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Meeting Report

Life is a gas

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Insect tracheae provide the most effective gas-exchange system in the animal kingdom. Until the last decade or so, we thought we understood its function reasonably well. Diffusion of oxygen is ~8000 times more rapid in the gas phase than in solution, so the air-filled tracheae allow rapid movement of oxygen into the animal, as well as exit of carbon dioxide. Based upon classic experiments using diapausing hawkmoth pupae, Schneidermann and colleagues in the 1950s and 1960s, demonstrated that insects open their spiracles to allow entry of oxygen, then close them. Closure reduces evaporative water loss from the tracheal system. Because CO_2 is much more soluble in tissues than O_2, a negative endotracheal pressure builds up, and insects "flutter" their spiracles to allow influx of air by bulk flow (convection) while minimizing water loss. Eventually, enough CO_2 accumulates that the spiracles are opened wide to get rid of it and bring in O_2. The fundamental reason for this respiratory pattern (discontinuous gas exchange; DGE) is that it reduces respiratory water loss.

This textbook model has received severe challenges in recent years;[1,2] diapausing pupae do not represent insect diversity, and we now know that many other respiratory patterns occur.[3] Furthermore, it is not clear that classical DGE even conserves water.[4] Because of the small size of most insects, active ventilation has been thought to be unnecessary because diffusion is so rapid. However, synchrotron X-ray imaging at the Argonne National Laboratory (Fig. 1) has revealed a far more dynamic tracheal system in which main trachea appear to deflate and reinflate.[5]

Insect respiration was a significant topic at the 49th Annual Drosophila Conference. Greg Beitel (Northwestern University) and Eric Johnson (University of Oregon) organized a workshop on gases, several posters featured measurements of metabolic rates, and the tracheal system continued to be a popular developmental system. There was even a vendor hawking respirometry systems specifically built for Drosophila. Fleischmannova et al. (University of South Bohemia, Czech Republic) described novel humidity-dependent respiratory patterns in adult flies, and work by Merkey et al., (University of Nevada, Las Vegas) revealed that, contrary to common belief, metamorphosis is metabolically inexpensive and pupal metabolic rates are relatively low. The drop in metabolic rates might be caused by oxygen limitation, as only one pair of spiracles is functional in the pupa. Merkey et al., tested this by exposing pupae to differing oxygen levels. Metabolism did not seem to be affected by low oxygen, and there was no evidence of anaerobic metabolism (e.g., no lactic acid accumulation) was seen.

Drosophila, however, can undergo anaerobic metabolism, as reported by Feala (University of California, San Diego) in the Gas Workshop. In addition to lactic acid, Drosophila produces acetic acid and alanine as anaerobic endproducts, much as other invertebrates do.[6] This metabolic flexibility contributes to the flies' ability to survive hypoxia, by reducing pH fluctuations associated with metabolite accumulation.[7] Naturally occurring hypoxic habitats (rotting fruit, burrows, etc.) are often also high in CO_2, so one might predict that hypoxia and hypercapnia would trigger at least some similar responses. However, at the Gas Workshop, Helenius (Northwestern University) reported that high CO_2 levels did not stimulate expression of hypoxia-related genes, nor were heat-shock proteins expressed. Instead, expression of immune response genes declined, with a concomitant increase in susceptibility to infection. At least some of these responses are caused by CO_2 itself, independently of the acidification associated with hypercapnia.

A third gas, NO, is now proving to have an important role in responses to hypoxia, as described by O'Farrell (University of California) and Morton (Oregon Health and Science University). One of nitric oxide's effects is activation of certain soluble guanylyl cyclases. There are five of these in Drosophila, two of which form an NO-binding heterodimer.[8] Morton reported that cGMP levels rise during hypoxia, and that larval mutants for one of the guanylyl cyclases displayed a slowed behavioral response to hypoxia. The NO-sensitive guanylyl cyclases are co-expressed in specific sensory neurons along the lateral wall and in the caudal sensilla; both places are exposed to atmospheric conditions.

Hypoxia itself is stressful, but so is the return to normoxic conditions. Reperfusion injury appears to be caused by production of reactive oxygen species.[9] In flies, damage increases with length of hypoxia and the number of reperfusion events.[10] Azad and Haddad (University of California, San Diego) described a microarray analysis of flies exposed to either constant or intermittent hypoxia for two hours. Differential expression was seen for protein kinase, alkaline phosphatase and cuticular protein gene families. Interestingly, some of the same genes were upregulated in one condition and

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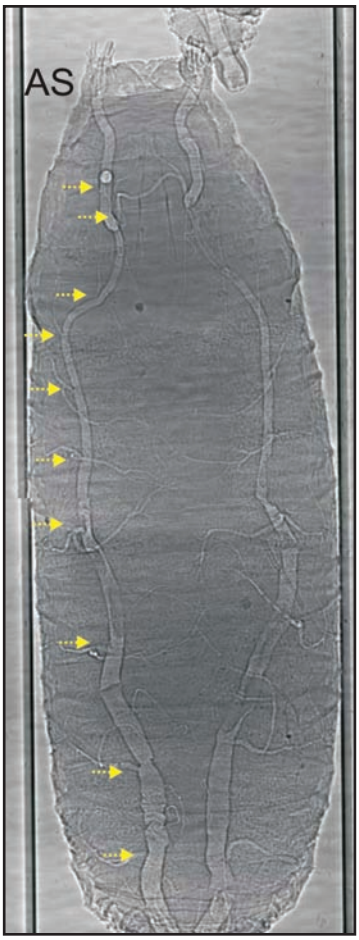

Figure 1. Synchrotron X-ray image of an Oregon-R white prepupa. Synchrotron X-ray imaging is based on density differences and provides a simple and rapid method for visualizing tracheae. Functional (i.e., air-filled) tracheae are easily distinguished from the surrounding tissues. In conjunction with fluorescent imaging, it should be possible to follow both the physical remodeling of the larval tracheae as they develop into the adult form and their gain of functionality when they are filled. In this image, the dorsal longitudinal tracheae run the entire length of a white prepupa, connecting the anterior spiracles (AS) with the posterior spiracles (out of the frame of the image). Ten nodes (arrows) are identified that correspond to branch points from which the secondary dorsal branches emerge (Hoshizaki DK, Merkey and Gibbs AG, unpublished).

downregulated in the other. These might be useful in defining mechanisms for reperfusion injury.

In a different microarray experiment, Zhou et al. (University of California, San Diego) compared a hypoxia-resistant line to a control line. They found downregulation of TCA cycle and other metabolic enzymes, consistent with a general reduction of metabolism. The *hairy* binding element was common to downregulated TCA cycle genes. Further evidence for *hairy*'s role in hypoxia tolerance came from the increased hypoxia sensitivity of a *hairy* mutant.

The interest highlighted by the Gas Workshop and the abundance of posters relating to tracheae and respiration are likely the beginnings of a more comprehensive approach in which studies of tracheal development will be integrated with the functional role of this highly efficient gas transport system. Certainly of importance are observations that the tracheal system exhibits plasticity in response to oxygen levels. Larvae reared in hypoxic conditions have wider dorsal main tracheae, thus compensating for low oxygen availability.[11] Surprisingly, these changes are heritable and stable even after two generations of rearing in normoxia. Heritability is the first requirement for a trait to be able to evolve. Indeed, tracheal dimensions may have played a central role in the evolution of insects on earth.[12]

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