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CORRECTION

Correction: Elucidating mechanisms for insect body size: partial support for the oxygen-dependent induction of moulting hypothesis (doi: 10.1242/jeb.166157)

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The author affiliations were incorrectly displayed. The correct version is shown above. This has been corrected in the online full-text and PDF versions.

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RESEARCH ARTICLE

Elucidating mechanisms for insect body size: partial support for the oxygen-dependent induction of moulting hypothesis

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ABSTRACT

Body size is a key life history trait, and knowledge of its mechanistic basis is crucial in life history biology. Such knowledge is accumulating for holometabolous insects, whose growth is characterised and body size affected by moulting. According to the oxygen-dependent induction of moulting (ODIM) hypothesis, moult is induced at a critical mass at which oxygen demand of growing tissues overrides the supply from the tracheal respiratory system, which principally grows only at moults. Support for the ODIM hypothesis is controversial, partly because of a lack of proper data to explicitly test the hypothesis. The ODIM hypothesis predicts that the critical mass is positively correlated with oxygen partial pressure (P_{O_2}) and negatively with temperature. To resolve the controversy that surrounds the ODIM hypothesis, we rigorously test these predictions by exposing penultimate-instar *Orthosia gothica* (Lepidoptera: Noctuidae) larvae to temperature and moderate P_{O_2} manipulations in a factorial experiment. The relative mass increment in the focal instar increased along with increasing P_{O_2}, as predicted, but there was only weak suggestive evidence of the temperature effect. Probably owing to a high measurement error in the trait, the effect of P_{O_2} on the critical mass was sex specific; high P_{O_2} had a positive effect only in females, whereas low P_{O_2} had a negative effect only in males. Critical mass was independent of temperature. Support for the ODIM hypothesis is partial because of only suggestive evidence of a temperature effect on moulting, but the role of oxygen in moult induction seems unambiguous. The ODIM mechanism thus seems worth considering in body size analyses.

KEY WORDS: Critical mass, Growth rate, Hyperoxia, Hypoxia, Larval instars, *Orthosia gothica*

INTRODUCTION

Body size is a key life history trait that is responsible for much of life history diversity (e.g. Roff, 1992). Understanding the evolution of this diversity requires knowledge of the mechanistic basis of body size. Holometabolous insects have become a model system for studying the mechanistic basis of body size (see e.g. Shingleton, 2011; Nijhout et al., 2014). Growth in holometabolous insects is determinate and all ontogenetic growth takes place in the larval stage, which consists of a series of instars that are separated by moults. At each moult, the exoskeleton is replaced with a larger one (Chapman, 1998) and the larva enters a new instar, except at the end of the final larval instar, where it instead moults into the pupal stage. The size at which the pupal moult occurs is a major determinant of adult body size (e.g. Nijhout et al., 2006, 2010; Davidowitz et al., 2016). However, the understanding of the mechanisms of moult induction, and consequent body size determination, in insects is still limited.

Studies on mechanisms determining body size in holometabolous insects have focused on the pupal moult (Shingleton, 2011; Callier and Nijhout, 2013; Nijhout et al., 2014; Gokhale and Shingleton, 2015). In the model organisms *Drosophila melanogaster* (Diptera: Drosophilidae) and *Manduca sexta* (Lepidoptera: Sphingidae), the timing and size at which pupation takes place is determined by: (1) the critical mass at which an endocrine cascade preceding the pupal moult is triggered; (2) the interval to the cessation of growth (ICG; that is, the time interval from the attainment of the critical mass until the cessation of growth for moulting); and (3) the growth rate that determines how soon the critical mass is attained and how much mass accumulates during the ICG (Nijhout et al., 2006, 2010; Shingleton, 2011; Gokhale and Shingleton, 2015; Davidowitz et al., 2016). The critical mass is a developmental checkpoint where some size-dependent mechanism triggers physiological processes that eventually result in pupation. ICG and growth rate are largely consequences of biological rates [e.g. rates of enzyme-catalysed reactions; ICG can be seen as a proxy for development rate, yet is also affected by photoperiodic control of endocrine events (e.g. Nijhout and Williams, 1974; Nijhoutet al., 2006, 2010; Davidowitz et al., 2016)], and are, thus, strongly temperature dependent (e.g. Davidowitz et al., 2004; Ghosh et al., 2013).

The critical mass is a central trait affecting moulting and thereby body size. There is, however, confusion around the concept of critical mass; the definition and the method of measuring it vary among studies. In *M. sexta*, the critical mass is a nutritionally defined developmental threshold (Nijhout and Williams, 1974; Nijhout et al., 2006; Callier and Nijhout, 2011), but this definition does not work as such in *D. melanogaster* (Stieper et al., 2008). Alternatively, the critical mass represents an energy allocation switch between larval and imaginal tissues (Hironaka and Morishita, 2017). These definitions pertain to the induction of the pupal moult, but the concept of critical mass can be extended to moults from one larval instar to the next (Callier and Nijhout, 2011). It remains unknown whether the alternative critical masses are estimates of the same thing, and what mechanism results in the critical mass. It has been hypothesized that a compromised capability of the respiratory system to support the increasing oxygen demand of growing tissues underlies critical mass [the oxygen-dependent induction of moulting hypothesis; Greenberg and Ar, 1996; Greenlee and Harrison, 2004, 2005; Callier and Nijhout, 2011]. The ODIM hypothesis presents a

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plausible general explanation for the size-dependent induction of moulting in holometabolous insects, but it has still not been rigorously assessed.

The basis of the ODIM hypothesis is the discontinuous growth of the tracheal respiratory system of insects. The tracheal system is an invagination of the exoskeleton (Chapman, 1998), which is why it primarily grows at moults, yet the very finest ends of the tracheal tubes (tracheoles) proliferate continuously (Jarecki et al., 1999). Some inter-moult tracheal growth has also been observed in final-instar *M. sexta* (Helm and Davidowitz, 2013), but not in any earlier instar (Callier and Nijhout, 2011). Even with tracheole proliferation and possible final-instar tracheal growth, the oxygen demand of tissues apparently increases faster than oxygen supply capacity of the tracheal system during a given larval instar. This is because tissue mass increases approximately exponentially during inter-moult growth. In accordance with this, the oxygen safety margin (i.e. excess oxygen supply capacity) of the tracheal system decreases towards the end of larval instars (Greenlee and Harrison, 2005; see also Greenlee and Harrison, 2004). Consequently, with continuing growth, tissue oxygen demand will eventually outrun the supply capacity. It can be hypothesized that oxygen limitation should be reflected in the growth trajectory and result in deceleration of growth at the end of larval instars, as implied by Callier and Nijhout (2011), although convincing evidence of oxygen limitation is lacking (see e.g. Callier and Nijhout, 2012). Nevertheless, the instar-specific growth trajectory can be used for determining the critical mass, and Nijhout et al. (2006) and Grunert et al. (2015) argued that the critical mass is at the inflection point of the instar-specific growth trajectory. We use this operational definition of critical mass throughout this article.

