

## Physiology

## A welcome shortage of breath

Thorsten Burmester

The respiratory systems of animals must guarantee an efficient oxygen supply. But it seems that, in some insects, they have evolved to restrict the flow of oxygen too.

Like most other animals, insects need to inhale oxygen and get rid of carbon dioxide. Oxygen fuels energy production in the cells' power plants, the mitochondria, and carbon dioxide is released as a waste product. On page 516 of this issue, Hetz and Bradley<sup>1</sup> show that, contrary to what might be expected, the insect respiratory system may limit rather than assist the uptake of oxygen.

In insects, the exchange of gas with the atmosphere is restricted by a mostly impermeable and inflexible outer layer — the cuticle. Therefore, insects have small openings called spiracles in their cuticle. These are connected to the inner organs by a system of highly branched, gas-filled tubes called tracheae<sup>2</sup> (Fig. 1, overleaf). Oxygen uptake and carbon dioxide release by the cells mainly occur at the tips of the smallest branches. In highly active organs,

such as flight muscle, the tracheal endings can even enter the cells and reach the mitochondria directly.

A common misconception is that the insect's tracheal system is a very inefficient transport pathway. In fact, oxygen and carbon dioxide are respectively delivered about 200,000 times and 10,000 times faster in tracheal air than in the aqueous environment of the blood<sup>2,3</sup>. Therefore, simple diffusion through the tracheae would probably be sufficient to supply adequate oxygen and remove carbon dioxide waste even in the largest insects known historically (for example, the dragonfly *Meganeura monyi*, which lived about 280 million years ago and had a wing-span of 70 cm).

The spiracles in the cuticle behave like valves, opening and closing to allow or restrict the insect's gas exchange. Physiologists have long been puzzled by a peculiar

rhythmic respiratory behaviour referred to as the discontinuous gas-exchange cycle (DGC)<sup>4</sup>. In insects exhibiting DGC, the spiracles close for long periods (up to several hours or even days) and open occasionally for only a few minutes. This unusual respiratory pattern has been observed in many adult insects, as well as in resting butterfly and moth pupae. Two main hypotheses have been proposed to explain why some insects display DGC: to reduce water loss through the spiracles, or to adapt to an underground lifestyle. But these ideas were disproved on closer inspection because DGC could be associated with neither the humidity<sup>5</sup> nor the carbon dioxide concentration<sup>6</sup> of the environment.

Hetz and Bradley<sup>1</sup> propose a different theory to explain DGC, and their hypothesis has far-reaching implications for how we view animal respiration. They provide compelling evidence that insects use DGC not to acquire but to avoid oxygen. Using the pupae of the moth *Attacus atlas* as a model system, the authors varied the environmental oxygen concentrations from partial pressures of 5 to 50 kPa (the normal atmospheric oxygen partial pressure at sea level is about 21 kPa). Nevertheless, the intra-tracheal oxygen levels in the resting pupae remained low, close to 4 kPa, across the whole range of partial

## Atmospheric physics

## Seeing the light

An aurora is not just a visual treat for Earth's latitudinally advantaged residents. These events also provide natural laboratories for physicists investigating the complex interplay of electromagnetic waves and ionized particles in plasmas. And we are not merely passive observers of such phenomena — we have the capability of manipulating these processes from the ground, as shown to striking effect by Todd R. Pedersen and Elizabeth A. Gerken elsewhere in this issue (*Nature* **433**, 498–500; 2005).

The playground for these luminous processes is the ionosphere — the ionized upper reach of the atmosphere that stretches from a height of around 100 km to the base of the magnetosphere far above. In Earth's polar regions, the geometry of the geomagnetic field is such that electrons and ions can occasionally be driven down from on high: an aurora (pictured) is the visual manifestation of the collision of these energetic particles with gases in the upper atmosphere and ionosphere.



With this basic understanding of the mechanism in place, researchers have shown previously that it is possible to induce such optical processes artificially. By pumping high-power radio waves into the ionosphere (the frequency of the waves being tuned to the local plasma environment), electrons can be locally energized to collide with atmospheric gases in a manner analogous to the natural auroral process. But the resulting optical effects are small, with emission intensities falling well below the detection limit of the human eye.

Now Pedersen and Gerken have shown that, if ionospheric conditions are just right, much stronger emissions can be generated by this approach — so strong, in fact, that they are in principle visible to the naked eye. The 'trick' underlying this demonstration was to choose a time when a natural aurora was already active (previous efforts were directed at quiet regions of the ionosphere). And rather than targeting the main ionospheric layer, the researchers tuned the radio waves to excite a much lower layer, which had been transiently ionized

by the inbound charged particles.

These observations raise many questions about the processes involved. For example, is an active aurora really a prerequisite for generating such bright emissions, or is it instead largely a picturesque bystander? Should the answer turn out to be the latter, we are left with the tantalizing (some would say disconcerting) possibility that such radio-fuelled emissions could form the basis of a technology for urban lighting, celestial advertising, and more...

