

Grasshopper calling songs convey information about condition and health of males

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Abstract Females of the grasshopper *Chorthippus biguttulus* invest much more in the offspring than do males. As a consequence, females are the more selective sex and exert a sexual selection on males by responding to the songs of certain conspecific males while rejecting others. What kind of information about the sender may a female obtain from a male's song, in addition to its species identity? We searched for correlations between a series of song features and morphometric parameters of individual males. In addition, also the immunocompetence of males was assessed by implanting small pieces of nylon thread. We found significant, positive correlations between certain song characteristics and indicators of male size and immunocompetence. Thus, grasshopper females may—in principle—be able to judge a male's condition and health from the acoustic signals he produces.

Keywords Sexual selection · Mating behavior · Song · Immunocompetence · Morphology

Introduction

In most animal species, females invest more in the offspring than do males. The number of progeny a female can pro-

duce is limited by her own nourishment and the resources she can invest in the energy supply of eggs. In contrast, a male's reproductive success is limited by the number of females he can fertilize. This imbalance in parental investment is the reason for differences in the mating behavior (Trivers 1972), in particular, why, as a rule, the females are the more selective gender (Anderson 1994). When trying to choose high-quality partners, or at least to avoid mating with low-quality males, females should assess honest indicators for male quality. Body size and mass are important criteria, since they strongly correlate with condition (Anderson 1994). Body mass influences directly or indirectly almost every physiological factor (e.g., Peters 1983; Whitman 2008). Bigger animals often increase the mating success in intrasexual competitions or due to female choice (Darwin 1871), and in general they have a higher reproductive rate (Wootton 1979; Thornhill and Alcock 1983; Honek 1993).

Another important aspect of male quality and thus a target of sexual selection is the immunocompetence. If the competence has a hereditary basis (Carton et al. 1992), it is useful for the females to assess clues for the immunocompetence of a potential mate, to be able to provide their offspring with a high resistance against pathogens. However, the immune response requires resources, which are then not available for other physiological processes. Sheldon and Verhulst (1996) reviewed that a reduced investment in the immune system reduces the average life span and decreases the fecundity in many species. The immunocompetence-handicap hypothesis proposes that costly characters may be indicators of a superior immune system (Hamilton and Zuk 1982; see also Zahavi 1975).

Many insect species produce acoustic communication signals in the context of mate attraction (Greenfield 1997; Bradbury and Vehrencamp 1998; Gerhardt and Huber

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2002). Parameters which convey the information about species and gender should be rather uniform among conspecifics and exhibit small variation coefficients (Gerhardt 1991). In contrast, good indicators for phenotypic condition which are under sexual selection tend to be variable within one species (“dynamic criteria” according to Gerhardt 1991; see, however Reinhold 2009). Elaborate songs and visual displays in grasshoppers belong to this category (von Helversen 1986; von Helversen and von Helversen 1994). These signals are long known to attract females (Walker 1975), and in general they are costly, from the view point of energy consumption or by attracting predators or parasitoids (Cade 1975; Prestwich 1994; Wagner 1996; Hoback and Wagner 1997; for review see Zuk and Kolluru 1998).

In some species of Orthoptera, for example in *Gryllus bimaculatus* and *Gryllus campestris*, specific features of calling songs correlate with body size (Simmons 1987, 1988a, b; Simmons and Zuk 1992; Bateman et al. 2001; Ferreira and Ferguson 2002), and female crickets seem to evaluate the size of a male by its song (Gerhardt and Huber 2002). In several investigated species the females prefer song parameters that are produced by bigger males (Forrest 1983; Simmons 1988a; Tuckerman et al. 1993; Simmons and Ritchie 1996; Brooks and Kemp 2001). As concerns immunocompetence, insects rely on an unspecific defensive strategy against pathogens (Cooper 1996). This unspecific response is genetically determined and not specialized on certain pathogens (Janeway et al. 2001). To take actions against invading foreign bodies and pathogens, insects have mainly two strategies available, i.e., humoral and cellular immune responses. A frequent strategy is the encrustation of foreign particles. This cellular response could happen with hemocytes and is very often associated with the deposit of melanin, by which the pathogen is separated from the hemocoel (e.g., Ratcliffe 1993). How efficiently an intruder is covered with a crust is not only genetically determined (e.g., Rizki and Rizki 1990; Carton et al. 1992) but also depends on the nutritional status of the insect (e.g., Sang and Burnet 1963). The maintenance of a cellular and humoral defense system is an important component of the immunocompetence, and it is generally believed that this maintenance is expensive (e.g., Connors and Nickol 1991; Keymer and Read 1991).