There is ample indirect support for the ODIM hypothesis. The excess oxygen supply capacity of the tracheal system diminishes along with growth (Greenlee and Harrison, 2004, 2005) and growth-associated changes in metabolic rate suggest that the respiratory system functions at its maximum capacity at the end of the majority of studied larval instars (Callier and Nijhout, 2011; Kivelä et al., 2016a). A negative correlation between the number of moults and oxygen partial pressure (P_{O_2}) in *Tenebrio molitor* (Loudon, 1988; Greenberg and Ar, 1996) is consistent with the ODIM hypothesis as well, given a threshold size for metamorphosis (see Grunert et al., 2015), yet the number of moults is unaffected by P_{O_2} in *M. sexta* and *D. melanogaster* (Harrison et al., 2006; Callier et al., 2013). Finally, a mathematical model derived from the ODIM hypothesis successfully explains moulting sizes in four butterflies (Kivelä et al., 2016b).

Direct tests of the ODIM hypothesis require experimental manipulations of P_{O_2}; decreasing and increasing P_{O_2} should decrease and increase the critical mass, respectively. As predicted, unnaturally low P_{O_2} (hypoxia) decreases the critical mass (Callier and Nijhout, 2011; Callier et al., 2013). Hypoxia also decreases size at the next moult, final body size and growth rate, and prolongs development (Loudon, 1988; Greenberg and Ar, 1996; Frazier et al., 2001; Henry and Harrison, 2004; VandenBrooks et al., 2012; Callier et al., 2013; Harrison et al., 2013; reviewed by Harrison et al., 2006, 2010; but Loudon, 1988 showed that hypoxia had no effect on final size). By contrast, the effect of unnaturally high P_{O_2} (hyperoxia) on the critical mass, moulting size and final body size is contradictory (Greenberg and Ar, 1996; Frazier et al., 2001; Henry and Harrison, 2004; Harrison et al., 2010; Callier and Nijhout, 2011; VandenBrooks et al., 2012; Callier et al., 2013). Furthermore, there are no consistent hyperoxia effects on growth and development rates (Greenberg and Ar, 1996; Frazier et al., 2001; Harrison et al., 2006, 2013; VandenBrooks et al., 2012; Callier et al., 2013). The contradictory results concerning hyperoxia effects may arise either because the ODIM hypothesis is unrealistic or because the applied hyperoxia manipulations have been unrealistically strong. Often, the hyperoxic manipulation has a P_{O_2} that is twice that of normal air (normoxia), potentially inducing toxic oxidative effects. Relaxing the oxygen constraint on growth with hyperoxia may also reveal some unknown constraints that then dominate moult induction. For testing the ODIM hypothesis, the design of most experiments manipulating P_{O_2} has not been ideal because the whole larval stage has been exposed to manipulations. Developmental plasticity of the tracheal system results in a negative correlation between tracheal diameter and P_{O_2} (Locke, 1958; Henry and Harrison, 2004; VandenBrooks et al., 2012; reviewed by Harrison et al., 2006), which may compensate for the changes in oxygen availability. A rigorous test of the ODIM hypothesis must eliminate developmental plasticity by exposing only a single instar to a manipulated P_{O_2} and measuring the relevant traits in that instar, which has rarely been done (Callier and Nijhout, 2011; Callier et al., 2013; see also Heinrich et al., 2011). Here, we conduct this kind of experiment with moderate hypoxia and hyperoxia manipulations to assess the validity of the ODIM hypothesis.

Temperature affects the balance between oxygen supply and demand, because increasing temperature increases metabolic rate (oxygen demand) faster than diffusion (oxygen supply) in general (e.g. Woods, 1999). Owing to this, it is important to consider temperature when testing the ODIM hypothesis, because the balance between oxygen supply and demand is at the heart of oxygen-dependent moult induction. In fact, the ODIM hypothesis predicts a decreasing critical mass with increasing temperature (Kivelä et al., 2016b), which has also been observed in *D. melanogaster* (Ghosh et al., 2013) but not in *M. sexta* (Davidowitz et al., 2004). Increasing temperature should thus affect moulting similarly as decreasing P_{O_2}. Moreover, temperature and P_{O_2} are predicted to affect juvenile development non-additively (Frazier et al., 2001), so an interaction between temperature and P_{O_2} on critical mass and moulting size is expected. Despite this, both temperature and P_{O_2} have, to our knowledge, been simultaneously manipulated only by Frazier et al. (2001), who investigated the whole larval stage. Consequently, the developmental consequences of a simultaneous manipulation of both P_{O_2} and temperature within a single instar remain un-investigated, which is why we address this issue here.

We test the ODIM hypothesis by assessing the effects of P_{O_2} and temperature on moulting, growth and development when the possibility for compensatory inter-instar developmental plasticity is eliminated. We expose penultimate-instar larvae of the moth *Orthosia gothica* (Linnaeus 1758) (Lepidoptera: Noctuidae) to P_{O_2} and temperature manipulations in a factorial experiment, and measure their relative mass increment [(instar peak mass)/(instar initial mass)], critical mass (estimated from the growth trajectory; cf. Grunert et al., 2015), growth rate and growth duration in the focal instar. This allows us to directly test predictions of the ODIM hypothesis stating that relative mass increment and critical mass are positively correlated with P_{O_2} and negatively correlated with temperature, and that there is a P_{O_2}×temperature interaction on these traits.

MATERIALS AND METHODS

The study species and acquisition of study animals

Orthosia gothica adults fly early in spring. The polyphagous larva feeds particularly on deciduous trees. The larva burrows into the ground for pupation, with metamorphosis to the adult stage initiating immediately after pupation. The adult overwinters within the pupal cuticle and ecloses in the following spring.

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We captured *O. gothica* adults from flowering willows (*Salix* spp.) from Oulu (65°00′N, 25°28′E), Finland. For oviposition, all females were placed individually, or with a male to ensure fertility, in 0.2 l plastic containers covered with a veil and moist moss (*Sphagnum* spp.) at the bottom. The containers were placed in a climatic room at 10°C, high humidity and very dim light. To delay oviposition, some females were kept at 5°C before moving them to the oviposition room. Hatching of some egg clutches was also delayed by keeping them at 5°C during nights. These delays were induced because our experimental design required completing the experiment in four temporally separate blocks (see below and Fig. S1), which necessitated extending the experiment over a longer time than the flight period of the study species. Delaying hatching of eggs resulted in a small size of the larvae of the last experimental block, part of them adding an extra instar in their larval stage (Fig. S2).

Hatched larvae were placed individually in 0.2 l containers with garden peat at the bottom and fresh leaves of the natural host plant *Prunus padus*. The containers were placed in a climatic room at 21°C and an 18 h:6 h light:dark cycle. The larvae were monitored daily and fed on *P. padus ad libitum*. Once the larvae reached the fourth instar, they were monitored at 2 h intervals when lights were on in the climatic room. Immediately after the moult to the penultimate (V) larval instar (*O. gothica* has six larval instars), the larva was weighed and exposed to the experimental manipulations explained below.

Experimental design

The experimental setup consisted of four temporally separate blocks arranged in an order of presumably decreasing larval development rate due to temperature and P_{O_2} manipulations to minimise the need to delay hatching of larvae for the later blocks (Fig. S1). Temperature (high/low) manipulations were applied to the blocks. Each block included the normoxic (20.9% O_2) control and one of the P_{O_2} manipulations (hyperoxia/hypoxia), because we had only two chambers where P_{O_2} manipulation was possible.

