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Reproductive behaviour of female *Chorthippus biguttulus* grasshoppers

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ABSTRACT

Female grasshoppers of acoustically communicating species assume series of reproductive states that are associated with particular behaviours. Studies on laboratory populations of *Chorthippus biguttulus* (L.) revealed that females of this species lack the period of 'passive copulatory readiness', increase their attractiveness to males by sound production and mate multiple times before their first oviposition. In particular, female *Ch. biguttulus* display a period of 'primary rejection' after their imaginal moult during which they reject male mating attempts followed by a period of 'active copulatory readiness' in which they produce acoustic signals and may copulate with courting males. Female stridulation generally stimulated male mating activity and stridulating females attracted more male mating attempts than mute females in the same cage, indicating that males preferentially court females that signal 'active copulatory readiness'. After receipt of a spermatophore, *Ch. biguttulus* females displayed periods of 'secondary rejection' followed by re-establishment of 'active copulatory readiness'. Acoustic responses of females to male songs, an indicator of reproductive readiness, were significantly reduced until 2 days after mating and remained slightly reduced in comparison to pre-mating levels. Some females mated multiple times before their first oviposition and cycled between 'secondary rejection' and 'active copulatory readiness'.

1. Introduction

In most gomphocerine grasshoppers both males and females produce acoustic communication signals by rhythmically rubbing their hind legs against the forewings (Elsner, 1974). Grasshopper acoustic signals are species- and sex-specific and serve to recognize, select, and localize reproductive partners. In some species including *Chorthippus biguttulus*, the mode of acoustic communication is determined by the reproductive state of the female which depends on maturation, sexual activity, and oviposition cycles (Renner, 1952; Kriegbaum and von Helversen, 1992; von Helversen, 1972). Based on experimental data derived from studies on a few species (see below), it has been assumed that the reproductive period of virgin grasshopper females can generally be subdivided in two parts, 'passive copulatory readiness' and 'active copulatory readiness'. In the state of 'passive copulatory readiness', females are mute and tolerate male mating attempts after courtship (Loher and Huber, 1964; von Helversen and von Helversen, 1983; Hartmann and Loher, 1996). This unilateral communication switches to bilateral communication when females enter 'active copulatory readiness' (Loher and Huber, 1964). In this state, the female sings spontaneously or answers to male calling songs which may initiate prolonged alternating duets with the male during which phonotaxis is performed by the male (von Helversen and von Helversen, 1983).

The course of female grasshopper reproductive states has been studied in a rather small number of species including *Gomphocerus rufus* (Loher, 1962; Loher and Huber, 1964; Riede, 1983), *Euthystira brachyptera* (Renner, 1952) and some specific aspects more recently in *Chorthippus parallelus* (Kriegbaum, 1988; Reinhardt and Köhler, 1999; Reinhardt et al., 2007). Although some differences between these species have been reported, the following scheme for the general course of female reproductive states has been widely accepted and used as a basis for studies with other grasshopper species (Fig. 1). During the first days after their imaginal moult, females do not stridulate and fend off male mating attempts. This behavioural state was named 'primary defence' by Loher and Huber (1964) but we suggest to denote it 'primary rejection' in order to discriminate it from the term used to describe predator–prey behaviour (Edmunds, 1974). After a few days, females assume the state of passive readiness during which they remain silent but may mate after male courtship. If females do not copulate during passive readiness, they enter the state of 'active copulatory readiness', in which they respond to male calling songs

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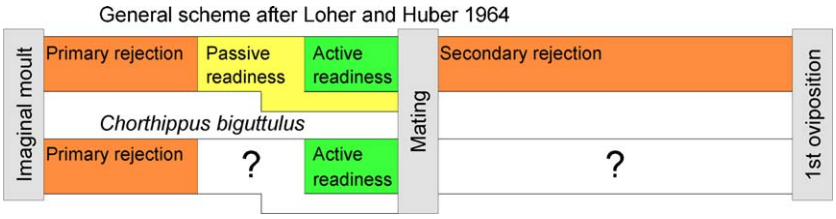

Fig. 1. Reproductive states of acoustically communicating grasshopper females between imaginal moult and first oviposition. Upper part summarises a scheme that was proposed by Loher and Huber (1964) based on experimental data from studies on *Gomphocerus rufus* and *Euthystira brachyptera*. Detailed explanation is provided in the text. Various studies on other grasshoppers including *Chorthippus biguttulus* indicated that this general scheme may not apply to all acoustically communicating species, questioning the occurrence of 'passive copulatory readiness' and the nature of behavioural states that follow mating in particular.

or even stridulate spontaneously and accept male mating attempts without prolonged periods of courtship. After mating, females either assume a state of 'secondary rejection' during which they reject male mating attempts (*G. rufus*; Hartmann and Loher, 1974, 1996) or, in species where subsequent copulations are prevented by the spermatophore plugging the spermathecal duct, display a period of 'passive copulatory readiness' that changes to 'secondary rejection' shortly before the first oviposition (*E. brachyptera*, Renner, 1952; *Chorthippus curtipennis*, Hartmann and Loher, 1974). Studies on other species reported that females copulated multiple times in rather short intervals (Kriegbaum, 1988; Reinhardt and Köhler, 1999), that females in the state of 'active copulatory readiness' rejected male mating attempts (Kriegbaum, 1988; Kriegbaum and von Helversen, 1992) and responsiveness of virgin females to male songs either increased (Loher and Huber, 1964; Kriegbaum and von Helversen, 1992) or decreased (Reinhold et al., 2002) with age. These observations suggested that the general scheme for the course of female reproductive states and associated behaviours cannot be directly extrapolated to all gomphocerine grasshoppers.