By preferring some songs over others, the females may choose mates with special characteristics. If the females prefer big males or males with a good immunocompetence, they increase the chance to pass on these characteristics to their offspring. Not only the song characteristics and morphological characters are inherited (e.g., Simmons 1987, for *Gryllus bimaculatus*; Vedenina et al. 2007, for the *Chorthippus albomarginatus* group; Klappert and Reinhold

2007 for *Chorthippus biguttulus*) but also the immunocompetence (Martinelli and Reichhart 2005). Thus, mating with immunocompetent males, by providing “good genes”, may yield indirect benefits for the females. In addition there may result a direct benefit as a healthy mate may reduce the risk of infections and thus lead to more viable eggs.

In our study species, *Chorthippus biguttulus*, the features of male songs that make a song attractive to females have been investigated in great detail (e.g., von Helversen 1972, 1997; von Helversen and von Helversen 1994, 1997). The males have to transmit three pieces of information with their songs: (1) most importantly, the species they belong to, (2) the gender, and probably, also (3) indicators about the quality of the sender. The temporal pattern of amplitude modulations, the song envelope, turned out to provide the most important cues for species recognition and mate choice by the females (von Helversen and von Helversen 1994; Balakrishnan et al. 2001; von Helversen et al. 2004; Schmidt et al. 2008). Indeed, females show preferential responses for the songs of certain males (Klappert and Reinhold 2003; Einhäupl et al. 2011). A particularly striking example of strong female preference is the rigid rejection of songs of males, who had lost one hind leg (von Helversen 1972; Ronacher and Stumpner 1988; Kriegbaum 1989; Klappert and Reinhold 2003). The ratio of syllable and pause durations (syllable-to-pause ratio) contains the information about the species (von Helversen 1972), while the shape of the sound pulses signals the gender—in this species both sexes do produce songs (von Helversen and von Helversen 1997). Recent studies by Balakrishnan et al. (2001) and von Helversen et al. (2004) demonstrated that females preferred model songs with accentuated syllable onsets and silent pauses (“offsets”, see Fig. 1 for definitions of song parameters). However, it is not known whether similar features also play a role in determining the attractiveness of natural songs.

In the present study we investigate whether some characteristics of natural songs of *C. biguttulus* males can provide clues about the condition and health of the sending male, so that a female can extract this information from the communication signals she receives. Conceivably, adult males that are bigger and healthier may produce more attractive songs. Indeed, body condition of *C. biguttulus* males correlated with song attractiveness (Klappert and Reinhold 2003). In the present study we searched for correlations between morphological characteristics and specific song features of individual males. As additional parameter, we included an indicator of the immunocompetence. An informative way to quantify immunocompetence in insects is to introduce a foreign body of definite size into the insect and to analyze the acquisition of the cellular encrustation of that intruder (Bailey et al. 2011).

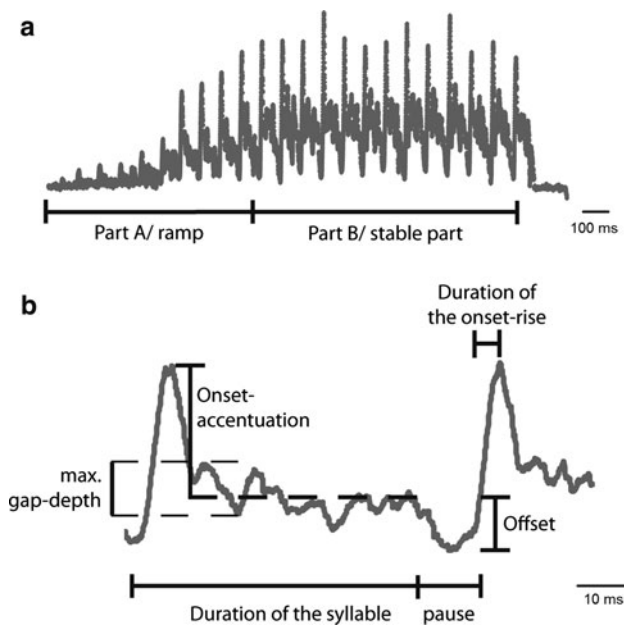


Fig. 1 The song of *Chorthippus biguttulus*. **a** Envelope of the song of a *C. biguttulus* male; **b** Definitions of song parameters indicated on the amplitude envelope of a song subunit

