Species Recognition Influences Female Mate Preferences in the Common European Grasshopper (*Chorthippus biguttulus* Linnaeus, 1758)

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Abstract

Species recognition and intraspecific mating preferences constitute two basic aspects of animal communication. Both can be considered as variations in response to signals and it has been suggested that they represent a continuum. Selection on species recognition could therefore influence intraspecific mating preferences. We show that females of the common European grasshopper *Chorthippus biguttulus* prefer conspecific male signals that can be distinguished more reliably from sympatrically occurring heterospecific signals. This suggests that in *C. biguttulus*, sexual selection might be influenced by pleiotropic effects from species recognition. The results show how the heterospecific signal environment could have determined why and in which direction specific traits become sexually selected.

Introduction

Mate-choice preferences are responsible for the evolution of some of the most fascinating and conspicuous secondary sexual signals. Their evolution is generally explained by hypotheses assuming direct or indirect benefits of mate choice such as: (i) Fisherian self-reinforcement processes, (ii) indicator mechanisms (good genes hypothesis), or (iii) direct phenotypic benefits (for explanations, see e.g. Andersson 1994). However, these hypotheses mask the fact that other processes can influence mate-choice preferences. Preferences could evolve as pleiotropic effects of other selection pressures on sensory systems (Ryan 1998); e.g. they could be shaped by species-recognition processes (Ryan 1990; Ryan et al. 1990). Sexual selection can be a driving force in reproductive isolation and enhance speciation (Dobzhansky 1937; Panhuis et al. 2001; Kirkpatrick & Ravigne 2002; Svensson et al. 2006). However, the avoidance of heterospecific matings also has the potential to shape the properties of signals and receivers in mate recognition systems (Pfennig 1998; Hankison & Morris 2002; Ryan et al. 2003; Phelps et al. 2006). Corroborating this argument, a theoretical study by Ryan & Getz (2000) showed that species recognition can drive intraspecific sexual selection.

Heterospecific matings often result in offspring with reduced fitness or no offspring, and thus an increase in the efficiency in mate-choice recognition and a simultaneous avoidance of heterospecific matings should enhance the fitness of an individual (e.g. Shapiro 2000; Rice & Chippindale 2002). Classically, it is thought that if a sex prefers traits that best allow species recognition, then it should prefer those that are most typical for its own species. In other words, it should prefer intermediate values around the population mean (Butlin et al. 1985). Such trait characteristics should vary little within and between individuals and represent the 'static' properties of a trait (Gerhardt 1991). According to this argument, species recognition leads to stabilizing selection

favouring such 'static' properties (Gerhardt 1991). However, costs of wrong decisions are high and if the opportunities for mistakes are substantial, the best strategy might not be to go for the most typical (i.e. population mean) but rather for the trait that is most distinct from that of the heterospecifics. An increased species-recognition efficiency resulting from a preference for extreme values in a population could thus cause directional selection instead of stabilizing selection.

Using *Chorthippus biguttulus*, a common European grasshopper, we investigated to what extent the requirement for species recognition may influence female mate choice, and therefore possibly lead to directional selection. In *C. biguttulus*, receptive females answer male calls with their own songs. The rate and intensity of this female acoustic response can be used as a bioassay to assess male attractiveness (Klappert & Reinhold 2003). Recent studies (Reinhold et al. 2002; Klappert & Reinhold 2003, 2005) have shown that male attractiveness estimated from female acoustic response rate reliably reflects the caller's mating success. In these previous studies, directional selection on male *C. biguttulus* signals was found. However, none of the measured male morphological or physiological properties were related to the attractiveness of the caller and the adaptive value of the female preference and the underlying mechanisms remained unclear (Reinhold et al. 2002; Klappert & Reinhold 2003, 2005). We tested to what extent species recognition allows the prediction of female mate-choice preferences in this species. To do so, we considered the male songs of 10 sympatrically occurring heterospecific orthopteran species in a multivariate approach and assessed whether the dissimilarity from the heterospecific signals and/or the compliance with the population average may explain female preference.

