

Respiratory Physiology: Strange Cycles and the Fruit-Fly's Tongue

Drosophila in flight show an unexpected cyclicity in gas exchange even at constant metabolic flux rates — perhaps because regular proboscis extensions assist in providing oxygen to the fly's brain.

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It is considered bad form for an author to invite his readers to kill themselves, especially in the first paragraph. So I won't suggest that you hold your breath for several minutes while meditating on the self-evident importance of respiratory gas exchange. Consider, however, that you, gentle reader, generate just $1 \text{ J s}^{-1} \text{ kg}^{-1}$ of metabolic flux; a flying insect's fire of life rages at up to six hundred times that figure [1]. How can such respiratory gas exchange rates be supported?

The answer, in part, lies in the most fundamental difference between vertebrate and insect gas exchange. Insects get their oxygen and eliminate their carbon dioxide wholesale. The costly, if colorful agent, hemoglobin, never enters the picture, and neither does the costly infrastructure, such as high-performance plumbing, required for its efficient transportation. Rather, air enters directly through spiracles into the tracheal system, which ramifies throughout the body with such obsessive thoroughness that no oxygen-hungry mitochondrion is more than a few microns away from a quick oxygen fix, plus an easy way to dump carbon dioxide.

Not so fast. Carbon dioxide and water vapor find the tracheal system a most excellent avenue of escape. The surface area to volume ratio of an insect is immense compared to that of most terrestrial vertebrates, making conservation of body water reserves a priority second only to exchanging respiratory gases. Accordingly, the insect exoskeleton has evolved remarkably low permeability to water vapor [2]. Respiratory water loss, in contrast, is controlled by the spiracles, the gateways

between the rude, dry external world and the insect's delicate, water-saturated *milieu interieur*. Thus an uneasy balance exists in all insects between efficient gas exchange and effective water conservation. The balancing act is even trickier for flying insects, as we shall see.

In most insects, and especially in small insects such as *Drosophila*, diffusion alone is sufficient to meet *all* gas exchange demands [3], and insects were thought to modulate spiracular area to accomplish a steady-state rate of gas exchange while minimizing loss of water vapor. The advent of sensitive gas analyzers showed that this was an oversimplification. It turns out that insects at rest orchestrate their spiracles' openings and closings in cunning ways, some showing rather dramatic patterns in response to the water/oxygen dilemma [4–7]. A single gas exchange cycle in an ant, for example, may last up to 20 minutes; a tick may 'hold its breath' for over an hour [5,6]. Cyclicities in external gas exchange, in any event, are now expected of insects at rest.

The kinetics of gas exchange in active insects are less well understood. We know that cyclicity is disrupted during activities such as terrestrial locomotion [5,6]. What about flight, during which oxygen demand is so extreme? Peter Miller and his Cambridge colleague Torkel Weis-Fogh studied gas exchange during flight in the plague locust *Schistocerca gregaria* (locust research in England at the time was coordinated not by a Locust Research Center, as might be expected, but by an *Anti-Locust* Research Center, a title that protected the participating

acridologists from charges of fraternizing with the enemy). Prior to that, observations were made of abdominal ventilation during insect flight, but Miller [8] and Weis-Fogh [9,10] showed that the flight motor was autoventilated by its own mechanical movements, which pulled air into and out of the thoracic spiracles adjacent to the flight motor. This tidal autoventilation was supplemented by unidirectional ventilation using abdominal contractions [8–10]. Lutz Wasserthal [11] later demonstrated an even more efficient, unidirectional air flow powered by autoventilation in the hawkmoth.

Whatever the particulars of gas exchange during flight, because of the mechanics of autoventilation it was now clear that wingbeat frequency was the primary determinant of airborne gas exchange kinetics. This is not to say that gas exchange is constant during flight; it can be modulated, for example, by varying the demand on the flight motor of *Drosophila* in a virtual reality arena [12]. But in a state of constant energy flux through the flight motor, it was assumed that the spiracles would remain open at close to their maximum conductance and thus, that the rate of CO₂ production would accurately parallel the energy flux rate of the flight motor, and, moreover, that this truism would hold in all flying insects.

Nothing, in my politically incorrect opinion, benefits a flock of pigeons more than a thrown cat. Using water vapor as a tracer of the conductance of the spiracles [13], Fritz Lehmann [14] showed that the consensus picture was an oversimplification. *Drosophila*, it turned out, is capable of modulating spiracular conductance during flight to minimize respiratory water loss rates. This was a novel and useful discovery. The field of insect respiratory physiology was not exactly in ferment, given that it has perhaps a dozen principal players scattered around the world, most of whom are perceived as relatively sane and decorous. But

from these troubled if shallow waters a further unexpected development has emerged.

Fritz Lehmann's latest paper [15], in collaboration with Nicole Heymann, demonstrates that flying *Drosophila* exhibit a cyclic variation of CO₂ output lasting about three seconds, even under constant flight motor load. That's about 600 wing beats per cycle – vastly in excess of anything autoventilatory in nature. Frankly, to anyone not looking specifically for periodic variations in CO₂ output while using a system tuned for fast response, these fluctuations would have been averaged out of existence. It is natural to object that these (relatively) slow oscillations simply reflect variations in flight motor energy flux, but the accompanying kinematic observations rule that out.

Obviously the cyclicity derived from patterns of abdominal pulsations? Lehmann and Heymann [15] decorated their flies' abdomens with tiny spots of paint, the better to track pulsations by video, but found no correlation between the abdominal pulsations and the CO₂ cycles. Next they modeled *Drosophila*'s four large thoracic spiracles as entities that opened or constricted independently in accordance with local gas levels.

Plainly one spiracle's activity could affect that of others, because it could alter gas concentrations in their vicinities to levels above or below the setpoints at which they would open or close. If the spiracles drifted into phase with each other while open, a peak of CO₂ emission occurred; if they drifted out of phase, a valley. Lehmann and Heymann [15] found that at certain specific ratios of spiracle threshold values to muscle partial pressures of respiratory gases, computer simulations produced results quite similar to their observations, but as they are quick to point out, their models make assumptions that are not yet proven and should be treated with caution.

Another, more charming, possibility exists for explaining the observed cyclicity. *Drosophila* stick out their probosces at

intervals during tethered flight, something that everyone who has worked with the venerable fly-on-a-stick preparation had noticed but none had investigated. These regular proboscis extensions prove to be highly correlated with the CO₂ cyclicity and, according to Lehmann and Heymann [15], may act to 'balance the local oxygen supply between different body compartments of the flying animal'.

Perhaps the fly brain needs additional oxygen during flight, for which the fruit fly's evolutionary history constrains it to stick out its tongue at intervals, the better to give its flight motor an intelligently chosen course. There is a moral here somewhere.

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Microtubule Flux: What Is It Good for?

During mitosis in a eukaryotic cell, microtubule subunits continuously move towards spindle poles. A new study has revealed that inhibiting this microtubule flux in mammalian cells has no major effects on chromosome movements; it does, however, increase the probability of erroneous chromosome segregation.

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The goal of mitosis is to segregate replicated chromosomes equally between the two daughter cells. This process must be reasonably fast and extremely efficient. Errors in chromosome partitioning are irreversible and aneuploidy is a hallmark of malignant transformation. To achieve the

requisite error-free chromosome segregation, eukaryotic cells have developed a marvelous self-organizing molecular machine called the mitotic spindle.

The microtubule-based mitotic spindle is highly dynamic in nature. Chromosome movement is accompanied and coordinated with changes in length of the 'K-fibers' that connect chromosomes to the spindle poles. In the