Materials and methods

Animals

The study species, the Gomphocerine grasshopper *Chorthippus biguttulus* L., is common in Europe. The animals can typically be found in dry grasslands, in the lowlands between June and August, while they occur till September in the Alps. Males were raised to adulthood in the lab from eggs obtained from females that were caught in the field (for details of the breeding procedures see Schmidt et al. 2008). The laboratory animals were kept in plastic cages with grass (*Dactylus glomerata*, *Bromus erectus*, *Poa annua* and others) ad libitum and a 14/10 light–dark cycle. All animals were marked with an individual three-point color code and were tested at the age of 2–3 weeks. For a subset of 20 animals the developmental time (from hatching to the final moult) was recorded. Since the temperature has a strong influence on the developmental time and potentially also on the size of insects (Atkinson 1994), we kept these animals as a cohort under identical rearing conditions (20–25°C).

Recording and analysis of male songs

The songs of the males were recorded 2 weeks after the final moult. For the recordings, individual males were kept in a sound-transparent cage (5 × 5 × 5 cm) which was placed in a compartment in a sound chamber covered with sound

attenuating foam. In this compartment a microphone was attached 3 cm above the cage. The microphone was connected to an amplifier and a computer, via a data acquisition board (PCI-MIO-16E-1, National instruments) that stored the digitized songs of the males. The songs of *C. biguttulus* males consist of 20–60 repetitions of stereotyped subunits, i.e., “syllables” that are separated by periods of relative quiet (“pauses”), see Fig. 1, terminology after von Helversen (1972). Since the amplitude modulation pattern of the songs is decisive for recognition and acceptance by females (von Helversen and von Helversen 1997, 1998), we extracted the song envelope (for details of the procedure see Machens et al. 2001 and von Helversen et al. 2004), and determined several song parameters from the envelope with a custom made LabView program (programmed by R. Matthias Hennig): onset, offset, period, length of the syllable and pause and syllable-to-pause ratio (see Fig. 1; following the definitions given by von Helversen et al. 2004). At least 30 syllables were analyzed per male in the plateau region of the song, from 2 to 5 songs per individual. For further analyses, the average values of a male were used. The temperature during the recordings was $30 \pm 1^\circ\text{C}$. This temperature was chosen to correspond to the temperature during behavioral experiments on females (e.g., von Helversen and von Helversen 1997, 1998; Balakrishnan et al. 2001; Schmidt et al. 2008); in addition high temperatures increase the males’ probability of spontaneous singing.

The morphological parameters

Soon after the song recordings, the males were killed and preserved in 70% alcohol until the morphometric measurements. Before measuring the dry weight and other morphological parameters, the animals ($N = 64$) were dried 3 days at room temperature. We used a high-accuracy weighting machine (ALT 100-5AM, Kern and Son GmbH, Balingen, Germany) to obtain the dry weight of the right femur. With a digital measuring slide (L826.1, Carl Roth GmbH + Co, Karlsruhe, Germany) the widths of the head capsule, the thorax and the femur, plus the length of the femur were measured. Each parameter of each male was measured thrice and the results were averaged to yield a single value per individual for further data processing. The discrepancies between those three repeated measurements were always less than 10%.

The immunocompetence

We tested a subset of $N = 20$ males for their immunocompetence by inserting a sterile nylon thread (0.35 mm diameter, Glorex, length 1.6–1.8 mm) into the thorax after completion of their song recordings. The same procedure was applied to a second cohort of $N = 15$ males that were

caught as last instar larvae in the field. The thread was brought into the body with sterile forceps through the inter-segmental skin between thorax and abdomen and was cautiously pushed until it was completely located in the thorax. The animals survived this treatment without any signs of illness. To control for possible damages of the nervous system, the songs of these males were recorded again (after 2 days): the song parameters of all males showed no change at all. Exactly after 1 week the thread was removed and carefully washed with distilled water to remove tissue from the grasshopper. Photos (Canon PowerShot S3 IS) were taken of two sides of the thread through a microscope (Zeiss 475022-9902). The encrustation was quantified by measuring the dark incrustated regions surrounding the thread at a length of 1.5 mm, with a program written in Matlab (R2010a, programmed by N. Stange); the encrustation values of the two sides were averaged ($CV < 15\%$).

