ANIMAL BEHAVIOUR, 2004, **68**, 131–144
doi:10.1016/j.anbehav.2003.10.020

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Acoustic communication in a duetting grasshopper: receiver response variability, male strategies and signal design

DAGMAR VON HELVERSEN*, ROHINI BALAKRISHNAN† & OTTO VON HELVERSEN‡
*Max-Planck-Institute für Verhaltensphysiologie, Seewiesen
†Centre for Ecological Sciences, Indian Institute of Science
‡Institute for Zoology II, University of Erlangen

(Received 11 April 2003; initial acceptance 27 May 2003; final acceptance 18 October 2003; MS. number: 7684)

In the duetting grasshopper *Chorthippus biguttulus*, a female's decision to reply to a conspecific male is based on the evaluation of a number of features of the male's song, which consists of uninterrupted syllables separated by pauses. Female responses are tuned to a restricted range of pause durations. However, males produce songs with noisy rather than silent pauses, which should make the measurement of pause durations more difficult for the female. We examined the adaptive value of these noisy pauses by testing female responses to (1) pairs of natural phrases, which differed only with respect to clear or noisy syllable pauses, and (2) synthetic phrases, in which the syllable onset accentuations and noise levels in the pauses were systematically varied. There was considerable variation between females, both in their preference for clear or noisy pauses in natural phrases, and in the optimal combinations of syllable onset accentuations and noise levels in pauses that they preferred in synthetic phrases. The response profiles of individual females were consistent. The experiments with synthetic phrases showed that, on average, females preferred more extreme values of syllable onset accentuations than were present in male songs. Noisy pauses increased the range of syllable pause durations accepted by females. The results suggest that noisy pauses could buffer signallers against the negative consequences of both signal degradation during transmission and extreme receiver choosiness.

Acoustic communication signals are used by many species of frogs and insects (particularly orthopterans) in the context of mate attraction (Bradbury & Vehrencamp 1998). Typically, males produce calling songs which attract conspecific females, which may respond either by approaching the male, as in gryllids, or by producing a reply song, as in some tettigoniid and acridid species (Huber 1983; Heller 1990). Calling songs convey information that allows conspecific recognition, which may facilitate reproductive isolation between closely related species living in sympatry (Perdeck 1957; Walker 1957; Hill et al. 1972; von Helversen & von Helversen 1994). These signals may also contain information on the quality of individual males, on the basis of which females could choose between males of the same species (Ryan 1985; Brown et al. 1996). Acoustic signals of males contain many different characteristics that are simultaneously evaluated by females, each of which may contribute to a greater or lesser extent towards the decision of the female to respond (Doherty 1985; Gerhardt 1992; von Helversen & von Helversen 1994). It is therefore not surprising that there exists considerable intraspecific variation, in both male signal structure and response functions of females (Gerhardt 1991; von Helversen & von Helversen 1994). Although there is an extensive literature on the former, individual differences in female preferences have not been studied in either an extensive or an exhaustive manner (Wagner 1998), even though these are expected to influence greatly the evolution of male signal structure.

The duetting gomphocerine grasshopper *Chorthippus biguttulus* provides a good example of a communication system in which several song characteristics of the male influence the female's decision to produce a reply song. The probability of replying is a good predictor of the female's subsequent acceptance of a male for mating (von Helversen & von Helversen 1994; Klappert & Reinhold

Correspondence: O. von Helversen, Institut für Zoologie II, Universität Erlangen, Staudtstrasse 5, 91058 Erlangen, Germany (email: helver@biologie.uni-erlangen.de). R. Balakrishnan is at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India.
This is one of the last papers that Dagmar von Helversen was able to complete before she passed away on 20 July 2003.

0003–3472/03/$30.00/0

2003). Male *C. biguttulus* songs consist of phrases 2–3 s long (Fig. 1). Each phrase has a distinctive syllable–pause structure, with syllable durations of 50–100 ms and pause durations of 8–25 ms, depending on the individual and the temperature of stridulation (von Helversen 1972; von Helversen & von Helversen 1997). As in other gomphocerine grasshoppers, *C. biguttulus* stridulates by rubbing a row of pegs on the inner side of the hind femur against a specialized vein on the forewing (see Fig. 1 for details of song structure and terminology). Each peg strike results in a very brief click of sound (250–450 μs long: Fig. 1) and each up or down stroke results in a series of clicks (at a rate of about 5–6 kHz). A syllable is composed of three down–up strokes with the two hindlegs moving in a phase-shifted manner, thereby masking the short gaps at the reversal points between strokes (Elsner 1974, 1983). Each syllable is followed by a short pause (10–15 ms at 30°C), during which the leading leg remains motionless

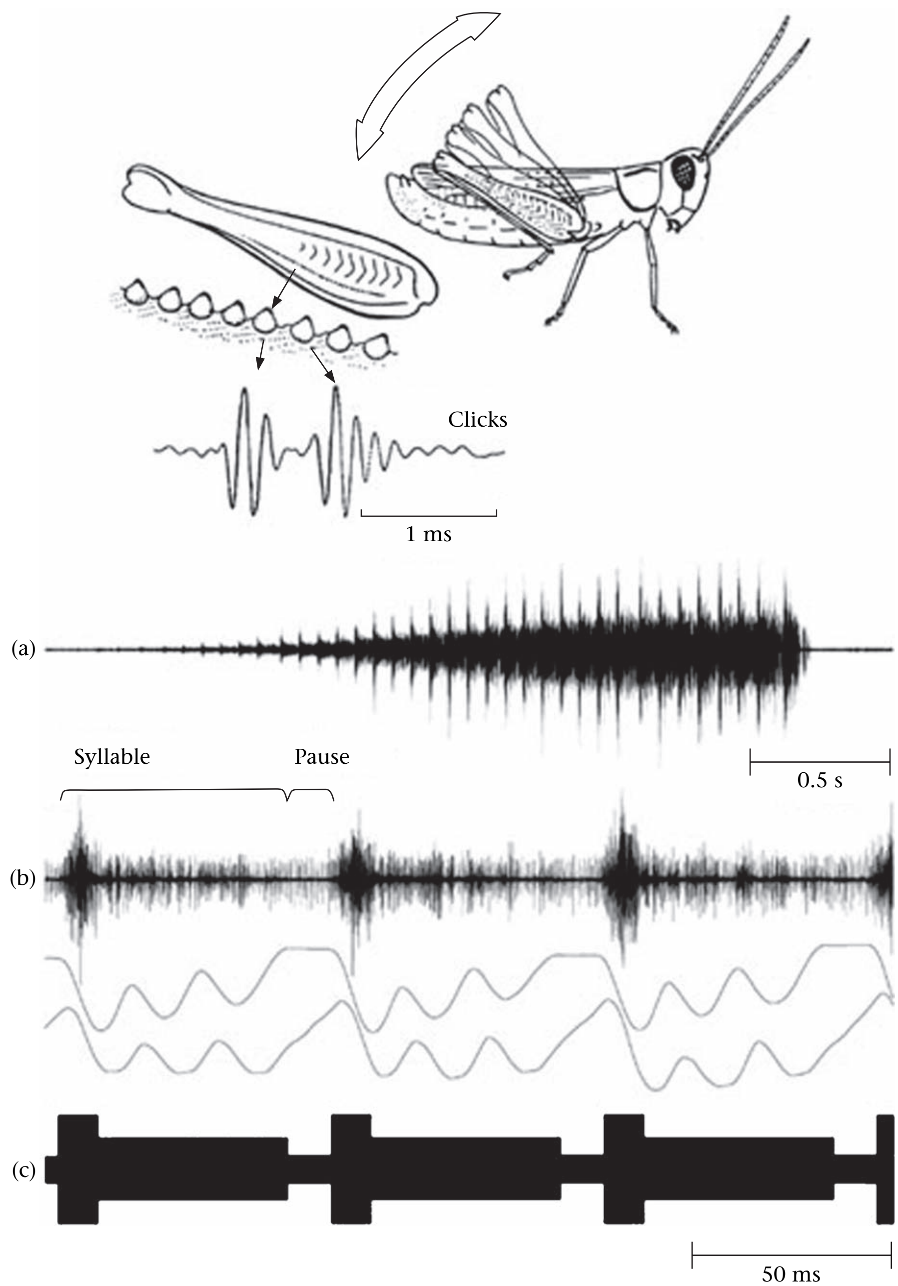

Figure 1. Production and structure of *C. biguttulus* male song. A syllable of *C. biguttulus* song is produced by three successive down–up strokes. Each peg strike produces a brief click of sound. (a) A phrase of male song. (b) Expanded timescale to show the structure of the syllables and noisy pauses. The two traces below this illustrate the simultaneous recordings of movements of the two hindlegs using an optoelectronic device. Note that the lagging leg continues to move during the pause. (c) Illustration of synthetic model song showing rectangular syllable onset accentuations and noisy pauses.

whereas the lagging one continues to move and engage the pegs against the wing vein (albeit at a lower rate than during the syllable: Fig. 1; Elsner 1974), resulting in noisy rather than silent pauses.

