

Original Article

Attractiveness of grasshopper songs correlates with their robustness against noise

Anne Einhäupl,^a Nicole Stange,^a R. Matthias Hennig,^{a,b} and Bernhard Ronacher^{a,b}

^aDepartment of Biology, Humboldt-Universität zu Berlin, Invalidenstr. 43, 10115 Berlin, Germany and

^bBernstein Center for Computational Neuroscience, Philipstr. 13, 10115 Berlin, Germany

Long-range communication signals commonly serve to attract mates. Evolution of such signals was channeled by 2 classes of constraints: signals have to be conspicuous against background noise and signals should enable the receiver—in particular females that invest heavily in offspring—to assess the sender's quality and attractiveness as a sexual partner. However, as noise in the transmission channel likely conceals quality cues present in signals, these goals may represent opposing selective forces. We explored how noise affects the preferences of choosy female grasshoppers toward the communication signals of individual males. Our prediction was that male signals would become less distinguishable with increasing noise levels and, hence, female preferences for attractive signals would disappear. Here, we show that, contrary to this prediction, the differences in attractiveness between natural male songs were preserved even at high noise levels: the most attractive signals were at the same time particularly robust against masking. We discuss these results in view of a sensory exploitation scenario and a potential reduction of females' costs of choosiness. *Key words:* acoustic communication, female choice, sensory exploitation, sexual selection, signal evolution. [*Behav Ecol* 22:791–799 (2011)]

INTRODUCTION

A ubiquitous problem for communication systems is signal degradation: Signals arriving at the receiver normally differ from those produced by the signaler. Signal degradation is particularly relevant for acoustic communication systems that serve to attract sexual partners from some distance (Gerhardt and Klump 1988; Römer 1992, 2001; Kroodsma and Miller 1996; Ryan and Kime 2002). A plethora of mechanisms, both on the sender's and the receiver's side, have been described, by which animals may improve the reliable transmission of signals (for an extensive review, see Brumm and Slabbekoorn 2005). Mechanisms at the sender's side range from increasing the loudness of the songs in response to high ambient noise levels, for example, in nightingales (Brumm and Todt 2002), over shifting to a less noisy frequency channel, for example, in concave-eared torrent frogs that use ultrasonic signals (Feng et al. 2006), to the use of time windows with reduced ambient noise in coqui frogs (Zelick and Narins 1985). At the receiver's side, frequency analysis (Schwartz and Wells 1984), comodulation masking release (Klump 1996), and spatial segregation of sound sources by the auditory pathway (Schwartz and Gerhardt 1989; Bee 2008) may be used to improve signal detection (Brumm and Slabbekoorn 2005).

These few examples underline how various communication systems strive to improve detection of relevant sound signals against a background of irrelevant energy impinging on their ears. Signal detection theory provides a framework which allows to separate the detectability of signals in background noise from changes of the receiver's response criterion (Wiley 2006). A fundamental conclusion of this theory is that a re-

ceiver cannot avoid to make erroneous decisions, as soon as the probability density functions of “background only” and “background plus signal” overlap. By lowering the response threshold, the probability of correct detections increases, but at the same time rises the probability of “false alarms,” that is, of responses to the background alone. Increasing the response threshold leads to a reduction of false alarms, however, at the expense of missed detections of the signal. The optimal choice of a threshold criterion will, of course, depend on the respective consequences of missed detections and false alarms (see Bradbury and Vehrencamp 1998).

Communication normally does not only involve detection of signals but also their classification, for example, the discrimination of conspecific versus heterospecific signals or a decision between signals differing in attractiveness (Klump 1996). Wiley (2006) gives a lucid account of how signal detection theory may be extended to cover also a discrimination and classification task—and what consequences this theory suggests for the evolution of signals and communication systems. A major conclusion is that “a receiver's performance in a task requiring classification is inevitably lower than in a comparable task requiring only detection” (Wiley 2006, p. 235). In line with this theoretical conclusion, Wollerman and Wiley (2002) found that natural background noise reduced the ability of female tree frogs to discriminate calls differing in carrier frequency content, although they were able to detect these calls. In zebra finches and budgerigars, further evidence has been found that discrimination of calls in noise is more demanding than call detection (Lohr et al. 2003).

Sexual selection theory predicts that females—due to their higher investment in offspring—are the more discriminating sex and should select mates that provide direct or indirect fitness benefits (Anderson 1994; Jennions and Petrie 1997; Anderson and Simmons 2006). But how do females judge the attractiveness of a potential mate and its mating signals? Sensibly, females should assess signal features that give honest clues about a male's condition, genetic quality, or

Address correspondence to B. Ronacher. E-mail: bernhard.ronacher@cms.hu-berlin.de.

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immunocompetence (Welch et al. 1998; Anderson and Simmons 2006; Tregenza et al. 2006). A male then has to provide resources and/or convey signals that make him attractive to females—an interdependence that in the long run can lead to ornaments like peacock tails or, in the acoustic domain, to elaborate song displays (Hasselquist et al. 1996; Todt and Hultsch 1998). Apart from the necessity to advertise the sender's quality to a potential mate, however, additional constraints may shape mating signals, for example, the distinction from sympatrically occurring heterospecific signals (Pfennig 2000; Deily and Schul 2006; Safi et al. 2006).

Most of the mechanisms, by which signal transmission may be improved (see above), are unattainable for our study subject, a gomphocerine grasshopper, because of limitations of its signal generation and auditory system. The generation of signals by rhythmically scraping a file on the legs over the wings is an innate behavior (von Helversen O and von Helversen D 1975; Gottsberger and Mayer 2007) allowing little freedom for short-term adjustments of the signal to varying environmental conditions. The calling songs of gomphocerine grasshoppers consist of amplitude modulations of a broadband carrier, with strong frequency overlap between different species (Meyer and Elsner 1996). The ears of these insects have a poor frequency resolution (Stumpner and von Helversen 2001; Hennig et al. 2004), leaving not much room for filtering noise by spectral analysis. Correspondingly, the primary cues for signal recognition reside in its species-specific pattern of amplitude modulations, that is, the sound envelope (von Helversen O and von Helversen D 1997; Schmidt et al. 2008).

