

## OXYGEN-SENSITIVE FLIGHT METABOLISM IN THE DRAGONFLY *ERYTHEMIS SIMPLICICOLLIS*

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Accepted 6 March; published on WWW 12 May 1998

### Summary

**Insect flight metabolism is completely aerobic, and insect resting metabolism is quite insensitive to atmospheric oxygen level, suggesting a large safety margin in the capacity of the tracheal system to deliver oxygen during flight. We tested the sensitivity of flight initiation and metabolism to atmospheric oxygen level in the libellulid dragonfly *Erythemis (Mesothemis) simplicicollis* using flow-through respirometric measurements of the rate of CO<sub>2</sub> emission ( $\dot{V}_{CO_2}$ ). Flight initiations were unimpaired in atmospheric oxygen levels as low as 10 %. However, flight metabolic rate was affected by ambient oxygen level. Flight  $\dot{V}_{CO_2}$  decreased in hypoxic mixtures (5 kPa or 10 kPa oxygen) and increased in hyperoxic atmospheres (30 kPa or 50 kPa oxygen), suggesting that ambient oxygen level**

**influences flight muscle oxygen partial pressure ( $P_{O_2}$ ) and the vigour of flight. These are the first data to show oxygen-limitation of flight metabolism in a free-flying insect. A low safety margin for oxygen delivery during dragonfly flight is consistent with a previous hypothesis that atmospheric hyperoxia facilitated gigantism in Paleozoic protodonates. However, allometric studies of tracheal morphology, and mechanisms and capacity of gas exchange in extant insects are necessary in order to test the hypothesis that the oxygen-sensitivity of aerobic metabolism increases with body size in insects.**

Key words: flight, metabolism, atmospheric composition, dragonfly, *Erythemis simplicicollis*, oxygen.

### Introduction

The tracheal system allows flying insects to achieve metabolic rates during flight that are among the highest in the animal kingdom. Because oxygen transport in tracheal systems occurs primarily in the gas phase, the ability of the tracheal system to deliver oxygen is generally considered to be in excess of the capacity of the tissues to consume oxygen. Evidence supporting this position includes the following: (1) the resting metabolism of insects is unaffected by decreasing the atmospheric content of oxygen to 5 kPa (Keister and Buck, 1964); (2) insect flight metabolism is believed to be completely aerobic (Kammer and Heinrich, 1978); and (3) studies of tracheal morphology support the notion that tracheolar diffusion can supply oxygen at a rate sufficient for flight (Weis-Fogh, 1964). However, direct tests of the safety margin of oxygen supply in flying insects are few and conflicting. During continuous, tethered flight, the rates of oxygen consumption of the blowfly *Lucilia sericata* and the fruitfly *Drosophila repleta* both decreased strongly with moderate (10 kPa) hypoxia; hyperoxia had no effect (Davis and Fraenkel, 1940; Chadwick and Gilmour, 1940). During free hovering flight, the metabolic rate of honeybees *Apis mellifera* is unaffected by variation in atmospheric oxygen content between 10 and 39 kPa (Joos *et al.* 1997). In the present study, we used flow-through respirometric measurements of the rate of carbon dioxide emission ( $\dot{V}_{CO_2}$ ) to

study the effect of atmospheric oxygen concentration on flight behaviour and metabolic rate in the libellulid dragonfly *Erythemis (Mesothemis) simplicicollis*.

The oxygen-sensitivity of the flight metabolism of dragonflies is of particular interest because of the well-known late Paleozoic gigantism of the related and morphologically similar Protodonata (Carpenter, 1943, 1947; May, 1982). Recent geological models suggest that the atmospheres of the late Paleozoic contained up to 35% oxygen (Berner and Canfield, 1989). Graham *et al.* (1995) have hypothesized that the increased atmospheric partial pressure of oxygen during the late Paleozoic may have permitted the evolution of larger insects because the increased gradient driving diffusion could allow adequate oxygen transport over greater distances. However, if the capacity of the tracheal system of modern insects to deliver oxygen far exceeds the capacity of the tissues to consume oxygen, it is difficult to argue that elevations in atmospheric oxygen levels today or in the past would enable an increase in insect size.

### Materials and methods

#### Animals

We studied *Erythemis simplicicollis* Say at the Desert

Studies Consortium Research Station (Zzyzx, CA, USA; latitude 35° 08' 34" N, longitude 116° 06' 14" W). At this site in the East Mohave desert, dragonflies commonly perch, mate and oviposit in several small ponds. This site has been described previously, and the thermal biology of *E. simplicicollis* has been studied in some detail (Polcyn, 1994). Dragonflies were captured from perches or in flight along pond edges, with an insect net, and quickly transferred to the respirometry apparatus, which was located outside under a shaded ramada (ambient temperature 32.1±0.88 °C, mean ± S.E.M., N=25).

