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Functional Hypoxia in Insects: Definition, Assessment, and Consequences for Physiology, Ecology, and Evolution

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Abstract

Insects can experience functional hypoxia, a situation in which O_2 supply is inadequate to meet oxygen demand. Assessing when functional hypoxia occurs is complex, because responses are graded, age and tissue dependent, and compensatory. Here, we compare information gained from metabolomics and transcriptional approaches and by manipulation of the partial pressure of oxygen. Functional hypoxia produces graded damage, including damaged macromolecules and inflammation. Insects respond by compensatory physiological and morphological changes in the tracheal system, metabolic reorganization, and suppression of activity, feeding, and growth. There is evidence for functional hypoxia in eggs, near the end of juvenile instars, and during molting. Functional hypoxia is more likely in species with lower O_2 availability or transport capacities and when O_2 need is great. Functional hypoxia occurs normally during insect development and is a factor in mediating life-history trade-offs.

1. INTRODUCTION

Hypoxia is a major focus of research in fields ranging from comparative physiology to biomedicine, yet general textbooks might lead one to conclude that hypoxia is rarely a problem for insects because of their efficient tracheal respiratory system. However, emerging research has revealed important roles for hypoxia in multiple physiological, developmental, and ecological functions in insects, and for many questions concerning hypoxia, insects have become leading models. Here, we begin by defining functional hypoxia and then discuss its pathological consequences and the various physiological changes occurring in response to functional hypoxia. In Section 4, we explore different approaches to assessing functional hypoxia, a topic with underappreciated complexity. Subsequently, we review how ecological, life history, and developmental factors affect the occurrence of functional hypoxia, with a particular focus on temperature. Finally, we propose future questions for the field, some of which are insect specific, but most of which are broadly applicable to animal ecophysiology, such as the interactions between oxygen signaling, development, and inflammation and the role of functional hypoxia in determining thermal responses of animals.

2. DEFINING FUNCTIONAL HYPOXIA

Environmental hypoxia can be simply defined as a partial pressure of oxygen (P_{O_2}) that is less than the current level of around 21 kPa (normoxia). Often, however, insects can maintain many functions at much lower environmental P_{O_2} values because of intrinsically high tracheal capacities and by use of compensatory processes, such as increasing ventilation. Conversely, there is evidence that sometimes insect tissues are limited in function by insufficient O_2 availability even in normoxic conditions. Furthermore, how insects respond to environmental hypoxia varies substantially, at least partly on the basis of species or life-stage ecology. Thus, we define functional hypoxia as the conditions under which an individual has a demand for O_2 that exceeds its capacity for supply despite compensations, with a resulting decrease in internal P_{O_2} and/or suppression of biological functions such as homeostasis, activity, and growth (**Figure 1*a***).

3. WHAT HAPPENS DURING FUNCTIONAL HYPOXIA

Like other animals (71), insects need to balance the opposing risks of oxygen toxicity and deprivation by matching their capacity for O_2 uptake to their demand for O_2, and they have many physiological mechanisms to do so (**Figure 1*a***) (53, 58). In response to either a decrease in ambient O_2 availability or an increase in O_2 demand due to increased growth, activity, or body temperature, insects can rapidly increase O_2 delivery to their tissues—for example, by opening spiracles and increasing abdominal pumping. Functional hypoxia occurs when these rapid response mechanisms to increase O_2 supply are insufficient or unsustainable, threatening oxidative ATP production. In response, insects can activate short-term mechanisms (e.g., suppressed activity) and long-term mechanisms (e.g., suppressed growth) to reduce ATP need. Additionally, insects have long-term methods for increasing ATP production—for example, by tracheal proliferation to improve O_2 supply and by recruiting anaerobic metabolic pathways for ATP production. Together, these responses may avert a major shortage of ATP.

Functional hypoxia is graded, with more detrimental effects occurring at lower internal P_{O_2} values and longer exposure times. Thus, finding unambiguous indicators of functional hypoxia is problematic. Clearly, when metabolic rate becomes O_2 limited, and ATP is also produced anaerobically, this provides a clear signal that ATP demand has exceeded that which can be supplied by oxidative catabolism. We describe this situation as severe functional hypoxia (**Figure 1*b***).

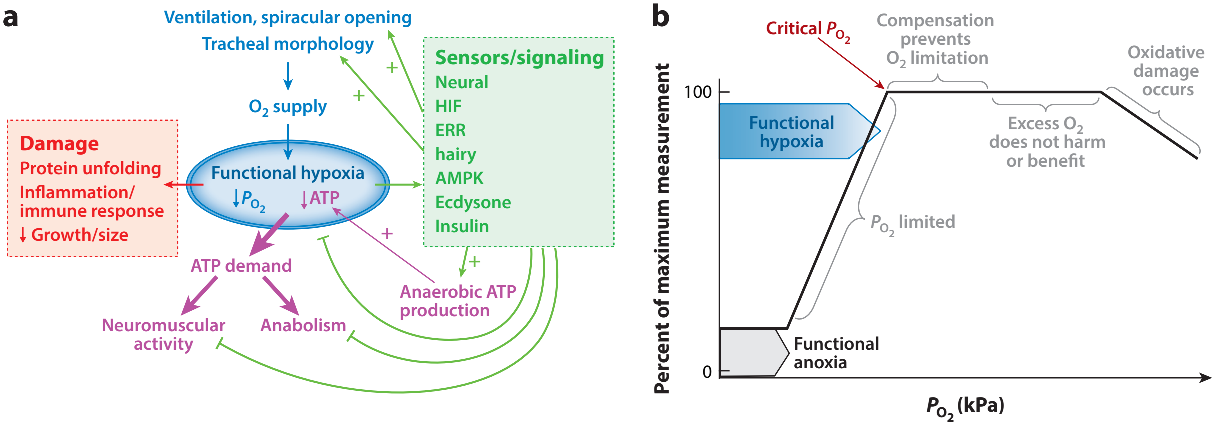


Figure 1

(*a*) Functional hypoxia occurs when O_2 supply is insufficient for oxidative metabolism to meet ATP demand. O_2 supply (*blue*) depends on tracheal morphology and physiological factors such as the degree of spiracular opening and ventilation. ATP turnover (*purple*) depends on ATP demand; during functional hypoxia, ATP can be supplemented by anaerobic ATP production. Functional hypoxia causes damage (*red*). A wide variety of sensing systems (*green*) respond to decreased internal P_{O_2} or ATP, acting to improve O_2 supply, decrease ATP demand, and increase anaerobic ATP production. (*b*) A hypothetical performance curve for O_2 effects on an organism. The P_{O_2} for the various transitions varies across and within species, life stages, temperatures, duration of exposure, and the dependent variable measured. Abbreviations: AMPK, adenosine monophosphate–activated protein kinase; ERR, estrogen-related receptor; HIF, hypoxia inducible factor; P_{O_2}, partial pressure of oxygen.

As described below, this often occurs in insects at ambient P_{O_2} values below 5 kPa. However, in many instances, insects respond to decreases in O_2 levels in a graded, neuroendocrine-mediated manner by suppressing behavior and growth at P_{O_2} values well above those that would lead to ATP limitation or accumulation of anaerobic end products. We describe this situation as mild functional hypoxia because of the O_2-dependent decrease in functions that are likely to be related to fitness. If the P_{O_2} is so low that aerobic metabolism is essentially zero, we term this functional anoxia; because of the high capacity of the tracheal system, this normally occurs at P_{O_2} values well below 1 kPa in insects (**Figure 1*b***).

3.1. Damage Resulting from Functional Hypoxia

We have a remarkably poor understanding of the very fundamental question of how hypoxia inflicts damage. With mild hypoxia (often at ambient P_{O_2} of 5–15 kPa), the primary responses demonstrated have been decreased survival, growth rate, body size, and reproduction (26, 57). Gene expression of heat shock proteins increases at 4-kPa P_{O_2} in *Drosophila* (89), which is approximately at the boundary between mild and severe functional hypoxia in this species (12). What are the mechanisms responsible for decreased survival during mild functional hypoxia? Perhaps, increased generation of reactive O_2 species (ROS) causes damage, but we are unaware of data to support this possibility in this range of P_{O_2} values. Another possibility is that ATP use is shifted toward compensatory responses, and decreased use of ATP for maintenance and/or repair causes damage. Possibly, the damage occurs at particular times (e.g., at the end of the instar or during molting) or in particular tissues. Hypoxia-induced decreases in growth rates and body size are known to be mediated by neuroendocrine signaling (**Figure 1*a***) (see Section 3.6). Although these compensatory responses may prevent more severe functional hypoxia, they may also be considered damage if they negatively impact fitness.

Even under very severe functional hypoxia/anoxia, the mechanisms of damage may seem obvious, but still many questions remain. Strong decreases in ATP levels will likely lead to ionic and osmotic disruptions that can cause damage to proteins. In many (141) but not all (66) insects, ambient anoxia quickly causes a rapid decrease in ATP levels, but whether and how this is causally linked to loss of ionic/osmotic homeostasis and damage to macromolecules has not been well demonstrated. Severe hypoxia/anoxia, especially when combined with reoxygenation, has been associated with an increase in production of ROS in mammals (25), so this represents an alternate or additional pathway of damage to nucleotides, proteins, and lipids (104). The expression of heat shock proteins in *Drosophila* strongly affects survival of severe hypoxia (3, 4, 89, 92), demonstrating the importance of protein unfolding in hypoxia-induced death.

Inflammation/immune response genes are strongly upregulated in both mild and severe hypoxia (4, 92). Whether this is a response to damage or alternatively is due to cross-talk of signaling pathways is unclear, because local hypoxia is commonly associated with infection (34). Hypoxia may make insects more susceptible to microbial infections if the insect's metabolism is more suppressed than that of the microbe. In support of this hypothesis, exposure to several days of hypoxia (5-kPa P_{O_2}) reduced the survival of *Tribolium* beetles infected with the entomopathogenic fungus *Beauveria bassiana* (93).

3.2. Short-Term Responses to Increase O_2 Supply

Behavioral escape from environmental hypoxia is often observed in insects. When faced with hypoxia, larval damselflies become positively phototaxic, moving toward the surface of the water, where oxygenation is likely better (1). Hypoxia-escape behavior observed in *Drosophila* larvae and adults is at least partially mediated by nitric oxide signaling (146) and a neuronal, O_2-sensing atypical guanylyl-cyclase (139).

Insects also respond to ambient hypoxia by increasing O_2 supply through increases in spiracular opening and ventilation. Opening of the spiracles and ventilatory movements, such as abdominal pumping, are both controlled by the central nervous system in response to O_2 (13, 15). In aquatic insects, the ventilatory response to hypoxia mainly serves to increase convection of external water. Most mayflies increase gill-beating rates, and damselflies move their abdominal gills and flex their wing pads to aid O_2 uptake (1). Similarly, in response to hypoxia, dragonflies increase rectal pumping, caddisflies initiate abdominal undulations, and stoneflies perform pushup behaviors (41, 111, 127, 145).