Plywood chambers (56×48×14 cm) with Plexiglas lids were used for manipulating P_{O_2}. In both chambers, air inflow first entered a small 3×48×14 cm chamber separated from the main chamber by a perforated wall to ensure spatially uniform inflow into the main chamber. At the other end, air outflow went through a similar small chamber. The chambers were under daylight spectrum light emitting LED lamps in a climatic room with 18 h:6 h light:dark cycle. Room temperature was set to 25 and 11°C at the high and low temperature manipulations, respectively. Temperature was measured in both chambers with temperature loggers (iButton Thermochron DS1922L, Maxim Integrated, Inc., San Jose, CA, USA) and oxygen content of the outflow was measured as explained below.

A line of dried and pressurized outdoor air was used for inflow. First, the line was split into two, with one line serving as a reference while the other was used to supply air to the experimental chambers. From the reference line (ca. 1 l min^{-1}), we took a sample (ca. 60 ml min^{-1}; SS4 Subsampler, Sable Systems, Las Vegas, NV, USA) from which CO_2 and potential humidity were removed with soda lime and silica gel. Then, a computer-directed valve directed the sample to a SERVOPRO 1440 gas analyser (Servomex, Brighton, UK) for the measurement of oxygen content. Air flowing to the experimental chambers was first moisturised by leading the airflow through a water column. Then, the line was split into two: one entering the control chamber and the other entering the manipulation chamber. Both of these lines went through Bronkhorst (Ruurlo, The Netherlands) Hi-Tec EL-Flow mass flow controllers set at 0.75 l min^{-1}, this flow rate being adequate to guarantee that oxygen consumption of the experimental larvae had only a negligible effect on ambient P_{O_2}. Extra oxygen (hyperoxia manipulation) or nitrogen (hypoxia manipulation) was added from a high-pressure gas bottle to the line entering the manipulation chamber. A constant level of hyper/hypoxia was ascertained with a low-volume mixing chamber preceding the actual experimental chamber. We aimed for 27 and 15% oxygen content in the hyperoxia and hypoxia manipulations, respectively. The correct inflow of extra oxygen and nitrogen was set before starting the experiment in a particular block to make sure that larvae experienced the intended P_{O_2} from the beginning of the experiment. We took samples (ca. 60 ml min^{-1}; SS4 Subsampler) from the air outflowing from the chambers. These samples entered the SERVOPRO 1440 gas analyser similarly via computer-directed valves as did the reference line. Oxygen content was recorded once a minute so that each of the three sample lines (experimental chamber, control chamber, reference) was measured for 10 min before switching to the next sample line. VEE Pro (Agilent Inc., Santa Rosa, CA, USA) software was used in directing the system and saving data on oxygen content.

Immediately after completing the moult to the penultimate (V) instar, the larvae were weighed with a Precisa 202A balance (Dietikon, Switzerland), moved to a 0.58 l container with walls that were very permeable to gases, and provided with *P. padus ad libitum*. The containers were randomised between the control and manipulation chambers so that five individuals per brood were placed into the control chamber and five into the manipulation chamber. Five broods were included in each block (i.e. 25 control and 25 manipulation larvae). The larvae were weighed twice a day with an interval of approximately 12 h, the host plant being simultaneously changed if needed. The lids of the chambers had to be opened for weighing the larvae, which resulted in flushing of the manipulation chamber with normal air. To rapidly fill the manipulation chamber with the intended gas mixture after weighing the larvae, we directed the gas flow into a 50 l plastic bag during weighing and, thereafter, compressed the gas mixture from the bag to the chamber.

Once the instar V larvae started moulting to the final (VI) instar, indicated both by (1) loss of mass since the previous measurement and (2) changed colour and/or head capsule slippage, they were removed from the chambers. Then, the larvae were moved back to 0.2 l containers and reared until pupation with *ad libitum P. padus* under the same conditions in which instar I–IV larvae were reared. Seven days after a larva had burrowed into the peat for pupation, it was excavated and sexed.

For each individual, we calculated the mean temperature and air oxygen content for the period in the experimental chamber. Then, we calculated the mean experienced temperature and oxygen content values across individuals in different manipulations (see Results).

Critical mass estimation

The instar-specific critical mass of moult induction is at the inflection point of the instar-specific growth trajectory (Grunert et al., 2015). Owing to low numbers of individual-specific observations (range: three to nine observations/individual) from instar V growth trajectories, we used a method based on absolute growth rates [(mass increment)/(time interval); i.e. slope of the growth trajectory] between successive mass measurements in estimating the individual-specific critical masses.

When growth accelerates, absolute growth rates increase monotonically from one measurement interval to the next, whereas

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the absolute growth rates decrease monotonically across measurement intervals under decelerating growth (Fig. 1A). Hence, the absolute growth rate between measurements i and i+1 [$g(i)$]=[$m_k(i+1)-m_k(i)$]/[$t_k(i+1)-t_k(i)$] (i=1, …, $n_{Obs,k}$−1) [$m_k(i)$, $t_k(i)$ and $n_{Obs,k}$ are mass of individual k at measurement i, time of measurement i and the number of growth observations from individual k, respectively] increases with increasing i under accelerating growth and, under decelerating growth, $g(i)$ decreases with increasing i. However, real growth trajectory data often deviate from this ideal pattern (Fig. 1B). Therefore, we adopted a conservative method for estimating the critical mass: (1) the growth rates between successive mass records must monotonically decrease until the instar-specific peak mass after the estimate, and (2) the estimate must occur after the highest (first) growth rate in the series of monotonically decreasing growth rates. The latter condition (2) ensured that empirically measured growth was decelerating all the way from the critical mass estimate until the cessation of growth in the focal instar. For 60 out of the 196 individuals with complete data, our method failed to find a critical mass that is lower than the peak mass. Hence, we had 136 individuals for analysing critical mass variation.

Analysed traits

We analysed four traits measured in the penultimate (V) instar: relative mass increment [(instar peak mass)/(instar initial mass)], critical mass (estimated as explained above), growth rate {[ln(peak mass)−ln(initial mass)]/(growth duration)} and growth duration (the duration of time from the beginning of the instar to the attainment of the instar peak mass). The relative mass increment is expected to be positively correlated with the critical mass, the correlation persisting across P_{O_2} manipulations. This is because observed P_{O_2} effects on growth and development rates (reviewed by Harrison et al., 2006) translate into such P_{O_2}-dependency of moulting size that parallels the predicted P_{O_2}-dependency of critical mass. Note that only growth and development rate changes occurring after the attainment of the critical mass (i.e. during the ICG) are of importance here because moulting size and critical mass can be decoupled across a gradient of P_{O_2} only if growth and development rate changes during the ICG counteract changes in critical mass. The expected negative temperature-dependency of both critical mass (Kivelä et al., 2016b) and final body size (Davidowitz and Nijhout, 2004; Grunert et al., 2015) should result in a positive correlation between relative mass increment and critical mass also across temperatures. Hence, relative mass increment is an appropriate trait for testing the ODIM hypothesis besides the critical mass.

Statistical analyses

We analysed all data (Kivelä et al., 2017) with linear mixed-effects models fitted with the maximum likelihood method by using the function ‘lme’ (package ‘nlme’; http://CRAN.R-project.org/package=nlme) in R version 3.2.2 (https://www.R-project.org/). To avoid overly simplified inferences and problems in model selection, we utilised the multimodel inference approach (e.g. Burnham and Anderson, 2002).