Materials and Methods

Chorthippus biguttulus is one of the most common grasshoppers in Germany. In a previous experiment, male signals were recorded and analysed to determine signal attractiveness, its reliability and whether it allows to predict mating success (Klappert & Reinhold 2003). To do so, 159 males and 169 females were collected in 1999, from which 100 males and 93 females were randomly selected for a playback experiment (Klappert & Reinhold 2003). We refer from now on to these 100 recordings of males as the test signals. In addition to these 100 test signals we used 10 randomly selected recordings of different

individuals recorded in the year 2000 for our study which will be referred to as the conspecific signals. And finally, in summer 2003 we collected males and recorded songs of as many different species of Orthoptera as possible that occurred sympatrically with *C. biguttulus* in the vicinity of Bonn, Germany and sang simultaneously. All recordings were made following the same standard recording procedure as described in Klappert & Reinhold (2003). We were able to obtain recordings of two males for each of 10 orthopteran species (Fig. 3). These 20 signals are referred to as heterospecific signals.

A playback experiment was used to determine the attractiveness of the test signals (Klappert & Reinhold 2003). To each of the 93 females, which were separated into two groups of approximately equal numbers (n = 41 and n = 52), 50 randomly selected male test signals were presented. The calculation of the attractiveness value for each male signal took into account how many females answered a male and how often they answered (for details, see Klappert & Reinhold 2003).

As the neuronal mechanism of sound perception in female call recognition relies on the temporal pattern, we analysed the time/amplitude domain (the rhythm) of the signals (von Helversen & von Helversen 1998; Balakrishnan et al. 2001; Machens et al. 2003). From all recordings, a 2-s interval covering the signal parts with maximum amplitude was manually selected. These intervals were transformed by calculating the average root mean square (RMS) values for a gliding 3-ms window and thus filtered out the high-frequency components of the amplitude modulation that are unlikely to be perceived.

We then calculated the power spectrum, i.e. the absolute value of the Fourier transformation (FFT) of the RMS signal, cutting off rhythmic components of the amplitude modulation higher than 2000 Hz (FFT frequency). The power spectrum represents a description of the temporal pattern (of the rhythm) of a 2-s interval. The components of the power spectrum (FFT frequencies) describe the amount of energy contained in a specific rhythmic component of the song and can also be used in order to reconstruct the amplitude modulation of the song (see Fig. 1). In Fig. 1, one of the rhythmic components shown (a) corresponds to a modulation in amplitude every 1/12th seconds (12 Hz). Finally, we reduced the dimensions of our data set (2000 FFT frequencies) using a principal component analysis (PCA). The purpose of PCA is to derive a small number of linear combinations (principal components) of a set of variables (FFT frequencies) that
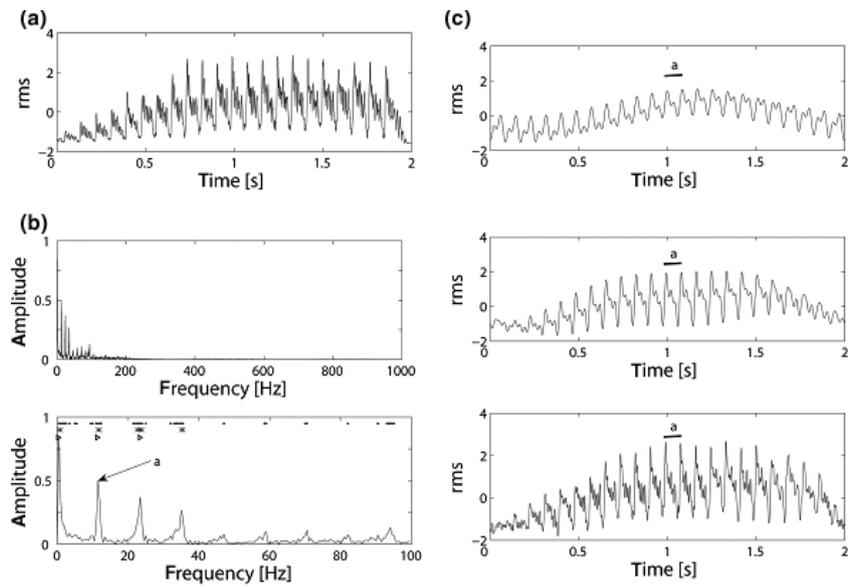
Fig. 1: (a) Temporal variation of RMS of one of the 10 positive training examples of *C. biguttulus*. (b) Corresponding power spectrum. Top: All frequencies. Bottom: Only frequencies up to 100 Hz. The arrow points to the peak at around 12 Hz, which is also prominently seen in the reconstructed traces. (c) Reconstruction of the RMS trace by using the three largest (top), 10 largest (middle), and 40 largest (bottom) FFT components containing most power as indicated in subfigure b. The small bar 'a' shows the wavelength, that corresponds to the peak shown in b

retain as much of the information in the original variables as possible. We therefore used the PCA to detect signal traits (principal components) that would facilitate distinction between the two classes of stimuli (between conspecifics and heterospecifics) by including only the calls of the conspecifics (n = 10) and the heterospecifics (n = 20). To estimate the dissimilarity between two signals p and q, we calculated the Euclidean distance over all 20 PCs (see equation 1).

