Fourier Analysis of Wing Beat Signals: Assessing the Effects of Genetic Alterations of Flight Muscle Structure in *Diptera*

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ABSTRACT A method for determining and analyzing the wing beat frequency in *Diptera* is presented. This method uses an optical tachometer to measure *Diptera* wing movement during flight. The resulting signal from the optical measurement is analyzed using a Fast Fourier Transform (FFT) technique, and the dominant frequency peak in the Fourier spectrum is selected as the wing beat frequency. Also described is a method for determining quantitatively the degree of variability of the wing beat frequency about the dominant frequency. This method is based on determination of a quantity called the *H* index, which is derived using data from the FFT analysis. Calculation of the *H* index allows computer-based selection of the most suitable segment of recorded data for determination of the representative wing beat frequency. Experimental data suggest that the *H* index can also prove useful in examining wing beat frequency variability in *Diptera* whose flight muscle structure has been genetically altered. Examples from *Drosophila* indirect flight muscle studies as well as examples of artificial data are presented to illustrate the method. This method fulfills a need for a standardized method for determining wing beat frequencies and examining wing beat frequency variability in insects whose flight muscles have been altered by protein engineering methods.

INTRODUCTION

The fruitfly *Drosophila melanogaster* has proven to be a useful organism for studying the relationship between structure and function of contractile proteins responsible for flight (Sparrow et al., 1991; Bernstein et al., 1993). In flies transformed with mutant genes, the functional consequence of altering protein structure can be assessed at a variety of levels, from in vivo measurements of flight ability to in vitro measurements of contractile performance of single flight muscle cells. Analysis of wing movement has been an important part of the functional assessment of the performance of the flight muscle, linking changes in flight ability to changes in flight muscle performance resulting from alterations of flight muscle ultrastructure and contractile protein structure (see, e.g., Drummond et al., 1990; Yamakawa et al., 1991; Warmke et al., 1992).

Here we present a standardized method for determining wing beat frequencies and frequency variability in *Drosophila* and other *Diptera*. The method uses a simple optical "tachometer" to record changes in ambient light intensity caused by disruption of a light beam by the movement of the insect's wings during flight. (For non-*Dipteran* insects, one set of wings can get out of phase with one another, thereby limiting the usefulness of the optical technique.) The light intensity fluctuation is translated into a voltage signal that can be sampled by computer and analyzed off-line using Fourier analysis. Fourier analysis is a powerful technique that allows determination of the dominant frequency components of a signal even in the presence of a substantial amount of random noise. From the Fourier spectrum, we derive the insect's mean wing beat frequency, *f*, from the dominant peak.

We also derive a measure of the deviation of the insect's wing beat about the mean wing beat frequency, which we call the *H* index. Often a recorded wing beat signal will contain moments when the insect will cease beating its wings or will suddenly increase or decrease its wing beat frequency. To obtain a reliable value, the wing beat frequency should be determined from a segment of the recorded data where the wing beat frequency is least variable. The selection process can be performed by visual inspection of spectral analysis plots obtained from different segments of the recorded data, but visual inspection of a large number of plots is time consuming. As will be shown, computation of the *H* index provides a convenient, computer-based method for determining the degree of variability about the dominant wing beat frequency (as determined from the Fourier spectrum of each data segment) and, thus, can be used for automatic selection of the most suitable data segment for determination of the representative wing beat frequency. The experimental data suggest that the *H* index can also be useful in examining possible correlations between increased variability in resonant wing beat frequency and genetic alteration of flight muscle structure.

MATERIALS AND METHODS

Data acquisition

In the optical tachometer method, *Diptera* are tethered and wing movements (during attempted flight) are recorded by an optical device based on the design of Unwin and Ellington (1979). The optical device converts the changes in ambient light intensity that result from the movement of the insect's wings into an amplified voltage. To prevent aliasing during sampling, the amplified voltage signal is passed through an active 5-pole Bessel low-pass filter (TTE Inc., Los Angeles, CA) with a -3 dB corner frequency of 5 kHz. The amplified voltage signal is sampled at 20 kHz by a personal computer using a high-frequency analog-to-digital (A/D) data acquisition

board (model DT-2828, Data Translation, Inc., Marlboro, MA). Wing beat data are captured until 65,536 (2^{16}) data points are acquired, for a total capture duration of 3.28 s. Fig. 1 illustrates a block diagram of the experimental setup used to collect wing beat signals for analysis.

