

DUET SINGING AND FEMALE CHOICE IN THE BUSHCRICKET *PHANEROPTERA NANA*

by

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Summary

In the phaneropterine bushcricket *Phaneropteranana* both males and females sing, producing a duet. The male's song is answered by a short female 'tick'. A male that receives a response in a defined interval after the end of his chirp usually initiates phonotaxis toward the female. Measurements of temporal, spectral, and energy characters of male chirps indicate that individual males vary considerably in several temporal song characters. Females exhibited equivalent response rates to solitary singing males in laboratory trials regardless of their song characters. However, when simultaneously presented with two singing males, females showed consistent preferences: They responded preferentially to relatively longer chirps produced by larger males. Male chirp rate had no influence on female response, suggesting that females were not influenced by a summation of acoustic input integrated over multiple chirps. Rather, female 'decisions' to respond were probably made following individual chirps.

The selective responses exhibited by *Ph. nana* females contradict some previous expectations that females in duet signalling systems should be indiscriminate. Selectivity may reflect

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the reliability with which females can assess male song at a distance and potential disadvantages of attracting multiple suitors.

Laboratory trials indicated that only males receiving a high female response rate actually approach and mate with the female. Thus, this duet signalling system may be relatively resistant to intraspecific exploitation.

Keywords: acoustic communication, duet, song characters, female choice, bushcrickets, *Phaneroptera nana*.

Introduction

In many orthopteran insects, including most Tettigoniidae and Gryllidae and some Acrididae, stationary males produce long-range advertisement calls that attract receptive females from a distance (Gwynne & Morris, 1983; Bailey, 1991; Greenfield, 1997). An acoustic signal, the calling song, is repeatedly produced by the male and may provide the female with information on the male's species identity, individual 'quality', and location. Various studies have shown that females may orient toward and mate with an individual male based on amplitude, temporal, or spectral features of his calling song (*e.g.* Gwynne, 1982; Latimer & Sippel, 1987; Ritchie, 1991; Tuckerman *et al.*, 1993; Ritchie *et al.*, 1995; Brown *et al.*, 1996; but see Gwynne & Bailey, 1988; Bailey *et al.*, 1990; recent review by Brown, 1999).

Several deviations from the above pair-forming protocol exist among phaneropterine tettigoniids in which the movement of the sexes entails a fundamental role-reversal from typical orthopteran pair-formation (Heller, 1990; Spooner, 1995). In these variations the female emits a weak acoustic signal in response to the male advertisement call, resulting in a duet. The latency of the female response, which can be as short as 15 ms (Heller & Helversen, 1986), may be required to occur during a precise interval following the end of the male's call: Responses not falling within this interval may fail to elicit a male response, but responses within the interval usually result in male phonotaxis toward the female (Heller & Helversen, 1986; Robinson *et al.*, 1986; Zimmermann *et al.*, 1989; Dobler *et al.*, 1994). Here, males may either (1) move the entire distance toward the responding female or (2) only a partial distance, with the females then making the final approach toward the male. Additionally, (3) responding females may move over most of the distance, with males making the final approach toward the female, or (4) both the male and female may move simultaneously toward each other.

Insects may be particularly vulnerable to predation during the phonotaxis stage of pair formation (Sakaluk & Belwood, 1984; Heller, 1992). In species with stationary calling males, females have to bear the risks of moving, and in some species they may forgo choosiness because of the increased exposure it entails at the onset of and during orientation movement (Hedrick & Dill, 1993; Farris *et al.*, 1998). However, in duetting species, in which females move little or not at all and call but only weakly, the situation is less predictable. On one hand, low costs for females favour high selectivity; nonetheless, females may be indiscriminate in their responses to calling males during the early stages of pair formation (Heller *et al.*, 1997). Females may attract all calling males and make a mating decision later on the basis of other cues which may be assessed more accurately at close range.

Duet singing in Orthoptera has been extensively used as a model system to study signal recognition and sound localisation. In these studies the female phonoresponse was used to bioassay male calls to explore the physiological processes of auditory localisation and call recognition (Helvesen, 1984; Robinson *et al.*, 1986; Helvesen & Helvesen, 1995; Tauber & Pener, 2000). Less attention has been devoted to female preference in duetting species (but see Tuckerman *et al.*, 1993). Here, we use phonoresponse bioassays to study the nature and level of female choice in the duetting phaneropterine tettigoniid *Phaneroptera nana* Fieber (= *Phaneroptera nana nana*, *sensu* Ragge, 1956). We report that in this duetting species, in which the males call as well as exhibit phonotaxis, females are selective in their choice of mates.

