DESCRIPTIONS OF THE CALLING AND COURTSHIP SONGS OF TIBICEN ESFANDIARIII DLABOLA, 1970 (AUCHENORRHYNCHA: CICADIDAE)

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The calling song of *Tibicen esfandiarii* Dlabola, 1970 is described for the first time. The calling song consists of a continuous train of syllables (3–9 ms) and short intersyllable intervals (0.4 to 1.9 ms). The song begins at a lower intensity, increasing intensity through a series of steps to the ultimate portion of the call that is produced for an extended period at this loudest intensity. The song terminates after a short pause (2 ms) that is followed by about 0.5 s of singing. The total syllable repetition frequency is 193.1±60.24 Hz (n=48 measurements from six individuals) and the frequency of the average peak energy of calling song is 4875±59.45 Hz (n=6 individuals) and courtship songs (n=15 parts of call from one individual) is 4875±0 Hz. The courtship song consists of alternative phrases usually starting after a short pause (average of 70.8±4.85 ms) and syllable frequency reducing from the average of 157.0±7.1 Hz to 128.4±1.67 Hz. The duration, frequency of syllables and the average RMS amplitude in different portions of the song and in different specimens are measured and the behavior of the specimen during song production is described. We show significant differences in the frequency and temporal components of *T. esfandiarii* from its congeneric species, *Tibicen plebejus* (Scopoli, 1763), with which is shares part of its range in Iran.

Key words: calling song, courtship song, *Tibicen esfandiarii*.

INTRODUCTION

*Tibicen esfandiarii* Dlabola, 1970 is a large, endemic cicada of southern Iran (Mozaffarian & Sanborn 2010, 2016). Although the largest of the Iranian cicadas, little is known about the biology of the species. Dlabola (1970) provided no information on the biology of the species with the description of the species and Abaii (2000) reported the species as a pest on *Quercus* spp., but most references to the species are in the listing of it as part of a broader study (Nast 1972, Mirzayans et al. 1976, Dlabola 1981, Mirzayans 1995). Distributional data showed the species only inhabits southern Iran and is found at altitudes between 210–2291 m above sea level and the species was reported to emerge in large densities in some years (Mozaffarian & Sanborn 2016). Information on the species call is currently lacking.
A detailed study (Mozaffarian & Sanborn 2016) of the morphology and distribution of *T. esfandiarii* and *T. plebejus* (Scopoli, 1763) inhabiting Iran showed that although the two species are very similar morphologically and there is some overlap in the extremes ranges of color and morphometric characters of the two species, there are highly significant ($p < 0.0001$) differences in the morphometric traits and distributions of the two species. The calling songs of cicadas are known to be species specific and analysis of the song would provide additional data to distinguish the species in Iran as well as basic information about *T. esfandiarii*. This study is a description and analysis of the calling and courtship songs of *T. esfandiarii*. We provide comparative analysis to *T. plebejus* from Iran in order to facilitate distinguishing the two Iranian species.

We have retained the usage of *Tibicen* Latreille, 1825 as the valid genus throughout the paper. There is currently a proposal under consideration by the International Commission of Zoological Nomenclature to determine whether *Tibicen* or *Lyristes* Horváth, 1926 should be used for members of the genus (Boulard & Puissant 2014, Marshall & Hill 2014, Sanborn 2014, Marshall 2015, Puissant et al. 2015). There is currently no decision by the Commission.

**MATERIAL AND METHODS**

The calling and courtship songs of *T. esfandiarii* were recorded in Khabr National Park and villages in the region of the park in Kerman province, Iran in 2013 and Kavar, Fars province, Iran in 2017. Songs were recorded with a Zoom Handy Recorder H4 at a sampling rate of 96 KHz and 24-bit resolution, microphones with ±3 db sensitivity up to 11–12 kHz whereupon the frequency response decreases. In 2013 (in Kerman province) the microphones were located closer than twice of the body length of the specimens. Therefore, the recordings were not included in the statistical analyses provided here due to the potential near field effect based on the proximity of the microphone recording. In 2017 (in Fars province), the microphones were located in the far field approximately 100 cm from the calling individuals and a total of 10 min and 1.569 s of the calling songs of 6 specimens and 6 min and 56.544 s of the courtship song of one specimen were recorded. Notes on the behavior of the cicadas during singing were also made. The recorded song files were analyzed using Adobe Audition CS6 Version 5.0, the durations of different portions of the songs, syllables and intersyllable intervals, pauses, the frequency of syllables in any individual and in different portions of the songs, the average peak call energy (frequency of maximum intensity) and the average RMS amplitude in different parts of the song were calculated. Sections of the call with minimum background noise were selected and scanned in order to calculate the amplitude for an individual song segment. No filters or other sound manipulating programs were used when recording or analyzing the calls. The collected specimens were deposited in Hayk Mirzayans Insect Museum, Tehran, Iran (HMIM). We apply standard bioacoustics terminology to the description of the songs. The song is produced as phrases are repeated for extended periods of time. Each phrase is composed of a series of repeating syllables, the time between syllables is the intersyllable interval. Individual syllables are produced by the buckling of the timbal and timbal ribs.
RESULTS

