Mosquito (*Aedes aegypti*) flight tones: Frequency, harmonicity, spherical spreading, and phase relationships

Benjamin J. Arthur,^{a)} Kevin S. Emr,^{b)} Robert A. Wyttenbach,^{c)} and Ronald R. Hoy^{d)}

Department of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853

(Received 25 April 2013; revised 13 October 2013; accepted 16 December 2013)

Mosquito flight produces a tone as a side effect of wing movement; this tone is also a communication signal that is frequency-modulated during courtship. Recordings of tones produced by tethered flying male and female *Aedes aegypti* were undertaken using pairs of pressure-gradient microphones above and below, ahead and behind, and to the left and right over a range of distances. Fundamental frequencies were close to those previously reported, although amplitudes were lower. The male fundamental frequency was higher than that of the female and males modulated it over a wider range. Analysis of harmonics shows that the first six partials were nearly always within 1 Hz of integer multiples of the fundamental, even when the fundamental was being modulated. Along the front-back axis, amplitude attenuated as a function of distance raised to the power 2.3. Front and back recordings were out of phase, as were above and below, while left and right were in phase. Recordings from ahead and behind showed quadratic phase coupling, while others did not. Finally, two methods are presented for separating simultaneous flight tones in a single recording and enhancing their frequency resolution. Implications for mosquito behavior are discussed.

© 2014 Acoustical Society of America. [<http://dx.doi.org/10.1121/1.4861233>]

PACS number(s): 43.80.Ka, 43.80.Ev [MJO]

Pages: 933–941

I. INTRODUCTION

Insect flight produces a continuous sinusoidal tone at the frequency of wing movement, although higher harmonics may dominate in some insects (Sotavalta, 1947; Williams and Galambos, 1950; Webb *et al.*, 1976). This flight tone is characteristic of a species and can sometimes be used to identify species or count individuals (Brogdon, 1998; Moore and Miller, 2002; Raman *et al.*, 2007). For mosquitoes, however, the flight tone is more than a mere by-product of locomotion; it is a critical communication signal.

It has long been known that male mosquitoes are attracted to real and artificial female flight tones (Maxim, 1901; Ross, 1901; Roth, 1948; Wishart and Riordan, 1959). Tones are detected by the antennae of the male, which are more plumose than those of the female, have a larger Johnston's organ, and are more sensitive to vibration (Göpfert *et al.*, 1999). More recently, it has been shown that female mosquitoes hear and respond to male flight tones. In several species that have been investigated so far, courting males and females modulate their flight tones to converge toward a common frequency. Males and females of *Toxorhynchites brevialpis* have similar flight tones and converge on the same wingbeat frequency during courtship, while same-sex pairs modulate wingbeat frequencies to avoid overlap (Gibson and Russell, 2006). In *Culex quinquefasciatus*, *Culex pipiens*, *Anopheles gambiae*, and *Aedes*

aegypti, the frequencies of male and female flight are so far apart that convergence on the same fundamental frequency may not be compatible with flight. Instead, these species converge on a shared harmonic, the male first and female second or the male second and female third (Cator *et al.*, 2009; Warren *et al.*, 2009; Cator *et al.*, 2010; Pennetier *et al.*, 2010). Following successful courtship, the pair remains together in flight during copulation.

Discussion of the mechanisms of convergence tends to focus on the properties of the receiver, with measurements of the sensitivity of the flagella to vibration and the electrical responses of Johnston's organ (Cator *et al.*, 2009; Warren *et al.*, 2009; Arthur *et al.*, 2010). Beyond basic measurements of frequency and amplitude (Belton and Costello, 1979; Brogdon, 1998; Wekesa *et al.*, 1998), there is little published data on the acoustic properties of mosquito flight tones themselves. The current study presents detailed analysis of flight tones recorded over a range of distances in the six cardinal directions around male and female mosquitoes flying on tethers. In addition, since flight tones of a courting male and female are normally recorded with a single microphone and have overlapping harmonics, methods for separating simultaneous flight tones in a single recording are presented.

II. METHODS

A. Mosquitoes

Ae. aegypti for this study came from a lab colony established from eggs collected in Tapachula, Mexico (14°54'N, 92°15'W) in 2006 and supplemented with field-collected eggs from the same region in 2008 and 2009. Mosquitoes were kept in an environmental chamber simulating natural conditions, with a 14:10 h light:dark cycle and 2 h of dawn

^{a)}Present address: Scientific Computing, Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147.

^{b)}Present address: SUNY Upstate Medical School, Syracuse, NY 13210.

^{c)}Present address: NBB Program, Emory University, 1462 Clifton Rd. NE, Suite 304, Atlanta, GA 30322.