Natural history and acoustic communication in *Ph. nana*

The genus *Phaneroptera* Serville is widespread in the Old World and Australia (Ragge, 1956). *Ph. nana* is found in areas of sclerophyllous vegetation throughout the Mediterranean basin. This species is common in Israel, where it occurs on a wide range of vegetation and feeds on foliage and flowers. Males sing during evening hours and are heard during all months of the year when evening temperatures exceed 14°C. Singing males may be closely spaced, with as many as 5-10 individuals occurring within 9 m². As in many tettigoniids (see Gwynne, 1997), *Ph. nana* males provide the females a 'nuptial gift', a spermatophylax attached to the sperm ampulla, which

the female consumes during copulation (Pener & Ayal, unpubl. data). Pair formation in *Ph. nana* is effected by male phonotaxis toward females who phonorespond to male calls (duetting type 1, as listed in the Introduction; Pener & Ayal, unpubl. obs.). Males produce ~ 70 -ms calls (chirps) at a chirp rate $\cong 1 \text{ s}^{-1}$ (Heller, 1988); the peak frequency of *Ph. nana* male calls is ~ 16 kHz (for more data on the song characters, see Results).

Methods

Rearing

We established a laboratory population of *Ph. nana* in 1995 from insects collected at three different localities in Jerusalem, Israel. The laboratory colony was reinforced several times by insects collected at the same localities (Jerusalem) to avoid inbreeding. The insects were kept under a 12:12 L:D photoperiod and at 31°C and 27°C during photophase and scotophase, respectively. The insects were fed on fresh plants (*Plumbago capensis* Tunb.) and flaked oats. Eggs were laid into strips of dry filter paper (see Grassé, 1924 under the name *Ph. quadripunctata*, and Ayal *et al.*, 1974) that were wetted within two weeks of oviposition and kept wet. Under these conditions, diapause does not occur and continuous generations can be obtained in the laboratory. One generation, from oviposition to oviposition, takes approx. 3 months.

After moulting to the adult, experimental males and females were kept in two acoustically isolated rooms to prevent mating and habituation of females to males' songs. Experimental insects were examined before testing to ensure that they were intact; those with injured or missing legs, incomplete antennae, or damaged wings were discarded. Individuals were uniquely marked on the pronotum with two small dots of oil-based markers of various colours 1-2 days before experimentation.

After completion of experiments, each male was anaesthetised with CO₂ and four morphological parameters were measured: weight (± 0.05 mg), hind femur length (± 0.05 mm), maximum pronotal width, and forewing length. We used principal component analysis (PCA) to incorporate all four morphometric measures; The first principal component, SIZE, although explaining only 48% of the variability, was still found to be a useful measure for assessing male size (see below).

Acoustic recording and playback

Experimental insects, 1-4 weeks past the adult moult, were placed individually in 4 × 4 × 4 cm mesh cages held in an acoustically insulated 2 × 1 × 1 m chamber. We made all experimental recordings during scotophase and at 24 ± 2°C, except where otherwise stated. Both male and female songs were recorded with LinearX model M53 microphones (± 1 dB from 10 Hz to 40 kHz) placed 0.5 cm above the mesh cages and oriented toward the insects. Sound pressure levels (SPLs) of the insects' calls were measured with a Brüel & Kjaer model 2203 sound pressure level meter (PEAK setting) fitted with a model 1613 octave filter centred on 16 kHz. Precise values of absolute SPL of the extremely short *Ph. nana* calls were obtained by calibrating oscilloscope readings of call peak amplitudes with peak equivalents of 16 kHz

continuous sound. We calculated means and standard deviations of SPLs by converting dB values to Pa, determining the statistics, and reconvertng the statistics to dB.

We split the microphone outputs and sent one channel of each to the input of an eight-channel soundcard (National Instruments model Lab PC+) installed in a desktop computer. These inputs were digitised in the soundcard's A : D converter at 30 or 60 kilosamples s^{-1} and stored as computer files for later analysis via custom software. The other channel split from the microphone outputs was recorded on a four-channel video cassette recorder (Neuro Data KD-484; sampling rate up to 44 kilosamples s^{-1}). The output of the microphones, as recorded on the video cassette recorder, was also observed in real time using a Tektronix four-channel oscilloscope. These oscilloscope observations allowed us to monitor the ongoing acoustic behaviour in our experiments.

Signals broadcast in playback trials testing female responses to male song models were synthesised using CoolEdit software (Syntrillium Corp.; Phoenix, AZ, U.S.A.) and converted to analogue via a 16-bit soundcard (Creative Labs, Inc. model CT2940) installed in a second desktop computer. Each of two outputs from the sound card were amplified (Revox Model 39 amplifier) and split, one signal driving a playback speaker (Utah Electronics, model H208 tweeter horn) while the other was sent to a separate channel in the recording computer described above. We used a 16 kHz pure tone for the song models; thus, the playback stimulus did not include the sidebands seen in the natural male call. However, females responded to the synthetic calls in a manner similar to their responses to the calls of live males (Tauber & Pener, 2000).

