Use of acoustic signals in mating in an eavesdropping frog-biting midge

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The sensory systems of organisms are shaped by selective pressures imposed by their performance in a variety of contexts. Female frog-biting midges use the mating calls of anurans to locate their host to obtain a blood meal. Although the use of sound in foraging is well documented in this group, it is unknown whether sound is used in other contexts. To investigate the ability to use sound in nonforaging contexts, we experimentally tested the prediction that frog-biting midges (*Corethrella appendiculata*) use sound in mating. We recorded their wing beats while swarming, in controlled tethered conditions and during different- and same-sex pair interactions. Our results show sexual differences in the acoustic properties of flight tones, with male midges having higher frequency wing beats than females. Wing beats of free-flying individuals were significantly higher in frequency than those recorded from tethered individuals, cautioning the interpretation of recordings obtained following this widely used technique. In addition, interacting, tethered opposite-sex pairs altered their flight tones to match their upper harmonics, converging at the third and fourth harmonic frequencies of males and females, respectively. In male—male interactions, however, the frequency of their wing beats diverged. Therefore, flight tones in frog-biting midges may function as courtship signals attracting conspecific females and deterring rival males. We discuss the use of sound in multiple contexts in these midges and potential factors leading to their ability to eavesdrop on anurans.

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Many animals depend on mating signals to attract individuals from the opposite sex. A large variety of elaborate mating displays has evolved across species, and adult males may increase the likelihood of attracting a mate by forming mating aggregations (Höglund & Alatalo, 1995). Insects are exceptional in their diverse mating strategies and often depend on sensory modalities that have traditionally received less attention (e.g. vibration: Cocroft & Rodríguez, 2005; near-field sounds: Santer & Hebets, 2008). Investigating the use of signals in such sensory modalities across different contexts provides a unique opportunity to ultimately examine the evolution of signal production and perception. In this study we investigate the acoustic mating signals used by frog-biting midges, a group in which females use sound when searching for a blood meal. Even though the use of sound in foraging in frog-biting midges is well documented (Bernal, Rand, & Ryan, 2006; McKeever, 1977; McKeever & French, 1991), the use of sound in nonforaging context has not yet been investigated.

Frog-biting midges belong to the monogeneric family Corethrellidae (Diptera) (Borkent, 2008; Borkent & Grafe, 2012). These midges are closely related to mosquitoes (Culicidae) (Stone, 1957; Wood & Borkent, 1989), and females also feed on blood to obtain protein required for egg development (McKeever, 1977; McKeever & French, 1991; Williams & Edman, 1968). In contrast to most haematophagous insects, frog-biting midges use acoustic cues to locate their host (Bernal et al., 2006; Borkent, 2008; McKeever, 1977). These midges are interspecific eavesdroppers specialized in exploiting the communication system of frogs and toads. Frog-biting midges require anuran calls to find their host and do not use other host-emitted cues commonly used by other haematophagous insects (Bernal & de Silva, in press). Given that female frog-biting midges use blood meals from anurans to support egg production, eavesdropping has critical consequences for their

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fitness. Fossil, cladistic and morphological evidence suggests frogs and frog-biting midges have been interacting probably at least since the early Cretaceous (Borkent, 2008).

Although most mosquitoes do not use sound to find their hosts (Borkent & Belton, 2006; but see Bartlett-Healy, Crans, & Gauger, 2008), the use of sound in mating is well known in Culicidae. Wing beats of mosquitoes generate air particle displacement that is detected in the near-field by conspecifics (Roth, 1948; reviewed in Clements, 1999). The use of wing beat signals in mating has been reported for a number of species across several genera of Culicidae (e.g. *Aedes*: Roth, 1948; *Anopheles*, *Culex* and *Toxorhynchites*: Downes, 1969; Clements, 1999). Both female and male mosquitoes produce courtship signals by actively modulating their flight tones to match their upper harmonics during their mating flight (Cator, Arthur, Harrington, & Hoy, 2009; Cator, Arthur, Ponlawat, & Harrington, 2011; Cator, Ng'Habi, Hoy, & Harrington, 2010; Gibson & Russell, 2005; Pennetier, Warren, Dabire, Russell, & Gibson, 2010; Warren, Gibson, & Russell, 2009). Although in mosquitoes the use of flight tones in mating has long been investigated (Roth, 1948), the role of acoustic signals in the reproductive behaviour of the closely related family of frog-biting midges has not been explored.