Compensatory responses to hypoxia are usually incomplete, and a decrease in tracheal P_{O_2} is not fully averted (98). During flight, increased tracheal conductance does not match the increase in O_2 need, and so flight muscle P_{O_2} drops from 10 kPa at rest to 6–8 kPa (83, 84). Thus, insects can tolerate considerable variation in internal P_{O_2}. If an insect has a critical tissue P_{O_2} for metabolic rate of 4 kPa, and normoxic tissue P_{O_2} is 10 kPa, there will be a 6-kPa P_{O_2} gradient that insects can "use" in response to hypoxia, either as a so-called safety margin or to increase O_2 supply during short bouts of activity (56) or spiracular closure (64). While the actual P_{O2} values undoubtedly vary among insects, this principle of permitting a decrease in internal P_{O_2} to increase the P_{O_2} gradient or cope with hypoxia is likely quite general among insects.

3.3. Longer-Term Developmental Responses to Increase O_2 Supply

Local hypoxemia causes the tracheal system to branch and invade regions of hypoxic tissue (144). Multiple studies have now demonstrated that chronic rearing in mild hypoxia increases the size and/or number of tracheae (73, 94, 130). The supply of O_2 can also be enhanced by reducing the distance from tracheole to mitochondria by reductions in cell size, as happens in *Drosophila melanogaster* reared under mild hypoxia (59, 110).

Effects of hypoxia on cell size and tracheal growth are mediated by hypoxia-inducible factor (HIF) signaling (reviewed in Reference 23). In contrast to mammalian systems, only one HIF has been identified in insects (85). HIF signaling is primarily regulated posttranslationally; however, in insects, hypoxic expression of HIF-α may vary at both the protein level and the transcript level (85, 95, 105). Prolyl hydroxylase (known as fatiga in *Drosophila*) uses O_2 to hydroxylate HIF-α (known as sima in *Drosophila*) for degradation. Under hypoxic conditions, HIF-α accumulates, dimerizes with HIF-β (tango in *Drosophila*), and enters the nucleus, where it acts as a transcription factor. HIF-signaling responses in *Drosophila* to hypoxia occur at higher P_{O_2} in the tracheae than in other tissues (85). HIF-stimulated increases in tracheation should increase tracheal system conductance and elevate the P_{O_2} of tissue, reducing the need for other tissue-level responses to ambient hypoxia.

HIF signaling plays important roles in development in normoxia (6, 107), suggesting that mild functional hypoxia during development may be normal. Sima-null *Drosophila* mutants (lacking HIF signaling) reared in normoxia differ strongly from control animals in the levels of many sugars and components of the pentose phosphate pathway (89). Tracheation decreases when insects are reared in a hyperoxic atmosphere (61, 81, 130), likely representing a decrease in the normal stimulation of tracheal development by HIF; this response may function to prevent O_2 poisoning or simply to reduce unnecessary investment in tracheae.

In many vertebrates and aquatic crustaceans, hemoglobin levels increase in hypoxia. Although hemoglobin has been identified in several insect orders, especially in the tracheae, its role in O_2 homeostasis and transport remains unclear in most species (14, 43). In *D. melanogaster*, knockdown of glob1 (one of three hemoglobins in this species, expressed in tracheae and fat body) reduces survival in hypoxia, supporting a role for the tracheal globin in oxygen transport (43). In the aquatic larvae of *Chironomus* spp., hemoglobin improves oxygen transport at low P_{O_2} (being nonfunctional at normoxia) (152), and exposure to mild (but not severe) hypoxia can induce an increase in hemoglobin concentrations (44). In contrast, for reasons that are unclear, hemoglobin gene expression decreases in response to hypoxia in *Drosophila* (42). Unfortunately, insufficient studies have been performed to determine what general patterns may exist for how insect hemoglobins are regulated by hypoxia.

3.4. Anaerobic ATP Production and Responses that Increase Anaerobic Capacities

When O_2 supply is inadequate to allow sufficient oxidative ATP production to meet demand, insects switch at least partially to anaerobic pathways for ATP production. In insects, lactate, alanine, succinate, malate, alpha-glycerol phosphate, glycerol, glycerol-3-P, acetate, and ethanol have been reported to accumulate as a result of anaerobic metabolism (67). Interestingly, succinate accumulation inhibits the HIF prolyl hydroxylase, which initiates a HIF-mediated hypoxia response (28). In most insects, anaerobic ATP production is likely to be a time-limited survival strategy, but, when coupled with metabolic depression, survival times of insects can be days or even weeks even in complete anoxia (52, 65).

With chronic exposure to mild or severe hypoxia, insects upregulate genes coding for proteins involved in glycolysis and anaerobic ATP production (89, 92). Hypoxia-induced induction of glycolytic genes can be either HIF dependent or HIF independent, in many cases with the involvement of the estrogen-related receptor (which binds to the inducible protein component of HIF) (89). Glycolytic enzymes upregulated by hypoxia (4-kPa P_{O_2}) in late-third-instar larval *Drosophila* include lactate dehydrogenase and phosphofructokinase (89). HIF signaling also induces amylase production during hypoxia, facilitating glycogen breakdown (89, 92). Activation of hydroxymethyl–coenzyme A synthase in mildly hypoxic larvae suggests possible shifts toward production of ketone bodies from fatty acids for use as fuel for the nervous system, similar to mammals (89).

3.5. Short-Term Behavioral Responses that Reduce O_2 Demand

Mild hypoxia can suppress many behaviors, such as general activity (129), feeding (38), and flight (115), reducing O_2 demand. At least some of these responses are mediated by neuronal O_2-sensing pathways (139).

3.6. Long-Term Developmental Effects that Reduce O_2 Demand

Perhaps the parameters most sensitive to mild hypoxia are growth and size. Most insects that have been tested grow more slowly and are smaller in 10-kPa P_{O_2} (57). Few have been tested between 21 and 10 kPa, but *Drosophila* are smaller even in 15-kPa P_{O_2} (110), so size may be very sensitive to hypoxia. In contrast, effects of hyperoxia are not consistent, enlarging size in some species but not others (40, 130). Because suppression of growth and size occurs at P_{O_2} levels above those that reduce short-term metabolic rates (57), and because hypoxic exposure at egg, larval, or pupal stages leads to reductions in size (59), these growth and size changes seem likely to be programmed responses rather than being due to direct ATP limitation.

How hypoxia reprograms the body size in insects is unclear, although such responses are likely mediated by neuroendocrine responses that may be both cell autonomous and systematic. Hypoxia appears to inhibit growth and anabolism via alterations in insulin and TOR (target of rapamycin) signaling (33, 117, 147). Additionally, chronic rearing in mild hypoxia (10-kPa P_{O_2}) elevates basal ecdysone levels possibly via HIF signaling (20), and ecdysone has been shown to suppress growth via alterations in insulin signaling (30). The O_2-sensitive, Cu^{2+}-dependent enzyme peptidylglycine-amidating monooxygenase regulates the bioactivity of several insect peptide hormones in response to hypoxia and is likely another important neuroendocrine response system for hypoxia (122).

Long-term exposure to hypoxia causes downregulation of mitochondrial systems. One transcription factor, *hairy*, is induced by both mild and severe hypoxia in adult *Drosophila* and acts to suppress tricarboxylic acid cycle enzymes, likely leading to metabolic suppression (92, 154). In overwintering gall insects, decreases in levels of cytochrome c oxidase mRNA (*COX*) in hypoxia without concomitant changes in mitochondrial DNA levels suggest that mitochondria were altered toward metabolic suppression (100).

4. ASSESSING FUNCTIONAL HYPOXIA: METHODOLOGICAL AND CONCEPTUAL CHALLENGES

Given the graded nature of functional hypoxia and the compensatory responses mounted by insects, assessing functional hypoxia is not straightforward. Below, we discuss challenges for different approaches, which range from taking a whole-organism perspective using critical P_{O_2} tests to molecular approaches, such as metabolomics.

4.1. Critical P_{O_2} Tests

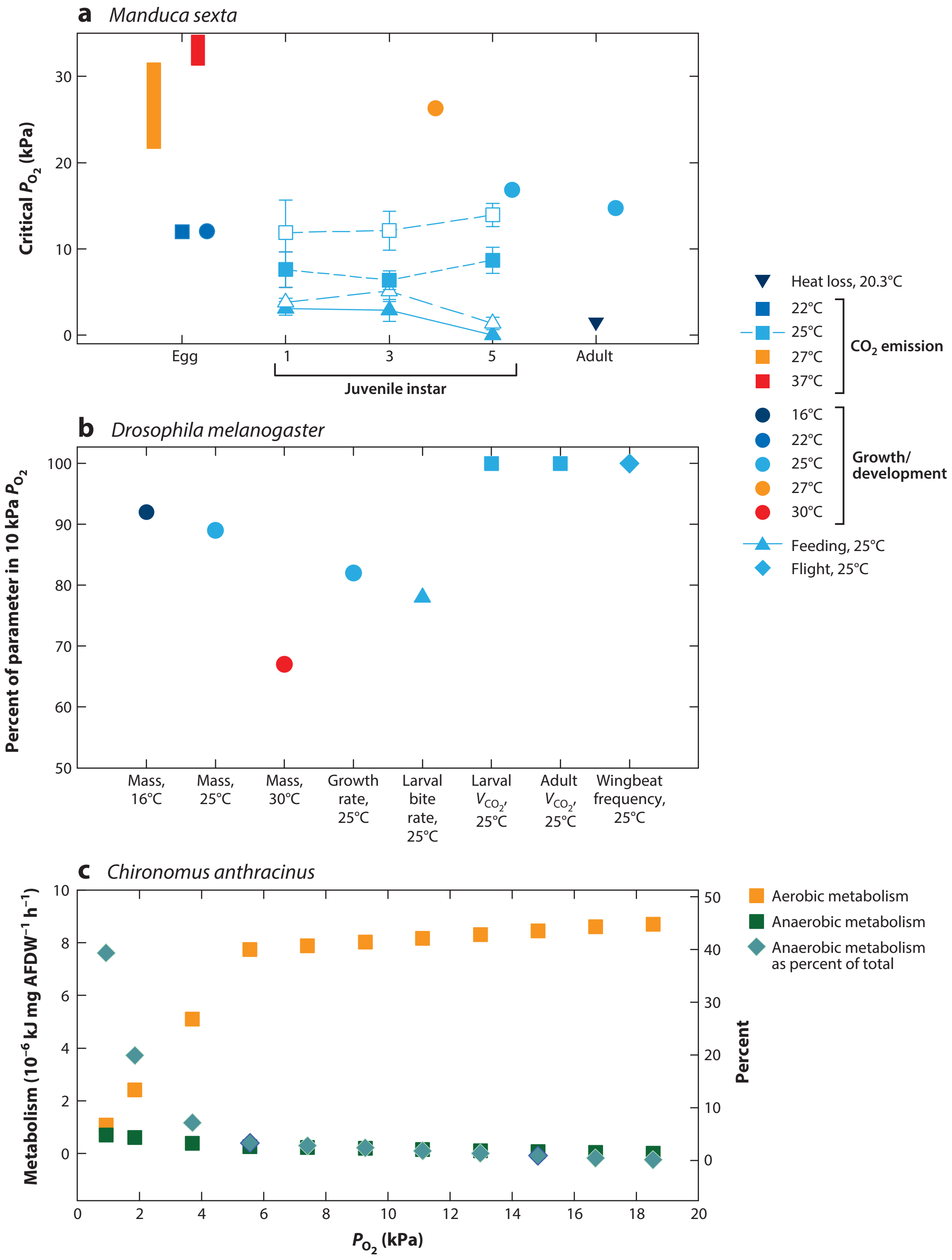
With a critical P_{O_2} test, atmospheric P_{O_2} is manipulated in a graded fashion, ideally above and below normoxia (21-kPa P_{O_2}), and consequences on performance (e.g., metabolic rate, activity, growth) are evaluated. Theoretically, performance at the lowest range of P_{O_2} need not be zero because of anaerobically supported metabolism but will increase gradually with increasing P_{O_2} over the range where P_{O_2} limits function in a graded manner, followed by an optimal range over which P_{O_2} has no effect (because of compensatory responses and saturation of O_2-using reactions) and a higher range in which hyperoxia causes damage due to reactive O_2 species production (**Figure 1*b***). The critical P_{O_2} is defined as the ambient P_{O_2} below which function declines (becomes O_2 limited) (**Figure 1*b***), which can be at or even above normoxia.