$$distance = \sqrt{\sum_{i=1}^{20}(p_i - q_i)} \qquad (1)$$

The calls of the 100 test signals whose attractiveness for female *C. biguttulus* were previously determined were then transformed into the multivariate space derived from those 30 initial signals. We subsequently correlated male attractiveness with the average Euclidean distance using the first 20 PCA components between test male signals and the 10 conspecific signals to test whether females prefer typical signals. Likewise, we correlated attractiveness and distance to heterospecific signals to check whether attractiveness can be predicted by dissimilarity to heterospecific calls. Thus we modelled attractiveness to be:

$$\text{Attractiveness} = \alpha \cdot distance_{conspecifics}$$
$$+ \beta \cdot distance_{heterospecifics} + \delta, \qquad (2)$$

In this equation, $distance_{conspecifics}$ represents the mean Euclidean distance to the 10 conspecific, and $distance_{heterospecifics}$ the respective distance to the 20 heterospecific signals.

Results

The PCA on the FFT-transformed calls of the 10 conspecific and 20 heterospecific signals (Figs 1 and 2) resulted in 30 PCA components. For the analysis, we used the first 20 PCA components that explain 96.1% of the original variance of the initial 10 conspecific and 20 heterospecific signals.

The temporal pattern of the acoustic signals clearly differs between *C. biguttulus* and the heterospecifics (pairwise Euclidean distance using the first 20 PCA components, mean ± standard deviation among conspecifics: 29.2 ± 10.1, between conspecifics and heterospecifics: 61.8 ± 13.0; Fig. 3).

The multiple correlation model (equation 2) explains a significant proportion of variance in male attractiveness (GLM: $r^2 = 0.11$, $F_{2,97} = 5.9$, p = 0.004) (Fig. 4). For the 100 test calls, the measured attractiveness was positively correlated with $d_{heterospecifics}$ (Fig. 4, $\bar{x} \pm SD = 59.3 \pm 2.4$, $\beta \pm SE = 0.05 \pm 0.02$, $F_{1,97} = 8.8$, p = 0.004), but there was no significant correlation with $d_{conspecifics}$ ($\bar{x} \pm SD = 36.8 \pm 3.2$, $\alpha \pm SE = -0.02 \pm 0.01$, $F_{1,97} = 2.1$, p = 0.15; intercept $\delta \pm SE = -1.5 \pm 1.2$, p = 0.22). This shows that female *C. biguttulus* prefer male signals with increasing dissimilarity to heterospecific signals but not with increasing compliance with the population mean (Fig. 3).