Experimental design

We recorded the solo calling of male *Ph. nana* to determine the variation among individuals in various temporal song characters. Five temporal song characters, number of pulses per chirp (PN), chirp duration (CD), pulse duration (PD), inter-pulse interval (IPI), and chirp period (CP) were measured from each of 23 males (see Fig. 1 for definitions of song characters). We measured both CD and PN because of the possibility that males differed in IPI. Recordings were made from each male for 100 s, and temporal data from all calls included in the recordings were analysed via custom computer programs.

Because individual males varied significantly in several song characters (see Results), we paired males and females to determine whether females responded differentially to the various males based on their signalling. Here, the male and female were separated by 1 m, and we placed a microphone above each individual's cage. The separate outputs from the two microphones were sent to two channels in the recording computer sound card and the video cassette recorder. Gains in the computer and the video cassette recorder were adjusted to low levels to minimize cross-talk between the male and female channels. We recorded 100 s of duetting from unique combinations of seven males and eight females (only 49 out of the 56 possible combinations were tested due to loss of some insects; the missing combinations were evenly distributed among the seven males). Recordings began after both the male and female began calling. A female call occurring < 100 ms after a male's chirp was construed as a positive response to that male. The female's response rate was calculated by dividing the number of her calls by the number of calls delivered by the male (see Helversen, 1984). Each individual was tested only once during an evening, and from four to six different combinations were recorded on a single evening. All duets were recorded over a 4-week period.

To determine whether male song characters changed between solo calling and duetting, we compared the songs recorded from the 23 solo males with those of the seven males used in male-female duets. We supplemented this comparison by recording the songs of five additional males while singing alone and then when paired with a female. A different female was paired with each of these five males.

Because females may be more discriminating when presented with the simultaneous choice of several males than when presented with males singly (see Doherty, 1985), we tested the responses of females to pairs of calling males in choice trials ('trio design'). Here, the two males were separated by 2 m, and the female was placed midway between the males. Each of the three insects were recorded for 100 s with a separate microphone whose output was split and sent to separate recording channels in the computer and the video cassette recorder. Trials in which one of the males called little or not at all were curtailed, and the non-calling male was replaced. Nine pairs of males, including 18 different individuals, were each tested with a different female.

The latency of female response that was observed earlier in recordings of male-female pairs was used as a criterion to ascribe the female's reply to one of the males: Female calls occurring within the 100-ms interval following the end of a given male's call were construed as positive responses to that male. Separate female response rates were calculated for each male in the trial and then compared using the binomial test between proportions (Zar, 1996, p. 552).

We could not measure the amplitude of male calls in our choice trials directly, because the microphones were positioned close to the males and any movement by them introduced excessive variation in microphone-male distance (1-4 cm). Therefore, we relied on the female microphone, which was positioned 100 cm from either male cage and oriented along a line bisecting the axis between the two male cages. Owing to this position the distance between the female microphone and a male varied little (98-102 cm), ensuring that the relative amplitudes of the males were represented accurately as crosstalk on the female recording (see Results).

Our choice trials indicated that females preferentially replied to males whose songs included more pulses (see Results), but males always varied in several song characters, including amplitude. To determine whether pulse number per se influenced female response, we presented long (6-pulse) and short (2-pulse) synthetic calls in an alternating sequence from the playback speakers. Amplitude and other song characters of these two stimuli were held at identical values (SPL = 60 dB; PD = 0.5 ms; IPI = 15 ms; CP = 1.2 s). We have already reported (Tauber & Pener, 2000) that 2-pulse male songs are clearly recognized by the female as conspecific songs; in no choice experiments they elicited similar rates of female response as songs composed from higher pulse numbers. Each of five test females was presented with the following three two-speaker playbacks: (1) long vs short calls; (2) same as (1) but with speakers reversed; (3) long calls from both speakers. The two speakers were 1 m apart, and the test female was placed between them. As above, a microphone was placed near the female, and its output was sent to a third channel in the recording computer, enabling us to monitor the female's response and the playbacks at her location. Each playback was presented for 200 s. We calculated female response rates toward each speaker and compared the rates as in the choice test using males.

To confirm that the males to which females phonoresponded were actually preferred as mates, we tested an additional 10 pairs of calling males as in the choice tests above except that the three insects were released from their cages following the 100-s acoustic recording. Thus, we observed the movements and approaches of the male(s) toward the responding female.

Here, each male pair was tested with a different female, and observations of insects moving freely on the floor of the acoustically insulated chamber continued until a given male and the female made physical contact or for 20 min. Observations were facilitated by illuminating the chamber with dim red light.

Results

Ph. nana song characters

Solo calling males produce well defined calls, onomatopoeically termed chirps (Fig. 1; for temporal characters see Table 1). Pulses at the end of the chirp are ~ 12 dB louder than those at the beginning (Fig. 1). The peak frequency of *Ph. nana* male calls is ~ 16 kHz. Energy within 6 dB of that at the peak frequency extends from ~ 14 –18 kHz (Fig. 2A). Sound pressure level (SPL) measured 28 cm from a solo calling male is 61 ± 8 dB (mean \pm SD; 30 chirps recorded from each of 7 males; temp. = $26 \pm 1^\circ\text{C}$; 0 dB re 20 μPa).