We recently reported that frog-biting midges form aerial mating swarms similar to mosquitoes and other closely related taxa (Clements, 1999; de Silva & Bernal, 2013). Male frog-biting midges aggregate under low-light conditions and perform characteristic mating flights. Female midges approach males in the swarm and copulation occurs in midair. In swarming species, male mosquitoes have more plumose antennae than females (Clements, 1999; Downes, 1955). The antennal hairs of males are resonantly tuned to the wing beat frequencies of female flight tones and transmit forces acting on them to the antennal flagellum, resulting in high acoustic sensitivity (Gopfert, Briegel, & Robert, 1999). Similar to mosquitoes, frog-biting midges have sexually dimorphic antennae (Felt, 1905), which suggests they also may depend on acoustic mating signals. Here we experimentally examine the wing beat frequencies and acoustic interactions among individuals of a frog-biting midge, *Corethrella appendiculata*.

To characterize the use of flight tones of frog-biting midges is necessary to examine their wing beats under natural mating conditions. In this study we investigate the flight tones of *C. appendiculata* during their mating swarms. We found differences in acoustic properties of flight tones of males and females, an indicator that this trait may be under sexual selection (Andersson, 1994). To investigate the role of flight tones in mating, we then examine acoustic interactions between same-sex and opposite-sex pairs. In particular, we study convergence on harmonically related frequencies among males and females, contrasting this behaviour with the interactions between males. We discuss the acoustic signals of frog-biting midges in relation to the wing beats of mosquitoes to provide insights about the evolution of acoustic mating signals in this group.

METHODS

Study Species

Corethrella appendiculata is a tree-hole breeding species, in which larvae are predacious and feed on aquatic insects including mosquito larvae (Lounibos, Makhni, Alto, & Kesavaraju, 2008). Adult females of *C. appendiculata* are facultative autogenous and thus do not require a blood meal to lay their first batch of eggs. We obtained *C. appendiculata* males and females from our colony maintained at Texas Tech University (Bernal Lab, Department of Biological Sciences). The colony was maintained in a

$20 \times 20 \times 20$ cm mosquito-rearing cage. Details about the colony are described in de Silva and Bernal (2013). Since adult midges are sexually mature and mating takes place 4 days after emergence (de Silva & Bernal, 2013), we only used 5-day-old virgin adults to guarantee including only sexually mature individuals in our experiments.

Flight Sound Recording in Free-flying Swarms

To examine the natural flight tones of frog-biting midges, we recorded the wing beat frequencies of natural-forming swarm, which consist of over 900 male midges, while mating in their rearing cage. In general, the sex ratio is close to 1:1 in our colony. We used a particle velocity microphone (Knowles NR-21358 electric condenser microphone, Itasca, IL, U.S.A.) with an integral FET amplifier to record the flight tones of individuals in the mating swarm. This microphone offers noise cancellation and a bandwidth of 100 Hz–4 kHz. The sensitivity of the microphone is −52 dB, relative to 10 V/Pa. The microphone was placed inside the rearing cage during 1800–1900 hours, when this species is known to swarm (de Silva & Bernal, 2013). A total of 5 h were recorded during 5 days, each day with a different swarm.

Flight Sounds of Individual Midges

We recorded the sounds emitted by tethered flying male and female frog-biting midges ($N = 10$ for each sex) following Göpfert et al. (1999). We anaesthetized individuals on a custom-made CO_2 bed at 68.95 kPa (Cornelius CO_2 Gas Regulator, Cornelius Inc., Glendale Heights, IL, U.S.A.) and tethered each individual to the blunted end of an insect pin (4 cm, size 000, Bioquip 1208B, Czech Republic) using superglue (Ross Products, Inc., Columbus, OH, U.S.A.). Tethers were positioned on the dorsal surface of the abdomen, posterior to the thoracic flight muscles, allowing individuals to fly naturally. Tethered individuals were suspended 2 cm in front of a Knowles NR-21358 microphone with a custom electronic circuit used to power the microphone and amplify the recordings. The head of each midge was pointed towards the particle velocity microphone, and all sounds were recorded using a Marantz PMD660 professional sound digital recorder (sampling rate of 44.1 kHz, 16-bit resolution). Following Cator et al. (2009), we recorded the wing beat sounds of suspended midges that sustained flight for at least 10 s.