^{d)}Author to whom correspondence should be addressed. Electronic mail: rrh3@cornell.edu

and twilight, at $75 \pm 7\%$ relative humidity, and at $22\text{--}30^\circ\text{C}$ fluctuating temperature. Eggs were vacuum-hatched in water to obtain simultaneous cohorts. Larvae were fed 1:1 lactalbumin and brewer's yeast. Male and female pupae were transferred to separate 2-l containers with mesh lids and offered a 20% sucrose solution upon eclosion. No more than 45 pupae were placed in a container. Containers of adult mosquitoes were kept in the environmental chamber until the day of an experiment. After recordings, wing length was measured as a proxy for body mass (Nasci, 1990), averaging 2.3 ± 0.2 mm (males) and 2.8 ± 0.2 mm (females). All mosquitoes used in these experiments were 7–28 days post eclosion.

B. Recordings

For recording, a mosquito was tethered to a stiff stainless steel wire with cyanoacrylate ester on the dorsal prothorax. An articulating arm mounted in the center of a vibration table (Technical Manufacturing Corp., Peabody, MA, USA) held the mosquito in a natural flight position with its abdomen 45° downward and antennae 15° upward. After tethering, the mosquito was allowed to rest in a quiet environment for approximately 5 min before the first recording. A pair of calibrated pressure-gradient microphones was positioned on opposite sides of the mosquito, with sequential trials simultaneously measuring sound radiating ahead (anterior) and behind (posterior), left and right, or above (dorsal) and below (ventral), at distances ranging from 1 to 19 cm from the midline between wing hinges. Side, distance, and sex were interleaved randomly within and across mosquitoes and days. Between recordings, flight was inhibited by allowing the mosquito to grasp a small piece of paper. Flight was initiated by removal of the paper and, when necessary, a gentle air puff. Data were acquired to a computer after digitizing (System III, Tucker Davis Technologies, Alachua, FL, USA) using custom software written in MATLAB (MathWorks, Natick, MA, USA). A 24 kHz sample rate permitted 35 s of flight tone to be recorded on each trial. Recordings took place at a temperature of approximately 23°C .

C. Microphones

Pressure-gradient microphones were used to measure air particle velocity (CMP-5247TF-K, CUI Inc., Tualatin, OR, USA). A custom electronic circuit powered the microphone and amplified the signal (Arthur *et al.*, 2013). Calibration

was performed relative to a factory-calibrated pressure microphone (4135 1/4 in., Brüel & Kjær, Denmark) in anechoic far-field conditions using procedures described elsewhere (Arthur *et al.*, 2010). Sensitivity was comparable to that of other pressure gradient microphones, except for a high-frequency roll off at 5 kHz due to a low-pass anti-aliasing filter in the amplifier (Fig. 1, cf. the Knowles NR-23158 in Fig. 1 of Arthur *et al.*, 2010).

D. Analysis

Spectrograms were created using Fourier transforms on overlapped segments of time. Log magnitudes of the coefficients were plotted using a gray scale that clipped the extreme one percent at both ends. Frequency modulation of the fundamental and its harmonics was quantified using the estimated phase change that would occur in the Fourier coefficients if the time segment were shifted by one sample (Charpentier, 1986; Brown and Puckette, 1993). This approach gives a more precise measure of frequency than would interpolation between peaks in the magnitude spectrum. The resulting one-dimensional time series also greatly simplified the analysis presented in Figs. 2, 3, 5, and 6. Amplitudes of the fundamental and its harmonics at an instant in time were determined by averaging Fourier coefficients across a few successive segments that exhibited minimal frequency modulation, interpolating the magnitude spectrum at the set of integer-related frequencies closest to the peaks, and correcting with the microphone calibration data. Time-varying phases of the fundamental and its harmonics were quantified by fitting sines and cosines at integer-related frequencies of interest, using the arctangent to compute the phase, and adjusting with the microphone calibration data. Decrease in amplitude with distance was fit with a power function, taking into account the 1-mm distance between the front of the microphone and the sensing diaphragm. Polyspectra were calculated on a line corresponding to the harmonic ratios of interest rather than over the full space (Brillinger, 1965; Mendel, 1991). Complex Fourier coefficients were conjugated as appropriate before plotting the log magnitude of their product. Bicoherence was computed by normalizing block-averaged bispectra [Eq. (29) of Kim and Powers, 1979].

III. RESULTS

Flight tones were recorded from 31 male and 28 female mosquitoes in the six cardinal directions at distances of 1 to 19 cm in 2-cm increments. Flight was initiated within 5 s of

FIG. 1. The (A) magnitude and (B) phase of the response to particle velocity of the two pressure-gradient microphones used in paired recordings. The dashed line in (B) shows the expected quarter-cycle phase lag between pressure and particle velocity.