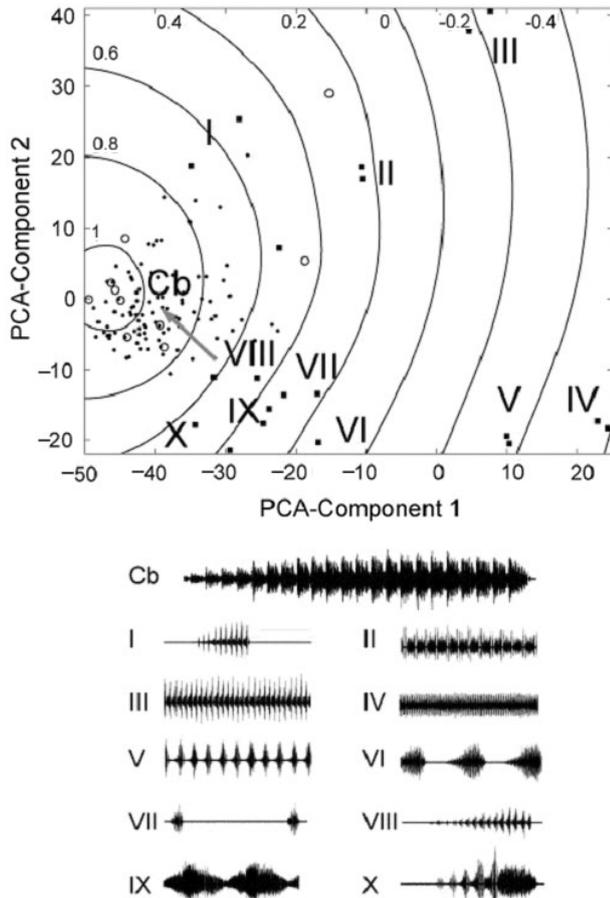We also checked the correlation between signal attractiveness and the signal mean distance to the
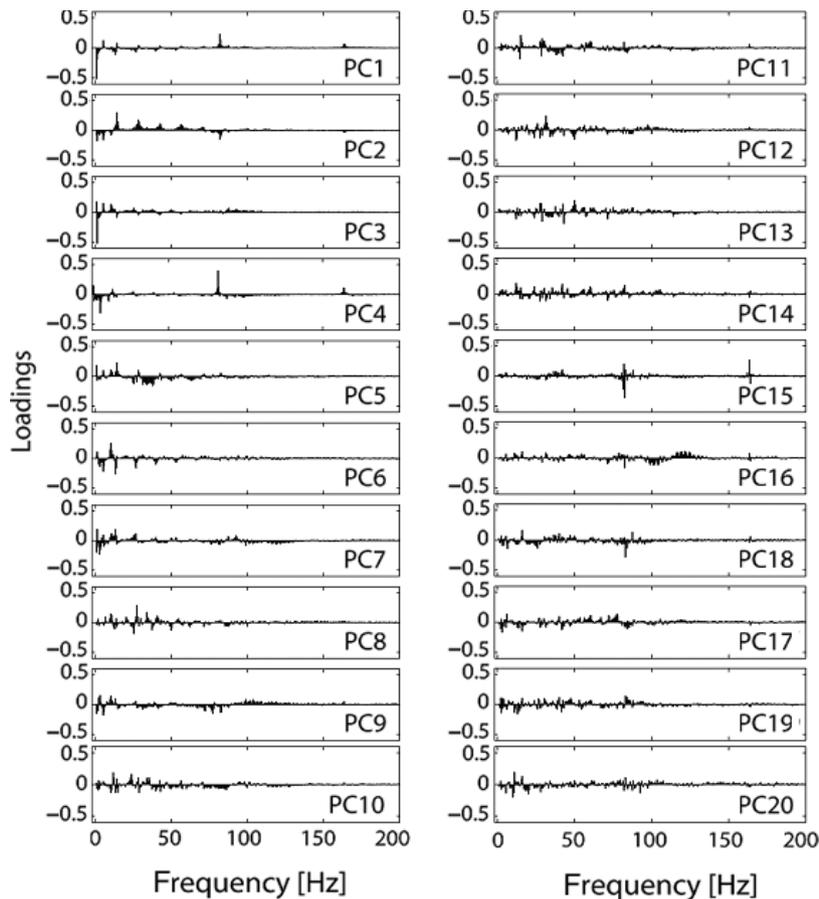
Fig. 2: The loadings of the FFT frequencies for the first 20 PCA components, which add up to a total of 96% explained variance of the original data

other 99 test samples to control for the fact that the 10 initial *C. biguttulus* conspecific calls might not reflect the true average of the population (using distance$_{test}$ as the average pair wise Euclidean distance of the 100 test samples instead of d$_{conspecifics}$; $\alpha \pm SE = -0.007 \pm 0.01$, $F_{1,99} = 0.4$, $p = 0.5$). This test also confirmed that similarity to the population average could not predict female preference.

Finally, we checked whether our unequal samples of 20 heterospecifics vs. 10 conspecifics could have potentially influenced the outcome of our analyses. In order to do so we randomly chose 500 times any 10 signals of the 20 heterospecific signals and ran all the analyses with the random subset of heterospecific signals again, from the PCA to the correlation of attractiveness of the test samples with the distance to the heterospecifics. In all 500 cases, the correlation between attractiveness and distance to heterospecific signals remained significant. These analyses thus show that our results are robust and can even be reproduced with incomplete sets of samples.
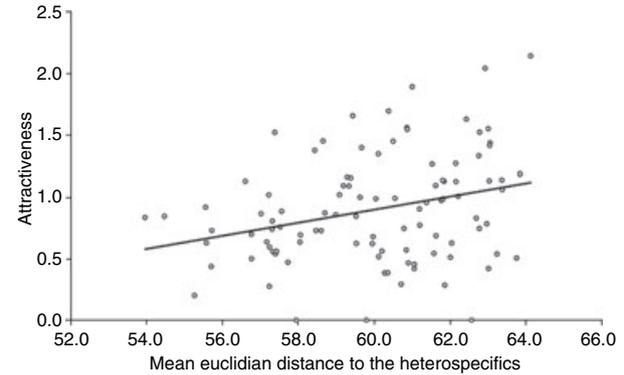
Discussion