Flight Sounds of Tethered Pairs

To investigate the acoustic interactions between the sexes of frog-biting midges, we recorded the flight tones of pairs by moving a tethered flying female midge past a stationary tethered flying male following the procedure described by Cator et al. (2009). A tethered *C. appendiculata* male was positioned 1.5 cm from the particle velocity microphone and, 5 s after they initiated flight, a tethered female was introduced into the arena (10 cm). We moved the female in and out of the male's acoustic range (within 2 cm) while recording the flight tones of both individuals for 10 s before and during their interaction. We examined the acoustic interactions of 35 male−female pairs, estimating the frequency of the first five harmonics of their wing beat sounds to determine changes in flight tones during their interactions. To further examine the context in which wing beat matching occurs, we also tested the acoustic interactions between same-sex pairs. We recorded the flight tones of 10 male−male pairs by moving a tethered flying male midge past a stationary tethered flying male.

Sound Analysis

For all recordings, temperature ($27 \pm 5 \,^{\circ}C$) and humidity ($75 \pm 5\%$ RH) were controlled, given that these factors are known to affect wing beat frequency in mosquitoes (Clements, 1999; Takken et al., 2006). Test individuals were exposed to light intensities known to elicit swarming behaviour (<2 lx, de Silva & Bernal, 2013). All recordings initially were viewed and normalized (70%) using CoolEdit Pro 2.1 (Syntrillium Software, Scottsdale, AZ, U.S.A.) to identify wing beat sounds. Selected sound clips (4—6 s long) were analysed using Raven 1.0 (Cornell University Laboratory of Ornithology, Ithaca, NY, U.S.A.). Based on the computed power spectra, we measured the frequency of harmonics 1—5 (discrete Fourier transform, DFT = 256 samples; frequency resolution = 43.2 Hz; time resolution = 11.6 ms; 50% frame overlap) for sound clips representing free-flying swarms ($N = 42$), female—male interactions ($N = 35$), male—male interactions ($N = 10$). A total of 87 sound clips were analysed.

Wing beat frequency data were normally distributed and thus parametric statistic tests were used (Zar, 1999). We examined the changes between fundamental frequencies of the flight tones of males and females using a Student t test. In addition, we compared the harmonics of wing beat frequencies of males and females using a repeated measures ANOVA. We compared the wing beat frequencies of a tethered midge when it was in flight by itself and the same individual when it was interacting with a midge of the opposite sex using paired t tests. The frequency of the wing beats produced during interactions between opposite-sex and same-sex pairs in which flight tones were matched were contrasted using a Fisher's exact test. Statistical analyses were conducted using SYSTAT 13 (Wilkinson, 1990) and MINITAB 16 statistical software (Ryan, Joiner, & Cryer, 2012).

Ethical Note

Experiments were conducted in accordance with animal welfare guidelines. Maintenance of the frog-biting midge colony was reviewed and approved by Animal Care and Use Committee at Texas Tech University (ACUC 12092-12) and Purdue University (PACUC 1401001015). We encountered no problems using carbon dioxide as an anaesthetic, and frog-biting midges effectively recovered from it. Handle and harm to frog-biting midges was minimized when tethering them by performing the procedure while they were anaesthetized. After the experiments, tethered individuals were euthanized under low temperatures following Bernal et al. (2006).

RESULTS

The characteristics of wing beat frequencies of male and female *C. appendiculata* midges are described in Table 1. Their wing beat tones consist of a stack of harmonics with energy detectable in recordings of tethered individuals to up to the fifth harmonic. There were differences in the characteristics of wing beat frequencies between the sexes (see Supplementary Audio S1, S2). The fundamental frequency of the wing beats of males was significantly higher than that of females (Student's t test: $t_9 = 4.781$, $P = 0.001$). Not surprisingly, the frequencies of other harmonics (second to fifth) also differed significantly between males and females (repeated measures ANOVA: $F_{4,54} = 147$, $P = 0.001$).

Recordings of individuals flying in the free-mating swarms resulted in flight tones with spectral features characteristic of low, female-like wing beats and high, male-like wing beats. While a few recordings contained wing beats with relatively low fundamental frequencies (648.1 ± 12.7 Hz, $N = 9$), the majority of recordings contained wing beats with high, male-like fundamental frequencies averaging 977.5 ± 40.3 Hz ($N = 30$; Table 1). The wing beat frequency of free-flying midges from both sexes, however, was significantly higher than that of tethered individuals (paired t test: males: $t_{60} = 3.46$, $P = 0.001$; females: $t_{39} = 3.55$, $P = 0.001$). Overall, free-flying individuals produced wing beats with fundamental frequencies that were about 90 Hz higher than those of tethered midges (males: 94.76 ± 3.77 Hz; females: 87.06 ± 6.26 Hz).