Location, time and vegetation – The calling song of *T. esfandiarii* was recorded from 6 specimens calling from *Amygdalus* sp. for a total of 18 min and 5.025 s in Kavar, 29°3.911′N 52°38.494′E, 1755 m, Fars province, 26 July 2017. Recording began at about 11:00 AM with the final call being recorded prior to the termination of the calling period at about 14:00 PM, in Firouzabad, Khergheh as well, close to the collecting site. Singing *T. esfandiarii* specimens were observed in Kerman province during July 2013 on *Juglans* sp. and *Prunus* sp.

Calling behavior and male density – In both localities, the cicadas were singing with their head upward with the body axis aligned along the trunks and branches of the trees. Intraspecific competition while calling significantly influences escape behavior in *T. esfandiarii*. After a mass emergence in Kerman province during 2013, several specimens were observed singing from the same tree. Their sensitivity to environmental threats was very low and they could be collected easily by hand a few days after the mass emergence began to decline. However, during the much lower density Fars province emergence in 2017, single male specimens were observed singing on any tree and they flew away when they heard any small noise while being approached.

Calling song – The calling song of *T. esfandiarii* is a continuous steady buzz without any wing clicks. The beginning of a song bout was recorded in two specimens, one in 2017 and one in 2013 (Figs 1a,b). The calling song started with a train of syllables with short intersyllable intervals increasing to maximum amplitude in only a few steps. At the beginning, the average relative RMS amplitude was equal to −31.4 dB for 2 s. The RMS amplitude increased to −19.78 dB for 4 s, the song continued with the average RMS amplitude of −15.44 dB for 7 s and finally continued with an average relative RMS amplitude of −11.44 dB for the remainder of the calling bout (Fig. 1a). A similar song pattern was recorded in 2013 from this species in Kerman province (Fig. 1b) illustrating consistency in song pattern over the geographic range of the species.

Detailed analysis of the audio files showed the calling song is a continuous train of syllables with lower amplitude oscillations at the end and short intersyllable intervals between them (Figs 2a–e). Every syllable consisted of two higher amplitude parts followed by lower amplitude oscillations to the pulse terminus (Figs 2a–c). The syllables are separated by intersyllable intervals of 0.4 to 1.9 ms (average 1.36±0.45, n = 42 measurements in 5 individuals). In the higher amplitude portions of the song (Figs 2c–e), the amplitude of the terminal parts of both sections of the syllable and intersyllable interval increased (Figs 2c–d) and intersyllable intervals in some cases became rather
unclear (Fig. 2e). The duration of intersyllables were decreased as the song amplitude increased. Intersyllable duration was determined to be 0.7–1.9 ms (average 1.53±0.36 ms, n = 32 measurements in 3 individuals) in lower amplitude portions of the call (as in Figs 2a–b), and 0.4–1.05 ms (average 0.8±0.19 ms, n = 10 measurements in 2 individuals) for higher amplitude portions of the call (as in Figs 2c–e). The change in amplitude during call initiation is illustrated with the following example. The range of average relative RMS amplitude in syllables with recognizable parts (Figs 2a–b) was measured between −17.67 to −12.02 dB with an average of −13.66±1.70 dB (n = 17 measurements in 3 individuals). The range of average relative RMS amplitude within syllables as shown in Figs 2c–e was −12.55 to −7.54 dB with an average of −9.83±1.68 dB (n = 10 measurements in 3 individuals). The range of syllable duration was 3 to 9 ms in all song segments but the average varied slightly from 4.56±1.59 ms (n = 16 measurements) for Figs 2a–b to 3.11±1.17 ms (n = 9 measurements) for the louder portions of the call (Figs 2c–e).

Syllable frequencies showed less variation within than between individuals but was sometimes reduced in short periods in the same individual (Fig. 4).

