INSECT MICROFLUIDICS: HEARING AND BREATHING WHILE TINY

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Summary Insects have evolved to handle fluids using strategies that vary significantly from those used by larger animals since, by virtue of their size, they interact with fluids in the microscale regime, where surface forces dominate. Here we discuss two examples from insect microfluidics: flow actuation in insect respiration, and the amplification of acoustic sound localization cues in a parasitoid fly. We review behavioral data and present the mathematical, computational, and microfluidic device models we have developed to represent idealized versions of these systems that retain the salient features. In both examples, the insects appear to make use of finely tuned structural and material properties in the organs that come into contact with the fluids in question. In the case of the fly, we show that a tympanal asymmetry of just 5% may lead to order-of-magnitude gains in its hearing abilities.

INTRODUCTION

Bioinspired engineering has been a flourishing field of research for decades. From a mechanics perspective, much bioinspired research occurs in the realm of bioinspired materials and locomotion. Bioinspired fluid dynamics has primarily dealt with external flows at the macroscale, where inertial and viscous forces are both important. There are fewer examples of bioinspired internal and microscale flows, in which viscous and surface tension forces dominate. As integrated circuit cooling and lab-on-a-chip technologies develop, it may be fruitful to look to nature for inspiration for handling fluids efficiently at the microscale. Insects provide a particularly relevant group of model organisms. Here, we present two examples from insect microfluidics that we have studied in our laboratory, insect respiratory pumps and the hearing apparatus of the parasitoid fly, *Ormia ochracea*.

Fluid pumping in insect respiratory systems

Insects have the highest metabolic range in the animal kingdom, owing to their unique respiratory systems that do not use blood as an intermediate oxygen carrier. Instead of using lungs, insects take air in through pairs of openings around their body called spiracles. The air passes through the spiracles into a complex network of tracheal tubes that ramify and eventually reach the tissues (See Figure 1). Although the size of the largest tracheal tubes depends on the size of the insect, the size of the smallest tracheal tubes, located next to the tissues, is fixed at around $0.5 - 1\ \mu$m.

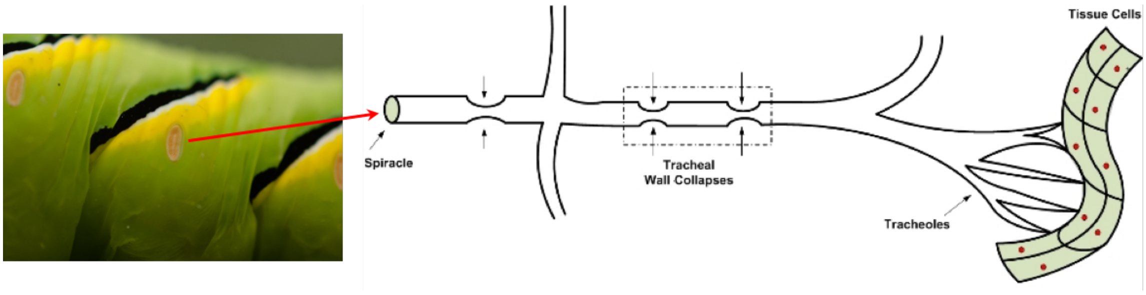

Figure 1: (Left) Close-up of spiracles on a *Manduca sexta* caterpillar. [Steve Begin, Spiracles, Creative Commons (2009); used in accordance with the Creative Commons Attribution (CC BY-NC-SA 2.0) license [1].] (Right) A schematic of the insect pathway for oxygen from spiracle to tissues. Modified with permission from [2].

It has been determined that in many insect species, portions of the tracheal tubes collapse rhythmically during active ventilation [3]. This is indicated by the boxed section labeled "Tracheal Wall Collapses" In Figure 1 (Right). We have developed mathematical, computational, and microfluidic device models that capture the salient features of tracheal-collapse driven respiratory flows (see, e.g., [2, 4]).

Sound localization in a parasitoid fly

Ormia ochracea is a parasitoid fly that relies on phonotaxis to locate its hosts in the horizontal plane. Gravid females locate their cricket hosts with astonishing precision, equal to that of horizontal sound localization in humans, though their small size should preclude this due to fundamental constrains from the physics of sound propagation [6]. Miles *et al.* demonstrated that the fly's two tympanal membranes (highlighted in Figure 2, Left) are mechanically coupled via a cuticular bridge, which increases the interaural time delay (ITD) between the tympana, allowing her to resolve nanosecond time differences, and greatly increasing the precision with which she can locate her larval hosts [5]. Below, we present new experimental and mathematical modeling results that demonstrate that small asymmetries in the tympanal system may lead to large gains in *O. ochracea*'s sound localization abilities.