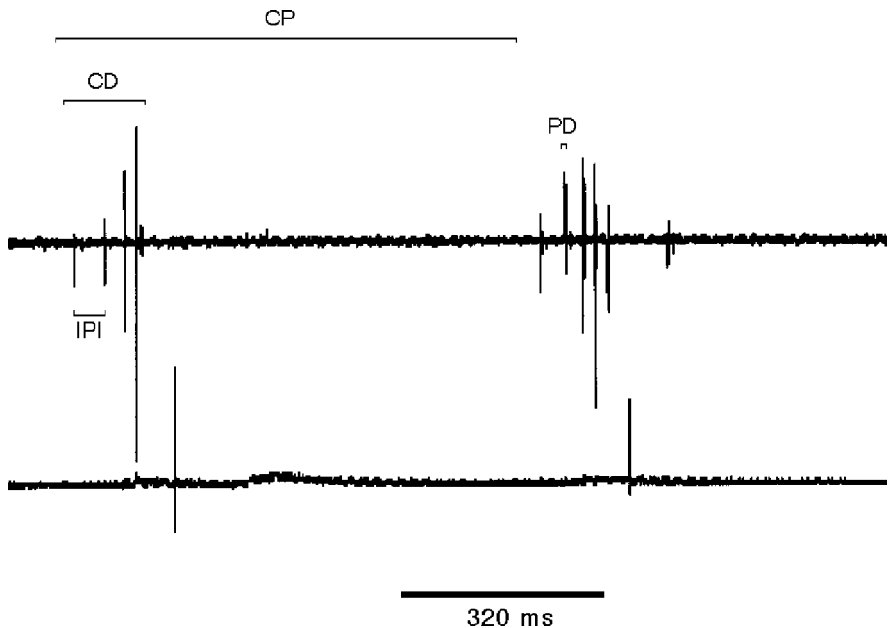


Fig. 1. Duet singing in *Ph. nana*. Oscillogram of male song (upper trace) and female response (lower trace) of *Ph. nana* ($T = 24^\circ\text{C}$). CP: chirp period, CD: chirp duration, IPI: inter-pulse interval, PD: pulse duration.

TABLE 1. *Calling song characters of Ph. nana males recorded in solo, while duetting (paired with a female), and in a trio (two males and a female)*

	PN	CD [ms]	PD [ms]	IPI [ms]	CP [s]
Solo	5 (4-7)	52 (30-82)	0.3 (0.2-0.4)	6.9 (5.8-11.0)	1.02 (0.85-1.14)
Duet	7 (6-8)	45 (41-49)	0.4 (0.2-0.7)	5.5 (5.0-7.1)	0.77 (0.59-0.85)
Trio	4 (3-5)	41 (38-60)	0.5 (0.4-0.6)	9.5 (5.7-11.9)	0.63 (0.56-0.68)

Medians and 25-75% percentiles of individual median values. Solo: $N = 23$ males, duet: $N = 12$ males, trio: $N = 18$ males. Abbreviations of song parameters: PN = number of pulses in a chirp, CD = chirp duration, PD = pulse duration, IPI = inter-pulse interval, CP = chirp period (see Fig. 1). One-pulse chirps were ignored in CD measurements.

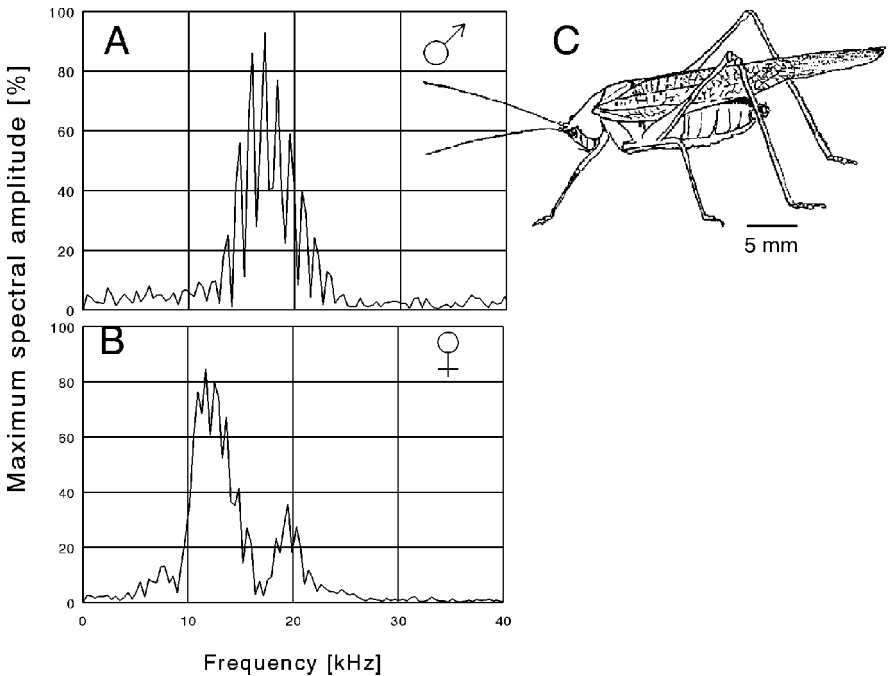


Fig. 2. Power spectra of *Ph. nana* song. **A:** The song of a male. **B:** The answer of the female. **C:** A drawing of a male (scale bar 5 mm).

