

*Review*

# Atmospheric oxygen level and the evolution of insect body size

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Insects are small relative to vertebrates, possibly owing to limitations or costs associated with their blind-ended tracheal respiratory system. The giant insects of the late Palaeozoic occurred when atmospheric PO<sub>2</sub> (aPO<sub>2</sub>) was hyperoxic, supporting a role for oxygen in the evolution of insect body size. The paucity of the insect fossil record and the complex interactions between atmospheric oxygen level, organisms and their communities makes it impossible to definitively accept or reject the historical oxygen-size link, and multiple alternative hypotheses exist. However, a variety of recent empirical findings support a link between oxygen and insect size, including: (i) most insects develop smaller body sizes in hypoxia, and some develop and evolve larger sizes in hyperoxia; (ii) insects developmentally and evolutionarily reduce their proportional investment in the tracheal system when living in higher aPO<sub>2</sub>, suggesting that there are significant costs associated with tracheal system structure and function; and (iii) larger insects invest more of their body in the tracheal system, potentially leading to greater effects of aPO<sub>2</sub> on larger insects. Together, these provide a wealth of plausible mechanisms by which tracheal oxygen delivery may be centrally involved in setting the relatively small size of insects and for hyperoxia-enabled Palaeozoic gigantism.

**Keywords:** body size; evolution; oxygen; gigantism

## 1. INTRODUCTION

If the insects had hit on a plan for driving air through their tissues instead of letting it soak in, they might have become as large as lobsters, though other considerations would have prevented them from becoming as large as man.

(Haldane 1926)

More than 300 Ma, giant insects up to 10-fold larger than those in similar groups alive today roamed the earth (Shear & Kukulova-Peck 1990; Grimaldi & Engel 2005). It has been proposed that atmospheric hyperoxia (defined here as atmospheric oxygen partial pressures (aPO<sub>2</sub>) greater than the current 21 kPa) in the Palaeozoic was the major factor allowing the evolution of giant insects and other animals, with subsequent hypoxia (defined here as aPO<sub>2</sub> below present day) being responsible for their disappearance (Graham *et al.* 1995; Dudley 1998; Berner 2006*b*; Ward 2006). While a number of evolutionary events have been tied to changes in atmospheric oxygen level (Berner *et al.* 2007), the hypothesis that variation of aPO<sub>2</sub> is responsible for historical changes in the body size of insects has recently been challenged owing to uncertainties in physiological mechanisms and in patterns of oxygen and body size variation through time (Butterfield 2009).

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## 2. GEOLOGICAL AND PALAEOONTOLOGICAL EVIDENCE FOR CHANGES IN ATMOSPHERIC OXYGEN LEVEL AS A CAUSE OF HISTORICAL INSECT GIGANTISM

Recent atmospheric modelling efforts suggest that oxygen levels have varied dramatically from the initial evolution of insects in the late Silurian (Grimaldi & Engel 2005) to the present day. These models are based on a number of approaches including the balance of sedimentary rock abundance (Berner & Canfield 1989), carbon and sulphur isotopic changes (Berner 2005, 2006*a,b*), and incorporating feedbacks from atmospheric oxygen levels and fire on biogeochemical cycling (Bergman *et al.* 2004). Although some aspects of the pattern of aPO<sub>2</sub> during the Phanerozoic have been controversial (e.g. Triassic hypoxia, Cretaceous hyperoxia and Tertiary oxygen stability), all the models agree on one major point—a period of hyperoxia spanning the Carboniferous and Permian reaching a maximum of 27–35 kPa (figure 1). The large-scale variation in oxygen modelling results during the Triassic to Tertiary (figure 1) should act as a general warning of associating oxygen variation in time periods other than the Permo-Carboniferous to evolutionary events.

In 1995, Graham *et al.* pointed out that the high oxygen spike in the Permo-Carboniferous was coincident with the rise of giant Palaeozoic animals in diverse taxa, including insects, millipedes, chelicerates and amphibians. Giant dragonflies of the extinct order Protodonata (*Meganeura*) had wingspans exceeding 70 cm in length, with over five times the length and twice the thoracic width of the largest extant