Statistics

To test for interdependencies between the measured parameters, we applied a principal component analysis (PCA) with the program Minitab (15.1.30.0). With the program Graphpad InStat (Version 3.06) the significance of correlations was explored. If the data were parametric we utilized the Pearson correlation, for nonparametric data the Spearman rank correlation was used. All tests were two tailed. A correction for multiple comparisons was performed following Bernoulli–Holm (Holm 1979).

Results

Range of song parameters and morphological characteristics of *Chorthippus biguttulus* males

The onset accentuations of the male songs (for definitions see Fig. 1) varied between 4.2 and 10.8 dB (mean value: 6.7 dB). The deepest offset amounted to -32.0 dB, while the smallest offset was -7.3 dB (mean -17.5 dB). The subunits consisted of syllables of 51.3–92.2 ms and pauses of 10.2–26.4 ms (periods 68–116 ms). The average durations of syllables, pauses and periods were 68.8, 18.2 and 86.9 ms, respectively, while the average syllable-to-pause ratio was 4.2. The parameters period and duration of the syllable (Pearson: $r = 0.861$, $P < 0.0001$) and the duration of the pause and the syllable-to-pause ratio were dependent (Spearman: $r = -0.804$, $P < 0.0001$).

The 64 males had an average femur length of 8.6 mm (range: 6.7–10.1 mm), and the femora had diameters of 1.98–2.23 mm. The weight of the femora varied between 5.4 and 10.2 mg, with an average value of 7.7 mg. The average widths of thorax and head capsules were 2.03 or 2.08 mm,

respectively (see supplementary table A for the range of parameters). All these parameters were independent, after analyzing the data with a PCA it was not possible to reduce parameters (see supplementary tables B, C and D).

Negligible effects of the developmental time

Because the developmental time, from hatching to final moult, may influence morphological features, as well as immunocompetence or song parameters, we recorded the developmental time of 20 males and searched for correlations with these parameters. On average these males were 17.5 days in the larval stage. However, individual males needed between 14 and 22 days after the eclosion until the final moult, in spite of experiencing identical rearing conditions in this cohort. We found no significant correlations between the developmental time and the morphology or the song parameters of the adult animals after the Bernoulli–Holm-correction (supplementary Table E). Neither was there a significant correlation between immunocompetence (i.e., size of encrustation) and the developmental time (Pearson: $r = 0.282$, $P = 0.229$; Table 1).

Significant correlations between morphological parameters and song features

The onset accentuation of song syllables was positively correlated with the femur length (Fig. 2a; Spearman: $r = 0.327$, $P = 0.007$, $P = 0.021$ after Bernoulli–Holm correction), the femur width (Spearman: $r = 0.363$, $P = 0.002$, after Bernoulli–Holm correction: $P = 0.008$), and the weight of the femur (Spearman: $r = 0.244$, $P = 0.045$; after Bernoulli–Holm correction: $P = 0.135$). A strong positive correlation existed between the width of the thorax and the onset accentuation (Fig. 2b; Spearman: $r = 0.671$, $P < 0.0001$, $P < 0.0005$ after Bernoulli–Holm correction). Males with broader femora produced longer pauses (Fig. 2c; Pearson: $r = 0.388$, $P = 0.001$, after Bernoulli–Holm correction: $P = 0.005$) and a smaller syllable-to-pause ratio (Fig. 2d; Spearman: $r = -0.438$, $P = 0.0002$, after Bernoulli–Holm correction: $P = 0.001$, all correlations are listed in supplementary Table F). In spite of the large variance visible in Fig. 2, these correlations demonstrate that certain song features may provide clues about the size of the sender for a listening female. On average large males produced particularly loud onsets compared to the mean amplitude of the syllable.