Female responses are usually lower in SPL than male chirps and occur ~ 60 ms after the end of the males' chirps. The responses include one or two 0.8-ms pulses (Fig. 1, lower trace) whose peak sound frequency is 13.2 ± 1.9 kHz ($N = 6$ females, with 3 samples per individual; Fig. 2B).

TABLE 2. *Correlation matrix of song and morphological characters*

	PN	CD	PD	IPI	CP
CD	0.44				
PD	0.51	0.16			
IPI	-0.23	0.49	-0.11		
CP	0.14	-0.28	-0.36	-0.48	
SIZE	0.36	0.34	0.59 ¹⁾	-0.09	0.40

Abbreviations of song characters (PN, CD, PD, IPI and CP) as in Table 1; SIZE = morphological size score according to the first principle component score (see text). Spearman correlation coefficients are shown. *P* values are adjusted using the Holm procedure. *N* = 23 males.

¹⁾ $p < 0.05$.

Variation and correlation among male song characters

Temporal song characters were not normally distributed (Shapiro-Wilk test for normality, $p < 0.001$ for all characters) and were not normalised by any standard transformation. We therefore rely on non-parametric tests in the following analyses.

Recordings of solo males indicated that individuals vary significantly in each of the five temporal song characters measured ($p < 0.05$; Kruskal-Wallis test). Thus, females may potentially respond toward individual males in a differential manner based on these characters. Of the five temporal song characters of the males (see Methods and Fig. 1), only pulse duration (PD) was significantly correlated with male size ($p < 0.05$; Holm adjustment for multiple tests [Rice, 1989]; Table 2).

Individual males sang considerably faster while duetting with a female than when singing alone: Male CPs averaged 750 ms in duetting, approximately 75% that of the solo CP value ($p = 0.01$, $U = 27$, Mann-Whitney U -test, two-tailed, on median values of 23 solo and 12 duetting males; Table 1). Otherwise, song characters of the duetting males were not distinguished from those of the solo males ($p > 0.15$ on median values for each character: PN, CD, PD, and IPI; Mann-Whitney U -tests, two-tailed). We found comparable similarity within the five males that were recorded both while calling in solo and duetting ($p = 0.06$ for CP; $p > 0.8$ for PN, CD, PD, and IPI; Wilcoxon matched-pairs signed-ranks test, two-tailed, on median values). Peak frequencies of these males were also equivalent ($p = 0.44$; Wilcoxon test, two-tailed).

Female choice for male song characters

Our male-female duets did not reveal any significant female preferences for individual males that were correlated with temporal male song characters or any other features. Although, as found earlier in solo singing, the seven males used in duets exhibited significant between-individual differences in song characters ($p < 0.001$ for each character; Kruskal-Wallis test), the females did not display significantly different response rates toward different males. Individual males were scored by tabulating the response rates they received from each of the females with which they were paired. Tabulated response rates were not significantly different among the males (Friedman 2-way ANOVA: $\chi^2 = 8.6$, $p > 0.07$).

However, when simultaneously presented with two calling males in choice trials ('trio design'), females exhibited strong preferences for a given individual that were correlated with some of his temporal song characters and morphological features. In all nine choice trials, one male received a significantly higher proportion of female responses (Table 3; difference between proportions test [Zar, 1996, p. 552], $p < 0.01$ for each pair). Over 90% of female responses consisted of one pulse only, and we did not assess the level of female response by its mean length or by its pulse number.

In order to determine whether each female response was an independent event or part of an entrainment toward a given male's calling rhythm, we employed one-sample runs tests (Zar, 1996) to evaluate the temporal sequence of female responses within individual trials. These tests showed that successive female responses during a choice trial did not occur as significant 'runs' of answers toward a given male and then toward the other ($p > 0.6$; one-sample runs test, two-tailed, for each of five trials that have been analysed). This consideration allowed us to tabulate a female's response rates toward the two males and compare them statistically.

