A test of the oxidative damage hypothesis for discontinuous gas exchange in the locust *Locusta migratoria*

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The discontinuous gas exchange cycle (DGC) is a breathing pattern displayed by many insects, characterized by periodic breath-holding and intermittently low tracheal O₂ levels. It has been hypothesized that the adaptive value of DGCs is to reduce oxidative damage, with low tracheal O₂ partial pressures (P₂ ~2–5 kPa) occurring to reduce the production of oxygen free radicals. If this is so, insects displaying DGCs should continue to actively defend a low tracheal P₂ even when breathing higher than atmospheric levels of oxygen (hyperoxia). This behaviour has been observed in moth pupae exposed to ambient P₂ up to 50 kPa. To test this observation in adult insects, we implanted fibre-optic oxygen optodes within the tracheal systems of adult migratory locusts *Locusta migratoria* exposed to normoxia, hypoxia and hyperoxia. In normoxic and hypoxic atmospheres, the minimum tracheal P₂ that occurred during DGCs varied between 3.4 and 1.2 kPa. In hyperoxia up to 40.5 kPa, the minimum tracheal P₂ achieved during a DGC exceeded 30 kPa, increasing with ambient levels. These results are consistent with a respiratory control mechanism that functions to satisfy O₂ requirements by maintaining P₂ above a critical level, not defend against high levels of O₂.

**Keywords:** discontinuous gas exchange; insect; hyperoxia

1. INTRODUCTION

Many insects at rest breathe using the discontinuous gas exchange cycle (DGC): an alternating pattern of gas exchange and breath-holding. This behaviour is known to occur among at least five [1], and possibly as many as seven [2], insect orders. It is characterized by the sequential repetition of three phases: a ‘closed phase’ when the spiracles are shut and tracheal P₂ drops continuously while PCO₂ rises; a ‘flutter phase’ when the spiracles rapidly open and close releasing minimal CO₂ while admitting some O₂ to the tracheal system; and an ‘open phase’ when both CO₂ and O₂ move freely between the atmosphere and tracheal system. Large fluctuations in tracheal PO₂, varying from near atmospheric (approx. 20 kPa) to severely hypoxic (2–5 kPa), are a peculiar characteristic of this pattern. The potential adaptive significance of the DGC has led to much debate, with suggestions that it is adaptive to reduce oxidative damage (oxidative damage hypothesis [3]), to reduce respiratory water loss (hygic hypothesis, [4]), or as a consequence of neural downregulation affecting respiratory control (neural hypothesis, [5]). The hygic and neural hypotheses both assume that the low tracheal PO₂ during the closed and flutter phases occurs inadvertently, either as a consequence of protracted spiracle closure reducing respiratory water loss, or due to a change in the behaviour of the insect’s respiratory control system. The oxidative damage hypothesis is based on the assumption that the DGCs primary function is the production of a low tracheal PO₂ during the closed phase that is then maintained during the flutter phase [3,6–8].

Oxygen is a toxic molecule, capable of damaging tissues through the production of reactive oxygen species (ROS) [9]. According to the oxidative damage hypothesis, DGCs evolved as a mechanism to reduce tracheal PO₂ periodically when the insect’s metabolic rate is low, thereby lowering ROS production. The respiratory behaviour of moth pupae supports this idea, as they regulate their tracheal PO₂ at approximately 5 kPa during the flutter phase of their DGC, even in hyperoxic atmospheres up to 50 kPa [3]. While the hygic and neural hypotheses do not require the maintenance of a low P₂ during DGCs, the question remains: is a low and constant tracheal P₂ during the flutter phase a universal feature of DGCs displayed by adult insects? Pupae are physiologically atypical, as they represent a life stage characterized not only by a very low metabolic rate [10], but also reduced neurological functions [11].

To determine whether tracheal P₂ during the flutter phase of a DGC is independent of ambient P₂ in adult insects, we implanted fibre-optic oxygen optodes into the tracheal systems of adult locusts exposed to hyperoxic, normoxic and hypoxic atmospheres.

2. MATERIAL AND METHODS

Adult migratory locusts *Locusta migratoria* (810 ± 72 mg) were reared at the University of Adelaide. Locusts were maintained in plastic terraria, at 33 ± 1°C, approximately 30 per cent RH, 12 L:12 D cycle and ad libitum food. They were fasted 24 h prior to gas exchange measurements and weighed to 0.1 mg (AE163, Mettler, Greifensee, Switzerland) immediately before each experiment. Flow-through respirometry was performed at 22–23°C using a LI-820 CO₂ analyser (Li-COR Biosciences, Lincoln, NE, USA) and gas mixes produced by mass flow controllers (Aalborg 0–500 ml min⁻¹ and 0–1000 ml min⁻¹) connected to cylinders of compressed O₂ and N₂. Gas mix composition was verified using a calibrated OXzilla II oxygen analyser (Sable Systems, Nevada, USA).