In view of background noise and signal degradation, male grasshoppers have to strive for 2 goals when producing their calling songs: to increase their broadcasting range (Römer 1998; Couldridge and van Staaden 2004) and to produce signals that are attractive to females (Kriegbaum 1989; von Helversen O and von Helversen D 1994; Gerhardt et al. 2000; Gerhardt and Huber 2002; Greenfield 2002; Klappert and Reinhold 2003; Greenfield and Rodriguez 2004). However, the most attractive signal would be useless if it is too susceptible against interference and therefore cannot be detected at some distance (Römer et al. 1989; Klump 1996). Can these 2 major functions of male long-distance signals, attractiveness and resistance against noise, be reconciled or do they represent opposing selective pressures? Conceivably, males may be forced to invest primarily in broadcasting range and robustness of signals (Römer et al. 1989; Ryan and Kime 2002) and to care about attractiveness only in the second place. Or do females consider only those songs as attractive that are robust against perturbations? We investigated these hypotheses in the grasshopper *Chorthippus biguttulus*. Males of this species do not provide any resources or direct benefits to females. Hence, the situation is typical for sexual selection, and females do indeed exhibit preferences for the songs of some conspecific males (Kriegbaum 1989; Kriegbaum and von Helversen 1992; Klappert and Reinhold 2003, 2007). We tested virgin *C. biguttulus* females with natural calling songs of 8 males under different levels of broad band masking noise. Our prediction was that with increasing noise levels, the differences between the individual signals would become less distinguishable and that females' discrimination between attractive and unattractive signals would break down.

MATERIALS AND METHODS

Animals

The animals used in the experiments were adult virgin females of *C. biguttulus* L. caught as nymphs in the field near Erlangen. Females were individually marked with a 3-point color

code. If they are inclined to mate, females respond to calling males with a song of their own. This allows us to perform the experiments in a fully automated computer controlled device, thus excluding observer biases (for details of the apparatus and the test procedures, see Schmidt et al. 2008). A female's response is a reliable predictor of the subsequent acceptance of the male as a sexual partner (von Helversen O and von Helversen D 1994, 1997; Klappert and Reinhold 2003, 2007). Thus, the percentage of responses to a male's song upon repeated presentation can serve as index for the attractiveness of a song.

Stimulation

Songs of 8 intact males in their unmasked version and under 3 levels of masking noise served as stimuli. The tested songs were chosen for relatively high attractiveness to observe possible reductions due to noise. The songs were originally recorded by Maria Bauer and Otto von Helversen (Erlangen) with a Bruel & Kjaer equipment (1/2 inch microphone type 4136; precision sound level meter 2231; stored on Racal instrumentation tape recorder, upper frequency limit 40 kHz). To eliminate potential differences in signal detectability due to differing carrier frequency spectra, the songs were rescaled and their envelopes were filled with the carrier frequency of one song. By the rescaling procedure, the period duration of song subunits (a syllable plus a pause) was rescaled to 100 ms (procedure described in Machens et al. 2003). The rescaling to a constant subunit duration was important for an electrophysiological study showing that these 8 songs could well be discriminated on the basis of spike trains of auditory receptor neurons (Machens et al. 2003). We decided to use the same stimuli in the behavioral tests to ensure comparability with the earlier study. However, preliminary tests with songs where subunit durations were not rescaled yielded similar results. The overall duration of the individual songs varied between 2.1 and 4 s. These differences were not compensated for, as earlier work has shown that responses of females saturate for song models of more than 1–1.5 s duration (von Helversen 1972). Because a positive correlation exists between sound pressure level (SPL) and attractiveness of songs (Klappert and Reinhold 2003), we adjusted the intensities of all 8 songs to the same maximal intensity (70 ± 1 dB sound pressure level; root mean square measurement; intensity calibration as in Schmidt et al. 2008). Natural songs exhibit a slow rise in amplitude (see top trace in Figure 2a); for this reason, we adjusted the intensities in the last part of the songs. These procedures eliminated subunit duration, intensity, and spectral differences as cues for detection and discrimination, whereas differences in the fine temporal pattern of amplitude modulations between songs remained as only cues for attractiveness.

As a masker, we used broad band white noise with a frequency range of 1–40 kHz; this range covers the main frequencies of male songs (von Helversen O and von Helversen D 1997). All songs were broadcast without a masker and in the presence of 3 different noise levels: 58, 64, and 70 dB SPL (noise levels measured over the entire 39 kHz band). The noise was “on” only during the stimulus presentations. The respective signal-to-noise ratios were 6, 3, and 0 dB, according to $\text{SNR} = 10 \log[\text{signal}/\text{noise}]$. An effective song model (rectangularly modulated 80 ms “syllables” separated by 12 ms pauses) served as positive control for sufficient motivation (see Schmidt et al. 2008), whereas unmodulated white noise served as a negative control for low selectivity. To ensure responsiveness of females, a stimulation cycle (32 test stimuli + 2 controls) started only if the female had responded to the positive control stimulus. With most females, the stimulation

cycle was repeated 18 times; in a few animals, the cycle was repeated 15 times. To avoid carry-over effects of attractive or unattractive stimuli, a pause of at least 30 s separated stimulus presentations. The sequence of stimuli was newly randomized for each stimulation cycle, in order to minimize carry-over effects or a potential influence of changes in a female's motivation. The complete stimulus set was tested with 27 females. One of these was excluded from the analysis because it responded too scarcely (<50% response) to all stimuli, another 3 females were excluded for responding too frequently (>15%) to the negative control stimulus, which indicates unselective responses. Inclusion of these animals in the data set would, however, not have markedly affected our results or conclusions.

With a different cohort of females ($N = 18$), we compared the responses to the unmasked songs of 6 males {m1, m2, m3, m5, m7, m8} and at noise levels of 46, 52, and 58 dB SPL. Response levels did not differ significantly between the unperturbed stimuli and stimuli presented at a masking level of 46 dB SPL (for all 6 songs: $P > 0.05$, Dunn's post hoc test after Friedman test); the responses to the signals with the 46 dB masker were on average only 3.6% lower than for the unmasked songs. Therefore, in Figure 2b, the unmasked stimuli were placed at a noise level of 46 dB SPL.

For the analysis of song parameters, the envelopes of the amplitude modulations were calculated with a custom made LabView program (programmed by R.M.H.), following the procedures described in Machens et al. (2001). From the envelopes, various parameters were extracted following the definitions of von Helversen et al. (2004): durations of syllables and pauses, syllable-to-pause ratio, onset accentuation, offset depth (for definitions, see Figure 2c). We analyzed between 7 and 14 syllables per song, all in the plateau region toward the end of a song (see Figure 2a) and averaged the values for each male song.

