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Plasticity of insect body size in response to oxygen: integrating molecular and physiological mechanisms

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The hypoxia-induced reduction of body size in *Drosophila* and *Manduca* is ideal for understanding the mechanisms of body size plasticity. The mechanisms of size regulation are well-studied in these species, and the molecular mechanisms of oxygen sensing are also well-characterized. What is missing is the connection between oxygen sensing and the mechanisms that regulate body size in standard conditions. Oxygen functions both as a substrate for metabolism to produce energy and as a signaling molecule that activates specific cellular signaling networks. Hypoxia affects metabolism in a passive, generalized manner. Hypoxia also induces the activation of targeted signaling pathways, which may mediate the reduction in body size, or alternatively, compensate for the metabolic perturbations and attenuate the reduction in size. These alternative hypotheses await testing. Both perspectives — metabolism and information — are necessary to understand how oxygen affects body size.

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Adult body size in insects is determined by the mechanisms that cause a larva to stop growing when a species-characteristic size is attained. The cessation of growth and pupation are physiological responses to an increase in the level of circulating ecdysteroids. Ecdysteroids are secreted by the prothoracic glands (PG), and their secretion is stimulated by the brain, via the secretion of the prothoracicotropic hormone (PTTH). Signaling through three pathways appears to be important in regulating the transcription and translation of the genes involved in ecdysteroid synthesis in the PG: the insulin/IGF signaling pathway, the TOR signaling pathway and the Ras/Raf/MAPK signaling pathway. Suppressing the activity of any of these pathways in the PG causes larvae to delay ecdysteroidgenesis, so larvae continue to grow and metamorphose into larger adults [1–3]. The physiological and endocrine events that lead to the secretion of PTTH and ecdysteroids, and hence to the cessation of growth, are initiated when larvae pass a well-defined critical weight. Hypoxia reduces the critical weight in *Manduca* and *Drosophila* and oxygen limitation has been shown to be the mechanism for the normal regulation of body size in *Manduca* [4•].

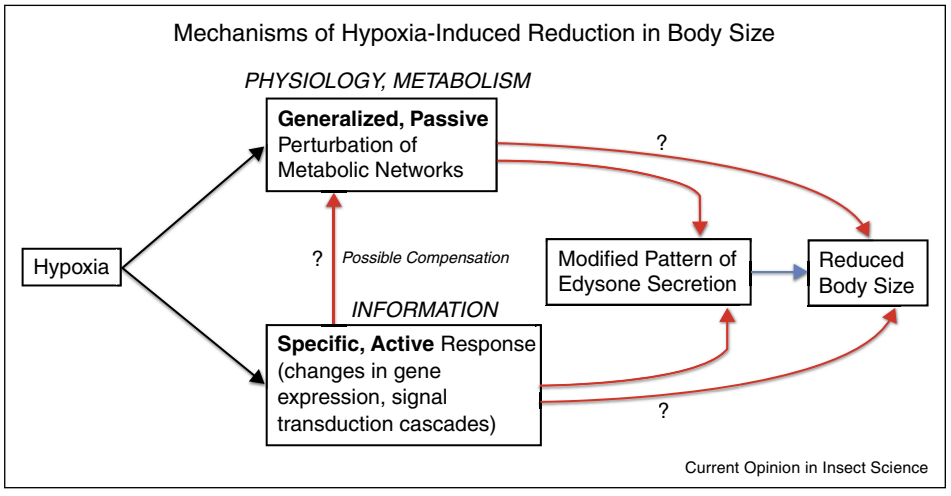
The hypoxia-induced reduction of body size in *Drosophila* and *Manduca* are ideal systems for understanding the mechanisms of phenotypic plasticity of body size. In *Drosophila*, the genetic and molecular mechanisms for oxygen sensing are fairly well elucidated [5–13]. At the other end, the effects of hypoxia on body size and development time have been documented [14–16] and are consistent across a variety of species [17,18]. What is missing is the link between the molecular mechanisms of oxygen sensing and the organismal-level phenotypic effects of hypoxia. These effects must come from the interaction of oxygen sensing pathways with the molecular and physiological mechanisms that regulate growth, developmental transitions, and body size. Fortunately, the mechanisms that regulate growth and the initiation of metamorphosis are well understood [19•], particularly in *Manduca* [4•,20–24] and *Drosophila* [2,3,25–29]. We integrate the molecular mechanisms of oxygen sensing discovered in *Drosophila* with what is known about the physiology of growth and size regulation in *Manduca* to outline a conceptual framework for understanding the hypoxia-mediated reduction in body size observed in diverse species.