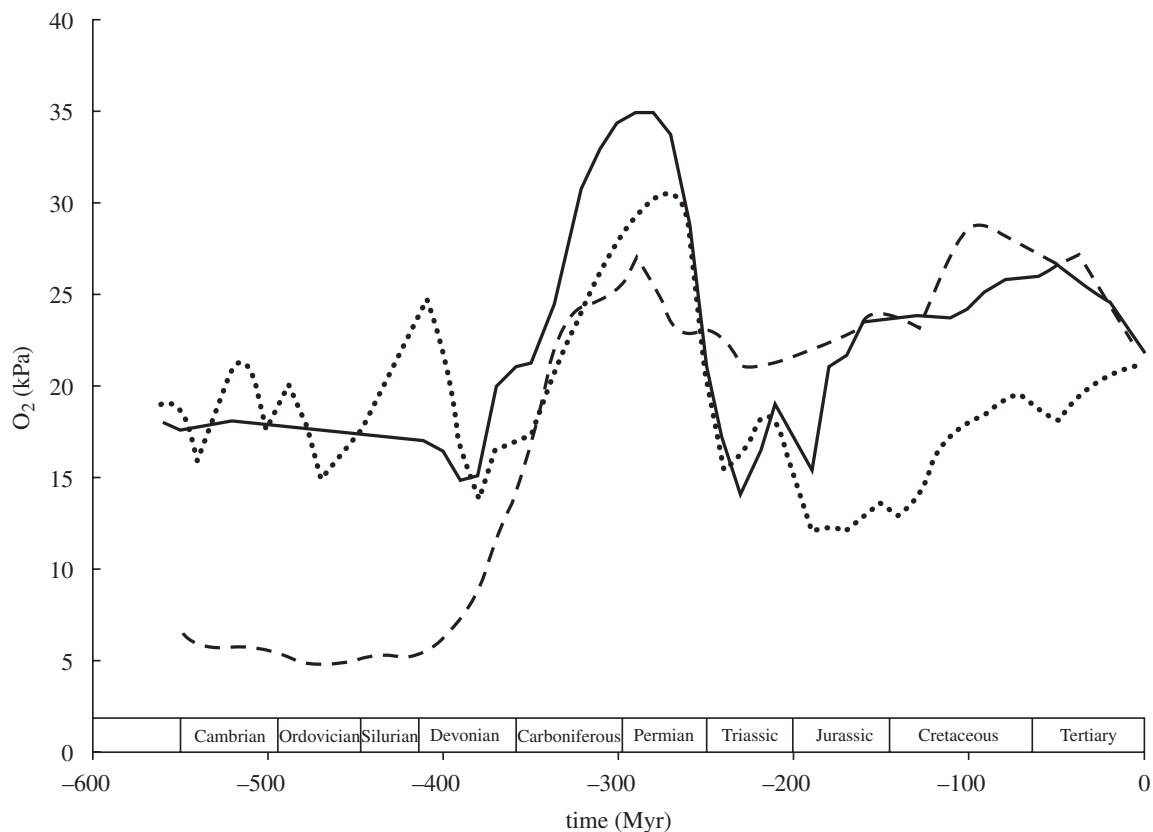


Figure 1. Various patterns of modelled  $aPO_2$  over the Phanerozoic. Solid line (Berner & Canfield 1989), dotted line (Berner 2006b), dashed line (Bergman *et al.* 2004).

dragonflies (May 1982; Shear & Kukalova-Peck 1990; Carpenter 1992). Among insects, gigantism in the Permo-Carboniferous has also been reported for Ephemeroptera, Diplura, Thysanura and the extinct order Paleodictyoptera (Briggs 1985; Kukalova-Peck 1985). *Arthropleura*, a group related to modern day millipedes, reached upwards of 2 m in length, almost six times the size of any extant millipede.

However, a critical analysis must conclude that the palaeontological evidence for a link between insect size and atmospheric oxygen levels is, at best, weakly correlational. The evidence for insect gigantism in the Permo-Carboniferous is based on a few documented large fossils, raising the possibility that this is a sampling artefact. Extant species represent a small proportion of all species that have ever occurred, and so it is not surprising that larger species than today's existed in the past; also larger species tend to be better-preserved (Grimaldi & Engel 2005). The loss of giant insects in the late Permian coincided with a major loss of insect diversity (Labandera & Sepkoski 1993), and it is not clear that larger species were differentially affected. While there are reports of gigantic Ephemeroptera in the late Cretaceous (Carpenter 1992); since some models predict hyperoxia and others hypoxia during that time (figure 1), it is presently unclear whether this observation supports a link between hyperoxia and insect gigantism (Dudley 2000). The majority of Permo-Carboniferous insects were not giants; plots of the size distributions of fossil insects from the Wellington formation of the Lower Permian demonstrate that most insects from this time period were moderately sized, with a few large species (Beckemeyer & Hall 2007). Similarly, extant insects often exhibit right-skewed size distributions with mostly

moderately sized species and a few very large ones (Allen *et al.* 2006; Chown & Gaston 2010). While the largest insects were clearly bigger in the Permo-Carboniferous than in any other time period, we do not know whether average insect size was larger. Because laboratory studies suggest strong effects of oxygen on both average and maximal body size (see below), statistical demonstration of positive correlations between modelled atmospheric oxygen level and average or maximal fossil size would be extremely useful for building the case for a link between oxygen and insect size.

The palaeontological data supporting effects of  $aPO_2$  on body size is somewhat stronger for other taxonomic groups. Falkowski *et al.* (2005) showed that mammalian body size over the last 65 Myr gradually increased with the  $aPO_2$  predicted by the Berner *et al.* models. Similarly, the average body and head size of Reptilomorph fossils ranging from the Upper Carboniferous to the Upper Permian was strongly positively correlated with modelled  $aPO_2$  (VandenBrooks 2007). Correlations between  $aPO_2$  and body size in multiple, independently evolved animal lineages certainly support the hypotheses that  $aPO_2$  affects body size through evolutionary time.

### 3. MECHANISMS FOR OXYGEN EFFECTS ON INSECT BODY SIZE

Perhaps the most relevant evidence would be long-term experiments on the effects of abnormally high or low oxygen partial pressures on the living representatives of the many groups which exist today.