Data evaluation

The attractiveness of unmasked male songs was measured as the percentage of the 18 (or 15) stimulus presentations that evoked at least one response song of the female (cf. Klappert and Reinhold 2003; von Helversen O and von Helversen D 1997). We determined "critical noise levels" to quantify resistance against noise on the basis of the individual response curves (see Figure 2b). A common criterion is the reduction to a fixed response level of 50% (Ronacher et al. 2000). This criterion is intuitive if the curves start from a high level (e.g., m1 in Figure 2b). It mimics a situation of competition that may occur in the field with several males present and considers the noise level at which a particular male's chance to get a positive response from a female is reduced to 50% in comparison to attractive songs of other males. However, this type of evaluation may introduce a bias against less attractive songs (m6 in Figure 2b). An alternative procedure is to search for the noise level at which the response to a given song drops to half of the response for the unmasked song (arrow in Figure 2b). In this example, the critical noise level obtained with the half-maximal criterion is shifted to a ~4 dB higher level as compared with the fixed 50% threshold. With the second evaluation procedure, one rather probes the specific resistance of each song against added noise, ignoring the potential influence of competitors. As it is not immediately obvious which is the more appropriate evaluation procedure, we applied both. However, both procedures yielded highly consistent results (cf. Supplementary Tables 1 and 2). This appears mainly due to the fact that 17 of the 23 females in our sample

responded to all original songs in over 66% of the stimulus presentations, which reduced the differences between the 2 evaluation procedures. Here, we show the data for the second criterion (threshold at half-maximal value); data obtained from the other evaluation is presented as Supplementary Material.

Statistics

All tests were 2-tailed. As not all the data of Figure 1a were normally distributed, we applied a nonparametric repeated measures analysis of variance (ANOVA) (Friedman test combined with Dunn's multiple comparisons as post hoc test) to compare attractiveness values. Critical noise levels of females determined for male songs were normally distributed, allowing for Tukey–Kramer's multiple comparisons test. To test for interdependencies between various song parameters, we performed a principal component analysis. Onset accentuation, offset, and duration of syllables and pauses turned out to be independent parameters. For correlation analysis, we applied either Spearman rank correlation in case of nonparametric data or Pearson correlation (parametric data). Statistical analyses were computed using Graphpad InStat Version 3.06 and Minitab 15.1.30.0.

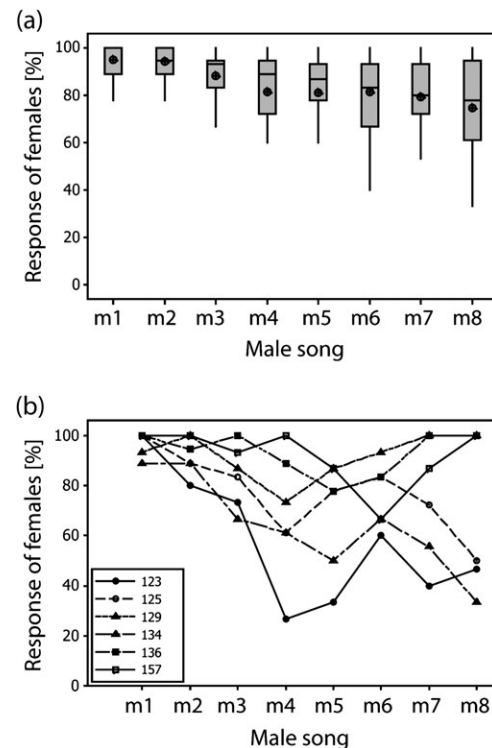


Figure 1

Attractiveness of male songs as determined from the responses of *Chorthippus biguttulus* females. (a) Attractiveness of 8 songs of *C. biguttulus* males (m1–m8), based on responses of 23 females. Ordinate: percentage of female responses to 18 (or 15) stimulus presentations; medians horizontal bars, gray boxes indicate interquartile ranges, whiskers 10 and 90 percentiles; means are shown as circles. For m1, the median was 100%. A Friedman test revealed highly significant differences ($N = 23$; $P < 0.0001$, 2-sided). According to Dunn's post hoc test, songs m1 and m2 differed significantly from all other songs except m3 ($P < 0.05$ to $P < 0.001$). The response to m3 was different from m8 ($P < 0.05$) but not from others, whereas attractiveness values of m4 to m8 did not differ significantly ($P > 0.05$). (b) Individual response profiles of 6 representative females.

Each of the 23 females was tested with the same set of 8 songs, which introduces an issue of pseudoreplication (Hurlbert 1984; Kroodsma 1990). As a consequence, we cannot conclude that the males from which the songs stem were “attractive” senders. To do so, we should have used, for example, 20 different songs from each male and test each female with a different exemplar from each male. This, however, was not feasible in this study. Nevertheless, statements about the attractiveness of the specific stimuli are possible without reservation.

RESULTS

As an indicator for each song’s attractiveness, we determined the average response level of females ($N = 23$) for the 8 unmasked male songs (Figure 1a; m1 being the most attractive and m8 the least attractive song—cf. Klappert and Reinhold 2003). Mean responses varied from $74.5 \pm 22.2\%$ to $94.9 \pm 7.4\%$, mean \pm standard deviation; medians between 77.8% and 100%. Although none of the songs was particularly unattractive, the differences in attractiveness were highly significant ($P < 0.0001$; Friedman repeated measures ANOVA; $N = 23$, $F_{7,22} = 59.76$); Dunns post hoc test indicated highly significant differences between the scores of {m1, m2} and {m7, m8} ($P < 0.001$), and significant differences between {m1, m2} and {m4, m5, m6} ($P < 0.05$). However, individual females differed substantially in their preference rankings (Figure 1b). Shown in this figure are response profiles of 6 females that are representative for the observed variation between females. Obviously, only a partial agreement exists among *C. biguttulus* females about who is an attractive or unattractive male: even the—on average—least attractive unmasked song {m8} scored a 100% response from some females (Figure 1b).