Since the critical P_{O_2} depends on both the supply and demand for O_2, in general, we predict that critical P_{O_2} will tend to be higher when metabolic rates are higher. As supporting examples, CO_2 emission during flight in the dragonfly *Erythemis simplicicollis* peaked at environmental P_{O_2} values of 30 and 50 kPa, suggesting that aerobic metabolism might be O_2 limited during flight in normoxia (55). Similarly, in stonefly nymphs (*Dinocras cephalotes*) exposed to extreme heat, environmental hyperoxia increased survival, and individual nymphs were worse at tolerating heat stress when their metabolic rate strongly increased with increasing temperatures (132).

4.1.1. Technical challenges of critical P_{O_2} tests. Although critical P_{O_2} tests seem straightforward, many potential technical challenges exist. In some aquatic insects, there is no clearly definable critical P_{O_2} because metabolism falls throughout all ranges of P_{O_2} tested, first with a low slope and then a steep slope (11, 88). This may indicate the two phases of mild and severe functional hypoxia, with the actual critical P_{O_2} being above normoxia. The order of exposure to the P_{O_2} values can affect critical P_{O_2}, owing to changes in insect performance over time (e.g., insects may change fuel sources or "settle" over time as they recover from handling stress). While exposing insects to ambient P_{O_2} in random order may control for such biases, exposure to very low P_{O_2} values early on may induce anaerobic activity and confound performance during the remainder of the test. Therefore, the least problematic protocol may be to use different individuals for each test P_{O_2} or to use progressive hypoxia, interspersed with regular assessment of function at normoxia. Because behavior can change over time, behavior should be monitored, quantified, and reported. Good flushing of the system is necessary (91); if the time constant of the respirometry system is slow relative to the rate of P_{O_2} manipulation, the measured gas exchange rate may be artificially elevated by the metabolism occurring at the prior P_{O_2}. Also, if the exposure duration is short, investigators should estimate internal O_2 stores to ensure these are not buffering hypoxic effects.

Another potential methodological problem arises from the fact that most studies of the critical P_{O_2} of terrestrial insects measure only CO_2 emission rate. Without knowledge of the respiratory exchange ratio, shifts in the metabolic fuel usage can lead to incorrect estimation of metabolic rate. In addition, during hypoxic exposure, insects often decrease tissue P_{CO_2} (partial pressure of carbon dioxide) and form CO_2 from bicarbonate, temporarily elevating CO_2 emission rate (45, 46), potentially biasing critical P_{O_2}. Studies measuring both CO_2 emission and O_2 consumption did not find differences in the corresponding critical P_{O_2} values for the locust *Schistocerca americana* (46) and the midge *C. anthracinus* (**Figure 2**) (51). This is a hopeful finding, but more tests of this potential problem should be conducted.

4.1.2. Conceptual challenges related to exposure time of critical P_{O_2} tests. Mild hypoxia (e.g., 10-kPa P_{O_2}) often has no significant effect on the metabolic rate of quiescent insects measured over the short term (hours) but does cause suppressed feeding, growth, and body size with chronic exposure (57). For example, in *D. melanogaster* (**Figure 2*b***), larval feeding is suppressed in 10-kPa P_{O_2} (38), while the critical P_{O_2} for larval metabolic rate is 1–2 kPa, when measured using progressive hypoxia over about two hours (80). These differences in O_2 sensitivity seem unlikely to


a Manduca sexta
Critical P_{O_2} (kPa)
0
10
20
30
Egg
1
3
5
Adult
Juvenile instar
Heat loss, 20.3°C
22°C
25°C
27°C
37°C
CO_2 emission
16°C
22°C
25°C
27°C
30°C
Growth/development
Feeding, 25°C
Flight, 25°C
b Drosophila melanogaster
Percent of parameter in 10 kPa P_{O_2}
50
60
70
80
90
100
Mass, 16°C
Mass, 25°C
Mass, 30°C
Growth rate, 25°C
Larval bite rate, 25°C
Larval V_{CO_2}, 25°C
Adult V_{CO_2}, 25°C
Wingbeat frequency, 25°C
c Chironomus anthracinus
Metabolism (10^{-6} kJ mg AFDW^{-1} h^{-1})
0
2
4
6
8
10
Percent
0
10
20
30
40
50
0
2
4
6
8
10
12
14
16
18
20
P_{O_2} (kPa)
Aerobic metabolism
Anaerobic metabolism
Anaerobic metabolism as percent of total

be due simply to elevation in metabolic rates in feeding animals since the larvae in the Klok et al. (80) study were measured immediately after removal from food. Additionally, longer exposure times potentially allow insects to make developmental, compensatory responses to hypoxia such as proliferation of tracheoles. Thus, our assessment of the O_2 sensitivity of the insect depends strongly on the parameter measured and the duration of exposure.

Only one limited study has measured the effect of exposure time on critical P_{O_2} for metabolic rate, and a significant and complex effect was observed. For adult *S. americana*, the calculated critical P_{O_2} increased from 1.8 to 3.25 to 4 kPa as the time of exposure to each P_{O_2} step rose from 3 to 15 to 60 min. However, the critical P_{O_2} was unaffected by exposure duration for first-instar animals (46). It would be very useful to measure the effect of exposure durations, from a few minutes of exposure up to the entire developmental period, on critical P_{O_2} values for metabolic rate and other functions in a range of insects.

4.2. Accumulation of Anaerobic End Products

As noted above (Section 3.4), with severe hypoxia, insects accumulate anaerobic end products, and measurement of this process is a useful way to assess severe functional hypoxia. Unfortunately, changes in the concentrations of anaerobic end products can occur for reasons other than functional hypoxia, including mitochondrial damage (e.g., by freezing), without any problems with O_2 delivery. In some rapidly growing insects, pyruvate, lactate, and other compounds may accumulate because of aerobic glycolysis, a condition in which elevation of the rate of glycolysis relative to mitochondrial substrate consumption occurs, providing substrates for the synthesis of nucleotides and membrane lipids. Lactate accumulates in insect embryos and *Drosophila* larvae, partly independent of P_{O_2}, possibly because of aerobic glycolysis (118, 124, 125).

Given the uncertainty in using anaerobic end products as indices of hypoxia, how well do measures of those compounds match with measures of critical P_{O_2} in insects? Data for larvae of the midge *C. anthracinus* show that the critical P_{O_2} for O_2 uptake matches well with the onset of anaerobic metabolism (**Figure 2*c***). Adult fruit flies showed some accumulation of anaerobic end products in 4-kPa P_{O_2} and much stronger changes in 0.5-kPa P_{O_2} (39), consistent with measures of the critical P_{O_2} for metabolic rate for adults of this species (3.4 kPa) (12). While these data suggest that accumulation of anaerobic end products can serve as good markers for severe functional hypoxia, this should be confirmed by also testing whether hyperoxia abolishes their accumulation.

4.3. Transcriptional Responses

4.3.1. Effects of dose on the transcriptional response to hypoxia.

Studies of transcriptional responses to hypoxia have not examined a wide range of P_{O_2} values, so exposure and dosage

Figure 2

(*a*) Critical P_{O_2} values for different developmental stages, temperatures, and parameters for *Manduca sexta*. Open symbols indicate late instar; closed symbols indicate early instar. Note that the greatest sensitivity to hypoxia (highest critical P_{O_2}) occurs in eggs or late in the instars and at higher temperatures. (*b*) Effect of reducing P_{O_2} to 10 kPa on various parameters for *Drosophila melanogaster*. Note that mild hypoxia strongly decreased growth, size, and feeding behavior without significant effects on metabolic rates and that higher temperatures exacerbated effects of hypoxia. (*c*) Effects of P_{O_2} on aerobic and anaerobic metabolism in the aquatic larvae of the midge *Chironomus anthracinus* (data from Reference 51). Note that the contribution of anaerobic metabolism is nonzero above the critical P_{O_2}, increases as P_{O_2} decreases, and increases sharply at the critical P_{O_2}. Abbreviations: AFDW, ash free dry weight; P_{O_2}, partial pressure of oxygen; V_{CO_2}, carbon dioxide production.

responses are poorly known. The best data are for *Drosophila*, in which transcription has been studied in the range of the critical P_{O_2} for aerobic metabolic rate (4–5-kPa P_{O_2}) and in severe hypoxia (1.5–0-kPa P_{O_2}). Li et al. (89) compared transcriptional responses to 0.5-kPa P_{O_2} after one or six hours of exposure to severe hypoxia and found that expression of many genes (such as heat shock proteins) increased with time and many more genes were upregulated with longer exposure duration. Fewer genes (mostly transcription factors and editing genes) were induced by mild hypoxia, and these did not include heat shock proteins, consistent with the hypothesis that insects primarily mount compensatory responses at mild hypoxia, while shifting primarily to damage control in severe hypoxia.

4.3.2. Transcriptional responses linked to functional hypoxia. Mild hypoxia increases expression of *HIF-α* (*sima*) and genes that stimulate tracheal growth and proliferation (e.g., *sequoia*, a fibroblast growth factor receptor gene) (89). HIF signaling also induces a set of genes that negatively regulate HIF signaling, including prolyl hydroxylase (fatiga in *Drosophila*, also referred to as EGL-9) and the von Hippel Lindau factor (89), which, somewhat ironically, are some of the best markers of activation of HIF signaling.