Correlations between immunocompetence and song features

The tests for immunocompetence were performed in two independent groups: males reared from eggs in the lab and

Table 1 Correlation between song parameters and morphology parameters and the encrustation of the nylon thread of 20 lab reared males of *C. biguttulus* and 15 males caught in the field (*r* correlation coefficient; *P* Pearson-correlation; *S* Spearman-correlation)

Parameters	Lab animals		Field animals	
	<i>r</i> (statistical test)	<i>P</i> value (after Bernoulli–Holm correction)	<i>r</i> (statistical test)	<i>P</i> value (after Bernoulli–Holm correction)
Onset (dB)	0.464 (P)	0.039 (0.390)	0.572 (P)	0.026 (0.260)
Offset (dB)	−0.677 (P)	0.001 (0.012)	−0.311 (S)	0.260
Period (ms)	0.593 (P)	0.006 (0.066)	−0.175 (P)	0.534
Syllable (ms)	0.380 (P)	0.098	−0.393 (S)	0.147
Pause (ms)	0.458 (P)	0.042 (0.387)	0.348 (P)	0.203
Syllable/pause	−0.297 (P)	0.203	0.154 (P)	0.584
Length femur (mm)	−0.265 (S)	0.258	0.756 (P)	0.001 (0.011)
Width femur (mm)	0.288 (P)	0.218	−0.024 (P)	0.932
Weight femur (mg)	0.055 (P)	0.818	0.247 (P)	0.375
Width thorax (mm)	−0.036 (S)	0.879	0.321 (P)	0.243
Width head capsule (mm)	0.376 (P)	0.103	0.136 (P)	0.629
Developmental time (days)	0.282 (P)	0.229	–	–

Bold values emphasize significant *P* values

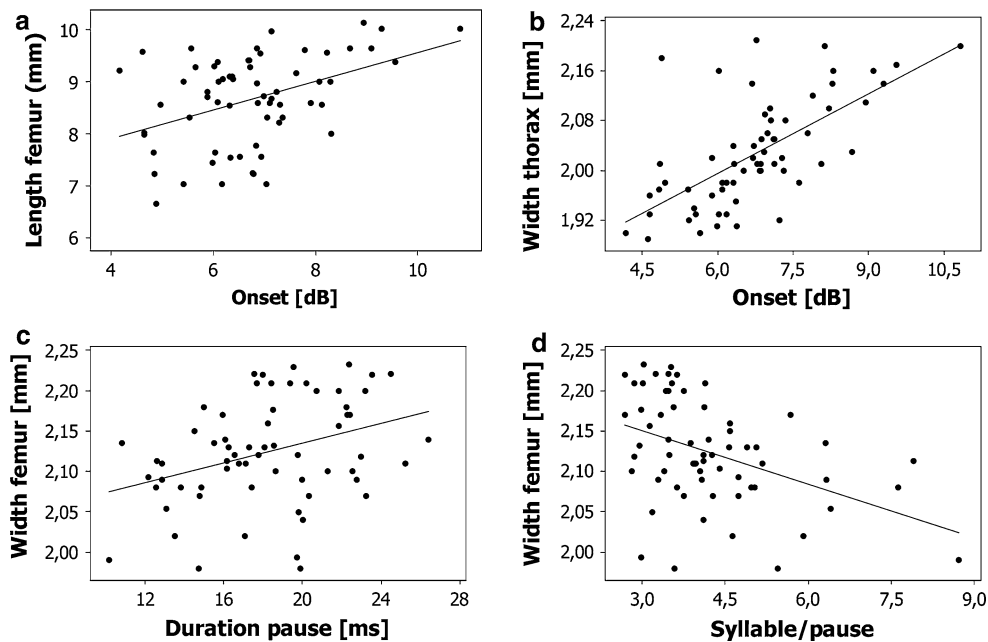


Fig. 2 Search for correlations between morphological characteristics and song parameters. **a** Correlation between the length of the femur and the onset (Spearman: $r = 0.327$, $P = 0.007$, $P = 0.021$ after Bernoulli–Holm correction); **b** correlation between the width of the thorax and the onset (Spearman: $r = 0.671$, $P < 0.0001$, $P < 0.0005$ after Bernoulli–Holm correction); **c** correlation between the width of the femur

and the duration of the pause (Pearson: $r = 0.388$, $P = 0.001$, $P = 0.005$ after Bernoulli–Holm correction); **d** correlation between the width of the femur and the syllable-to-pause ratio (Spearman: $r = -0.438$, $P = 0.0002$, $P = 0.001$ after Bernoulli–Holm correction); for definitions of song parameters, see Fig. 1. Correlation tests were two tailed, $N = 64$ males

males caught as subadults in the field. The inserted nylon threads induced an average encrustation of 0.29 mm^2 (see inset in Fig. 3a), however with a large inter-individual spread: the males with the lowest and highest encrustation produced values of 0.02 and 0.61 mm^2 , respectively.