The 8 songs were then presented under different levels of masking noise (e.g., Figure 2a). We used the reduction of female responses at different noise levels to assess the robustness of male songs against masking. The procedure is exemplified with the responses of one female to the 8 male songs under different masking levels (Figure 2b). We determined

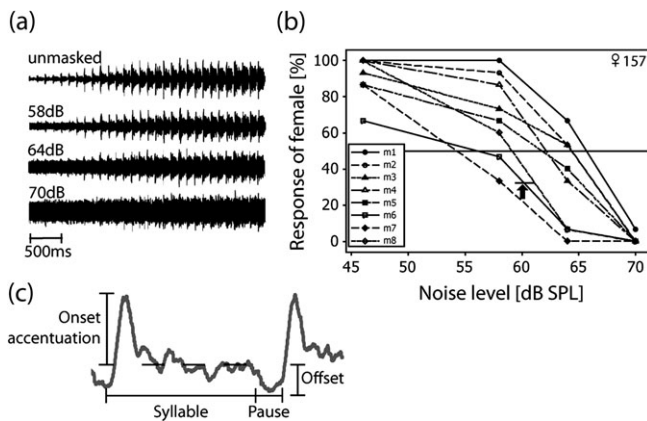


Figure 2

Measuring resistance of male songs against masking by noise. (a) Unmasked and masked versions of the song of an attractive male (m2); levels of broadband noise: 58, 64 and 70 dB SPL. (b) Responses of one female ($N = 157$) to the 8 male songs at different masking levels. Maximal signal intensities were kept constant at 70 ± 1 dB SPL (root mean square measurement, see MATERIALS AND METHODS). Abscissa: masker level (at 46 dB unmasked signal); ordinate: response of female; at 100% the female responded to each of the 18 stimulus presentations. Arrow points at the critical noise level as determined for the song m6 (see MATERIALS AND METHODS for criterion). (c) Definitions of song parameters indicated on the amplitude envelope of a song subunit.

a “critical noise level” as that noise level at which a female’s response dropped to 50% of her response to the unmasked song. For the song m6, starting at 66% response, the critical noise level was 60 dB, determined at the intersection of the curve with 33%, see arrow in Figure 2b. Even for the curves starting from a high response percentage, there was a considerable spread of critical noise levels over a range of ~ 10 dB (Figure 2b). With this criterion, we determined for each song the mean critical noise levels across females and arranged them in Figure 3a according to their attractiveness measured in the unmasked version (Figure 1a). Attractive songs resisted higher masking levels than less attractive ones (Figure 3a). When presented at 70 dB SPL, the 2 most attractive songs (m1, m2) resisted noise levels around 62.5 dB SPL, whereas the 3 least attractive songs tolerated only 58–59 dB. A repeated measures ANOVA indicated significant differences in noise resistance ($F_{7,22} = 14.349$; $P < 0.0001$), and Tukey–Kramer multiple comparisons post hoc test revealed 2 homogeneous groups of songs {m1, m2} and {m6, m7, m8} whose critical noise levels were highly significantly different ($P < 0.001$, $N = 23$); the songs m3 to m5 occupy an intermediate position (see Supplementary Table 1). Comparing mean critical noise levels and female responses (Figure 1a) revealed a significant correlation between attractiveness and robustness against masking (Figure 3b; $r = 0.87$, $P = 0.0097$; $N = 8$, Pearson correlation). Applying a different evaluation, criterion (with fixed threshold, see MATERIALS AND METHODS) yielded very similar results (see Supplementary Figure S1 and Table 2).

The data of Figure 1 reveal large interindividual variations between female responses, in particular to the songs m6 to m8. Could it be that the differences in noise resistance visible

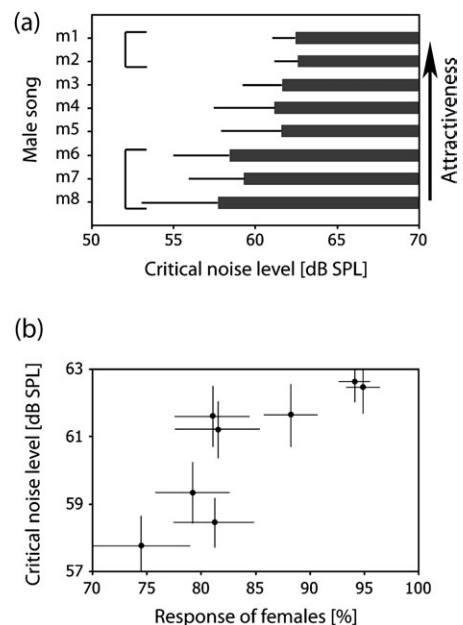


Figure 3

Correlation between a song’s attractiveness and its robustness against masking. (a) Critical noise levels (mean \pm standard deviation) as determined according to Figure 2b. Male songs are arranged according to their median attractiveness, derived from Figure 1a. A repeated measures ANOVA revealed highly significant differences between the 2 encircled groups of songs ($N = 23$, $P < 0.001$). For the complete statistics, see Supplementary Table 1 in supporting information. (b) Correlation between mean attractiveness and critical noise levels; bars indicate standard error of the mean. $r = 0.870$, $P = 0.0097$; $N = 8$, Spearman rank correlation.

in Figure 3 were produced only by those females that responded particularly weakly to the less attractive songs {m6, m7, m8}? We checked for this possibility by repeating the data evaluation of Figure 3a and splitting our sample of females into 2 cohorts: 11 females that responded less than average to songs {m6, m7, m8} and those that responded to these songs rather well. In spite of the reduced sample size, the picture remained essentially the same for the 12 females that responded well to songs m6 to m8. The songs {m6, m7, m8} formed a homogeneous group, which tolerated only a 4.5 dB lower noise level than the songs of males {m1, m2, m3} (differences significant at $P = 0.05$ to 0.001, Tukey–Kramer post hoc test). In the group of females with low response to songs {m6, m7, m8}, the songs {m1, m2} were by 7 dB more robust against noise than songs {m6, m8}, (differences significant at $P = 0.05$ to 0.001), whereas the difference to m7 was only 3 dB higher (difference not significant). This additional data evaluation suggests that the differences in noise resistance were general properties of the male songs and cannot be attributed to individual preferences of only a small subset of females.

As a further control, we also tested whether the total duration of the songs, which varied between 2.1 and 4 s, was crucial in determining noise resistance or attractiveness. However, neither the correlations between song duration and critical noise levels nor between song duration and attractiveness approached significance ($r = 0.484$, $P = 0.225$; $r = 0.418$,

$P = 0.303$, respectively; $N = 8$, Spearman rank correlation). Song duration and attractiveness scores were further investigated with a different large sample of male songs (15 females, each tested with 27 songs; in these tests, the differences between the individual carrier frequency spectra were not removed). Again no significant correlation between the song duration and attractiveness scores was found ($r = 0.0099$, $P = 0.961$, Spearman; Stange N, unpublished data). Therefore, overall song duration does not appear to be a critical determinant of attractiveness, at least in the range of durations tested here.