The female's decision to reply involves the evaluation of a number of signal features, which differ in their relative contribution to the response. Some of these characteristics fulfil necessary criteria: songs lacking them are rejected by most females. These criteria include phrases of a minimum length of about 1 s, built up of uninterrupted syllables of at least 40 ms duration, and intersyllabic pauses not exceeding 30 ms (von Helversen & von Helversen 1994, 1997). Songs containing gappy or interrupted syllables, which are typical of males that have lost a hindleg, are rejected by most females (Kriegbaum 1989; von Helversen & von Helversen 1994; Klappert & Reinhold 2003). Females also evaluate the shape of the syllable: songs with syllables that increase in intensity towards the end are strongly rejected (von Helversen & von Helversen 1983). In addition, several other signal characteristics contribute to the attractiveness of the signal, including spectral characteristics (presence of both low (6–8 kHz) and high (25–30 kHz) components in the frequency spectrum), amplitude modulation of the phrase and relative sound level between syllables and pauses (von Helversen & von Helversen 1997; Balakrishnan et al. 2001). Optimal values of these characters vary widely between females.

Syllables with enhanced onsets (higher sound intensity at the beginning than in the rest of the syllable: Fig. 1c), typical of male songs, are often preferred over rectangular syllables (von Helversen & von Helversen 1983), but not necessarily by all females. Females also vary in their requirement for a sound level difference between the pause and the end of the preceding syllable (relative offset level): some females require clearly defined syllable offset cues (silent or less noisy pauses), whereas others tolerate and may even prefer stimuli without pauses (Balakrishnan et al. 2001). Experiments with model song stimuli (phrases consisting of rectangular noise syllables separated by silent pauses) have shown that females are sharply tuned to a restricted range of pause durations (von Helversen & von Helversen 1997). It is therefore intriguing that the natural male songs contain noisy pauses, which are expected to make the measurement of pause durations more difficult for the female.

We examined how two particular features of the male song structure of *C. biguttulus* (syllable onset accentuations and noisy pauses) are matched with the response properties of females in the context of high variation between females in their responses to these features. We also investigated whether the production of noisy pauses (rather than silent ones) by *C. biguttulus* males confers any advantage or disadvantage in mate attraction. Both of our objectives were addressed in the context of the interactions between syllable onset accentuations/noisy pauses and other simultaneously evaluated song features (such as pause durations) during signal processing within receivers (females). The duetting response of the female was used as a measure of the attractiveness of male songs.

GENERAL METHODS

Study Animals

We collected adult *C. biguttulus* males and last-instar female larvae in the field during July–October 1996–2000 from grassy areas around Erlangen in Germany and from the Bavarian and Austrian Alps. Females were collected as larvae to ensure virginity. Males and females were housed in separate cages (50 × 50 × 40 cm) and fed on fresh grass (*Dactylis glomerata*). The animals were on a 14:10 h light: dark cycle and maintained at a temperature of 25–30°C. Cages were monitored every day and each freshly moulted adult female was individually marked with a paint marker pen (Edding 750) using a three-point colour code. Thus, the age of every female was known to within 1 day at the time of testing. Females were tested between 5 and 25 days of the final moult.

Song Recording and Analysis

We recorded songs with a ½ inch Bruel & Kjaer microphone (B & K Type 4136) and Precision Sound Level Meter (B & K Type 2231) and stored them on a Racal instrumentation tape recorder (upper frequency limit: 40 kHz). Simultaneously, we recorded movements of both hindlegs with an optoelectronic device (von Helversen & Elsner 1977). Sound signals were then digitized (sampling rate: 100 kHz) and analysed with Turbolab signal analysis software (Bressner Technology, Gröbenzell, Germany). The leg movement recordings were also digitized and analysed with Turbolab software.

Syllable Analyses

We calculated the syllable onset accentuations and offset levels in male songs by extracting the amplitude envelope of a phrase with an integration time of 4 ms. The value of 4 ms was chosen based on the results of our unpublished experiments aimed at determining the integration time of the female during the evaluation of syllable structure. We squared the value at each sampling point, integrated the squared values over the entire segment and then determined the square root of this value. This is equivalent to the RMS (root mean square) value of the sound pressure level with an integration time of 4 ms. We then determined the maximum amplitude of the syllable (A_{onset}), the minimum amplitude in the following pause (A_{pau}) and the average amplitude (A_{syl}) over the rest of the syllable (defined as the section of the syllable between 10 ms after A_{onset} and 20 ms before A_{pau}). The syllable onset accentuation in decibels (S_{acc}) was calculated as

$$S_{acc} = 20 \times \log (A_{onset}/A_{syl})$$

and the syllable offset level in decibels (S_{off}) was calculated as

$$S_{off} = 20 \times \log (A_{pau}/A_{syl})$$

Onset accentuations and offset levels were calculated as above for five syllables of each male phrase.

Song Models

Song models were synthesized with Turbolab (Bressner Technology) software. The stimuli were played back with custom-built power amplifiers and Dynaudio (D21/2) dome tweeter loudspeakers (flat frequency response: 2–40 kHz). Each stimulus was calibrated to the desired intensity level of playback in a sound-attenuated chamber with a Bruel & Kjaer Sound Level Meter (Type 2231). In addition, the loudspeaker output of most stimuli was recorded as described above, then digitized and analysed with Turbolab software to confirm the relative amplitude levels and durations of syllables and pauses. The natural and stereotyped phrases were played back at 82 dB sound pressure level (SPL, peak); the synthetic stimuli used for the measurement of female response profiles to syllable onset accentuation and offset levels were played back at 64 or 70 dB SPL (peak, for the nonamplitude-modulated part of the syllables, so that an onset accentuation of 18 dB resulted in a peak value of 82 or 88 dB SPL).

Playback Experiments

Virgin females were placed in a small gauze cage in a sound-attenuated chamber (29 ± 1 °C) and a computer-controlled set-up automatically played back phrases of song models. The female's reply to a song phrase was typically completed within 10 s. The next song model (one phrase) was played back 30 s after the end of the female's response to the previous one or, in the absence of a reply, 30 s after the end of the previous stimulus phrase. Female responses (reply songs) were also automatically recorded by the computer-controlled set-up.

We selected responsive females by placing a singing male (confined in a small gauze cage) into a cage containing several virgin females and choosing a female that gave consistent replies to the male's calling song. Approximately 75% of females selected in this manner remained motivated enough to duet throughout a given test session (which often lasted 3–4 h). A single test session typically consisted of at least 20 and mostly 30–50 cycles, where each cycle involved playback of 15–45 different song models to a single female in a random order (i.e. 15–45 presentations of each song model in a cycle). A given test session involved playback of song models of only one of the three types (natural, stereotyped or synthetic). Although the order of presentation of stimuli was randomized, this was not changed between cycles of a test session.

We calculated the percentage response to each song model for each female as the percentage of presentations of that song model during a test session to which reply song was elicited.

Analysis of Female Response Profiles

To analyse female responses to natural and stereotyped phrases we used repeated measures ANOVA. The female response frequencies were arcsine transformed for this analysis. Post hoc pairwise comparisons were performed with paired *t* tests. Individual female response profiles were analysed with the Wilcoxon signed-ranks test. Two-tailed tests of significance were used in all cases. To investigate whether there was a significant correlation of female response profiles with female age for natural and stereotyped phrases, we calculated for each female the difference in response between noisy and clear versions of each model song. We then examined this set of response differences for a significant correlation with female age, using the Pearson correlation coefficient. Statistical tests were performed with Statistica software (Statsoft Incorporated, Tulsa, U.S.A.).

NOISY VERSUS CLEAR PAUSES

Experiment 1: Natural Phrases

Methods

To examine whether females prefer noisy pauses to clear ones in natural male songs, we tested the responses of females to eight pairs of phrases (from eight different males) in the presence or absence of noise (clicks) in the pauses. We played back natural phrases either unedited (with noisy pauses) or with clear (silent) pauses. Pauses in eight phrases of natural male song were defined with the help of the simultaneous optoelectronic recordings of the leg movements and the change in click rate between syllables and pauses. We then edited the pause segments and deleted the clicks in the noisy pauses with a custom-built program (J. Schul, Columbia, U.S.A.) to produce the song models with clear pauses, which were identical to the natural ones in all respects except for the absence of noise in the pauses. Each female was presented with eight pairs of male phrases, where each pair consisted of the natural (noisy) version and the version with clear pauses.