#### Protocols and manipulation of atmospheric oxygen level

Animals were placed in a 21 clear plastic respirometry chamber, which was then flushed with the test gas. We mixed gases of varying oxygen and nitrogen concentration by regulating the flow from nitrogen and oxygen tanks; excurrent oxygen level from the chamber was measured with an Ametek (Pittsburg, PA, USA) S3A oxygen analyzer to an accuracy of ±0.3%. The dragonflies sat quietly on the bottom of the chamber when undisturbed. We stimulated the animals to fly by gently shaking the chamber for 1–2 s. After the disturbance, animals flew for varying periods (range 4–23 s, mean 8 s), which we timed using a stopwatch (±0.1 s).

To control for changes in animal motivation or physiological state with time, we flew each animal in a normoxic atmosphere (21 kPa oxygen, 79 kPa nitrogen) before and after flight in each test gas. Each animal experienced all test gases. The test gases used were 5, 10, 30 and 50 kPa oxygen, balance nitrogen (all atmospheres were normobaric). Half of the animals were exposed to test gases which increased in oxygen level (test gas order: 5, 10, 30, 50 kPa oxygen), while the other half were exposed to test gases which decreased in oxygen concentration. Sample sizes were 18–20 animals for each test gas. Statistical analyses were performed using SYSTAT, version 5.2, SYSTAT Inc., Evanston, IL, USA.

#### Flight behaviour

Flight behaviour was quite variable, with some animals exhibiting stationary hovering, most flying repeatedly into the sides of the chamber and others unable to fly. For this study, we simply rated flight behaviour as able to fly or unable to fly (unable to lift the abdomen off the substratum). Data are reported for the 13 individuals for which ratings were made for all test atmospheres.

#### Thoracic temperature

Thoracic temperatures were measured at the end of respirometry using a Physitemp (Clifton, NJ, USA) MT-29/1 hypodermic microprobe (time constant 0.025 s) and a BAT-1 thermocouple thermometer. All dragonflies had been most recently flown in a 21 kPa oxygen atmosphere. Data are reported for the 13 individuals for which thoracic temperatures were obtained within 10 s of opening the respirometry chamber.

#### Measurement of flight $\dot{V}_{CO_2}$

We measured the carbon dioxide fraction of the dehydrated

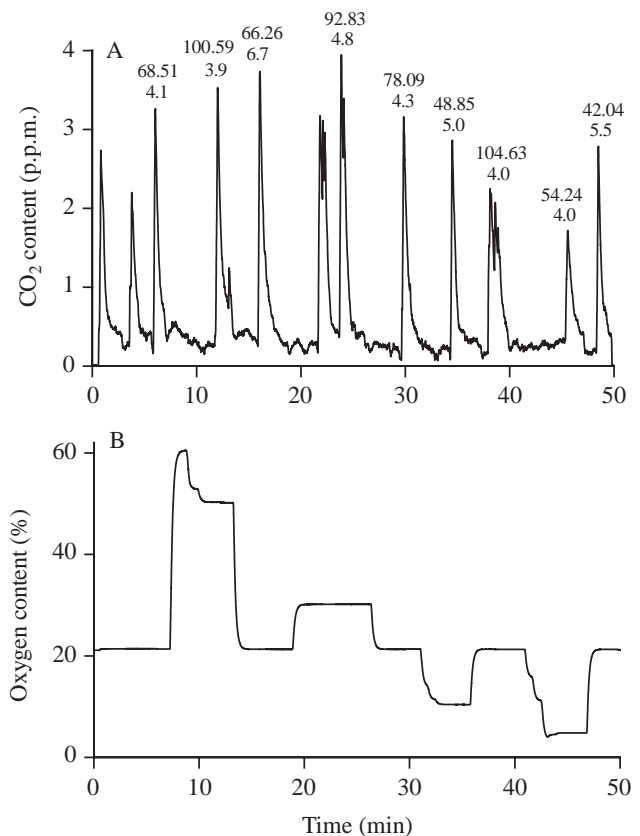


Fig. 1. The carbon dioxide content of excurrent air from the respirometry chamber (A) at varying atmospheric oxygen levels (B) for an individual *Erythemis simplicicollis*. In A, the upper inset numbers indicate  $\dot{V}_{CO_2}$  (in ml g<sup>-1</sup> h<sup>-1</sup>) and the lower inset numbers indicate the hovering time (in s) during the flight bout. Note that for this and some other animals, there were occasional spontaneous bouts of flight for which we did not obtain hovering times or metabolic rates.