FIG. 2. (A) Spectrogram of the sound generated by a tethered flying male mosquito as recorded by a pressure-gradient microphone 1 cm in front of the head. (B) Histograms of fundamental frequencies as they varied throughout frontal recordings. Solid lines show data from 10 males; dashed lines are from 11 females. From bottom to top, individuals within each sex are sorted from lowest to highest mean frequency. (C) Spectral analysis of the fundamental frequencies in (B) treated as time-varying waveforms. The amplitude of modulation in Hz is plotted against the frequency of modulation in Hz.

the beginning and lasted beyond the 35-s recording duration in 20 of the 70 recordings at 1 cm. The median flight duration in all 70 recordings was 17 s. In general, flight tones consisted of a fundamental tone and overtones up to at least 12 kHz, the Nyquist frequency of our system, that were synchronously modulated in time [Fig. 2(A)]. As reported previously (Göpfert *et al.*, 1999; Cator *et al.*, 2009), the male fundamental frequency was higher than that of the female, with both sexes showing considerable variability [Fig. 2(B)]. Mean frequencies of males ranged from 571 to 832 Hz (overall male mean and standard deviation, 711 ± 78 Hz, $n = 10$), while females ranged from 421 to 578 Hz (overall 511 ± 46 Hz, $n = 11$). Males and females differed significantly (t -test, $p < 10^{-7}$). The standard deviation of individuals was much less than that of the group, 29 Hz for males and 15 Hz for females, resulting in individual coefficients of variation of 0.042 ± 0.015 and 0.028 ± 0.017 , respectively; the latter distributions were not significantly different (t -test, $p > 0.05$). Males and females modulated their wingbeat frequencies at similar rates, with the largest changes occurring slowly [Fig. 2(C)]. Novel findings reported here include measurement of inharmonicity, the rate of spherical spreading loss, and the phase relationships between directions and partials. We also present two methods for separating a pair of flight tones recorded simultaneously with a single microphone.

A. Inharmonicity

The extent to which overtones were integer multiples of the fundamental was assessed by extracting the time-varying

frequency of each overtone using successive overlapped short-time Fourier transforms (Brown and Puckette, 1993). The algorithm was manually seeded with the initial value of the fundamental, which was refined using the estimated phase derivative of the nearest spectral maximum. Maxima were then sought near integer multiples of the fundamental and their precise frequency quantified with the phase derivative. Each subsequent time segment was seeded with the fundamental frequency of the previous segment. This analysis showed that overtones were within a few Hz of being harmonic over time scales tens of seconds long, even while undergoing frequency modulation [Figs. 3(A) and 3(B)]. For the exemplar stack of Fig. 2(A), all overtones were within 1 Hz of integer multiples of the fundamental 97% of the time [Fig. 3(C)]. Over the entire data set, all overtones were within 1 Hz of integer multiples 90% of the time and within 2 Hz 98% of the time [Fig. 3(D)].

B. Attenuation with distance

Flight tones were the loudest ahead and behind, quietest to the right and left, and decreased rapidly with distance [Figs. 4(A) and 4(C)]. Each successively higher harmonic was also quieter than the preceding one [Figs. 4(B) and 4(D)]. Although individual harmonics could not always be seen in spectrograms at the furthest distances recorded, a harmonic stack was often salient to the eye due to synchronous frequency modulations. To avoid systematic bias, fits of attenuation with distance were limited to distances for which the fundamental flight tone could be seen in all

FIG. 3. (A) Modulated frequency of the lowest six partials in the stack of Fig. 2(A) after division by the partial number. Inset shows the extent of overlap. (B) The maximum frequency difference between all possible pairs of the divided partials in (A). (C) A histogram of the frequency differences in (B), with counts on a log scale. (D) Same as (C) for the data set analyzed in Figs. 2(B) and 2(C).

mosquitoes. Given this limitation, only recordings ahead of and behind mosquitoes provided enough points for curve fitting to be meaningful. In these two directions, exponential fits gave R^2 values >0.99 and p values <0.0001 in all cases. Particle velocity attenuated slightly faster than the square of distance, with exponential coefficients of 2.3 ± 0.2 for

recordings ahead of and behind males and 2.3 ± 0.1 and 2.5 ± 0.1 for recordings ahead of and behind females, respectively (mean $\pm 95\%$ confidence interval). To yield a cubic relation, distance would have to be 9 mm greater than measured; to yield a quadratic, it would have to be 3 mm less. Either one is greater than our estimated maximum error

FIG. 4. (A) Average instantaneous amplitudes of the fundamental frequency of nine males as a function of distance along the six cardinal directions. (B) Average instantaneous amplitudes of the lowest six harmonics at a distance of 1 cm ahead, for the same nine males. (C) and (D) Same measurements for nine females. Long dashed lines in each panel approximate the noise floor of the recording apparatus. Amplitude is given in dB re 5×10^{-8} m/s.

of ± 1 mm, based on divisions of the ruler used to measure distance.

