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1‘Singing on the wing’ as a mechanism for species recognition in the malarial mosquito *Anopheles gambiae*

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Summary

Anopheles gambiae, responsible for the majority of malaria deaths annually, is a complex of seven known species and several chromosomal/molecular forms. The complexity of malaria epidemiology and control is due, in part, to its remarkable genetic plasticity, enabling its adaptation to a widening range of human-influenced habitats, which leads to rapid ecological speciation as soon as reproductive isolation mechanisms start to develop [1-6]. Although reproductive isolation is essential for speciation, little is known about how it occurs in sympatric populations of its incipient species [2]. We show that in such a population of the ‘M’ and ‘S’ molecular forms a novel mechanism of sexual recognition (male-female pairs of mosquitoes match flight-tones [7-9]), also confers the capability of mate recognition, an essential precursor to assortative mating; frequency-matching occurs more consistently in same-form pairs than in mixed-form pairs ($P > 0.001$). We also show that the key to frequency-matching is ‘difference tones’ produced in the nonlinear vibrations of the antenna by the combined flight-tones of a pair of mosquitoes, and detected by the auditory Johnston’s organ. Through altering their wing-beat frequencies to minimise these difference tones, mosquitoes can match flight-tone harmonic-frequencies above their auditory range. This is the first description of close-range mating interactions between males and females of incipient An. gambiae species.
**Results and Discussion**

*Anopheles gambiae* s.l. has become a focus of research on the evolution of species complexes to understand how populations diverge and become distinct species [4]. The essential mechanism leading to speciation is the evolution of reproductive isolation between diverging populations. Within the *Anopheles gambiae* complex, several degrees of reproductive isolation among its members can be observed in field populations. On one hand, formally recognized species such as *An. gambiae* s.s. and *An. arabiensis* have evolved strong reproductive isolation, although a permeable species barrier still exists leading to a small degree of introgressive hybridization[1, 555]. On the other hand, within *An. gambiae* s.s. cryptic incipient speciation has led to the recognition of two molecular forms, named ‘M’ and ‘S’[6], that assortatively mate[10] at different frequencies across different eco-geographical settings[3, 11]. The mechanisms responsible for reproductive isolation between M and S are not fully understood and appear to vary across populations. In Mali, for example, unknown behavioural cues used by the two forms to identify swarm sites have diverged and, since they mate in segregated swarms, hybrids are rarely produced [12]. In Burkina Faso, only 500 km away, M and S form mosquitoes can be found in the same swarm[6, 10, 13, 14] and yet hybrids are also rare, indicating the potential existence of a close-range barrier to interbreeding.

There are no published reports of close-range mate recognition in the *An. gambiae* complex, and attempts to demonstrate mate recognition in the field with volatile pheromones have not been successful (J.D. Charlwood, personal communication, 2009). In this paper we report the first evidence of form-specific, close-range (~ 2 cm) interactions between males and virgin females, characterized by
continuously monitored audio-motor feedback between individual mosquitoes. This
behaviour, which provides the capability of mate recognition in mosquitoes, may
contribute to the observed assortative mating between M and S form mosquitoes
where they meet in mixed swarms.

75 **Behavioural Interactions**

We recorded the flight tones and flight tone interactions produced by tethered wild
male and virgin female M and S form mosquitoes, individually and in same- and
mixed-form pairs under semi-natural conditions in Bobo Dioulasso, Burkina Faso
(Figure 1 inset). Individual male and female mosquitoes flew at mean fundamental
wing-beat frequencies (WBFs) similar to those reported previously [15], with males
flying at significantly higher WBFs (mean ± SD; M males = 704 ± 25 Hz, n=4; S
males = 682 ± 27 Hz, n=5) than their conspecific females (M females = 467 ± 31 Hz,
S females = 460 ± 26 Hz, n=5; P < 1.0 x 10^{-7}, Tukey’s honest significant
difference (HSD), Experimental Procedures] for flight records of mean length = 8.7 s.

