

INSIDE JEB

Breath-holding locusts don't keep spiracles open when they exhale



A locust with EMG electrodes. Photo credit: Stav Talal.

For animals equipped with a pair of lungs and a circulatory system to deliver oxygen throughout the body, inhalation is a relatively straightforward process. However, insects depend on a mixture of gas diffusion and active pumping by muscles to drive airflow through the intricate network of air tubes that plumb their bodies. One specific insect breathing pattern, discontinuous gas exchange, has fascinated scientists for decades: Stav Talal from Tel Aviv University, Israel, explains that butterfly pupae hold their breath for hours at a time, exhaling CO₂ intermittently while waiting out winter (diapausing). The airflow is regulated by the spiracles – pores where the trachea (ventilation tubes) reach the surface of the body – which open for periods of several minutes to release CO₂. However, it was becoming clear that other resting insects may use modified versions of the intermittent breathing pattern and no one had confirmed that the spiracles remained continually open as the insects exhaled: ‘It is often assumed, based on CO₂ emission traces’, says Eran Grefen

from the University of Haifa-Oranim, Israel. Explaining that it is more than six decades since anyone directly monitored spiracle opening while an insect was breathing using discontinuous gas exchange, Talal and his supervisors, Grefen and Amir Ayali, also from Tel Aviv University, decided to investigate the spiracle opening and closing patterns of resting locusts.

‘At the beginning, it was a great challenge, because nobody had ever combined respirometry with EMG recording from respiratory muscles in such small animals’, says Talal, describing how it took a year to devise a technique where he could gently insert an EMG electrode into the tiny muscle that controls spiracle opening through a minute hole in the insect’s exoskeleton. He also attached electrodes to the abdominal muscles that pump air through the resting locust’s body, to record when they were active, before placing the locust in an enclosed dark chamber where he could monitor the CO₂ produced by the insect as he varied the

oxygen content of the air between 40% and 5%.

However, when the team compared the CO₂ emission trace with the spiracle muscle activity, they were astonished to find that instead of remaining open while the locust exhaled CO₂, the spiracle was opening and closing rapidly in time with the abdominal muscles, which pump air from the front of the body to the rear. ‘Throughout our experiments, this full synchrony was never broken’, says Ayali. ‘[Discontinuous gas exchange] involves not only spiracle action but also ventilation, which is tightly coupled to the spiracle activity’, says Talal; ‘It is more complicated than we thought’, he adds. The trio also suspects that a CO₂ sensor triggers rapid opening and closing of the spiracle that accompanies CO₂ release and they say, ‘Our results are consistent with previous studies in suggesting a dominant role for CO₂ sensing’.

Having discovered that the locust discontinuous gas exchange pattern is so different from that of butterfly pupae, Talal says, ‘This means that the dominant adaptive hypotheses for the evolution and the maintenance of discontinuous gas exchange ... are not relevant to locust discontinuous gas exchange’, and he warns, ‘As a classic discontinuous gas exchange model, the pupa is a unique case and not a good representative of insect discontinuous gas exchange’.

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