C. Phase relationships

Simultaneous paired recordings enabled comparison of phases on opposite sides of the mosquito. While the sound radiating to the right was in phase with that to the left, ahead versus behind and above versus below recordings were both out of phase [Fig. 5(A)]. Analyzing the lowest six harmonics separately with phase spectra showed that ahead-behind and above-below recordings were out of phase for each harmonic as well [Fig. 5(B)]. The left-right recordings were in phase at their lowest harmonics, and had more-or-less uniform distributions at higher ones.

A comparison of phase between harmonics within each recording was undertaken to determine whether they arose from a quadratic nonlinearity. Sum and difference frequencies generated by such nonlinearities have phases equal to the sum of the phases of the original frequencies (Brillinger, 1965; Dubnov and Rodet, 1997). For example, the phases of harmonics 1 and 2 would sum to the phase of harmonic 3. We examined all such combinations of the lowest six harmonics for evidence of quadratic phase coupling (Fig. 6). Recordings ahead of and behind mosquitoes had bicoherence magnitudes near unity and hence satisfied the sole criterion for quadratic phase coupling of Kim and Powers (1979). However, only those from behind had a bicoherence phase near zero, satisfying the additional stricter criterion of Fackrell and McLaughlin (1995). Recordings from the right, left, above, and below had no clear pattern across the data set.

D. Separating simultaneous flight tones

During courtship, pairs of *Ae. aegypti* typically modulate their flight tones toward the male second and female third harmonic [Fig. 7(A)]. It is possible to separately assess

their frequency modulations by looking at the fundamental frequencies, which are distinctly separated. However, frequency resolution at low frequencies is relatively poor in discrete Fourier transforms [Figs. 7(C) and 7(D), lower panels]. The estimated phase derivative method used above to analyze inharmonicity can also be used to enhance frequency resolution [Fig. 7(B)]. For signals that are too obscured by noise to accurately estimate phase, polyspectra can achieve similar results. Frequency modulation of higher harmonics is multiplied by the harmonic number, while the frequency resolution of the Fourier transform remains the same across the spectrum [Figs. 7(C) and 7(D), upper panels]. The effective higher frequency resolution of these higher harmonics can be utilized by multiplying spectrograms across the non-overlapping harmonics [Fig. 7(E)]. In effect, a polyspectrum collapses harmonics together to increase time-frequency resolution. At the same time, noise is reduced by the root of the number of terms in the product, yielding results similar to that obtained with the estimated phase derivative method [Figs. 7(F) and 7(G)].

IV. DISCUSSION

The mosquito flight tone is an unusual communication signal in that its production is directly linked to locomotion. Unlike other insects, which advertise species and sex via temporal pulse patterns (Gerhardt and Huber, 2002), a mosquito can vary only the carrier frequency of its signal. The current study analyzed several aspects of the flight tone in order to better understand the acoustics of these courtship signals. Key findings were as follows. (1) Overtones are exact harmonics. (2) Intensity decreases with both distance and harmonic number. (3) Flight tone recordings ahead of and behind the mosquito are out of phase, as are those above and below, while those to the left and right are in phase. (4) Phases of harmonics are quadratically related only in recordings from ahead and behind. Given these acoustic properties, there are two methods of obtaining better resolution and/or

FIG. 5. (A) Simultaneous paired recordings of male flight tones with microphones 1 cm distant, expanded to show phase relationships. Solid lines represent recordings from ahead, right, and above; dashed lines represent recordings from behind, left, and below. (B) Cumulative histograms of the phase differences between simultaneous recordings at 1 cm. Each of the lowest six harmonics is plotted separately. Data are combined from full-length recordings of 10 males and 11 females (ahead-behind), 12 males and 11 females (right-left), and 13 males and 13 females (above-below); median duration of each recording was 17 s.

FIG. 6. (A) Phases of the first three harmonics of the flight tone shown in Fig. 2(A). (B) Summed phases of harmonics 1 and 2 minus the phase of harmonic 3. (C) Histograms of the phases in (A) and (B). The peak is for $(\Phi_1 + \Phi_2) - \Phi_3$; histograms for the single harmonics are nearly flat and barely visible because their phases are random. (D) The complex bicoherence of $(\Phi_1 + \Phi_2) - \Phi_3$ plotted on a unit circle in polar coordinates. Orientation of the arrow indicates the average phase in (B); length reflects variance in phase. (E) Bicoherence data for all mosquitoes reported in Fig. 5(B), showing all six cardinal directions and seven harmonic combinations. Each dot represents the vector from one mosquito; each arrow is the average vector from all mosquitoes.

sensitivity in recordings of harmonic convergence. First, time derivatives of the phases of the fundamentals provide an order of magnitude or more improvement in resolution in frequency modulation. Similarly, higher-order spectral analysis such as a polyspectrum increases the signal-to-noise ratio by collapsing information across harmonics. The latter technique may also extend the effective range of microphones, which is presently inadequate to record convergence of freely flying mosquitoes in the field.