In addition to HIF signaling, functional hypoxia changes the transcription of a variety of metabolic genes (see Sections 3.4 and 3.6), and potentially, glycolytic/mitochondrial gene expression ratios could be used to indicate functional hypoxia. Additionally, mild hypoxia tends to induce genes that code for enzymes that use O_2 as a substrate, and these seem to be excellent candidates for markers of functional hypoxia. These include the *Drosophila* genes coding for phenylalanine hydroxylase, multiple cytochrome P450 genes, spermine oxidase, choline dehydrogenase, flavin-linked sulfhydryl oxidase, desaturases, and, as described above, prolyl hydroxylase (89, 92). Assuming that enzyme protein and activity levels are also increased, upregulation of these may serve to maintain function of these O_2-dependent enzymatic functions at lower tissue P_{O_2}.

Functional hypoxia also appears to upregulate expression of genes and enzymes that are related to protection from oxidative stress. For example, the enzyme spermine oxidase produces spermidine, which was reported to alleviate oxidative stress in *Bombyx mori* (151). Another group of genes that are strongly induced in adult *Drosophila*, in both mild and severe hypoxia, are those coding for periredoxins (92). Often considered as antioxidants (119), periredoxins may plausibly be produced to cope with the oxidative damage associated with severe hypoxia (116). However, the effects of dose and duration of functional hypoxia on mitochondrial ROS production remain poorly understood. Most tissues probably do not increase ROS production in response to mild hypoxia, but O_2-sensing tissues likely do, contributing to O_2 sensing (99). As periredoxins appear to be broadly expressed, an alternative explanation for the increased induction of periredoxins in mild hypoxia is that they are involved in the inflammatory responses occurring in mild hypoxia (121).

4.4. Overview of Assessment Approaches

Because severe functional hypoxia can cause limitations on mitochondrial ATP production, it may share signaling pathways activated by other environmental factors that also affect mitochondrial ATP production (e.g., thermal or xenobiotic damage to mitochondria, starvation). In general, experimental approaches that directly manipulate O_2 level (and in particular test whether exposure to mild hyperoxia reverses observed effects) are most useful for distinguishing responses to functional hypoxia from responses to other kinds of cellular stress. However, because hyperoxia can both cause damage and stimulate signaling pathways by increasing ROS production (18, 118), and because tracheal system limitations might not allow hyperoxia to relieve all functional hypoxia, results of gas manipulations should be viewed with some caution. While biochemical and transcriptional assessment approaches have the potential to falsely implicate functional hypoxia, they provide detailed mechanistic insights critical for understanding the graded consequences of and responses to functional hypoxia and potentially allow for tissue-specific investigations. Thus, integrated biochemical, transcriptional and gas-manipulation studies are recommended (138).

5. WHEN DOES FUNCTIONAL HYPOXIA OCCUR? ECOLOGICAL AND LIFE HISTORY ASSOCIATIONS WITH FUNCTIONAL HYPOXIA

As the occurrence of functional hypoxia results from the interplay between environmental availability of oxygen and the insect's capacity for taking up and delivering oxygen to its tissues, functional hypoxia is more likely in species that experience lower O_2 availability or have reduced oxygen transport capacities (e.g., water breathers, life stages such as eggs that depend on diffusion) and when O_2 need is great (e.g., high temperatures, flight).

5.1. Environmentally Hypoxic Habitats

5.1.1. Aquatic habitats. Aquatic insects that perform gas exchange underwater encounter environmental hypoxia much more frequently than air-breathing insects. Inherent to the low capacitance of water for O_2, processes that consume or generate O_2 quickly result in large changes in aquatic P_{O_2}, especially in small bodies of water. Further confounding this problem, aquatic P_{O_2} takes much longer to equilibrate with atmospheric P_{O_2} because of the much slower rates of diffusion (32). Similar considerations may apply to the habitats of species that are essentially aquatic (e.g., endoparasites, endophytic species, rotten-wood borers, rotten-fruit specialists, etc.). In small eutrophic waters, hypoxic events tend to grow more severe during the night when respiration is not counteracted by photosynthesis, but during daytime, the reverse can happen, exposing insects to hyperoxia. For example, aquatic P_{O_2} was found to vary tenfold in a shallow, sun-exposed pool (**Figure 3**), where increased temperature and consequent reduction in the solubility of O_2 and the increase in dissolved O_2 worked in tandem to create a hyperoxic P_{O_2} during the daytime (135). The few studies investigating direct effects of ambient hypoxia on aquatic insects have shown shifts in time budgets, with increased time spent on ventilation and concomitant reductions on other activities such as feeding (127, 140). Aquatic insects differ widely in their ability to survive hypoxic exposure (127, 133), and such differential survival likely underlies shifts in assemblage composition. Some are able to tolerate prolonged, extreme hypoxia, establishing ecological dominance (51). Combined field (transplant) and experimental approaches show reductions in insect activity coupled with mortality in response to hypoxia (96) and hypoxic stress being aggravated by warming both in the lab and the field (136).

5.1.2. Terrestrial hypoxic habitats: soils, flooded burrows, etc. Although less common, terrestrial insects can also experience environmental hypoxia. This generally occurs in habitats where equilibration rates with the atmosphere are low and where microbial activity is high, including soils, dung, grain piles, or in burrows that become flooded (67). There is some evidence that insects in life stages that live in such habitats have adaptations that enable them to cope with environmental hypoxia and have a greater capacity to survive anoxia, but the data are equivocal and phylogenetically controlled analyses are missing (22, 53, 65). Dung beetles, which likely encounter both hypoxia and hypercapnia in their dung-filled burrows, have been suggested to have particularly low critical P_{O_2} values for metabolism (69), but the cavity-nesting bee *Megachile rotundata* had critical P_{O_2} values similar to other insects (109). Pupae of *Manduca sexta*, which are soil dwellers, were better than fifth-instar larvae at surviving flooding (150), but in the semiterrestrial

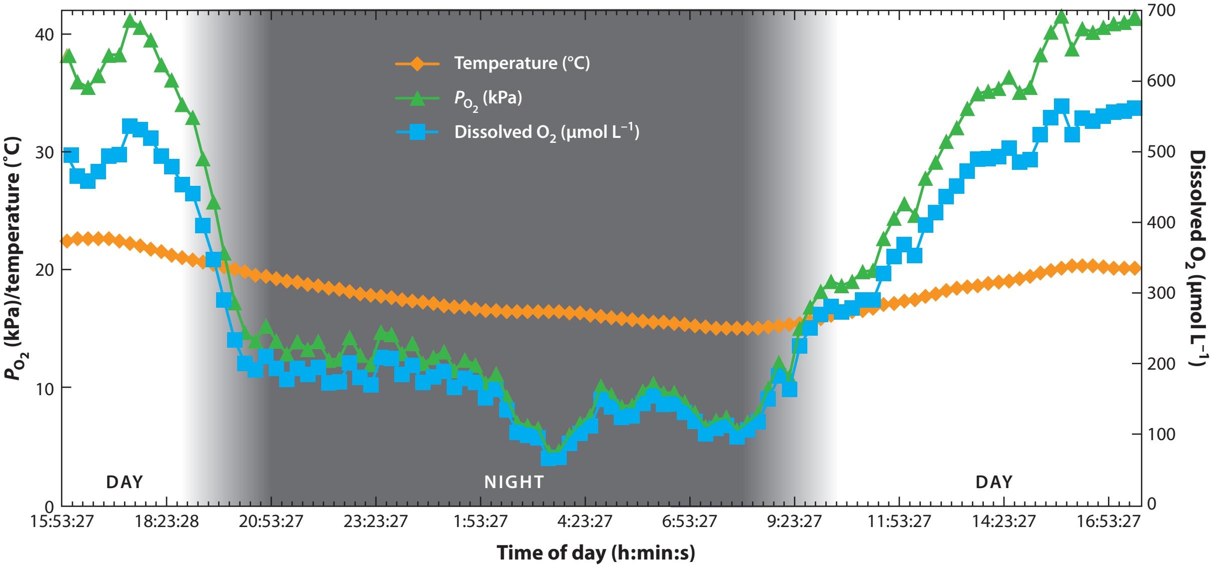


Figure 3

Diurnal fluctuations in temperature, partial pressure of oxygen (P_{O_2}), and dissolved O_2 in a small, freshwater pond dominated by insects (e.g., the mayfly *Cloeon dipterum* and the hemipteran *Plea minutissima*), measured on a sunny day during the fall equinox of 2016 (9).

caddisfly *Ironoquia plattensis*, pupae are more sensitive than larvae to flooding, and both stages can experience flooding (22).

5.1.3. High-altitude habitats. In high-altitude habitats, the lower air density causes lower P_{O_2} but also a proportional increase in the diffusivity of O_2; so altitude has no effect on diffusion of gases in air but will affect advective gas transport. In general, colder temperatures and shorter growing seasons at high altitude are thought to limit insects more strongly than hypoxia (36). However, these conclusions are generally based on analysis of short-term effects, such as the finding that hypoxia does not affect the walking performance of flies until P_{O_2} falls below those they are likely to experience (35). High-altitude hypoxia might have more important effects on growth and size, but this has not been studied. Hypoxia begins to affect flight metabolism of honey bees at approximately the P_{O_2} at the altitude above which they do not occur (74). Migratory locusts from high elevation in Tibet had increased hypoxia tolerance, decreased mitochondrial injury, and increased catalytic efficiency of electron transport compared to lowland locusts, providing direct evidence for high-altitude adaptation (153).

In water at high altitude, the lower P_{O_2} results in lower diffusion rates of O_2. The diffusivity of oxygen actually decreases because of lower temperatures at high altitude (133). The diversity of stream insects decreased along an altitudinal cline and was found to be best explained by oxygen availability (72).

5.2. Effect of Respiratory Mode and Tracheal System Capacities on Functional Hypoxia

Insects that exchange gases via systems with low transport capacity either for phylogenetic or environmental reasons are likely to be more susceptible to functional hypoxia. Verberk & Bilton

(133) compared pairs of species of aquatic insects across four different insect orders to investigate the link between respiratory mode and susceptibility to functional hypoxia. Species that were poor at regulating O_2 uptake (i.e., relying on gas exchange across the integument or aquatic gill respiration) were consistently more vulnerable to the synergistic effects of warming and hypoxia than those that had better regulatory capacity (i.e., species that pump water across enclosed gills or are air breathers). Differences in respiratory mode may also underlie differences across life stages in their susceptibility to functional hypoxia (Section 5.4).

