

Duetting Behaviour in the Leafhopper *Aphrodes makarovi* (Hemiptera: Cicadellidae)

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Abstract Mate recognition and location in Cicadellidae is mediated exclusively via substrate-borne vibrational signals. In the present study we investigated vibrational signals and mate searching behaviour of the leafhopper *Aphrodes makarovi*. We studied mating behaviour and exchange of vibrational signals between live insects and in playback experiments. Males emitted long and complex calling signals composed of several sections. Female reply was long and always overlapped the end of the male call. The exchange of male and female vibrational signals was a complex and dynamic interaction during which both partners modified their signals according to partner's reply. The duration of female reply was influenced by the duration of the male call to which she was responding, while the duration of male call was influenced by the duration of the previous female reply. Such relationship suggests the role of sexual selection in the evolution of male vibrational signals.

Keywords Vibrational communication · mate recognition · searching behavior · leafhopper · duet · sexual selection

Introduction

Communication between potential partners is an essential part of reproductive behaviour (Bradbury and Vehrencamp 1998). Many insect species use air-borne or substrate-borne sounds to recognize and locate conspecific mates (reviewed in Greenfield 2002; Gerhardt and Huber 2002; Virant-Doberlet and Čokl 2004). Air-borne sound

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communication has been described in only a few insect groups but it has been, nevertheless, extensively studied (Greenfield 2002; Gerhardt and Huber 2002). In contrast, vibrational signalling is prevalent among insects (Cocroft and Rodríguez 2005), however, until recently most studies focused primarily on the use of species-specific vibrational signals in resolving taxonomic problems (reviewed in Claridge 1985b; Čokl and Virant-Doberlet 2003). Given that vibrational communication is, in estimation, used by at least 195,000 insect species in 19 orders (Cocroft and Rodríguez 2005), detailed studies of signalling and mating behaviour in such diverse groups should reveal mechanisms that are important for our understanding of evolution of communication strategies in general.

Vibrational communication is common in Hemiptera (Virant-Doberlet and Čokl 2004; Cocroft and Rodríguez 2005). While vibrational signals have been described in a great number of species (reviewed in: Čokl and Virant-Doberlet 2003; Gogala 2006; Cocroft and McNett 2006; Tishechkin 2006; Kanmiya 2006; Percy et al. 2006), vibrational signalling and reproductive strategies have been studied in detail in only a few of them. Leafhoppers (Cicadellidae) comprise more than 22,000 species (Dietrich 2004). In this group mate recognition and location is mediated exclusively via vibrational signals and communication has been studied in a number of species (reviewed in Claridge 1985a, b; Čokl and Virant-Doberlet 2003; Percy and Day 2005; Nuhardiyati and Bailey 2005; Tishechkin 2006; Percy et al. 2008; Mazzoni et al. 2009b).

Due to differences in experimental set-up and recording techniques direct comparison between studies is not possible, however, the general pattern emerging from these studies is as follows: (a) males use “call-fly” strategy (Hunt and Nault 1991) to increase their signalling space; (b) pair formation begins with emission of male calling signals; (c) male signals are more complex than female calls; and (d) after vibrational contact between potential partners is established, the exchange of signals continues and male searches for a replying stationary female. On the other hand, it seems that leafhopper species vary greatly in their repertoire, duration and complexity of male signals, as well as in duration and temporal precision of female response. The duration of male calling signals can be less than 1 s (Saxena and Kumar 1984; Heady et al. 1986; Nuhardiyati and Bailey 2005) or longer than 20 s (Hunt et al. 1992; Tishechkin 2000). Regardless of the duration, male calling signals could either be composed of several sections (Claridge and Nixon 1986; Heady et al. 1986; Gillham 1992; Hunt et al. 1992; Tishechkin 2000; Nuhardiyati and Bailey 2005; Percy and Day 2005; Percy et al. 2008) or of series of single pulses (Tishechkin 2000; Mazzoni et al. 2009b). In some species, male signals associated with later stages of courtship that differ from calling signals have been described (Saxena and Kumar 1984; Claridge and Nixon 1986; Heady et al. 1986; Tishechkin 2000; Nuhardiyati and Bailey 2005; Mazzoni et al. 2009b). The female reply can be a single pulse (Tishechkin 2000; Mazzoni et al. 2009a, b), a series of single pulses (Inoue 1982; Heady and Nault 1991) or a signal with defined structure and duration (Saxena and Kumar 1984; Heady et al. 1986; Tishechkin 2000; Nuhardiyati and Bailey 2005; Percy et al. 2008). Not taking into account the duration of single pulses, the duration of reported female replies ranges from around 0.2 s (Heady et al. 1986) to more than 4 s (Tishechkin 2000; Percy et al. 2008). In some species, specific female signals associated with later stages of courtship have been described (Tishechkin 2000).

The exchange of male and female signals itself has rarely been studied in leafhoppers. While the results of several studies indicate that the exchange has a

stereotyped temporal pattern (Heady et al. 1986; Tishechkin 2000), the constant latency of the female reply has been reported only for *Balclutha incisa* (Matsumura) (Nuhardiyati and Bailey 2005), *Homalodisca liturata* Ball and *Graphocephala atropunctata* (Signoret) (Percy et al. 2008) and *Scaphoideus titanus* Ball (Mazzoni et al. 2009a, b). Predictable temporal association between the male call and the female reply characterizes a proper duet (Bailey 2003). The duet form differs between leafhopper species. The female reply in *S. titanus* is reduced to only one pulse that closely resembles the male pulses and a female pulse is sporadically inserted between pulses in the male signal with the mean latency 0.24 s (Mazzoni et al. 2009a, b). In *B. incisa*, *H. liturata* and *G. atropunctata* the female reply follows after the male call with the latency of around 0.135, 0.25 and 1.7 s, respectively (Nuhardiyati and Bailey 2005; Percy et al. 2008).

In the present study we investigated vibrational signals and mate searching behaviour in the leafhopper *Aphrodes makarovi* Zachvatkin. These leafhoppers are relatively big (males around 6 mm, females around 7 mm in length), locally abundant and widely distributed over the Palaearctic and they appear also in North America (Hamilton 1983; Tishechkin 1998; Nickel and Remane 2002). *Aphrodes makarovi* is a common species in grassland leafhopper communities (Eyre 2005; Maczey et al. 2005). This species is usually associated with nettles (*Urtica* sp.) (Tishechkin 1998), however, it can also be found on thistles (*Cirsium* sp.), dandelion (*Taraxacum* sp.) (Nickel and Remane 2002) and on various fabaceous plants (De Groot M., Pavlovčič P., Virant-Doberlet M., personal observations). A brief description of vibrational signals of *A. makarovi* has been provided by Tishechkin (1998, 2000).