A. Frequency

The flight-tone frequencies reported here are slightly higher than those reported elsewhere for tethered *Ae. aegypti* [Fig. 2(B); cf. Göpfert *et al.*, 1999; Cator *et al.*, 2009]. However, field recordings of free flight show significantly higher fundamental frequencies than those reported here, 982 vs 721 Hz for males and 664 vs 514 Hz for females (Cator *et al.*, 2011). In general, insect wingbeat frequency increases with temperature (Unwin and Corbet, 1984). Temperature may explain the some of the difference between field recordings (33°C) and our lab recordings

(23°C), but the greatest slope reported by Unwin and Corbet (1984), 5.4 Hz/°C for *D. melanogaster*, can only account for 54 Hz of the 150–260 Hz difference. We suspect an effect of tethering itself, since we have measured higher frequencies from high-speed video of freely flying *Ae. aegypti* of the same size as those used in the present work (S. Iams, unpublished data). Although video analysis of *D. melanogaster* shows distorted wing stroke patterns in tethered flight, the effect on wing beat frequency is not clear (Fry *et al.*, 2008).

Higher frequencies in free flight may affect mechanisms of convergence behavior. While mosquitoes flying at 721 and 514 Hz might converge on a shared harmonic of 1400–1500 Hz, mosquitoes at 982 and 664 Hz would have to converge on a shared harmonic of 1800–2000 Hz. The smaller field potentials produced by the latter frequencies make it less likely that convergence is based on directly hearing the shared harmonic (as suggested by recordings of Cator *et al.*, 2009) and more likely that beats between shared harmonics are involved (Warren *et al.*, 2009; Arthur *et al.*, 2010).

While males flying solo showed a greater absolute range of modulation than did females, modulation range as a

FIG. 7. (A) Spectrogram of the flight tones of a tethered male and female held approximately 2 cm apart (data from [Cator et al., 2009](#)). Blue and red labels indicate the male and female harmonics, respectively. Green box highlights an instance of convergence behavior. (B) The highlighted convergence from (A), with male (blue) and female (red) harmonics separated using estimated spectral phase changes of the non-overlapping fundamentals; the two time series were then overlaid. (C) Male harmonics 1, 3, 5, and 7 during the convergence, the four lowest that do not overlap with any female harmonics. (D) Female harmonics 1, 2, 4, and 5 during convergence, the four lowest that do not overlap with any male harmonics. (E) The product of the spectrograms in (C) and (D). Note the better frequency resolution and signal-to-noise ratios of these polyspectrograms compared to the single spectrograms above them. (F) The panels of (E) put into the red and blue channels of an RGB image, with contrast enhanced by raising each pixel to the fourth power. (G) The panels of (E) colored red and blue and then overlaid, omitting pixels that fell below an arbitrary threshold, illustrating another way to prepare the display.

fraction of the fundamental did not differ significantly between the sexes. Moreover, the rate of modulation was similar for male and female *Ae. aegypti* (Fig. 2). Male and female *T. brevipalpis* have similar flight tone frequencies; opposite-sex pairs converge during courtship, while same-sex pairs diverge their flight tones. It has been reported that

male-male pairs modulate their tones more rapidly than female-female pairs ([Gibson and Russell, 2006](#)). It is not known whether males of *T. brevipalpis* also modulate more rapidly during solo flight, or whether same-sex pairs of *Ae. aegypti* modulate at all.

B. Amplitude

The flight tone amplitudes reported here are much lower than others have found, 75 dB re 5×10^{-8} m/s [0.3 mm/s; Figs. 4(A) and 4(C)] as compared to 95 dB [3 mm/s; [Göpfert et al., 1999](#), Fig. 6(C)]. Reasons for this difference are not clear. Both studies used tethered mosquitoes with a microphone 1 cm in front of the head. The two studies used pressure-gradient microphones of different dimensions from different manufacturers but similar procedures for calibration and measurement.

Several recent studies have modeled the air movement and acoustic effects produced by hovering flight, forward flight, and wing dynamics ([Aono et al., 2008](#); [Bae and Moon, 2008](#); [Manela, 2012](#)). Insect flight proves to be more acoustically less straightforward than might be expected from a set of wings vibrating in unison. Furthermore, frequency composition of the flight tone varies between hovering and forward flight. During hovering, a drag dipole at the wingbeat frequency is generated along the front-back axis while a lift dipole at the second harmonic is generated along the left-right axis. In contrast, forward flight generates lift and drag dipoles at the wingbeat frequency without clear directivity differences ([Bae and Moon, 2008](#)).