Relative mass increment and growth rate were ln-transformed to improve model goodness of fit. The ‘global model’ was identical for each trait, and included P_{O_2} manipulation (control/hyperoxia/hypoxia), temperature (high/low), sex and all possible interactions among them as fixed effects. Initial mass of instar V was included as a covariate in the global model to take into account size variation among and within experimental blocks (Fig. S2). Brood was set as a random effect.

The global models explaining variation in relative mass increment, critical mass and growth duration were heteroscedastic so that residual variance was higher under low temperature than in high temperature. Hence, we included weights by the variance function ‘varIdent’ (http://CRAN.R-project.org/package=nlme) in models for these variables. The global model explaining variation in growth rate was also heteroscedastic, with residual variance increasing with increasing fitted value. This heteroscedasticity was modelled by adding weights with the variance function ‘varExp’ (http://CRAN.R-project.org/package=nlme) by using the fitted value of the model as a variance covariate in models for growth rate.

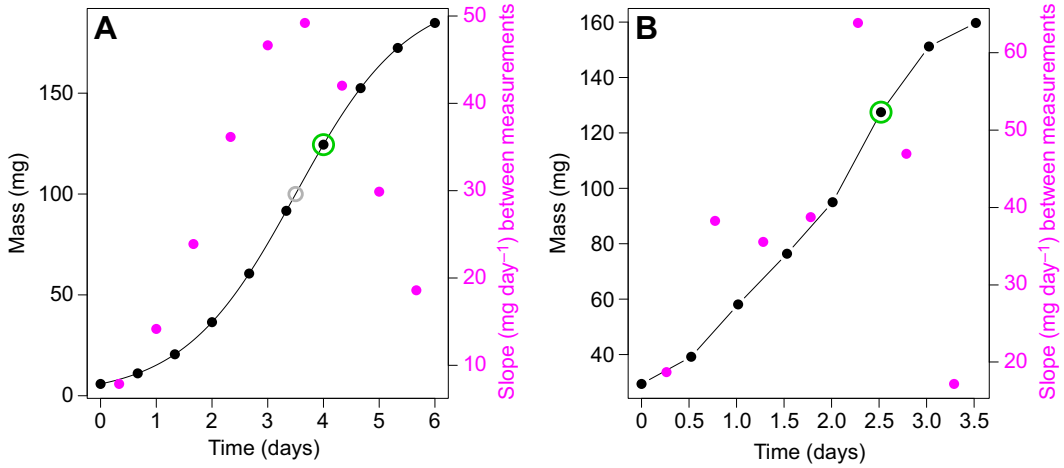

Fig. 1. Illustration of the method used for critical mass estimation from the instar V growth trajectories. (A) An ideal example based on a hypothetical growth trajectory. (B) A real-data example. Growth trajectories are depicted with black lines, with black points being the observations. Slopes (i.e. growth rates) between successive observations i and i+1 {[$m_k(i+1)-m_k(i)$]/[$t_k(i+1)-t_k(i)$]; i=1, …, $n_{Obs,k}$−1, with $m_k(i)$, $t_k(i)$ and $n_{Obs,k}$ being mass of individual k at the measurement i, time of the measurement i and the number of growth observations from individual k, respectively} are illustrated with magenta points. The critical mass (i.e. the inflection point of the instar-specific growth trajectory) was estimated to be the observed mass that fulfilled the conditions: (1) the growth rates between successive observations monotonically decrease until the instar-specific peak mass after the estimate, and (2) the estimate occurs after the highest growth rate in the series of monotonically decreasing growth rates. The critical mass estimates are depicted with the green circles. The grey circle in A depicts the true inflection point of the growth trajectory that is known in this hypothetical example.

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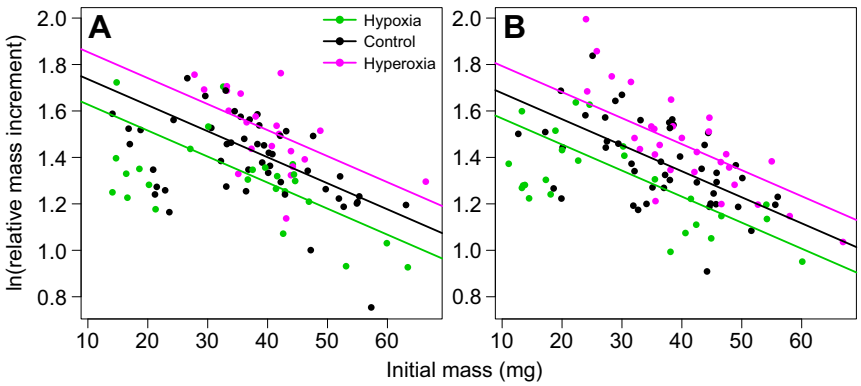

Fig. 2. Observed relative instar V mass increment [(instar peak mass)/(instar initial mass); unitless] in relation to instar V initial mass and oxygen partial pressure (P_{O_2}) manipulation in female and male *Orthosia gothica* (points). (A) Females; (B) males. Sex- and P_{O_2}-manipulation-specific regression lines based on the model-averaged (full average) fixed effects are also presented. For this and subsequent figures, only those fixed effects with $P<0.05$ (see Table 1) were taken into account when drawing the regression lines. There are 49 control, 21 hyperoxic and 25 hypoxic females, with the respective numbers of males being 47, 29 and 25.

By starting from the global models explained above, we derived the set of all meaningful simpler models for each of the four traits analysed with the function 'dredge' (package 'MuMIn'; http://CRAN.R-project.org/package=MuMIn) and averaged these models with the function 'model.avg' (http://CRAN.R-project.org/package=MuMIn). We investigated model goodness of fit separately for the global model, the best model [i.e. the model with the lowest small-sample-size corrected version of Akaike's information criterion (AIC_c)] and all models within two units of ΔAIC_c from the best model for each of the four traits analysed. In each case, model goodness of fit was excellent. We base inferences on the parameters averaged over the full sets of models included in the analyses (see Table S1), including those models that do not include a particular parameter to avoid biasing estimated effect sizes upwards. We consider model-averaged parameters with $P<0.05$ as important in explaining variation in the analysed traits and use them in inferences. These parameters were associated with terms having a relative importance (i.e. sum of Akaike weights of all models including the term in question; $0 \leq$relative importance≤ 1) of at least 0.89, which further highlights the statistical support for the reported effects.

RESULTS

P_{O_2} and temperature manipulations were successful. On average, individuals exposed to normoxia (control), hyperoxia and hypoxia manipulations experienced an oxygen content of 20.92 [95% confidence interval (CI): 20.91–20.93], 25.96 (25.89–26.04) and 15.82% (15.69–15.95), respectively. Individuals exposed to a high or low temperature experienced, on average, an ambient temperature of 30.51 (30.45–30.57) and 16.80°C (16.76–16.84), respectively.

Relative mass increment in the penultimate instar was negatively correlated with initial mass of the instar, increased with increasing P_{O_2} and was lower in males than in females (Table 1, Fig. 2). P_{O_2} had essentially an additive effect on the relative mass increment because hyperoxia (5.1% increase in average P_{O_2}) increased the relative mass increment by $e^{0.117} \approx 1.12$ (+12.4%) in relation to normoxia, whereas hypoxia (5.1% decrease in average P_{O_2}) decreased the relative mass increment practically by the same amount ($e^{-0.109} \approx 0.900$; −10.0%) in relation to normoxia, which is consistent with the prediction. Contrary to predictions, there was no interaction between P_{O_2} and temperature on the relative mass increment and only suggestive evidence (relative importance of 0.87) of a main effect of temperature (Table 1).