Female phaneropterine tettigoniids respond to males after a characteristic latency reflecting the male's sensitive time window (Heller & Helversen, 1986; Robinson *et al.*, 1986; Zimmermann *et al.*, 1989), and this feature in *Ph. nana* afforded us unambiguous recognition of the preferred male in choice trials. In the 49 male-female duets, this latency was 59 ± 9 ms (mean \pm SD; $N = 2671$ responses) following the end of a male's chirp (Fig. 3). The interval between a given female's successive responses (excluding double-pulse answers) was highly variable (2.30 ± 0.81 s; mean \pm SD; grand mean

TABLE 3. *Female rate of response and size measures of males, in two-choice test ('trio' design)*

Set No.	Proportion of answers received by male		Z ¹⁾	Length of hind femur [mm] of male		Length of fore-wing [mm] of male		Width of pronotum [mm] of male	
	no. 1	no. 2		no. 1	no. 2	no. 1	no. 2	no. 1	no. 2
1	0.54	0.46	5.49	mm ²⁾	mm ²⁾	20.5	19.6	2.1	2.3
2	0.68	0.32	5.28	16.8	16.0	22.0	19.4	2.4	2.3
3	0.69	0.31	14.8	16.1	15.6	20.8	19.3	2.1	2.4
4	0.81	0.19	21.1	15.5	14.9	19.8	18.7	2.4	2.2
5	0.67	0.33	20.4	16.2	17.0	19.6	19.5	3.2	2.4
6	0.73	0.27	7.71	mm ²⁾	mm ²⁾	20.8	19.6	2.5	2.4
7	0.84	0.16	2.42	16.3	16.1	20.2	20.0	3.5	3.0
8	0.70	0.30	2.06	17.9	14.6	19.5	19.3	2.8	2.8
9	0.76	0.24	4.31	16.5	15.9	19.4	18.6	3.3	2.6

¹⁾ We compared proportions of males calls that have been answered by the female using the binomial test between proportions (Zar, 1996, p. 552). The male receiving a higher proportion of female answers is arbitrarily designated as male no. 1. Z is the test statistics.
²⁾ mm = not measured.

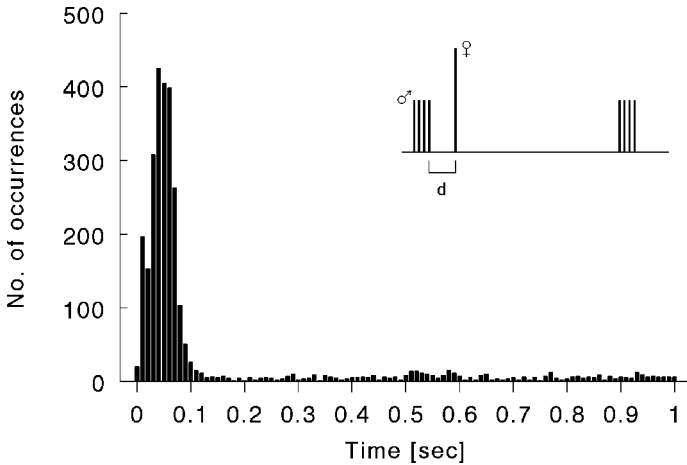


Fig. 3. Timing of the response of *Ph. nana* females. Distribution of the latencies of female responses. The latency is defined as the time interval between the end of the male's song and the female answer (see *d* in insert).

of 8 females), indicating that females do not merely adjust an underlying rhythm to that of the males. Signal interactions between the two males also facilitated our recognition of the preferred males. *Ph. nana* males alternate their songs, and for a mean chirp period of 600-700 ms (Table 1, 'trio' column, CP), usually more than 150 ms will elapse between successive songs of two singing males. This is much longer than the female latency, which is approximately 60 ms (Fig. 3). Thus, female responses do not overlap the songs of either male in the choice trials and are clearly associated with one of the two males (Fig. 4).

The higher level of female preference observed in choice trials (trios) than in (male-female) duets probably reflects the discrimination that occurs when the opportunity for simultaneous comparison is present rather than greater variance in song characters among males in trios. For all five temporal characters, the ranges of individual median values were comparable between males used in duets and males used in trios (Table 1). Moreover, distributions of these median values did not differ significantly among males in duets and in trios ($p > 0.11$ for each of the five temporal characters; Kolmogorov-Smirnov two-sample test, two-tailed).

We had the opportunity to determine correlations between female preference and temporal male song characters because paired males within trios differed significantly ($p < 0.05$; for one or more characters in all 9

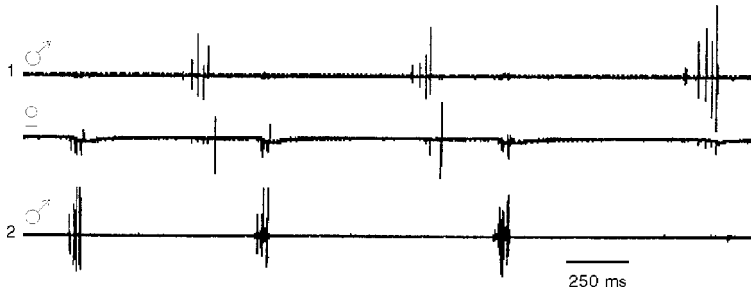


Fig. 4. Songs of two *Ph. nana* males recorded in the presence of an answering female (the 'trio' design). Some 'cross-talk' from the male songs is recorded on the female channel. In this example the female answered two chirps of male no. 1 and ignored male no. 2.

pairs; Mann-Whitney U -test, two-tailed). The song of the preferred male had a significantly higher number of pulses per chirp (PN) in all nine pairs ($p = 0.0019$; binomial test). Similarly, the preferred male consistently had longer chirps (CD) in eight of these nine pairs ($p = 0.019$). We found no correlation, however, between female preference and the two temporal song characters CP and IPI or the product of $\text{PN} \cdot \text{CP}^{-1}$ (summation of pulses) ($p > 0.25$ for each), but this could also be a result of the low power of the these tests ($p = 0.15$).