Fourier analysis

The 65,536 data points of captured data are divided into segments that are each 16,384 (2^{14}) points in length. Each segment overlaps the previous segment by 75%. The data is thus divided into a total of 12 segments, each 0.8192 s in duration. The data series corresponding to the ith segment of data is denoted by $x_i(n)$. This data segment is multiplied by a Blackman-Harris window function to reduce sidelobe leakage before the Fourier transform is computed. A Fast Fourier Transform technique based upon a radix-2 Cooley-Tukey algorithm is then applied to each segment of the Blackman-Harris windowed data, $x_i(n)$, to determine its corresponding set of Fourier coefficients, $F_i(k)$ (Proakis and Manolakis, 1988). For each of these data segments, $x_i(n)$, and each corresponding Fourier spectrum, $F_i(k)$, a value called the H index is determined. From the values of the H index obtained from each of the 12 segments, the segment of data determined to be best representative of the *Diptera* wing beat is selected (i.e., the segment with the highest value of H is selected).

The equations relating each data segment series, $x_i(n)$, to its set of Fourier coefficients, $F_i(k)$, $k = 0, 1, \cdots, N/2$, are given by Eqs. 1–3 (Proakis and Manolakis, 1988):

$$x_i(n) = \sum_{k=0}^{N/2} F_i(k) \exp(j\omega_k n), \quad \text{where} \quad \omega_k = \frac{2\pi k}{N} \quad (1)$$

$$F_i(0) = \frac{1}{N} \sum_{n=0}^{N-1} x_i(n), \quad F_i\left(\frac{N}{2}\right) = \frac{1}{N} \sum_{n=0}^{N-1} x_i(n)\exp(-j\pi n) \quad (2)$$

$$F_i(k) = \frac{2}{N} \sum_{n=0}^{N-1} x_i(n)\exp(-j\omega_k n), \quad \text{where} \quad k = 1, 2, \cdots, \frac{N}{2}-1. \quad (3)$$

It can be seen from Eq. 1 that the relative strength of each discrete frequency ω_k in $x_i(n)$ is given by the corresponding Fourier coefficient, $F_i(k)$. A sampling frequency of 20 kHz and an FFT length of 16,384 result in an FFT frequency resolution of 1.2207 Hz. Therefore, each Fourier coefficient, $F_i(k)$, gives the relative strength for the frequency component at 1.2207 × k Hz.

Blackman-Harris window

Digital sampling of a continuous analog signal results in a discrete, finite-length data series with abrupt discontinuities at both the beginning and the end of the series. A data series thus obtained can be viewed as a discrete, infinite-length representation of the original analog signal multiplied by a "rectangular" window (Proakis and Manolakis, 1988). The abrupt discontinuities caused by the rectangular window, however, result in "sidelobe leakage" in the Fourier spectrum. To reduce sidelobe leakage, we apply a Blackman-Harris window to the data series before computing the Fourier transform. The Blackman-Harris window, unlike the rectangular window,

is a window function with a smooth taper. This smooth taper helps diminish spectral leakage in the Fourier transform caused by discontinuities at the ends of the data (Proakis and Manolakis, 1988).

Window functions with different tapers such as Hanning or Hamming windows can be used and give similar results, but the Blackman-Harris window was chosen because it yields the smallest sidelobes in the Fourier spectrum (Proakis and Manolakis, 1988). Although use of the Blackman-Harris window results in smaller sidelobes, it achieves them at the expense of the sharpness of the primary spectral peak. As we will demonstrate later, however, computation of the H index requires small sidelobes but not a sharp primary peak. Therefore, for our method of analysis the Blackman-Harris window is an excellent choice for reducing sidelobe leakage. The Blackman-Harris window function has the form given in Eq. 4.

$$w(n) = a_0 - a_1\cos\left(\frac{2\pi n}{N}\right) + a_2\cos\left(\frac{4\pi n}{N}\right) - a_3\cos\left(\frac{6\pi n}{N}\right),$$

$$n = 0, 1, \cdots, N. \quad (4)$$

where $a_0 = 0.35875$, $a_1 = 0.48829$, $a_2 = 0.14128$, $a_3 = 0.01168$.

