THE EFFECT OF FIBRIL ERECTION ON HEARING IN MALE AEDES TOGOI: AN OPEN AND SHUT CASE

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1. INTRODUCTION

Mosquitoes hear with their Johnston's organs, complex sensory structures in the large second segment of their antennae. These organs detect the direction and amplitude of the movement of the distal part of the antenna as it is displaced by sound waves. In many species, the long fibrils on the male flagellum are erect when the insects swarm at dusk and dawn, and recumbent during the rest of the day (Clements 1999). It has been claimed that when the fibrils are recumbent, males cannot hear (Nijhout and Sheffield, 1979, and references therein). To test this, we used laser vibrometry to examine the movement of the antennae of male *Aedes togoi* with erect and recumbent fibrils to sound stimuli of frequency sweeps and clicks. From the sweeps, we determined the resonant frequency of the antennae. We hypothesized that: a) males with recumbent fibrils would be able to detect sound; b) the resonant frequency of males with open fibrils and their selectivity would be different from those with recumbent fibrils, and c) females would not change their resonant frequency when males had open or recumbent fibrils.

2. METHODS

2.1 Experimental Insects

A colony of *Aedes togoi* was cultured from larvae collected from rock pools in West Vancouver, BC, Canada. Larvae were fed fish flake food, and maintained at 25° C on a 16:8 light:dark photoperiod. Adults were fed sugar water and allowed a bloodmeal. All insects were 3 to 5 days old. Males with recumbent fibrils were tested during the light photoperiod when their fibrils were naturally closed; males were tested in the erect fibril state during the dark photoperiod, when their fibrils were expanded naturally. Females were tested along with males at both times.

2.2 Sound generation

A 10 Hz to 1 kHz logarithmic sweep, 5.1 s long was generated with a Brunelle Instruments model 3050 sweep generator. Sound was kept at 65 dB at the insect antenna by running the signal through a General Radio 1569 Automatic Level Regulator that monitored sound level through a Radio Shack Realistic sound level meter (SLM) 33-1028 or a Brüel and Kjaer 2204 SLM placed directly next to the mosquito. The sound was played from a Sennheiser headphone placed approximately 10 cm from the antenna.

2.3 Laser vibrometry

Laser vibrometer recordings from the base of the flagellum were made with a Polytec OFV-2500 with a Polytec Vib-E 220 data acquisition system. The sampling rate was set at 24 kHz for 10.92 s with 2 mm/s sensitivity. The sweep was recorded concurrently with the antennal response by recording the output of the SLM on the second channel of the Vib-E system. All recordings were made at a temperature of 24-25°C.

2.4 Data analysis

To determine the resonant frequency of the antennae, the laser recordings were analyzed in Adobe Audition 3.0. The time of the phase change between antennal response and the sound played to the antenna was noted, and corresponding frequency determined using Raven Pro 1.4. The directional hypothesis that the erect fibrils of males have a higher resonant frequency than recumbent fibrils was tested with a Student's t-test in JMP 7.0.2, 90% confidence. An ANOVA was also run in JMP 7.0.2 to test for differences between the four tested categories of antennae, 95% confidence.

3. RESULTS

Male antennae with recumbent and erect fibrils and female antennae recorded in the same times as the males all showed a resonant peak between 200 and 400Hz. Examples are shown in Figure 1. Males with erect fibrils had significantly higher resonant frequencies than males with recumbent fibrils when tested with the directional t-test ($p < 0.08$), and were significantly different from the two categories of female antennae when tested in an ANOVA. This test showed no statistically significant difference between the resonant frequencies of female antennae during the active and inactive times (Figure 2).

