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Courtship behaviour and vibrational communication of the planthopper *Apartus michalki* (Wagner, 1948)

(Hemiptera: Fulgoromorpha: Cixiidae)

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Summary: Cixiidae require a set of clues in order to recognize and localize potential conspecific partners for mating (specific mate recognition system = SMRS). The use of acoustic and/or vibrational signals as part of the SMRS is ubiquitous in Hemiptera. However, the general knowledge of the mating behaviour of Cixiidae is still patchy. Here we report for the first time evidence for vibrational communication in *Apartus michalki* along with observations of the courtship behaviour.

Key words: specific mate recognition system (SMRS), bioacoustics, biotremology

1. Introduction

Within the Hemiptera, the largest hemimetabolous insect taxon with more than 100.000 described species (Zhang 2013) the family Cixiidae is a comparatively diverse group comprising around 2500 species (Hoch 2013). Despite the worldwide distribution and enormous morphological and ecological diversity and the fact that some species are of economic importance (Alma 2002, Holzinger et al. 2002, Mori et al. 2012, Wilson & Turner 2010) still little is known about the behaviour of cixiids. Like all sexually reproducing organisms Cixiidae require a set of clues (optical, mechanical, or a combination thereof) in order to recognize and localize potential conspecific partners for mating – the so-called specific mate recognition system (SMRS) (sensu Paterson 1985, see also Claridge 1993). One ubiquitous feature in Hemiptera as part of the SMRS is the production and use of acoustic and vibrational signals (Ossiannilsson 1949; Claridge 1985, 1993; Drosopoulos & Claridge 2006; Hoch et al. 2006; Wessel et al. 2014). But so far only a limited number of studies have documented vibrational communication in Cixiidae: Tishechkin (1997, 1998, 2003, 2012), Howarth et al. (1990), Hoch & Howarth (1993), Hoch & Wessel (2006), Hoch et al. (2011, 2013), Mazzoni et al. (2010). The best-studied examples of intraspecific communication in Cixiidae are from highly modified, cave-dwelling lineages, e.g. the Hawai'ian cave planthopper *Oliarus polyphemus* Fennah, 1973 and the Australian *Solonaima* Kirkaldy, 1909 (for review see Hoch & Wessel 2006). Under special conditions, as for example after the colonization of new habitats, sexual selection acting directly upon the mating behaviour may even drive superfast speciation processes in cave planthoppers (Hoch & Howarth 1993, Wessel & Hoch 1999, Wessel et al. 2013).

Though, the general knowledge of the mating behaviour of epigeic species of Cixiidae still remains patchy (Tishechkin 1997, 1998, 2003, 2012; Mazzoni et al. 2010). One reason is due to the fact that Cixiidae are somehow difficult to handle study objects. The nymphs are

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subterranean and live in the soil or leaf litter and emerge only as adults. Therefore they are not easy to rear (Müller 1942, Hoch & Howarth 1993). Moreover, the adults of many species occur usually only in low abundance and are very fugitive.

One of the few European cixiid species that may occur in higher abundance is *Apartus michalki* (Wagner, 1948) (Fig. 1). It is endemic to the southern limestone Alps and adjacent areas between 250 and 1200 meters above sea level in southern Austria, Northeast Italy and Slovenia feeding on pine trees in thermophilous *Erica carnea*-Pine forests (=Erico-Pinetea) (Fig. 2). Adults of *A. michalki* appear quite early in the year and are locally often the dominating species during spring (Holzinger 1999, 2002; Holzinger et al. 2002). Here we provide for the first time evidence for vibrational communication in this species.

Fig. 1: Habitus pictures of *Apartus michalki* (male) (Photo by Gernot Kunz).

Fig. 2: Habitat of *A. michalki*. Schütterwald, Dobratsch Mt., 23.03.2007 (Photo by Gernot Kunz).