Two groups of locusts were measured: a control group and a treatment group. Individuals in the control group were placed within 10 ml syringe barrels and gas exchange was measured over a 2–8 h period following 1 h of acclimation. They were measured in normoxia (n = 9) using outside air scrubbed of CO₂ and water vapour, as well as hypoxia (7 kPa; n = 4) and hyperoxia (40 kPa; n = 4). Briefly, the gas mix was regulated at 350 ml min⁻¹ using a mass flow controller, passed through the syringe containing the rest-ing locust, through a small column of desiccant, and into the CO₂ analyser. The analogue outputs of the mass flow controller and CO₂ analyser were recorded at 1 s intervals to a computer with a
for all treatments was 29.8 min. The only significant effect of ambient $P_{O_2}$ on mean DGC duration was found between 21.3 kPa (33.2 ± 4.1 min) and 40.5 kPa $P_{O_2}$ (18.3 ± 2.4 min; $p < 0.05$). The reduced duration in 40.5 kPa is attributable to a decrease in the closed and open durations and the virtual elimination of the flutter phase (table 1 and figure 2). The lowest tracheal $P_{O_2}$ during a DGC occurred at the transition from the closed to the flutter phase (figure 1). This minimum $P_{O_2}$ varied between 3.41 ± 0.43 kPa in normoxia, dropping slightly to 3.13 ± 1.52 and 1.21 ± 0.22 kPa in atmospheres between 15.2 and 5.1 kPa, respectively (figure 1). In hyperoxia, however, the minimum tracheal $P_{O_2}$ increased with ambient $P_{O_2}$ with a slope of 1.38, rising to 17.66 ± 1.56 kPa in 30.4 kPa $O_2$, and 30.10 ± 1.21 kPa in 40.5 kPa $O_2$ (figure 1).

4. DISCUSSION

The minimum tracheal $P_{O_2}$ in adult locusts displaying DGCs is dependent on ambient $P_{O_2}$ in hyperoxia (figure 1). This is in contrast to a tracheal $P_{O_2}$ maintained at low levels in hyperoxia, as expected by the oxidative damage hypothesis [3]. Why then do moth pupae display a consistently low tracheal $P_{O_2}$ in hyperoxia during their flutter phase, when locusts do not? The answer lies in the relative tracheal and body fluid volumes of the two insects. Current research indicates that spiracles flutter open and closed in response to internal hypoxia and open fully in response to high $CO_2$ [12,13]. Owing to its high solubility, most of the $CO_2$ produced during the closed and flutter phases of the DGC is sequestered in the insect’s body fluids, while the bulk of the $O_2$ used during the closed phase is obtained from air in the tracheal system [14,15]. Therefore, a flutter phase and its associated low and stable $P_{O_2}$ can occur only if the tracheal $O_2$ store is depleted to a hypoxic threshold before $CO_2$ accumulation in the body fluids initiates an open phase. This is determined by the ratio of the insect’s $O_2$ store (tracheal system volume) to its $CO_2$ sink (body fluid volume and buffer capacity).

Moth pupae possess a tracheal system that contains approximately 140 μl of air per gram of body mass, while a locust’s tracheal system holds approximately 310 μl g$^{-1}$ (for references, see the electronic supplementary material). As such, moth pupae have approximately half the volume of $O_2$ on which to draw during the closed phase of their DGC relative to their $CO_2$ sink (body fluid volume; proportional to mass) compared with a locust. Additionally, moth pupae have higher whole body buffer capacity (75 mmol HCO$3_-$ pH$^{-1}$ kg$^{-1}$) than those of a locust (28–43 mmol HCO$3_-$ pH$^{-1}$ kg$^{-1}$; electronic supplementary material) and, therefore, have a greater capacity to store $CO_2$ relative to pH change. These differences mean that a pupa in hyperoxia can deplete its tracheal $O_2$ reserve to approximately 5 kPa during the closed phase of its DGC and transition to the hypoxia-initiated flutter phase before it has accumulated sufficient $CO_2$ to initiate an open phase. This accounts for the consistently low tracheal $P_{O_2}$ displayed by moth pupae in hyperoxic atmospheres. In contrast, a locust’s substantial $O_2$ reserve in hyperoxia

3. RESULTS

Locusts displayed both continuous and discontinuous gas exchange patterns. During continuous ventilation, tracheal $P_{O_2}$ matched ambient levels from hypoxia across hyperoxia, increasing with a slope of 0.94 (figure 1). There was no significant difference between mean DGC duration of optode-implanted and control locusts ($p > 0.05$). The overall mean DGC duration

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**Table 1.** Mean DGC duration (min) during exposure to hyperoxia.

<table>
<thead>
<tr>
<th>$P_{O_2}$ (kPa)</th>
<th>Mean DGC duration (min)</th>
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<tbody>
<tr>
<td>5.1</td>
<td>22.1 ± 3.2</td>
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<tr>
<td>10.1</td>
<td>20.3 ± 2.1</td>
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<tr>
<td>15.2</td>
<td>18.5 ± 1.8</td>
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<tr>
<td>21.3</td>
<td>16.7 ± 1.5</td>
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<tr>
<td>30.4</td>
<td>14.9 ± 1.3</td>
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<tr>
<td>40.5</td>
<td>13.1 ± 1.1</td>
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**Figure 1.** Intratracheal $P_{O_2}$ (± 95% CI) in locusts breathing continuously (open circles) and the minimum tracheal $P_{O_2}$ in locusts displaying DGCs (filled circles) during exposure to hypoxia and hyperoxia. Tracheal $P_{O_2}$ in *Atticus atlas* pupa during the flutter phase (filled diamonds) are included for comparison (data from Hetz & Bradley [3]). The solid black line indicates unity.
means that its tracheal $PO_2$ cannot drop to the hypoxic flutter threshold during the closed phase before excessive accumulation of $CO_2$ initiates an open phase. This ensures that the locust’s tracheal $PO_2$ remains close to ambient levels (figures 1 and 2). We conclude that the apparent independence of tracheal $PO_2$ in moth pupae can be explained as the by-product of a comparatively small tracheal volume coupled with a large $CO_2$ buffer, and the interaction between a hypoxic threshold initiating the flutter phase and a high $CO_2$ threshold initiating the open phase.

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