In recordings on the azimuth plane around tethered flying blowflies, the first harmonic shows a dipole-like radiation, constricted at the sides, while the second harmonic has a more rounded monopole-like pattern. As a result, the second harmonic dominates at the sides while the first harmonic dominates at the front and back ([Sueur et al., 2005](#)). These results match the predictions for hovering flight. However, that study also found consistently greater amplitudes behind than ahead of the fly, as might be expected from the thrust component of forward flight in a tethered fly, along with a noisy non-periodic waveform at the rear, neither of which is predicted of hovering flight ([Bae and Moon, 2008](#)).

In the current study, the fundamental wingbeat frequency dominated in all directions [Figs. 4(B) and 4(D)] and there was no noisy waveform behind the mosquito (nor is there behind flying *Drosophila melanogaster*; [Bennet-Clark and Ewing, 1968](#)). Tethered mosquitoes also differed from tethered blowflies in that recordings ahead of and behind the mosquito were of the same amplitude [Figs. 4(A) and 4(C)]. These results may be more consistent with the predictions for forward than for hovering flight. Although tethering alters wing kinematics in consistent ways ([Fry et al., 2008](#)), it is difficult to know what type of flight is attempted by a tethered fly.

All of our recordings took place in the near field of the first few harmonics of the wingbeat frequency (defined as a distance $< 1/6$ of a wavelength from the source). Over the range of 1–9 cm in front of or behind the mosquito, amplitude attenuated slightly faster than the square of distance,

with an exponent of 2.3–2.5. This exponent lies between that expected of near-field particle velocity from a dipole source ($1/r^3$) and that expected from a monopole source ($1/r^2$) (Bennet-Clark, 1998). However, the near field of a simple dipole is complicated and particle velocity is not entirely radial (Dowling, 1997). The presence of several dipoles may complicate things further (Bae and Moon, 2008).

C. Phase

Phase relations between ahead-behind, right-left, and above-below recordings were as expected for a pair of wings moving in unison to provide lift and thrust (Fig. 5). Not surprisingly, analysis of overtones shows that flight tones were highly harmonic (Fig. 3), on par with most musical instruments (Brown, 1996). However, phase relations among those overtones were complicated, with only the recordings from behind showing strict quadratic phase coupling (Fig. 6). Quadratic phase coupling is often used along with other data to determine whether sounds come from a common source (e.g., Persson *et al.*, 2000). In the case of mosquito flight, co-modulation of harmonics makes the strongest case for a common source. If quadratic phase coupling were more prevalent in all directions, it might be useful for detecting flight tones in noisy recordings. To our knowledge, neither models of flight nor data from other flying insects have addressed phase relations in flight tones.

D. Analysis

We used two methods to separate simultaneous flight tones during convergence behavior. First, the frequency resolution of non-overlapping fundamentals can be enhanced with the phase derivative, resulting in a pair of one-dimensional time series [plotted together in Fig. 7(B)]. Alternatively, polyspectra of non-overlapping harmonics can produce a single two-dimensional image [Fig. 7(G)]. Each method has its uses. Measuring the slope of frequency modulation or the difference between two tones that are close in frequency would be simpler with a pair of one-dimensional time series, while a two-dimensional image that is not severely thresholded preserves more of the raw data. Although flight-tone convergence during courtship is a striking behavior, it is not precise. Even in the clearest examples, male and female flight tone harmonics are several Hz away from a true match (Warren *et al.*, 2009). This was suspected from examination of regular spectrograms and becomes even more evident with enhanced frequency resolution [Figs. 7(B) and 7(G)]. This imprecision may favor explanations of convergence based on sexual selection (Cator *et al.*, 2010; Cator and Harrington, 2011) over those suggesting that flying at harmonically related frequencies offers mechanical benefits to joined flight during copulation.

A mosquito can vary the carrier frequency, but not the temporal pattern, of its courtship signal. However, even that variability is limited by the need to remain airborne. If a mosquito flying alone beats its wings at the frequency most efficient for flight, modulation of that frequency is likely to incur some cost. Thus large modulations could indicate fitness in terms of ability to fly and maneuver at suboptimal

wingbeat frequencies. Although there is evidence that modulating to convergence is an indicator of male fitness (Cator and Harrington, 2011), it is not known whether, during courtship, males tend to alter wingbeat frequency more than females, as might be predicted by sexual selection hypotheses. More detailed quantitative investigation of modulation during courtship would be facilitated by some of the methods applied in the current study (Kim and Powers, 1979; Brown and Puckette, 1993).