When male-female pairs of same-form and mixed-form were flown within auditory
range (~2 cm) of each other’s flight tones, their flight behaviour altered significantly;
males and females of both molecular forms significantly increased their mean WBFs
(ANOVA; F=5.103; df=1,101; P = 0.026, for solo v. paired flight), with males
continuing to fly at significantly higher mean WBFs (M males = 771 ± 42 Hz, n= 30;
S males = 715 ± 55 Hz, n = 14) than their conspecific females (M females = 489 ± 33
Hz, n = 24; S females = 475 ± 28 Hz, n = 20; P < 1.0 x 10^{-7} for both comparisons,
Tukey’s HSD), irrespective of whether they were in same- or mixed-form pairs. All
types of mosquito also significantly increased the variability of their respective WBFs
(mean interquartile range, IQR) when flying in pairs (F = 20.137; df =1, 101; P = 1.9
95X $10^{-5}$) from a mean value for males of 10 Hz for solo flights to 27 Hz for paired flights, and for females from 5 Hz to 22 Hz, irrespective of the form they were paired with.

The phenomenon of frequency-matching is, however, the most remarkable feature of auditory interactions we observed in pairs of *An. gambiae* mosquitoes. Frequency-matching is defined here as the maintenance of a relatively constant ratio (+ 1%; Experimental Procedures) between the fundamental WBFs of two mosquitoes through continuous audio-motor feedback interactions between them, as shown in Figure 1. The closest audible frequency shared by females and males of both molecular forms occurs at the 3rd harmonic of the female and the 2nd harmonic of the male, given that the basic ratio between male and female WBFs is ~ 1.5 and the range of sensitivity of *An. gambiae* antennae is < 2,000 Hz when they are flying ~ 2 cm apart (see below, Frequency Tuning). On the basis of our definition for frequency-matching, 92% of matching sequences in our records occurred at the 3:2 harmonic frequency, with matching frequencies that differed by < 22 Hz (see discussion of ‘difference tones’ below).

Samples of male-female pairs of M and S form mosquitoes matching at a ratio of 3:2 shown in Figure 1A & B illustrate our finding that the absolute mean matching frequency is variable, unique to each interaction, and can change during a matching sequence with one mosquito frequency-tracking the other. For example, in Figure 1A & B the pairs of mosquitoes frequency-match for a few seconds at a time (light coloured regions), reducing the variability in their respective WBFs when the ratio between them is close to 3:2, but when they come back together after breaking apart, the mean matching frequency has generally changed. Fine time-scale interactions are shown in Figure 1C & D to illustrate the ability of mosquitoes to respond to changes
in each other’s WBF on a moment-to-moment basis with a brief (~50 – 60 ms) delay. It is worth noting that both males and females actively respond to the other during these interactions.

To accommodate this variability in behaviour between individual mosquitoes, we developed a set of criteria for scoring the frequency-matching status of each record, based on a minimum proportion of the record with matching and a minimum duration of matching (frequency-match for > 20% of a record and for > 1s, Experimental Procedures). Hence, based on the definition of frequency-matching, the M-form pair in Figure 1 A matched for 25.5% (2.8 s) of the 11.0 s record, the S-form pair in Figure 1B matched for 38.0% (4.9 s) of the 13.0 s record, the mixed-form pair (S female-M male) in Figure 1E matched for only 5.4 % (0.7 s) of the 13.0 s record, and the M female-S male pair in Figure 1F matched for 4.0% (0.5 s) of the 13.0 s record. Based on our set of scoring criteria, the pairs in A and B scored ‘positive’ and the pairs in E and F scored ‘negative’ for frequency-matching.

The results of this analysis show that frequency-matching occurred significantly more often in same-form pairs (14 out of 24 pairs) than in mixed-form pairs (2 out of 20 pairs) ($\chi^2 = 11.013; df =1; P=0.001$), thus demonstrating the capability of M and S form mosquitoes to discriminate between ‘same’ and ‘other’ form to a greater level of accuracy than any other adult phenotype assay described so far [16].

Why does frequency-matching occur more often in same-form pairs? We have evidence of physiological and behavioural factors that may potentiate sustained frequency-matching in same-form pairs. The relative wing-beat frequencies of M and S males and females at higher harmonics may constrain the range of possible WBF ratios within mixed-form pairs. M-form pairs frequency-matched at significantly
higher frequencies than S-form pairs (Fig 2A thick symbols, M-form = 1510 ± 78 Hz
v. S-form 1440 ± 72 Hz, $F=9.347; \text{df}= 1,10; P=0.0121$, Experimental Procedures).