Estimated critical mass (i.e. the inflection point of the instar-specific growth trajectory) in the penultimate instar was positively correlated with initial mass of the instar, and the effect of P_{O_2} was sex specific as indicated by the interaction between sex and P_{O_2} (Table 1, Fig. 3). The predicted positive effect of hyperoxia on the estimated critical mass was found only in females, whereas the predicted negative effect of hypoxia on the estimated critical mass was male specific (Table 1, Fig. 3). There was no evidence of P_{O_2}×temperature interaction or a main effect of temperature on the critical mass (Table 1), contradicting the predictions. Estimated critical masses were, however, positively correlated with relative mass increment (Pearson's $r=0.241$, d.f.=134, $t=2.88$, $P=0.0047$) as predicted, supporting the use of relative mass increment in testing the ODIM hypothesis.

Growth rate in the penultimate instar was negatively correlated with initial mass of the instar, negatively affected by hypoxia and much higher at high than at low temperature (Table 1, Fig. 4). Even though hypoxia reduced growth rate, hyperoxia did not have a positive effect on it, and there was no strong evidence of a P_{O_2}×temperature interaction on growth rate (Table 1).

Growth duration in the penultimate instar was longer at low than at high temperature, and this temperature effect was less pronounced

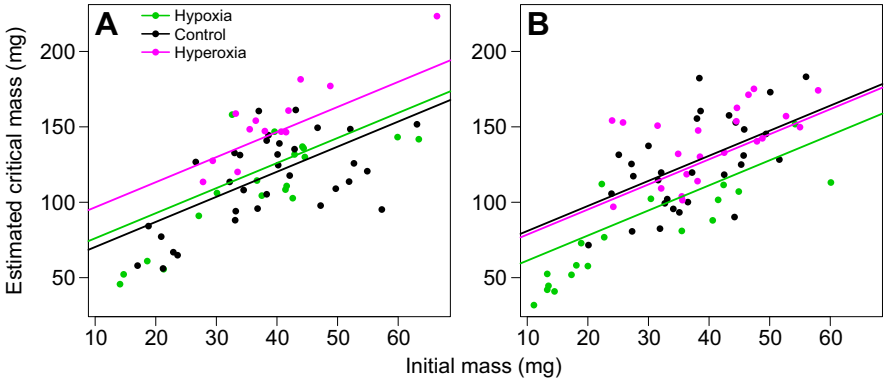

Fig. 3. Estimated instar V critical masses (i.e. inflection points of the growth trajectories of the instar V; see Materials and methods, 'Critical mass estimation' section for details) in relation to instar V initial mass and P_{O_2} manipulations in female and male *O. gothica* (points). (A) Females; (B) males. Sex- and P_{O_2}-manipulation-specific regression lines based on the model-averaged (full average) fixed effects are also presented. There are 33 control, 13 hyperoxic and 19 hypoxic females, with the respective numbers of males being 30, 22 and 19.

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Table 1. Model-averaged (full average) fixed effects of linear mixed-effects models explaining variation in ln-transformed relative mass increment and growth rate as well as in untransformed critical mass and growth duration in the penultimate (V) larval instar in *Orthosia gothica* in relation to oxygen partial pressure manipulation (P_{O_2}; control/hyperoxia/hypoxia), temperature (temp.; high/low), sex and initial mass of the instar (covariate)

Trait	Term (relative importance)	Factor levels	Averaged estimate	Adjusted s.e.	*z*-value	*P*-value
Relative mass increment		Intercept	1.85	0.0680	27.2	**<0.0001**
	Initial mass (1)		−0.0112	0.00137	8.18	**<0.0001**
	P_{O_2} (1)	Hyperoxia	0.117	0.0294	3.96	**<0.0001**
		Hypoxia	−0.109	0.0287	3.80	**0.00014**
	Sex (0.96)	Male	−0.0602	0.0287	2.10	**0.036**
	Temp. (0.87)	Low	−0.0759	0.0526	1.44	0.15
	Sex×temp. (0.57)	Male×low	0.0472	0.0522	0.904	0.37
	P_{O_2}×temp. (0.3)	Hyperoxia×low	0.0157	0.0383	0.411	0.68
		Hypoxia×low	−0.0193	0.0425	0.455	0.65
	P_{O_2}×sex (0.13)	Hyperoxia×male	−0.00512	0.0218	0.234	0.81
		Hypoxia×male	−0.00183	0.0179	0.102	0.92
	P_{O_2}×sex×temp. (<0.01)	Hyperoxia×male×low	7.65×10^{-5}	0.00572	0.013	0.99
		Hypoxia×male×low	-1.05×10^{-4}	0.00586	0.018	0.99
Critical mass		Intercept	53.7	11.5	4.66	**<0.001**
	Initial mass (1)		1.66	0.233	7.14	**<0.001**
	P_{O_2} (1)	Hyperoxia	26.3	6.40	4.12	**<0.001**
		Hypoxia	5.77	5.32	1.09	0.28
	Sex (1)	Male	10.6	4.90	2.16	**0.031**
	Temp. (0.44)	Low	−3.05	7.00	0.436	0.66
	Sex×temp. (0.13)	Male×low	0.694	3.09	0.225	0.82
	P_{O_2}×temp. (0.11)	Hyperoxia×low	0.10	3.05	0.033	0.97
		Hypoxia×low	−1.44	5.14	0.281	0.78
	P_{O_2}×sex (1)	Hyperoxia×male	−28.7	8.02	3.58	**0.0003**
		Hypoxia×male	−25.4	7.75	3.28	**0.0011**
	P_{O_2}×sex×temp. (<0.01)	Hyperoxia×male×low	−0.00538	0.864	0.006	1.00
		Hypoxia×male×low	−0.00462	0.854	0.005	1.00
Growth rate		Intercept	0.225	0.0815	2.76	**0.0058**
	Initial mass (1)		−0.00728	0.00162	4.50	**<0.0001**
	P_{O_2} (0.94)	Hyperoxia	0.0412	0.0542	0.760	0.45
		Hypoxia	−0.136	0.0672	2.03	**0.043**
	Sex (0.47)	Male	−0.00638	0.0251	0.254	0.80
	Temp. (1)	Low	−0.749	0.0620	12.1	**<0.0001**
	Sex×temp. (0.16)	Male×low	−0.00762	0.0264	0.288	0.77
	P_{O_2}×temp. (0.85)	Hyperoxia×low	0.0348	0.0618	0.563	0.57
		Hypoxia×low	0.161	0.0867	1.86	0.063
	P_{O_2}×sex (0.08)	Hyperoxia×male	0.00456	0.0236	0.193	0.85
		Hypoxia×male	−0.00121	0.0177	0.068	0.95
	P_{O_2}×sex×temp. (<0.01)	Hyperoxia×male×low	5.36×10^{-4}	0.0115	0.047	0.96
		Hypoxia×male×low	5.76×10^{-4}	0.0113	0.051	0.96
Growth duration		Intercept	1.61	0.162	9.90	**<0.0001**
	Initial mass (0.47)		−0.00228	0.00342	0.668	0.50
	P_{O_2} (0.90)	Hyperoxia	0.0284	0.0784	0.362	0.72
		Hypoxia	0.126	0.0903	1.40	0.16
	Sex (0.65)	Male	−0.0578	0.0710	0.815	0.415
	Temp. (1)	Low	1.46	0.130	11.3	**<0.0001**
	Sex×temp. (0.49)	Male×low	0.111	0.139	0.793	0.43
	P_{O_2}×temp. (0.89)	Hyperoxia×low	−0.0222	0.133	0.167	0.87
		Hypoxia×low	−0.447	0.210	2.13	**0.033**
	P_{O_2}×sex (0.11)	Hyperoxia×male	−0.00570	0.0470	0.121	0.90
		Hypoxia×male	0.00936	0.0518	0.181	0.86
	P_{O_2}×sex×temp. (0.02)	Hyperoxia×male×low	−0.00688	0.0611	0.112	0.91
		Hypoxia×male×low	−0.00661	0.0594	0.111	0.91

