same method to allow comparison of the results. We also employed some males in several trials, so that some active males were able to court different females, and the possibility cannot be excluded that these males were especially attractive for females because of their dominant status, evaluated on the basis of pheromones. Thus, we assume that narrower selectivity occurred than would have been the case if mated females were used and if each male was used only once.

According to Gerhardt & Huber (2002), the between-male variability of some acoustic properties of advertisement calls in frogs exceeds within-male variability, providing a possible substrate for sexual selection. In crickets, the between-male variability in the dominant frequency of ticks was 1.7 times higher than within-male variability (Table 1). Female preferences based on this character were, however, neither directional, nor stabilising: females responded readily to all stimuli, independent of the carrier frequency of ticks. Taking into account the above data, we suggest the existence of some signature of sexual selection acting on this character.

We found that the courtship song of *G. bimaculatus* in the majority of specimens consisted of two distinct elements, ticks and pulses, although pulses were obscure in some specimens. This finding has not been reported by other authors, who have indicated that the courtship song of *G. bimaculatus* always contains two elements. In general, the courtship songs of all studied *Gryllus* species have been shown to contain two elements (Nocke 1972; Zhantiev & Dubrovin 1974; Rheinlaender et al. 1976; Ragge & Reynolds 1998; Wagner & Reiser 2000; Fitzpatrick & Gray 2001; Vedenina & Pollack 2012), although the function of the low-amplitude pulses remains unclear (Libersat et al. 1994; Rantala & Kortet 2003). In our experiments, the mounting latencies for responses to stimuli without low-amplitude pulses were even lower than

for stimuli with low-amplitude pulses. Because the songs that only included ticks (of rare occurrence) appeared to be more effective than the songs with both ticks and pulses (of frequent occurrence), we consider female preferences to be directional. The relative amplitude of pulses and ticks was shown to be the most variable parameter of the courtship song. Therefore, we share the opinion that variable traits are important for intraspecific competition and could serve as a substrate for sexual selection (Gerhardt & Huber 2002) if between-male variability in this character were higher than within-male variability. However, the variability in this character between and within males was similar (Table 1). Thus, the support for this hypothesis is not sufficiently strong.

One of the relatively variable characters in the *G. bimaculatus* song was the tick duration. Changing the duration from 12 ms (as in averaged song) to 30 ms did not result in a decrease in the mounting response, but it did result in an increase in latency. Changing the duration of ticks from 30 to 36 ms resulted in a crucial decrease in the mounting response compared with that in the negative control. These results are inconsistent with the idea that traits that are important for song recognition exhibit little variability (Popov & Shuvalov 1977; Gerhardt 1991).

The pulse dominant frequency was shown to be a rather stable parameter (Table 1). This is a surprising result considering the higher effectiveness of stimuli without pulses compared with stimuli containing pulses. We can explain high stability of this parameter based on the constraints originating from the limitations of the stridulating apparatus in crickets. When emitting low-amplitude pulses, which are very soft sounds, males of *G. bimaculatus* might be unable to produce the pronounced higher harmonics. In several species of *Gryllus*, the higher harmonics in courtship pulses have been shown to be much less pronounced than the fundamental frequency (Nocke 1972; Rheinlaender et al. 1976; Fitzpatrick & Gray 2001). It is only in the courtship song of *G. assimilis* that pulses that are sometimes as loud as ticks include highly pronounced higher harmonics (Vedenina & Pollack 2012).

The carrier frequency of the song has been suggested to be one of the key parameters used for species recognition and mate choice in crickets (Rheinlaender et al. 1976; Pollack et al. 1984; Popov 1985). There are two principal ascending auditory neurons that have been shown to play a critical role in cricket acoustic behaviour. AN1 is tuned to low frequencies (e.g. 5 kHz in *G. bimaculatus*), while AN2 shows broad tuning to high frequencies (e.g. Wohlers & Huber

1982; Hennig 1988). AN1 has been studied with respect to its role in relaying information about calling songs to the brain (e.g. Schildberger & Hörner 1988), whereas the behavioural roles proposed for AN2 are recognition of courtship song (Rheinlaender et al. 1976; Harrison et al. 1988) and bat evasion (Nolen & Hoy 1986). Taking this into account, the results obtained by Libersat et al. (1994) appear logical: the courtship song containing the high-frequency component resulted in a high success rate, whereas the courtship song containing the low-frequency component was ineffective. However, we suggest that this point of view is simplified. In *G. assimilis*, the frequency selectivity of AN1 and AN2 was shown to be sufficiently broad to tolerate the spectral variability of courtship chirps (Vedenina & Pollack 2012). In this previous report, we assumed that simultaneous inputs from both neurons received by the 'chirp recogniser' might constitute one component of the courtship song recognition mechanism. We offered the hypothesis of 'sensory permissiveness', in which broad tuning of the sensory system allows relaxation of selection pressure for precise control of the sound frequency. If we suppose a similar mechanism in *G. bimaculatus*, courtship ticks with different spectral maxima are also expected to stimulate both neurons and may therefore be equally effective in generating an appropriate behavioural response.

Summarising the available studies on insects and anurans, Gerhardt & Huber (2002) showed that the carrier frequency and fine temporal structure of the advertisement song appear to be stable characters more often than other, gross-temporal properties, which the authors explained based on physiological constraints. The carrier frequency and pulse structure show limited variation because they are usually tied to the size of the individual and the mechanics and morphology of the peripheral sound-producing structures, respectively. By contrast, characters such as the call rate and call duration are correlated with physiological and behavioural traits such as temperature, energetic costs and signalling interactions. Our study does not provide strong support for this idea; the dominant frequency of ticks and the tick duration, which could be attributed to fine temporal properties, appeared to be unstable characters. However, the relative amplitude of ticks and pulses, which may reflect differences in the energetic contribution, was quite variable, consistent with the generalisation put forth by Gerhardt & Huber (2002). The partial inconsistency of our results with the data of other authors could arise from different patterns of past

and current selection on long-distance and short-distance acoustic signals. As most of the conclusions reported thus far were made based on studies conducted on long-distance signals, it is worth studying short-distance calls and the mechanisms underlying their evolution in more detail.

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