Evidently there is a mechanism, or behavioural strategy yet to be identified, that favours same-form frequency-matching. For example, having increased their mean WBFs on hearing the sound of a nearby mosquito, if M-form males then decrease and M-form females further increase their respective mean WBFs, they would increase the likelihood of frequency-matching, whereas the reverse is true for S-form mosquitoes (after the initial increase in WBFs, females decrease and males further increase their respective WBFs to match). Were each type of mosquito to respond always as if it were flying in a same-form pair, the chance of frequency-matching in mixed-form pairs would be much reduced because the difference between their respective 3:2 WBFs would increase.

Previous attempts to detect potential mate recognition characteristics in the mean WBFs of *An. gambiae* species may have failed because WBFs were measured only in solo flying mosquitoes. Our findings that mosquitoes increase the overall frequency and variability of their wing beats when encountering others and the potential importance of the relative WBFs of males and females at higher harmonic ratios had not yet been appreciated [15, 17].

The interactive aspect of frequency-matching appears to be essential; presentation of pure tones or pre-recorded mosquito flight tones to individual tethered-flying *Anopheles* mosquitoes did not elicit frequency-matching in either form. Analysis of factors controlling frequency-matching and subsequent mating behaviour must be undertaken in free-flight experiments.

Frequency-matching may have evolved due to a selected advantage of mating in free-flight; males are known to chase females by localising the source of their
flight tone[17, 18], and frequency-matching at close-range would enable the relatively small male to form a copula with the larger female in mid-flight by synchronising with the potentially turbulent air stream generated by her wing beats[19, 20]. The findings presented here represent the first breakthrough in furthering our understanding of mosquito mating interactions since Belton’s analysis of male mate localisation by sound > 35 years ago [18]. They are also the first documentation of form-specific close-range interactions related to mating behaviour since Coluzzi first put forward his theory of the evolution of reproductive isolation in diverging populations [4, 21].

Frequency Tuning and Sensitivity of Mosquito Hearing

The physiological mechanism that controls frequency-matching is based on the characteristics of one of the most sensitive hearing organs in the animal kingdom[7, 18322-24]. Sounds are detected by the complex arrangement of sensillae (~15,000 in males, ~7,500 in females) of the Johnston’s organ (JO) in the pedicel of the antenna (Figure 3A). The sensillae mechanoelectrically transduce and amplify the nanometre displacements of the flagellum caused by the near-field component of sound[23, 24].

There is evidence for three species of mosquito, *Culex quinquefasciatus*[8], *Aedes aegypti*[9] and now *An. gambiae*, that frequency-matching of flight tones occurs at frequencies that are about three times higher than the fundamental WBF of females. How do these high frequencies compare with the frequency bandwidth and tuning of the flagellum and the JO? Male *An. gambiae* mosquitoes hydraulically extend and collapse the fibrillae of their antennae[25] (Figure 3A, photo) on a diurnal cycle linked to the swarming periods at dusk and dawn when mating occurs[17, 26]. These mechanical changes in the antennae alter the response characteristics of the JO[27].
Accordingly, we obtained antennal-mechanical and JO-receptor-potential frequency-tuning-curves both during their diurnal phase of inactivity, when the fibrillae were collapsed and at dusk when they were extended.

Mechanical threshold-tuning curves (0.2 nm criterion, noise floor 0.13 nm R.M.S) measured with a laser-diode interferometer directed at the base of the flagellum[28] from two male An. gambiae are shown in Figure 3B. With fibrillae collapsed (solid symbols), the minima is at a frequency of 235 ± 14 Hz and at a particle velocity of 4.1 x 10^{-6} ± 2.0 x 10^{-7} ms^{-1} (n = 7). With fibrillae extended (open symbols), tuning shifts significantly upwards in frequency (P = 0.003) to 540 ± 45 Hz, but sensitivity is decreased to 1.8 x 10^{-5} ± 5.7 x 10^{-6} ms^{-1} (n = 5), largely through loss of the sensitive minima at ~ 200 Hz. Extension of the fibrillae is, therefore, associated with an upwards shift in the most sensitive frequency of the antennae at the expense of low-frequency mechanical sensitivity. Similar measurements from female An. gambiae (red symbols, Figure 3B) did not reveal diurnal shifts in the sensitivity and tuning of the flagellum (tuning frequency minima = 209 ± 33 Hz; particle velocity = 1.4 x 10^{-5} ± 6.0 x 10^{-6} ms^{-1}, n = 5). The sensitivity and tuning of the female flagellum, which was similar to that of the male’s with collapsed fibrillae, had noticeable and repeatable notches of sensitivity around the 1st and 2nd harmonics of the male’s flight tone (arrows, Figure 3B), similar to that reported for Ae. aegypti [22].