It is difficult to identify the particular song features that were responsible for the differences in attractiveness or noise resistance. Due to the small number of songs that could be investigated in one behavioral test series, the meaningfulness of a multidimensional analysis of song features is rather limited. In experiments with artificial model songs, the syllable-to-pause ratio, the accentuation of syllable onsets, and the depth of the pause after a syllable (offset) were found to be features that strongly influenced the attractiveness of the songs (von Helversen 1972; von Helversen O and von Helversen D 1994; Balakrishnan et al. 2001; see Figure 2c). In particular, onset accentuation may be a feature that improves detection of song syllables under masking conditions. We therefore assessed these parameters in the 8 songs (see MATERIALS AND METHODS) and tested for possible correlations with attractiveness and noise resistance. There was a weak positive

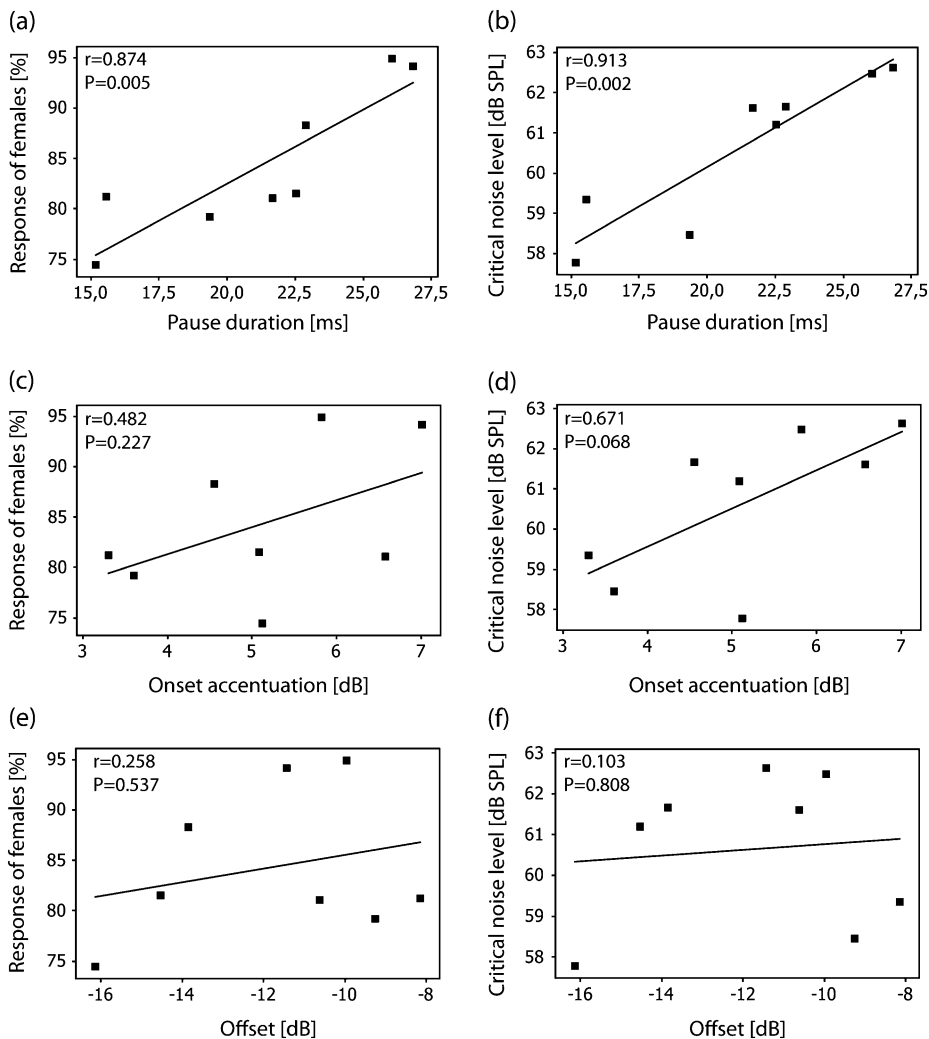


Figure 4

Search for song features mediating attractiveness: (a, b) Correlations between pause duration and attractiveness (i.e., mean response of females according to Figure 1) and critical noise level. (c, d) Correlation between onset accentuation and attractiveness and critical noise level. (e, f) Correlations between offset and attractiveness and critical noise levels. For definitions of song parameters, see Figure 2c. Correlation coefficients and significance values are indicated in the graphs, Pearson, 2-tailed, $N = 8$ songs.

correlation between onset accentuation and attractiveness which was, however, not significant (Figure 4c, $r = 0.482$, $P = 0.227$). Offset value had an even smaller predictive value (Figure 4e, $r = 0.258$, $P = 0.537$). Unexpectedly, the best predictor for attractiveness was a long duration of the pauses between syllables (since the period duration of subunits was rescaled to 100 ms, see MATERIALS AND METHODS, longer pauses corresponded to shorter syllable durations and a low syllable-to-pause ratio). The strong correlation between attractiveness and pause duration ($r = 0.874$, $P = 0.005$) remained significant even after Bonferroni correction for multiple testing (Figure 4a). A similar strong correlation also existed between pause duration and critical noise levels (Figure 4b, $r = 0.913$, $P = 0.002$). Whereas the correlation between onset accentuation and noise resistance approached significance (Figure 4d, $r = 0.671$, $P = 0.068$), offset cues had only a minimal predictive value for noise resistance (Figure 4f, $r = 0.103$, $P = 0.808$).

DISCUSSION

This study yielded 3 major results: 1) Among our sample of 8 male songs, we found significant differences in attractiveness as judged by females (Figure 1a). These differences must reside in the pattern of amplitude modulations because intensity cues and differences in the carrier frequency spectra were eliminated. 2) Individual females exhibited large differences in their preference profiles (Figure 1b). Although the songs of 2 males (m1, m2) were attractive for most females, there was no such consensus among females about the other songs. Such differences in female preferences likely will soften the selective pressure upon signal production of males. 3) Despite these differences between female responses, a clear trend in noise resistance was obvious for the 8 songs. The 2 songs that were most attractive in their unmasked version elicited female responses up to a 4 dB higher noise level compared with the 3 least attractive songs (Figure 3).

Our null hypothesis was that noise will spoil cues of natural signals that may be used by the females to assess the songs and, indirectly, the sender's quality. Hence, the expectation was that signals would become less distinguishable and that female preferences for attractive signals observed with unmasked songs would disappear at higher noise levels. Contrary to this expectation, we found that the differences in attractiveness were preserved with increasing noise (Figure 2b). Apparently, this grasshopper species has mastered the problem of retaining attractiveness cues in spite of signal degradation: attractive signals were at the same time particularly robust against masking (Figure 3).

Attractiveness or detectability?