Results

Playback of these stimuli revealed no significant overall effect of clear versus noisy pauses on female response probability (repeated measures ANOVA: $F_{1,23} = 0.008$, $P = 0.93$; Fig. 2a). There was, however, a highly significant interaction between the identity of the phrase (male) and its clear or noisy status ($F_{7,161} = 9.75$, $P = 0.0001$). Post hoc paired comparison *t* tests revealed that, for two of the eight phrases (males 3 and 5 in Fig. 2a), females showed a significant preference for the version with clear pauses to the natural, noisy version (male 3: $t_{23} = -4.38$, $P = 0.0002$; male 5: $t_{23} = -2.06$, $P = 0.05$). This suggests that the attractiveness of clear or noisy songs may depend on other features of the male phrase.

We then examined the responses of individual females to the eight pairs of male phrases (Table 1), to ascertain whether they showed consistent and significant preferences for clear or noisy pauses. Of 24 females, 14 showed no consistent and significant preference for clear or noisy pauses (Fig. 2b), five preferred songs with clear pauses and five preferred noisy pauses (Fig. 2b). Females varied

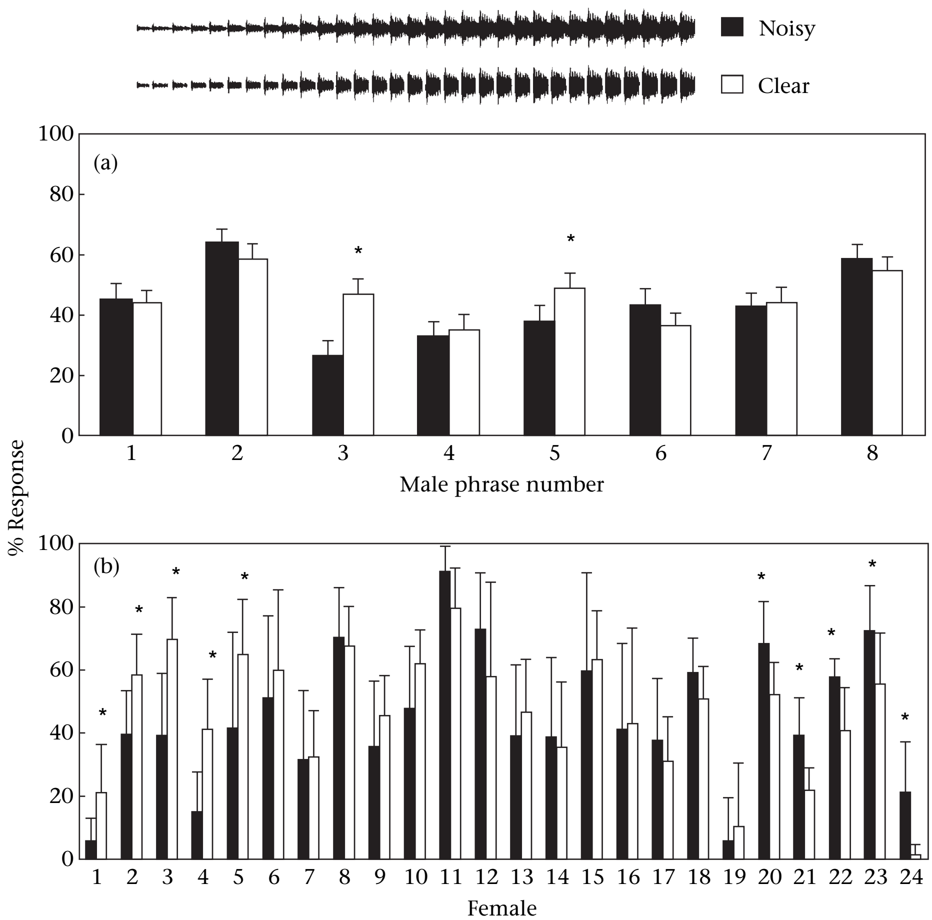

Figure 2. The attractiveness of male phrases with noisy and clear syllable pauses. (a) Mean female response + SD ($N = 24$ females) to each of eight male phrases. Two versions of each phrase were played back: the natural phrase with noisy syllable pauses and a version with clear (silent) syllable pauses. (b) Individual responses of 24 females ($\bar{X}$ + SD, $N = 8$ phrases) to noisy and clear versions of male phrases. Asterisks denote significant preferences ($P < 0.05$, paired *t* test) for clear (□) and noisy (■) versions of male phrases. The response to each stimulus (in this and all subsequent figures) was measured as the percentage of the total number of presentations of that stimulus that evoked a reply song from the female.

markedly, with some consistently preferring clear or noisy pauses across different male phrases. There was no significant correlation between response profile and age at which the females were tested (Pearson correlation: $R^2_{22} = -1.96$, $P = 0.948$).

Experiment 2: Stereotyped Phrases

The male phrase is a complex stimulus consisting of many different song characters that influence the female's response probability. These characters could influence the preference for clear or noisy pauses in unknown ways. The strength of the preference for clear or noisy pauses may thus be more clearly revealed in the absence of variation in the other song characters. We therefore re-examined female preferences for clear or noisy pauses in the context of seminatural stereotyped phrases in which we reduced the overall variability by holding the phrase length and amplitude constant over the entire stimulus series.

Methods

The stimuli consisted of 14 different pairs of model song phrases, which were played back to each female. For each of the pairs of phrases, the version with noisy pauses consisted of 33 iterations of a single natural syllable and succeeding noisy pause derived from a phrase of male song. The version with clear pauses consisted of 33 iterations of the same syllable separated by clear pauses (produced by deleting the clicks). The syllable and pause durations and syllable shapes were held constant within any pair of stimuli. Syllables were 50–107 ms long and pauses were 10–20 ms long (between different pairs of song models). The 14 syllables used to construct the phrases were derived from the phrases of seven different males (seven of the eight males used in experiment 1, two syllables from each phrase). We tested 24 females, different from those used in experiment 1.

Results

As in experiment 1, playback of these stimuli revealed a large variation between females in their preference for

Table 1. Statistical analysis of preferences of individual females for clear or noisy pauses in natural and stereotyped song phrases (Wilcoxon signed-ranks test)

	Natural phrases		Stereotyped phrases	
Female	*Z*	*P*	*Z*	*P*
1	2.207	<u>0.027</u>	3.300	<u>0.001</u>
2	2.524	<u>0.012</u>	2.480	<u>0.013</u>
3	2.524	<u>0.012</u>	2.667	<u>0.008</u>
4	2.524	<u>0.012</u>	3.360	<u>0.001</u>
5	1.960	<u>0.050</u>	2.824	<u>0.005</u>
6	1.609	0.108	3.170	<u>0.002</u>
7	0.000	1.000	2.831	<u>0.005</u>
8	−0.001	0.441	2.270	<u>0.023</u>
9	1.400	0.161	2.231	<u>0.025</u>
10	1.680	0.093	2.790	<u>0.005</u>
11	−1.778	0.075	3.100	<u>0.002</u>
12	−1.752	0.08	3.289	<u>0.001</u>
13	0.841	0.400	2.824	<u>0.005</u>
14	−1.016	0.310	2.455	0.145
15	0.351	0.726	1.667	0.096
16	0.421	0.674	0.105	0.916
17	−1.122	0.262	1.483	0.138
18	−1.682	0.092	0.405	0.686
19	1.511	0.131	1.750	0.08
20	−2.243	<u>0.025</u>	−2.480	<u>0.013</u>
21	−2.383	<u>0.017</u>	−3.181	<u>0.001</u>
22	−2.375	<u>0.017</u>	−3.230	<u>0.001</u>
23	−1.960	<u>0.05</u>	−2.201	<u>0.028</u>
24	−2.366	<u>0.018</u>	−2.201	<u>0.028</u>

Underlined *P* values are significant at a level of 5%.

clear or noisy pauses. Thirteen females consistently preferred clear pauses (Table 1, Fig. 3), five consistently preferred noisy pauses and six females showed no significant preference for either (Fig. 3). Three of these six females showed a reversal of preference for clear or noisy pauses, depending on the syllable that was used to construct the phrase. As in experiment 1, there was no significant correlation between response profile and age at which the females were tested (Pearson correlation: $R^2_{22} = 0.016$, $P = 0.55$).

Natural versus Stereotyped Phrases

The response profiles of individual females in experiments 1 and 2 suggested that the strength of the preference (difference in response between the noisy and clear version for each pair of stimuli) for clear or noisy pauses was greater in the case of stereotyped phrases. We therefore examined the distribution of the response differences between noisy and clear versions.

