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A CHARACTERISATION OF THE PAIR FORMING ACOUSTIC SIGNALS OF *ISOPHYA HARZI* (ORTHOPTERA, TETTIGONIOIDEA, PHANEROPTERIDAE)*

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This study gives the first description of the male calling song and male-female duet of *Isophya harzi*, an Eastern European bush-cricket species known as an endemism of the Cozia Mountains (Southern Carpathians, Romania). The male calling song is a long sequence of syllable groups. Each group is composed of two syllable types and the song can be formulated as A...A – BA...A – BA...A – B (where "A...A" means a varying number of "A" syllables, and "–" means a longer inter-syllable interval). Females emit their short response songs after the "B" syllables of the male song with a response delay of 125–186 ms from the beginning of the male syllable. The male calling song has a wide band frequency spectrum with intensive components between 15–30 kHz. The oscillographic pattern of the male song resembles that of the songs of *Isophya beybienkoi* and *I. posthumoidalis*. However, syllables are distinctively longer, repeated at a lower rate and "A...A" syllable groups contain less syllables in *Isophya harzi* than in the other two species. The stridulatory file consists of 100–130 pegs. Our results support the validity of the specific status of this narrow range, vulnerable bush-cricket.

Key words: song pattern specificity, oscillogram, male-female duet, *Isophya posthumoidalis*, *Isophya beybienkoi*

INTRODUCTION

With a number of species approaching 50 *Isophya* is the second most species rich bush-cricket genus in Europe (HELLER *et al.* 1998). Most species are rather similar to each other regarding their morphology (HARZ 1969, HELLER *et al.* 2004). Morphological uniformity makes identification rather difficult in species with sympatric distribution and rise doubts about the taxonomic validity of some of the species with allopatric areas. Contrary to morphology the rhythmic structure of the sexual acoustic signals of males often shows clear differences even when

* To the memory of BÉLA KIS (1924–2003), the excellent investigator of the Transylvanian Orthoptera.

comparing the morphologically most closely related species (HELLER 1988, INGRISCH 1991, HELLER *et al.* 2004, ORCI *et al.* 2005). As usual in phaneropterid bush-crickets pair formation is achieved during an acoustic duet of the male and female (ZHANTIEV & DUBROVIN 1977, ZHANTIEV & KORSUNOVSKAYA 1990). Females respond to males with their simple clicks within a strictly defined, specific time-window (in relation to the male song) (HELLER & VON HELVERSEN 1986, ROBINSON *et al.* 1986, ZHANTIEV & KORSUNOVSKAYA 1986). Female preferences for specific male song characters together with male song specificity seem to be significant components of the species specific mate recognition system of these insects (ZHANTIEV & KORSUNOVSKAYA 1977, 1990, ORCI 2007). Hence examining the acoustic signals of these bush-crickets is often the most effective way to overcome problems in identification and to clarify taxonomic relations.

An other important aspect why describing acoustic signals in *Isophya* is useful is the acoustic detection of animals. It is often rather difficult to find specimens in the field because insects may be missing from many habitat patches that looks suitable for them, and if they are present, population density may be rather low. Moreover, the standard sampling methods used in orthopterology are often inappropriate to collect *Isophya*, because there are a number of species that prefer tall, dense vegetation (BAUER & KENYERES 2006) where collecting either by sweeping net or by dish traps is rather ineffective (NAGY *et al.* 2007).

The most comprehensive overview of our knowledge on the acoustic signals of *Isophya* can be found in HELLER (1988). His work have been followed by numerous papers presenting descriptive and comparative studies of the songs of previously unexamined species of the genus (INGRISCH 1991, ORCI *et al.* 2001, ORCI & HELLER 2004, SEVGILI *et al.* 2006, CHOBANOV 2009) However, there are still a number of species where we know nothing about the features of the acoustic signals. *Isophya harzi* KIS, 1960 was one of those species.