V. CONCLUSIONS

Recordings of courtship in natural settings are hard to obtain. In *Ae. aegypti*, mating occurs in swarms around host species. A single microphone may record snippets of courtship convergence as a pair flies past, along with the flight tones of any other nearby mosquitoes. There has been some success recording mating swarms with microphone arrays (Cator *et al.*, 2011), but recordings are brief, tens of ms at most. Combining recording arrays with the analyses reported here may make it possible to address several questions of interest to mosquito natural history and control: How precise is convergence? Does one sex tend to modulate more than the other during convergence? How does convergence depend on such factors as mating receptivity and environmental conditions?

ACKNOWLEDGMENTS

We thank Sylvie Pitcher, Melissa Orteza, and others in the lab of Professor Laura Harrington (Department of Entomology, Cornell University) for mosquito rearing and maintenance, and Yngve Birkelund (Department of Physics and Technology, University of Tromsø) for commenting on a draft of the manuscript. This research was supported by National Institutes of Health Award No. 5R01DC103-38 to R.R.H.

- Aono, H., Liang, F., and Liu, H. (2008). "Near- and far-field aerodynamics in insect hovering flight: An integrated computational study," *J. Exp. Biol.* **211**, 239–257.
- Arthur, B. J., Sunayama-Morita, T., Coen, P., Murthy, M., and Stern, D. L. (2013). "Multi-channel acoustic recording and automated analysis of *Drosophila* courtship songs," *BMC Biol.* **11**, 11.
- Arthur, B. J., Wytenbach, R. A., Harrington, L. C., and Hoy, R. R. (2010). "Neural responses to one- and two-tone stimuli in the hearing organ of the dengue vector mosquito," *J. Exp. Biol.* **213**, 1376–1385.
- Bae, Y., and Moon, Y. J. (2008). "Aerodynamic sound generation of flapping wing," *J. Acoust. Soc. Am.* **124**, 72–81.
- Belton, P., and Costello, R. A. (1979). "Flight sounds of the females of some mosquitoes of Western Canada," *Entomol. Exp. Appl.* **26**, 105–114.
- Bennet-Clark, H. C. (1998). "Size and scale effects as constraints in insect sound communication," *Philos. Trans. R. Soc. London Ser. B* **353**, 407–419.
- Bennet-Clark, H. C., and Ewing, A. W. (1968). "The wing mechanism involved in the courtship of *Drosophila*," *J. Exp. Biol.* **49**, 117–128.
- Brillinger, D. R. (1965). "An introduction to polyspectra," *Ann. Math. Stat.* **36**, 1351–1374.
- Brogdon, W. G. (1998). "Measurement of flight tone differentiates among members of the *Anopheles gambiae* species complex (Diptera: Culicidae)," *J. Med. Entomol.* **35**, 681–684.
- Brown, J. C. (1996). "Frequency ratios of spectral components of musical sounds," *J. Acoust. Soc. Am.* **99**, 1210–1218.