2. Material and Methods

2.1 Animals

To obtain data on the mating behaviour, a total of about 200 adult specimens were collected at the slopes of Dobratsch Mt., Carinthia, Austria (Figs. 2-3; locality No. 3 in Holzinger 1999) in the years 2000 (March 3, April 5) and 2001 (April 6). They were kept in the laboratory in cages (size 25cm x 25 cm x 75 cm and 25cm x 45cm x 100cm) on twigs of their host plants (*Pinus sylvestris*) and observed all day.

For the purpose of recording courtship signals, adults were collected at the same locality as mentioned above on March 23, 2007. Adults were immediately separated by sex to ensure unmated males and females. The insects were then transferred together with their host-plant to a refrigerated box (5° Celsius) and shipped to the bioacoustics laboratory of the Museum für Naturkunde Berlin (Germany).

2.2 Acoustic recordings

Vibrational signals were recorded in the bioacoustics laboratory (Museum für Naturkunde Berlin) between March 26-28, 2007. The average room temperature was 20° Celsius. The recording device used was a magneto-dynamic system (Fig. 4) as designed and described by Strübing & Rollenhagen (1988). For recording purposes the insects were kept in a small plastic box (Fig. 5). Twigs of *Pinus sylvestris* and *P. nigra* respectively served as feeding plants and substrate for the acoustic experiments. The plastic box with the animals was then placed in a bigger box filled with sand to reduce external vibrations (Fig. 5). The signals received were registered directly on magnetic tapes (Sony UX Position Chrome 60, IEC II/Type II, High Bias 70 µs EG) using a Sony audio recorder (TC-D5 M) (Fig. 4). The sound records were analysed using Raven Pro 1.5 (Bioacoustics Research Program, 2011) and Avisoft-SAS Lab Pro, v. 5.1.16 (Specht 2011). Sample sound files are deposited in the Tierstimmenarchiv (TSA, Animal Sound Archive; Museum für Naturkunde, Berlin, Germany); the TSA accession number is Apartus_michalki_DIG0168_04.

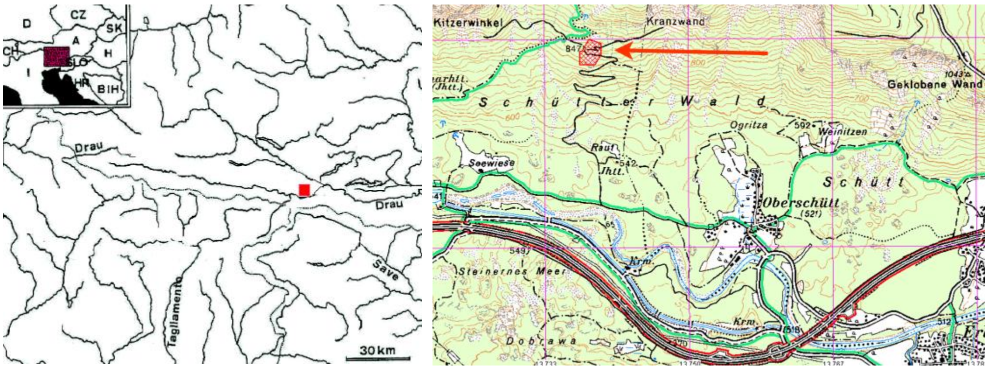

Fig. 3: Distribution area of *A. michalki* (left) and collecting area (right). The red square and red arrow is indicating the collecting area of the present study (after Holzinger 1999).

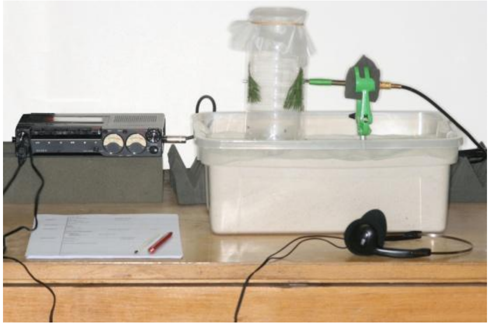

Fig. 4: Acoustic recording setup.

