The Advanced Photon Source

Insect Aerobics

Insects use oxygen to drive the reactions that produce the energy needed for life. But insects don't have lungs and their blood doesn't carry much oxygen. Insects have a separate respiratory system that allows passive diffusion of gas into and out of their bodies through holes, called "spiracles," in their exoskeletons. Diffusion is sufficient for the needs of small or low-activity insects, but larger or more active insects have developed another method, termed "abdominal pumping," that uses body movements to generate gas-moving pressure changes in the body. Pupal forms of developing insects, because they are immobile and have lower energy needs, require less oxygen and should not need to use ventilation. Yet, they employ abdominal pumping. It is just not clear why they the make the effort.

To understand more about the role of abdominal pumping in developing insects, researchers from Virginia Tech used x-ray phase contrast imaging at X-ray Science Division beamline 32-ID-B,C at the U.S. Department of Energy's Advanced Photon Source (APS) to view the tracheal system of the pupal form of the darkling beetle *Zophobas morio*. Their insights into how insects produce flows at very small scales that could lead to innovations in bio-inspired microfluidic engineering.

The researchers designed a system that would allow them to monitor circulatory system pressure changes, airflow, and abdominal and tracheal movements, all at the same time in a living beetle pupa. They mounted pupae of the *Zophobas morio* beetle onto a small platform and inserted a hydrostatic pressure sensor into the body of the pupa. This let them assess pressure changes in the circulatory system that are associated with airflow. To measure airflow, they built a customized respirometry chamber to monitor carbon dioxide coming out of the spiracles. These carbon dioxide bursts can be correlated with pressure changes to identify airflow events. For imaging abdominal movements, they used infrared sensors or a video camera on the outside of the animal; tracheal movements inside the animal were monitored using x-ray phase contrast imaging at the APS beamline. The team was able to monitor carbon dioxide bursts to determine whether spiracles were open (carbon dioxide burst) or closed (no burst), and whether these respiratory activities correlated with compression (air movement) or relaxation of the tracheae. Finally, these respiratory activities were correlated to abdominal pumping events (Fig. 1).

The team's hypothesis was that when there was no carbon dioxide burst, and spiracles were closed, the magnitude of abdominal pumping would be low and the trachea would not collapse. If the spiracles were open, abdominal pumping would produce active ventilation by increasing the pressure in the circulatory system and leading to tracheal collapse and air exchange.

However, what they observed was somewhat different. Active ventilation events, those with a carbon dioxide burst correlated to a tracheal collapse and circulatory pressure increase, were always associated with an abdominal pump. But 63.7% of abdominal pumps occurred without an associated tracheal collapse and actually showed higher

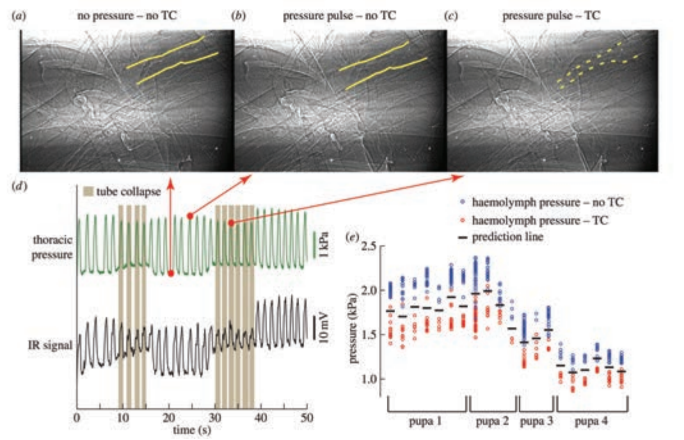

Fig. 1. Correlation of abdominal pumping with tracheal compression and haemolymph pressure. X-ray video (a–c) shows that tracheal compression occurs during only some abdominal pumping/pressure cycles (d). Here, the IR signal indicates movement of the abdomen only; the magnitude does not represent absolute displacement. The results of clustering of pressure pulses can be used to predict tracheal compression (e); the points below the black bar are predicted to be associated with tube collapse. Each group represents a different temporal sequence. TC, tracheal compression. From H. Pendar et al., Bio. Lett. 11(6), 20150259 (2015).

pressures. The team was able to conclusively demonstrate that pupae do indeed use abdominal pumping to facilitate active respiration. All of the active airflow events they observed were associated with an abdominal pump. This finding was facilitated by the set-up at the APS, which allowed them to visualize for the first time the associated tracheal collapse in a pupa. Second, this work strongly suggests that pupae also perform abdominal pumping for other reasons. Over half of the abdominal pumps occurred when the spiracles were closed and did not result in respiration.

The team hypothesizes that other roles for abdominal pumping may include internal gas mixing, support of blood circulation, or management of water loss; answering this question will be the focus of future work. *— Sandy Field*

See: Hodjat Pendar*, Melissa Kenny, and John J. Socha, "Tracheal compression in pupae of the beetle *Zophobas morio*," Bio. Lett. **11**(6), 20150259 (17 June 2015). DOI: 10.1098/rsbl.2015.0259

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Call for APS General-User Proposals

The Advanced Photon Source is open to experimenters who can benefit from the facility's high-brightness hard x-ray beams.
General-user proposals for beam time during Run 2016-1 are due by Friday, October 30, 2015.
Information on access to beam time at the APS is at https://www1.aps.anl.gov/Users-Information/About-Proposals/Apply-for-Time or contact Dr. Dennis Mills, DMM@aps.anl.gov, 630/252-5680.

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