Automated Classification of Morphologically Identical Mosquito Sibling Species Using Wingbeat Harmonics

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INTRODUCTION

Wingbeat frequency has been used as a character for differentiating insect species. Here, we show that harmonic patterns associated with wingbeat frequency provide additional species-specific information which can be used to differentiate closely related species with overlapping wingbeat frequencies.

Here, we report how we used harmonic patterns to differentiate female *Culex pipiens* mosquitoes from *Cx. quinquefasciatus*. These sibling species are morphologically identical. Previously, they could be differentiated only by molecular methods.

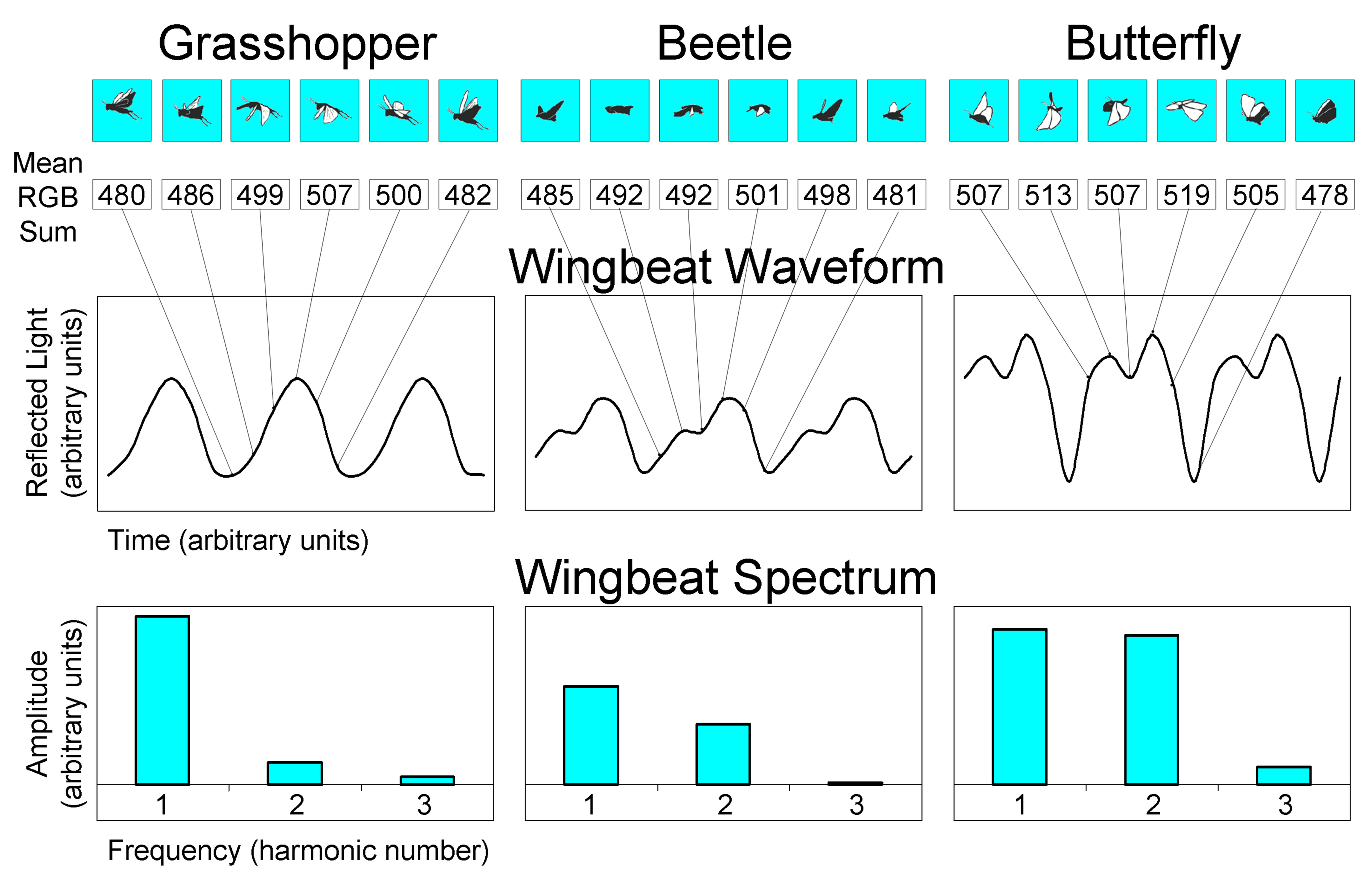

Figure 1. Origin of harmonics in optically-sensed wingbeat waveforms. Images are from a web page entitled "How Insects Fly" (http://park.org/Canada/Museum/insects/flight/flapping.html). To