Formally we can distinguish 3 different tasks the animals had to solve in the present experiment: 1) the detection of the signals against a background noise, 2) the identification of the signals as stemming from a conspecific male, and 3) a classification into more or less attractive signals. Steps 2 and 3 may be combined as a single step when we assume that signals from a foreign species attain very low scores on an attractiveness scale. However, as elaborated in the excellent review by Wiley (2006), the observed responses will depend on 2 variables, the detection success plus the classification score. These 2 factors cannot easily be disentangled in the presence of masking noise (see also Bee and Schwartz 2009).

However, we here argue that the detectability of the unmasked signals was far beyond threshold, and the responses of the grasshopper females to unmasked songs therefore reflect primarily the attractiveness score. The arguments are as

follows: 1) We used unmasked songs to determine attractiveness, and all stimuli were presented at 70 dB SPL. This intensity is at least 15–20 dB above behavioral threshold and lies in the optimum of female response curves (von Helversen D and von Helversen O 1997). 2) The data yield no indication that detectability was a limiting factor for the unmasked songs. The 3, on average, least attractive songs (m6, m7, m8) each scored a 100% response by 4 females (see Figure 1b for examples), and additional 94% responses by another 2 or 3 females (high scores of a total of 9 females). Such high response levels are not in accord with signal detection near threshold. An additional argument is that 17 of 23 females responded to every unmasked song in more than 66% of the stimulus presentations. 3) In addition to the uniform intensity, differences in carrier frequency spectra among songs were eliminated as potential discriminating features (see MATERIALS AND METHODS). Because intensity and spectral composition are major factors in signal detection, it is unlikely that female preferences for unmasked songs were dominated by their detectability. The same songs were also used in an earlier electrophysiological study on auditory receptors which demonstrated that these songs could be discriminated very well on the basis of single spike trains of auditory receptors (Machens et al. 2003). Thus, the different response scores of Figure 1 likely reflect the females' assessment of the quality of male songs, which is inferred from details of the amplitude modulation patterns (Kriegbaum 1989; von Helversen O and von Helversen D 1994; Klappert and Reinhold 2003).

Under conditions of masking, however, the distinction between detectability and quality judgment may fade, if we go a step further and focus upon the neuronal messages available for the animals' decision center located in the brain, that is., spike trains of ascending auditory neurons (Ronacher et al. 1986; Bauer and von Helversen 1987; Ronacher et al. 2008). A highly "attractive" song will produce a neuronal signal that stimulates the female's recognition mechanism in an optimal way, whereas an unattractive signal will produce a less strong input to the recognition mechanism, which is therefore less likely to surmount a neuronal detection threshold. Thus, in the presence of background noise, the classification problem—to discriminate between attractive versus unattractive signals—may be in the end primarily a neuronal detection problem (cf. Wiley 2006). Nevertheless, the data presented here show that attractive signals retain their detectability in the face of masking noise better than less attractive songs.

Features of attractiveness?

Experiments with song models revealed that a female's decision to reply to a communication signal is based on the combination of a number of signal features, as syllable-to-pause ratio, absence of gaps in the syllables, presence of accentuated syllable onsets, and others (von Helversen 1972; Kriegbaum 1989; von Helversen O and von Helversen D 1994, 1997; Balakrishnan et al. 2001; Schmidt et al. 2008). In line with our observation of interindividual differences in response profiles among females (Figure 1b), Balakrishnan et al. (2001) did distinguish between different response types among *C. biguttulus* females, depending on their acceptance of onset and offset cues in model songs (Balakrishnan et al. 2001; von Helversen et al. 2004). One may intuitively expect that the accentuation of syllable onsets would strongly contribute to noise resistance and possibly also to attractiveness of songs. However, the respective correlations were not very strong and not significant (Figure 4). This may be due to the small number of songs that could be investigated in one behavioral test series, which delimits the meaningfulness of a multidimensional feature analysis. Remarkably, significant

positive correlations existed between pause duration and both attractiveness and noise resistance (Figure 4), indicating that longer pauses may facilitate the detection of the subunit structure of a song. The duration of pauses found in the most attractive songs was, however, longer than the optimal pause durations observed in tests with rectangular model songs without onset accentuation (26 and 27 ms compared with 12–15 ms, cf. von Helversen D and von Helversen O 1997). Interestingly, added noise in the pauses of song models (with onset accentuation) led to a marked extension of the acceptance range toward long pause durations (von Helversen et al. 2004). This effect corroborates the observed high attractiveness of the songs with long pauses used in the present study. The high attractiveness of long pauses is in contrast to the study of Klappert and Reinhold (2003), who found a reduced attractiveness of songs with large pauses. However, there was a huge variance of attractiveness values among their large sample of songs (Figure 3b in Klappert and Reinhold 2003), and it is conceivable that the difference between the 2 studies is partly due to the specific choice of our small sample of songs.

Evolutionary links between attractiveness and noise resistance?

What evolutionary scenario may have produced the link between attractiveness and robustness of signals against noise? Most likely a communication signals that resists noise will convey an advantage to both, the sender and the receiver (Wiley 2006). Hence, we can conceive of several—not mutually exclusive—potential explanations. From a male's point of view, it is important to produce long-range communication signals that elicit strong and reliable responses in the auditory pathway of females, in spite of masking and signal degradation (e.g., Gerhardt 1994; Klump 1996; Kroodsmas and Miller 1996; Wiley 2006). An obvious way to improve signal transmission is to increase signal intensity in the presence of noise, and there are several reports demonstrating this strategy termed “Lombard effect” in birds and mammals (e.g., Brumm and Todt 2002; Brumm and Slabbekoorn 2005). However, physiological limitations may prevent animals from such adjustments of signal intensity. Gray treefrogs, for example, did not increase call amplitude in the presence of noise but rather produced longer calls (Love and Bee 2010). The authors concluded that the strong competition in choruses forces the frogs always to call as loud as possible. In grasshoppers, the production of loud sounds appears to be limited by physical restrictions of their stridulatory apparatus (Bennet-Clark 1998; Gerhardt and Huber 2002). An alternative means to produce strong and reliable responses in the auditory pathway of females are large amplitude modulations (Machens et al. 2001; Hennig et al. 2004), as found in the accentuated syllable onsets of *C. biguttulus* (Figure 2a). Although the ability to produce a song with strong amplitude modulations may correlate with a male's condition or fitness parameters, this is not a necessary precondition for the evolution of female preferences. Most sensory systems are endowed with a bias favoring high contrasts that facilitate detection of relevant signals against noisy backgrounds (Guilford and Dawkins 1993; Machens et al. 2001; Ryan and Kime 2002; Gomez et al. 2009). A male whose signals exploit such preexisting biases (e.g., through powerful amplitude modulations) will elicit stronger and more reliable responses in the auditory pathway of females (Machens et al. 2001) and may increase his mating success simply because his signals are more conspicuously represented in the auditory pathway of the receiver. Thus, the attractiveness of noise-resistant songs could be an indirect result of sensory exploitation (Ryan and Kime 2002). At the same time, female preferences for such