In this study we describe the male and female vibrational signals as well as duetting and searching behaviour of *A. makarovi*. We provide a detailed description of the structure of the male call emitted by isolated males and during signal exchange with the female. We examined the within- and between-individual variation (Gerhardt 1991) for several parameters of vibrational signals. Signals used in sexual communication not only convey the message but also provide the information about the sender and its location (Bradbury and Vehrencamp 1998; Gerhardt and Huber 2002). When signals show low within- and high between-individual variation, they may be used by potential mates to assess the quality of a potential partner at an early stage of the mating sequence (Gerhardt 1991). In natural male–female duets and in playback experiments we examined whether duration of the male call and the female reply are correlated. By evaluating the differences between the structure of the male call in isolated males and during call exchange with the female we addressed the question of which signal parameters may be under sexual selection by female choice.

Materials and Methods

Insects

In Slovenia *Aphrodes makarovi* is ubiquitous (Seljak 2004) and adult leafhoppers were collected using a sweep net from alfalfa (*Medicago sativa*) and nettle (*Urtica dioica*) from end of May until mid June at ten localities in southwest of the country.

In the laboratory, males and females were kept separately in plastic boxes (38×26×27 cm) at 23–28°C, 50–70% humidity and photoperiod 16:8 (L:D) h. Two days before the experiments males were put individually in plastic cups (14 cm high, 6 cm in diameter at the bottom and 10 cm in diameter at the top). Leafhoppers were fed with cut alfalfa and red clover (*Trifolium pratense*) placed in vials filled with water.

Male Calling Signals

Calling signals are defined as signals that are produced spontaneously by isolated males (de Vrijer 1984). Twenty-five behavioural tests with *A. makarovi* males from all ten localities were performed on a knapweed (*Centaurea jacea*) cutting (30 cm high) with two to three leaves at the room temperature (20–25°C) and 50% relative humidity. The bottom of the stem was put into a vial filled with water to prevent wilting and placed upright into a jar filled with moist artificial substrate. The plant stem was positioned within a circular opening (10 cm diameter) of the custom made wooden tripod. To cover the hole, overlapping incised paper and cardboard circles were positioned around the stem, thus creating a platform and separating the upper and lower half of the plant. A single male was placed on the upper half of the plant and a transparent plastic cup (size as above) was put over the plant to prevent him from escaping. Vibrational signals were recorded from the plant stem 3 cm below the platform with a laser vibrometer (controller 2200-L, sensor head OFV-353, Polytech, GmbH, Waldbronn, Germany) and stored in a computer using a Sound Blaster Audigy 4 sound card (Creative Labs Inc.) and Cool Edit Pro 2 software at the sampling rate of 48-kHz and 16-bit resolution. Signal recordings were analyzed using Raven 1.3 (Charif et al. 2008) and Sound Forge (Sonic Foundry, Madison, WI, USA) software. We observed the male for 15 min or we stopped recording if the male jumped off the plant.

For males that emitted at least one signal and stayed on the plant for more than 2 min ($n=20$), we calculated the call rate (number of calls per minute). In the detailed signal analyses, we included recordings of ten males from six (out of 10) localities that emitted at least 5 calling signals during the recording session. The male call can be divided into several sections (see [Results](#)) and we measured the following parameters: total duration of the male call, duration of each section within the call, number of units (chirps) in the 4th section, pulse repetition time in the 1st, 4th and 5th section of the call and dominant frequency of each section. Pulse repetition in the 5th section of the call was measured from ten consecutive pulses in the middle part of the section.

Female Calling Signals

Since females did not emit vibrational signals spontaneously, we induced their emission by playback stimulation with pre-recorded male calling signals. Eighteen females from all ten localities were tested. We used the experimental set-up as described above. The plant stem was vibrated approximately 6 cm below the platform with the conical tip of a 5-cm metal rod (4 mm in diameter) screwed firmly into the head of a vibration exciter (Minishaker type 4810, Brüel and Kjaer, Naerum, Denmark). Vibration exciter was driven from the computer with pre-recorded male

calling signals by Cool Edit Pro 2 program. From a library of recordings we randomly chose five males, each from one of the localities from which the females originated. For each male we randomly chose one calling signal. Females were stimulated with a random sequence of the five male signals. The amplitude of stimulation was adjusted to the level of naturally emitted male signals at the point of recording. Stimulation signals and female vibrational signals were recorded from plant stem approximately 3 cm below the cardboard platform with a laser vibrometer (PDV 100, Polytech, GmbH, Waldbronn, Germany), stored in a computer and analysed as described above.

We measured the following parameters: duration of the female call, pulse repetition time, and dominant frequency. The pulse repetition time and dominant frequency were measured from ten consecutive pulses following immediately after the end of male call. Since females always start to emit their calling signal during the male call (see [Results](#)), we also monitored the starting position of the female reply and the duration of female call after the end of the male call (non-overlapping duration). The relative position of the onset of female reply within the last section of the male call was expressed as a ratio between non-overlapping duration of the last section of the male call and total duration of this section.

Mating Behaviour

Six pairs from four (out of 10) localities were used. A male and a female were placed on a red clover cutting (height 30 cm) that was fixed in the jar as described above. To prevent leafhoppers escaping a Perspex cylinder (height 50 cm; diameter 30 cm) with a small opening for the laser beam was put over the jar. Vibrational signals were recorded with a laser vibrometer (controller 2200-L, sensor head OFV-353, Polytech, Waldbronn, Germany) and stored in a computer and analysed as described above. Their behaviour was recorded with a 3CCD video camcorder (Canon DM XM2) fitted with a 4.2–84 mm zoom lens, together with the vibrational signals. This enabled us to associate vibrational signals with particular behaviour. Video recordings were transferred into the computer with Microsoft Windows Movie maker 5.1.

We finished the observations when the male located the female. In addition to parameters mentioned above, the following parameters were monitored in males: number of males searching (defined as walking during female reply), search latency (time needed to start moving after the end of male call) and searching time (defined as time needed to locate the female after the onset of searching). In females, we monitored the number of female replies, the duration of non-overlapping part of the female call and pulse repetition time.