Accordingly, it can be observed from Figure 3B that the frequencies at which the mosquitoes frequency-match are within the frequency range of the vibrations of the flagellum (i.e., up to ~ 2,000 Hz at the particle velocity expected of mosquito wings beating 2 cm away[20, B. Warren, unpublished]).

Voltage responses recorded from the JO are dominated by receptor currents from the sensory cells (supplemental material S1) and henceforth, in this paper they
will be referred to as compound phasic receptor potentials. The phasic receptor
potentials are twice the frequency (2f) of the applied sound stimulus\[29-31\] and
preserve the temporal information necessary for frequency-matching\[8\]. Threshold
receptor-potential frequency-tuning curves (criterion = 1.4 times recording noise
floor, 19.3 µV R.M.S) are shown in Figure 3C. With fibrillae collapsed (solid
symbols), the minima frequency is 200 ± 15 Hz (particle velocity = 1.0 x 10^{-6} ± 9.1 x
2610^{-7} \text{ ms}^{-1}, n = 4). With fibrillae extended (open symbols), tuning shifts upwards (300 ±
2725 Hz) with increased sensitivity (1.5 x 10^{-7} ± 6.2 x 10^{-8} \text{ ms}^{-1}, n = 4). In contrast to the
frequency range of the flagellum vibrations, the frequencies at which the mosquitoes
match their flight-tones is outside the bandwidth of the JO phasic receptor potentials
and thus outside the auditory range of An. gambiae mosquitoes. It has been reported
for Ae. aegypti \[9\] that the auditory range of the DC component of the JO receptor
potential extends far above that of the phasic response and encompasses the
frequency-matching range. We measured DC components of the receptor potential
and plotted DC frequency tuning curves (insets to Figure 3C). We also plotted DC
component tuning curves for Cx. pipiens mosquitoes (supplemental material S2). It is
clear from our findings that DC component frequency tuning curves are bounded by
the phasic receptor potential tuning and do not extend the auditory range of the JO.
Changes in the frequency tuning and sensitivity of the JO during extension of
fibrillae are complex and may not entirely be due to mechanical changes in the
flagellum. The electrical responses of the JO and mechanical responses of the
flagellum are metabolically vulnerable when the fibrillae are extended, and can
collapse within 5 min when disturbed by experimental procedures. It would be
interesting to discover if there is metabolic enhancement of the sensitivity of the JO
during the increased hydrostatic pressure that causes erection of the fibrillae.
We conclude that An. gambiae match their flight tones at frequencies that are outside the bandwidth of the JO’s phasic responses to acoustic stimulation. It appears that the near-field auditory systems of Anopheles and Culex species[8] are similar. Each consists of a broadly-tuned, non-linear, detector (flagellum) that oscillates spontaneously at frequencies close to the female’s WBF and can detect, through distortion, the higher harmonics of the flight tones. When pairs of tones (frequencies f1 and f2, or the mosquito’s own flight tones and those of the other) are presented simultaneously, the flagellum generates distortion products, including one at the difference frequency (f2 – f1), as can be seen in the amplitude spectra measured from the vibrations of the flagellum (Figure 4A,C,D). This difference tone is detected by the receptors of the JO even though the stimulus tones are beyond the frequency range of the JO and cannot be detected by it (Figure 4B,E,F). It is essential for the purpose of frequency-matching that difference tones can be generated at low frequencies by the flagellum and be detected by the JO. The JO can, for example, generate a strong difference tone at 12 Hz in response to pairs of tones at 1399 and 1411 Hz (Figure 4C), which is within the frequency-matching range when in free flight and at stimulus levels equivalent to the flight tones mosquitoes produce when 10 mm apart[22]. We detected difference tones at 22 Hz in the receptor potentials (Figure 4E), which is similar to the magnitude of the differences in frequency between two mosquitoes when frequency-matching. Difference tones at lower frequencies were masked by low-frequency electrical noise that is generated in the JO. The basis of this noise was beyond the scope of this study, but a strong candidate is the pulsating antennal heart[32].

The detection of difference tones provides mosquitoes with a strategy for matching the harmonic components of their flight tones at frequencies they cannot
hear (Figure 3B). By analogy with violinists who tune their instruments by detecting
beats, mosquitoes adjust their wing-beat frequencies to within a few Hz of each other
until the difference tones drop in frequency and disappear when the harmonics are
perfectly matched.