The H index

From spectral analysis of periodic data such as sine, square, and sawtooth waves, we found that, when a Blackman-Harris data window is applied before computing the FFT, the dominant peak in the spectrum corresponding to the first harmonic is composed of seven points for our standard FFT of 16,384 points. These seven points comprised the point at the peak maximum and three points on either side of the maximum point. The relatively large breadth of the peak is caused by the effects of the Blackman-Harris window. For an FFT with a length other than 16,384 points or when a different window function is applied, the number of points that comprise the peak will in general be different.

Because the sine, square, and sawtooth waves we tested were periodic signals with a fixed frequency, we found that a measure of deviation of the wing beat frequency from a fixed frequency was provided by comparing the relative strength of the seven points comprising the first harmonic peak with the relative strength of frequency components just outside the first harmonic peak. Equation 5 defines the parameter H, which provides a reliable measure of spread about, or deviation from, the dominant frequency (the frequency at k = max):

$$H = \frac{\sum_{k=\max-3}^{\max+3} F(k)}{\sum_{k=\max-14}^{\max+14} F(k)}. \quad (5)$$

The values $F(k)$ are the Fourier coefficients. Because the frequency resolution of the FFT is 1.2207 Hz, the numerator represents a range of approximately ±3.7 Hz and the denominator represents a range of approximately ±17.1 Hz. The range in the denominator was chosen arbitrarily, but it is large enough to include all reasonable deviations about the peak frequency within the limited period of time sampled (0.82 s).

As we have described, the H index provides a measure of wing beat frequency variability and, thus, can be used to determine a suitable segment of data for determination of the wing beat frequency from the Fourier spectrum. After determination of the Fourier spectrum for each segment of data, the H index is determined and the segment with the highest H value is selected for determination of the wing beat frequency.
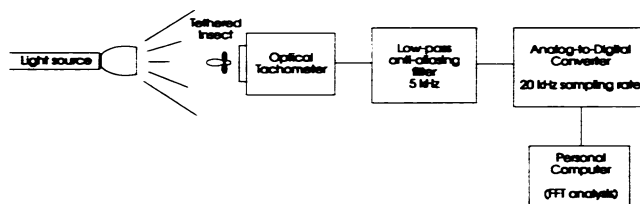
RESULTS AND DISCUSSION

The *Drosophila* flight system

Drosophila wing movement is powered indirectly by two antagonistic groups of muscle fibers. Both groups of fibers are attached to the thoracic cuticle, as are the wings, so that forces developed by the two groups of fibers are transmitted indirectly to the wings via deformation of the cuticle. Both

FIGURE 1   Block diagram of the basic setup for obtaining wing beat signals using the optical tachometer method.

fiber groups are activated by calcium, which is released intracellularly in response to asynchronous neural stimulation. After calcium activation, each muscle group contracts alternately in response to a stretch produced by the other, an oscillatory or "click-like" process that powers wing movement and flight (Pringle, 1949; Chaplain and Frommelt, 1968). The wing beat cycle is probably triggered by an auxiliary set of muscles or deformation of the cuticle during the "jump" response before flight. The dominant frequency of the wing beat is determined by the mechanical characteristics of the entire flight system, including the size and mass of the wings, the stiffness of the thoracic cuticle, and the stiffness and kinetic properties of the flight musculature.

Windowing effects on the H index

Before discussing the results of our method applied to actual wing beat signals, the results of our method applied to a few selected artificial signals will be presented. The discussion of artificial signals will help illustrate the important aspects of the technique when applied to real data.

As described previously, a window function (Blackman-Harris) with a smooth taper is applied to the data before spectral analysis to provide a reduction in sidelobe leakage. It is important to the calculation of the H index to apply a tapered window function such as the Blackman-Harris window before computing the FFT. For this reason, a brief description will be presented of how the Blackman-Harris window affects the Fourier spectrums of several artificial signals.

Fig. 2 illustrates sidelobe leakage and the differing effects of the rectangular and Blackman-Harris windows on sidelobe leakage. The FFT spectrum of a rectangular-windowed, pure sine wave whose frequency exactly matches a discrete frequency ω_k will result in a single, very narrow peak as shown in Fig. 2 a. As shown in Fig. 2 c, however, the FFT spectrum of another rectangular-windowed, pure sine wave whose frequency lies between two discrete FFT frequencies has a considerable amount of spectral leakage into neighboring frequencies.