To analyse in more detail the parameters of the male call during a duet, 15 males from 5 (out of 10) localities were tested in playback experiments in which we simulated a male–female duet. Experiments were performed on a stinging nettle (*Urtica dioica*) at the room temperature (20–25°C) and 40–50% relative humidity. The upper part (height 30 cm) of a wild nettle plant stem was cut and planted into a peat soil in plastic cups (cup dimensions as above). All leaves, except the top ones and the fourth pair counting from the top, were cut off. The junction of the 4th leaf pair was located approximately 7 cm below the tip of the plant. A single male was

placed on the top of the plant. To prevent males from escaping a rectangular net cage (65×65×50 cm) with openings for the laser beam and the camera lens was put over the plant. After 2 min we applied pre-recorded male–female duet in order to induce male calling. When the male on the plant emitted a call, we presented him with a female reply. The application of a female reply was triggered manually and we timed the onset of a female reply with the typical decrease in amplitude at the end of the last section of the male call (Fig. 1). Artificial duetting was maintained for 13 min or until the male located the source of vibrations, whichever came first. We applied

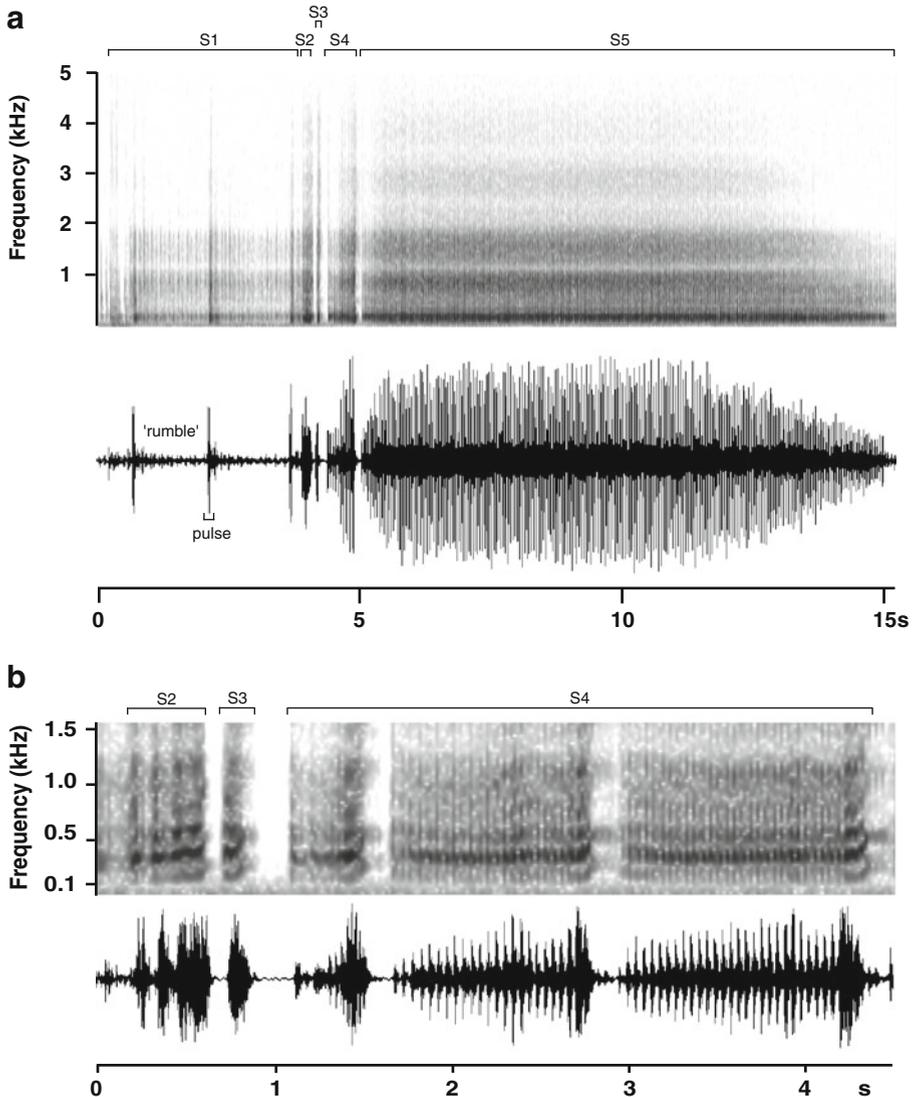


Fig. 1 Male calling signal of *Aphrodes makarovi*. **a** a representative signal; **b** detailed structure of chirps in sections 2, 3 and 4. Both signals were recorded on knapweed (*Centaurea jacea*). Sonogram (*above*) and oscillogram (*below*) are shown for each signal

vibrational stimuli to the tip of the leaf using a vibration exciter (Minishaker type 4810, Brüel and Kjaer, Naerum, Denmark) and the side of stimulation was changed four times a day. The female call used in playback experiments was composed of natural pulses assembled by the use of Cool Edit Pro 2 computer program. Four pulses were taken from a natural female call which was recorded on an alfalfa plant 5 cm from the calling female with the laser vibrometer (controller 2200-L, sensor head OFV-353, Polytech, Waldbronn, Germany). The pulse repetition time (52 ms) in the composed female call corresponds closely to the mean repetition time determined in natural female calls (see [Results](#)). The duration of the composed signal was 10.4 s, which corresponds to the non-overlapping duration of the female call determined in natural duets and in playback experiments (see [Results](#)). The vibrational stimuli and responses of the leafhoppers were recorded from the main stem 1 cm above the branching point with a laser vibrometer (controller 2200-L, sensor head OFV-353, Polytech, GmbH, Waldbronn, Germany). The amplitude of stimulation was adjusted to the level of the recorded female signals. Signals were digitized at 48 kHz sample rate and 16-bit resolution and stored onto a hard drive of a PC with an external sound card (Sound Blaster Extigy, Creative Labs Inc.) using Cool Edit Pro 2 software. The behaviour was filmed and vibrational signals were analysed as described above.

The playback experiments were finished when a male located the source of vibrations. We monitored the same parameters as mentioned above in observations of natural mating behaviour.

Terminology and Analyses

Pulse was defined as a unitary homogenous parcel of sound of finite duration (Broughton 1963). Chirp was defined as a sound consisting of a group of pulses that is identifiable to the human ear as a unitary event (Booij 1982; Hunt et al. 1992).