Fig. 5: Plastic box with twigs of the food plant.

2.3 Experimental setup

Sound production of males and females was tested in different combination. The following constellations have been used:

M = several males (no females) to identify the male calling signal.

F = several females (no males) to identify the female signals.

D = duetting couple: 1 male and 1 female to document the acoustic interactions between both sexes.

G = several males and several females (roughly equal numbers) mainly to identify acoustic interactions between a group of individuals.

3. Results

3.1 Mating behaviour

Caged adults did not show mating behaviour during daytime (the cages were kept in direct sunlight), but started mating only during dawn, between 19:00 and 22:00. Mating behaviour started with motionless sitting of males and females on the same twig in distances of few centimetres for some time. Then the male often moved towards the female and started to touch it with his fore legs. For this purpose, he sat parallel to the female and put his leg or legs on the thorax of the female. Then, in most cases, the female just walked away. In those cases when the female stayed, the male climbed onto the back of the female and mounted his genitalia towards the female's genitalia. Then he tilted backwards and stayed there, upside down, with his head pointing away from the female (retroconjugate copula, see Fig. 10 in Holzinger 2002).

3.2 Vibrational signals

Spontaneous calls produced by males could be observed and registered in all setup combinations where males were present (M, D and G, for details see Tab. 1). Female signals occurred only in the presence of several males (setup combination G). Duetting males and females could be recorded several times in combination G (Fig. 6). The duets were always initiated by the common male calling signal, which has an average length of 1.90 seconds (range 1.70-2.70 seconds) and a frequency between 300-900 Hz (mean frequency 375 Hz). The females replied immediately (0.02 seconds; range 0.02-0.03 seconds) after the male signal stopped and the female responding signal (mean frequency 750 Hz, range 700-800 Hz;

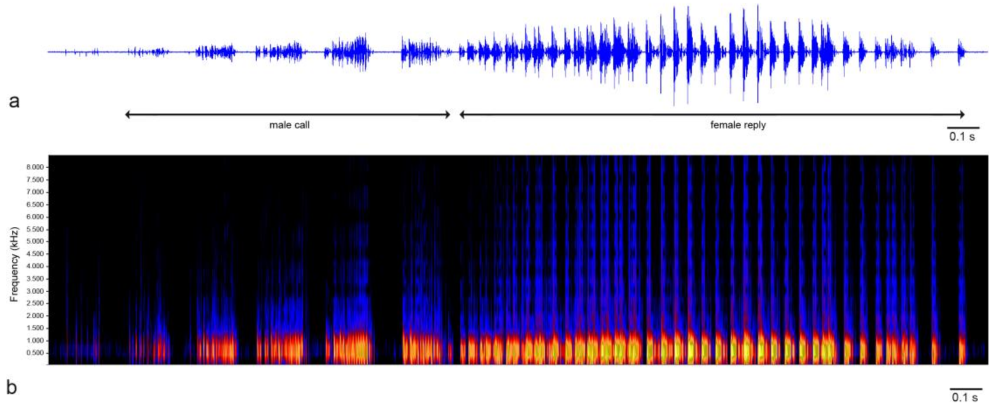

Fig. 6: Oscillogram(a) and sonogram (b) of a duetting male and female of *Apartus michalki*.

Table 1: Log of the accoustic recordings of *A. michalki*. For abbreviations see text (2.3 Experimental setup).