Methods

We calculated the difference in percentage response between the noisy and clear version for each female for each pair of stimuli and constructed a frequency histogram of the pooled data (Fig. 4). This was done separately for both experiments.

Results

The variance of the strength of the preference was higher for stereotyped phrases than for natural ones (*F* test: $F_{271,191} = 1.741$, $P \leq 0.001$; Fig. 4), that is, females were more likely to show strong preferences when presented with stereotyped phrases than with natural ones. To avoid the problem of pseudoreplication, we reanalysed the data using the mean difference in response (to noisy and clear pauses) of each female and obtained similar results. Again, the variance in preference strength was significantly higher with stereotyped phrases (*F* test: $F_{23,23} = 2.584$, $P = 0.013$). These results suggest that the natural male phrases are in some way 'buffered' against extreme values of female preference strength for clear or noisy pauses.

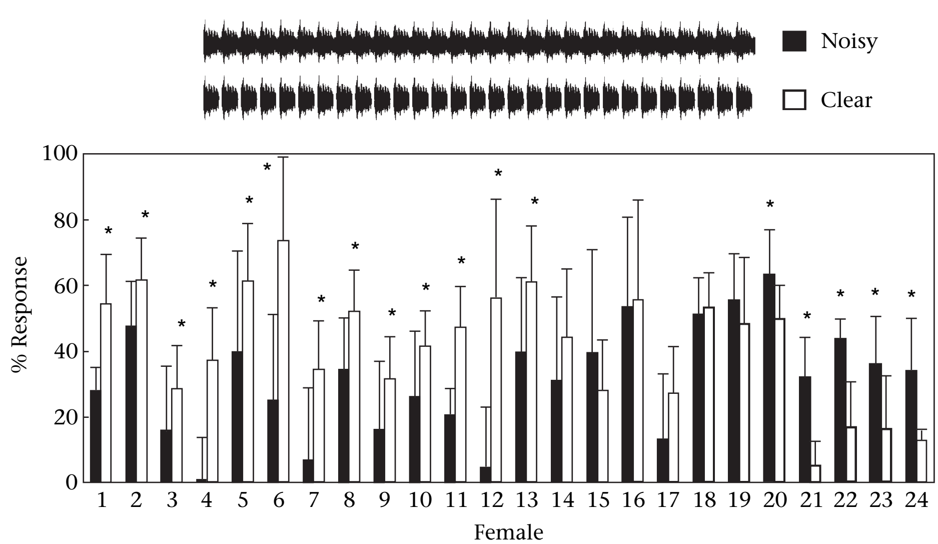

Figure 3. The attractiveness of stereotyped phrases with noisy and clear syllable pauses. Each pair of bars illustrates the mean responses of a female to 14 pairs of stimuli, where each pair consisted of a noisy and a clear version of a stereotyped phrase (produced by repetition of a single syllable from a natural phrase: see text for details). An asterisk above a white bar indicates a significant preference ($P < 0.05$) for the version with silent pauses; an asterisk above a black bar indicates a significant preference for the version with noisy pauses.

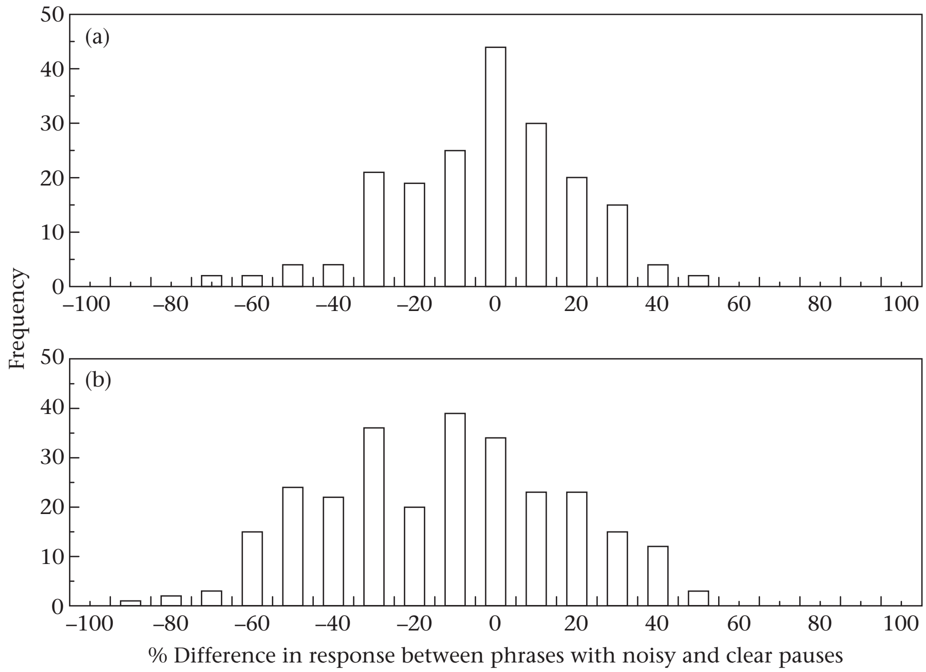

Figure 4. The strength of female preference for noisy or clear syllable pauses in natural and stereotyped phrases. The figure shows frequency histograms of the difference in percentage response towards (pairs of) phrases with noisy pauses and those with clear ones. Each data point in the histogram is the response difference between the noisy and clear versions of a given stimulus pair for a given female. (a) Natural phrases ($N = 192$ data points derived from 24 females). (b) Stereotyped phrases ($N = 272$ data points derived from 24 females, different from those tested for natural phrases).

SYLLABLE ONSET ACCENTUATIONS AND OFFSET LEVELS

Experiment 3: Synthetic Phrases

We know from previous work (Balakrishnan et al. 2001) that syllable−pause discrimination by *C. biguttulus* females occurs on the basis of the independent determination of the relative sound levels of syllable onset and offset. In addition, the shape of the syllable (which is essentially the modulation of sound intensity over the course of the syllable) may contribute to the perception of the level of noise in the pauses. Male *C. biguttulus* songs typically contain syllables that are enhanced in amplitude during the first 10−12 ms. In experiment 3, we therefore covaried the syllable onset accentuations (the relative sound level between the first 10 ms and the rest of the syllable) and relative offset levels in a systematic manner. To remove entirely the effects of variation in the other song features, we used synthetic song models.

Methods

The syllables of synthetic song models consisted of segments of white noise (80 ms in duration) recorded from a random noise generator and then digitized at 200 kHz. A phrase of synthetic model song consisted of around 33 iterations of a single 'syllable (80 ms) plus pause' (12 ms, either silent or filled with noise) segment and was typically 3 s long. The standard 80/12 stimulus used during playback consisted of a 3-s phrase composed of rectangular white noise syllables (80 ms long) separated by silent pauses (12 ms).

We varied the syllable onset accentuation (the sound level of the beginning of the syllable with respect to the rest of the syllable) by increasing the amplitude of the first 10 ms of the syllable and we varied the relative offset level (the sound level of the pause with respect to the preceding syllable) by attenuating the noise in the pauses. We varied both the syllable onset accentuations and the relative offset levels from 0 to 18 dB through a stimulus series (Fig. 5e).

Results

The optimal values of onset accentuations and relative offset levels varied widely between females. Of 23 females, seven responded well over a wide range of onset and offset levels (example shown in Fig. 5a); five required either exceptionally high onset accentuations (12 dB and higher: example in Fig. 5b) or offset levels (−12 dB and higher: Fig. 5c) and five responded best to continuously noisy stimuli with high onset accentuations and no pauses to mark the offsets (Fig. 5d). Finally, the other six females responded optimally to specific combinations of onset accentuation and offset levels.