Data are presented as means, ranges and SD together with the number of signals analyzed (n) and the number of leafhoppers (N) from which the signals were obtained. Dominant frequency data are presented as medians together with ranges. For all measured parameters of the male and female call we also analyzed within- and between-individual variation (coefficient of variation_{intra} and CV_{inter}, respectively) (Gerhardt 1991). The differences in duration, dominant frequency and pulse repetition time were analyzed with a non-parametric Wilcoxon test for comparison between two groups and Kruskal-Wallis test followed by a Steel-Dwass multiple comparison test (Steel 1959) for comparison between more than two groups. The differences in intra-individual variation in different experimental situations were analysed by Steel-Dwass test (Steel 1960). Proportion of searching with the number of female calls was analysed using GLMM with a log-normal link function after assuming binomial error distribution fitted with a Laplace approximation with the individual leafhopper included as a random factor (McCullagh and Nelder 1989). In a natural duet, the duration of male and female calls was analyzed with a linear mixed model (McCullagh and Nelder 1989). In each case the duration was related to the previous call of the opposite sex (in females this is the male call to which she was responding). Durations of all calls of each sex ($n=66$) emitted during vibrational duet from six pairs were included in the analysis. In the model the repeats with

different pairs were used as a random effect in order to reveal a general pattern. The violation of heterogeneity in the model with male call duration was solved by including variance structure in which variance was changed per individual (Zuur et al. 2009). In the model for female call duration two outliers were removed. In both models the pattern of the explanatory variables was checked and transformed if necessary. All analyses were done with R version 2.10.1 (R Development Core Team 2010).

Results

Male Calling Signals Emitted by Isolated Males

Males readily emitted calling signals within a few minutes after being placed on a plant. There was a great variation in calling behaviour between males (Tables 1, 2, 4) and calls were emitted either as sporadic, isolated signals or as a series of successive signals. Males were often moving around the plant and called from different positions or they jumped off the plant and began calling again on the platform or on the cup.

Male calling signal had a complex, stereotyped structure and could be divided into five sections (Figs. 1a, 3). The first section (S1) was formed by low amplitude sound onomatopoeically termed ‘rumble’ and higher amplitude pulses emitted at regular intervals (Table 1). Both sound elements had broad-band characteristics and similar median dominant frequency around 150 Hz (Table 3). In ‘rumble’ section most energy was contained in the frequency range below 2,000 Hz, whereas in pulses the frequency band extended up to 5,000 Hz (Fig. 1a).

Section 2 (S2) following immediately after S1 was short (Figs. 1, 3, Table 1) and it was characterized by high amplitude frequency modulated chirps. Chirps had broad-band frequency characteristics and dominant frequency sweep between 100 and 370 Hz and contained frequencies up to 5,000 Hz (Fig. 1a).

Section 3 (S3) was separated from S2 by a silent gap (60–100 ms) and consisted of a single high amplitude chirp (Figs. 1, 3) that often differed from the chirps in S2 in more pronounced frequency modulation (Fig. 1b). In few recorded signals this section was missing.

Section 4 was separated from the single chirp in section 3 by a 100–200 ms silent gap (Fig. 1). It consisted of up to 4 units separated by gaps (25–150 ms) within which a few low amplitude pulses could sometimes be discerned (Fig. 1). Each unit was formed by regularly repeated pulses that progressively increased in amplitude and finished with a high amplitude, frequency modulated chirp. The duration of this section showed the highest intra- and inter-individual variability (Table 2). The median number of units per signal was 1, however, in few recorded signals this section was missing.

The last section (S5) was separated from the previous one by a silent gap (60–150 ms). S5 was the longest section of the male call and consisted of a series of regularly repeated pulses (Figs. 1, 2, 3, Table 1). The pulse amplitude was progressively increasing in the beginning and decreasing at the end of the section. Duration of the last section, pulse repetition time within this section and total duration of the male call showed the lowest intra- and inter-individual variability (Table 2).

Table 1 Temporal parameters of calling signals of *Aphrodes makarovi* obtained from isolated males and females in natural duets and playback induced duets. The grand mean, standard deviation (SD), the number of animals (N) and total number of signals (n) are shown

Signal parameter	Isolated males			Natural duet			Playback induced duet			
	Mean	SD	n	Mean	SD	n	Mean	SD	n	
Male call	S1 duration (s)	4.09	0.95	10	3.58	2.09	6	2.80	1.23	15
	S2 duration (s)	0.55	0.16	10	0.41	0.13	6	0.44	0.10	15
	S3 duration (s)	0.16	0.03	10	0.19	0.10	6	0.25	0.05	15
	S4 duration (s)	1.42	0.99	10	1.60	0.95	6	0.99	0.39	15
	S5 duration (s)	12.73	2.17	10	6.96	3.02	6	9.07	2.41	15
Total duration (s)	19.11	1.69	10	12.75	3.97	6	13.43	3.29	15	
Pulse repetition time, S1 (s)	1.46	0.36	10	1.87	1.09	6	1.36	0.41	15	
Pulse repetition time, S4 (s)	0.052	0.004	10	0.042	0.009	6	0.048	0.004	15	
Pulse repetition time, S5 (s)	0.051	0.004	10	0.049	0.006	6	0.047	0.003	15	
Total duration (s)	–	–	–	–	–	–	15.42	6.10	18	
Non-overlapped duration (s)	–	–	–	8.44	7.48	6	12.43	5.33	18	
Pulse repetition time (s)	–	–	–	0.050	0.01	6	0.050	0.01	18	

Table 2 The within (CV_{intra}) and between individual variation (CV_{inter}) of the temporal parameters of *Aphrodes makarovi* calling signals of isolated males and males and females in natural duets and playback induced duets. The mean, minimum and maximum of the within-individual and between-individual variation are shown

Signal parameter	Isolated males					Natural duet					Playback induced duet					
	CV_{intra}	Min	Max	CV_{inter}		CV_{intra}	Min	Max	CV_{inter}		CV_{intra}	Min	Max	CV_{inter}		
Male call																
S1 duration (s)	16.49	8.13	24.41	18.61	56.88	30.18	97.36	25.02	30.24	13.96	46.65	29.23				
S2 duration (s)	20.10	13.32	24.79	27.22	25.91	17.33	32.48	17.41	18.24	8.53	26.70	13.08				
S3 duration (s)	13.56	5.09	25.65	14.68	33.94	9.01	115.84	50.96	11.11	2.61	46.96	10.04				
S4 duration (s)	48.66	7.61	98.00	34.07	61.14	34.89	97.04	46.45	17.51	3.89	44.38	20.88				
S5 duration (s)	11.88	3.78	21.34	13.75	22.99	12.94	38.25	33.46	12.20	4.85	37.82	23.68				
Total duration (s)	5.37	3.23	9.72	7.43	29.82	9.20	48.51	13.20	11.94	3.91	33.20	21.69				
Pulse repetition time, S1 (s)	20.45	3.80	32.95	11.95	29.96	17.97	70.41	34.43	23.00	7.37	34.90	24.11				
Pulse repetition time, S4 (s)	4.64	1.86	11.11	7.53	14.58	3.15	30.89	16.37	3.56	1.77	5.91	8.19				
Pulse repetition time, S5 (s)	2.37	1.14	6.16	6.78	3.58	2.33	6.52	10.72	1.77	0.88	4.43	6.65				
Total duration (s)	–	–	–	–	–	–	–	–	–	–	–	–				
Non-overlapped duration (s)	–	–	–	–	59.32	20.37	91.30	40.67	29.15	13.80	55.75	33.60				
Pulse repetition time (s)	–	–	–	–	7.13	4.25	26.00	7.57	9.64	2.10	27.01	10.33				