Record	Date	Time	Group	No. males	No. females	Observed vibrational communication
1	26.03.2007	13:00-14:30	D	1	1	Male calling signal
2	26.03.2007	16:20-17:05	D	1	1	Male calling signal
3	26.03.2007	17:10-18:05	D	1	1	Male calling signal
3	26.03.2007	18:15-18:50	G	6	2	Male calling signal
4	26.03.2007	19:15-20:15	G	8	10	Male calling signal, duet
5	26.03.2007	20:15-21:00	G	8	10	Male calling signal, duet
6	27.03.2007	15:30-15:55	D	1	1	-
6	27.03.2007	15:58-16:30	D	1	1	-
6	27.03.2007	16:35-17:10	D	1	1	-
6	27.03.2007	17:30-18:25	M	10	-	Male calling signal
6	27.03.2007	18:40-19:37	F	-	12	-
7	27.03.2007	19:45-20:32	G	1	12	Male calling signal
7	27.03.2007	19:45-20:32	G	1	2	Duet?
8	27.03.2007	20:36-21:06	G	1	12	Male calling signal, duet
8	27.03.2007	21:06-21:29	G	2	13	Male calling signal
9	28.03.2007	12:25-12:45	F	-	8	-
9	28.03.2007	12:52-13:45	G	1	8	Male calling signal
10	28.03.2007	13:51-14:21	G	1	8	-
10	28.03.2007	15:26-16:18	G	2	8	Male calling signal, female call, duet
11	28.03.2007	16:20-17:20	G	2	8	Duet
12	28.03.2007	17:13-17:53	G	2	8	Male calling signal
12	28.03.2007	17:55-18:40	G	10	8	Male calling signal

total length 2.20 seconds, range 1.59-2.80 seconds) consisted of around 40 short bursts. The females always stayed in place during the dueting, while the males approached them.

No spontaneous female signals could be observed. But one female was emitting a different signal while a male was approaching (distance about 3 centimetres). This signal was 2.60 seconds long with a frequency between 700-800 Hz.

4. Discussion

Our observations on the mating behaviour of *Apartus michalki* can only be regarded as preliminary. Although it is the first evidence that this species uses intraspecific vibrational communication. It seems remarkable that in some cases copulations were observed without any obvious previous vibrational communication. This fact may be a hint that vibrational communication is only one aspect of the specific mate recognition system in this species. In well-studied blind cave cixiids on the other hand successful mating could only be observed in connection with of vibrational signals (Howarth et al. 1990, Hoch & Howarth 1993, Hoch & Wessel 2006). Very likely visual, or even chemical communication might play a crucial role in successful mating in *A. michalki*.

The signal pattern is relatively simple compared to other Fulgoromorpha (e.g. Delphacidae) but our results corroborate hitherto documented song pattern in other Cixiidae species (e.g. Tishechkin 1997, 1998, 2003, 2012, Howarth et al. 1990, Hoch & Howarth 1993, Hoch & Wessel 2006, Hoch et al. 2013, Mazzoni et al. 2010). According to our observations the courtship behaviour is always initiated by males. They are calling spontaneously waiting for a response of a congeneric partner. If the partner, in our case the female, is responding, the male repeats his call while trying to locate the female which remains stationary. This behaviour is well known in Hemiptera and often referred to the "call-and-fly" strategy (Gwynne 1987, Hunt & Nault 1991). If the female and male are close to each other some other recognition signals might be important such as visual, tactile or possibly also chemical signals.

5. Zusammenfassung

Paarungsverhalten und Vibrationskommunikation von *Apartus michalki* (Wagner, 1948) (Hemiptera: Fulgoromorpha: Cixiidae). – Cixiidae benötigen, wie alle sich sexuell reproduzierenden Taxa, eine Reihe von Erkennungsmerkmalen, um potentielle Paarungspartner zu identifizieren und zu lokalisieren (specific mate recognition system = SMRS). Zwar ist der Einsatz von akustischen und/oder substratgebundenen Signalen als Teil des SMRS bei Hemipteren bekannt und weit verbreitet, die Kenntnisse zum Paarungsverhalten bei Cixiiden sind jedoch noch lückenhaft. In diesem Beitrag dokumentieren wir zum ersten Mal die Vibrationskommunikation bei *Apartus michalki* zusammen mit weiteren Beobachtungen zum Paarungsverhalten dieser Art.

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