This problem is mitigated by use of a tapered window function such as the Blackman-Harris window. Fig. 2, b and d show the FFTs of the same two sinusoids as in Fig. 2, a and c, but this time a Blackman-Harris window has been

applied to the data to reduce spectral leakage. The spectrum peak of the sinusoid has been broadened somewhat, but its H index is actually increased by a small amount (0.99988 vs. 0.99979). This demonstrates that for our method of analysis, the Blackman-Harris window, with its properties of greatly reduced sidelobes at the expense of peak broadening, is an excellent choice for reducing spectral leakage. The spectral leakage of the other FFT has been reduced considerably ($H = 0.99914$ vs. 0.69257). As shown by Fig. 2, b and d, the spectrum peaks of the two sinusoids are very similar when windowing is applied. This is important for proper determination of the H index.

Additional effects on the H index

As can be seen from Figs. 2 and 3, H is essentially unity for all Blackman-Harris windowed sine waves, square waves, and sawtooth waves tested. In fact, H should be nearly unity for a wing beat signal of constant frequency regardless of the waveform shape. Waveform shape only effects the magnitude of the higher order harmonics of the main frequency and not the breadth of the 1st harmonic peak as shown by Fig. 3, a and b, for the square ($H = 0.99979$) and sawtooth ($H = 0.99987$) waves, respectively.

Fig. 4 shows the Fourier spectrum of a sinusoidal data series where three abrupt changes in sinusoidal frequency were imposed during the time course of the series. This example illustrates the utility of computing H when an insect's wing beat frequency changes during acquisition of the wing beat signal. Not only is there peak broadening but there are (in this case) two separate peaks. If only the breadth of the tallest peak were used as a measure for frequency variability (as in the quality factor "Q" described in the next section), one might conclude that because this peak is narrow, there is minimal frequency variation. Clearly, this is not the case. This example shows how H decreases with an increase in variability of wing beat movement.

The Q factor versus the H index

In engineering applications a quantity resembling the H index, referred to as the quality factor or "Q", is used to quan-

FIGURE 2 The Fourier spectrums of sinusoidal data series with a rectangular window applied (a and c) and a Blackman-Harris window applied (b and d). (a) The spectrum of a sinusoidal data series with a frequency that is exactly equal to a characteristic Fourier frequency ω_k. (b) The spectrum of the same data series when a Blackman-Harris window is applied before computing the spectrum. (c) In this case, the frequency of the sinusoidal data series lies halfway between two characteristic Fourier frequencies ω_{k-1} and ω_k, and spectral leakage is present. (d) The spectrum of the same data series after a Blackman-Harris window is applied.
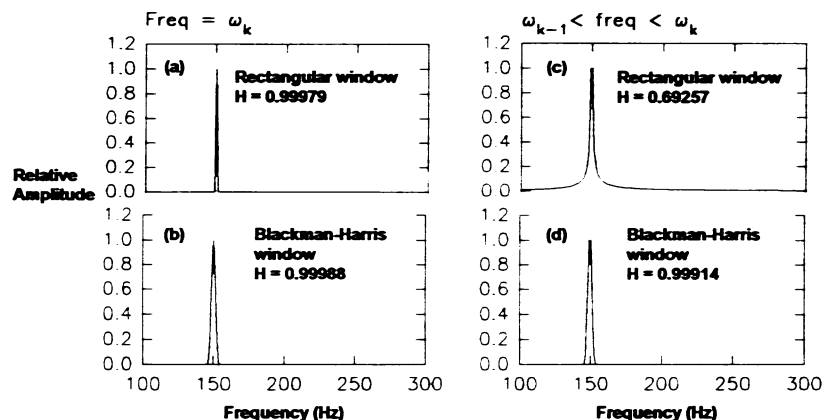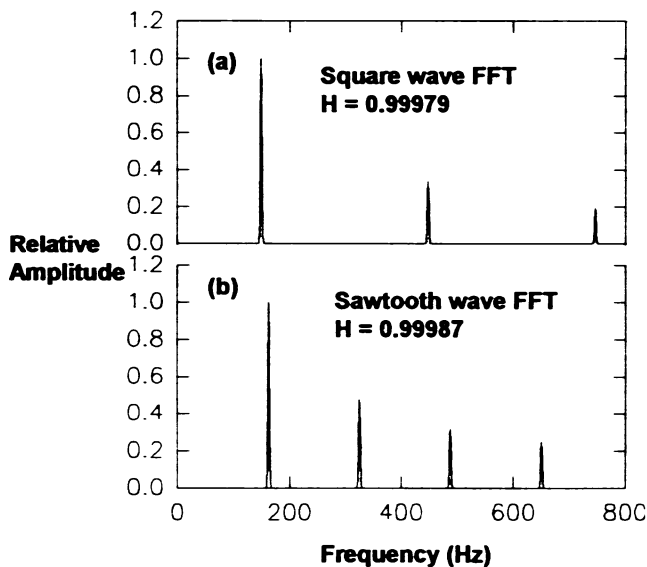
FIGURE 3   The Fourier spectrums of a square wave (a) and a sawtooth wave (b). (a) In the case of the square wave spectrum, only odd-numbered harmonics are present. (b) In the sawtooth wave spectrum, all harmonics are present. Note that in both cases the value of the H index is still near unity.
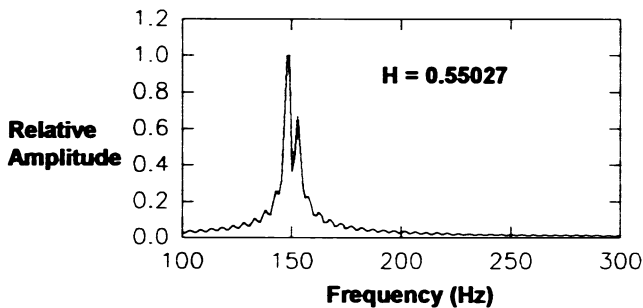