Table 3 The dominant frequency of calling signals of *Aphrodes makarovi*. Values for different sections of the male call and of the female call for calls of isolated individuals and natural duets and playback induced duets are shown. The median, minimum, maximum, the number of animals (*N*) and total number of signals (*n*) are shown

	Dominant frequency (Hz)														
	Isolated male					Natural duet					Playback induced duet				
	Median	Min	Max	<i>N</i>	<i>n</i>	Median	Min	Max	<i>N</i>	<i>n</i>	Median	Min	Max	<i>N</i>	<i>n</i>
Male call S1	141	117	1,237	10	70	396	100	1,416	6	66	152	70	275	15	193
Male call S2	144	105	369	10	70	223	102	1,073	6	67	163	76	656	15	196
Male call S3	173	105	1,278	10	68	382	121	1,713	6	63	182	70	1,788	15	196
Male call S4	170	73	1,255	10	69	398	119	1,298	6	50	170	88	610	15	181
Male call S4	170	117	1,255	10	70	397	119	1,265	6	68	182	94	686	15	196
Female call	–	–	–	–	–	520	118	1,166	6	86	574	381	761	18	86

Female Calling Signal

Female calling signal was a series of regularly repeated single pulses (Fig. 2a). Although the onset of the female reply would occur during every section of the male call, the great majority appeared in the last section and most females started to respond during the last third of this section (Fig. 4). Female reply was long—up to 30 s—and it always continued after the end of the male call (Fig. 2b). Both, the total duration of female reply and the duration of non-overlapping part the female call varied substantially within and between individuals (Tables 1, 2). Pulse repetition time had the lowest intra- and inter-individual variability (Table 2). There was no significant difference between the pulse repetition time in male and female call (Wilcoxon test; $P < 0.05$). The female call had broad-band frequency characteristics with dominant frequency of around 570 Hz and most energy was contained in the range below 2,000 Hz (Fig. 2, Table 3). Often the second prominent frequency peak was observed around 730 Hz. The amplitude of the female call was always low and the highest observed level was around 6 dB below the highest amplitude of the male call.

Mating Behaviour

Natural Exchange of Male and Female Vibrational Signals and Searching Behaviour

As in experiments with single leafhoppers, males emitted calling signals shortly after they were placed on the plant. Females often did not respond to the first male call, however, after the first reply they responded to every male call. The highest observed amplitude of female response was around 10 dB lower than the amplitude of the male call (Fig. 2b). After the males stopped emitting calling signals their behavioural response to a continuing female reply was immediate locomotion associated with searching for the female. The shortest observed searching latency was 240 ms, which indicates that males needed to evaluate at least five pulses in the female reply to commence

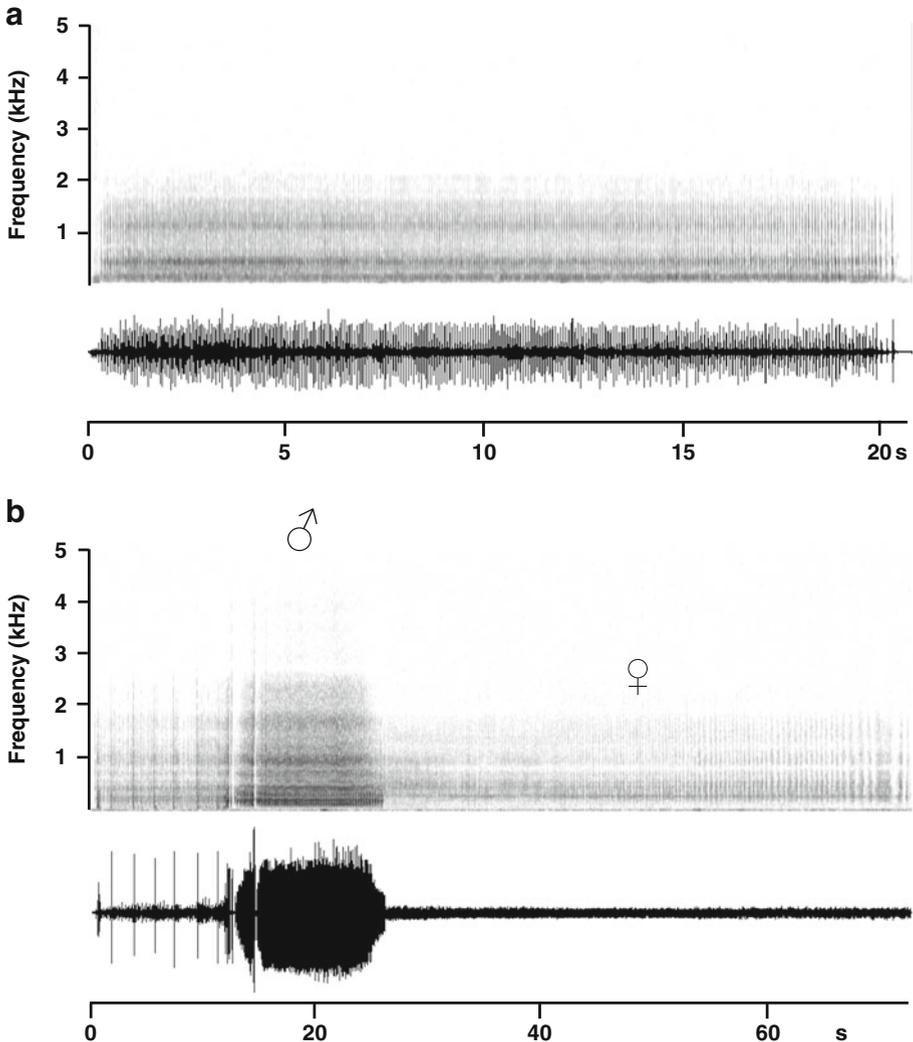


Fig. 2 Female calling signal (**a**) and a natural male–female duet (**b**) in *Aphrodes makarovi*. Female signal shown in **a** was obtained by stopping the playback before male call was finished. Female call shown in **a** and duet shown in **b** were recorded on knapweed (*Centaurea jacea*) and red clover (*Trifolium pratense*), respectively. Sonagram (*above*) and oscillogram (*below*) are shown

searching. Males were searching during 85% of female replies (Table 4). Males were moving only during the female reply and when the female call ended, males stopped and called again. In comparison with isolated males, the calling rate was relatively stable (Table 4). Males needed approximately 4 min to locate the female.