(McAlester 1970)

Body size is affected by a wide range of proximate and ultimate factors. Proximally, adult body size is the product of growth rate and development time. Genes affect growth rate by influencing capacities to consume, process and assimilate food, and the hormonally regulated transitions to a sexually mature state that determine development time. These intrinsic factors are modulated by environmental factors such as food availability, food quality and temperature. A variety of hormones and signalling cascades regulate growth rate and development time, including insulin, target of rapamycin, ecdysone and juvenile hormone (Nijhout 2006; Mirth & Riddiford 2007).

Many ultimate factors affect the evolution of animal size (Allen *et al.* 2006; Chown & Gaston 2010). In a variety of insects, reproductive success, including fecundity in females and competitive ability of males, increases with individual size, owing to positive correlations between adult size and egg size and number, foraging ability, fighting ability and capacities to survive resource dearths (Brown & Mauer 1986). Nonetheless, most species have relatively small sizes, as smaller animals tend to be more viable, requiring less space, development time and nutrients to reach adulthood (Blanckenhorn 2000). Ecological factors such as temperature, nutrient availability, community interactions (competition and predator–prey), the match between the size of animals and their food and season length also affect body size distributions (Allen *et al.* 2006; Chown & Gaston 2010). Taxon-specific physiological or biomechanical constraints can influence body size distributions; certainly, terrestrial arthropods in general, and insects in particular, are usually smaller than vertebrates, a pattern that remains to be definitively explained.

Multiple mechanisms can be suggested for how changing aPO<sub>2</sub> can affect insect body size. At the individual level (figure 2a), positive correlations between PO<sub>2</sub>, metabolic rates and performance (e.g. locomotion, feeding) could increase food intake, growth and survival. Positive correlations between aPO<sub>2</sub> and oxidative stress could reduce growth and survival. Insects alter the resistance of their tracheal system in compensatory responses to aPO<sub>2</sub> (e.g. greater ventilation and spiracular opening at lower aPO<sub>2</sub>), affecting respiratory water loss rates, growth and survival. Changing internal PO<sub>2</sub> will also affect developmental processes mediated by signalling pathways such as insulin and hypoxia inducible factor (HIF) that influence protein synthesis, cell size and number, and body size (Gorr *et al.* 2006; Dekanty *et al.* 2007). HIF-signalling mediates compensatory changes in tracheal diameters and branching in response to aPO<sub>2</sub>, dampening effects of aPO<sub>2</sub> on internal PO<sub>2</sub> and altering the allocation of body materials, energy and space between the respiratory and non-respiratory system. Because HIF and hypoxic regulation of growth are widespread in the animal kingdom (Gorr *et al.* 2006), it is reasonable to suggest that similar responses would have been present in Palaeozoic insects.

Changing oxygen level over multiple generations may affect just the maximal size of the largest species, but could also affect the average size of most species. As one example, if higher aPO<sub>2</sub> generally improves viability and fecundity for individuals, but the body size that optimizes fitness is unaffected, an increase in abundance and

range of body sizes is probable, increasing maximal without affecting mean size (figure 2b). Alternatively, intrinsically larger animals may benefit more from higher aPO<sub>2</sub>, leading to evolutionary changes in size. For example, individuals may vary in their genetically determined body and jaw sizes, with larger size allowing greater feeding, growth, size and fecundity under good environmental conditions, but reducing survival under poor conditions. If higher aPO<sub>2</sub> improves environmental conditions (e.g. by enabling more foraging or reducing costs of respiration), individuals with larger bodies and jaws may benefit more. A significant size\*oxygen interaction favouring intrinsically larger animals will tend to shift the size distribution, increasing mean and maximal population body size (figure 2c). Finally, aPO<sub>2</sub> might only affect the largest individuals within a species or the largest species. For example, higher aPO<sub>2</sub> might relieve some upper constraint on body size, leading to increases in size of the very largest individuals (figure 2d).

The remainder of this section will examine the empirical data related to the various possible pathways in figure 2. We begin by examining the capacity of insects to dampen the effect of aPO<sub>2</sub> by behavioural, physiological and morphological compensation. Next we review the evidence that aPO<sub>2</sub> has acute effects on insect performance that could affect size or fitness. We continue by considering developmental responses to aPO<sub>2</sub> that affect body size distributions. Finally, we examine the existing data that aPO<sub>2</sub> affects insect size evolution. Empirical support for specific mechanisms (arrows in figure 2) helps build support for the hypothesis that changing aPO<sub>2</sub> affects insect size evolution, but the multiple plausible pathways in series and parallel (figure 2) make it challenging to determine cause and effect.

#### (a) *Acute respiratory compensation to varying aPO<sub>2</sub>*

Most animals, including insects, exhibit compensatory changes in respiratory function in response to changing oxygen levels. In insects, these changes include adjusting the degree and duration of opening of spiracular valves, alteration of fluid levels in tracheoles, and changes in ventilation (Harrison *et al.* 2006). In all cases, these responses are compensatory, with tracheal system resistance increasing at higher aPO<sub>2</sub>, dampening changes in internal PO<sub>2</sub>. The positive correlation between aPO<sub>2</sub> and tracheal system resistance leads to a negative correlation between aPO<sub>2</sub> and respiratory water loss (Lighton *et al.* 2004).