excurrent air from the respirometry chamber using a LICOR (Lincoln, NE, USA) 6251 CO<sub>2</sub> analyzer, with the analog output digitized and analyzed with Sable Systems (Las Vegas, NV, USA) Datacan V software and hardware. Flow rates (mean 14.41 min<sup>-1</sup>) were measured using an Omega (Stamford, CT, USA) FMA-5613 mass flow meter. These high flow rates promoted rapid chamber flushing, so that each flight or attempted flight bout was accompanied by a distinct peak in CO<sub>2</sub> emission (Fig. 1). Metabolic rate during flight or attempted flight was high relative to periods when the animal was quiescent in the respirometry chamber (Fig. 1), so it was possible to calculate the amount of CO<sub>2</sub> evolved during the flight period from the integrated area under each CO<sub>2</sub> emission peak, subtracting the baseline 'resting' CO<sub>2</sub> emission. Carbon dioxide emission rates during flight or attempted flight ( $\dot{V}_{CO_2}$ , ml g<sup>-1</sup> h<sup>-1</sup>) were determined from the fraction of carbon dioxide in the excurrent air and the hovering time for each animal (Fig. 1). We changed the gas flowing through a chamber after the dragonfly had landed, but not always after the CO<sub>2</sub> washout had been completed (Fig. 1). However, since

the flow rate through the chamber was constant, this had no effect on the shape of the CO<sub>2</sub> washout, allowing us to proceed as rapidly as possible through the experimental sequence for each animal.

#### Respiratory exchange ratios and muscle-mass-specific metabolic rates

We calculated whole-body and flight-muscle mass-specific metabolic rate under normoxic conditions from the respiratory exchange ratio, the body mass and the flight muscle mass of each individual. Respiratory exchange ratios were measured using closed-system respirometry in normal air. The 21 chamber was flushed for 2 min at 101 min<sup>-1</sup>, initial oxygen and carbon dioxide fractions were measured, and the chamber was sealed. Animals were then agitated to fly for approximately 10 min, during which flight was sporadic. We then collected chamber gases using a 60 ml syringe and measured post-flight oxygen and carbon dioxide gas fractions by injecting the dehydrated gas sample sequentially through the carbon dioxide and oxygen analyzers. Carbon dioxide production rates were converted to watts assuming carbohydrate catabolism, since our respiratory exchange ratios did not differ significantly from 1.0 (see Results). Flight muscle mass was estimated from the masses of thoraxes before and after NaOH digestion (Marden, 1989).

## Results

### Flight behaviour and thoracic temperatures

Flight behaviour in normoxic atmospheres was vigorous, as indicated by the high thoracic temperatures (40±0.8 °C, mean ± S.E.M., N=12), which were similar to those observed for this species in free flight (Polcyn, 1994). In the five trials in normoxic atmospheres, 83–100 % of the dragonflies exhibited successful flight, and the ability to fly did not vary among the five trials in 21 kPa oxygen atmospheres (Cochran's *Q*-test, *Q*<sub>4</sub>=2.5, *P*>0.5; Sokal and Rohlf, 1995), suggesting that flight behaviour did not degrade with time in the respirometry system.

Atmospheric oxygen level significantly affected flight behaviour in *E. simplicicollis* (Fig. 2; Cochran's *Q*-test, *Q*<sub>4</sub>=30.78, *P*<0.001). In atmospheres containing 10 kPa oxygen or greater, at least 80 % of the animals hovered successfully, while only 30 % could sustain flight in 5 kPa oxygen.

### Atmospheric oxygen level and flight $\dot{V}_{CO_2}$

Flight metabolic rates of dragonflies in 21 kPa *P*O<sub>2</sub> atmospheres did not vary significantly with time in the respirometry chamber (repeated-measures analysis of variance, ANOVA, *F*<sub>4,56</sub>=1.30, *P*=0.28), and  $\dot{V}_{CO_2}$  was not correlated with flight duration (*r*<sup>2</sup>=0.11). Also, metabolic rates at the four test atmospheres did not differ significantly between the group that experienced ascending oxygen levels and the group that experienced descending oxygen levels (ANOVA, all values of *P*>0.25). Atmospheric oxygen level strongly affected  $\dot{V}_{CO_2}$  during flight or attempted flight (Fig. 3A, Huynh-Feldt