The mean total duration of male calling signal emitted during exchange with the female was significantly (Steel-Dwass test; $P < 0.01$) shorter than in experiments with isolated males (Table 1) and there was a significant increase in within-individual variation (Wilcoxon test; $P < 0.001$) (Table 2). In natural duets, the duration of non-overlapping part of the female call was not significantly shorter than in the calls

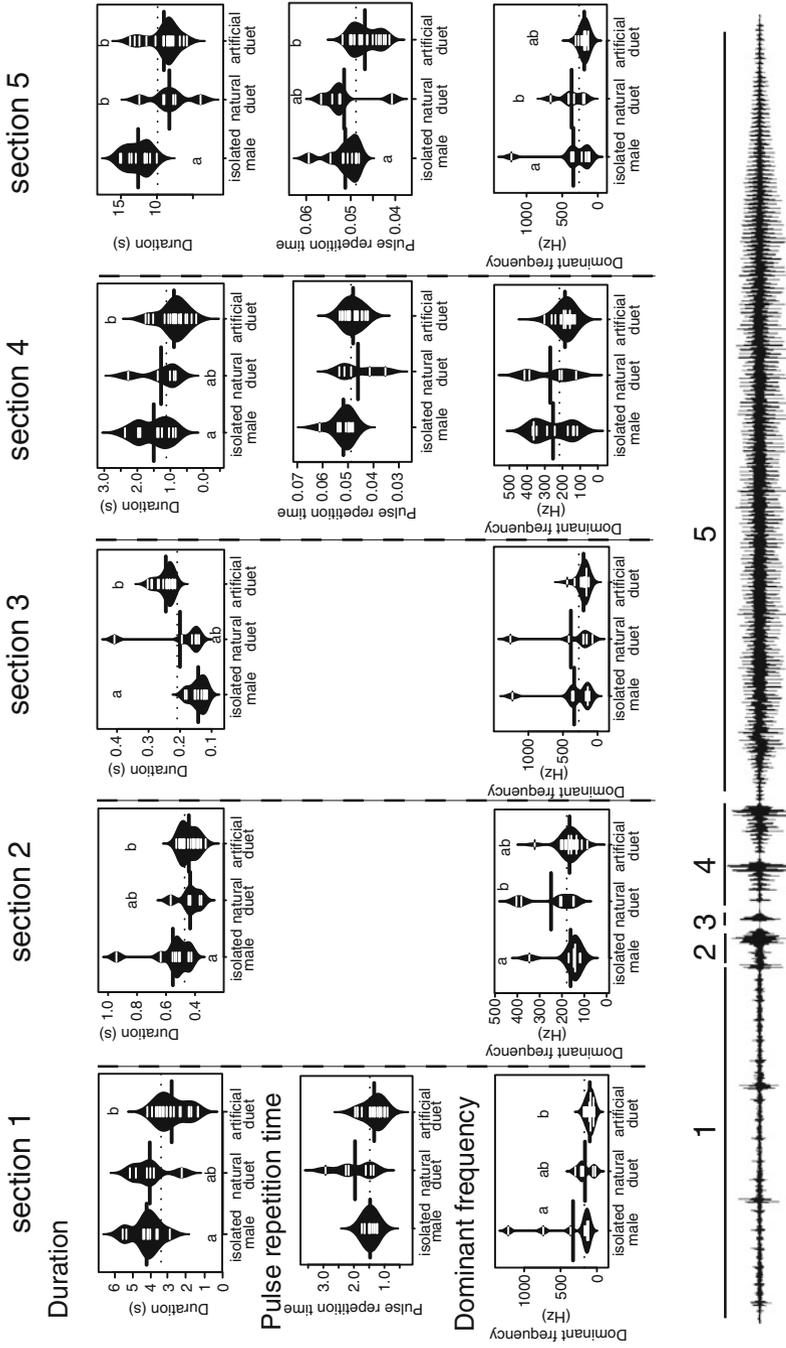
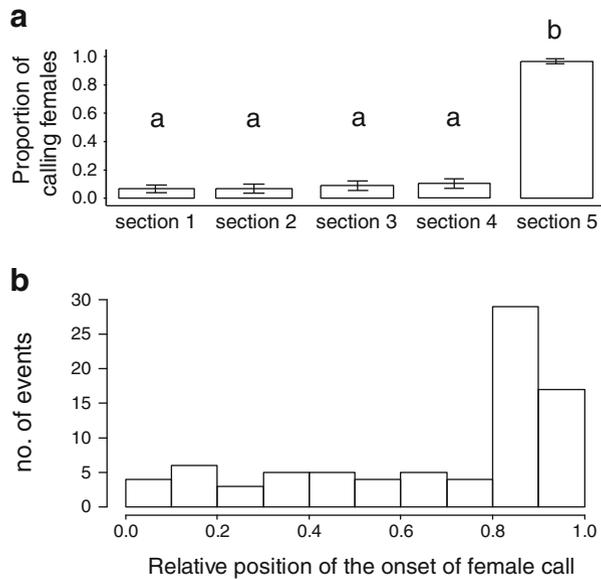


Fig. 3 Comparison of call parameters between isolated males, males in natural duets and in playback induced duets. Means for each tested individual (*white lines*), means per group (*black solid line*) and overall means (*dashed line*) are shown. The shape of the beanplot reflects the density of observations. Isolated male, $N=10$; natural duet, $N=6$; playback-induced duet, $N=15$. Groups with *different letters* are statistically significantly different (Steel-Dwass test, $P<0.05$). Note differences in scales

Fig. 4 Distribution of the onset of the female reply in *Aphrodes makarovi*. **a** proportion of females calling during each section of the male call. The average proportion (\pm sd) obtained from proportions for 18 females is shown. Groups with *different letters* are statistically significantly different (GLMM, $P < 0.001$). **b** The relative positions of the onset of the female reply within the last section of the male call



induced by a playback of male call (Steel-Dwass test; $P = ns$) however, there was a significant increase in within-individual variation (Wilcoxon test; $P < 0.05$) (Table 2). Furthermore, the analysis showed that both, the duration of male and female call, were positively influenced by the partner’s signals. The duration of the female call was influenced by the duration of the male call to which she was responding, while the duration of male call was influenced by the duration of the previous female reply (Table 5).