Repeatability

Methods

To test whether the observed variations in response profiles reflected consistent differences between females, we retested nine of the 23 on a different day with the same stimulus combinations. The interval between two test sessions for a female was 1−12 days (mean = 5.8 days). To

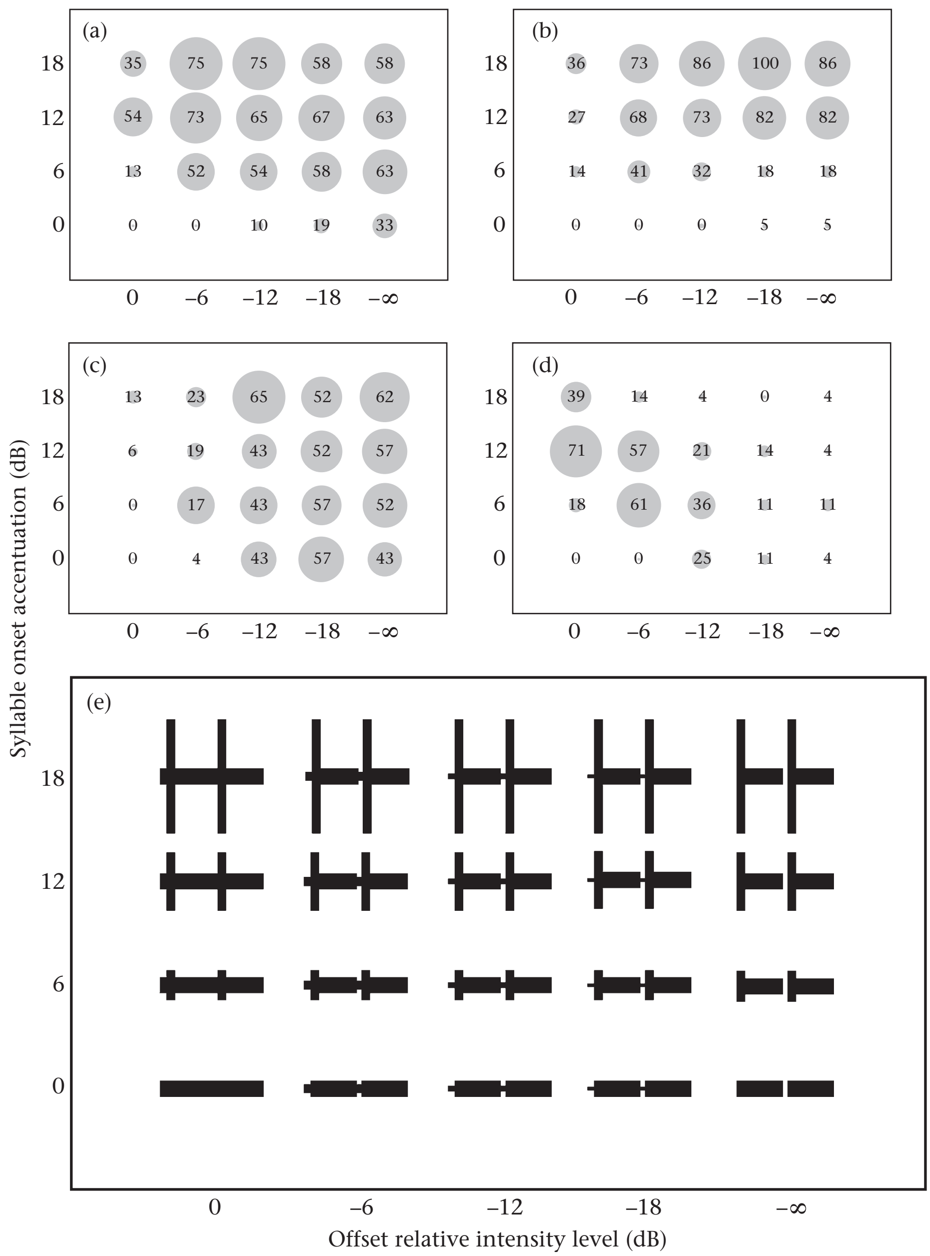

Figure 5. The optimal values of syllable onset accentuations and offset levels for individual females. Each panel illustrates the response of an individual female to a series of song models consisting of 80-ms-long white noise syllables separated by 12-ms pauses: the syllable onset accentuations and offset levels were varied from 0 to 18 dB through the stimulus series. The relative offset level of infinity indicates stimuli with silent pauses. The relative offset level of 0 dB indicates stimuli with no pauses. The values in the circles indicate the percentage response to each stimulus. (a) Female that responded over an exceptionally wide range of onset and offset levels; (b) female that preferred high onset levels; (c) female that preferred high offset levels; (d) female that responded maximally at high levels of onset accentuation in the absence of syllable pauses; (e) schematic illustration of song models.

test for the consistency of the preference patterns of individual females, we calculated a distance measure between the two replicates of each female's response profile. Before calculating the distances, we normalized each response profile, with the maximum response of a female during a test session being set to 100%. This was done to control for differences in motivational levels between test sessions and between females. Response profiles in which the absolute maximum response (before normalization) was less than 40% were not analysed. We calculated the distance between any two response profiles as the sum of squares of the difference in response to each stimulus combination within a profile. For each female, we compared the distance of its response profile to its own replicate with the mean of its distances to the other 22 females (for females tested twice, only one of the response profiles was used at random) using a Z test at a 1% level of significance.

We did not use the more conventional measures of repeatability (Boake 1989; Reinhold et al. 2002) which are applicable to comparisons between single stimulus points or single response curves because our response profiles consisted of 20-point two-dimensional matrices. The simple measure of distance we used adequately summarized the response profiles and was therefore used to examine the consistency of individual responses.

Results

For each of the nine females, the distance to the response pattern of its own replicate was significantly less than the mean of its distances to the response patterns of the other 22 females ($P < 0.01$; Table 2). Furthermore, in eight of nine cases, the distance to the response profile of a female's own replicate was either the smallest or second smallest of the 23 distance measures (one to its own replicate and 22 to the response profiles of other females). Thus, individual females did show consistent response profiles.

Female Preferences and Song Features

Methods

We measured the syllable onset accentuations and offset levels in phrases of male song derived from 26 males (five syllables per phrase: see General Methods). We then compared the values of the mean onset accentuations and offset levels of the different male phrases with the mean female response profile derived from experiment 3.

Results

Although there were highly significant differences between males in both mean syllable onset accentuations and offset levels (one-way ANOVA: onsets: $F_{25,104} = 8.8$, $P \leq 0.0001$; offsets: $F_{25,104} = 22.1$, $P \leq 0.0001$), the range of the mean syllable onset accentuation (5.6–10.7 dB) was relatively narrow compared with that of the mean syllable offset level (−7.2 to −23 dB; Fig. 6).

Female response increased with increasing values of both onset accentuation and offset levels and then showed saturation (Fig. 6). The average female response increased with increasing values of syllable onset accentuation up to +12 dB. The values of syllable onset accentuations in male songs ($\overline{X} \pm SE = 7.04 \pm 1.03$ dB) were, on average, significantly lower than those that elicited a maximal response from females (14.6 ± 3.5 dB; *t* test: $t_{47} = 9.416$, $P \leq 0.0001$). This can also be seen from an examination of the response profiles of individual females tested in experiment 3: in 12 of the 23 females, the response level continued to increase with syllable onset accentuation, with an increase in response of 10–25% between 12 and 18 dB accentuation (mean increase in response level = 17%, compare Fig. 6 with individual females in Fig. 5). Thus, the values of syllable onset levels in male songs, while lying within the range preferred by females, are suboptimal, since at least 50% of the females tested had a stronger preference for onset levels beyond the range produced by males.

Relative offset levels in male songs were well matched to the mean of the onset of saturation levels of females (Fig. 6). When individual response profiles were compared with the range of variation of the offset level in male songs, in 13 of 23 cases, the optimal values of female response were well matched to the offset levels in male songs. Six females preferred pauses that were less noisy than those found in male songs and four preferred noisier pauses.

ARE NOISY PAUSES ADVANTAGEOUS TO MALES?

Experiment 4: Enhanced Onsets and Noisy Pauses

The results of the analysis of female preferences suggest that males would be selected to enhance their syllable onset accentuations: this could be achieved either by enhancing the syllable onsets or by lowering the amount of noise in the pauses. If noisy pauses do not confer any advantage on males, it is surprising that they are retained, since lowering the noise in the pauses could enhance the perceived intensity level of the syllable onset (Balakrishnan et al. 2001). This suggests that noisy pauses may confer some advantage on males.

Balakrishnan et al. (2001) showed that females that responded to model songs lacking pauses nevertheless showed a narrowly tuned response to a restricted range of pause durations when presented with stimuli containing

Table 2. Statistical analysis of repeatability of individual female response profiles

Female	Distance to its own replicate profile	Mean distance to response profiles of 22 other females	*Z*	No. of distances smaller than the self-distance
1	77.20	142.73	−7.34*	1
2	112.54	175.11	−6.75*	1
3	77.77	152.07	−9.07*	0
4	76.59	152.56	−8.95*	1
5	99.42	202.22	−21.16*	0
6	56.39	142.01	−8.50*	0
7	72.43	156.74	−10.08*	0
8	53.54	140.75	−10.40*	0
9	108.12	147.24	−4.17*	4

*$P \leq 0.01$.