All model-averaged fixed effects are presented, and those with $P<0.05$ are indicated in bold. Brood was set as a random effect. Models for relative mass increment, critical mass and growth duration included varIdent variance functions to take into account higher residual variance under low than high temperature, varExp variance function being included in the model for growth rate to take into account positive correlation between residual variance and fitted value.

under hypoxia than under normoxia or hyperoxia as indicated by the P_{O_2}×temperature interaction (Table 1, Fig. 5). Contrary to the other analysed variables, instar initial mass did not explain variation in growth duration (Table 1).

DISCUSSION

In this test of the ODIM hypothesis (Greenberg and Ar, 1996; Greenlee and Harrison, 2004, 2005; Callier and Nijhout, 2011), P_{O_2} had exactly the predicted effect on the relative mass increment in penultimate-instar *O. gothica*. Relative mass increment increased essentially additively with increasing P_{O_2} across moderate P_{O_2} manipulations when developmental plasticity in response to the experimental manipulations was eliminated. In the estimated critical mass, the effect of P_{O_2} was sex specific, the predicted hypoxia and hyperoxia effects being found only in males and females, respectively. The less clear-cut results in critical mass than

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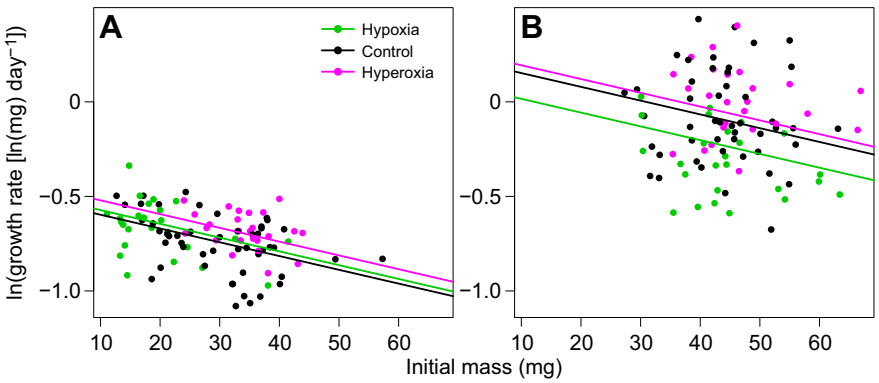

Fig. 4. Observed instar V growth rate in relation to instar V initial mass and P_{O_2} manipulation in *O. gothica* at low and high temperature (points). (A) Low temperature; (B) high temperature. Temperature- and P_{O_2} manipulation-specific regression lines based on the model-averaged (full average) fixed effects are also presented. There are 49 control, 25 hyperoxic and 25 hypoxic individuals at the low temperature, with the respective numbers of high-temperature-exposed individuals being 47, 25 and 25.

in relative mass increment, and the sex specificity of the P_{O_2} effects on critical mass, may be a consequence of exaggerated measurement error combined with a smaller data set for the critical mass analysis. Even though results concerning P_{O_2} support the ODIM hypothesis, we found only suggestive evidence of a temperature effect on moult induction, and no evidence of the expected P_{O_2}×temperature interaction.

Although the hypoxia effect on growth and moulting predicted by the ODIM hypothesis have been found practically universally (Loudon, 1988; Greenberg and Ar, 1996; Henry and Harrison, 2004; Callier and Nijhout, 2011; VandenBrooks et al., 2012; Callier et al., 2013; Harrison et al., 2013; reviewed by Harrison et al., 2006, 2010), the expected hyperoxia effect found in the present study is exceptional in comparison to the controversial results reported in earlier studies (Greenberg and Ar, 1996; Frazier et al., 2001; Henry and Harrison, 2004; Harrison et al., 2006, 2010; Callier and Nijhout, 2011; VandenBrooks et al., 2012; Callier et al., 2013). In combination with earlier studies, the present results suggest that it is important to eliminate developmental plasticity and to use only moderate P_{O_2} manipulations when testing the ODIM hypothesis, especially the hyperoxia effect. Developmental plasticity was also eliminated by Callier and Nijhout (2011), who found that strong hyperoxia (40% O_2) had a slight positive effect on moulting size in fourth- but not in fifth-instar *M. sexta* larvae. Yet, no positive effect on peak larval size was found in *M. sexta* even under moderate hyperoxia (25% O_2) when developmental plasticity of the respiratory system was not eliminated (Harrison et al., 2013). In studies where developmental plasticity has not been eliminated consistently, the predicted hyperoxia effects have only occasionally been reported even when hyperoxic manipulations have been moderate (Greenberg and Ar, 1996; Frazier et al., 2001; Harrison et al., 2006; VandenBrooks et al., 2012). Developmental plasticity of the tracheal system (Locke, 1958; Henry and Harrison, 2004; VandenBrooks et al., 2012; reviewed by Harrison et al., 2006) and potentially harmful effects of strong hyperoxia thus seem to be confounding factors for testing the ODIM hypothesis by manipulating P_{O_2}.

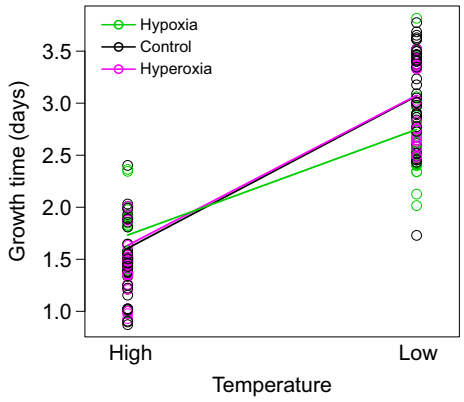

Fig. 5. Observed instar V growth durations (i.e. the time interval from the beginning of the instar to the attainment of the instar peak mass) in relation to temperature and P_{O_2} manipulations in *O. gothica* (points). The lines connect temperature-specific model-averaged fitted values for each P_{O_2} manipulation. Numbers of individuals in the different temperature–P_{O_2} manipulation combinations are the same as in Fig. 4.