In both cohorts, the strength of encrustation correlated positively with the onset accentuation (Table 1; see also Fig. 3a, for the data of lab males). Although after the Bon-

ferroni correction the significance level of 0.05 was missed, we consider this effect as real, since it was observed in two independent experiments. A strong correlation with the offset was observed in the lab cohort (Fig. 3b), while in the field cohort the significance level was clearly missed; however, its correlation had the same sign as for the lab cohort—see Table 1). The correlation between encrustation and subunit period was significant for the lab cohort;

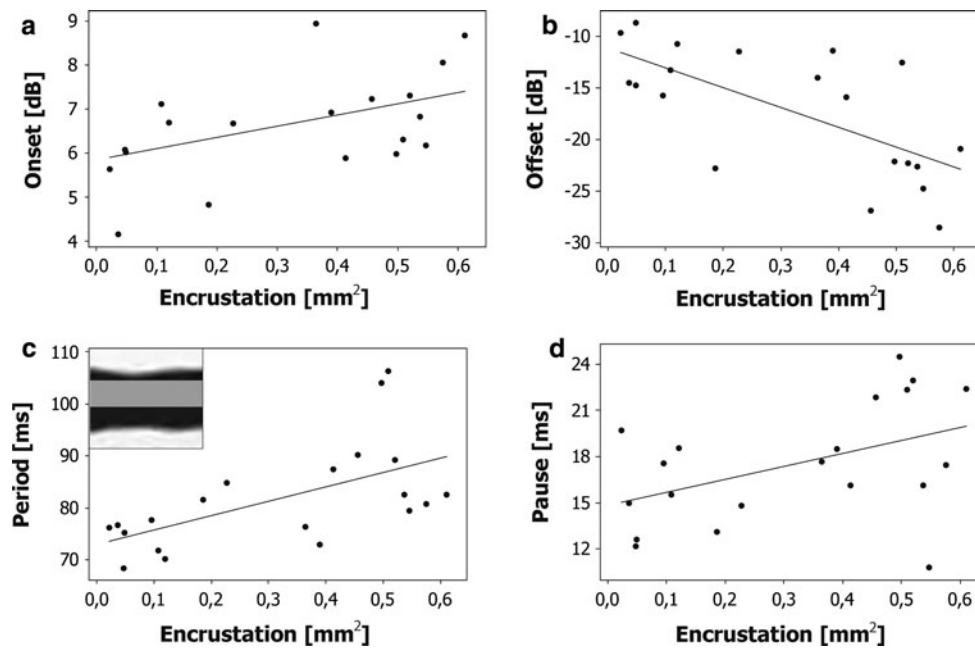


Fig. 3 Search for correlations between the encrustation and song parameters. **a** Correlation between the encrustation and the onset (Pearson: $r = 0.464$, $P = 0.039$, $P = 0.390$ after Bernoulli–Holm correction); **b** correlation between the encrustation and the offset (Pearson: $r = -0.677$, $P = 0.001$, $P = 0.012$ after Bernoulli–Holm correction); **c** correlation between the encrustation and the period

(Pearson: $r = 0.593$, $P = 0.006$, $P = 0.066$ after Bernoulli–Holm correction); **d** correlation between encrustation and the pause (Pearson: $r = 0.458$, $P = 0.042$, $P = 0.387$ after Bernoulli–Holm correction); for definitions of song parameters, see Fig. 1. Pearson correlation tests were two-tailed, $N = 20$ males; *Inset* shows the encrustation of a nylon thread, *light grey* the pure thread

nevertheless this relationship appears doubtful since the correlation coefficients had opposite signs in the two cohorts (Fig. 3c; Table 1). The same is true for the correlation with femur length, which was significant for the field cohort, but had the opposite sign in the lab cohort (Table 1). A correlation with the pause duration (Fig. 2d, Table 1) is only weakly supported.