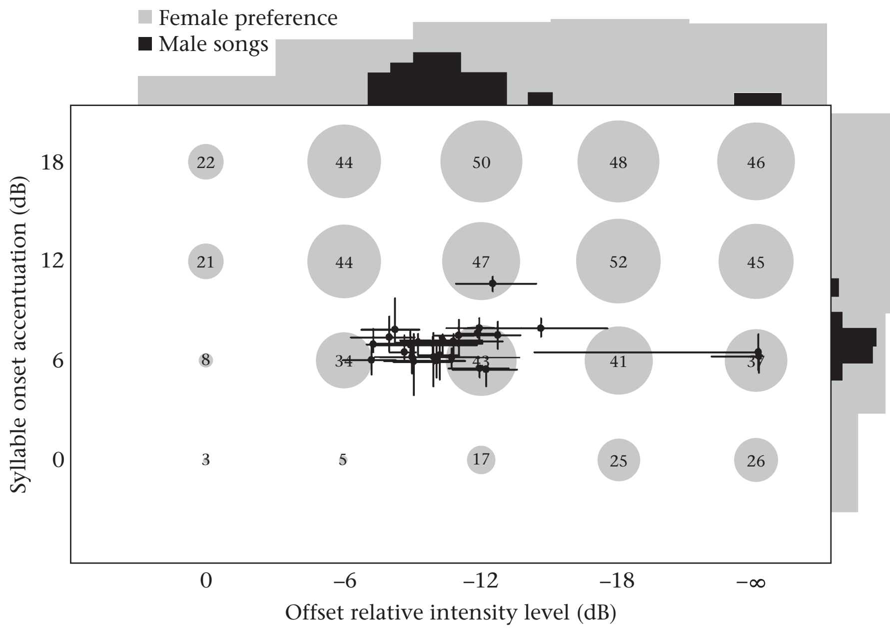

Figure 6. The match between the mean syllable onset accentuations and relative offset levels in male songs and mean female preference. Numbers within grey circles show the mean female responses ($N = 23$ females) to song models with different combinations of syllable onset accentuations and relative offset levels. The values of mean ± SD syllable onset accentuation and relative offset level are shown for the songs of 26 males (small black circles). Histograms above the graph: mean female responses to offset relative intensity levels of the synthetic model songs (grey) and the distribution of offset relative intensity levels in male songs (black). Histograms to the right of the graph: mean female responses to syllable onset accentuations in model songs (grey) and the distribution of onset accentuations in male songs (black).

clear pauses. This suggested that noisy pauses may increase the ambiguity in the pause duration perceived by the female, resulting in responses from a greater number of females. We therefore expected that enhancement of syllable accentuations and the presence of noisy pauses would both increase the attractiveness of model song phrases.

Methods

We compared the response functions of 20 females to syllable pause durations for (1) phrases containing rectangular or accentuated syllables and (2) phrases with and without noise in the syllable pauses (see illustration of stimuli in Fig. 7). To ensure that observed differences in response were not merely due to changes in overall sound level, we played the model song with rectangular syllables at two sound pressure levels (64 and 76 dB SPL, representing the range over which the experiment was conducted).

Results

With respect to the response profiles, we found a high variability between individuals, which was only partly the result of differences in the sensitivity of individual females. Observed preferences seemed not to be correlated with each other. Therefore, in the following, we list the general results, and we give examples to demonstrate the variation between females in the details of their response profiles (Fig. 7a–f). These serve to illustrate the complex interactions between onset accentuations, noise in the pauses and the range of acceptable pause durations.

(1) Confirming results from previous studies, all 20 females responded to rectangular syllables separated by clear pauses and showed a tuned response to a restricted range of pause durations. According to the sensitivity of the individual females, some females responded better at the lower sound pressure level (examples: Fig. 7a, b, response curves with open and closed circles), some at the higher sound level (Fig. 7e, f) and others responded nearly equally at both sound levels (Fig. 7c, d).

(2) There was a clear preference for song models with accentuated onsets of syllables (16 of 20 females). The average response of 20 females to the song model with accentuated syllable onsets and silent pauses (median 37%) was significantly higher than that to rectangular syllables and silent pauses (median 20.5%; Wilcoxon test for matched samples: $Z = 2.90$, $P = 0.003$, analysed for stimuli with the optimal pause duration of 12.5 ms). To examine the effect of accentuation independent of the effect of overall sound level, we examined those females that showed similar response levels to the rectangular stimuli at the two sound pressure levels ($N = 7$). We found that onset accentuation enhanced the attractiveness (see also Fig. 5), but to a variable extent, increasing responses markedly in some females (Fig. 7b, d: compare curves with open circles and open squares).

(3) The onset accentuation could increase the acceptability of stimuli with very short or no pauses (0 and 5 ms), so that some females responded even in the absence of pauses (Fig. 7c, e). The average response of the 20 females to song models with short pauses of 5 ms or less was significantly higher (median 22%) if the

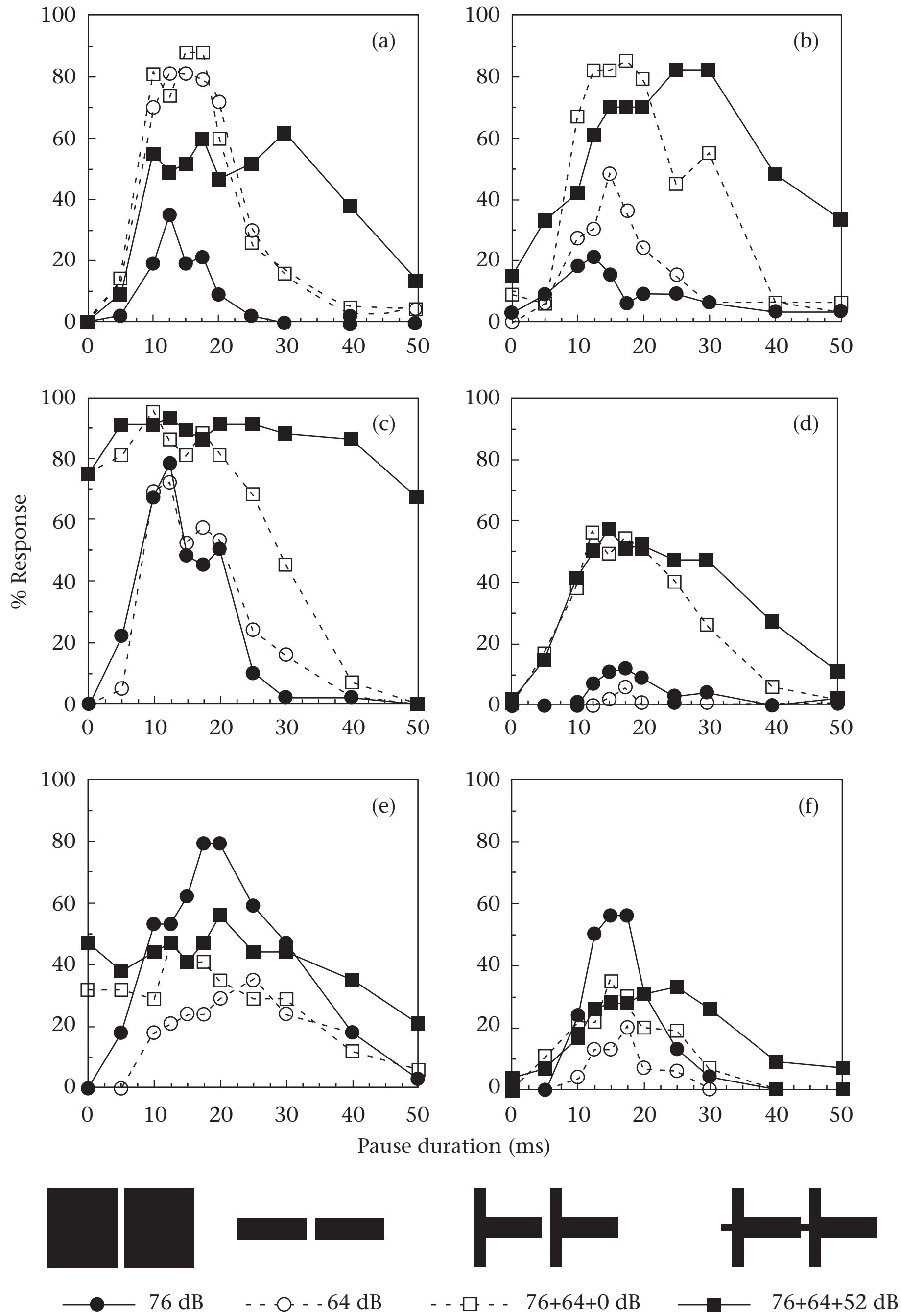

Figure 7. The effect of syllable onset accentuation in model songs with silent (□) and noisy (■) pauses in comparison with model songs consisting of rectangularly modulated syllables separated by silent pauses. To test for the effects of sound intensity, the latter song models were presented at two sound pressure levels, 64 (○) and 76 (●) dB SPL. The figure shows examples of the responses of six females to the four series of model songs, selected such that the response to the rectangular stimuli at 76 dB SPL was less than (a, b), equal to (c, d) or greater than (e, f) that for 64 dB SPL.

syllables contained accentuated onsets than if they were rectangular (median 0%; Wilcoxon test for matched samples: $Z = 3.17$, $P = 0.002$, analysed for song models with 5-ms pauses). In 15 of 20 females, we found the response range enlarged towards smaller pauses (less than 5 ms), whereas in five females enhanced syllable onsets did not serve to accept stimuli with no pauses (Fig. 7a, d). Figure 7a shows a case where the enhanced onsets increased neither the attractiveness nor the range of pause durations.