Although recordings of the swarms had low signal-to-noise ratio given the high densities of individuals flying together, we detected three cases (7% of sound clips from the swarm) in which convergence of wing beat frequencies between individuals seemed to occur. The third harmonic of individuals with high-frequency fundamental flight tones, suspected to be males, converged with the fourth harmonic of the wing beat of individuals with lower-frequency fundamental flight tones, potential females. Such harmonic convergence occurred at around 2900 Hz, with a difference between harmonics of 6.4 ± 23.2 Hz (Table 1). Wing beat convergence lasted about 3—5 s when individuals were flying by in the swarm.

In the presence of a midge from the opposite sex, tethered pairs of males and females significantly altered their fundamental wing beat frequencies (paired t test: $t_{34} = 3.65$, $P = 0.0001$; Table 1, Supplementary Audio S3). Harmonic convergence occurred in 22 of 35 (62%) pairs of *C. appendiculata* tested, and in the majority of those cases (17 of 22), males and females differentially increased the frequency of their flight tones until their harmonics matched (by 111.7 ± 46.9 Hz in males and 52 ± 40.4 Hz in females). This frequency change resulted in a close match (6.8 ± 30.1 Hz) between the third and fourth harmonic of males and females, respectively (Fig. 1). Most pairs converged in the frequency of their upper harmonics at about 2675 Hz (Table 1). In a few pairs (11%), however, harmonic convergence occurred between the male's second harmonic (1912.2 ± 39.9 Hz) and the female's third harmonic (1926.6 ± 62.2 Hz). Whether convergence occurred between the third and fourth harmonic or between the second and third harmonic was not influenced by how close in frequency those harmonics were before their interaction. The shift in frequency required for harmonic convergence differed between the sexes depending on the specific harmonics they matched (paired t test: males: $t_{34} = 3.65$, $P = 0.025$; females: $t_{34} = 3.65$, $P = 0.009$). For males, converging at the third and fourth harmonic required a smaller frequency shift (232.6 ± 96.4 Hz) than when converging at the second and third harmonics (427.7 ± 74.2 Hz). In contrast, for females, the frequency shift was smaller when converging at the second and third harmonics (114.7 ± 98 Hz versus 325.9 ± 103 Hz). In 22% of the pairs, the individuals did not shift their wing beat frequencies to converge at any harmonic frequency.

When pairs of tethered same-sex individuals were interacting, the frequency of the two males diverged. Although the wing beat frequencies of same-sex pairs converged in a few cases (3 out of 10), it lasted only a brief period (<1 s). Those cases of convergence were significantly less frequent (30%) than convergence resulting in interactions between opposite-sex pairs (62%) (Fisher's exact test: $N = 10$, $P = 0.036$). Divergence of wing beat frequencies in male—male pairs was common (70%), with each individual increasing or decreasing its flight tones compared with that of solo males (Fig. 2). Such shifts resulted in differences of 67.42 ± 65.9 Hz ($N = 7$) between the fundamental frequencies of the males.

DISCUSSION

Our results revealed and characterized the acoustic mating interactions of a species of frog-biting midge, *C. appendiculata*. We

Table 1
Average flight tone frequencies (Hz) of *Corethrella appendiculata*

Recording	N	Harmonic				
		Fundamental	Second	Third	Fourth	Fifth
Swarming individuals						
Single individuals						
'Male'	30	977.5±40.3	1962.1±49.6	2911.0±151.6	3942.8±187.6	4807.2±218.8
'Female'	9	648.1±12.7	1250.6±65.1	1973.3±54.8	2918.2±96.7	3891.6±68.8
Acoustic interactions						
'Male'	3	1010.7±20.4	2043.9±37.6	**2931.1±26.7**	3934.5±28.4	4119.3±52.1
'Female'	3	658.2±10.3	979.5±17.7	1958.0±47.5	**2937.5±49.9**	3953.0±46.0
Tethered individuals						
Single individuals						
Male	10	762.4±19.0	1498.4±50.3	2417.7±53.6	3312.9±49.2	4582.9±123.9
Female	10	561.6±10.9	1228.3±71.3	1820.3±50.2	2361.6±39.1	3005.4±73.8
Acoustic interactions						
Male–Female	35	882.7±44.1	1772.5±69.5	**2670.9±56.4**	3455.5±79.6	4430.4±82.7
Female–Male	35	620.9±36.9	1209.9±40.7	1876.8±99.1	**2679.3±113.8**	3171.5±122.6
Male₁–Male₂	7	972.0±33.8	2006.9±66.6	2857.8±60.8	3584.3±139.2	4716.6±151.1
Male₂–Male₁	7	904.7±49.5	1680.7±64.8	2610.2±212.9	3305.4±138.1	4312.4±180.3