Discussion

This study was guided by the question of how much information about the quality of the calling male a female may extract from a song she hears. Therefore, we searched for potential correlations between morphology, immunocompetence, developmental time, and the song parameters in males of *Chorthippus biguttulus*. We found that males with longer and broader hind leg femora and broader thorax produced more accentuated onsets and a smaller syllable-to-pause ratio (caused by longer pauses). Our data suggest that bigger males and males with a higher immunocompetence produce a louder onset. A female thus could, in principle, be able to extract this information from a male song she perceives.

The song parameters we investigated were selected on the basis of results provided by von Helversen and von Helversen (1997) and von Helversen et al. (2004), showing that

the temporal pattern of amplitude modulations is decisive for signal recognition in *C. biguttulus*. Special attention was given to the accentuation of syllable onsets, since (1) onset accentuation may be a good way to improve signal transmission in a noisy habitat, (2) accentuated onsets are produced by especially deep down stroke movements of the hind legs, and hence might be energetically costly, and (3) females exhibited individual differences in their preferences for onset and offset cues when tested with artificial song models (von Helversen et al. 2004). The range of onset accentuations, as determined here for male songs (~ 4 – 9 dB, see Figs. 2, 3), is within the range of onset levels that are discriminated by grasshopper females when tested with model songs: a weak onset accentuation of 4.5 dB led to a marked increase of the female response (von Helversen 1979), and a 3-dB difference in onset accentuation was sufficient to change the female responses drastically (Balakrishnan et al. 2001). In general, song models with accentuated onsets and deep offsets were particularly attractive for females (Balakrishnan et al. 2001). We therefore asked whether these song features might be honest indicators of a male's size and health condition.

Developmental time

We had expected that the developmental time may influence morphological features and, possibly, also song

parameters of the males. However, our results gave no hints that the duration of the larval stage had an influence on the tested morphological parameters, nor on song parameters. The developmental time of individual males varied between 14 and 22 days. Such a high variance in the development time was surprising, since this cohort experienced identical rearing conditions. Thus, individual differences likely reflect differences in genetic background. A field study on two other *Chorthippus* species reports longer developmental times of a few weeks (Kiel 2003; unfortunately no precise data were given). These durations were very variable, depended on the factors food, temperature, day length and inter- and intraspecific conditions (competition and population density). The rearing conditions in our lab presumably caused the rather short period of development.

As we found no differences in the morphological parameters and the immunocompetence between the late developers and fast developing males, there seems to be no disadvantage of a shorter developmental time, at least given our rearing conditions. Our data on song features did not reveal reliable clues that females could use to infer the speed of post hatching development. Maybe both fast and slow post-hatching development may confer advantages, depending on the yearly weather conditions, so that there is no strong incentive for females to extract this kind of information from male songs, nor for males to provide (or hide) the respective cues in their songs.

Morphology and song parameters

The males of our large sample showed several correlations between song features and morphological parameters, mostly size indicators. The longer and wider and heavier the femur was, the higher was the onset, the longer was the pause and the smaller the syllable-to-pause ratio (Fig. 2). Significant positive correlations between femur length and onset accentuation were also found in four different populations of *C. biguttulus* (N. Stange, unpublished observations), confirming the data presented here. The relationships between the morphology and an intensity cue, i.e., the strength of amplitude modulations, are of particular interest, because the onset accentuation is one of the parameters, which appeared to be affected by sexual selection (Balakrishnan et al. 2001). However, since we do not know the heritability of these song characters, at present the question remains open whether selection by females would result in evolutionary changes of male song traits.

Former studies on Ensifera revealed correlations between morphology and song parameters. The quality of songs, for example the intensity, frequency, pulse rate and song duration, often correlates with the body size (Judge et al. 2008; Morris 2008). Significant correlations between body size of

males and the pulse rate were found in *Grylloides sigillatus* (Champagnon and Cueva del Castillo 2008). In *Gryllus campestris* the body size was negatively correlated with the call frequency (Simmons and Ritchie 1996), while *Oecanthus niveus* showed a negative relationship between call duration and body size (Ponce-Wainer and Cueva del Castillo 2008). Such correlations between morphology and song parameters allow the females to discriminate between males with different morphological features. Often bigger males are preferred and this preference may lead to an increased reproductive success (Greenfield 1997; Couldridge and van Staaden 2006; Mhatre and Balakrishnan 2008; Ponce-Wainer and Cueva del Castillo 2008). Bigger males exhibit advantages in other physiological properties, too. Bigger males (*Dactyloctenium aegyptium*) show higher levels of lipids and glycogen (De Bano 2008), and seem to have a higher metabolic efficiency (Fielding and Defoliati 2008).