Despite the effects of P_{O_2} on growth and moulting being in accordance with the ODIM hypothesis, we did not find the predicted temperature effect on critical mass (Kivelä et al., 2016b). The only evidence of a temperature effect on moulting-related traits is the high relative importance (0.87; third highest of all variables) of temperature for the relative mass increment (see Table 1; Burnham and Anderson, 2002), yet the model-averaged coefficients for temperature did not attain statistical significance. The lack of any evidence of a temperature effect on critical mass probably reflects the smaller amount of data with a higher measurement error in critical mass than in relative mass increment. Our data may be insufficient for detecting the temperature effect if the magnitude of temperature effect is much smaller than that of P_{O_2}. An alternative mutually non-exclusive explanation for the lack of clear evidence of temperature effect may be the extremity of our temperature manipulations. This explanation derives from the observed peaked thermal reaction norm of body size (pupal mass) in *O. gothica* in our study population (P.V. and N.K., unpublished data). A qualitatively similar thermal reaction norm is predicted for peak larval mass in *M. sexta* (Davidowitz and Nijhout, 2004; Grunert et al., 2015). Therefore, we might expect a peaked thermal reaction norm also for the relative mass increment and critical mass in *O. gothica* because these traits are positively correlated with peak mass of the focal (V) instar (relative mass increment versus peak mass: Pearson's r=0.207, d.f.=194, t=2.95, P=0.0036; critical mass versus peak mass: Pearson's r=0.909, d.f.=134, t=25.3, P<0.0001). If our temperature manipulations were on the opposite sides of the peak of the thermal reaction norm, then no temperature effect on relative mass increment and critical mass may be detected. Hence, additional studies are needed for evaluating the predictions of the ODIM hypothesis concerning the effect of temperature on growth and moulting.

The temperature effects on growth rate and duration were exactly as expected because the strong and positive temperature dependency of biological rates translates into a high growth rate and a short growth duration at high temperatures within a

population's thermal limits (e.g. Angilletta, 2009). In addition to temperature, P_{O_2} also affected growth rate and duration in *O. gothica*. Hypoxia had a negative effect on growth rate independently of temperature, which is in accordance with other data on P_{O_2} effects on insect growth rate (Loudon, 1988; Greenberg and Ar, 1996; Frazier et al., 2001; VandenBrooks et al., 2012; Harrison et al., 2013; reviewed by Harrison et al., 2006, 2010). Hypoxia affected growth duration only at low temperature, where it reduced growth duration. A short duration of growth and development is usually considered to be of high fitness value (e.g. Roff, 1992), including for insects in seasonal environments (e.g. Kivelä et al., 2013). This result thus contradicts the expectation that the adverse effect of hypoxia translates into prolonged development, which has also been reported in many earlier studies (Loudon, 1988; Greenberg and Ar, 1996; Frazier et al., 2001; Harrison et al., 2006; VandenBrooks et al., 2012). The unexpected hypoxia effect may, however, be explained by the shorter growth duration of larvae having an extra instar compared to larvae not having it in the last experimental block [growth duration without an extra instar=3.01 (95% CI: 2.80–3.22) days; growth duration with an extra instar=2.64 (2.51–2.77) days], where the combination of low temperature and hypoxia was applied, and which was the only experimental block where extra instars were observed. Moderate hyperoxia had no effect on growth rate or duration in *O. gothica*, which is consistent with many earlier results (Frazier et al., 2001; VandenBrooks et al., 2012; Harrison et al., 2006, 2013), although strong hyperoxia, in particular, tends to adversely affect these traits (Greenberg and Ar, 1996; Frazier et al., 2001; Harrison et al., 2006; VandenBrooks et al., 2012; but see Callier et al., 2013).

Excluding the temperature by P_{O_2} interaction on development rate, P_{O_2} effects on growth rate and duration were very similar in *O. gothica* and other species studied in this respect. This implies that the general physiology of *O. gothica* is comparable to other insects studied so far, and that the observed P_{O_2} effects on moulting-related traits (critical mass, relative mass increment) may be a general phenomenon in holometabolous insects. The lack of the hyperoxia effect in many other studies may be due to the confounding effects of developmental plasticity of the tracheal system and overly strong hyperoxia manipulations. Therefore, great care is needed when drawing inferences concerning the ODIM hypothesis, especially when using data not primarily designed to test the hypothesis. A direct and rigorous test of the ODIM hypothesis requires a manipulative experiment designed for that purpose. Owing to the above reasons, the contradictory results concerning hyperoxia effects on moulting in the literature (Greenberg and Ar, 1996; Frazier et al., 2001; Henry and Harrison, 2004; Harrison et al., 2006, 2010; Callier and Nijhout, 2011; VandenBrooks et al., 2012; Callier et al., 2013) should not be treated as evidence against the ODIM hypothesis. Still, more work on multiple species is required for a rigorous assessment of the generality and validity of the ODIM hypothesis. It is also worth noting that, besides ODIM, a direct influence of P_{O_2} on growth and development rates may also affect moulting size and relative mass increment. This mechanism is mutually non-exclusive with ODIM. The relative contributions of these alternative mechanisms to moulting size and relative mass increment remain to be resolved.

As a general implication, our work suggests that oxygen supply has a role in moult induction in holometabolous insects as stated by the ODIM hypothesis (Callier and Nijhout, 2011). Because moulting has a strong influence on body size, this kind of mechanistic knowledge of moult induction is crucial for understanding the determination of insect body size (Shingleton, 2011; Callier and Nijhout, 2013; Nijhout et al., 2014; Gokhale and Shingleton, 2015). The present results thus encourage the development and use of mathematical descriptions of moult induction derived from the ODIM hypothesis, in life history analyses (Kivelä et al., 2016b, Hironaka and Morishita, 2017). Adding these types of mechanisms into life history models is necessary for deriving realistic and quantitative predictions, which is becoming increasingly important due to the anthropogenic environmental change, and the consequent effects on the selective pressures on the life histories of organisms.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.M.K., N.K., K.G., E.H., P.V.; Methodology: S.M.K., N.K., K.G., E.H., P.V.; Validation: S.M.K.; Formal analysis: S.M.K.; Investigation: S.M.K., S.V., N.K., P.V.; Resources: S.M.K.; Data curation: S.M.K.; Writing - original draft: S.M.K.; Writing - review & editing: S.M.K., S.V., N.K., K.G., E.H., P.V.; Visualization: S.M.K.; Supervision: S.M.K.; Project administration: S.M.K.; Funding acquisition: S.M.K., N.K., K.G.

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Data availability
Data are available from the Dryad Digital Repository (Kivelä et al., 2017): http://dx.doi.org/10.5061/dryad.sf2bt

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.166157.supplemental

References
Angilletta, M. J., Jr. (2009). *Thermal Adaptation, a Theoretical and Empirical Synthesis*. New York: Oxford University Press.
Burnham, K. P. and Anderson, D. R. (2002). *Model Selection and Multimodel Inference, A Practical Information-Theoretic Approach*, 2nd edn. New York: Springer.
Callier, V. and Nijhout, H. F. (2011). Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis. *Proc. Natl. Acad. Sci. USA* **108**, 14664-14669.
Callier, V. and Nijhout, H. F. (2012). Supply-side constraints are insufficient to explain the ontogenetic scaling of metabolic rate in the tobacco hornworm, *Manduca sexta*. *PLoS ONE* **7**, e45455.
Callier, V. and Nijhout, H. F. (2013). Body size determination in insects: a review and synthesis of size- and brain-dependent and independent mechanisms. *Biol. Rev.* **88**, 944-954.
Callier, V., Shingleton, A. W., Brent, C. S., Ghosh, S. M., Kim, J. and Harrison, J. F. (2013). The role of reduced oxygen in the developmental physiology of growth and metamorphosis initiation in *Drosophila melanogaster*. *J. Exp. Biol.* **216**, 4334-4340.
Chapman, R. F. (1998). *The Insects, Structure and Function*, 4th edn. Cambridge: Cambridge University Press.
Davidowitz, G. and Nijhout, H. F. (2004). The physiological basis of reaction norms: the interaction among growth rate, the duration of growth and body size. *Integr. Comp. Biol.* **44**, 443-449.
Davidowitz, G., D'Amico, L. J. and Nijhout, H. F. (2004). The effects of environmental variation on a mechanism that controls insect body size. *Evol. Ecol. Res.* **6**, 49-62.
Davidowitz, G., Roff, D. and Nijhout, H. F. (2016). Synergism and antagonism of proximate mechanisms enable and constrain the response to simultaneous selection on body size and development time: an empirical test using experimental evolution. *Am. Nat.* **188**, 499-520.