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METHODS

The classical model for hearing in *O. ochracea* can be written as:

$$\begin{bmatrix} k_1+k_3 & k_3 \\ k_3 & k_2+k_3 \end{bmatrix} \mathbf{x} + \begin{bmatrix} c_1+c_3 & c_3 \\ c_3 & c_2+c_3 \end{bmatrix} \dot{\mathbf{x}} + \begin{bmatrix} m & 0 \\ 0 & m \end{bmatrix} \ddot{\mathbf{x}} = \mathbf{f}, \tag{1}$$

where $\mathbf{x} = (x_1(t), x_2(t))$ is the unknown tympanal amplitude vector, the force applied from the incident sound pressure wave is $\mathbf{f} = (f_1(t), f_2(t))$, and $\dot{( )}$ represents differentiation with respect to time, t. The parameters k_i and c_i are spring stiffnesses and dash-pot constants, respectively, and the parameter m is the effective mass of all the moving parts of the auditory system and is assumed to be concentrated at the ends of the intertympanal cuticular bridges. The subscripts 1 and 2 refer to the ipsi- and contralateral tympanal membranes, respectively. [5].

We introduced a size asymmetry between the left and right tympanal membranes into the mathematical model of hearing in *O. ochracea*. Additionally, we made 38 tympanal measurements of 19 *O. ochracea* specimens obtained from the Virginia Tech Insect Collection and the collection of the Smithsonian Museum of Natural History. The specimens were decapitated in order to expose the tympana, photographed with a high resolution camera (Canon 6D dSLR camera with a 65mm MP-E macro lens) and the tympanal areas were measured using ImageJ analysis software [7].

RESULTS

Measurements of the 38 *O. ochracea* tympana demonstrated a mean left-right asymmetry in tympanal area of approximately 5.6% (Figure 2, Center). With the modified mathematical model, we demonstrated that an asymmetry of just 5% in tympanal area can increase the ITD by a factor of 15 compared to the symmetric case (see Figure 2, Right). An additional result from the model, not shown here, is that a 5% asymmetry can also introduce interaural amplitude differences (IAD) four times greater than for the symmetric case, depending on the incident angle of the sound, θ.

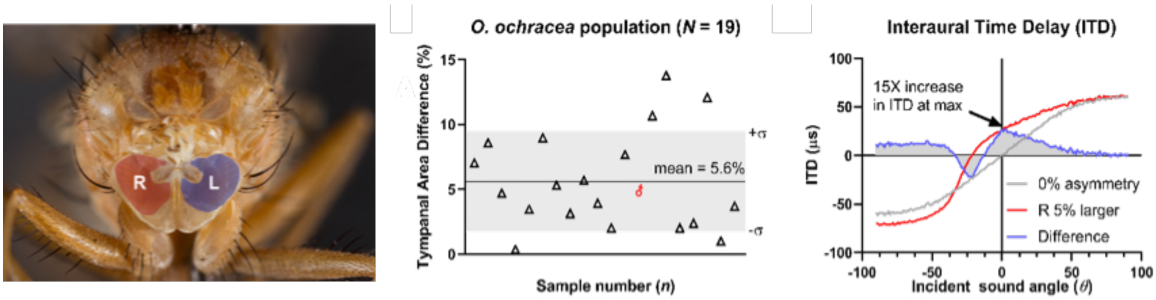

Figure 2: (Left) A headless *O. ochracea* fly with left (L) and right (R) tympanal membranes highlighted. (Center) Percent difference between left and right tympanal membrane areas for 19 *O. ochracea* samples. (Right) Interaural time delay (ITD) between the two tympana as a function of incident sound angle. An asymmetry of just 5% in tympanal area can increase the ITD by a factor of 15.

CONCLUSIONS

The mechanical coupling of the tympana in *O. ochracea* provides a significant advantage to the animal, allowing it to overcome fundamental physical constraints in locating its prey with precision. Here we demonstrate that 1) *O. ochracea*'s tympanal membranes have an average asymmetry of approximately 5.6% in area, and 2) that this asymmetry may allow for an order-of-magnitude additional increase in *O. ochracea*'s ITD and a similarly significant increase (factor of 4) in O. ochracea's interaural amplitude differences (IAD), the two quantities used by the fly to determine its prey's azimuthal location.

References

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