FIGURE 4   The Fourier spectrum of a sinusoidal data series where, during the time course of the series, there were three changes in sinusoidal frequency. Each change in frequency was small (less than ±1.2207 Hz from a base frequency of 150.15 Hz), evenly spaced (a change every 4096 points), and randomly produced. The peak is broadened and, in this case, separates into two distinct peaks. The H index is also substantially decreased.

tify the deviation in frequency about the dominant peak in a frequency spectrum. Q is a dimensionless quantity given by the equation $Q = f/\Delta f$, where f is the frequency of the dominant peak and Δf is the width of the peak at the -3 dB point. The Q factor is usually used by engineers and scientists to analyze resonant systems to determine the degree of sharpness of the resonance (French, 1971). A large value for Q generally indicates the resonant peak is narrow, i.e., the system is highly selective for the resonant frequency. Conversely, a small Q indicates a broad peak and less selectivity for the resonant frequency. Yamakawa and Maughan (1991) suggested that a parameter q, defined as Q is above, could serve as an index of flight system efficiency for Diptera, where low q reflects a highly damped, less efficient system.

Computation of the H index is similar in form to computation of the Q factor, but a single well defined peak at the

resonant frequency is not assumed in computation of H. For many wing beat spectra, side peaks are present around the main peak frequency, indicating marked deviation about the resonant frequency of the flight system. These side peaks are not included in the calculation of q, but they are included in the calculation of H. These side peaks would be present, e.g., when the wing beat frequency shifts suddenly to another slightly lower or higher frequency (as demonstrated in Fig. 4).

Drosophila studies

Fig. 5 a illustrates a typical wing beat frequency spectrum for wild-type (Canton Special) Drosophila melanogaster. Fig. 5 b shows a spectrum for a mutant fly, $Mlc2^-/+$, a heterozygous hypomorph in which only half of the wild-type level of the regulatory light chain of myosin (MLC-2) is expressed (the null mutant $Mlc2^-$ is denoted $Ifm(3)99Eb^{E38}$ in Warmke et al., 1992). MLC-2 is one of the subunits of the myosin molecule that is required for normal myofibrillogenesis and flight muscle function in Drosophila (Warmke et al., 1992). These data illustrate the type of differences that can result from flight muscle mutations. For the $Mlc2^-/+$ mutant, both wing beat frequency and H are significantly less than those of wild-type flies. Table 1 summarizes results from a set of wild-type and $Mlc2^-/+$ mutant flies of both sexes.

