Hypoxia in insects

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All living beings require a minimal amount of oxygen for their daily survival and to ensure proper functioning of all their body parts. Hypoxia is described as a phenomenon where there is a deficit in sustainable amounts of oxygen at tissue levels, required for carrying out normal physiological actions. Hypoxia occurs when there is less than normal levels of oxygen pressure (as opposed to normoxia or normal conditions). Like almost all other living beings, insects regulate their uptake of oxygen by carefully balancing out oxygen toxicity and deprivation. An increase or decrease in ambient oxygen levels results in suspension of homeostasis, activity and growth. This in turn incites physiological responses in the insect body. When response mechanisms are unable to keep up with the growing demand of the tissues, it results in either hypoxemia (excess amounts of oxygen) or hypoxia (insufficient amount so oxygen). When faced with hypoxia, insects often try to counteract by activating both short and long-term mechanisms. It is a common belief amongst many scientists that the mass extinction of insect species during the late Permian period came about due to induced hypoxic stress as a result of low atmospheric oxygen and rising temperatures in the environment.

Types of hypoxia in insects

Hypoxia in insects manifests itself in two forms, *i.e.,* functional and environmental hypoxia. In functional hypoxia, the supply of oxygen falls short to meet the oxygen demand. The occurrence of this type of hypoxia is normal early insect development and is a factor in mediating life-history trade-offs. When oxygen levels drop below the ambient oxygen level of 21 kPa is known as environmental hypoxia (Harrison et al., 2018). Hypoxia in insects have been observed in different types of habitats, ranging from aquatic, terrestrial, subterranean and high altitudes. A key observation in insects when exposed to hypoxia is decrease in ATP production which results from a decrease in oxygen in the living tissues. Hypoxia also manifests itself in the form of protein unfolding, inflammation and immune response. In order to deal with the drop in ambient oxygen levels, insects often try to increase the oxygen supply by opening their spiracles and increasing abdominal pumping. The effects of hypoxia can be seen in insects in the form of reduced body size, decreased growth and survival in *Drosophila melanogaster*, decreased egg production and reduced adult emergence in *Callosobruchus maculates*, and developmental abnormalities at ecdysis (*Manduca sexta*) (Harrison et al., 2006). In certain instances, hypoxia has also induced susceptibility in *Tribolium castaneum* to microbial infection *Beauveria bassiana* (Lord, 2009).

Response of insects to hypoxia

Insects have developed remarkable ways to deal with hypoxia, these changes may be morphological, physiological or behavioural in nature and may vary based on the severity of hypoxia and habitat of the insect.

Exposure to hypoxia typically brings about a slew of behavioural changes, this often starts with an immediate increase in frequency of spiracular openings, ventilatory movements and activity. Terrestrial insects like American bird grasshopper (*Schistocerca americana*) respond to hypoxia by increasing tracheal conductance and entering quiescence. Insects also adopt escape measures like damsel fly nymphs have shown an increase in their rate of ventilatory movements. Aquatic insects survive hypoxic waters by spreading their gills and exposing their respiratory structures to the surface air. The latter are less tolerant to hypoxic conditions than terrestrial insects (Hoback et al., 2001). Insects often reside in unusual microhabitats, to avoid excess competition and predators. These microhabitats may be severely hypoxic or anoxic, which results in the development of several physiological adaptations. The adult scarab dung beetles show quiescence at lower oxygen concentrations (<1-2% oxygen). The beetles are capable of movement and seem to measure hypoxia and even move to oxygen-rich areas of the ding pat, when necessary (Holter and Spangenberg, 1997). Similar behaviour has also been observed in calliphorid flies, *Phormia regina* and *Calliphora vomitoria*, where the larvae move from anoxic to oxygenated areas of decaying tissues while feeding (Brand, 1946).