#### (b) *Acute effects of aPO<sub>2</sub> on insect performance*

Critical PO<sub>2</sub> (the aPO<sub>2</sub> below which a process is oxygen-limited) values are highest for highly aerobic behaviours such as flight (Harrison *et al.* 2006). Hyperoxia increases flight metabolism and performance in one dragonfly species (Harrison & Lighton 1998), but had no effect relative to normoxia in honey bees (Joos *et al.* 1997) or grasshoppers (Rascón & Harrison 2005). Critical PO<sub>2</sub> values for feeding rates are 5 kPa in *Manduca sexta* caterpillars (Greenlee & Harrison 2005) and 10 kPa in *Drosophila melanogaster* larvae (Frazier 2007). For oxygen delivery in resting insects, critical PO<sub>2</sub> values average 6 kPa (Harrison *et al.* 2006). Thus the empirical data available suggest that moderate hypoxia probably

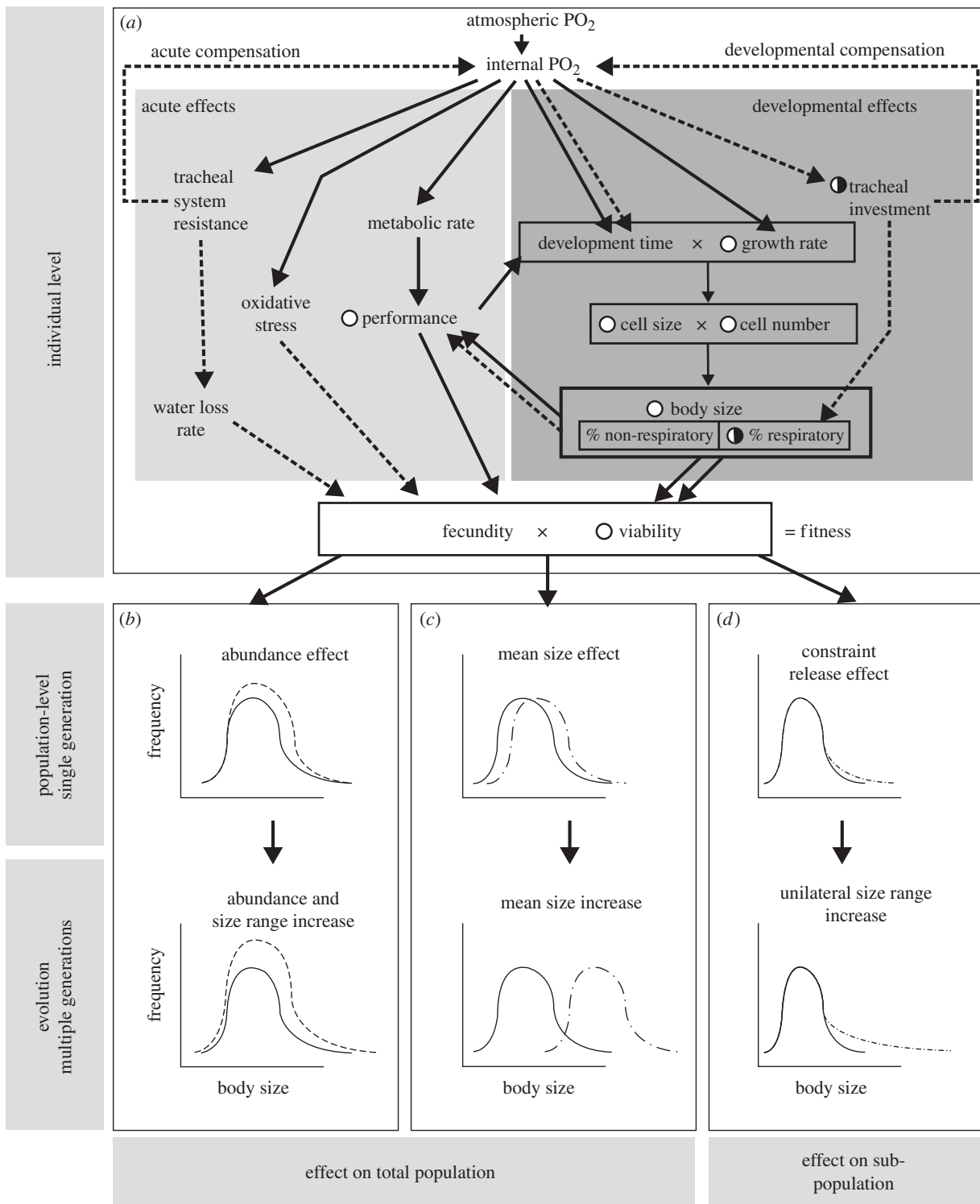


Figure 2. Plausible mechanisms by which atmospheric oxygen partial pressure ( $aPO_2$ ) might affect the evolution of insect size. Straight arrows indicate positive correlations, dashed negative, and both arrows together indicate nonlinear effects. Circles next to a trait indicate empirical support for an effect of hypoxia (open circles), or both hypoxia and hyperoxia (semi-filled circles) in *Drosophila melanogaster*. At the individual level (a), changes in  $aPO_2$  result in changes in internal  $PO_2$  that are damped relative to the environment owing to changes in the resistance of the tracheal system. Changing tracheal system resistance affects water loss rates, altering fitness in some environments. Internal  $PO_2$  causes direct, acute effects (left box), altering levels of oxidative stress and affecting performance variables such as flight or feeding. Changes in performance can directly affect fitness; for example, changing agility might improve foraging or the ability to escape predators. Internal  $PO_2$  also causes developmental effects (right box) via oxygen-sensitive signalling systems that control development time, cell size and number, that then affect body size and fitness. Developmental processes allow compensatory changes in respiratory structures; altering relative investment in non-respiratory structures such as muscles and ovaries that may affect fitness. The within-individual effects may influence average or maximal size of all or just the largest species. Some of the multiple possibilities include (b) alterations in abundance that affect the range but not mean size, (c) evolutionary shifts in mean size that increase over generations owing to  $size \times aPO_2$  interactions or (d) effects only on the largest individuals or species, such as relief of a constraint on maximal size. Over multiple generations, individuals within the new population cycle through the changed  $aPO_2$ , causing enhanced evolution of body size.

depresses peak aerobic performance of many insects, while moderate hyperoxia generally has few acute effects. Similar results have been found for most air-breathing vertebrates, with only the most aerobic mammals (e.g. racehorses) showing a stimulation of metabolism and performance by hyperoxia (Jones 1994).