Karl Ziemelis

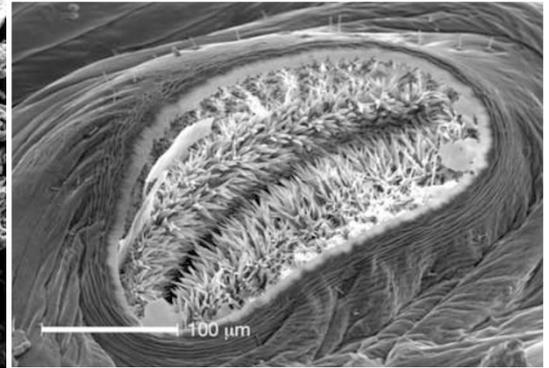
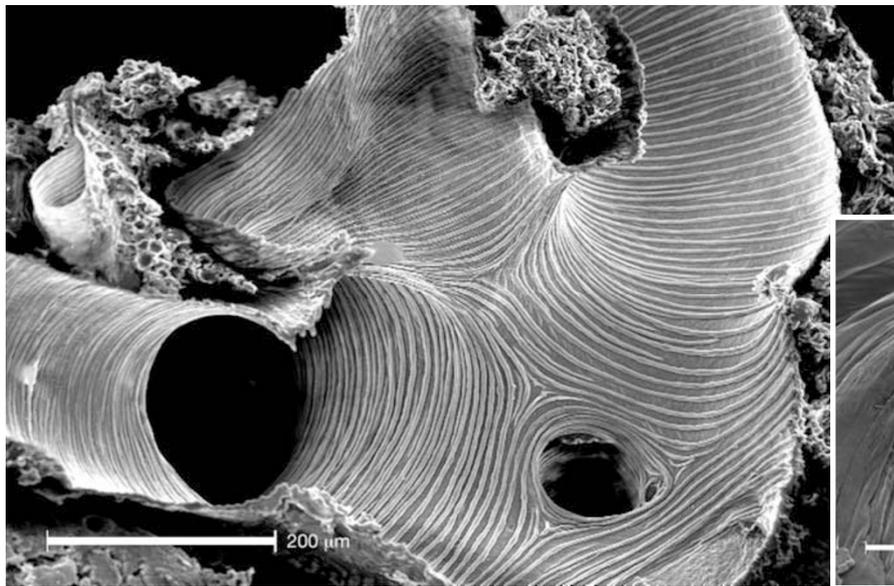


Figure 1 Insect breathing apparatus. Left, the tracheal system of the beetle *Zophobas rugipes*; below, a spiracle of the same species.

pressures. Thus, the moth pupa limits the amount of oxygen taken in by keeping the spiracles closed for as long as possible, and opening them only to get rid of the accumulated carbon dioxide.

At first glance, the idea that an air-breathing animal should try to limit apparently normal oxygen levels seems perplexing. But oxygen is a double-edged sword: although required to fuel energy production, it is also a potent source of toxic compounds known as reactive oxygen species (ROS), which can damage proteins, DNA and lipids<sup>7</sup>. In recent years, ROS have been recognized as a major threat to cell survival, and toxic ROS effects are suggested to underlie ageing and cell death. Therefore, it is advantageous to keep cellular oxygen levels just high enough for efficient mitochondrial respiration, and as low as possible to minimize oxidative damage. Obviously, the critical oxygen concentration in moth pupae is far below the normal atmospheric level of about 21%. This is probably true for other animals too; for instance, quite low oxygen levels (0.4–5 kPa) are also found in mammalian tissues<sup>8</sup>.

But if atmospheric oxygen concentrations are toxic to resting pupae, why aren't they noxious to the insects that rarely or never close their spiracles? The answer probably lies in differences in metabolic activity. The insects' tracheal system is well designed for efficient oxygen supply during periods of high activity, when oxygen never accumulates to critical concentrations in the cell because it is rapidly converted into water by the respiratory chain. However, in periods when respiration falls, such as in the resting butterfly pupa, oxygen consumption is too low to prevent it building up to harmful levels. It seems that a particular breathing pattern, the DGC, has evolved to ensure oxygen homeostasis. ■

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#### RNA interference

## Methylation mystery

Michael Ronemus and Rob Martienssen

Tiny RNA molecules called microRNAs are important in development, and are thought to function by causing the degradation of matching messenger RNAs. That may not be their only mode of action, however.

RNA molecules come in various sizes, ranging from the very long to the very short. The smaller RNAs fall into two major classes: microRNAs (miRNAs), which guide development in an organism by regulating target genes<sup>1</sup>; and small interfering RNAs (siRNAs), which target viruses, inserted genes and mobile genetic elements — a significant function being defence of the genome<sup>2</sup>. One way in which siRNAs work is by guiding the modification (by methylation) of DNA strands, as well as the modification of the histone proteins around which DNA is wrapped, thus silencing gene expression<sup>3</sup>. Writing in *Developmental Cell*, Bao *et al.*<sup>4</sup> suggest that miRNAs might also — contrary to expectation — contribute to DNA methylation.

At a casual glance, miRNAs and siRNAs don't seem all that different. Both are short, single-stranded RNA molecules, generally 21–22 nucleotides in size. Both are processed from true or transiently double-stranded precursor RNA molecules, by specialized enzymes called Dicers. And both programme the activity of Argonaute proteins in RNA-

induced silencing complexes (RISCs)<sup>5</sup>.

But look more closely, and it seems that any similarity is nothing more than a consequence of an ancient common origin. A miRNA precursor originates from a non-protein-coding gene and is processed in a multi-step pathway that is coupled to its export from the cell nucleus. Once channelled to the cytoplasm, the miRNA interacts with a target messenger RNA (mRNA), via the RISC, to trigger the destruction of the mRNA, or to prevent it from being translated into protein<sup>1</sup>. This, then, effectively silences the gene that encodes the mRNA.

In contrast, the precursor of an siRNA is any double-stranded RNA molecule, a feature that allows for multiplication of the original siRNA through reiterative dicing and synthesis<sup>6</sup>. siRNAs target complementary RNAs for degradation and also influence the modification of repetitive DNA and the proteins that package it. Adding to the differences between the two RNAs, certain miRNA sequences are conserved throughout the animal kingdom or between distantly related plant species, whereas siRNA