Our results suggest that recognition of species-specific traits in *C. biguttulus* had, or still has an influence on the evolution of female preference. The lack of female preference for average male signals indicates that females do not necessarily base their preference on the compliance of a male signal with the average signal (Machens et al. 2003). One adaptive explanation is that females are expected to prefer signals that maximise the efficiency of species recognition. Such increased recognition efficiency, as our study shows, could result from a preference of females of *C. biguttulus* for conspecific signals that are more reliably recognized from heterospecifics (see also Ryan & Getz 2000). An increased efficiency in mate recognition can enhance the individual fitness by reducing subtle costs of mate choice, besides direct benefits of avoidance of heterospecific matings. Such costs could, for example, result from futile time investment or phonotaxis towards incompatible mates. In environments where conspecific and heterospecific signals can be confounded, signals that

Fig. 4: Correlation of the dissimilarity of male signals to heterospecific signals with attractiveness. The dissimilarity was measured as the mean of the Euclidean distances to the heterospecific signals for the male individuals using the first 20 PCA components. Attractiveness was determined in playback experiments

Fig. 3: Scatter plot of 10 conspecific signals (circles), 20 heterospecific signals (boxes), and 100 test signals (dots) in the first two PCA dimensions (33% of the original variance). The arrow points to the location of the conspecific average call. The contour lines are estimates of attractiveness from the model (equation 2). Predicted maximum of preference: (PC1, PC2) = (−46, 1). Mean of conspecific signal = (−39, −1). Below are the oscillograms of: Cb, *Chorthippus biguttulus*; I, *Chrysochraon dispar*; II, *Gomphocerus rufus*; III, *Omocestus viridulus*; IV, *Metrioptera roeseli*; V, *Chorthippus vagans*; VI, *Myrmeleotettix maculatus*, VII, *Chorthippus brunneus*; VIII, *Chorthippus parallelus*; IX, *Stenobothrus lineatus*; X, *Chorthippus dorsatus*. The time scale for all oscillograms is 2 s

are most reliably distinguished from heterospecific signals or ambient noise should be preferred (Pfennig 1998; Baker 2006). Thus, for individuals trying to increase efficiency in species recognition, traits within a population that are most dissimilar from heterospecifics should elicit stronger responses (Nelson & Marler 1990; Ryan & Getz 2000).

Although it appears unlikely that species-recognition processes can lead to the evolution of sexually selected traits far beyond what should be needed for sex or species recognition (Andersson 1994), it can initiate and influence the direction of selection for such traits. The initiation and direction of female preference therefore need not be mere products of chance, but can trigger directional selection. Thus, under the influence of species recognition, traits can become directionally selected instead of stabilized. In *C. biguttulus*, the heterospecific signal environment could predict the direction in which female preference moved ahead of the population average trait. The heterospecific signal environment could however also influence the direction of selection irrespective of the potential forces driving and maintaining directional selection by limiting and stipulating the direction in which selection can move. Species-recognition processes should thus lead to an increased divergence of sexually selected traits between species and such mechanisms could explain why closely related species often differ extensively in sexually selected traits (Andersson 1994; Panhuis et al. 2001).

As far as we are aware, this study includes more sympatric heterospecific species than other studies, and reconstructs the heterospecific environment in near complete manner. The species-recognition approach was able to unravel a possible explanation for a mechanism that leads to directional selection on male calls. Other mechanisms, however, cannot be ruled out and final conclusion will need further experiments. If these proposed processes shaped female mate preferences, then we would expect differences in populations with different heterospecific signal environments. Future studies should include experimental examination of the influence of species recognition on female preference. Finally, presenting females experimentally to heterospecific calls could help to evaluate the incurred costs of errors in species recognition.

Acknowledgements

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