If larger insects have higher critical  $PO_2$  values, this could provide a mechanism driving evolution of larger size in hyperoxia (figure 2*c,d*). However, three ontogenic studies on resting grasshoppers (Greenlee & Harrison 2004*a*), jumping grasshoppers (Kirkton *et al.* 2005) and feeding caterpillars (Greenlee & Harrison 2005) have found no evidence for an increase in the critical  $PO_2$  values with size across developmental instars, suggesting that the capacity of the tracheal respiratory delivery system is at least matched to tissue needs as insects grow (but see Kirkton *et al.* (2005) for some contrary evidence). In fact the highest critical  $PO_2$  values may occur in the smallest, earliest developmental stages in grasshoppers (Greenlee & Harrison 2004*a*). Similarly, the greatest oxygen sensitivity measured for *M. sexta* occurs in the eggs, whose metabolic and development rates are oxygen-limited at temperatures of 27°C and higher, and improved by hyperoxia (Woods & Hill 2004). Interestingly, critical  $PO_2$  values rise dramatically late within each instar, as metabolic rates increase with tissue growth while tracheal systems may be compressed before moulting (Greenlee & Harrison 2004*b*, 2005). Together these studies provide evidence that critical  $PO_2$  does not generally increase with size, but it would be useful to study this question during specific behaviours or life stages when oxygen is most limiting.

#### (c) *Developmental plasticity in response to changes in aPO<sub>2</sub>*

Recent studies indicate that developmental plasticity causes most insects to be smaller when reared in moderate to severe hypoxia, and some species to grow larger when reared in hyperoxia (Harrison *et al.* 2009). The developmental responses of growth and size to atmospheric oxygen levels affect both the mean and maximal size of populations, and can be caused by both changes in growth rates and development time. In addition, compensatory morphological changes in the tracheal respiratory system to variation in a $PO_2$  are striking.

##### (i) *Responses to hypoxia*

Hypoxia has strong effects on growth and size of insects (developmental plasticity), reducing body size in eight of nine examined insect species: in the fruitfly *D. melanogaster*, the mealworm beetles *Zophobas morio* and *Tenebrio molitor*, the scarabaeid beetle *Cotinus texana*, the cockroach *Blattella germanica*, the tobacco hornworm moth *M. sexta* and the mayfly *Hexagenia limbata* (Winter *et al.* 1996; Harrison *et al.* 2009). The exception was the grasshopper, *Schistocerca americana*, whose body size is not affected by rearing in oxygen levels as low as 5 kPa (Harrison *et al.* 2006). Even small insects such as *D. melanogaster* can be limited by relatively mild hypoxia, with the size of these flies decreasing linearly below 15 kPa a $PO_2$  (Peck & Maddrell 2005). Hypoxia extends development times and decreases mean and maximal size, growth rates and survival (Loudon 1988;

Greenberg & Ar 1996; Frazier *et al.* 2001; Klok *et al.* 2009). Hypoxic rearing reduces both cell size and number in *Drosophila* wings (Peck & Maddrell 2005). Similar developmental effects of oxygen on body size have been found in a wide variety of animals including humans (Bailey *et al.* 2007), alligators (Owerkowicz *et al.* 2009) and crustaceans (Seidl *et al.* 2005).

The morphology of the respiratory system of animals often compensates for changes in a $PO_2$  (Sollid *et al.* 2003; Owerkowicz *et al.* 2009). Insects compensate for hypoxia by increasing tracheal diameters and the number of tracheoles (Jarecki *et al.* 1999; Henry & Harrison 2004). Many other compensatory changes occur in response to hypoxia, including down-regulation of metabolic enzymes (Zhou *et al.* 2007, 2008). Together, these physiological and developmental responses probably reduce negative effects of hypoxia on fitness.