Preferred males were also relatively larger individuals (Fig. 5). In all pairs tested, preferred males had higher SIZE values, and the difference in SIZE was a significant predictor for the difference between the rate of response (preference) that each male elicited from the female ($F = 6.5$, $\text{df} = 9$, $p < 0.034$).

In five out of the nine male pairs used in choice trials, the songs of both males were loud enough to be detected by the female microphone (*e.g.*, see crosstalk in Fig. 4). In two of these five pairs, the male song preferred by females was approx. 6 dB lower in SPL; in the three other pairs both male songs were approx. equal in SPL, as judged by the relative amplitudes of male crosstalk traces on the female channel.

Our playback trials of synthetic male songs further confirmed that song characters other than amplitude could influence female preference greatly. Each of the five females tested, exhibited significantly higher response rates toward the longer (6-pulse) chirp when presented with a choice of this and the short (2-pulse) chirp in an alternating sequence; female rate of response was 27-44% vs 8-18% for long and short chirps respectively ($p < 0.001$ for each female; difference between proportion test, two-tailed). As in the

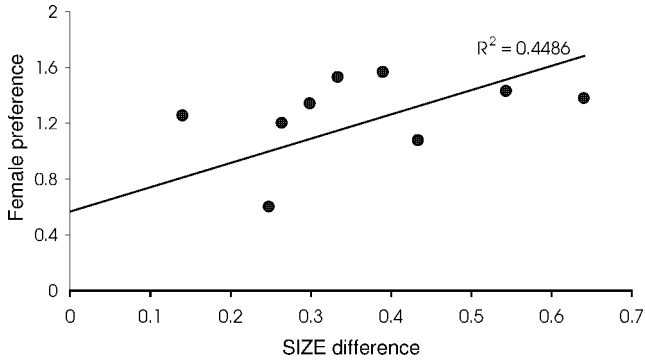


Fig. 5. Female preference for larger males. Female preference as a function of the SIZE difference (log-transformed) between males of each pair. Female preference was calculated as the difference in rate of response (log-transformed) that each of the males elicited from the female. The linear regression is also depicted ($F = 6.5$, $df = 9$, $p < 0.05$).

choice tests using males, female responses toward a given speaker did not occur in significant runs ($p > 0.63$; one-sample runs test towards each of the speakers, two-tailed). The females showed no preference for either speaker when both produced long chirps ($p > 0.19$ for each female).

Releasing of males and females from their mesh cages following the recording of choice trials confirmed that the males designated as preferred actually paired with females. In all 10 trials wherein the insects were released, the preferred male approached the female. When copulation occurred, females consistently copulated with preferred males (eight out of eight copulations: $p = 0.0039$; binomial test). In the 9th trial, the preferred male moved to within 10 cm of the female (in the remaining trial none of the males approached the female during the observation). In contrast, non-preferred males moved relatively little. Six non-preferred males remained within 10 cm of their cage. The remaining four were located about 0.5 m from the copulating pair. Females moved very little in all 10 trials, and all copulations occurred near their cages.

Discussion

Ph. nana males may incur considerable risk and energy expenditure during pair formation. Males signal loudly, perform all of the phonotactic movement, and provide the spermatophylaces as a nuptial gift. In contrast to

species where the cost of female choice may be high (Hedrick & Dill, 1993; Farris *et al.*, 1998), *Ph. nana* females may incur relatively little cost by responding to a given male and exhibiting high selectivity. However, in some duetting species females are indiscriminate and respond to all perceived males (Heller *et al.*, 1997). In these cases, they may gain somatically or genetically from acquiring spermatophylaxes and sperm from multiple males (Heller & Helversen, 1991). In addition, females may choose among potential mates at later stages. It was interesting to find in our work that *Ph. nana* females discriminate strongly among signalling males when presented with a simultaneous choice. And, given the densities of *Ph. nana* in the field, such choices should be commonplace in natural populations. Possibly, female discrimination in *Ph. nana* reflects the reliability with which male 'quality' can be assessed at a distance and/or that the conspicuousness of and harassment by multiple males are thereby avoided. Similar discrimination was recently reported in an analogous dialogue signalling system in the North American firefly *Photinus consimilis* (Coleoptera: Lampyridae). In this species stationary females produce exaggerated flash responses toward certain (rapidly flashing) males (Branham & Greenfield, 1996).