5.3. Locomotion

Flight is completely aerobic in insects, and flight muscles have extensive tracheation and ventilation (58). However, the high rates of O_2 consumption during flight result in much higher critical P_{O_2} values than at rest (54). The critical P_{O_2} for flight metabolic rate was above 21 kPa in the dragonfly *Erythemis simplicicollis* (55), and flight behavior was decreased by mild hypoxia in multiple dragonfly species (62) and tethered flying grasshoppers (114). An important role for functional hypoxia in mediating flight traits has been revealed for populations of the fritillary butterfly, which vary in dispersal capacities and in allozymes for succinate dehydrogenase (143). Variation in these allozymes affected tracheation of the flight muscles and was coupled to performance and postflight muscle damage. This allozyme effect is likely mediated by variation in accumulation of succinate during functional hypoxia, producing variable inhibition of prolyl hydroxylase, HIF signaling, and tracheal proliferation (97). The adaptive significance of variation in dispersal capacity may relate to trade-offs with other life-history traits such as water loss and survival, suggesting that functional hypoxia may have an important role during development in mediating life-history trade-offs (97).

5.4. Ontogeny

5.4.1. Life-stage differences. Life stages may differ in their sensitivity to functional hypoxia. This relates at least partly to differences in respiratory mode and capacities across life stages (Section 5.2). In grasshoppers, hypoxia sensitivity was highest in the youngest instars, possibly because of their lack of air sacs and their concomitant low capacity for convective ventilation (46, 49). In contrast, in larval *Ma. sexta*, hypoxia sensitivity of metabolic rate did not vary across the juvenile instars (**Figure 2*a***) (48). In both *S. americana* (46) and *Ma. sexta* (**Figure 2*a***) (142), the critical P_{O_2} for quiescent metabolic rate of adults is lower than for juveniles, probably because of the high tracheal capacities of the flying adults (49, 77). As further evidence of the diversity of responses across species, in the solitary bee *Me. rotundata*, critical P_{O_2} did not vary throughout the prepupal, pupal, or adult stages (109).

Although eggs have received little attention, they may be one of the most interesting stages from the standpoint of functional hypoxia because of their relatively limited capacity for behavioral and physiological modulation of O_2 supply. Consequently, eggs could be a particularly sensitive stage, often constituting the weakest link when assessing the vulnerability of a species (128). In multiple insects, lactate accumulates in the egg and can be partly decreased by hyperoxia, indicating some functional hypoxia (118). Eggs of *Ma. sexta* experience delayed development and reduced survival in mild hypoxia (9–15-kPa P_{O_2}) (149), whereas juveniles reared in 10-kPa P_{O_2} have no developmental delays, although they are smaller than normoxic controls (**Figure 2*a***). Similarly, the strongest evidence for a role of O_2 limitation in setting thermal tolerance in an air-breathing insect to date comes from eggs. The O_2 consumption of eggs increases more than 4× during development in *Ma. sexta* (148), and as temperature increases, critical P_{O_2} rises above normoxic levels, suggesting that warm eggs are O_2 limited in normal conditions (**Figure 2*a***). In some insects, eggs are the only life stage in which hemocyanin is expressed (14), and knockdown experiments with *Locusta migratoria* have demonstrated that hemocyanin is important for egg development and survival (27), further corroborating the high sensitivity of eggs to functional hypoxia.

Molting may be a time in which functional hypoxia is routine, as during the molt process, the tracheal system is briefly disrupted (21). Blocking O_2-sensing atypical guanylyl-cyclases caused substantial lethality during ecdysis in *Drosophila* (106). By contrast, the relative activities of anaerobic and aerobic catabolic enzymes in the midgut do not vary through the molt in *Ma. sexta*, arguing against the occurrence of O_2 limitations (24).

5.4.2. At the end of larval instars. There is considerable evidence that O_2 supply does not increase in proportion to the rise in demand as an insect progresses through juvenile instars. Most insects experience large (2–10×) increases in body mass from the beginning to the end of each instar, with consequent large increases in O_2 demand (17, 47), and an increasing mismatch between supply and demand of O_2 over time has been hypothesized to induce molting (19, 47, 78, 126). How the morphology of the tracheal system, especially the tracheoles, changes within the instar remains poorly studied. However, the sclerotized spiracles and likely the largest tracheal tubes of the tracheal system can only increase in size at the molt. Helm & Davidowitz (60) demonstrated that in *Ma. sexta*, the mass of the tracheal system increases substantially within the final larval instar, but to what extent this represents increases in wall thickness versus internal diameters is not clear. Tracheal volumes of *Ma. sexta* decrease within the third and fourth instars and increase only marginally within the fifth instar (16). In grasshoppers, tracheal volumes decrease strongly within the instar, likely because of air sac compression caused by tissue growth (29, 77, 87). Late-stage grasshoppers have higher ventilation frequencies and lower tidal volumes than early stage animals, supporting the hypothesis that compression of the tracheal system later within the instar affects the physiological performance of the tracheal system (47).

As predicted by these trends in metabolism, tracheal morphology, and ventilation, the critical P_{O_2} for some functions rises as insects grow within an instar. For example, in *Ma. sexta*, the critical P_{O_2} values for both feeding and CO_2 emission rise late in the instar (**Figure 2*a***) (48). In grasshoppers, the critical P_{O_2} for CO_2 emission increased with time within the instar for adults but not juveniles, while the critical P_{O_2} for abdominal pumping increases strongly later in the instar (47). A wide range of transcriptional responses to hypoxia occur in late-third instar but not mid-second instar *Drosophila*, suggesting that O_2 demand approaches or outstrips supply in this life stage (89). Tests of within-instar hypoxia are clearly needed in other species of insect to determine whether the occurrence of functional hypoxia late within instars is widespread.

5.5. Body Size Variation Across Species and Populations

For terrestrial insects, evidence that larger insect species are more likely to experience functional hypoxia is lacking. The critical P_{O_2} for multiple parameters has been shown to be independent of body size in resting beetles (86), grasshoppers (50), and a suite of different insect species (54), as well as in flying dragonflies (62). This preservation of the match between O_2 supply and demand across size may be partially attributed to increased mass-specific investment in the tracheal system in larger insects (75).

For aquatic insects, O_2 uptake is more challenging, and plausibly, the interactions between body size and functional hypoxia may differ (131). For aquatic nymphs of the stonefly *D. cephalotes*, hypoxia limited their ability to survive heat stress in a size-dependent manner, with larger animals being more likely to succumb to heat, especially under hypoxia (132, 138).

5.6. Temperature

Given that energy metabolism increases with temperature in ectotherms, functional hypoxia may be more likely in insects under high temperatures. However, functional hypoxia need not be an inevitable outcome of higher temperatures, as insects can and do compensate for increased O_2 demand by increasing O_2 uptake and transport.

5.6.1. Acute effects of temperature. Energy metabolism increases with temperature in an exponential manner. Functional hypoxia may limit thermal performance windows (112) and possibly survival of heat stress (reviewed in Reference 137). While hypoxia and its signaling pathways clearly can induce thermal stress responses, such as the expression of heat shock proteins (7), there is an active debate on whether insufficient O_2 delivery is a primary driver of upper thermal limits or, alternatively, one of several physiological processes that are compromised near thermal limits (37, 113). Disentangling cause and effect is difficult. While insufficient oxygen delivery may sometimes be a primary cause for an animal to succumb to acute heat stress, heat stress itself may impair performance, including uptake and transport of oxygen (137). In terrestrial insects, several studies demonstrate adequate O_2 delivery near upper thermal limits for survival under normoxic conditions (90, 103, 108). Klok et al. (82) argued that O_2 delivery is less likely to become rate limiting at higher temperatures in terrestrial insects compared to other animal groups because of the high capacity of their tracheal system. In support of an oxygen transport capacity hypothesis, evidence for O_2 limitation of upper thermal tolerance is more abundant for aquatic compared to air-breathing insects (82, 133, 137). Experimentally reducing the capacity of a bimodal breather to perform aerial gas exchange by denying it access to air caused upper thermal tolerance to become O_2 limited (133). Thus, it appears that insects or certain insect life stages with lower O_2 regulatory capacities are more susceptible to whole-organism O_2 limitation at extreme heat.

In contrast, those studies that addressed how lower critical temperature is affected by O_2 availability found no influence of O_2 levels on lower thermal limits in the beetle *Tenebrio molitor* (123) or in the moth *Thaumatotibia leucotreta* (8). Cold has been reported to increase metabolic markers associated with anaerobic metabolism, such as alanine in the fly *Sarcophaga crassipalpis* (102), lactate in moths *T. leucotreta*, and succinate in the midge *Belgica antarctica* (101). However, such metabolic markers and the onset of anaerobic metabolism do not necessarily imply that functional hypoxia is involved. If cold induces mitochondrial failure or inhibition of mitochondrial function, then the falling ATP production rates may be compensated for by anaerobic metabolism, even if the mitochondria are fully oxygenated (8, 137).

5.6.2. Chronic effects of temperature. From an ecological perspective, whether oxygen limitation or some other mechanism limits survival at extreme temperatures may be a moot point if these extreme temperatures are not experienced in the field and if distribution limits associated with warmer temperatures are more related to sublethal effects of chronically higher temperatures (10). At the chronic high temperatures that reduce survival in the mayfly *Neocloeon triangulifer*, there is no evidence for O_2 limitation, suggesting chronic thermal limits are not related to functional hypoxia (76). However, hypoxia amplified the negative effects of rising water temperatures in the field for two different species of mayfly for which lab experiments also suggested oxygen limitation of upper thermal limits (136). Interactive effects of hypoxia and warming were equally strong if not stronger in the field compared to a lab setting, suggesting that over longer time periods, insects might experience functional hypoxia even under moderate warming or mild hypoxia. The difference between these two studies may relate to species differences, biotic interactions, and episodic hypoxia that were present in the field but not in the lab, or perhaps functional hypoxia was undetected in the lab (e.g., if it only occurred in molting larvae).

5.6.3. Oxygen and the temperature–size rule. Oxygen limitation has also been suggested to explain the temperature–size rule, whereby ectothermic animals, such as insects, grow faster but to a smaller size when reared under warmer temperatures (70). If O_2 limitation becomes stronger near the end of the instar under warmer conditions, this could be a proximate mechanism, preventing an insect from growing to a larger size under warm conditions. Functional hypoxia in developing animals may mediate the trade-off between growth and development that is thought to underlie the temperature–size rule. Indeed, the graded nature of functional hypoxia, how it is likely exacerbated by warm conditions, and the observation that already mild hypoxia can impair growth well before the onset of anaerobic metabolism suggest that there is certainly much scope for such an oxygen perspective on the temperature–size rule. Warmer temperatures also tend to reduce cell size in ectotherms, including insects (5, 31), adding further support to the idea that insects experience onset of functional hypoxia under warm conditions and the resulting reductions in cell size help to maintain aerobic metabolism and reduce body size (2, 63).

Both experimental and comparative work support a role for O_2 in size determination. Experimentally rearing animals under different temperature and O_2 conditions showed that animals grown under warm conditions are smaller (i.e., a temperature–size rule response) and this was exacerbated by rearing in hypoxia (40, 68). Comparative work shows that size responses to temperature differ between terrestrial and aquatic insects (70), a finding that could be linked to decreased O_2 availability in water or to respiratory advantages to being bigger in cold water to overcome viscosity problems (131). If indeed sensitivity to hypoxia is promoted by larger size only in some insects, this could also explain the divergent thermal responses among different insects (79). The observation that clines in body size tend to be stronger along latitudinal than in altitudinal gradients, where they may be absent or even reversed (120), also deserves further study since both altitudinal clines and latitudinal clines encompass a similar thermal gradient but a different P_{O_2} gradient.