##### (ii) *Responses to hyperoxia*

The effects of hyperoxia on growth and body size are less consistent and often nonlinear (Harrison *et al.* 2009). Body size increases in the giant mealworm, *Z. morio* (27%  $O_2$ ; Harrison *et al.* 2009) and in the scarabaeid beetle *C. texana* (40%  $O_2$ ; Harrison *et al.* 2009). However, in five tested species, there is no increase in body size at any tested level of hyperoxia, and *M. sexta* are smaller in hyperoxia (Harrison *et al.* 2009). *Drosophila melanogaster* reared in population bottles are larger when reared in hyperoxia (Frazier *et al.* 2001), but neither mean nor maximal sizes are larger when flies are reared individually, suggesting that hyperoxic effects on size in the population bottles are owing to evolutionary changes occurring in a single generation (Klok *et al.* 2009). The species showing the most positive responses of body size to hyperoxia are ground-dwelling beetles like *Z. morio*, suggesting that species which develop in occasionally hypoxic environments like soils may be more likely to benefit from atmospheric hyperoxia. In *Z. morio*, there is a distinct  $\cap$ -shaped response to hyperoxia, with mild hyperoxia increasing size and more extreme hyperoxia reducing size. The nonlinear and diverse developmental responses to hyperoxia in insects suggest that negative effects associated with oxidative damage overlap with stimulatory effects of higher a $PO_2$ . When hyperoxia increases body size, it does so primarily by extending development time rather than increasing growth rate (Greenberg & Ar 1996). Hyperoxic rearing leads to decreasing tracheal diameters and a reduced number of tracheoles (Jarecki *et al.* 1999; Henry & Harrison 2004). The effects of chronic hyperoxia have received little attention in vertebrates, but recent studies suggest that hyperoxia can increase the size and growth of embryonic and juvenile alligators, and reduce investment in cardiorespiratory structures (Owerkowicz *et al.* 2009).

#### (d) *Evolutionary responses to aPO<sub>2</sub>*

##### (i) *Laboratory selection studies*

Rearing *D. melanogaster* for multiple generations in hyperoxia results in evolution of increased mean and maximal body mass (Klok *et al.* 2009). The heaviest flies are obtained when flies reared for multiple generations in 40 kPa  $PO_2$  are returned to normoxia, suggesting involvement of parental effects. Rearing for multiple generations

in hypoxia (either in 10 kPa PO<sub>2</sub> or with stepwise decreasing aPO<sub>2</sub> down to 4 kPa) causes flies to be smaller, but this effect is completely owing to developmental plasticity, as these flies are the same size as control flies when returned to normoxia (Zhou *et al.* 2007; Klok *et al.* 2009). *Drosophila melanogaster* may commonly encounter hypoxia in its natural habitat, which may explain why it has well-developed plastic responses to hypoxia. By contrast, hyperoxia is probably a novel situation for this species, and thus may be more likely to elicit evolutionary responses. The mechanisms that elicit evolution of larger size in *D. melanogaster* in response to hyperoxia remain unclear. Since survival is not affected (Frazier *et al.* 2001) and both mean and maximal sizes increase (Klok *et al.* 2009), it is plausible that larger flies gain fecundity advantages at higher oxygen levels.

Evolution of large size in the field is probably driven by natural or sexual selection. Does aPO<sub>2</sub> interact with such selection, potentially synergistically, to affect evolution of size? At least in *D. melanogaster*, the answer appears to be 'no'. Populations selected for large size do not differ in mean or maximal size when reared for multiple generations in 21 or 40 kPa PO<sub>2</sub> (Klok & Harrison 2009). However, hypoxia (10 kPa PO<sub>2</sub>) strongly limited size even when flies are selected for large size (Klok & Harrison 2009). This effect is owing to plasticity, as these flies selected for large size in 10 kPa PO<sub>2</sub> also have identical sizes as control flies when returned to normoxic conditions.

Lastly, *D. melanogaster* evolve larger tracheae when reared for multiple generations in hypoxia, and smaller tracheae when reared in hyperoxia (Henry & Harrison 2004), providing evidence for selection for such a compensatory strategy. This result suggests that tracheal investment has significant costs (materials, energy or space) that result in selection against excess tracheal structure.

#### (ii) Comparative studies

##### *Habitat PO<sub>2</sub> and insect body size*

In a variety of aquatic invertebrates, habitat PO<sub>2</sub> correlates positively with maximal and average body sizes (Peck & Chapelle 2003; Chapelle & Peck 2004), suggesting that oxygen availability has a strong effect on size in these groups. Terrestrial insects do not appear to exhibit consistent trends in body size across altitude-related aPO<sub>2</sub>, but changes in altitude are accompanied by potentially confounding changes in temperature, air density and growing season length (Dillon *et al.* 2006). Unfortunately, there is little data on how other natural hypoxic environments (i.e. organic soils, burrows) influence insect body size.

##### *The relationship between species body size and critical PO<sub>2</sub>*

Theoretically, oxygen delivery could be more challenging in larger species of insects owing to their longer tracheae, which could limit maximal insect size, providing a simple mechanism for oxygen effects on size evolution. However, empirical data indicate no effect of size on the critical PO<sub>2</sub> values for oxygen delivery across resting grasshopper (Greenlee *et al.* 2007) or beetle species (Lease 2008). These and comparative data in other animal groups, like fishes (Nilsson & Östlund-Nilsson 2008), mammals

(Weibel *et al.* 1998) and sea spiders (Woods *et al.* 2008) suggest that oxygen supply is morphologically and physiologically adjusted to demand across species body sizes. Large size might only be a problem for oxygen delivery during periods of higher oxygen consumption rates, such as running or flying at high temperatures. In support of this hypothesis, performance of larger bivalves becomes oxygen-limited at elevated temperatures that stimulate metabolism (Peck 2007). Perhaps, critical constraining effects of oxygen on large size occurs only during extreme environmental or physiological events.