Means ± SE are shown for free-flying swarming individuals, tethered individuals of each sex flying independently and tethered same-sex and opposite-sex pairs acoustically interacting. Values in bold denote frequencies at which the wing beats converged in the swarm and in tethered pairs. Wing beats recorded in the swarm that were identified as being produced by each sex based on their spectral properties are in quotes ('Male' and 'Female').

found that both same-sex and opposite-sex pairs interacted in flight prior to mating. While pairs of males and females modulated their wing beats so their upper harmonic frequencies converged (at the male's third and the female's fourth harmonic), pairs of interacting males diverged (third harmonic). Such acoustic interactions occurred both when the individuals were tethered and when they were freely flying in mating swarms.

Our results show that flight tones from tethered individuals have lower frequencies than those from individuals flying in swarms. Although similar differences between swarming and
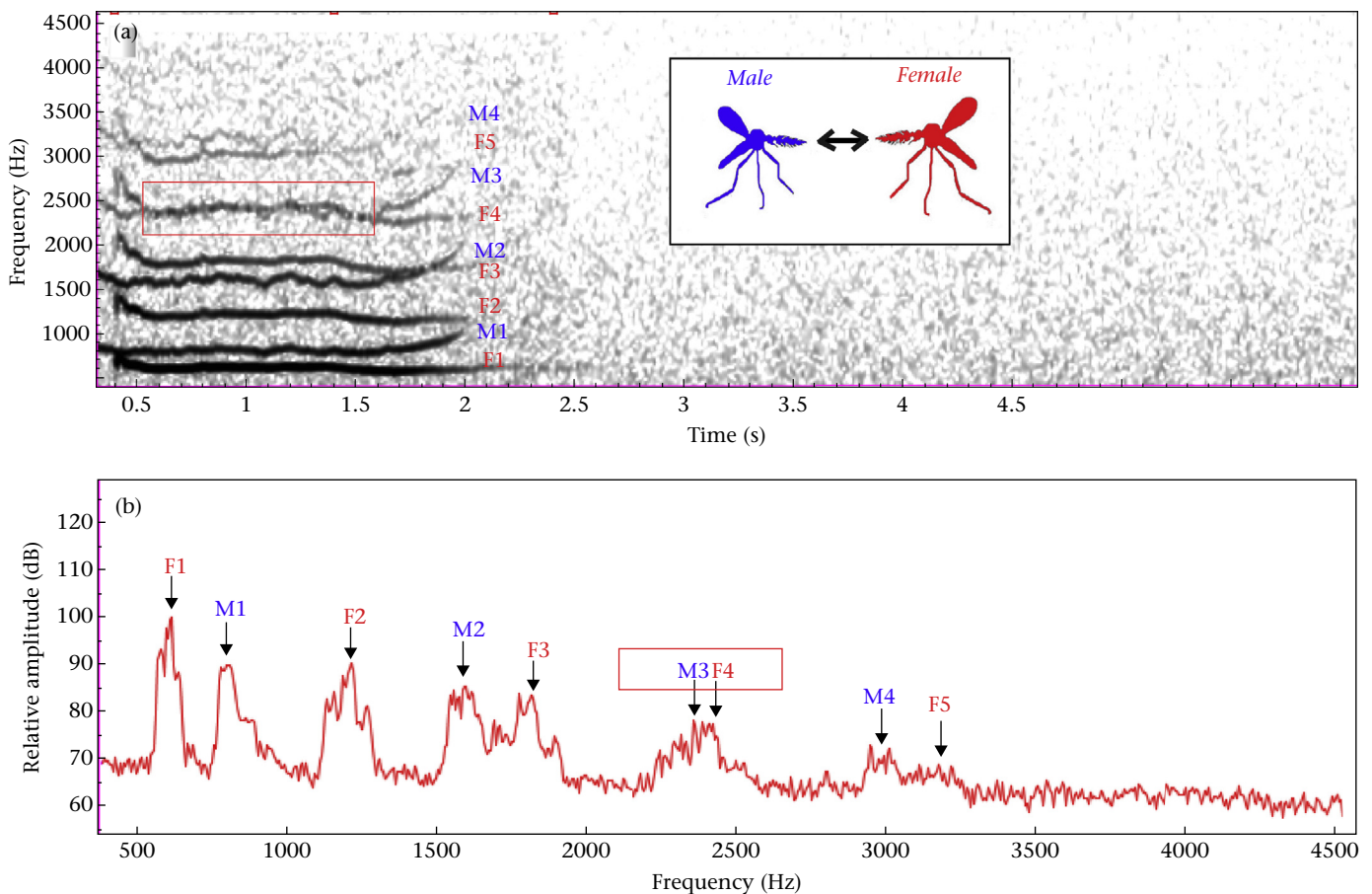
Figure 1. Representative sound clip illustrating the acoustic interactions between tethered male and female *C. appendiculata*. Spectrogram (a) and power spectra (b) depicting the convergence in the upper harmonics in the male's third (M3) and the female's fourth (F4) harmonic frequencies. Harmonics are indicated by arrows in (b). Boxes highlight the harmonic convergence in the spectrogram and the power spectra, respectively.
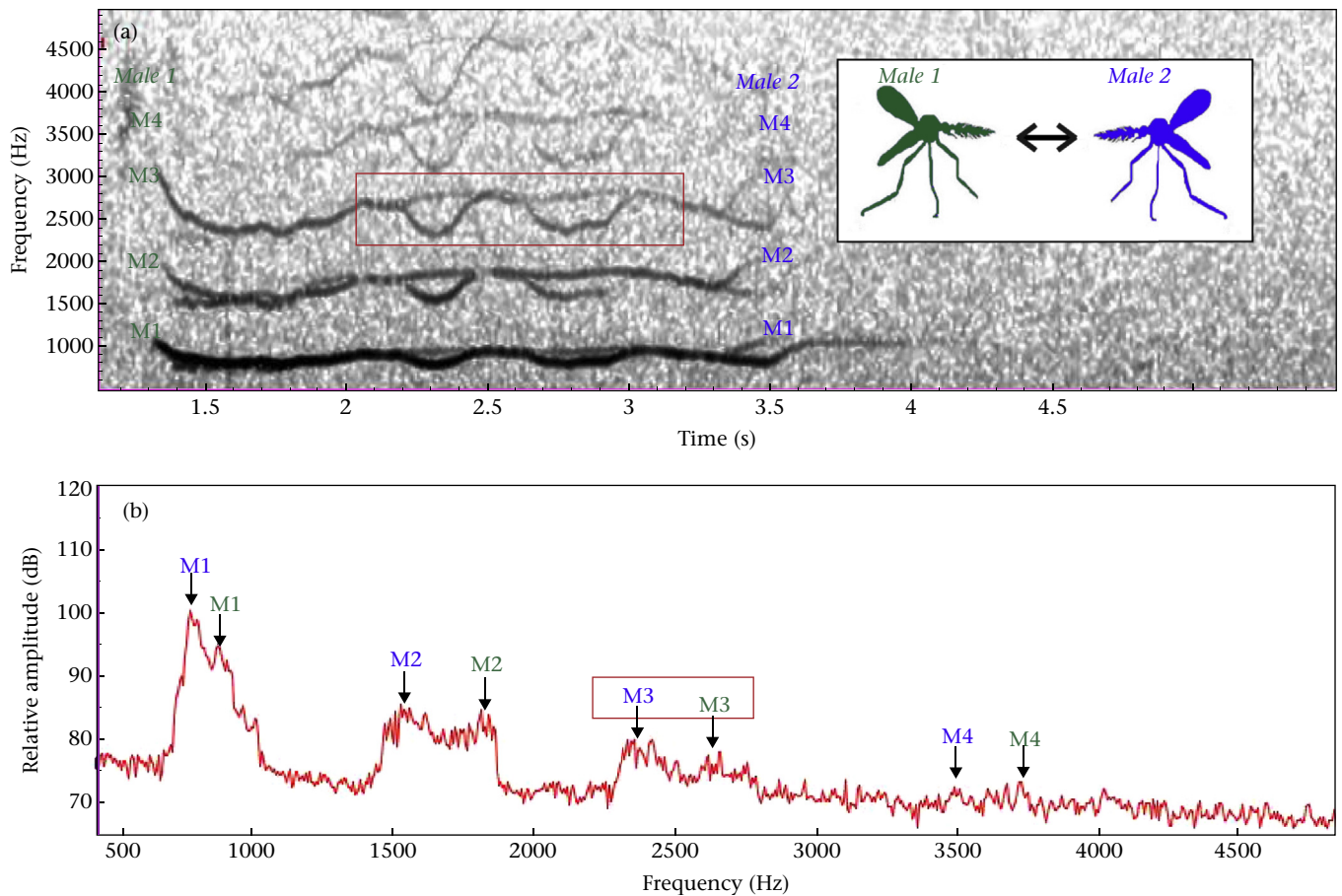
Figure 2. Representative sound clip illustrating the acoustic interactions between tethered males of *C. appendiculata*. Spectrogram (a) and power spectra (b) depicting the divergence of the third harmonic of the flight tones of two males. Harmonics are indicated by the arrows in (b). Boxes highlight the frequency divergence in the spectrogram and the power spectra, respectively.