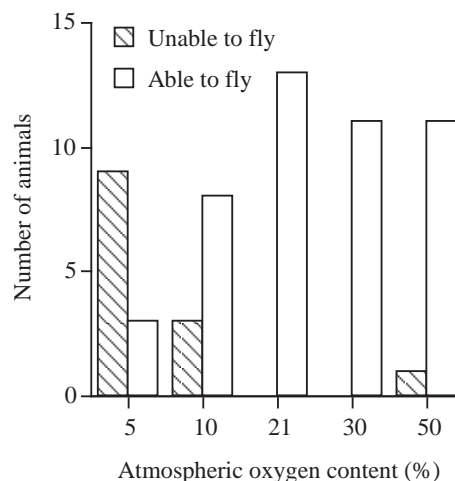


Fig. 2. The effect of atmospheric oxygen concentration on the flight behaviour of *Erythemis simplicicollis*.

corrected repeated-measures ANOVA, *F*<sub>4,64</sub>=3.49, *P*=0.03). There was a significant linear effect of atmospheric oxygen level on flight or attempted flight  $\dot{V}_{CO_2}$  (linear single degree-of-freedom test of order, *F*<sub>1,16</sub>=5.49, *P*=0.032). When flight or attempted flight  $\dot{V}_{CO_2}$  values in each test gas were compared with the mean of their normoxic controls (Fig. 3B), both hypoxic gas mixtures were associated with significantly lower  $\dot{V}_{CO_2}$  values (*post-hoc* comparisons, repeated-measures ANOVA, *P*<0.05) while both hyperoxic gas mixtures were associated with significantly elevated  $\dot{V}_{CO_2}$  values (*post-hoc* comparisons, repeated-measures ANOVA, *P*<0.05). Flight or attempted flight  $\dot{V}_{CO_2}$  values were similar at 5 kPa and 10 kPa oxygen because of the effect on the mean of the few animals that could sustain flight in 5 kPa oxygen. In 5 kPa oxygen, the mean  $\dot{V}_{CO_2}$  of animals that could fly was 113 ml g<sup>-1</sup> h<sup>-1</sup> (range 85–122 ml g<sup>-1</sup> h<sup>-1</sup>, N=3), while the mean  $\dot{V}_{CO_2}$  for animals that could not fly was 70±6.0 ml g<sup>-1</sup> h<sup>-1</sup> (mean ± S.E.M., N=9).

### Respiratory exchange ratios and flight-muscle mass-specific metabolic rates

The respiratory exchange ratio of flying *E. simplicicollis* at 21 kPa oxygen was 0.94±0.033 (mean ± S.E.M., N=6), suggesting primarily carbohydrate-based flight metabolism. For the dragonflies we used, body mass averaged 195.1±4.51 mg (N=20), thorax mass averaged 105.9±2.31 mg (N=20) and flight muscle mass averaged 99.6±3.12 mg (means ± S.E.M., N=19). Metabolic rate in an atmosphere of 21 kPa oxygen averaged 1152±74.5 W kg<sup>-1</sup> flight muscle (mean ± S.E.M., N=19).

## Discussion

### Oxygen-sensitivity of flight behaviour

The ability to fly is quite oxygen-insensitive in these dragonflies. Most *E. simplicicollis* were able to fly in atmospheres containing as little as 10 kPa oxygen; some could even fly in 5 kPa oxygen (Fig. 2). Thus, there is at least a