robust signal traits could have been stabilized by a reduction of her costs of choosiness if a female could discriminate between males from some distance (Gerhardt et al. 2000; Gerhardt and Huber 2002; Brumm and Slabbekoorn 2005). In such a scenario, grasshopper females may have preferred signal features that provided direct benefits, in terms of better detection or localization cues (Michelsen and Rohrseitz 1997). Another incentive to prefer particular signal features may have been cues that facilitate discrimination against heterospecifics (Pfennig 2000; Deily and Schul 2006; Safi et al. 2006; Gottsberger and Mayer 2007). Females may benefit particularly from song features that yield robust indications about a sender's condition or genetic quality (cf. Welch et al. 1998; Greenfield and Rodriguez 2004). In this case, a female may earn not only indirect—good genes—benefits but also direct benefits by reduced costs of choosiness, if she can judge a potential mate's quality from some distance. In this scenario, the females initially had to detect those song features that gave hints to the sender's quality, or provided direct benefits, also in a masked song version. Evidently, such traits will also be attractive in their unmasked version. The males, on the other hand, could have improved their mating success by including such traits (e.g., strong onsets) in their songs. Indeed, attractiveness of male songs was found to correlate with body condition in *C. biguttulus* and to predict mating success (Klappert and Reinhold 2003). Our data suggest that this connection is valid over larger communication distances. Hence, by assessing reliable long-distance signals extant grasshopper females could, in principle, obtain information about a potential mating partner's condition and health, and thereby reduce their costs of choosiness. Remarkably, grasshopper males solved the problem of preserving attractiveness badges in their long-distance signals in spite of the leveling effects of noise and signal degradation.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>.

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REFERENCES

- Anderson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.
- Anderson M, Simmons LW. 2006. Sexual selection and mate choice. *Trends Ecol Evol.* 21:296–302.
- Balakrishnan R, von Helversen D, von Helversen O. 2001. Song pattern recognition in the grasshopper *Chorthippus biguttulus*: the mechanisms of syllable onset and offset detection. *J Comp Physiol A.* 187:255–264.
- Bauer M, von Helversen O. 1987. Separate localisation of sound recognizing and sound producing neural mechanisms in a grasshopper. *J Comp Physiol A.* 165:687–695.
- Bee MA. 2008. Finding a mate at a cocktail party: spatial release from masking improves acoustic mate recognition in grey treefrogs. *Anim Behav.* 75:1781–1791.