tethered individuals have been attributed to differences in temperature between field and laboratory recordings (*Aedes aegypti*: Cator et al., 2011), in both conditions in our study the temperature was identical. Tethering is a standard and widely used technique to record flight tones in mosquitoes. This process of immobilizing the insect and/or the use of carbon dioxide to anaesthetize it could potentially change their flying pattern. Although it has previously been suggested that tethering may change the wing beat frequencies compared to those produced by free-flying individuals (Cator et al., 2011), this is the first study that compares the flight tone of free-flying and tethered individuals under the same environmental conditions. Studies that control for differences in age, body size and the effect of the anaesthesia are necessary to evaluate whether these factors also contribute to changes in wing beat frequencies. The difference reported here, however, is relevant for studies investigating signal recognition among sympatric species (e.g. Cator et al., 2010; Gibson, Warren, & Russell, 2010; Tripet, Dolo, Traore, & Lanzaro, 2004) or for those interested in implementing flight tones for mosquito control programmes (e.g. Ikeshoji, 1985; Ikeshoji et al., 1987; Ikeshoji & Ogawa, 1988; Stone, Tuten, & Dobson, 2013). Use of field techniques to record flight tones in swarming mosquitoes as developed by Cator et al. (2011), or laboratory recordings of swarms as described here, are necessary to expand current work to understand the behaviour of free-flying individuals.

Harmonic convergence of the flight tones of male and female frog-biting midges during mating is a behaviour previously known

only for a few mosquito species. This study complements recent studies showing the active role in mating of female mosquitoes and now, frog-biting midges. In these midges, wing beats are also used during male–male interactions, probably to deter rival males. In most of the acoustic interactions between males, individuals adjusted their wing beat frequencies to diverge in their upper harmonics rather than to converge as they do in interactions between opposite-sex pairs. This divergence is consistent with observations of interactions between male mosquitoes of *Toxorhynchites brevipalpis* and *Culex quinquefasciatus* (Gibson et al., 2010; Warren et al., 2009). This behaviour probably allows them to maintain their spatial separation to avoid jamming in the swarm (Gibson et al., 2010). As in many mating signals in other taxa, flight tones serve a dual function: they are used to attract females and to avoid rival males.

The wing beats of frog-biting midges cover a wide frequency range (0.5–5 kHz) that matches the frequency range of calls produced by frogs and toads (Gerhardt & Huber, 2002). Although mosquito wing beats also cover a similar range that partially overlaps with the spectral domain of anuran calls (0.3–3 kHz; Göpfert & Robert, 2001), behavioural responses to conspecific wing beats suggest that mosquitoes are sensitive to lower frequencies than frog-biting midges. When interacting with conspecifics, mosquitoes modulate their wing beats to match the male's second harmonic frequency and the female's third harmonic frequency, converging at about 1.2 kHz (Cator et al., 2009, 2010; Warren et al., 2009). Our results show that *C. appendiculata* modulate their wing

beats so that their upper harmonic frequencies converge when interacting with individuals of the opposite sex. In these midges, however, convergence occurs at a higher frequency than previously described for mosquitoes. In frog-biting midges, harmonic convergence occurs between the third harmonic of the male wing beat and the fourth harmonic of the female wing beat, at frequencies more than twice as high as those known for mosquitoes (about 2.7 kHz and 2.9 Hz in tethered and swarming individuals, respectively). This harmonic matching suggests that frog-biting midges perceive higher frequencies than mosquitoes, better matching the frequency range of anuran calls, which could predispose them to exploit the advertisement calls of anurans.