While non-preferred *Ph. nana* males did receive some female responses (Table 3), none of these males reached the female in the trials wherein males and females were released from their mesh cages. Perhaps, males evaluate their attractiveness relative to that of their neighbour(s) and behave accordingly: If relatively unpreferred, they avoid approaching the female, as such phonotaxis would not result in mating. That is, females may only mate with males with whom they have been interacting acoustically throughout the entire pair-forming sequence. A consequence of this signalling system is that non-signalling males, 'satellites' who might otherwise track the movements of males engaged in dialogues with females, are apparently absent. In the analogous signalling systems in fireflies, signalling males do occasionally exhibit various forms of interloping in the flash dialogues established between neighbouring males and responding females (reviewed by Buck, 1988). Similar events may also occur in some duetting phaneropterine tettigoniids (see Galliard & Shaw, 1991).

A recent study of male: female duetting in the Australian tettigoniid *Elephantodeta nobilis* (Phaneropterinae) revealed satellite male tactics in which individuals may insert short calls within the latency periods of neighbouring callers (Bailey & Field, 2000). Presumably, calls inserted by

satellites may divert the female's attention from the neighbour. Interestingly, *E. nobilis* also differ from *Ph. nana* in their phonotaxis pattern, whereby the task of searching and approaching in *E. nobilis* carried out by the females.

Ph. nana females apparently discriminate among males on the basis of their song characters, specifically the temporal characters pulse number and chirp duration. The specific reliance on temporal song characters by females is not unexpected. Coefficients of variation of the temporal characters are relatively high among individuals (Table 1), suggesting that these characters are 'dynamic' (*sensu* Gerhardt, 1991) and involved in mate choice rather than mere species recognition. Moreover, these temporal characters likely provide more reliable information about the male signaller than song frequency and amplitude, as they are influenced little by distance and obstructions en route to the female receiver (Simmons, 1988). Frequency is apparently a 'static' character, as behavioural tuning curves of *Ph. nana* females (Tauber & Pener, 2000) show that females are maximally sensitive to the mean carrier frequency of the calling song of the male (16 kHz).

Our results from choice tests and playback trials should not be construed to imply that only pulse number (or chirp length) influences female choice and that song amplitude is unimportant. The loudness of a signal, as perceived by a female, depends on the amplitude at emission and the distance. If the difference in loudness of males' signals is sufficiently high, females would likely respond to the louder male, regardless of the number of pulses in his chirps. Some critical difference in the amplitude at perception may override the attractiveness of the higher pulse number. For example, females of the tettigoniid *Ephippiger ephippiger* show a preference for their own 'song race' (Ritchie, 1991), but this preference was overridden by amplitude when songs of another race were ≥ 10 dB louder.

Our finding that preferred males are not distinguished by a higher chirp rate (CP^{-1}) suggests that females 'decide' the manner in which they respond to a male following each individual male chirp. That is, we found no indication that a female's responses are influenced by a summation of acoustic input integrated over successive chirps. This interpretation is consistent with the assumption that female phaneropterine tettigoniids employ fast, short-thoracic neural circuits for mate recognition (Robinson *et al.*, 1986).

Heller *et al.* (1997) have implied that the extremely brief interval between male song and female response in phaneropterines may not allow for evaluation of the male's signal by the female. If females do not evaluate

male songs, they would forfeit acoustic species recognition. This loss may occur in some cases (Heller *et al.*, 1997), but it would not be expected to evolve when several acoustic species sharing similar sound frequencies co-occur or certain abiotic noises are present. Additionally, when females evaluate male song in the context of species recognition, mate choice may represent an extension of such evaluation (Ryan & Rand, 1993). Clearly, female *Ph. nana* evaluate male songs (Table 3), and similar evaluation has been reported in other duetting phaneropterines (Tuckerman *et al.*, 1993; Galliard & Shaw, 1992, 1996).

As larger males may produce and transfer a larger spermatophylax, females may use such acoustic assessment to obtain a more substantial nuptial gift (review by Gwynne, 1997). In our study of *Ph. nana*, preferred males were also larger, and they produced longer chirps (CD) that included higher numbers of pulses (PN). However, we did not measure the size of spermatophore in this study. We have also failed to detect a significant relationship between male size and CD or PN, the preferred song characters (Table 2). Pulse duration (PD) seemed to be the best predictor for male size (Table 2). Unfortunately, however, because of the sampling rate limitation in the trio recording, the PD could not be estimated reliably and we cannot provide information about possible difference in the PD between preferred and non-preferred males.

In another study on *Ph. nana* (Tauber & Pener, 2000) we found that a two-pulse song was both necessary and sufficient to activate female response; songs with different number of pulses, when presented individually (no-choice design), elicited the same rate of female response. Here, we show that females prefer responding to males that produce higher number of pulses. The discrepancy between our previous and present results may be accounted by the differences between no-choice and two-choice designs (*e.g.* Doherty, 1985).

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