(4) The addition of noise in the pauses enhanced the range of acceptable pause durations in nearly all cases examined (18 of 20). Song models with noisy pauses of long duration (30–50 ms) were more attractive (median 32%) than those with clear pauses of that range (median 15%; Wilcoxon test for matched samples: $Z = 3.17$,

$P = 0.002$, analysed for song models with 30-ms pauses). The increase in range was not correlated with a general preference for noisy pauses: in the 'acceptable' range of syllable pauses (5–25 ms), over 50% of the females tested (13 of 20) showed no preference for clear or noisy pauses.

DISCUSSION

The relative attractiveness of male *C. biguttulus* songs with clear or noisy pauses depended both on various other characteristics of the song and on the individual female. There was high variation between females in their preferences for clear or noisy pauses, as well as for strong or weak syllable onset accentuations. Individual response profiles were, however, consistent. The addition of noise in silent syllable pauses of synthetic model phrases increased the range of pause durations accepted by females. The variability of male songs for the same parameters was also considerable. What are the advantages and disadvantages for males, in view of this variability, of producing more or less noisy syllable pauses?

Response Variability and Signal Features

When multiple features of a signal are evaluated simultaneously, females may weight them differently (Doherty 1985). Some features of *C. biguttulus* song, such as minimum phrase and syllable length, uninterrupted syllables and the absence of long pauses may be termed necessary. These tend to be weighted heavily and in a similar manner by different females. This could be because these criteria would help to prevent mismating with closely related species with which *C. biguttulus* is often sympatric (von Helversen & von Helversen 1994).

On the other hand, for features such as spectral properties and relative levels of syllable onset accentuation and offset, the optimal values of the features and their relative weighting differed widely between females. The response profiles of females to syllable onset accentuation and offset levels, as measured using synthetic song models lacking variation in other song characters, varied greatly between females but were repeatable within females (Fig. 5, Table 2). There was also a large variation between females in their preference for clear or noisy pauses in experiments with natural phrases (Fig. 2). In other words, the requirement for optimal levels of noise in the pauses was weighted differently by different females.

Our results revealed that, for two of the eight male phrases tested, females significantly preferred the version with clear pauses to the natural phrases with noisy pauses. This suggests that the relative contribution of any single song character to the overall attractiveness depends on the values of the other song characters evaluated by the female. The variation in interactions of signal features at the level of neuronal processing in the receiver, together with individual variation in their relative weighting, may make it difficult to predict receiver responses to the complete signal (Gerhardt 1992).

Among females that showed consistent preferences for clear or noisy pauses, the strength of the preference was significantly lower for natural phrases than for stereotyped ones, which contained less variation both within and between song features. A possible reason for this is that, in systems where several characters are simultaneously evaluated and contribute additively to the response, deficits in some characters may be compensated for by optimal values of others.

Observations of such trade-offs were first made by early ethologists such as Seitz (1940, 1941) who formulated the 'Reizsummenregel' or 'Rule of Stimulus Summation.' For acoustic stimuli, studies on both crickets (Doherty 1985) and frogs (Gerhardt et al. 1996) have revealed the existence of trade-offs, wherein suboptimal values of some signal features may be compensated for by optimal values of others. The additivity of these characteristics in the neuronal processing circuitry of the receiver, together with the high interindividual variation in their differential weighting, would result in trade-off phenomena, allowing the maintenance of high levels of variation in these features, while at the same time decreasing the variance in the receiver response to the overall signal. Thus, in the system discussed here, responses based on multiple features that are weighted differently by individual receivers may allow signallers to be 'buffered' against extreme receiver choosiness for specific features.

Females tolerated unnaturally long pauses when they had been filled with low-level noise (Fig. 7). Most probably this is a side-effect of the structure of the neuronal network responsible for syllable–pause recognition of the species-specific song. As suggested by Balakrishnan et al. (2001), two different pathways are integrated in the neuronal network: the first measures the period of syllable onsets, whereas the second is best activated by uninterrupted, noisy stimuli and is responsible for the rejection of long syllable pauses. The preference for noisy pauses observed in some females may be caused by a high level of activation of the second pathway.

Match between Signal and Receiver

Acoustic signals can simultaneously convey several different messages, such as the species, sex and condition of the signaller (Gerhardt 1992). As mentioned, features that convey information on the species and sex of the signaller tend to be weighted heavily and in a similar manner by females. This would exert a strong selection pressure leading to a decrease in variation of such signals, both within and between individuals ('static properties': Gerhardt 1991). These features are characterized by low variance in both signal and receiver response, and a good match between receiver preferences and signal.

Syllable offset levels in male songs were well matched with female preferences, insofar as they peaked just above the point where saturation started in females (Fig. 6). Both signal and receiver, however, showed high levels of variation between individuals, indicating that syllable offset levels, on their own, may not be a feature under strong selection at the population level.

Signal features with high intra- and interindividual variation have been classified as 'dynamic' criteria (Gerhardt

1991). Examples of such features include song intensity, duration and frequency of calling bouts or phrases. Such features are typified by high variation in the signal and predictable responses in the receiver, and are often under strong directional selection. In *C. biguttulus* also, females prefer song phrases with higher intensity, duration and repetition rate (von Helversen & von Helversen 1994). All of these features provide some index of signal 'quantity' and, being energetically expensive, could provide information on the condition or quality of the signaller (Hedrick 1986; Forrest & Green 1991). On the other hand, it may sometimes be difficult to disentangle this function from that of simply increasing the range and probability of detection (Römer 1993).

A comparison of the mean values of syllable onset accentuations preferred by females and the range of these characteristics in male songs (Fig. 6) showed that the latter lay below the point of response saturation for onset. Thus, females respond more strongly to onset accentuations that are greater than those produced by males, being 'super-responsive' for this parameter, which may therefore be under directional selection (von Helversen & von Helversen 1994 and references therein; Ryan 1998).

Signal Design and Male Strategies

Syllable onset accentuation appears to be a feature under directional selection: a male that produces an onset accentuation of 12 dB (which is above the range of values present in male songs) should elicit responses from a greater number of females. Males should thus be expected to enhance their syllable onsets further and to produce less noisy pauses (the latter would increase the perceived syllable onset level).

Constraints on syllable structure

Males may be physiologically constrained from producing very high syllable onset accentuations, since there is an obvious limit to both the height to which the leg can be raised and the force and velocity of the movements over the pegs during stridulation (which in turn determine the intensity of sound produced). The onset accentuation may thus be a good candidate for a sexually selected trait, and it may reveal honest information on the quality of the signaller.

Males could also enhance the effectiveness of the song by reducing the intensity of the rest of the syllable (with respect to the first 10–12 ms). This strategy would, however, confer other disadvantages: (1) it would reduce the overall intensity of the song, which would in turn reduce the effective range of the signal, resulting in a lower probability of finding a responsive female; (2) a steep decline in intensity over the course of the syllable might increase the duration of the perceived syllable pause causing females to reject these songs.

Males are, however, unlikely to be physiologically constrained from producing silent pauses: a number of related grasshopper species are capable of producing silent pauses of the order of 5–20 ms by synchronizing the movements of the two stridulating hindlegs (Elsner 1974; Elsner & Wasser 1995; Fries & Elsner 1996).

The advantage of noisy pauses

Experiment 4, in which the syllable pause durations were systematically varied (Fig. 7), revealed that the range of acceptable pause durations was greatly extended by the presence of noise in the pauses. In the presence of clear pauses, individual females are sharply tuned to a restricted range of pause durations. One would therefore expect that the pause durations of male songs would be under stabilizing selection if the pauses were silent. The range of pause durations measured as stops of the movement of the leading leg (pattern I, Elsner 1974) is narrow and well matched to the optimum (silent) pause durations preferred by females at a given temperature (von Helversen & von Helversen 1994). This indicates that the syllable pause duration is, or has been in the past, a character under stabilizing selection (but see Klappert & Reinhold 2003).