Our results indicate that acoustic interactions in flight prior to mating extend beyond Culicidae. Other studies have presented circumstantial evidence suggesting that other related families, such as Chironomidae (Fedorova & Zhantiev, 2009; Fyodorova & Azovsky, 2003) and Ceratopogonidae (Blackwell & Mordue, 1992; Downes, 1969), may also use flight tones in mating. The use of acoustic mating signals is likely to be widespread taxonomically in the infraorder Culicomorpha, a clade that includes both mosquitoes (Culicidae) and frog-biting midges (Corethrellidae) together with Chaoboridae, Chironomidae, Ceratopogonidae, Dixidae, Simuliidae and Thaumalidae. Studies that further investigate the use of flight tones in mating are necessary, however, to better understand the evolution of this behaviour across families in this infraorder.

Sensory systems evolve under strong selective pressures from multiple contexts such as predation, foraging and mating (Bradbury & Vehrencamp, 1998; Endler & Basolo, 1998). When a sensory system has evolved due to selection in one context, it may allow organisms to exploit signals and cues for other purposes. Studies investigating this pre-existing sensory bias hypothesis have focused on signals and cues that are incorporated from other contexts into mating (Proctor, 1991; Weller, Jacobson, & Conner, 1999). Less attention, however, has been given to how sensory systems that evolve for mating may guide the use of signals or cues to avoid predators, or to forage. In frog-biting midges, this pre-existing sensory bias hypothesis proposes that eavesdropping on anuran calls was co-opted from the ability to use sound in mating. The phylogenetic relationships of frog-biting midges (Corethrellidae), mosquitoes (Culicidae) and other Culicomorpha suspected to use sound in mating (Chironomidae and Ceratopogonidae) suggest that the use of wing beats as acoustic mating signals is an ancestral trait in this clade. Thus, consistent with the pre-existing sensory bias hypothesis, our results framed in a phylogenetic context, show that the use of sound in mating in frog-biting midges evolved before their eavesdropping behaviour.

If the ability to eavesdrop on anurans evolved from the use of flight tones in mating, the antennae of the midges should be able to detect anuran calls. It is unknown, however, how frog-biting midges perceive acoustic stimuli. McKeever (1988) proposed that their antennal sensilla pediconica act as sound receptors but, given their rigid structure and their absence in some species of frog-biting midges attracted to frog calls (A. Borkent, personal communication; X. E. Bernal, personal observation), these structures are unlikely to have such function. The setose antennae of the midges and their communication using flight tones suggest that, similar to mosquitoes, antennal hearing is used in this group. Although use of their antennae to perceive conspecific wing beats is expected, it is of debate whether this mechanism allows them to detect anuran calls. Field experiments indicate that frog-biting midges respond to frog calls in the far field where particle displacement is substantially diminished (Bernal et al., 2006). Antennal hearing in mosquitoes, however, is assumed to be restricted to perceiving sounds in the near field (Clements, 1999; Göpfert & Robert, 2001). While the sensitivity of mosquito

antennae to sounds in the far field has not been investigated, anecdotal evidence has been invoked to suggest that they may perceive sounds in the far field (Borkent, 2008; Roth, 1948). Given that studies of antennal hearing in mosquitoes have recently challenged common assumptions of antennal sensitivity (Cator et al., 2009; Nadrowski, Effertz, Senthilan, & Göpfert, 2011), it is possible that the antennae and the associated Johnston organ of mosquitoes and frog-biting midges are more sensitive than previously thought. Ongoing studies investigating the sensory mechanisms underlying the response of frog-biting midges to conspecific wing beats and anuran calls will determine how they hear and will further evaluate the pre-existing sensory bias hypothesis for eavesdropping in this group.

To conclude, our results revealed that frog-biting midges alter the overall frequency of their wing beats when interacting with conspecifics, providing robust evidence for the use of acoustic signals in mating in a species of frog-biting midges. Our work provides a framework to understand the use of sound in multiple contexts in this eavesdropping midge and complements recent studies investigating the acoustic mating signals of mosquitoes (Cator et al., 2009; Gibson & Russell, 2005; Gibson et al., 2010; Pennetier et al., 2010; Warren et al., 2009). Harmonic convergence at higher frequencies suggests higher acoustic sensitivity in frog-biting midges than in the closely related family of mosquitoes. Ultimately, this study provides the first line of support to the preexisting sensory bias hypothesis for eavesdropping in frog-biting midges. Further studies that examine the sensory mechanism used to hear sound by frog-biting midges are necessary to further test this hypothesis.

Supplementary Material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.anbehav.2015.02.002.

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