The nightingale grasshopper *Ch. biguttulus* (L.) is a frequently used species for neurobiological, behavioural, ecological, evolutionary and hormone physiological studies. Male calling and courtship songs and female response songs that serve as crucial elements in the reproductive behaviour of *Ch. biguttulus* (von Helversen and von Helversen, 1997) have been employed for studies intended to understand bioacoustic phenomena in the field (Lang, 2000; Gilbert and Elsner, 2000), criteria for female choice (Kriegbaum, 1989; Kriegbaum and von Helversen, 1992; Reinhold et al., 2002; Safi et al., 2006), localization and recognition of acoustic patterns (von Helversen and von Helversen, 1997; Balakrishnan et al., 2001; Schmidt et al., 2008), mechanisms of auditory information coding in the nervous system (Machens et al., 2001, 2003; Neuhofer et al., 2008; Ronacher et al., 2008), and the neuropharmacological basis of motivational states (Heinrich et al., 2001; Hoffmann et al., 2007; Weinrich et al., 2008). In contrast to numerous studies on the production and processing of reproduction-related acoustic signals of *Ch. biguttulus*, general information about its reproductive states and associated behaviours are merely derived from observations on other species (e.g. *G. rufus*, *E. brachyptera*, *Ch. parallelus*, *Ch. curtipennis*) although these studies revealed considerable differences (mentioned above) in the course of female reproductive states. In order to provide information about the reproductive behaviour of this important grasshopper species we studied the course of reproductive behavioural states of individually labelled *Ch. biguttulus* females in small laboratory populations between their imaginal moult and first oviposition. In particular, we asked the questions, whether *Ch. biguttulus* females express a period of 'passive readiness' between 'primary rejection' and 'active copulatory readiness', whether they re-establish copulatory readiness after matings and whether they copulate more than once prior to first oviposition. The results are compared with data from earlier laboratory and field studies on *Ch. biguttulus* and other grasshopper species.

2. Materials and methods

2.1. Animals

Studies were performed with adult male and female grasshoppers of the species *Ch. biguttulus* (Orthoptera: Acrididae; L. 1758). All females used in this study were reared from eggs that derived from grasshoppers collected in the vicinity of Göttingen, Germany in the previous summer (the summers of 2007 and 2008). Mixed populations of these grasshoppers were kept for up to 3 weeks in the laboratory enabling females to lay eggs into dishes filled with moistened vermiculite. Male grasshoppers used in our study either derived from the same clutches or were caught as subadults in grassland areas around Göttingen. Eggs were kept at 4 °C for at least 4 months. Hatching occurred after approximately 1 week at 26 °C and the nymphs were fed with grass and supplemental food for crickets (Nekton, Pforzheim, Germany) ad libitum. After moulting into the fourth nymphal instar, fully intact females were randomly selected from the rearing cages and separated from males. The grasshoppers were reared and maintained at 20–26 °C and photoperiods of 16 h light and 8 h darkness.

2.2. Determination of males' mating readiness

In order to select *Ch. biguttulus* males with high mating readiness as potential reproductive partners in subsequent experiments we stimulated 14 mature males on 6 consecutive days after their isolation from females with the recording of a female song. Individual male grasshoppers were transferred to an arena that was heated by a heating lamp from above to constant 30 °C. The record of female stridulation was presented at an intensity of 60 dB SPL to the right or to the left side of the body via a loudspeaker (Sound Craft Conrad DT 25 P). The acoustic stimulus consisted of 27 repetitions of a female song sequence of 1.5 s duration separated by 3.3 s long pauses. Recognition of female song elicits a uniform series of male reactions (von Helversen and von Helversen, 1983). We rated the intensities of the males' reactions to stimulation with female song with the following scores: 0, no reaction; 1, stridulation; 2, turning toward the sound source; 3, jumping or walking toward the sound source. The maximal response of each individual male on each day following separation from females was determined and the days were ranked with respect to the maximal response intensities (higher ranks accord to higher maximal responses; equal ranks were assigned to days with equal maximal responses). After an overall Friedman test, a post hoc Wilcoxon–Wilcox test for multiple comparisons of matched samples was applied to compare the rank sums of the different experimental days. Males used in this experiment were not included in the experiments with females described above.