Immunocompetence

A major result of the present study was that some song parameters—i.e., the onset accentuation of syllables and the offset were correlated with the encrustation rate of implanted nylon threads, which is generally accepted as an indicator of the immunocompetence. Remarkably, neither developmental time nor most of the measured morphological parameters correlated with the encrustation (Table 1).

Correlations between the immunocompetence and other physiological or morphological parameters were found in several species (Wedekind and Jakobsen 1998; Kurtz et al. 2000). But the majority of reports refer to trade-offs between life-history and immunocompetence in birds (Norris et al. 1994; Munoz et al. 2010). The activation of the immune system is fundamentally more costly in vertebrates than in invertebrates (Janeway et al. 2001). In several species we find strong differences between the immunocompetence of males and females. This difference is explained by the handicap-immunocompetence hypothesis. Because of the high competition for females, males may invest less energy in other physiological systems that do not directly improve mating success (Sheldon and Verhulst 1996; Zuk 1996; Zuk and McKean 1996). Even if this life-history “decision” may increase the mortality, the relative fitness of males may increase provided that they live long enough to produce more offspring than the competitors. Several studies were devoted to this trade-off problem (Zuk 1996; Zuk and McKean 1996; Adamo et al. 2001; Schmid-Hempel 2003). *Drosophila melanogaster* shows less fecundity and decreased interspecies competitiveness for resources if it successfully encrusted a pathogen (Kraaijeveld and Godfray 1997; Fellowes et al. 1999). Similar effects were observed in the yellow fever mosquito, *Aedes aegypti*: males and

females with high resistance against nematodes had reduced reproductive success (Koella and Boete 2002). *Apis mellifera* and the flour moth *Plodia interpunctella* exhibit similar trade-offs (Rothenbuhler and Thompson 1956; Sutter et al. 1968; Boots and Begon 1993). In scorpion flies (Kurtz and Sauer 1999), dragonflies (Rantala et al. 2000) and beetles (Rantala et al. 2000) characters under sexual selection are positively correlated with the immunocompetence. The ability to encrust a foreign body increases with the size of the wings and the body size in the beetle *Euoniticellus intermedius* (Pomfret and Knell 2006). The immunocompetence in male wolf spiders *Hygrolycosa rubrofasciata* is correlated with the drumming frequency during the courtship (Ahtiainen et al. 2004). Ryder and Siva-Jothy (2000) found a correlation between the number of hemocytes and the quality of the courtship song in *Acheta domesticus*, and females of *Gryllus bimaculatus* prefer the songs of males with a high immunocompetence (Rantala and Kortet 2003).

Adamo et al. (2001) investigated the immunocompetence and age dependence in *Gryllus texensis*. After their final moult the males stop investing resources in their immune system. This results in a big difference in the immunocompetence of adult males and females as well as between subadult and adult males. In *Chorthippus biguttulus* we found an increased immunocompetence correlated with higher values in song parameters under sexual selection (onset, offset). The detection of encrustation in adult males demonstrates that this grasshopper does not turn down its immune system after the final moult, as reported for male crickets (Adamo et al. 2001). Thus, the males of *Chorthippus biguttulus* seem to be able to invest energy in both: Immunocompetence and mating behavior. Perhaps the risk of infection is higher in grasshoppers, so a powering down of the immune system would be too risky. Female *C. biguttulus* show preferences for accentuated onsets and deep offsets of song models (Balakrishnan et al. 2001; von Helversen et al. 2004). By this preference females may select males, which are able to resist parasites and diseases, and thereby pass these features to their offspring.

Conclusion

Specific features of the song of *C. biguttulus* males allow inferences regarding the aptitude in morphology and immunocompetence. Thus, a female may—in principle—be able to extract this information from a calling song she hears. These results could be used as the basis for a study that tests whether and how females benefit from choosing males with preferred song types.

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