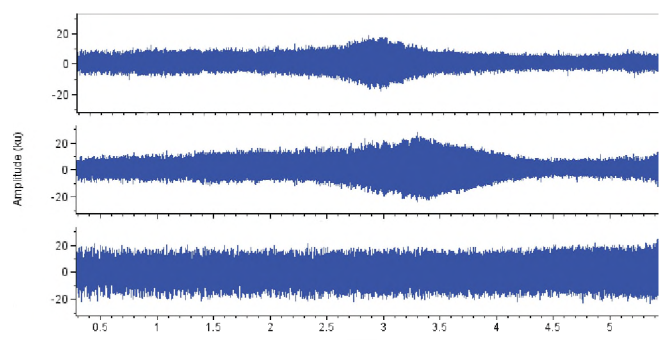

Figure 1. Responses of male *Aedes togoi* with recumbent (top), and erect fibrils (middle) to a flat 65 dB logarithmic sweep, from 10 Hz to 1 kHz (bottom).

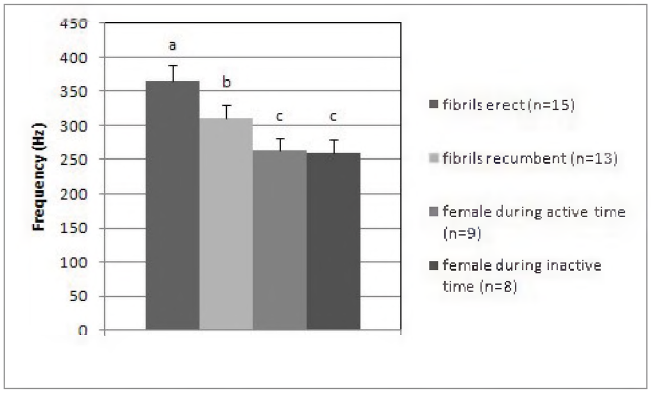

Figure 2. Mean resonant frequency +SE of male antennae with recumbent or erect fibrils (t-test) and of female antennae during active or inactive times (ANOVA, *p* value set at 0.05).

4. DISCUSSION

Laser vibrometry of male antennae with erect or recumbent fibrils and female antennae show very similar resonant responses. The antennae of females have only 10% the surface area of typical 'bottlebrush' male antennae of the same species (Clements 1999), and this makes it unlikely that the physical characteristics of the flagellum play a major role in the sensitivity and tuning of the antenna. Clearly, long fibrils are not essential to detect the wingbeat of nearby flying mosquitoes of either sex. As recently as 1999, Clements in his admirable monograph concluded that "female mosquitoes are believed not to respond behaviourally to sound" but since then, female mosquitoes in four different genera have been shown to change their wingbeat frequency to match harmonics with a nearby flying male (Gibson and Russell, 2006 Cator et al, 2009; Warren et al, 2009; and Pennetier et al, 2010) and one species has been shown to be attracted by the call of its frog food source (Borkent and Belton, 2006). Nevertheless the ultra-sensitive Johnston's organs of male mosquitoes are tuned closely to the wingbeat frequency of a female of the same species, to which they are attracted during swarming. In *Aedes togoi* we measured mean wingbeat frequencies of 523 Hz in males and 306 Hz in females, the latter within the range of resonance we measured in male antennae.

Verticillate (bottlebrush) antennae evidently give male flies that are attracted from swarms by the sound of a female some selective advantage because they have evolved in at least six different families, and in the Ceratopogonid biting midges, not closely related to mosquitoes, the tiny males of most species erect their long antennal fibrils before they swarm and mate.

It is not obvious why the resonant frequency of the male antennae should increase when the fibrils expand because viscous drag would tend to reduce it (Fletcher, 1992). However, our results are similar to those of Pennetier et al. (2010) who found that the antennae were less sensitive but tuned to a higher frequency when the fibrils were extended in *Anopheles gambiae*, another species that erects its fibrils before swarming. Perhaps the contractile properties of the sense cells in Johnston's organ (Gӧpfert, and Robert, 2001) somehow increase the frequency of its tuning. In *Anopheles*, an alpha-adrenergic transmitter substance is thought to trigger extension of the fibrils and it could possibly also affect the sense cells (Nijhout and Martin, 1978).

We believe ours is the first examination of resonant frequencies in an *Aedes* species known to expand its fibrils, and we show that the antennae of males with recumbent fibrils vibrate in response to sounds much like those with them erect. Female antennae are resonant much like males but their antennae do not have long fibrils and they do not change their resonant frequency before mating.

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