##### *Temperature and body size*

Global temperatures were low during the late Carboniferous when some insects were gigantic (Royer *et al.* 2004), and interactions between temperature and oxygen could be part of the historical oxygen-size linkages. Lower temperatures tend to cause insects to be larger, via both direct developmental effects and by evolutionary changes in mean size (Kingsolver & Huey 2008; Chown & Gaston 2010). Rising temperatures exacerbate oxygen delivery problems and size responses to oxygen owing to exponential effects on metabolic rates (Frazier *et al.* 2001; Woods & Hill 2004). Lower temperatures could shorten the growing season, favouring evolution of larger insects more resistant to starvation (Chown & Gaston 2010). Lower temperatures and higher aPO<sub>2</sub> could have expanded the available habitat for insects (Huey & Ward 2005); and since larger insects require a larger geographical range, this could have favoured larger species (Chown & Gaston 2010).

##### *Tracheal investment and insect body size*

Larger insects have lower mass-specific metabolic and gas exchange rates (Chown *et al.* 2007). Thus, we would predict that the tracheal system should scale hypometrically, as observed for mammalian capillary density and fish gills, or perhaps isometrically as observed for mammalian lungs (Weibel *et al.* 1998). However, the three studies that have investigated the scaling relationship of the tracheal system to date suggest that tracheal investment is hypermetric, with greater proportional investment in larger insects. During ontogeny of *S. americana*, tracheal investment increases in the leg muscle (Hartung *et al.* 2004) and at the whole-body level, with tracheal volumes and ventilation scaling approximately with mass<sup>1.3</sup> (Lease *et al.* 2006; Greenlee *et al.* 2009). Similarly, across four tenebrionid beetle species, tracheal volumes scale with mass<sup>1.29</sup> (Kaiser *et al.* 2007). Such a trend appears to be general for insects: tiny stick insects have tracheal volumes of around 2 per cent (Schmitz & Perry 1999), while giant scarabaeid beetles have tremendous air sacs (Miller 1966). Theoretical calculations suggest that the observed hypermetry is consistent with a need to overcome reduced rates of diffusive gas exchange in longer, blind-ended tracheoles (Harrison *et al.* 2009).

Trends in compensation by differential increase of area-dependent structures cannot be continued indefinitely without producing structural absurdities.

(Gould 1966)

Increasing investment in a structure with size imposes constraints on larger animals. For example, larger

vertebrates must have proportionally larger and thicker skeletons; this leads to more upright postures and reduced agility (Biewener 1989). Similarly, tracheal hypermetry may constrain insects. Tracheal hypermetry can potentially have multiple consequences on insect morphology and physiology that could result in selection against large insects including decreased density, increased cost of the respiratory system, displacement of other tissues and exhaustion of internal space available for tracheae.

Increasing animal volume per unit mass could affect many aspects of performance, e.g. increasing drag, lever arms for locomotion, or niche space required. Based on the scaling of the tracheal system in grasshoppers, a 1 kg grasshopper would have a volume of 3.7 l, greatly increasing the mass-specific need for nutrient investment in the exoskeleton and tracheal system, and probably the susceptibility to breakage (Greenlee *et al.* 2009). Hypermetry of the tracheal system could also lead to displacement of other tissues, reducing the performance of animals relative to those with a similar mass. The per cent of body volume occupied by non-respiratory tissues falls quadratically with mass, from 92 per cent in 10 mg grasshoppers to 59 per cent in 10 g animals, and extrapolated to 27 per cent in theoretical 1 kg grasshoppers (Greenlee *et al.* 2009). Potentially, survival or reproduction of larger grasshoppers could decrease relative to smaller individuals owing to reduced locomotory, digestive or reproductive capacities associated with such volume-specific decreases in functional tissue content.

Increasing tracheal hypermetry could also directly limit maximal insect size by filling all available space within key regions of the body that cannot be expanded for biomechanical reasons. In interspecific comparisons of beetles, the most dramatic example of hypermetry occurred at the connection between legs and body (Kaiser *et al.* 2007). Across four species of beetles, the cross-sectional area of the leg orifice scales with mass<sup>0.77</sup>, but the tracheal diameter penetrating the orifice scales with mass<sup>1.02</sup> (above the 0.67 predicted by isometry), occupying increasingly larger fractions of the exoskeleton in larger animals. Extrapolating these trends of tracheal hypermetry indicates that in the largest extant beetle, the leg will be already 90 per cent full of trachea, suggesting a spatial limitation (Kaiser *et al.* 2007). Studies of the scaling of respiratory and non-respiratory structures in the largest extant insects are needed to test these extrapolations.