Analysis of variance of f and H within the population of 100 wild-type flies shows that f depends significantly on wing length (WL) and ambient temperature (T) over the limited range tested ($f = 157 - 20WL + 5.3T$). The average wing beat frequency of male Canton S flies was ~7% higher than that of female Canton S flies of similar age and temperature; this is very likely because the smaller male flies have wing lengths ~11% shorter than females. The value of H was independent of sex, wing length and temperature, suggesting that flies of both sexes have similar variability in their wing beat frequency.

The lower wingbeat frequency of $Mlc2^-/+$ is caused by the reduced level of expression of MLC-2 and the concomitant disruption of the peripheral region of flight muscle myofibrils, which amounts to about half the total number of myofilaments (Warmke et al., 1992). A proportional reduction in active muscle stiffness likely occurs under these circumstances. Ultrastructural and mechanical studies of isolated skinned indirect flight muscle fibers (Warmke et al., 1992) indicate that the disorganized lattice of the peripheral region is unstable and, therefore, unlikely to support normal levels of active tension development. The studies also suggest that MLC-2-deficient fibers have slower contraction kinetics than wild-type fibers. Both factors would contribute to a net reduction in active stiffness.

If most of the stiffness of the flight system is within the flight muscles, then the resonance frequency of the flight system will be determined primarily by both the active muscle stiffness (K) and the inertia of the wings (I) according to the relationship (Pringle, 1957): $f = (2\pi)^{-1}K^{1/2}I^{-1/2}$. Wing inertia is proportional to wing length, and frequency

FIGURE 5 The Fourier spectrums and raw signals from the *Drosophila melanogaster* type Canton S (a) and $Mlc2^-/+$ mutant (b). Both are typical cases and illustrate the use of the H index as a measure of variability of the wing beat about the dominant frequency.
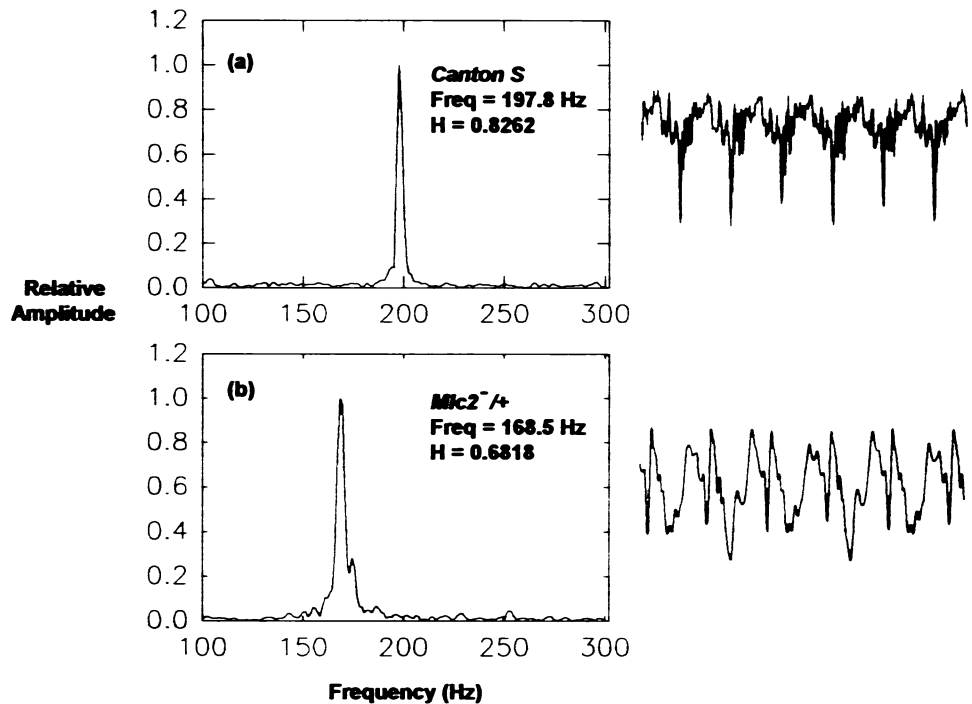
TABLE 1 Wing beat data for tethered wild-type and mutant flies

Strain	Sex	WL (mm)	T (°C)	n	f (Hz)	H
CS	Male	2.09 ± 0.11	21 ± 1	50	211 ± 13	0.87 ± 0.05
	Female	2.33 ± 0.15*	21 ± 1	50	200 ± 10*	0.86 ± 0.04
	Male		12 ± 1	10	156 ± 4*	0.84 ± 0.16
$Mlc2^-/+$	Male	2.09 ± 0.08	23 ± 1	12	152 ± 14*	0.81 ± 0.11*
	Female	2.36 ± 0.09*	23 ± 1	22	147 ± 16*	0.79 ± 0.12*

CS = Canton Special (inbred wild-type fly); $Mlc2^-/+$ = regulatory light chain hypomorph (Warmke et al., 1992). WL = wing length, measured from tip to insertion into thorax (mm). T = ambient temperature (°C).