6. SUMMARY AND FUTURE ISSUES

In summary, functional hypoxia is widespread in insects and plays important roles in many aspects of function, as found for other animals. Many important questions remain, but here, we propose some critical research questions for this field.

1. How does O_2 reprogram body size?
2. What is the role of O_2 signaling in normal development?
3. What are the major mechanisms of damage during mild and severe hypoxia?
4. Why is inflammation tightly linked with hypoxia?
5. Does functional hypoxia explain the temperature–size rule?
6. Do O_2-supply limitations explain maximal limits on insect size, and do these limitations vary in aquatic and terrestrial insects?
7. How important is functional hypoxia to determining the distributions of aquatic insects and their sensitivity to environmental stress and xenobiotics?
8. How commonly, and by what mechanisms, does functional hypoxia give rise to developmental life history trade-offs?
9. What are the major adaptations to environmental hypoxia in different habitats and clades?

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LITERATURE CITED

1. Apodaca CK, Chapman LJ. 2004. Larval damselflies in extreme environments: behavioral and physiological response to hypoxic stress. *J. Insect. Physiol.* 50:767–75
2. Atkinson D, Morley SA, Hughes RN. 2006. From cells to colonies: At what levels of body organization does the 'temperature-size rule' apply? *Evol. Dev.* 8:202–14
3. Azad P, Ryu J, Haddad GG. 2011. Distinct role of Hsp70 in *Drosophila* hemocytes during severe hypoxia. *Free Radic. Biol. Med.* 51:530–38
4. Azad P, Zhou D, Russo E, Haddad GG. 2009. Distinct mechanisms underlying tolerance to intermittent and constant hypoxia in *Drosophila melanogaster*. *PLOS ONE* 4:e5371
5. Azevedo RBR, French V, Partridge L. 2002. Temperature modulates epidermal cell size in *Drosophila melanogaster*. *J. Insect. Physiol*. 48:231–37
6. Azevedo SV, Caranton OAM, de Oliveira TL, Hartfelder K. 2011. Differential expression of hypoxia pathway genes in honey bee (*Apis mellifera* L.) caste development. *J. Insect. Physiol.* 57:38–45
7. Baird NA, Turnbull DW, Johnson EA. 2006. Induction of the heat shock pathway during hypoxia requires regulation of heat shock factor by hypoxia-inducible factor-1. *J. Biol. Chem.* 281:38675–81
8. Boardman L, Sørensen JG, Koštál V, Šimek P, Terblanche JS. 2016. Chilling slows anaerobic metabolism to improve anoxia tolerance of insects. *Metabolomics* 12:176
9. Bohm S, ter Haar S, Ludwig K, Mathijssen D. 2016. *Het verband tussen de ademhalingsstrategieën van macroinvertebraten en de zuurstofcondities in duinplassen*. Course Report Systeemecologie, Radboud Univ. Nijmegen, Neth.
10. Bozinovic F, Calosi P, Spicer JI. 2011. Physiological correlates of geographic range in animals. *Annu. Rev. Ecol. Evol. Syst.* 42:155–79
11. Brodersen KP, Pedersen O, Walker IR, Tranekjaer Jensen M. 2008. Respiration of midges (Diptera; Chironomidae) in British Columbian lakes: oxy-regulation, temperature and their role as palaeo-indicators. *Freshwater Biol.* 53:593–602
12. Burggren W, Souder BM, Ho DH. 2017. Metabolic rate and hypoxia tolerance are affected by group interactions and sex in the fruit fly (*Drosophila melanogaster*): new data and a literature survey. *Biol. Open* 6:471–80
13. Burkett BN, Schneiderman HA. 1967. Control of spiracles in silk moths by oxygen and carbon dioxide. *Science* 156:1604–6
14. Burmester T. 2015. Evolution of respiratory proteins across the Pancrustacea. *Integr. Comp. Biol.* 55: 792–801
15. Bustami HP, Harrison JF, Hustert R. 2002. Evidence for oxygen and carbon dioxide receptors in insect CNS influencing ventilation. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 133:595–604
16. Callier V, Nijhout HF. 2011. Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis. *PNAS* 108:14664–69
17. Callier V, Nijhout HF. 2012. Supply-side constraints are insufficient to explain the ontogenetic scaling of metabolic rate in the tobacco hornworm, *Manduca sexta*. *PLOS ONE* 7:e45455

18. Callier V, Nijhout HF. 2013. Body size determination in insects: a review and synthesis of size- and brain-dependent and independent mechanisms. *Biol. Rev.* 88:944–54
19. Callier V, Nijhout HF. 2014. Plasticity of insect body size in response to oxygen: integrating molecular and physiological mechanisms. *Curr. Opin. Insect. Sci.* 1:59–65
20. Callier V, Shingleton AW, Brent CS, Ghosh SM, Kim J, Harrison JF. 2013. The role of reduced oxygen in the developmental physiology of growth and metamorphosis initiation in *Drosophila melanogaster*. *J. Exp. Biol.* 216:4334–40
21. Camp AA, Funk DH, Buchwalter DB. 2014. A stressful shortness of breath: Molting disrupts breathing in the mayfly *Cloeon dipterum*. *Freshwater Sci.* 33:695–99
22. Cavallaro MC, Hoback WW. 2014. Hypoxia tolerance of larvae and pupae of the semi-terrestrial caddisfly (Trichoptera: Limnephilidae). *Ann. Entomol. Soc. Am.* 107:1081–85
23. Centanin L, Gorr TA, Wappner P. 2010. Tracheal remodelling in response to hypoxia. *J. Insect. Physiol.* 56:447–54
24. Chamberlin ME, Gibellatio CM, Noecker RJ, Dankoski EJ. 1997. Changes in midgut active ion transport and metabolism during larval-larval molting in the tobacco hornworm (*Manduca sexta*). *J. Exp. Biol.* 200:643–48
25. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, et al. 2000. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1α during hypoxia: a mechanism of O_2 sensing. *J. Biol. Chem.* 275:25130–38
26. Charette M, Darveau C-A, Perry SF, Rundle HD. 2011. Evolutionary consequences of altered atmospheric oxygen in *Drosophila melanogaster*. *PLOS ONE* 6:e26876
27. Chen B, Ma R, Ma G, Guo X, Tong X, et al. 2015. Haemocyanin is essential for embryonic development and survival in the migratory locust. *Insect. Mol. Biol.* 24:517–27
28. Chinopoulos C. 2013. Which way does the citric acid cycle turn during hypoxia? The critical role of the α-ketoglutarate dehydrogenase complex. *J. Neurosci. Res.* 91:1030–33
29. Clarke KU. 1957. On the role of the tracheal system in the post-embryonic growth of *Locusta migratoria* L. *Proc. R. Entomol. Soc. Lond. A* 32:67–79
30. Colombani J, Bianchini L, Layalle S, Pondeville E, Dauphin-Villemant C, et al. 2005. Antagonistic actions of ecdysone and insulins determine final size in *Drosophila*. *Science* 310:667–70
31. Czarnoleski M, Cooper BS, Kierat J, Angilletta MJ. 2013. Flies developed small bodies and small cells in warm and in thermally fluctuating environments. *J. Exp. Biol.* 216:2896–901
32. Dejours P. 1975. *Principles of Comparative Respiratory Physiology*. Amsterdam, Neth.: North-Holland
33. Dekanty A, Centanin L, Wappner P. 2007. Role of the hypoxia–response pathway on cell size determination and growth control. *Dev. Biol.* 306:339
34. D'Ignazio L, Bandarra D, Rocha S. 2015. NF-κB and HIF crosstalk in immune responses. *FEBS J.* 283:413–34
35. Dillon ME, Frazier MR. 2006. *Drosophila melanogaster* locomotion in cold thin air. *J. Exp. Biol.* 209: 364–71
36. Dillon ME, Frazier MR, Dudley R. 2006. Into thin air: physiology and evolution of alpine insects. *Integr. Comp. Biol.* 46:49–61
37. Ern R, Huong DTT, Phuong NT, Madsen PT, Wang T, Bayley M. 2015. Some like it hot: thermal tolerance and oxygen supply capacity in two eurythermal crustaceans. *Sci. Rep.* 5:10743
38. Farzin M, Albert T, Pierce N, VandenBrooks JM, Dodge T, Harrison JF. 2014. Acute and chronic effects of atmospheric oxygen on the feeding behavior of *Drosophila melanogaster* larvae. *J. Insect. Physiol.* 68:23–29
39. Feala JD, Coquin L, Zhou D, Haddad GG, Paternostro G, McCulloch AD. 2009. Metabolism as means for hypoxia adaptation: metabolic profiling and flux balance analysis. *BMC Syst. Biol.* 3:91
40. Frazier MR, Woods HA, Harrison JF. 2001. Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* 74:641–50
41. Genkai-Kato M, Nozaki K, Mitsuhashi H, Kohmatsu Y, Miyasaka H, Nakanishi M. 2000. Push-up response of stonefly larvae in low-oxygen conditions. *Ecol. Res.* 15:175–79
42. Gleixner E, Abriss D, Adryan B, Kraemer M, Gerlach F, et al. 2008. Oxygen-induced changes in hemoglobin expression in *Drosophila*. *FEBS J.* 275:5108–16