simulate data from a non-imaging photosensor, we calculated the mean sum of the red, green, and blue values for pixels in each image. This values were plotted as wingbeat waveforms. Fast Fourier transform of these waveforms generate very different spectra, even though wingbeat frequencies are identical.

METHODS

Wingbeat waveforms were recorded using FAST-ID instrumentation developed by APTIV Inc. Waveforms were recorded when female mosquitoes from two colonies of *Cx. pipiens* (CT, MA) and two colonies of *Cx. quinquefasciatus* (CA, FL) flew between an infrared LED array and a miniature solar cell (Fig. 2). For each waveform, wingbeat frequency was estimated using a YIN estimator, frequency spectrum was calculated using a fast Fourier transform, and a harmonic pattern was derived by integrating area under the frequency spectrum adjacent to each harmonic (Fig. 3).

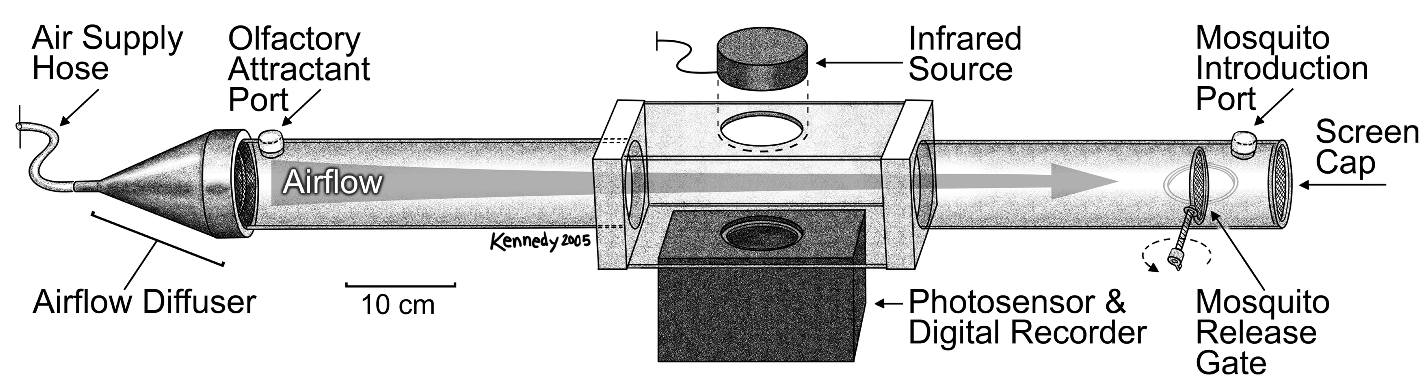

Figure 2. Apparatus.