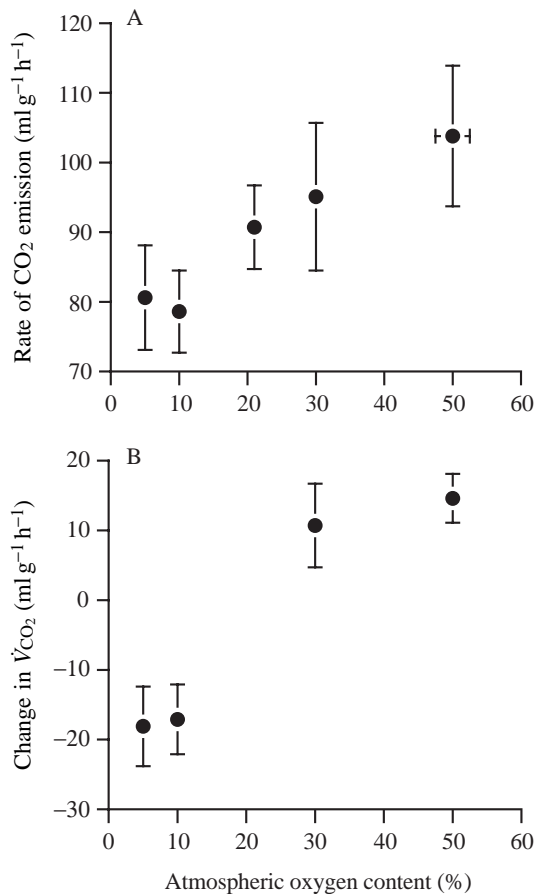


Fig. 3. (A) Body-mass-specific carbon dioxide emission rates ( $\dot{V}_{\text{CO}_2}$ ) during flight or attempted flight as a function of atmospheric oxygen level. (B) The difference between flight or attempted flight  $\dot{V}_{\text{CO}_2}$  at a test atmospheric oxygen level and the mean flight  $\dot{V}_{\text{CO}_2}$  measured in 21% oxygen before and after measurement in the test atmosphere for that individual. Means and S.E.M. shown.

10 kPa  $P_{\text{O}_2}$  (50%) safety margin in the ability of the tracheal system to deliver oxygen at a rate sufficient to support flight. The oxygen-sensitivity of the ability to attain flight is very similar to that documented in honeybees *A. mellifera* (Joos *et al.* 1997) and flies *L. sericata* (Davis and Fraenkel, 1940) and *D. repleta* (Chadwick and Gilmour, 1940).

#### Metabolic rates in comparison with other studies

The muscle-mass-specific metabolic rates achieved by *E. simplicicollis* during flight in this study were very high, consistent with strong selection on flight performance in dragonflies (Marden, 1989). These flight metabolic rates were 2–3 times higher than those recorded for dragonflies during warm-up (May, 1979), 30–40% higher than those estimated from the heat budget of flying *Anax junius* dragonflies (May, 1995), 70% higher than those estimated from a heat budget for *Sympetrum sanguineum* dragonflies (Wakeling and Ellington, 1997) and approximately 80% of the flight metabolic rate reported for similarly sized, hovering orchid bees, which have metabolic rates among the highest reported for any aerobic

animal tissue (Casey *et al.* 1985). *Erythemis simplicicollis* may be capable of even higher metabolic rates, since increases in mechanical power output of over 50% can be achieved by orchid bees hovering in low-density air (Dudley, 1995).

#### Oxygen-sensitive flight metabolism

The observation that  $\dot{V}_{\text{CO}_2}$  increases with hyperoxia and decreases with hypoxia suggests that oxygen delivery limits metabolic rate to some extent (approximately 10%) during escape flight for *E. simplicicollis*. The rise in  $\dot{V}_{\text{CO}_2}$  with higher oxygen partial pressures cannot be explained by changes in air density, since substituting oxygen for nitrogen slightly increases air density and decreases the power requirements for flight (Dudley, 1995). Interpretation of the effect of oxygen on flight metabolism in this experiment is difficult, given that the highest flight metabolic rates we measured may not represent maximal rates. However, the observations (1) that the dragonflies appeared to be attempting to escape from the chamber, (2) that body temperatures were near those measured for animals in flight in the field (Polcyn, 1994), (3) that the metabolic rates we measured were the highest yet recorded for dragonflies, and (4) that flight metabolic rate was oxygen-sensitive, all support the conclusion that *E. simplicicollis* were attempting to fly at a high, near-maximal metabolic rate. Since animals lost the ability to fly at atmospheric oxygen  $P_{\text{O}_2}$  values between 5 and 10 kPa, the metabolic rate in 10% oxygen may represent a value near the minimal metabolic rate necessary to achieve flight, while the higher metabolic rates at greater atmospheric oxygen levels may be due to increased acceleratory flight and more vigorous flight behaviour. Direct tests of the effect of atmospheric oxygen levels on flight performance are necessary to test this hypothesis.

Flight metabolic rates only varied by 25% with a sixfold change in atmospheric oxygen concentration (Fig. 3), indicating that compensatory changes limited the effect of decreasing oxygen concentration on mitochondrial ATP production. During hypoxia, these compensatory changes might include increasing convective ventilation (Joos *et al.* 1997), decreasing fluid levels in the tracheoles (Wigglesworth, 1983) or changing levels of regulators or substrates of mitochondrial oxidation (Connett *et al.* 1990). Under hyperoxic conditions, the increase in metabolic rate with increasing atmospheric  $P_{\text{O}_2}$  may be limited by other factors such as fuel availability (Weis-Fogh, 1964), ATP consumption capacity of the flight muscle, or perhaps by behavioural motivation. The approximately 10% stimulation of flight metabolic rate during hyperoxia is similar to the increase in maximal oxygen consumption during hyperoxia in athletic mammals (reviewed by Jones and Lindstedt, 1993; Wagner, 1996).