Male Vibrational Signals and Searching Behaviour in Response to a Playback Female Reply

As in natural duets, males responded to a female reply presented in response to their calling signals with immediate searching. None of the measured parameters of male calling signals or male behaviour differed significantly from the values obtained in natural duets (Fig. 3). In comparison with calling signals emitted by isolated males, the durations of all sections (except of S3) and consequently also the total duration

Table 4 Male calling and searching behaviour determined for isolated males and males in natural duets and playback induced duets. The mean, standard deviation and the number of animals (N) and total number of signals (n) are shown

Parameter	Isolated male			Natural duet				Playback induced duet			
	Mean	SD	N	Mean	SD	N	n	Mean	SD	N	n
Call rate (no./min)	0.89	0.90	20	3.69	1.59	6	–	2.50	0.88	15	–
Proportion searching	–	–	–	0.85	0.13	6	–	0.64	0.39	15	–
Search latency (s)	–	–	–	0.89	0.64	6	68	0.83	1.14	15	158

Table 5 Model statistics (linear mixed model) of the influence of the duration of partner's vibrational signals on the duration of male and female call in natural duet. Before testing, the explanatory male and female call duration were log and square root transformed, respectively

Response variable	Parameter	Estimate	se	df	t	P
Female call duration	Intercept	-8.47	4.76	59	-1.78	ns
	Mall call duration	6.37	1.80	59	3.53	<0.001
Male call duration	Intercept	9.53	0.56	57	16.93	<0.001
	Previous female call duration	0.73	0.12	57	5.89	<0.001

of the call were significantly shorter (Fig. 3). In contrast, the duration of S3 was significantly longer. The changes in the duration of a male call during the searching phase are also reflected in a significant increase in within-individual variation in duetting males (Wilcoxon test; $P < 0.05$) (Table 2). The duration of section 4 however, was significantly less variable than in isolated males or in natural duets (Steel-Dwass test; $P < 0.01$ and $P < 0.05$, respectively) (Table 2). Males needed approximately 4 min to locate the female.

Discussion

Results of the present study showed that vibrational communication in *A. makarovi* follows the general pattern described in other leafhoppers (Claridge 1985a, b; Nuhardiyati and Bailey 2005; Tishechkin 2006; Percy et al. 2008; Mazzoni et al. 2009a, b). However, more detailed analyses revealed that the exchange of male and female vibrational signals was a complex and dynamic interaction during which both partners modified their signals according to partner's reply.

The onset of the female response in relation to the male call was not as precise as in the duetting bushcrickets communicating via air-borne sound (reviewed in Bailey 2003), however, the temporal association in *A. makarovi* was nevertheless stereotyped and predictable. Females always started to reply during the male call and therefore the reply delay after the end of male signal depended on the pulse repetition time in female signal and was 50 ms or shorter. In duetting bushcrickets, when male signal is long and complex, female reply is usually triggered by a particular sound element signifying the conclusion of the male call (Heller 1990; Dobler et al. 1994; Bailey and Field 2000; Stumpner and Meyer 2001). Vibrational signals of male Auchenorrhyncha (leafhoppers, planthoppers and treehoppers) are often long and complex (e.g. den Bieman 1986; de Vrijer 1986; Hunt et al. 1992; Coccoft and McNett 2006; Mazzoni et al. 2009a) and the duration of these signals often shows substantial variation (den Bieman 1986; de Vrijer 1986; Hunt et al. 1992). There is, however, very little information about triggering of female response. In the leafhopper *Graminella nigrifrons* (Forbes) female reply is triggered by particular elements (essential pulses) within the complex male call and female starts to respond before the sequence of a regularly repeated pulses and chirps is finished (Hunt et al. 1992). In the treehopper *Enchenopa binotata* (Say) females use the end of the whine element in male signal to trigger the emission of their vibrational reply

and they respond with a species-specific response delay (Rodríguez and Cocroft 2006). In the planthopper *Javesella pellucida* (F.) majority of females start to reply before the conclusion of male signal and the timing of the female reply in this species is highly variable and, at least to some extent, temperature dependent (de Vrijer 1984). The facts that *A. makarovi* females always start to respond before the male signal is finished and that the majority start replying in the last section indicate that female response may be triggered by the elements in the previous parts or by the beginning of the last section. Although analyses of the current data did not reveal any correlation between different components of male call and the timing of female response, further detailed studies are needed to elucidate the links between properties of the male call and onset of the female reply. Females may use more than one element within the male call for timing their reply (Bailey and Hammond 2004) or they may respond when male call duration reaches a critical duration (see below).

Is there any adaptive significance to the overlap of female reply with the end of the male call? One possible explanation is that female has to respond within a defined time window to ensure the recognition and to trigger male searching behaviour. Such species-specific time window is characteristic for a duet in phaneropterine bushcrickets (Heller and von Helversen 1986; Tauber and Pener 2000; Bailey 2003; Bailey and Hammond 2004). It has been noted in some of these species that females can reply also before the male stops calling (Tauber and Pener 2000; Bailey and Hammond 2004). In *Caedicia* sp. the tendency to begin replying before the conclusion of the male call has been attributed to high motivation of the females and to maximizing the chance that the reply appears in the male's critical recognition time-window (Bailey and Hammond 2004). It is conceivable that in species with long male call that lacks a reliable trigger indicating the end of the signal and with short and narrow male's sensitive time window it is advantageous that females start to respond before the male call is finished. This may be especially important in plant-dwelling insects when partners are not in the vicinity of each other, since due to the relatively low propagation velocities of vibrational signals (Michelsen et al. 1982; Barth 1998) and unpredictability of the distance between partners and pathway over which the signals are travelling, the resulting transmission time delays can be large in comparison with air-borne sound. Up to now, the critical time window of the female reply in vibrational communication systems has been demonstrated only in the genus *Sialis* (Neuroptera) in which male searching behavior is triggered only when female replies within 400 ms after the end of male signal (Rupprecht 1975). In another neuropteran *Chrysoperla plorabunda* (Fitch) partners closely match their response latencies and appropriately timed responses are needed to maintain a stable courtship duet important in maintaining reproductive isolation (Henry and Wells 2006). However, in these species overlapping of male and female signals has not been observed.