43. Gleixner E, Ripp F, Gorr TA, Schuh R, Wolf C, et al. 2016. Knockdown of *Drosophila* hemoglobin suggests a role in O_2 homeostasis. *Insect Biochem. Mol. Biol.* 72:20–30
44. Grazioli V, Rossaro B, Parenti P, Giacchini R, Lencioni V. 2016. Hypoxia and anoxia effects on alcohol dehydrogenase activity and hemoglobin content in *Chironomus riparius* Meigen, 1804. *J. Limnol.* 75: 347–54
45. Greenlee KJ, Harrison JF. 1998. Acid-base and respiratory responses to hypoxia in the grasshopper *Schistocerca americana*. *J. Exp. Biol.* 201:2843–55
46. Greenlee KJ, Harrison JF. 2004. Development of respiratory function in the American locust *Schistocerca americana* I. Across-instar effects. *J. Exp. Biol.* 207:497–508
47. Greenlee KJ, Harrison JF. 2004. Development of respiratory function in the American locust *Schistocerca americana* II. Within-instar effects. *J. Exp. Biol.* 207:509–17
48. Greenlee KJ, Harrison JF. 2005. Respiratory changes throughout ontogeny in the tobacco hornworm caterpillar, *Manduca sexta*. *J. Exp. Biol.* 208:1385–92
49. Greenlee KJ, Henry JR, Kirkton SD, Westneat MW, Fezzaa K, et al. 2009. Synchrotron imaging of the grasshopper tracheal system: morphological components of tracheal hypermetry and the effect of age and stage on abdominal air sac volumes and convection. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297:1343–50
50. Greenlee KJ, Nebeker C, Harrison JF. 2007. Body size-independent safety margins for gas exchange across grasshopper species. *J. Exp. Biol.* 210:1288–96
51. Hamburger K, Dall PC, Lindegaard C. 1994. Energy metabolism of *Chironomus anthracinus* (Diptera: Chironomidae) from the profundal zone of Lake Esrom, Denmark, as a function of body size, temperature and oxygen concentration. *Hydrobiologia* 294:43–50
52. Hamburger K, Dall PC, Lindegaard CL, Nilson IB. 2000. Survival and energy metabolism in an oxygen deficient environment. Field and laboratory studies on the bottom fauna from the profundal zone of Lake Esrom, Denmark. *Hydrobiologia* 432:173–88
53. Harrison JF. 2015. Handling and use of oxygen by pancrustaceans: conserved patterns and the evolution of respiratory structures. *Integr. Comp. Biol.* 55:802–15
54. Harrison JF, Klok CJ, Waters JS. 2014. Critical PO_2 is size-independent in insects: implications for the metabolic theory of ecology. *Curr. Opin. Insect. Sci.* 4:54–59
55. Harrison JF, Lighton JRB. 1998. Oxygen-sensitive flight metabolism in the dragonfly *Erythemis simplicicollis*. *J. Exp. Biol.* 201:1739–44
56. Harrison JF, Philips JE, Gleeson TT. 1991. Activity physiology of the two-striped grasshopper, *Melanoplus bivittatus*: gas exchange, hemolymph acid-base status, lactate production and the effect of temperature. *Physiol. Zool.* 64:451–72
57. Harrison JF, Shingleton AW, Callier V. 2015. Stunted by developing in hypoxia: linking comparative and model organism studies. *Physiol. Biochem. Zool.* 88:455–70
58. Harrison JF, Wasserthal LT, Chapman RF. 2013. Gaseous exchange. In *The Insects: Structure and Function*, ed. SJ Simpson, AE Douglas, pp. 501–45. New York: Cambridge Univ. Press
59. Heinrich EC, Farzin M, Klok CJ, Harrison JF. 2011. The effect of developmental stage on the sensitivity of cell and body size to hypoxia in *Drosophila melanogaster*. *J. Exp. Biol*. 214:1419–27
60. Helm BR, Davidowitz G. 2013. Mass and volume growth of an insect tracheal system within a single instar. *J. Exp. Biol.* 216:4703–11
61. Henry JR, Harrison JF. 2004. Plastic and evolved responses of larval tracheae and mass to varying atmospheric oxygen content in *Drosophila melanogaster*. *J. Exp. Biol*. 207:3559–67
62. Henry JR, Harrison JF. 2014. Body size effects on the oxygen-sensitivity of dragonfly flight. *J. Exp. Biol.* 217:3447–56
63. Hessen DO, Daufresne M, Leinaas HP. 2013. Temperature-size relations from the cellular-genomic perspective. *Biol. Rev.* 88:476–89
64. Hetz SK, Bradley TJ. 2005. Insects breathe discontinuously to avoid oxygen toxicity. *Nature* 433:516–19
65. Hoback WW. 2012. Ecological and experimental exposure of insects to anoxia reveals surprising tolerance. In *Anoxia: Evidence for Eukaryotic Survival and Paleontological Strategies*, ed. A Altenbach, JM Bernhard, J Seckback, pp. 169–88. New York: Springer

66. Hoback WW, Podrabsky JE, Higley LG, Stanley DW, Hand SC. 2000. Anoxia tolerance of con-familial tiger beetle larvae is associated with differences in energy flow and anaerobiosis. *J. Comp. Physiol. B* 170:307–14

67. Hoback WW, Stanley DW. 2001. Insects in hypoxia. *J. Insect. Physiol.* 47:533–42

68. Hoefnagel KN, Verberk WCEP. 2015. Is the temperature-size rule mediated by oxygen in aquatic ectotherms? *J. Therm. Biol.* 54:56–65

69. Holter P, Spangenberg A. 1997. Oxygen uptake in coprophilous beetles (*Aphodius*, Geotrupes, Sphaeridium) at low oxygen and high carbon dioxide concentrations. *Physiol. Entomol.* 22:339–43

70. Horne CR, Hirst AG, Atkinson D. 2015. Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species. *Ecol. Lett.* 18:327–35

71. Hsia CCW, Schmitz A, Lambertz M, Perry SF, Maina JN. 2013. Evolution of air breathing: oxygen homeostasis and the transitions from water to land and sky. *Compr. Physiol.* 3:849–915

72. Jacobsen D. 2008. Low oxygen pressure as a driving factor for the altitudinal decline in taxon richness of stream macroinvertebrates. *Oecologia* 154:795–807

73. Jarecki J, Johnson E, Krasnow MA. 1999. Oxygen regulation of airway branching in *Drosophila* is mediated by branchless FGF. *Cell* 99:211–20

74. Joos B, Lighton JRB, Harrison JF, Suarez RK, Roberts SP. 1997. Effects of ambient oxygen tension on flight performance, metabolism, and water loss of the honeybee. *Physiol. Zool.* 70:167–74

75. Kaiser A, Klok CJ, Socha JJ, Lee W-K, Quinlan MC, Harrison JF. 2007. Increase in tracheal investment with beetle size supports hypothesis of oxygen limitation on insect gigantism. *PNAS* 104:13198–203

76. Kim KS, Chou H, Funk DH, Jackson JK, Sweeney BW, Buchwalter DB. 2017. Physiological responses to short term thermal stress in mayfly larvae (*Neocloeon triangulifer*) in relation to upper thermal limits. *J. Exp. Biol.* 220:2598–605

77. Kirkton S, Hennessey L, Duffy B, Bennett M, Lee W-K, Greenlee K. 2012. Intermolt development reduces oxygen delivery capacity and jumping performance in the American locust (*Schistocerca americana*). *J. Comp. Physiol. B* 182:217–30

78. Kivelä SM, Friberg M, Wiklund C, Leimar O, Gotthard K. 2016. Towards a mechanistic understanding of insect life history evolution: oxygen-dependent induction of moulting explains moulting sizes. *Biol. J. Linn. Soc.* 117:586–600

79. Klok CJ, Harrison JF. 2013. The temperature size rule in arthropods: independent of macro-environmental variables but size dependent. *Integr. Comp. Biol.* 53:557–70

80. Klok CJ, Kaiser A, Lighton JRB, Harrison JF. 2010. Critical oxygen partial pressures and maximal tracheal conductances for *Drosophila melanogaster* reared for multiple generations in hypoxia or hyperoxia. *J. Insect. Physiol.* 56:461–69

81. Klok CJ, Kaiser A, Socha JJ, Lee WK, Harrison JF. 2016. Multigenerational effects of rearing atmospheric oxygen level on the tracheal dimensions and diffusing capacities of pupal and adult *Drosophila melanogaster*. In *Hypoxia*, ed. RC Roach, PD Wagner, PH Hackett. New York: Springer

82. Klok CJ, Sinclair BJ, Chown SL. 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *J. Exp. Biol.* 207:2361–70

83. Komai Y. 1998. Augmented respiration in a flying insect. *J. Exp. Biol.* 201:2359–66

84. Komai Y. 2001. Direct measurement of oxygen partial pressure in a flying bumblebee. *J. Exp. Biol.* 204:2999–3007

85. Lavista-Llanos S, Centanin L, Irisarri M, Russo DM, Gleadle JM, et al. 2002. Control of the hypoxic response in *Drosophila melanogaster* by the basic helix-loop-helix PAS protein similar. *Mol. Cell. Biol.* 22:6842–53

86. Lease HM, Klok CJ, Kaiser A, Harrison JF. 2012. Body size is not critical for critical P_{O_2} in scarabaeid and tenebrionid beetles. *J. Exp. Biol.* 215:2524–33

87. Lease HM, Wolf BO, Harrison JF. 2006. Intraspecific variation in tracheal volume in the American locust, *Schistocerca americana*, measured by a new inert gas method. *J. Exp. Biol.* 209:3476–83

88. Lencioni V, Bernabò P, Vanin S, Di Muro P, Beltramini M. 2008. Respiration rate and oxy-regulatory capacity in cold stenothermal chironomids. *J. Insect. Physiol.* 54:1337–42

89. Li Y, Padmanabha D, Gentile LB, Dumur CI, Beckstead RB, Baker KD. 2013. HIF- and non-HIF-regulated hypoxic responses require the estrogen-related receptor in *Drosophila melanogaster*. *PLOS Genet*. 9:e1003230
90. Lighton JRB. 2007. Hot hypoxic flies: Whole-organism interactions between hypoxic and thermal stressors in *Drosophila melanogaster*. *J. Therm. Biol*. 32:134–43
91. Lighton JRB. 2017. Limitations and requirements for measuring metabolic rates: a mini review. *Eur. J. Clin. Nutr*. 71:301–05
92. Liu G, Roy J, Johnson EA. 2006. Identification and function of hypoxia-response genes in *Drosophila melanogaster*. *Physiol. Genom*. 25:134–41
93. Lord JC. 2009. Efficacy of *Beauveria bassiana* for control of *Tribolium castaneum* with reduced oxygen and increased carbon dioxide. *J. Appl. Entomol.* 133:101–7
94. Loudon C. 1989. Tracheal hypertrophy in mealworms: design and plasticity in oxygen supply systems. *J. Exp. Biol.* 147:217–35
95. Ma E, Haddad GG. 1999. Isolation and characterization of the hypoxia-inducible factor 1β in *Drosophila melanogaster*. *Mol. Brain Res*. 73:11–16
96. Madsen PB, Morabowen A, Andino P, Espinosa R, Cauvy-Fraunie SC, et al. 2015. Altitudinal distribution limits of aquatic macroinvertebrates: an experimental test in a tropical alpine stream. *Ecol. Entomol.* 40: 629–38
97. Marden JH, Fescemyer HW, Schilder RJ, Doerfler WR, Vera JC, Wheat CW. 2012. Genetic variation in HIF signaling underlies quantitative variation in physiological and life-history traits within lowland butterfly populations. *Evolution* 67:1105–15
98. Matthews PGD, Snelling EP, Seymour RS, White CR. 2012. A test of the oxidative damage hypothesis for discontinuous gas exchange in the locust *Locusta migratoria*. *Biol. Lett*. 8:682–84
99. McElroy GS, Chandel NS. 2017. Mitochondria control acute and chronic responses to hypoxia. *Exp. Cell Res*. 356:217–22
100. McMullen DC, Storey K. 2008. Mitochondria of cold hardy insects: responses to cold and hypoxia assessed at enzymatic, mRNA and DNA levels. *Insect Biochem. Mol. Biol.* 38:367–73
101. Michaud MR, Benoit JB, Lopez-Martinez G, Elnitsky MA, Lee REJ, Denlinger DL. 2008. Metabolomics reveals unique and shared metabolic changes in response to heat shock, freezing and desiccation in the Antarctic midge, *Belgica antarctica*. *J. Insect. Physiol.* 54:645–55
102. Michaud MR, Denlinger DL. 2007. Shifts in the carbohydrate, polyol, and amino acid pools during rapid cold-hardening and diapause-associated cold-hardening in flesh flies (*Sarcophaga crassipalpis*): a metabolomic comparison. *J. Comp. Physiol. B* 177:753–63
103. Mölich AB, Förster TD, Lighton JRB. 2012. Hyperthermic overdrive: oxygen delivery does not limit thermal tolerance in *Drosophila melanogaster*. *J. Insect Sci*. 12:109
104. Monaghan P, Metcalfe NB, Roxana T. 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* 12:75–92
105. Morin P Jr., McMullen DC, Storey KB. 2005. HIF-1α involvement in low temperature and anoxia survival by a freeze tolerant insect. *Mol. Cell. Biochem* 280:99–106
106. Morton DB, Stewart JA, Langlais KK, Clemens-Grisham RA, Vermehren A. 2008. Synaptic transmission in neurons that express the *Drosophila* atypical soluble guanylyl cyclases, Gyc-89Da and Gyc-89Db, is necessary for the successful completion of larval and adult ecdysis. *J. Exp. Biol.* 211:1645–56
107. Mukherjee T, Kim WS, Mandal L, Banerjee U. 2011. Interaction between Notch and Hif-α in development and survival of *Drosophila* blood cells. *Science* 332:1210–13
108. Neven LG, Lehrman N, Hansen LD. 2014. Effects of temperature and modified atmospheres on diapausing 5th instar codling moth metabolism. *J. Therm. Biol.* 42:9–14
109. Owings AA, Yocum GD, Rinehart JP, Kemp WP, Greenlee KJ. 2014. Changes in respiratory structure and function during post-diapause development in the alfalfa leafcutting bee, *Megachile rotundata*. *J. Insect. Physiol.* 66:20–27
110. Peck LS, Maddrell SHP. 2005. Limitation of size by hypoxia in the fruit fly *Drosophila melanogaster*. *J. Exp. Zool. A* 303A:968–75