Isophya harzi was described by KIS (1960) from the Cozia Mountains (Southern Carpathians, Romania), and since that time the species was not reported anywhere outside that area (IORGU *et al.* 2008). The species is considered as a recently isolated local endemism by KENYERES *et al.* (2009). KIS described the species clearly on a morphological basis. He noticed its close relationship to *I. camptoxypha* (FIEBER, 1853) (named *I. brevipennis* BRUNNER VON WATTENWYL, 1878 at that time, see HELLER *et al.* 2004) regarding the morphology of males, and emphasized that *I. harzi* has a larger body and longer ovipositor. We decided to examine acoustic communication in this interesting species to clarify its taxonomic status and to help its recognition and detection outside the type locality. We hope our study will help efforts to protect and save this interesting insect species listed in the IUCN Red List of Threatened species.

MATERIAL AND METHODS

The Cozia Mountains are situated at the southern feet of Masivul Fogarasului (near the village Călimăneşti, Southern Carpathians, Romania). We visited the type locality on 27th July 2003 and found a population of the species at the top region of the mountain at an elevation of 1300–1400 m a.s.l. Specimens were collected from a steep rocky slope covered by grassland patches mixed with bushes (*Juniperus*) and trees (*Pinus, Betula, Carpinus*). Specimens were found by visual and acoustic direct searching. The male calling songs were studied in six males, female response songs were recorded and examined in three females. Sound recordings were made using a Sony TCD-D8 DAT recorder (working at 48 kHz sampling rate and 16 bit amplitude resolution) connected to a Monacor ECM 921 electret condenser microphone or to a Pettersson D240X ultrasound detector. The latter device was used in its time expansion (10×, sampling frequency 307 kilosamples/s) mode to obtain wide-band recordings containing frequency components up to 120 kHz. Male song recording were made both in the field and indoors. To elicit response songs from females male calling songs were played back from a computer through an active PC speaker (Philips A 1.1; the upper limit of the frequency response of that system was 22 kHz).

Sound recordings were analysed and song parameters were measured using the software Adobe Audition 1.5.

Bioacoustic terminology

The bioacoustic terminology of this study is adopted mainly from RAGGE and REYNOLDS (1998). Calling song: spontaneous song produced by an isolated male.

Syllable: the song produced by one opening-closing movement cycle of the tegmina.

Impulse: a simple undivided transient train of sound waves (here: the highly damped sound impulse arising as the impact of one tooth of the stridulatory file).

Click: an isolated, distinct impulse.

Functional unit of the song: the shortest part of the song, which contains all necessary song elements and in the appropriate order to elicit female response.

Sonometric characters

The songs of these bush-crickets are predominantly amplitude modulated signals. Therefore mostly rhythmic characters, that are durations and counts (repetition rates can be calculated from these) were examined in details.

The following characters were measured. Abbreviations refer to Table 1. The duration of syllables were measured from the beginning of their first pulse to the beginning of their last pulse (D7, D8), the last pulse was included in the number of pulses per syllable (N2, N3) but after-clicks were not included. The delay of after-clicks were measured from the beginning of the last pulse of the preceding syllable (DaclA, DaclB). The durations of the inter-syllable intervals were measured from the beginning of the last pulse of the preceding syllable to the beginning of the first pulse of the following syllable (D3, D4, D5, D6) and the duration of "A...A" sequence from the beginning of its first syllable to the beginning of its last syllable (D2). The duration of the presumed song unit was measured from the beginning of its first syllable to the beginning of the next unit (D1). The delay of the female response was measured from the beginning of the preceding B-syllable of the male to the beginning of the response song of the female. (For a graphic presentation most of the measured characters see ORCI *et al.* 2001: Fig 1.)

RESULTS

Males produce their song by tegmino-tegminal stridulation. The stridulatory files of three examined specimens were 2.8, 2.9, 3.0 mm long and contained 101, 114, 128 stridulatory pegs. Singing activity is typical during afternoon and night.

The male calling song of *Isophya harzi* is a long series of syllable groups (Figs 1–3). Each syllable group is composed of two syllable types. One group contains one "B" and 1–4 "A" type syllables (see the formulation under the oscillogram Fig. 1). The two syllable types differ from each other only slightly: "B" syllables have longer duration and are composed of a higher number of impulses (Table 1). Moreover, after-clicks are produced with a longer delay after the main impulse series in "B" syllables than in "A" syllables (Table 1). Nevertheless, both syllable type has the same basic oscillographic structure: a main impulse series is followed by an after-click (Figs 4–8). In the main impulse series impulses are repeated evenly, however, in one male impulse repetition rate decreased gradually toward the end of the impulse series (Fig. 5).