Fig. 1. Initiation of a calling song of *T. esfandiarii*: a = Kavar (Fars province, July 2017), b = a similar song recorded in Godar-e Sabz (Kerman province, July, 2013)
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Fig. 2. Variation in the shapes of syllables by increasing the average RMS amplitude in any portion of the calling (a, c, d, e) and courtship song (b) of T. esfandiarii: a = –20.00 dB, b = –11.23 dB, c = –10.95 dB, d = –9.01 dB, e = –8.20 dB (Fars province, Kavar, July 2017). Time is indicated on the upper margin of each image.
The individual variation of syllable frequency (with total recording time) was determined to be: specimen no. 1 (4.747 s): 146±0 Hz (n = 3 measurements); specimen no. 2 (10 min and 15.031 s): 135 Hz to 147 Hz, but after 17 s, the frequency reduced to 106 Hz to 110 Hz, the average for all syllables being 137.37±12.43 Hz (n = 30 measurements); specimen no. 3 (4 min and 20.224 s): 191 Hz to 293 Hz, the average 232.6±33.54 Hz (n = 7 measurements); specimen no. 4 (4 min and 23.026 s): 198 Hz to 325 Hz, the average 256.4±44.64 Hz (n = 8 measurements). In specimens no. 5 (30.5 s) and no. 6 (2 min and 28 s), the interval oscillations grew and pulses could not be recognized easily for counting. The total syllable frequency average was calculated as 193.1±60.24 Hz (specimens no. 1 to 4). Within a single specimen, there was an increase in amplitude along with the increasing syllable frequency. However, comparing different specimens, a more intense song was not correlated with an increasing syllable frequency. The average peak call frequency in the 6 specimens was 4875±59.45 Hz (range of 4781–4969 Hz).

Calling song termination was recorded only in one specimen. The song paused for 2 ms prior to song termination phrase. The frequency of the sound syllables was consistent at 260 Hz 0.1 s before and after the pause. After the short pause, the song continued for 453 ms and then terminated.

Courtship song – While recording the calling song of one specimen, a female flew in near the calling male and sat on a branch of the same tree (Fig. 4). As soon as the female arrived, the continuous rhythm of the calling song shifted to an alternative courtship song composed of similar phrases (Fig. 5). The courtship song was recorded for 6 min and 56.544 s. Initially, the song paused for 113 ms. The pause altered the frequency of syllables, reducing it from 147 Hz to 129 Hz. In the next second, the syllable frequency returned.

Fig. 3. Variation of syllable frequencies among the songs of 4 specimens
to 149 Hz and then the syllable frequency reduced gradually over 6 s to 128 Hz. The reduction of syllable frequency was accompanied by a reduction of syllable amplitude. This courtship call was repeated as different phrases (from one pause to the next pause) as long as the female remained on the tree. The pattern of one of the courtship phrases is shown in Fig. 6. The two first phrases consisted of 5 cycles of alternative increasing and decreasing intensity before reaching the next pause at the beginning of the next phrase. However, in the following phrases, the intensity was reduced gradually towards the end of the phrase, sometimes with small short increases in intensity during the reduction in intensity. The duration of the pause at the beginning of the first courtship phrase (113 ms) was longer than the pauses at the beginning of the following phrases. The pause was reduced in the next phrases but it could be recognized at the beginning of all courtship phrases with the minimum of 64 ms and a maximum of 78 ms with an average of 70.6±4.85 ms. The average of the maximum and minimum syllable frequencies in phrases was calculated as 157.0±7.04 Hz (n = 5 mea-
measurements) and 128.4±1.67 Hz (n = 5 measurements) respectively. The average peak sound energy frequency in all courtship phrases did not vary and was equal to 4875±0 Hz (n = 15 measurements in one individual). The duration of the courtship phrases ranged from 15.3 s to 47.4 s with an average of 28.7±7.99 s (n = 13 measurements). The first phrase lasted 33.4 s and the second phrase lasted 47.4 s, each phrase consisting of 5 cycles. The range and the average of the cycle durations increased in the second phrases: In the first phrase, cycle durations ranged from 5.5 to 8.1 s with an average of 6.7±1.06 s (n = 5 measurements). In the second phrase, the cycle durations ranged from 8.2 to 11.9 s with an average of 9.5±1.47 s (n = 5 measurements). The following phrases lacked the obvious cycles of increasing and decreasing intensity. The duration for the third phrase was 15.3 s and increased rather irregularly to 36.9 s in the

Fig. 6. An expansion of a courtship song phrase of a male *T. esfandiarii* in the presence of a female on the same tree

Fig. 7. Changing pattern of the courtship song cycles with prolonged courtship song production in a male *T. esfandiarii* in the presence of a female on the same tree