There are several reasons why the pause durations finally perceived by a female cannot be exactly matched to the optimum of the female tuning curve. On the receiver side, both the height (indicative of response strength) and the width of the tuning curve of female response to (silent) pauses are strongly influenced by song intensity, females being more sharply tuned to a narrower range of pause durations with increasing intensity (von Helversen 1972). Such an intensity-dependent sharpening of female response functions has also been reported in the field cricket *Teleogryllus oceanicus* (Doolan & Pollack 1985). Unpredictable combinations of factors such as temperature differences between sender and receiver would also influence the perceived duration of a pause. In addition, the degradation of temporal cues and the attenuation during transmission of the signal through the vegetation may cause the pauses to be perceived as much longer at the position of the female. All these phenomena would decrease the probability of female response to stimuli with clear pauses.

The fact that noise in the pauses results in the broadening of the range of acceptable pause durations for females at high sound intensities might therefore confer an advantage on males. By producing noisy pauses, males would increase the probability of eliciting responses from a greater number of females over a wide range of intensities. By exploiting the sensory biases of females, noisy pauses may thus represent a male strategy to maximize the number of responding females and thereby of potential mates.

Acknowledgments

This article is dedicated to Professor Norbert Elsner who over many years insisted that we investigate 'the paradox of noisy pauses.' We are much indebted to Professor N. V. Joshi, who spent many hours exploring different methods of measuring the consistency of female response profiles. We gratefully acknowledge Maria Bauer for help in carrying out some experiments and her skilful preparation of the figures. We also acknowledge Johannes Schul for writing the program to delete clicks from syllable pauses, and we thank an anonymous referee for critical comments. This research project was funded in part by the DFG (He 2412/2-1).

References

Balakrishnan, R., von Helversen, D. & von Helversen, O. 2001. Song pattern recognition in the grasshopper *Chorthippus biguttulus*: the mechanism of syllable onset and offset detection. *Journal of Comparative Physiology A*, **187**, 255–264.

Boake, C. R. B. 1989. Repeatability: its role in evolutionary studies of mating behaviour. *Evolutionary Ecology*, **3**, 173–182.

Bradbury, J. & Vehrencamp, S. 1998. *Principles of Animal Communication*. Sunderland, Massachusetts: Sinauer.

Brown, W. D., Wideman, J., Andrade, M. C. B., Mason, A. C. & Gwynne, D. T. 1996. Female choice for an indicator of male size in the song of the black-horned tree cricket *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). *Evolution*, **50**, 2400–2411.

Doherty, J. A. 1985. Trade-off phenomena in calling song recognition and phonotaxis in the cricket, *Gryllus bimaculatus* (Orthoptera, Gryllidae). *Journal of Comparative Physiology*, **156**, 787–801.

Doolan, J. M. & Pollack, G. S. 1985. Phonotactic specificity of the cricket *Teleogryllus oceanicus*: intensity-dependent selectivity for temporal parameters of the stimulus. *Journal of Comparative Physiology A*, **157**, 223–233.

Elsner, N. 1974. Neuroethology of sound production in gomphocerine grasshoppers. I. Song patterns and stridulatory movements. *Journal of Comparative Physiology*, **88**, 67–102.

Elsner, N. 1983. A neuroethological approach to the phylogeny of leg stridulation in gomphocerine grasshoppers. In: *Neuroethology and Behavioural Physiology* (Ed. by F. Huber & H. Markl), pp. 54–68. Berlin: Springer.

Elsner, N. & Wasser, G. 1995. Leg and wing stridulation in various populations of the gomphocerine grasshopper *Stenobothrus rubicundus* (Germar 1817). *Zoology*, **98**, 179–190.

Forrest, T. G. & Green, D. M. 1991. Sexual selection and female choice in mole crickets (*Scapteriscus*: Gryllotalpidae): modelling the effects of intensity and male spacing. *Bioacoustics*, **3**, 93–109.

Fries, G. & Elsner, N. 1996. Transection of intraganglionic connective causes synchrony of hindleg stridulation in the gomphocerine grasshopper *Stenobothrus lineatus*. *Naturwissenschaften*, **83**, 284–287.

Gerhardt, H. C. 1991. Female mate choice in tree frogs: static and dynamic acoustic criteria. *Animal Behaviour*, **42**, 615–635.

Gerhardt, H. C. 1992. Multiple messages in acoustic signals. *Seminars in Neurosciences*, **4**, 391–400.

Gerhardt, H. C., Dyson, M. L. & Tanner, S. D. 1996. Dynamic properties of the advertisement calls of gray tree frogs: patterns of variability and female choice. *Behavioral Ecology*, **7**, 7–18.

Hedrick, A. V. 1986. Female preferences for male calling bout duration in a field cricket. *Behavioral Ecology and Sociobiology*, **19**, 73–77.

Heller, K.-G. 1990. Evolution of song pattern in east Mediterranean Phaneropterinae: constraints by the communication system. In: *The Tettigoniidae: Biology, Systematics and Evolution* (Ed. by W. J. Bailey & D. C. F. Rentz), pp. 130–151. Berlin: Springer-Verlag.

von Helversen, D. 1972. Gesang des Männchens und Lautschema des Weibchens bei der Feldheuschrecke *Chorthippus biguttulus* (Orthoptera, Acrididae). *Journal of Comparative Physiology*, **81**, 381–422.

von Helversen, D. & von Helversen, O. 1983. Species recognition and acoustic localization in an acridid grasshopper: a behavioral approach. In: *Neuroethology and Behavioral Physiology* (Ed. by F. Huber & H. Markl), pp. 95–107. Berlin: Springer-Verlag.

von Helversen, D. & von Helversen, O. 1997. Recognition of sex in the acoustic communication of the grasshopper *Chorthippus biguttulus* (Orthoptera, Acrididae). *Journal of Comparative Physiology A*, **180**, 373–386.

von Helversen, O. & Elsner, N. 1977. The stridulatory movements of acridid grasshoppers recorded with an opto-electronic device. *Journal of Comparative Physiology*, **122**, 333–340.

von Helversen, O. & von Helversen, D. 1994. Forces driving coevolution of song and song recognition in grasshoppers. *Fortschritte der Zoologie*, **39**, 253–284.

Hill, K. G., Loftus-Hills, J. J. & Gartside, D. F. 1972. Pre-mating isolation between the Australian field crickets *Teleogryllus commodus* and *T. oceanicus* (Orhtoptera: Gryllidae). *Australian Journal of Zoology*, **20**, 153–163.

Huber, F. 1983. Neural correlates of orthopteran and cicada phonotaxis. In: *Neuroethology and Behavioral Physiology* (Ed. by F. Huber & H. Markl), pp. 108–135. Berlin: Springer-Verlag.

Klappert, K. & Reinhold, K. 2003. Acoustic preference functions and sexual selection on the male calling song in the grasshopper *Chorthippus biguttulus*. *Animal Behaviour*, **65**, 225–233.

Kriegbaum, H. 1989. Female choice in the grasshopper *Chorthippus biguttulus*: mating success is related to song characteristics of the male. *Naturwissenschaften*, **76**, 81–82.

Perdeck, A. C. 1957. The isolating value of specific song patterns in two sibling species of grasshoppers (*Chorthippus brunneus* Thunb. and *Ch. biguttulus* L.). *Behaviour*, **12**, 1–75.

Reinhold, K., Reinhold, K. & Jacoby, K. J. 2002. Dissecting the repeatability of female choice in the grasshopper *Chorthippus biguttulus*. *Animal Behaviour*, **64**, 245–250.

Römer, H. 1993. Environmental and biological constraints on the evolution of long-range signalling and hearing in acoustic insects. *Proceedings of the Royal Society of London, Series B*, **226**, 179–185.

Ryan, M. J. 1985. *The Tungara Frog: a Study of Sexual Selection and Communication*. Chicago: University of Chicago Press.

Ryan, M. J. 1998. Sexual selection, receiver biases, and evolution of sex differences. *Science*, **281**, 1999–2003.

Seitz, A. 1940. Die Paarbildung bei einigen Cichliden I. *Zeitschrift für Tierpsychologie*, **4**, 40–84.

Seitz, A. 1941. Die Paarbildung bei einigen Cichliden II. *Zeitschrift für Tierpsychologie*, **5**, 74–100.

Wagner, W. E., Jr. 1998. Measuring female mating preferences. *Animal Behaviour*, **55**, 1029–1042.

Walker, T. J. 1957. Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. *Annals of the Entomological Society of America*, **50**, 626–636.