 Table 1. Descriptive statistics for some sonometric characters of the male calling song of *Isophya* harzi (durations were measured in 3 specimens, counts were measured in 5 specimens; durations are given in milliseconds).

		Ν	ne	mean	min.	max.	intraSV
Duration of the [AA-B] unit	D1	3	25	1240.8	1001	1554	133.99
Number of "A" syllables in "A" sequences	N1	5	25	2.2	1	4	0.45
Duration of "A" sequences	D2	3	25	288.3	205	530	91.98
Duration of A-A interval	D3	3	25	154.7	122	182	12.70
Duration of the last A-A interval in "A"	D4	3	25	155.2	122	182	13.69
sequence							
Duration of A-B interval	D5	3	25	566.1	392	765	82.26
Duration of B-A interval	D6	3	25	226.6	206	261	14.08
Duration of "A" syllable	D7	3	54	91.3	78	107	7.01
Number of impulses in "A" syllable	N2	5	54	39.5	25	53	3.24
Delay of after-click 1. following "A" syllable	DaclA	3	12	43.3	24	75	17.92
Duration of "B" syllable	D8	3	25	109.3	99	120	3.79
Number of impulses in "B" syllable	N3	5	25	47.0	37	57	1.64
Delay of after-click 1. following "B" syllable	DaclB	3	25	119.57	71	183	23.39

N- the number of speciemens examined; Ne- the number of the song elements measured; Mean – the overall mean calculated from the means obtained for each specimen; IntraSV – intra-specimen variability -the mean of standard deviations obtained for each specimen; Min. – the minimum value amongst the original data measured for that character; Max. – the maximum value amongst the original data measured for that character

B AAA B... 50 msBAA BAA BAA ö BAA BAA BA A.A. 50 ms 100 ms $100 \, \mathrm{ms}$ 100 ms 2 S ä ä ÷ ق ï ö



The male calling song is basically an amplitude modulated signal. The carrier wave has its most intensive components between 20–30 kHz, but frequency components with detectable sound pressure spread over the frequency range between 10 kHz and 60 kHz (Fig. 9).

Females emitted their short response songs exclusively after the "B" syllables of the male song (Fig. 16). The response delay of female songs from the beginning of the male "B" syllable varied between 125–186 ms (mean 158.3 ms; based on the measurement data of 39 response songs from 3 females, ambient air temperature varied between 25.7–26.3). In two females, where more than 10 answers could be recorded, response delay was negatively correlated with male syllable duration, if the delay was measured from the end of the male syllable (female 1: r = -0.773, p < 0.000, n = 23; female 2: r = -0.623, p = 0.041, n = 11), but it was independent of syllable duration, when measured from the beginning of the male syllables (female 1: r = -0.207, p = 0.342, n = 23; female 2: r = -0.232, p = 0.492, n = 11). Those results suggest that female response is timed to the beginning of the "B" syllables. Female responses divide the male song into functional units, which can be formulated as [A...A–B] in contrast to the apparent [BA...A] syllable groups of the song (Fig. 16).



Fig. 9. Frequency spectrum of the male calling song of *Isophya harzi* (FFT size 2048, window-function Blackmann-Harris) based on a sound sample recorded using a Pettersson D240x ultrasound detector in the field (ambient temperature 19.7 °C).