Fig. 8. Return of song pattern from the courtship song to the calling song by a male *T. esfandiarii* after the female being courted flew away. The red line locates the short pause (55 ms) after female departed prior to resuming the calling song.
last phrase. The duration of syllables within the phrases was 6 to 9 ms as it was in the calling song. The duration of syllables increased as the amplitude of the phrase decreased. In the second minute of the male singing the courtship call in the presence of female, the duration of the phrases reached 31 s and while the syllable frequency and amplitude were reducing both syllable frequency and amplitude twice increased temporarily (Fig. 7) as the male moved slowly toward the female. The male extended his wings and the tip of his abdomen moved outward as the amplitude of the song increased. In the last phrase, the duration reached 38 s, temporarily increasing amplitude and syllable frequency three times as the male movements towards the female were repeated but the female flew away. As soon as the female departed, a short pause (55 ms) appeared in the male song, the syllable frequency reduced from 146 Hz to 133 Hz, then increased to 142 Hz as the song continued with the earlier continuous rhythm of the calling song (Fig. 8).

Comparison to T. plebejus song – The temporal pattern, syllable duration, and intersyllable intervals of T. esfandiarii and T. plebejus within Iran can be compared to T. plebejus using the data of Medhipour et al. (2015). The calling song of T. esfandiarii differs from the calling song previously described for T. plebejus in the temporal pattern (constant with phrases lasting for periods of minutes vs. amplitude modulated with phrases lasting for 12.19±3.63 s, n = 17 calls), syllable duration (3.11±1.17 ms, n = 9 vs. 54.57±7.53, n = 17, t = 20.182, d.f. = 24, p < 0.0001), syllable frequency (193.1±60.24 Hz, n = 48 vs. 12.24±0.89, n = 17, t = 12.314, d.f. = 63, p < 0.0001) and intersyllable interval (1.36±0.45 ms, n = 42 vs. 25.82±9.83, n = 17, t = 16.294, d.f. = 57, p < 0.0001) (Medhipour et al. 2015).

DISCUSSION

The song of T. esfandiarii is a relatively simple call when compared to many cicada calls. There are some initial modifications to increase intensity at the beginning of a call bout but is constant in its parameters after the song reaches maximum intensity. Cicada song frequency is determined by the resonant frequency of the timbal organ (Pringle 1954, Bennet-Clark 1995). The peak frequency of the T. esfandiarii song determined here is less than 50 Hz from the predicted value (4828 Hz) and well within the 95% confidence interval of the relationship between body size and call frequency determined by Bennet-Clark and Young (1994).

Each syllable is composed of two large components. This may represent the individual sound pulses from the two timbals so that the timbals may be activated at slightly different times. It appears as if the timbals are operating slightly out of phase (Fig. 2). Further experimentation would be necessary to confirm this hypothesis (e.g. Aidley 1969, Young & Josephson 1983a, b, Fonseca 1996, Sanborn 2001).
The call of *T. plebejus* has been described multiple times (e.g., Popov 1971, 1975, 1985, Lodos & Boulard 1987, Boulard 1988, Quartaau & Rebelo 1994, Boulard 1995, Boulard & Mondan 1996, Gogala 1998, Boulard 2000, Gogala 2002, Sueur et al. 2004, Boulard 2006) with Joermann and Schneider (1987) providing the most detailed temporal and frequency component analysis. The calling song of Iranian specimens was described by Mehdipour et al. (2015). The song of *T. plebejus* begins with a short buzz followed by a long period of amplitude modulations produced as the abdomen is alternately raised and lowered rather than the continuous constant amplitude call found in the calling song of *T. esfandiarii*. The peak frequency of Iranian *T. plebejus* calls is around 6.1 kHz (Mehdipour et al. 2015) which is due to the smaller size (Mozaffarian & Sanborn 2016) and the relationship of size to resonant frequency in cicadas (Bennet-Clark & Young 1994).

The calling song of *T. esfandiarii* differs significantly from the calling song previously described for *T. plebejus* (Mehdipour et al. 2015). The differences in the song parameters of the two species represent further evidence along with the morphology and biogeography (Mozaffarian & Sanborn 2016) that the species are distinct.

The observations provided here increase the number of potential host species for *T. esfandiarii*. The species had been associated previously only with *Quercus* spp. (Abaii 2000). The species was observed calling from *Amygdalus* sp., *Juglans* sp., and *Prunus* sp. here so that a greater variety of host plants may exist or the species will call from a variety of sites that have some other characteristic the males find beneficial for calling. Oviposition of the females in these new plant associations will be necessary to determine if they represent potential host plants or are simply calling sites.

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