Our data are consistent with analyses of tracheal function in dragonfly flight muscle. From studies of the tracheal morphology of *Aeshna* dragonflies, Weis-Fogh (1964) concluded that a 13 kPa gradient for oxygen was required for adequate diffusion from the primary trachea to supply all the muscle mitochondria. While the oxygen level in the primary

trachea of dragonflies is unknown, in flying locusts it is approximately 16 kPa (Weis-Fogh, 1967) suggesting a 3 kPa safety margin. However, the oxygen consumption rates we measured for *E. simplicicollis* were approximately 1.7 times higher than those estimated by Weis-Fogh (1967) for *Aeshna* dragonflies from thoracic heat production. If the tracheal morphologies of *E. simplicicollis* and *Aeshna* sp. are similar, the higher oxygen consumption rate of *E. simplicicollis* will require a 22 kPa (1.7×13 kPa) gradient for oxygen from primary trachea to muscle mitochondria, consistent with oxygen limitation of flight metabolic rate in normal 21% oxygen atmospheres.

Flight metabolism in dragonflies appears to be completely aerobic in 21 kPa oxygen atmospheres, based on the respiratory exchange ratio and the observation that adult dragonfly flight muscle does not accumulate lactate (Dennison and Marden, 1994). Why might dragonfly flight muscle metabolism be aerobic and yet limited by oxygen delivery? One possibility, since flight durations were relatively short, is that the increase in flight  $\dot{V}_{CO_2}$  we observed in this species at higher atmospheric oxygen levels might represent evidence of a supra-aerobic ATP-utilization capacity which is used during burst performance and is normally powered by hydrolysis of high-energy phosphates. Another possible interpretation is that the small (10%) increase in flight metabolic rate with hyperoxia indicates a degree of imperfection in the match in capacity between oxygen delivery and mitochondrial oxygen consumption.

Why does the sensitivity of flight metabolism to atmospheric oxygen level vary among insects? In none of the four previous studies of the effect of atmospheric oxygen on insect flight metabolism (Davis and Fraenkel, 1940; Chadwick and Gilmour, 1940; Chadwick and Williams, 1949; Joos *et al.* 1997) was flight metabolism stimulated by hyperoxia, despite the fact that the oxygen consumption rates of flies in continuous flight were decreased by moderate hypoxia. Since none of the studies to date (including ours) has used a protocol in which it is known how near the insects are to maximal flight performance, it is possible that previous studies failed to show stimulation of flight metabolic rate by hyperoxia because behavioural motivation and flight performance were constant and submaximal.

Another intriguing possibility is that insects dependent on autoventilation to drive convective ventilation during flight might be more sensitive to atmospheric oxygen levels than those that utilize abdominal pumping. In many large flying insects, autoventilation drives convective ventilation: contractions of the flight muscles cause volume and pressure changes which induce convective air flow (Weis-Fogh, 1967). In dragonflies, locusts (Weis-Fogh, 1967) and cerambycid, elaterid and anthribid beetles (Miller, 1966), abdominal pumping is of minor importance relative to autoventilation for driving convective ventilation during flight. Insects, such as dragonflies, which are dependent on autoventilation can increase convective air flow by increasing wingbeat frequency or stroke amplitude (Miller, 1966; Weis-Fogh, 1967); however,

such changes will increase flight metabolic rate (Ellington, 1984; Dickinson and Lighton, 1995) and therefore may not provide the increase in the ratio of tracheal oxygen conductance to metabolic rate that is required to allow compensation for atmospheric hypoxia. In contrast, autoventilation is believed to be of minor importance in dipterans and hymenopterans (Weis-Fogh, 1964, 1967). In tethered flying wasps *Vespa crabro* (Weis-Fogh, 1967) and honeybees *Apis mellifera* (Bailey, 1954), abdominal pumping is strong and is believed to drive most or all of bulk oxygen flow during flight. Abdominal pumping rate is well-known to be oxygen-sensitive in non-flying insects (Miller, 1960; Arieli and Lehrer, 1988; Gulinson and Harrison, 1996) and may allow increasing ventilation to compensate for decreasing atmospheric oxygen levels in flying hymenopteran insects. In support of this contention, when honeybees are exposed to atmospheric hypoxia, the constant flight metabolic rate is accompanied by a 40% rise in evaporative water loss, suggesting that honeybees compensate for decreasing atmospheric oxygen content by enhancing tracheal conductance and perhaps abdominal pumping (Joos *et al.* 1997).