Conclusions

We report here the first quantifiable means of discriminating two molecular forms of
adult *An. gambiae* s.s. on the basis of an observed behaviour. This behaviour uses the
detection of difference tones as the basis of audio-motor interactions that occur
reliably between a male and a virgin female of the same form. The discovery of this
potential mate recognition mechanism constitutes the first evidence of a critically
necessary, albeit not sufficient, step in the process of assortative mating at close-
range, which is known to occur in this species complex. Our discovery of a mating-
related phenotype that is associated with genotype in the *An. gambiae* complex also
represents a breakthrough in research on how reproductive isolation can occur in
sympatric populations of incipient species.

Experimental Procedures

Mosquito larvae were collected from breeding sites typical of the respective forms; M
form from rice paddies (VK7 village) and S form from rain-fed pools (Soumousso
village), and identified to form-level by PCR[33] at the end of experiments. Flight
tones were recorded with a particle velocity microphone located within 2.0 cm of tethered mosquitoes and equidistant between them when two mosquitoes were flown together (Figure 1). Factors known to affect wing-beat frequency were controlled for. Behavioural and biophysical experiments were conducted on 4 – 7-day old males and virgin females only during the 2 hours preceding dusk (period of inactivity) and the 2 hours following the onset of dusk (peak of maximum activity).

Methods for generating stimulus tones, recording flight tones from tethered flying mosquitoes, making and analysing mechanical measurements of the flagellum of the antenna with a self-mixing laser diode interferometer and electrophysiological measurements from the JO, were as described previously.

Measurements were made within half an hour of preparation because sensitivity, distortion products and spontaneous emissions usually deteriorated or disappeared after this period.

Fundamental WBFs were digitized from recordings of duration = 4–18 s, (mean = 8.7 s), and analysed by three-way ANOVA (df= 1,101), followed by Tukey’s honest significant difference (HSD) test for multiple comparisons of means to test effect of sex, form and solo vs paired flight on the mean WBF and associated interquartile ranges (IQR). Mean WBF data for frequency-matching same-form pairs were analysed using a linear model in R, which produced a significant difference between the weighted WBF means the M and S form pairs (F=9.347, d.f.= 1,10, P=0.0121. A Q-Q plot for the model of standardised residuals against theoretical quantiles showed a reasonable fit to the straight line, and a symmetrical distribution of points above and below the line.

‘Frequency-matching’ defined as a harmonic-based integer ratio between the fundamental WBFs of two mosquitoes ± 1% (i.e., ± 0.02; since the range of values =
‘Positive’ score for frequency-matching (> 20 % and >1 second) based on analysis of the frequency distributions of the proportion and duration of records that contained frequency-matching, which showed two overlapping curves for same- and mixed-form data in proportion frequency-matching, with a clear breakpoint at ‘20% of record matching’. To avoid false positives when scoring frequency-matching due to multiple short bursts and crossing-over, a second criterion was added, that required matching for > 1 s, based on the frequency distribution of matching duration, which showed that all but two records had matching sequences that lasted more than 1 s, and matching in these two records was mainly due to cross-over matching.
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Figure captions

Figure 1 Auditory interactions between tethered flying mosquitoes.

Inset: Arrangement of particle velocity microphone and tethered mosquitoes during sound recordings; A - F) Spectrograms (reconstructed from digitised fundamental
frequencies) of flight tones with harmonics of males (blue) and females (red) and periods of frequency matching (grey: male, green: female). A & B) Same-form pairs of M-form and S-form mosquitoes, respectively, showing extended frequency-matching (pale colours), when the female’s 3rd and the male’s 2nd harmonics converge, at a ratio between their fundamental wing beat frequencies of 3:2 (i.e., 1.5, a harmonic-based ratio); C & D) Expanded views of 4 s of the spectrograms of A and B, respectively, showing periods of frequency-matching between the female’s 3rd and male’s 2nd harmonics of their flight tones. E & F) Mixed-form pairs, S female & M male and M male & S female, respectively, showing only transient periods of frequency-matching between harmonics. The ratio between their fundamental wing-beat frequencies does not stabilise at a harmonic-based value.
Figure 2  Comparison of the mean wing-beat frequencies of same-form and mixed-form pairs of *Anopheles gambiae* M and S form mosquitoes at the 3:2 harmonic frequencies where most matching occurs.