Because insect tracheal diameters are smaller and tracheoles are fewer when animals are reared at higher oxygen levels (Harrison *et al.* 2006), a higher aPO<sub>2</sub> would allow insects to achieve larger size with smaller proportional tracheal volumes, with less material or spatial or energy cost of the respiratory system, with less displacement of other tissues, and before reaching spatial constraints. It is plausible that a reduced investment in the tracheal system at higher aPO<sub>2</sub> is a benefit for insects of all sizes, but because insects experience tracheal hypermetry, it seems to be particularly important for larger insects, and thus a reasonable mechanism by which aPO<sub>2</sub> might drive evolution of larger insect sizes. Further studies on the impact of oxygen on tracheal systems in a wider range of taxonomic groups and sizes of insects are necessary to support this hypothesis.

#### 4. ALTERNATIVE EXPLANATIONS FOR GIGANTISM IN THE LATE PALAEOZOIC AND THE SMALL SIZE OF INSECTS RELATIVE TO VERTEBRATES

Multiple alternative explanations are possible for Palaeozoic gigantism in insects; however, in general these have not been tested empirically. One possible explanation for Palaeozoic insect gigantism is niche displacement. Since the body sizes of animals respond to predation and competition (Blackburn & Gaston 1994), it is plausible that giant insects evolved into open niches, but that these species were later displaced by vertebrates filling the same ecological roles. Evolution of these giants has also been hypothesized to have occurred as a response to stable, optimal environmental conditions, and the availability of coal-swamp forests as a new habitat with little competition or predation (Briggs 1985). Such improved environmental conditions could directly increase insect abundances and/or shift body size distributions. The large size of some of the Palaeozoic insects could have been the result of size-selection by predators, or the need for greater force generation in ground litter filled with tree branches (Shear & Kukalova-Peck 1990).

Although the distribution of sizes of insects and vertebrates overlap, the mean and maximal sizes of terrestrial insects are smaller than for terrestrial vertebrates. As noted above, for many years it has been hypothesized that the small size of insects is partially owing to their use of a tracheal respiratory system; evidence that atmospheric hyperoxia might enable larger insects would substantially strengthen this argument. However, the observation that terrestrial crustaceans and arachnids lacking tracheae are also small relative to vertebrates suggests that other constraints may exist. Two possible constraints are the open circulatory system and the exoskeleton of arthropods.

In terrestrial species, blood must be pumped against gravity, suggesting that larger species would require higher pressures or reduced peripheral resistance. The open circulatory systems of terrestrial arthropods generate low pressures relative to the closed circulatory systems of vertebrates (Burggren *et al.* 1990; Ichikawa 2009). Such open circulatory systems might be unable to produce sufficient pressures to drive adequate flows in arthropods comparable in size to large vertebrates. Insects, however, possess accessory pulsatile organs that enhance blood flow in critical anatomical locations like legs, wings and antennae (Pass 2000), so it is possible that the possession of those accessory hearts renders high blood pressure unnecessary. Unfortunately, no study has yet tested the scaling of cardiovascular pressures or resistances in terrestrial arthropods for such limitations.

If the exoskeleton of larger arthropods should be as strong and durable as smaller animals, a greater proportion of their body needs to be dedicated to exoskeletal material, if the material properties do not change (Price 1997). For similar reasons, larger vertebrates have relatively thicker bones (McMahon 1973). Biomechanical constraints imposed by the exoskeleton on large insects may be particularly important during moulting, when the force of gravity on soft exoskeletons has the potential to 'produce an animal like a great tough pancake' (Currey 1970). The larger mass of aquatic arthropods (e.g. lobsters) compared with

terrestrial ones supports a biomechanical constraint hypothesis, since aquatic organisms do not have to support their mass and thus experience less stress on their exoskeleton, especially during moulting (Smock 1980; Sabo *et al.* 2002). Exoskeletal thickness and energy storage of the leg does increase with size during ontogeny in locusts (Katz & Gosline 1992), but the mass of the exoskeleton scales isometrically with body mass in interspecific comparisons across insects (Lease 2008). As yet there are no data indicating that larger insects are more susceptible to exoskeletal damage, and as larger insect species do not invest more in the exoskeleton (Lease 2008), empirical support for a biomechanical constraint on size is lacking.

## 5. CONCLUSIONS

Insects larger than humans populate our movies and nightmares, but it seems unlikely that they will ever actually exist on earth. However, insects as large as sea gulls did exist in the Palaeozoic, and they stimulate our imagination. The complex interactions between aPO<sub>2</sub>, organisms and communities (figure 2) make it challenging to reject or accept the hypothesis that extant insects are small owing to possession of a tracheal respiratory system, and that hyperoxia enabled their Palaeozoic gigantism. Nonetheless, recent empirical data provide a wealth of plausible mechanisms by which aPO<sub>2</sub> may play a critical role in the evolution of insect size. Although alternate hypotheses exist for both Palaeozoic gigantism and the small size of insects relative to vertebrates, these have received minimal experimental investigation. Improved palaeontological data, and multi-generational selection experiments examining effects of oxygen on size in environments that require activity and flight may be particularly useful in addressing these questions. As we contemplate potential major future changes in the Earth's ecosystem, it is of concern that we do not yet understand the mechanisms for such fundamental and striking biological patterns. Answers will come from a renewed focus on animal systems physiology, in collaboration with evolutionary biologists, ecologists, palaeontologists and geologists.

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