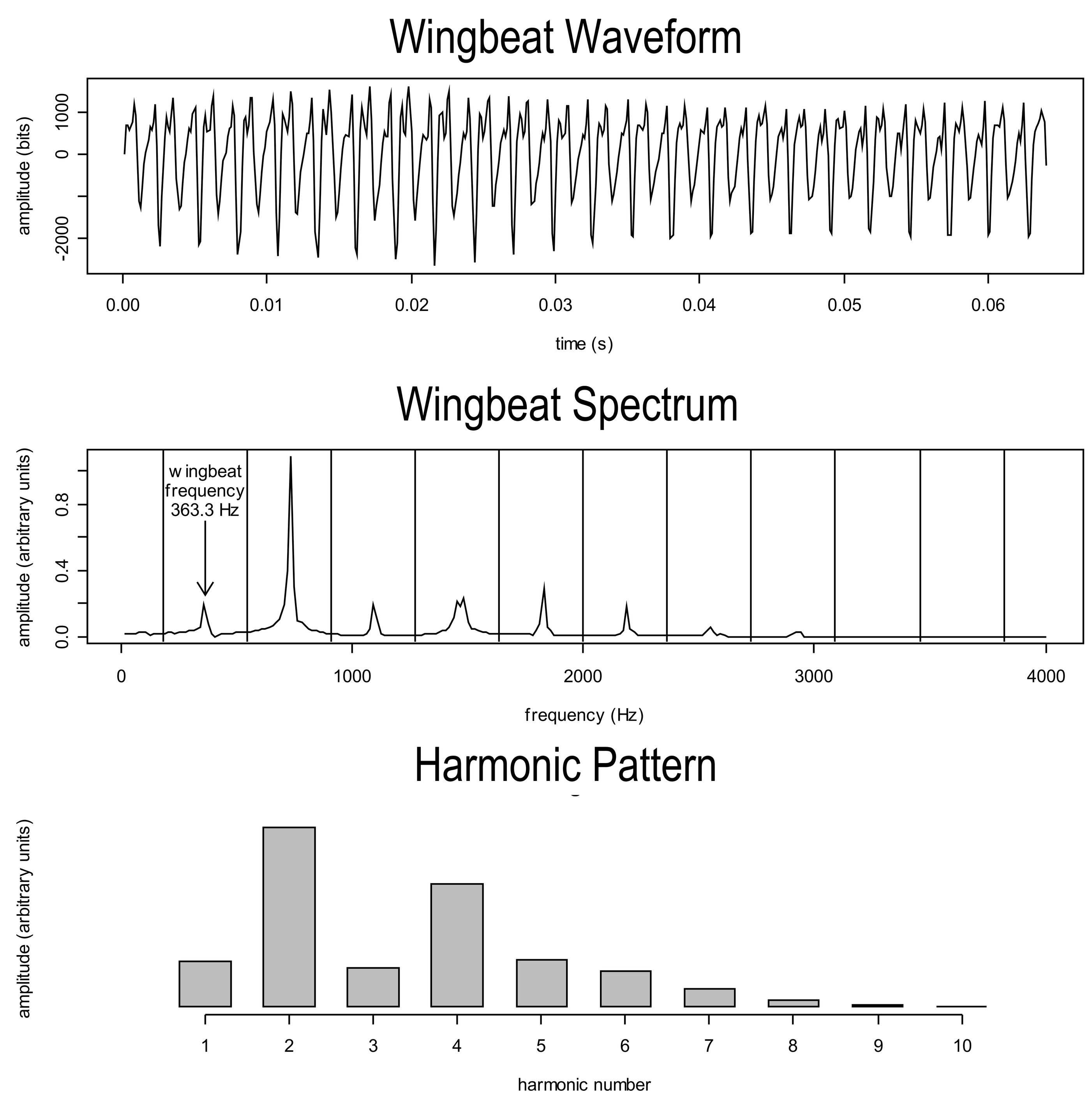

Figure 3. Digital signal processing.

RESULTS

Wingbeat frequencies of female *Cx. pipiens* and *Cx. quinquefasciatus* were not different (Table 1). However, harmonic patterns for waveforms within each frequency bin were significantly different (Fig. 4). *Cx. quinquefasciatus* produced stronger higher order harmonics than *Cx. pipiens.* In a cross-classification test with a nearest neighbor classifier using wingbeat frequencies and harmonic patterns, 65% of the mosquitoes were classified correctly, 31% were unclassified, and only 4% were misclassified.

Table 1. Number of waveforms in each frequency bin.