111. Philipson GN, Moorhouse BHS. 1974. Observations on ventilatory and net-spinning activities of larvae of the genus *Hydropsyche Pictet* (Trichoptera, Hydropsychidae) under experimental conditions. *Freshwater Biol.* 4:525–33

112. Pörtner H-O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213:881–93

113. Pörtner H-O, Giomi F. 2013. Nothing in experimental biology makes sense except in the light of ecology and evolution—correspondence on *J. Exp. Biol.* 216, 2771–2782. *J. Exp. Biol.* 216:4494–95

114. Rascón B, Harrison JF. 2004. Atmospheric oxygen effects on metabolic rate and behavior of tethered flying locusts. *Integr. Comp. Biol.* 44:627

115. Rascón B, Harrison JF. 2005. Oxygen partial pressure effects on metabolic rate and behavior of tethered flying locusts. *J. Insect. Physiol.* 51:1193–99

116. Rascón B, Harrison JF. 2010. Lifespan and oxidative stress show a nonlinear response to atmospheric oxygen level in *Drosophila*. *J. Exp. Biol.* 213:3441–48

117. Reiling JH, Hafen E. 2004. The hypoxia-induced paralogs *Scylla* and *Charybdis* inhibit growth by down-regulating S6K activity up stream of TSC in *Drosophila*. *Genes Dev.* 18:2879–92

118. Reynolds JA, Hand SC. 2009. Decoupling development and energy flow during embryonic diapause in the cricket, *Allonemobius socius*. *J. Exp. Biol.* 212:2065–74

119. Rhee SG, Woo HA, Kil IS, Bae SH. 2012. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. *J. Biol. Chem.* 287:4403–10

120. Shelomi M. 2012. Where are we now? Bergman's rule senso lato in insects. *Am. Nat.* 180:511–19

121. Shichita T, Hasegawa E, Kimura A, Morita R, Sakaguchi R, et al. 2012. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. *Nat. Med.* 18:911–17

122. Simpson PD, Eipper BA, Katz MJ, Gandara L, Wappner P, et al. 2015. Striking oxygen sensitivity of the peptidylglycine α-amidating monooxygenase (PAM) in neuroendocrine cells. *J. Biol. Chem.* 290: 24891–901

123. Stevens MM, Jackson S, Bester SA, Terblanche JS, Chown SL. 2010. Oxygen limitation and thermal tolerance in two terrestrial arthropod species. *J. Exp. Biol.* 213:2209–18

124. Tennessen JM, Baker KD, Lam G, Evans J, Thummel CS. 2011. The *Drosophila* estrogen-related receptor directs a metabolic switch that supports developmental growth. *Cell Metab.* 13:139–48

125. Tennessen JM, Bertagnolli NM, Evans J, Sieber MH, Cox J, Thummel CS. 2014. Coordinated metabolic transitions during *Drosophila* embryogenesis and the onset of aerobic glycolysis. *G3* 4:839–50

126. Välimäki P, Kivelä SM, Raitanen J, Pakanen V-M, Vatka E, et al. 2015. Larval melanism in a geometrid moth: promoted neither by a thermal nor seasonal adaptation but desiccating environments. *J. Anim. Ecol.* 84:817–28

127. van der Geest HG. 2007. Behavioural responses of caddisfly larvae (*Hydropsyche angustipennis*) to hypoxia. *Contrib. Zool.* 76:255–60

128. Van der Have TM. 2002. A proximate model for thermal tolerance in ectotherms. *Oikos* 98:141–55

129. Van Voorhies WA. 2009. Metabolic function in *Drosophila melanogaster* in response to hypoxia and pure oxygen. *J. Exp. Biol.* 212:3132–41

130. VandenBrooks JM, Munoz EE, Weed MD, Ford CF, Harrison MA, Harrison JF. 2012. Impacts of paleo-oxygen levels on the size, development, reproduction, and tracheal systems of *Blatella germanica*. *Evol. Biol.* 39:83–93

131. Verberk WCEP, Atkinson D. 2013. Why polar gigantism and Palaeozoic gigantism are not equivalent: effects of oxygen and temperature on the body size of ectotherms. *Funct. Ecol.* 27:1275–85

132. Verberk WCEP, Bilton DT. 2011. Can oxygen set thermal limits in an insect and drive gigantism? *PLOS ONE* 6:e22610

133. Verberk WCEP, Bilton DT. 2013. Respiratory control in aquatic insects dictates their vulnerability to global warming. *Biol. Lett.* 9:20130473

134. Verberk WCEP, Bilton DT. 2015. Oxygen-limited thermal tolerance is seen in a plastron-breathing insect and can be induced in a bimodal gas exchanger. *J. Exp. Biol.* 218:2083–88

135. Verberk WCEP, Bilton DT, Calosi P, Spicer JI. 2011. Oxygen supply in aquatic ectotherms: Partial pressure and solubility together explain biodiversity and size patterns. *Ecology* 92:1565–72

136. Verberk WCEP, Durance I, Vaughan IP, Ormerod SJ. 2016. Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. *Glob. Change Biol.* 22:1769–78
137. Verberk WCEP, Overgaard J, Ern R, Bayley M, Wang T, et al. 2016. Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 192:64–78
138. Verberk WCEP, Sommer U, Davidson RL, Viant MR. 2013. Anaerobic metabolism at thermal extremes: a metabolomic test of the oxygen limitation hypothesis in an aquatic insect. *Integr. Comp. Biol.* 53:609–19
139. Vermehren-Schmaedick A, Ainsley JA, Johnson WA, Davies S-A, Morton DB. 2010. Behavioral responses to hypoxia in *Drosophila* larvae are mediated by atypical soluble guanylyl cyclases. *Genetics* 186:183–96
140. Walshe BM. 1950. The function of haemoglobin in *Chironomus plumosus* under natural conditions. *J. Exp. Biol.* 27:73–95
141. Wegener G. 1993. Hypoxia and posthypoxic recovery in insects: physiological and metabolic aspects. In *Surviving Hypoxia: Mechanisms of Control and Adaptation*, ed. PW Hochachka, PL Lutz, T Sick, M Rosenthal, G van den Thillart, pp. 417–34. Boca Raton, FL: CRC Press
142. Wegener G, Moratzky T. 1995. Hypoxia and anoxia in insects: microcalorimetric studies on two species (*Locusta migratoria* and *Manduca sexta*) showing different degrees of anoxia tolerance. *Thermochim. Acta* 251:209–18
143. Wheat CW, Fescemyer HW, Kvist J, Tas EVA, Vera JC, et al. 2011. Functional genomics of life history variation in a butterfly metapopulation. *Mol. Ecol.* 20:1813–28
144. Wigglesworth VB. 1983. The physiology of insect tracheoles. *Adv. Insect Physiol.* 17:85–149
145. Williams DD, Tavares AR, Bryant E. 1987. Respiratory device or camouflage? A case for the caddisfly. *Oikos* 50:42–52
146. Wingrove JA, O'Farrell PH. 1999. Nitric oxide contributes to behavioral, cellular, and developmental responses to low oxygen in *Drosophila*. *Cell* 98:105–14
147. Wong DM, Shen Z, Owyang KE, Martinez-Agosto JA. 2014. Insulin- and warts-dependent regulation of tracheal plasticity modulates systemic larval growth during hypoxia in *Drosophila melanogaster*. *PLOS ONE* 9:e115297
148. Woods HA, Bonnecaze RT, Zrubek B. 2005. Oxygen and water flux across eggshells of *Manduca sexta*. *J. Exp. Biol*. 208:1297–308
149. Woods HA, Hill RI. 2004. Temperature-dependent oxygen limitation in insect eggs. *J. Exp. Biol.* 207:2267–76
150. Woods HA, Lane SJ. 2016. Metabolic recovery from drowning by insect pupae. *J. Exp. Biol.* 219:3126–36
151. Yerra A, Challa S, Valluri SV, Mamillapalli A. 2016. Spermidine alleviates oxidative stress in silk glands of *Bombyx mori*. *J. Asia-Pac. Entomol*. 19:1197–202
152. Zebe E. 1991. In vivo studies on the function of hemoglobin in the larvae of *Chironomus thummi* (Insecta, Diptera). *Comp. Biochem. Physiol. A: Physiol.* 99:525–29
153. Zhang Z-Y, Chen B, Zhao D-J, Kang L. 2013. Functional modulation of mitochondrial cytochrome c oxidase underlies adaptation to high-altitude hypoxia in a Tibetan migratory locust. *Proc. R. Soc. B* 280:20122758
154. Zhou D, Xue J, Lai JCK, Schork NJ, White KP, Haddad GG. 2008. Mechanisms underlying hypoxia tolerance in *Drosophila melanogaster*: *hairy* as a metabolic switch. *PLOS Genet*. 4:e1000221

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