- Brown, J. C., and Puckette, M. S. (1993). "A high resolution fundamental frequency determination based on phase changes of the Fourier transform," *J. Acoust. Soc. Am.* **94**, 662–667.
- Cator, L. J., Arthur, B. J., Harrington, L. C., and Hoy, R. R. (2009). "Harmonic convergence in the love songs of the dengue vector mosquito," *Science* **323**, 1077–1079.
- Cator, L. J., Arthur, B. J., Ponlawat, A., and Harrington, L. C. (2011). "Behavioral observations and sound recordings of free-flight mating swarms of *Ae. aegypti* (Diptera: Culicidae) in Thailand," *J. Med. Entomol.* **48**, 941–946.
- Cator, L. J., and Harrington, L. C. (2011). "The harmonic convergence of fathers predicts the mating success of sons in *Aedes aegypti*," *Anim. Behav.* **82**, 627–633.
- Cator, L. J., Ng'Habi, K. R., Hoy, R. R., and Harrington, L. C. (2010). "Sizing up a mate: Variation in production and response to acoustic signals in *Anopheles gambiae*," *Behav. Ecol.* **21**, 1033–1039.
- Charpentier, F. J. (1986). "Pitch detection using the short-term phase spectrum," in *Proceedings of the International Conference on Acoustics, Speech, and Signal Processing*, pp. 113–116.
- Dowling, A. P. (1997). "Steady-state radiation from sources," in *Encyclopedia of Acoustics*, edited by M. J. Crocker (Wiley, New York), pp. 107–125.
- Dubnov, S., and Rodet, X. (1997). "Statistical modeling of sound aperiodicities," in *Proceedings of the International Computer Music Conference* (International Computer Music Association, San Francisco), pp. 43–50.
- Fackrell, J. W. A., and McLaughlin, S. (1995). "Quadratic phase coupling detection using higher order statistics," in *IEE Colloquium on 'Higher Order Statistics in Signal Processing: Are They of Any Use?'*, pp. 9/1–9/8.
- Fry, S. N., Sayaman, R., and Dickinson, M. H. (2005). "The aerodynamics of hovering flight in *Drosophila*," *J. Exp. Biol.* **208**, 2303–2318.
- Gerhardt, H. C., and Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions* (University of Chicago Press, Chicago).
- Gibson, G., and Russell, I. J. (2006). "Flying in tune: Sexual recognition in mosquitoes," *Curr. Biol.* **16**, 1311–1316.
- Göpfert, M. C., Briegel, H., and Robert, D. (1999). "Mosquito hearing: Sound-induced antennal vibrations in male and female *Aedes aegypti*," *J. Exp. Biol.* **202**, 2727–2738.
- Kim, Y. C., and Powers, E. J. (1979). "Digital bispectral analysis and its applications to non-linear wave interactions," *IEEE Trans. Plasma Sci.* **7**, 120–131.
- Manela, A. (2012). "Vibration and sound of an elastic wing actuated at its leading edge," *J. Sound Vib.* **331**, 638–650.
- Maxim, H. S. (1901). "Mosquitoes and musical notes," in *The Times* (London), p. 11.
- Mendel, J. M. (1991). "Tutorial on higher-order statistics (spectra) in signal processing and system theory: Theoretical results and some applications," *Proc. IEEE* **79**, 278–305.
- Moore, A., and Miller, R. H. (2002). "Automated identification of optically sensed aphid (Homoptera: Aphidae) wingbeat waveforms," *Ann. Entomol. Soc. Am.* **95**, 1–8.
- Nasci, R. S. (1990). "Relationship of wing length to adult dry weight in several mosquito species (Diptera: Culicidae)," *J. Med. Entomol.* **27**, 716–719.
- Penner, C., Warren, B., Dabiré, K. R., Russell, I. J., and Gibson, G. (2010). "'Singing on the wing' as a mechanism for species recognition in the malarial mosquito *Anopheles gambiae*," *Curr. Biol.* **20**, 131–136.
- Persson, L., Lennartsson, R. K., Robinson, J. W. C., and McLaughlin, S. (2000). "Quadratic phase coupling analysis of passive sonar data using biphasic techniques," in *OCEANS 2000 MTS/IEEE Conference and Exhibition*, Vol. 1052, pp. 1053–1057.
- Raman, D. R., Gerhardt, R. R., and Wilkerson, J. B. (2007). "Detecting insect flight sounds in the field: Implications for acoustical counting of mosquitoes," *Trans. ASABE* **50**, 1481–1485.
- Ross, R. (1901). "Mosquitoes attracted by sounds," *Br. Med. J.* **2**, 979.
- Roth, L. M. (1948). "A study of mosquito behavior. An experimental laboratory study of the sexual behavior of *Aedes aegypti* (Linnaeus)," *Am. Midl. Nat.* **40**, 265–352.
- Sotavalta, O. (1947). "The flight-tone (wing-stroke frequency) of insects (contributions to the problem of insect flight I)," *Acta Ent. Fennica* **4**, 1–114.
- Sueur, J., Tuck, E. J., and Robert, D. (2005). "Sound radiation around a flying fly," *J. Acoust. Soc. Am.* **118**, 530–538.
- Unwin, D. M., and Corbet, S. A. (1984). "Wingbeat frequency, temperature and body size in bees and flies," *Physiol. Entomol.* **9**, 115–121.
- Warren, B., Gibson, G., and Russell, I. J. (2009). "Sex recognition through midflight mating duets in *Culex* mosquitoes is mediated by acoustic distortion," *Curr. Biol.* **19**, 485–491.
- Webb, J. C., Sharp, J. L., Chambers, D. L., and Benner, J. C. (1976). "Acoustical properties of the flight activities of the Caribbean fruit-fly," *J. Exp. Biol.* **64**, 761–772.
- Wekesa, J. W., Brogdon, W. G., Hawley, W. A., and Besansky, N. J. (1998). "Flight tone of field-collected populations of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae)," *Physiol. Entomol.* **23**, 289–294.
- Williams, C. M., and Galambos, R. (1950). "Oscilloscopic and stroboscopic analysis of the flight sounds of *Drosophila*," *Biol. Bull.* **99**, 300–307.
- Wishart, G., and Riordan, D. F. (1959). "Flight responses to various sounds by adult males of *Aedes aegypti* (L.) (Diptera: Culicidae)," *Can. Entomol.* **91**, 181–191.