Wingbeat Frequency (Hz)	*Cx. pipiens*		*Cx. quinquefasciatus*	
	CT	MA	CA	FL
321–333	10	1	4	1
334–348	16	10	12	4
349–364	27	73	21	1
365–381	67	151	7	2
382–400	67	173	28	11
401–421	40	18	45	25

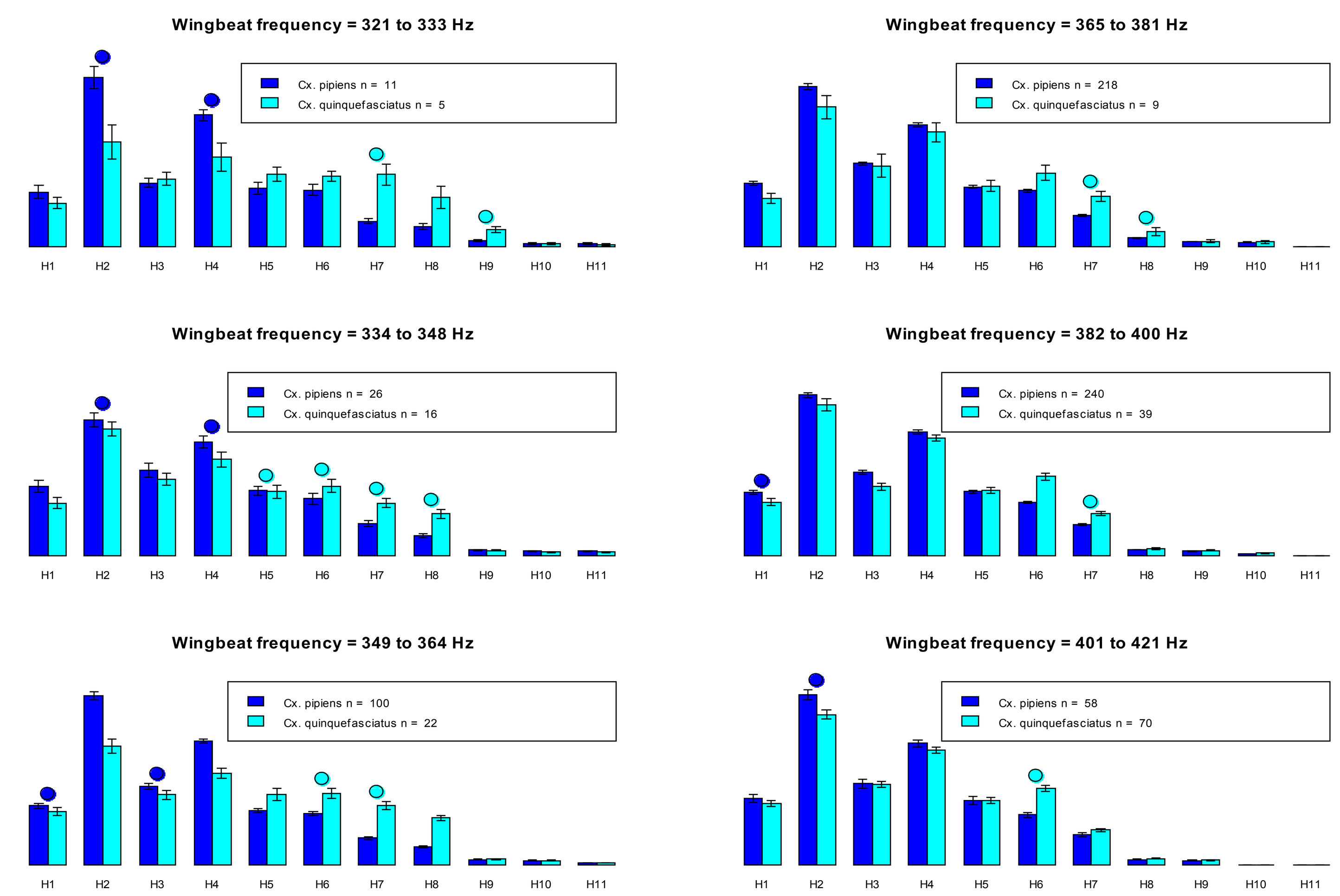

Figure 4. Harmonic patterns. Each bar represents relative energy associated with each harmonic. Error bars are SEM. Filled circles indicate significant differences.