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DISCUSSION

Regarding its basic oscillographic structure the male calling song of Isophya harzi resembles to the songs of Isophya posthumoidalis BAZYLUK, 1971 and I. beybienkoi MAŘAN, 1958 (Figs 10-15). All of those species produce two syllable types (Figs 13-15) during their songs ("A" and "B" syllables) in a pattern that can be formulated as AAA...A – BAAA...A – BAAA (ORCI et al. 2001, HELLER et al. 2004). Beside that similarity in basic structure the song of *I. harzi* differs clearly from the songs of those two species in several characters. In Isophya harzi the "AAA...A-B" song units contain less A-syllables (only 1-4, Table 1) than in I. beybienkoi or I. posthumoidalis. Both "A" and "B" syllables are longer containing a higher number of impulses, syllable repetition rate is lower and inter-syllable intervals are longer in I. harzi than in the other two species (for sonometric data on the songs of I. beybienkoi and I. posthumoidalis see ORCI et al. 2001 and HELLER et al. 2004). Moreover, "A" and "B" syllable-types are less differentiated, and contrary to the other two species here it is the "A" syllable, which tends to be shorter (Table 1), while in the other two species "B" syllables are approximately equal in length (I. beybienkoi, Fig. 14) or shorter (I. posthumoidalis, Fig. 15) than A-syllables. Regarding male morphology I. harzi is close to Isophya camptoxypha as it was noted by KIS (1960), but that species produce a relatively simple calling song containing only one type of syllable repeated evenly in long sequences (NAGY et al. 2003, HELLER et al. 2004, ORCI 2007). Moreover, the syllables of I. camptoxypha are much shorter (half of the duration of the I. harzi "A" syllables). A recently described species I. fatrensis CHLÁDEK, 2007 is also close to I. harzi regarding its morphology, but a verbal description of the song suggests, that the male signal of that species is similar to I. stysi CEJCHAN, 1958 or I. modestior BRUNNER VON WATTENWYL, 1882 regarding the syllable repetition pattern of the song (CHLÁDEK 2007). Those species produce short syllable-sequences (2–8 syllables) containing one syllable type and separated by long (several seconds) intervals (ORCI et al. 2005).

The stridulatory file of *I. harzi* contains a higher number of stridulatory pegs (100–130) than the stridulatory files of closest relatives (*I. beybienkoi*: 60–75, *I. posthumoidalis*: 70–80, *I. camptoxypha*: 50–80 [see HELLER *et al.* 2004 for SEM photos and more detailed data on the files of those species], *I. fatrensis*: 70–90 [CHLÁDEK 2007]). This finding is interesting especially because it shows that the number of stridulatory pegs can be used as a differential character when identifying dead specimens, where acoustic examination is impossible. Similar examples, where stridulatory file bears the the most reliable differential characters in mor-

phology comparing closely related species have been reported in other groups of singing Orthoptera also (e. g. RAGGE & REYNOLDS 1988, HELLER 1988, VEDENINA & VON HELVERSEN 2009, VEDENINA *et al.* 2009).

Females emitted their response songs exclusively after the "B" syllables of the males during the B–A inter-syllable intervals, just like as it is in *I. beybienkoi* (ORCI *et al.* 2001) and *I. posthumoidalis* (our unpublished results). That response pattern suggests, that the male songs of those three species are not only structurally similar, but the function of song elements may be the same. However, the functional similarity does not necessarily means close phylogenetic relatedness, because that male song structure and female response pattern can also be found in other phaneropterid genera such as *Ancistrura* and *Barbitistes* (DOBLER *et al.* 1994, STUMPNER & MEYER 2001). Therefore we think a thorough molecular phylogenetic examination would be needed to clarify the evolutionary relationships of those three *Isophya* species.

Unfortunately we do not have well established results on the function of "A" and "B" syllables in either of those species. We expect that their function is similar to that found in *Ancistrura nigrovittata* BRUNNER VON WATTENWYL, 1878 by DOBLER *et al.* (1994). In that bush-cricket species "A...A" syllable groups convey information, which is important for species recognition and the main function of "B" syllables is to trigger female response. Play-back experiments with modified male song samples would be needed to reveal the function of male song elements in these *Isophya* species.

Our results confirm the validity of the specific status of *Isophya harzi*, since the clear differences of the male calling songs from those of the morphologically related species suggest the incompatibility of their mate recognition systems. The presented song description may be used during taxonomic works dealing with morphologically similar members of the genus. We hope our study will help orthopterologists to identify and detect this interesting species during faunistic or ecological studies and also during research activities (e. g. population censuses or data collection for fine scale distribution maps etc.) aimed at protecting this insect species.

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