A) Same-form pairs: male (square) mean ± standard deviation wing-beat frequency plotted against female (triangle) mean win beat frequency, for M form (black) and S form (red) pairs during frequency-matching (thick lines) and non-matching (thin) sequences. Dotted line denotes slope=1, i.e., perfect frequency-matching; 

Figure 3 Mechanical and receptor-potential tuning-curves, from the flagellum and Johnston’s organ, respectively, of *Anopheles gambiae* (M form) mosquitoes.

A) Schematic cross-section of mosquito antenna; flagellum (F) inserted into cup-shaped pedicel that houses complex arrangement of cuticular processes (C) and attached, mechanosensory scolopidia (S) of the Johnston’s organ (JO) [31] and a photomontage of male *An. gambiae* mosquito head; fibrillae extended (left, active phase; dusk) and collapsed (right, inactive phase); B) Mechanical threshold frequency tuning curve (mean and ± standard deviation, vertical bars) measured from base of flagellum in male mosquitoes (blue) with collapsed (solid) and extended (open).
symbols) fibrillae and female mosquitoes (red, solid). Arrows indicate sensitivity
peaks at 700 and 1400 Hz. Dotted lines indicate flight tone at highest frequency
mosquitoes are likely to encounter, and to which antennae can respond [23];

C) Main figure: Compound phasic (2f) receptor potential frequency tuning curves
(mean and ± standard deviation, vertical bars) measured from JO of male mosquito
with collapsed (solid) and extended (open symbols) fibrillae; inset: receptor potential
(gray) with DC component (red) from a male with collapsed fibrillae in response to a
300 Hz tone, particle velocity 0.0011 ms\(^{-1}\); right: receptor potential tuning curves
derived from the 2f component (black) and DC component (red) of the receptor
potential.
Figure 4  Difference tones generated in the vibrations of the flagellum and detected in the receptor potentials of the Johnston’s organ of *Anopheles gambiae* (M form) mosquitoes.

A) Recordings from male *An. gambiae* of amplitude spectra of flagellum vibrations and B) JO compound receptor potentials (lower) in response to a pair of tones at 1399 Hz (f1) and 1499 Hz (f2), both at a particle velocity of 0.0011 ms\(^{-1}\). C – F) Difference tones in mechanical (flagellum) (C, D) and electrical (JO) (E,F) spectra in response to tones at the frequencies indicated with particle velocities of 0.005 ms\(^{-1}\). Responses to the primary tones (f1 and f2) are seen in the mechanical but not in the electrical responses. Difference tone (f2 – f1) and spontaneous oscillation (SO) responses are seen in both the mechanical and electrical spectra. The tones f1 and f2 also interact with the SOs to produce further distortion products (A).
The effect, on the compound JO potential of an M-form male *Anopheles gambiae*, of injecting TTX into the thoracic haemolymph sufficient to block the mosquito’s observable motor activity.

Voltage responses recorded from JO of male M-type *Anopheles gambiae* mosquito in response to a 300.3 Hz tone (particle velocity 0.56 mms\(^{-1}\)) before (black) and after (red) injecting 1 µM TTX in insect saline (ref) into the thorax. We assumed that the TTX had blocked neural activity when we no longer observed spontaneous, reflexive, or mechanically evoked motor responses from the palps, legs and wings. Note the phasic (2f) and DC (negative baseline shift) components of the voltage response remains unchanged but the initial negative peak (arrow) is greatly reduced following TTX injection. We conclude that the voltage response recorded from the JO is dominated by the compound receptor current of the sensory cells but the compound potential at the onset of the voltage response to the tone is dominated by compound neural responses. Extracellular potentials in response to tones recorded from the JO are, therefore, similar to those recorded from the cochlea. Extracellular
voltage responses recorded from the cochlea are dominated by receptor currents from the outer hair cells and synchronised firing of nerve fibre contribute to a compound action potential at the onset of the voltage response.
DC and phasic voltage responses and frequency tuning curves recorded from the Johnston’s organ of a male *Culex pipiens* mosquito (A). The red, superimposed trace is the DC component of the voltage response. C) Compound receptor potential (2f) (black) and DC receptor potential (red) threshold tuning curves recorded from the JO of a male mosquito based on the particle velocity necessary to produce a receptor potential 10 dB above the recording noise floor.