Phenotypic plasticity

Phenotypic plasticity is defined as the responsiveness of an organism's phenotype to environmental inputs, and a reaction norm (which can be graded or switch-like) describes the quantitative relationship between an environmental variable and the phenotype for a particular genotype [30•]. Plasticity can be active and adaptive, as in the case of seasonal polyphenisms in butterflies, but also passive and non-adaptive, for example when homeostatic mechanisms in physiology and development fail to buffer against genetic or environmental disruption [30•]. Currently, it is unknown whether the hypoxia-induced reduction in body size is an active and adaptive response or whether it is passive and non-adaptive.

Hypoxia could have its effects in two principal ways (Figure 1). First, oxygen levels might affect biophysical

Figure 1



Mechanisms of hypoxia-induced reduction in body size. The arrows in blue are interactions that are fairly well understood, whereas the arrows in red highlight areas that require further investigation. Both 'metabolic' and 'informational' perspectives are required to understand the mechanisms that mediate the hypoxia-induced reduction in body size.

processes and biomolecular interactions in a generalized manner, in the same way that temperature affects the rates of all enzymatic processes. For example, low oxygen levels might limit the rate of ATP production and thus slow *all* cellular processes, including those that regulate growth and development. This view focuses on the biochemical conversion of metabolites from one pool to another, and the control of flux as the critical variable that determines which perturbations ultimately impact the phenotype [31,32]. Hypoxia affects the control flux because some enzymes are oxygen-dependent [33], and some reactions use oxygen as a substrate (e.g. oxygen is the final electron acceptor in oxidative phosphorylation). These passive perturbations of metabolism will have effects on growth and size and would be non-adaptive.

Alternatively, specific signaling pathways could elicit targeted, adaptive responses that mediate the effects of hypoxia on growth and size. There is evidence that the response to oxygen is specific and that there are dedicated signaling pathways such as Nitric Oxide Synthase (NOS) and Hypoxia-Inducible Factor (HIF) signaling that regulate growth and development in response to low oxygen, for example by promoting the proliferation of tracheoles [6].

In contrast to the metabolic view of biology, the informational view focuses on the information contained in the genome, and how it is translated into 'developmental programs' [34]. This perspective also includes the transduction of external stimuli into cellular responses via kinase signaling cascades and gene regulatory networks. Hypoxia is sensed by several signaling pathways including HIF, which induces changes in the expression of suites of genes, many of which control growth and developmental transitions.

A third possibility is that signaling pathways may respond dynamically to perturbations that low oxygen causes in metabolic pathways (e.g. oxidative phosphorylation and ATP production) by activating compensatory processes. The dynamic responses could ensure that growth and development remain coordinated across the whole body and thus produce a functional albeit smaller adult body size. The active response could imperfectly *compensate* for a reduction in body size caused by the passive mechanisms, thus minimizing the passive effects of hypoxia on growth and size.

The passive effect of hypoxia reflects the fact that hypoxia affects metabolic networks that require oxygen as a substrate or co-factor for enzymatic reactions and this would affect growth and size passively and in a generalized manner. In contrast, an active response to hypoxia requires the activation of new physiological and molecular compensatory mechanisms that may involve new signaling pathways and new patterns of gene expression. Both the metabolic and informational perspectives are necessary to understand the mechanism by which hypoxia causes a reduction in body size.

Energy: oxygen as fuel for growth and metabolism

Manduca larvae undergo a metabolic switch in the middle of their final instar. Not only do late-instar *Manduca* larvae exhibit reduced oxygen-delivery safety margins [35], but also, remarkably, after larvae pass the critical weight, their rate of oxygen consumption levels off. The larvae