#### *Relevance of our data to the oxygen-sensitivity of flight metabolism and behaviour in giant dragonflies*

Graham *et al.* (1995) have recently suggested that the evolution of the giant insects of the late Paleozoic was made possible by atmospheric hyperoxia. Their hypothesis suggests that, at least in some large active, extant insects, the safety margin for oxygen delivery should be small. Our study is the first to provide evidence that the safety margin for oxygen delivery can be small or non-existent during free flight of an insect. These data contradict the often-held assumption that oxygen supply is always in excess for insects, because of the ability of the tracheal system to deliver oxygen to the cells in the gas phase. The sensitivity of growth, survival and tracheal development to levels of hypoxia that do not affect metabolic rate in mealworm *Tenebrio molitor* larvae (Loudon, 1988, 1989; Greenberg and Ar, 1996) also suggests that the match between tracheal capacity and tissue metabolic requirements may be close and tightly regulated in insects generally. The finding that flight metabolic rate is oxygen-limited in a living moderately sized dragonfly is consistent with the hypothesis of Graham *et al.* (1995) that atmospheric hyperoxia may have facilitated historical insect gigantism.

The hypothesis of Graham *et al.* (1995) also suggests that larger body size in extant insects should be associated with greater difficulty in gas exchange, because the final step in tracheal oxygen delivery is believed to be diffusion down a blind-ended tracheole. However, there are many mechanisms that larger insects might utilize to overcome diffusion limitations imposed by their larger size, such as increased use of convection, increased tracheal densities, etc. As yet, there are no comparative data to indicate how tracheal morphology or density varies with body size in insects or whether larger extant insects are more oxygen-sensitive than smaller forms.

We thank Rob Fulton of the Desert Studies Consortium Research Station for invaluable help with field logistics, Dave Polcyn for advice on dragonflies and Kendra Greenlee, Glenn Walsberg, Steve Roberts and Barbara Joos for help with the manuscript. This research was partially funded by NSF grant IBN-9317784 to J.F.H. and by NSF grant BSR 9306537 to J.R.B.L., and by a David and Lucile Packard Fellowship to J.R.B.L.