- Bee MA, Schwartz JJ. 2009. Behavioral measures of signals recognition thresholds in frogs in the presence and absence of chorus-shaped noise. *J Acoust Soc Am.* 126:2788–2801.
- Bennet-Clark HC. 1998. Size and scale effects as constraints in insect sound communication. *Philos Trans R Soc Lond B Biol Sci.* 353:407–419.
- Bradbury JW, Vehrencamp SL. 1998. Principles of animal communication. Sunderland (MA): Sinauer Associates Inc.
- Brumm H, Slabbekoorn H. 2005. Acoustic communication in noise. *Adv Study Behav.* 35:151–209.
- Brumm H, Todt D. 2002. Noise-dependent song amplitude regulation in a territorial song bird. *Anim Behav.* 63:891–897.
- Couldridge VCK, van Staaden MJ. 2004. Habitat-dependent transmission of male advertisement calls in bladder grasshoppers (Orthoptera; Pneumoridae). *J Exp Biol.* 207:2777–2786.
- Deily JA, Schul J. 2006. Spectral selectivity during phonotaxis: a comparative study in *Neoconocephalus* (Orthoptera: Tettigoniidae). *J Exp Biol.* 209:1757–1764.
- Feng AS, Narins PM, Xu CH, Lin WY, Yu ZL, Qiu Q, Xu ZM, Shen JX. 2006. Ultrasonic communication in frogs. *Nature.* 440:333–336.
- Gerhardt HC. 1994. Selective responsiveness to long-range acoustic signals in insects and anurans. *Am Zool.* 34:706–714.
- Gerhardt HC, Huber F. 2002. Acoustic communication in insects and anurans. Chicago (IL): Chicago University Press.
- Gerhardt HC, Klump GM. 1988. Masking of acoustic signals by the chorus background noise in the green treefrog: a limitation on mate choice. *Anim Behav.* 36:1247–1249.
- Gerhardt HC, Tanner SD, Corrigan CM, Walton HC. 2000. Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). *Behav Ecol.* 11:663–669.
- Gomez D, Richardson C, Lengagne T, Plenet S, Joly P, Lena J-P, Thery M. 2009. The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (*Hyla arborea*). *Proc R Soc B Biol Sci.* 276:2351–2358.
- Gottsberger B, Mayer F. 2007. Behavioral sterility of hybrid males in acoustically communicating grasshoppers (Acrididae, Gomphocerinae). *J Comp Physiol A.* 193:703–714.
- Greenfield MD. 2002. Signalers and receivers: mechanisms and evolution of arthropod communication. Oxford: Oxford University Press.
- Greenfield MD, Rodriguez RL. 2004. Genotype-environment interaction and the reliability of mating signals. *Anim Behav.* 68:1461–1468.
- Guilford T, Dawkins MS. 1993. Receiver psychology and the design of animal signals. *Trends Neurosci.* 16:430–436.
- Hasselquist D, Bensch S, von Schantz T. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature.* 381:229–232.
- Hennig RM, Franz A, Stumpner A. 2004. Processing of auditory information in insects. *Microsc Res Tech.* 63:351–374.
- Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol monogr.* 54:187–211.
- Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev.* 72:283–327.
- Klappert K, Reinhold K. 2003. Acoustic preference functions and sexual selection on the male calling song in the grasshopper *Chorthippus biguttulus*. *Anim Behav.* 65:225–233.
- Klappert K, Reinhold K. 2007. Indirect benefits for choosy female grasshoppers (*Chorthippus biguttulus*)? *Zoology.* 110:354–359.
- Klump GM. 1996. Bird communication in the noisy world. In: Kroodsma DE, Miller EH, editors. Ecology and evolution of acoustic communication in birds. Ithaca (NY): Cornell University Press. p. 321–338.
- Kriegbaum H. 1989. Female choice in the grasshopper *Chorthippus biguttulus*: mating success is related to song characteristics of the male. *Naturwissenschaften.* 76:81–82.
- Kriegbaum H, von Helversen O. 1992. Influence of male songs on female mating behavior in the grasshopper *Chorthippus biguttulus* (Orthoptera, Acrididae). *Ethology.* 91:248–254.
- Kroodsma DE. 1990. Using appropriate experimental designs for intended hypotheses in 'song' playbacks, with examples for testing effects of song repertoire sizes. *Anim Behav.* 40:1138–1150.
- Kroodsma DE, Miller EH, editors. 1996. Ecology and evolution of acoustic communication in birds. Ithaca (NY): Cornell University Press.
- Lohr B, Wright TF, Dooling RJ. 2003. Detection and discrimination of natural calls in masking noise by birds: estimating the active space of a signal. *Anim Behav.* 65:763–777.
- Love EK, Bee MA. 2010. An experimental test of noise-dependent voice amplitude regulation in Cope's grey treefrog, *Hyla chrysoscelis*. *Anim Behav.* 80:509–515.
- Machens CK, Schütze H, Franz A, Stemmler MB, Ronacher B, Herz AVM. 2003. Auditory receptor neurons preserve characteristic differences between conspecific communication signals. *Nat Neurosci.* 6:341–342.
- Machens CK, Stemmler MB, Prinz P, Krahe R, Ronacher B, Herz AVM. 2001. Representation of acoustic communication signals by insect auditory receptor neurons. *J Neurosci.* 21:3215–3227.
- Meyer J, Elsner N. 1996. How well are frequency sensitivities of grasshopper ears tuned to species-specific song spectra? *J Exp Biol.* 199:1631–1642.
- Michelsen A, Rohrseitz K. 1997. Sound localization in a habitat: an analytical approach to quantifying the degradation of directional cues. *Bioacoustics.* 7:291–313.
- Pfennig KS. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. *Behav Ecol.* 11:220–227.
- Römer H. 1992. Ecological constraints for the evolution of hearing and sound communication in insects. In: Webster DB, Fay RR, Popper AN, editors. Evolutionary biology of hearing. Berlin: Springer. p. 79–93.
- Römer H. 1998. The sensory ecology of acoustic communication in insects. In: Hoy RR, Popper AN, Fay RR, editors. Comparative hearing: insects. New York: Springer. p. 63–96.
- Römer H. 2001. Ecological constraints for sound communication. from grasshoppers to elephants. In: Barth FG, Schmid A, editors. Ecology of sensing. Berlin: Springer. p. 59–77.
- Römer H, Bailey W, Dadour I. 1989. Insect hearing in the field. III Masking by noise. *J Comp Physiol A.* 164:609–620.
- Ronacher B, von Helversen D, von Helversen O. 1986. Routes and stations in the processing of auditory directional information in the CNS of a grasshopper, as revealed by surgical experiments. *J Comp Physiol A.* 158:363–374.
- Ronacher B, Krahe R, Hennig RM. 2000. Effects of signal duration on the recognition of masked communication signals by the grasshopper *Chorthippus biguttulus*. *J Comp Physiol A.* 186:1065–1072.
- Ronacher B, Wohlgemuth S, Vogel A, Krahe R. 2008. Discrimination of acoustic communication signals by grasshoppers (*Chorthippus biguttulus*): temporal resolution, temporal integration, and the impact of intrinsic noise. *J Comp Psychol.* 122:252–263.
- Ryan MJ, Kime NM. 2002. Selection on long-distance acoustic signals. In: Simmons AM, Popper AN, Fay RR, editors. Acoustic communication. New York: Springer. p. 225–274.
- Safi K, Heinze J, Reinhold K. 2006. Species recognition influences female mate preferences in the common European grasshopper (*Chorthippus biguttulus* L.). *Ethology.* 112:1225–1230.
- Schmidt A, Ronacher B, Hennig RM. 2008. The role of frequency, phase and time for processing of amplitude modulated signals by grasshoppers. *J Comp Physiol A.* 194:221–233.
- Schwartz JJ, Gerhardt HC. 1989. Spatially mediated release from auditory masking in an anuran amphibian. *J Comp Physiol A.* 166:37–41.
- Schwartz JJ, Wells KD. 1984. Interspecific acoustic interactions of the neotropical treefrog *Hyla ebraccata*. *Behav Ecol Sociobiol.* 14:211–224.
- Stumpner A, von Helversen D. 2001. Evolution and function of auditory systems in insects. *Naturwissenschaften.* 88:159–170.
- Todt D, Hultsch H. 1998. How songbirds deal with large amounts of serial information: retrieval rules suggest a hierarchical song memory. *Biol Cybern.* 79:487–500.
- Tregenza T, Simmons LW, Wedell N, Zuk M. 2006. Female preference for male courtship song and its role as a signal of immune function and condition. *Anim Behav.* 72:809–818.
- von Helversen D. 1972. Gesang des Männchens und Lautschema des Weibchens bei der Feldheuschrecke *Chorthippus biguttulus* (Orthoptera, Acrididae). *J Comp Physiol.* 81:381–422.
- von Helversen D, Balakrishnan R, von Helversen O. 2004. Acoustic communication in a duetting grasshopper: receiver response variability, male strategies and signal design. *Anim Behav.* 68:131–144.
- von Helversen D, von Helversen O. 1975. Verhaltensgenetische Untersuchungen am akustischen Kommunikationssystem der Feldheuschrecken (Orthoptera, Acrididae) I Der Gesang von Artbastarden zwischen *Chorthippus biguttulus* und *C. mollis*. *J Comp Physiol A.* 104:273–299.
- von Helversen O, von Helversen D. 1994. Forces driving coevolution of song and song recognition in grasshoppers. In: Schildberger K,

- Elsner N, editors. Neural basis of behavioural adaptations. Stuttgart (Germany): G. Fischer. p. 253–284.
- von Helversen D, von Helversen O. 1997. Recognition of sex in the acoustic communication of the grasshopper *Chorthippus biguttulus* (Orthoptera, Acrididae). *J Comp Physiol A*. 180:373–386.
- Welch AM, Semlitsch RD, Gerhardt HC. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. *Science*. 280:1928–1930.
- Wiley RH. 2006. Signal detection and animal communication. *Adv Study Behav*. 36:217–247.
- Wollerman L, Wiley RH. 2002. Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Anim Behav*. 63:15–22.
- Zelick R, Narins PM. 1985. Characterization of the advertisement call oscillator in the frog *Eleutherodactylus coqui*. *J Comp Physiol A*. 156:223–229.