Frazier, M. R., Woods, H. A. and Harrison, J. F. (2001). Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* **74**, 641-650.
Ghosh, S. M., Testa, N. D. and Shingleton, A. W. (2013). Temperature-size rule is mediated by thermal plasticity of critical size in *Drosophila melanogaster*. *Proc. R. Soc. B* **280**, 20130174.
Gokhale, R. H. and Shingleton, A. W. (2015). Size control: the developmental physiology of body and organ size regulation. *WIREs Dev. Biol.* **4**, 335-356.
Greenberg, S. and Ar, A. (1996). Effects of Chronic Hypoxia, Normoxia and Hyperoxia on larval development in the beetle *Tenebrio molitor*. *J. Insect Physiol.* **42**, 991-996.
Greenlee, K. J. and Harrison, J. F. (2004). Development of respiratory function in the American locust *Schistocerca americana* II. Within-instar effects. *J. Exp. Biol.* **207**, 509-517.
Greenlee, K. J. and Harrison, J. F. (2005). Respiratory changes throughout ontogeny in the tobacco hornworm caterpillar, *Manduca sexta*. *J. Exp. Biol.* **208**, 1385-1392.
Grunert, L. W., Clarke, J. W., Ahuja, C., Eswaran, H. and Nijhout, H. F. (2015). A quantitative analysis of growth and size regulation in *Manduca sexta*: the physiological basis of variation in size and age at metamorphosis. *PLoS ONE* **10**, e0127988.
Harrison, J., Frazier, M. R., Henry, J. R., Kaiser, A., Klok, C. J. and Rascón, B. (2006). Responses of terrestrial insects to hypoxia or hyperoxia. *Respir. Physiol. Neurobiol.* **154**, 4-17.
Harrison, J. F., Kaiser, A. and VandenBrooks, J. M. (2010). Atmospheric oxygen level and the evolution of insect body size. *Proc. R. Soc. B* **277**, 1937-1946.
Harrison, J. F., Cease, A. J., VandenBrooks, J. M., Albert, T. and Davidowitz, G. (2013). Caterpillars selected for large body size and short development time are more susceptible to oxygen-related stress. *Ecol. Evol.* **3**, 1305-1316.
Heinrich, E. C., Farzin, M., Klok, C. J. and Harrison, J. F. (2011). The effect of developmental stage on the sensitivity of cell and body size to hypoxia in *Drosophila melanogaster*. *J. Exp. Biol.* **214**, 1419-1427.
Helm, B. R. and Davidowitz, G. (2013). Mass and volume growth of an insect tracheal system within a single instar. *J. Exp. Biol.* **216**, 4703-4711.
Henry, J. R. and Harrison, J. F. (2004). Plastic and evolved responses of larval tracheae and mass to varying atmospheric oxygen content in *Drosophila melanogaster*. *J. Exp. Biol.* **207**, 3559-3567.
Hironaka, K.-I. and Morishita, Y. (2017). Adaptive significance of critical weight for metamorphosis in holometabolous insects. *J. Theor. Biol.* **417**, 68-83.
Jarecki, J., Johnson, E. and Krasnow, M. A. (1999). Oxygen regulation of airway branching in *Drosophila* is mediated by Branchless FGF. *Cell* **99**, 211-220.
Kivelä, S. M., Välimäki, P. and Gotthard, K. (2013). Seasonality maintains alternative life-history phenotypes. *Evolution* **67**, 3145-3160.
Kivelä, S. M., Lehmann, P. and Gotthard, K. (2016a). Do respiratory limitations affect metabolism of insect larvae before moulting? An empirical test at the individual level. *J. Exp. Biol.* **219**, 3061-3071.
Kivelä, S. M., Friberg, M., Wiklund, C., Leimar, O. and Gotthard, K. (2016b). Towards a mechanistic understanding of insect life history evolution: oxygen-dependent induction of moulting explains moulting sizes. *Biol. J. Linn. Soc.* **117**, 586-600.
Kivelä, S. M., Viinamäki, S., Keret, N., Gotthard, K., Hohtola, E. and Välimäki, P. (2017). Data from: Elucidating mechanisms for insect body size: partial support for the oxygen-dependent induction of moulting hypothesis. *Dryad Digital Repository* http://dx.doi.org/10.5061/dryad.sf2bt.
Locke, M. (1958). The co-ordination of growth in the tracheal system of insects. *Q. J. Microsc. Sci.* **99**, 373-391.
Loudon, C. (1988). Development of *Tenebrio molitor* in low oxygen levels. *J. Insect Physiol.* **34**, 97-103.
Nijhout, H. F. and Williams, C. M. (1974). Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): growth of the last-instar larva and the decision to pupate. *J. Exp. Biol.* **61**, 481-491.
Nijhout, H. F., Davidowitz, G. and Roff, D. A. (2006). A quantitative analysis of the mechanism that controls body size in *Manduca sexta*. *J. Biol.* **5**, 16.
Nijhout, H. F., Roff, D. A. and Davidowitz, G. (2010). Conflicting processes in the evolution of body size and development time. *Philos. Trans. R. Soc. B* **365**, 567-575.
Nijhout, H. F., Riddiford, L. M., Mirth, C., Shingleton, A. W., Suzuki, Y. and Callier, V. (2014). The developmental control of size in insects. *WIREs Dev. Biol.* **3**, 113-134.
Roff, D. A. (1992). *The Evolution of Life Histories: Theory and Analysis*. New York, US: Chapman & Hall.
Shingleton, A. W. (2011). Evolution and the regulation of growth and body size. In *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs* (ed. T. Flatt and A. Heyland), pp. 43-55. Oxford, UK: Oxford University Press.
Stieper, B. C., Kupershtok, M., Driscoll, M. V. and Shingleton, A. W. (2008). Imaginal discs regulate developmental timing in *Drosophila melanogaster*. *Dev. Biol.* **321**, 18-26.
VandenBrooks, J. M., Munoz, E. E., Weed, M. D., Ford, C. F., Harrison, M. A. and Harrison, J. F. (2012). Impacts of paleo-oxygen levels on the size, development, reproduction, and tracheal systems of *Blatella germanica*. *Evol. Biol.* **39**, 83-93.
Woods, H. A. (1999). Egg-mass size and cell size: effects of temperature on oxygen distribution. *Am. Zool.* **39**, 244-252.