Although detailed information is in most cases lacking, the onset of the female vibrational reply before the conclusion of the male call has been observed in the planthopper genus *Javesella* (Strübing and Hasse 1975; de Vrijer 1984), in *G. nigrifrons* (Hunt et al. 1992), *E. binotata* (Rodríguez and Cocroft 2006), psyllid genera *Schedotrioza* and *Trioza* (Percy et al. 2006) and in stoneflies (Stewart and Sandberg 2006). In the treehopper *E. binotata* and in psyllids male calls are relatively short (up to 1.5 s). In *E. binotata* the occasional overlap of the last part of

the male call may be explained by the triggering of female response by the end of the first part of the male signal and longer-than-usual duration of the second section of the male call (Rodríguez and Cocroft 2006). While such overlap has no influence on female response, there is no available information about its potential influence on male signalling behaviour. In psyllids, the species with low variability in male calls engaged in tightly synchronised duets with female reply latencies less than 30 ms and majority of female replies started before the end of the male call (Percy et al. 2006). In contrast, in the stonefly *Paraperla wilsoni* Ricker a male call is several seconds long, continuous signal and the female reply is completely embedded within it (Stewart et al. 1995). Similarly, in the planthopper *J. pellucida* (F.) and in the leafhopper *G. nigrifrons* male vibrational calls are long (10–25 s) and females regularly start responding before the male signal is finished (de Vrijer 1984; Hunt et al. 1992). Although it has been noted that in the presence of a responding female, the leafhopper male calls continuously as he walks to the female (Heady and Nault 1991), due to the lack of detailed information about male behaviour, the influence of the timing of female reply on male searching behaviour in these two species it is not clear.

A reply at least partly overlapping with a male call may also provide some advantage for the female. Sexual and natural selection may be interacting through the trade-off between maximising sexual communication while minimising exposure to exploitation by predators. It is possible that the male–female duet in which calls are overlapping is perceived by predators as one vibrational signal arriving from spatially separated sources, making location more difficult. It has been shown that spiders are significant predators of *A. makarovi* and that they use vibrational signals of male leafhoppers as a cue during foraging and, as a result, kill significantly more males and females (Virant-Doberlet et al. 2011). However, although in the presence of the theridiid spider *Enoplognatha ovata* (Clerck) the decline in the numbers of males was significantly more rapid than of females, this spider also significantly reduced the number of female *A. makarovi*. Taking into account the eavesdropping by predators, the low amplitude of female vibrational signals may also be advantageous.

Females of *A. makarovi* varied the duration of their reply according to the duration of the male call to which they were responding. Such relationship suggests the role of sexual selection in the evolution of male vibrational signals (Bailey 2003). It has been shown in *E. binotata* that female preference is the strongest source of selection shaping male vibrational signals (Sullivan-Beckers and Cocroft 2010). In communication systems in which the duration of the female reply may be crucial for quick localization of the female, variation in duration may indicate female preference and choice and it has been shown in playback experiments that *A. makarovi* males located the source of longer female replies faster than the short female call (de Groot et al. 2011). The fact that *A. makarovi* females often did not respond to the first male call suggests that they may evaluate the male before choosing to reply (Jennions and Petrie 1997). On the other hand, the fact that they reply before the male call is finished might indicate that they do not discriminate between males based on the evaluation of call duration. It has also been suggested that evaluation of a long and complex male calls results in long female reply latency (Bailey and Hammond 2003). However, it is possible that *A. makarovi* females may listen to a high amplitude male call also while they are singing and then extend or

shorten their reply accordingly. Females may choose to respond when male call reaches a critical duration and then adapt the duration of the non-overlapping reply according to the duration of the overlapping part of the response. Such strategy may represent an advantage in a situation where the risk of rejecting a potential mate is high due to the unpredictability of the next encounter with a male (i.e. when another male will land on the plant with the female). However, mating preferences vary within and between individuals (Jennions and Petrie 1997) and female preference may depend on the social context in which she evaluates the male (Bateson and Healy 2005). However, it is not clear to what extent females assess males at long range by their vibrational signals (Virant-Doberlet and Žežlina 2007). Most properties of male calling signals as perceived by females depend on the relative positions of prospective partners and not necessarily on the quality of the male. It has been shown in the treehopper *Umbonia crassicornis* (Amyot & Serville) that in the context of long distance vibrational communication, variation in male advertisement signals does not influence female choice (De Luca and Cocroft 2009). However, the data obtained in the current playback study were not sufficient to reveal any correlation between the duration of different components of male call and duration of female reply. Additional studies are needed to test whether duration of male call is under sexual selection through female call preference.

However, the male calling strategy may be in conflict with female preferences (Bailey 2003). Calling of *A. makarovi* males is costly due to eavesdropping of predators (Virant-Doberlet et al. 2011). In addition, it is likely that male–female duet is vulnerable also to eavesdropping by rival males. Although there is currently no information about rivalry behaviour in *A. makarovi*, male–male vibrational interactions have been described in other leafhopper species (Heady et al. 1986; Hunt and Morton 2001; Nuhardiyati and Bailey 2005). The leafhopper *S. titanus* has a well developed intrasexual competition and intruding males use alternative tactics such as disturbance vibrational signals to disrupt an ongoing duet and satellite behaviour to approach silently a duetting female (Mazzoni et al. 2009a, b). In this species courting males shortened the duration of their vibrational signals in the presence of rivals, presumably to reduce time needed for localization of the female. In *A. makarovi* there may be a selection pressure to reduce the duration of the call after an initial contact with the female has been established to reduce risks imposed by predators and sexual competitors. In contrast, a longer duration of female reply seems to provide better information for localization (de Groot et al. 2011). If costs to increase the duration of female reply by emitting longer calls are too high, then the tendency in males may be to reduce the duration of their vibrational signals in order to achieve an increase in the duration of non-overlapping part of the reply. This is supported by our observation that although in induced playback duets the duration of female reply was constant, the duration of male call was significantly shorter than in isolated males, while the intra-individual variation was significantly higher. In addition, in natural duets the duration of male call was influenced by the duration of the previous female reply.

The current study provides the first detailed information about duet structure in Cicadellidae. Despite its relatively simple form, it suggests complex interactions. Further studies should provide more insight into mechanisms that are involved in the evolution of such communication strategy. In particular, detailed studies are needed

to test directly whether in *A. makarovi* there is a selection through female call preference and choice on duration and complexity of male call. It is likely that duetting (stereotyped and predictable temporal association between male call and female reply) (Bailey 2003) is common and widespread within Auchenorrhyncha and studies in other species may reveal mechanisms underlying evolution of duetting behaviour. Investigations in a complex social environment (Cocroft 2003) and in the presence of biotic (de Groot et al. 2010) and abiotic noise (McNett et al. 2010) may reveal important sources of selection on evolution of vibrational communication.

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