* Significant difference (p < 0.01: Bartlett chi-square statistic) from CS males; means and SDs given. Flies were tested 2–5 days after eclosion. The wing beat frequency f and frequency fluctuation index H depended on wing length (1.64–2.69 mm) and temperature (19–24°C), but not on ambient light or relative humidity (range, 12.5–14 E.V. and 36–75%, respectively).

is inversely related to wing length as predicted (Table 1). But because the wing length of $Mlc2^-/+$ is not significantly different than that of the wild-type fly of the same sex, inertial differences cannot explain the pronounced difference in f between $Mlc2^-/+$ and the wild-type fly. However, if K is proportional to the number of myofilaments constituting the well organized wild-type myofilament lattice (which in $Mlc2^-/+$ represents approximately half of the total myofilaments present in each myofibril), then most of the 0.73–0.75 reduction in wing beat frequency of $Mlc2^-/+$ can be explained by the putative reduction in active muscle stiffness ($f_{Mlc2^-/+}/f_{CS} = 0.5^{1/2} = 0.71$).

At 21–23°C, untethered wild-type flies generally flew upward, whereas untethered $Mlc2^-/+$ flies were flight-impaired. $Mlc2^-/+$ flies either glided or dropped downward despite a demonstrable (albeit slower) wing beat. Because power output is proportional to wing beat frequency (Ellington, 1984), it is possible that $Mlc2^-/+$ had insufficient power to support its weight in flight. If so, one should be able

to induce a similar flight impairment in Canton S with an experimentally reduced wing beat frequency, assuming of course that the uniformity of wing beat movement is unchanged. Table 1 supports such a prediction. Canton S males at 12°C had wing beat frequencies (~155 Hz) comparable with those of the flightless $Mlc2^-/+$ males at 23°C (~152 Hz), and they were similarly flight-impaired.

Although the uniformity of wing beat movement (H) was significantly less in the flight-impaired $Mlc2^-/+$ than in wild-type Canton S, H does not necessarily correlate with flight ability. For example, flight-impaired Canton S males at 12°C had an H index that was not significantly different than that of flighted Canton S males at 21°C. Nevertheless, H was demonstrably lower in the mutant flies. One might speculate that a mixture of organized and disorganized myofibrillar lattice structures gives rise to a mixed population of crossbridges with different kinetic properties that, acting nonuniformly and perhaps asynchronously, are unable to maintain a uniform wing beat frequency. In essence, the resonant fre-

quency of the flight system might be mismatched with the kinetics of the flight muscles.

CONCLUSION

We have shown that optical tachometry used to gather wing beat signals, in combination with Fourier spectral analysis of those signals, provides a simple, fast, and efficient way to collect and analyze wing beat frequencies. The dominant frequency peak in the Fourier spectrum (f) corresponds to the wing beat frequency (i.e., the primary resonant frequency of the flight system). A measure of the degree of variability of the wing beat frequency about f (i.e., the parameter H) is derived using data from the Fourier analysis. The advantage of using H, unlike q derived previously (Yamakawa and Maughan, 1991), is that it does not assume a single resonant peak and integrates information in a broader range about the primary spectral peak.

Exemplar data from *Drosophila* studies shows that wing beat frequency f, determined over the interval that exhibits the highest value of H, is abnormally low in mutant flies whose flight muscles have reduced levels of myosin regulatory light chain protein. Thus, f (representing a short segment of recorded data selected on the basis of the H index) is a useful index of flight system function that can serve to link flight ability with alterations in flight muscle structure and function. The H index is also significantly lower in the mutant flies than in the wild-type, reflecting greater wing beat frequency variability in mutant flies compared with wild-type. Thus H, although not a predictor of flight ability, can still prove useful for assessing the effects of genetic alterations of flight muscle structure on wing beat frequency variability.

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