### References

- ARIELI, R. AND LEHRER, C. (1988). Recording of locust breathing frequency by barometric method exemplified by hypoxic exposure. *J. Insect Physiol.* **34**, 325–328.
- BAILEY, L. (1954). The respiratory currents of the tracheal system of the adult honey-bee. *J. exp. Biol.* **31**, 589–593.
- BERNER, R. A. AND CANFIELD, D. E. (1989). A new model for atmospheric oxygen over phanerozoic time. *Am. J. Sci.* **289**, 333–361.
- CARPENTER, F. M. (1943). Studies on Carboniferous insects from Comentry, France: Part I. Introduction and families Protargridiidae, Meganeuridae and Campylopteridae. *Bull. geol. Soc. Am.* **54**, 527–554.
- CARPENTER, F. M. (1947). Lower Permian insects from Oklahoma. Part I. Introduction and the orders Magaseoptera, Protodonata and Odonata. *Proc. Am. Acad. Sci.* **76**, 25–54.
- CASEY, T. M., MAY, M. L. AND MORGAN, K. R. (1985). Flight energetics of euglossine bees in relation to morphology and wing stroke frequency. *J. exp. Biol.* **116**, 271–289.
- CHADWICK, L. E. AND GILMOUR, D. (1940). Respiration during flight in *Drosophila repleta* Wollaston: oxygen consumption considered in relation to the wing-rate. *Physiol. Zool.* **13**, 398–410.
- CHADWICK, L. E. AND WILLIAMS, C. M. (1949). The effects of atmospheric pressure and composition on the flight of *Drosophila*. *Biol. Bull. mar. biol. Lab., Woods Hole* **97**, 115–137.
- CONNETT, R. J., HONIG, C. R., GAYESKI, T. E. J. AND BROOKS, G. A. (1990). Defining hypoxia: a systems view of  $\dot{V}O_2$ , glycolysis, energetics and intracellular  $P_{O_2}$ . *J. appl. Physiol.* **68**, 833–842.
- DAVIS, R. A. AND FRAENKEL, G. (1940). The oxygen consumption of flies during flight. *J. exp. Biol.* **17**, 402–407.
- DENNISON, K. L. AND MARDEN, J. H. (1994). Muscle metabolism in a dragonfly that undergoes an ontogenetic shift in thermal sensitivity of flight performance. *Physiologist* **37**, A74.
- DICKINSON, M. H. AND LIGHTON, J. R. B. (1995). Muscle efficiency and elastic storage in the flight motor of *Drosophila*. *Science* **268**, 87–90.
- DUDLEY, R. (1995). Extraordinary flight performance of orchid bees (Apidae: Euglossini) hovering in heliox (80% He/20% O<sub>2</sub>). *J. exp. Biol.* **198**, 1065–1070.
- ELLINGTON, C. P. (1984). The aerodynamics of hovering insect flight. I. The quasi-steady analysis. *Phil. Trans. R. Soc. Lond. B* **305**, 1–15.
- GRAHAM, J. B., DUDLEY, R., AGUILAR, N. M. AND GANS, C. (1995). Implications of the later Palaeozoic oxygen pulse for physiology and evolution. *Nature* **375**, 117–120.
- GREENBERG, S. AND AR, A. (1996). Effects of chronic hypoxia, normoxia and hyperoxia on larval development in the beetle *Tenebrio molitor*. *J. Insect Physiol.* **42**, 991–996.
- GULINSON, S. L. AND HARRISON, J. F. (1996). Control of resting ventilation rate in grasshoppers. *J. exp. Biol.* **199**, 379–389.
- JONES, J. H. AND LINDSTEDT, S. L. (1993). Limits to maximal performance. *A. Rev. Physiol.* **55**, 547–569.
- JOOS, B., LIGHTON, J. R. B., HARRISON, J. F., SUAREZ, R. K. AND ROBERTS, S. P. (1997). Effects of ambient oxygen tension on flight performance, metabolism and water loss of the honeybee. *Physiol. Zool.* **70**, 167–174.
- KAMMER, A. E. AND HEINRICH, B. (1978). Insect flight metabolism. *Adv. Insect Physiol.* **13**, 133–228.
- KEISTER, M. AND BUCK, J. (1964). Respiration: some exogenous and endogenous effects on rate of respiration. In *Physiology of the Insecta*, vol. 3 (ed. M. Rockstein), pp. 618–658. New York: Academic Press.
- LOUDON, C. (1988). Development of *Tenebrio molitor* in low oxygen levels. *J. Insect Physiol.* **34**, 97–103.
- LOUDON, C. (1989). Tracheal hypertrophy in mealworms: design and plasticity in oxygen supply systems. *J. exp. Biol.* **147**, 217–235.
- MARDEN, J. H. (1989). Bodybuilding dragonflies: costs and benefits of maximizing flight muscle. *Physiol. Zool.* **62**, 505–521.
- MAY, M. L. (1979). Energy metabolism of dragonflies (Odonata: Anisoptera) at rest and during endothermic warm-up. *J. exp. Biol.* **83**, 79–94.
- MAY, M. L. (1982). Heat exchange and endothermy in Protodonata. *Evolution* **36**, 1051–1058.
- MAY, M. L. (1995). Dependence of flight behavior and heat production on air temperature in the green darner dragonfly *Anax junius* (Odonata: Aeshnidae). *J. exp. Biol.* **198**, 2385–2392.
- MILLER, P. L. (1960). Respiration in the desert locust. I. The control of ventilation. *J. exp. Biol.* **37**, 224–236.
- MILLER, P. L. (1966). The supply of oxygen to the active flight muscles of some large beetles. *J. exp. Biol.* **45**, 285–304.
- POLCYN, D. M. (1994). Thermoregulation during summer activity in Mojave desert dragonflies. (Odonata: Anisoptera). *Func. Ecol.* **8**, 441–449.
- SOKAL, R. R. AND ROHLF, F. J. (1995). *Biometry: The Principles and Practice of Statistics in Biological Research*. New York: W. H. Freeman and Company.
- WAGNER, P. D. (1996). Determinants of maximal oxygen transport and utilization. *A. Rev. Physiol.* **58**, 21–50.
- WAKELING, J. M. AND ELLINGTON, C. P. (1997). Dragonfly flight. III. Lift and power requirements. *J. exp. Biol.* **200**, 583–600.
- WEIS-FOGH, T. (1964). Diffusion in insect wing muscle, the most active tissue known. *J. exp. Biol.* **41**, 229–256.
- WEIS-FOGH, T. (1967). Respiration and tracheal ventilation in locusts and other flying insects. *J. exp. Biol.* **47**, 561–587.
- WIGGLESWORTH, V. B. (1983). The physiology of insect tracheoles. *Adv. Insect Physiol.* **17**, 85–149.