



The limits of respiratory function: External and internal constraints on insect gas exchange



Introduction

Insects must obtain sufficient oxygen from their environment in order to satisfy their aerobic requirements. Balancing the risks associated with too little oxygen (asphyxiation) and too much oxygen (poisoning), requires that oxygen supply needs to be matched to oxygen demand. This balancing act needs to be maintained across different levels of activity, across ontogeny and in the face of fluctuations in external conditions such as hypoxia and temperature which affect oxygen supply and demand. In a symposium held at the XXV International Congress of Entomology in Orlando, Florida, in September 2016, researchers presented their work focusing on the common question how the tracheal system of insects can maintain respiratory function in the face of external and internal constraints. The papers presented in this special issue of the *Journal of Insect Physiology*, and the talks delivered at the symposium, testify to the breadth and depth of this topic, with various factors, from environment to molecule and morphology to behavior, influencing an insect's 'limits of respiratory function'.

External constraints on gas exchange are perhaps most easily seen in insects that breathe under water, since breathing water poses some unique challenges compared to breathing air. Jones et al. (in this issue) investigated the performance of the plastron of *Aphelocheirus aestivalis* (Fabricius 1794), one of the classic 'model' species for this type of aquatic gas exchange. Using fiber-optic oxygen probes they were able to measure the PO_2 in the plastron of these aquatic bugs under different temperatures, flow conditions and activity patterns. This allowed them to delineate the conditions under which oxygen supply may become limiting, explaining why these bugs prefer cool, well-oxygenated flowing waters. The diving beetles studied by Verberk et al. (in this issue) can exchange oxygen at the water's surface but can also extract oxygen directly from the water using tracheated hairs on their elytra. Species that relied strongly on these 'setal tracheal gills' preferred cool well-oxygenated flowing waters, just like the aquatic bug *Aphelocheirus aestivalis*, but also showed reduced thermal tolerance and a lower capacity for acclimation, suggesting that this mode of breathing leaves insects more vulnerable to external constraints. Keeping with aquatic insects but focusing on a different constraint, Ubhi and Matthews (in this issue) asked whether aquatic dragonfly nymphs responded differently to hypoxia and hypercapnia compared to their terrestrial, adult counterparts. Water's high capacity for CO_2 but low oxygen solubility mean that ventilation in aquatic animals tends to be driven more by

lack of oxygen than by the need to excrete CO_2 . Ventilatory responses to hypoxia and hypercapnia indeed differed between the aquatic nymphs and the terrestrial adults and matched the expectation that water-breathing nymphs would not respond to hypercapnia. Boardman et al. (in this issue) addressed how responses to multiple stressors may differ from those to single stressors in a terrestrial setting by exposing fruit flies to warm and cold conditions, while also changing the oxygen levels. They found that mortality was greatly aggravated at high temperatures when combined with hypoxia. Global gene expression profiling showed that stressful treatments changed the expression of genes involved in catabolic processes (proteases, hydrolases, peptidases) and membrane function, identifying several important candidate genes for future research.

While the external environment determines the availability of oxygen, it is the conductance of the insect's tracheal system, as determined by its morphology and by behavioural changes in convective ventilation, that determines the rate of oxygen delivery that is available to meet an insect's aerobic demands. As such, insects should vary their ventilation (in the short-term) and tracheal morphology (over development) in response to environmental oxygen levels, as well as to their own aerobic demands. Using fruit flies as a model species, Harrison et al. (this issue) studied how conductance of the tracheal system was modified in response to rearing oxygen. They found compensatory changes in tracheal investment mostly at the level of the small tracheae and tracheoles and much less at the major tracheal trunks. So tracheal conductance matters, but insects are hypothesized to increase tracheal conductance during molts only. In between molts, increases in body size that occur in larval insects, could therefore limit oxygen uptake. Lundquist et al. (in this issue) tested this prediction and demonstrated that the large tracheae changed during molts but did not increase in diameter within the first four instars of the tobacco hornworm. Moreover, HIFs were present at the end of an instar, suggesting that late instars may experience hypoxia which could be a signal for molting. The gas exchange patterns of gregarious brown locusts were recorded by Duncan and Hanrahan (in this issue), who showed that discontinuous gas exchange was a feature of all stages of locust development except the 1st instar, while 4th instar locusts and beyond increased their use of convective ventilation, likely associated with their increasing aerobic demands. In addition to tracheal conductance (i.e. the supply side), mitochondrial networks that meet energy demand (i.e. the demand side) are also dynamic and VandenBrooks et al. (in this issue) compared both systems in fruit flies reared under different oxygen conditions. They demonstrate parallel changes in both tracheal and

mitochondrial investment, across oxygen conditions, with hypoxia having opposite and somewhat stronger effects compared to hyperoxia.

Keeping with fruit flies but taking a molecular approach, [Burmester et al. \(in this issue\)](#) investigated how hemoglobin helps cells to balance asphyxiation risks and risks of oxygen poisoning. Fruit flies have three distinct hemoglobin genes (glob1, glob2 and glob3) and these may differ in their function. Using transfection to overexpress different globin genes in *Drosophila Schneider* 2 (S2) cells, the research team showed that glob1 may help cells to deal with hypoxia, whereas glob2 safeguards the cells from reactive oxygen species during spermatogenesis. The rate at which cells use energy, and hence require oxygen, is strongly related to body mass, yet the reasons for this ubiquitous empirical pattern of metabolic scaling is strongly debated. [Schramm et al. \(in this issue\)](#) set out to investigate why energy use differs with body mass, testing the hypothesis that lower mass-specific metabolic rates in larger organisms reflect disproportionate increases in body components with low metabolic activity, such as storage and skeletal tissues. Across 15 species of carabid beetles, differences in the relative content of metabolically inert components did change with body mass but had only small effects on the allometric scaling of metabolic rate with body mass. Phylogenetic relationships were found to have a strong effects on the mass-scaling exponent, suggesting that metabolic rate is not dictated by body mass alone, but that it is also an ancestral characteristic shared by related species.

The studies published in this special issue, as well as the talks presented at the conference, testify to the great diversity in insect respiratory function. They show how a wide range of environmental and physiological conditions place limits on an insect's respiration, but equally, they demonstrate that the insects are very versatile in circumventing or resolving most of these constraints. Given the enormous biodiversity of this group, much can be learned from studying them.

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