Insect tracheal systems are highly efficient due to Fick's law of diffusion, according to which the rate of diffusion within tracheal systems is linearly at par with the cross-sectional volume. This aids in the maintenance of the rate of oxygen uptake by insects between 1-2%. Hypoxic conditions interrupt the movement of oxygen, halting its flow to the tissues. Morphological changes in the respiratory system of developmental stages helps insects accommodate hypoxic conditions. For example, enlargement of the cross- sectional tracheal volume allows greater intake of air into the tissues. Yellow mealworms (*Tenebrio molitor*), displayed a 40% increase in the cross-sectional volume when reared in 15% oxygen (Loudon, 1988). An increase in the number and frequency of tracheoles and tracheal branches has also been observed in fruit flies, where tracheolar branching seems to be induced due to the secretion of fibroblast growth factor from oxygen starved tissue (Wingrove and O'Farell, 1999). Intertidal species like *Pemphigus treherni* maintain low metabolic rates which are supported by cuticular gas exchange, when submerged (Forster and Treherne, 1976). However, *Anurida maritima,* survives submergence by trapping air-bubbles, which allow respiration for at least three hours (Zinkler et al., 1999). Nitric oxide/cyclic GMP pathway are mostly responsible for tracheole growth (Wingrove and O'Farell, 1999). Over-expression of nitric oxide synthase results in greater hypoxia response while its inhibition decreases such responses. In many instances, insects have also been reported to depend upon biochemical mechanisms to develop a suitable mechanism to withstand hypoxia. Long-term responses of hypoxia include activation of hypoxia-inducible factor (HIF) pathway in insects. Although the consequences of the induction of HIF have been poorly studied, HIF has been linked to induction of a homologue of fibroblast growth factor (FGF), which stimulates production of new branches in local tracheoles (Maxwell, 2004).

Schistocerca americana showed an increase in pH values of hemolymph which might be a result of intracellular pH regulation activities. Low oxygen supply often results in oxidative damage in insects, tiger beetle larvae prevent oxidative stress by lowering their metabolism while goldenrod gall insects, *Eurosta solidaginis* and *Epiblema scudderiana* accommodate ruperfusion (Harrison et al., 2018). Under anoxic conditions, a broad spectrum of metabolic events may take place which inhibit key aerobic pathways. Insects then switch to anaerobic metabolism to continue the production of ATP. This switch is often regulated by cellular signalling pathways that sense oxygen levels and trigger metabolic changes. In *Chironomus thummi* for example, exposure to anoxia led to severe decline in ATP levels, on the other hand ADP, AMP and IMP increased rapidly (Redecker and Zebe, 1988). In some cases, anoxic conditions in aquatic insects have led to accumulation of glycerol or conversion of carbohydrate to lactic acid, providing results similar to cold-acclimation (Heslop et al., 1963).

Role in insect pest management

Gaseous fumigants are often used as insecticides in storage conditions. Since the gases enter the body of the insect through the respiratory system, factors affecting respiration such as concentration of O_2, CO_2 and other gases play a potential role in efficiency of the fumigant toxicity (Lu et al., 2009). Modified or controlled atmospheres (MAs or CAs), which involve increasing or lowering the levels of atmospheric gases such as oxygen (O_2), carbon dioxide (CO_2), ozone (O_3), and nitric oxide (NO), offer a cost-efficient way to eliminate specific pests and safeguard stored products. Aerobic organisms depend on specific levels of oxygen for their survival. Manipulation of the level of oxygen present for utilization by the insects can greatly impact their normal physiological and biological conditions, resulting in high mortality rates. Insect tolerance to hypoxia has a crucial role to play in insect control (Cui et al., 2017). In case of *Sitophilus zeamais,* reduction of oxygen levels to 0% in 6–9 days in hermetic conditions, led to a significant decrease in the number of offspring when compared to weevils in non-hermetic conditions (Moreno-Martinez et al., 2000).

Conclusion

Continuous dearth of oxygen in living tissues leads to severe hypoxia or anoxia, conditions where there is a complete lack of oxygen. Insects, unlike vertebrates have a much developed although obscure, mechanism which enables them to recover from such conditions. Hypoxia is a well-studied phenomenon, with applications in various fields ranging from basic and comparative biology to biomedical sciences. Given their efficient tracheal system and their ability to escape hypoxic conditions through multiple developmental, physiological and ecological aspects, insects have become model organisms in the study of hypoxia. Hypoxia is a common occurrence in insect development and is a crucial factor in life-history trade-offs but the various mechanisms of functional hypoxia still remain an enigma. Despite all the research in this area of interest, many questions pertaining to species-specific adaptations to hypoxia still remain unanswered. Studies on the role of oxygen signalling in insect development and effects of hypoxia would provide a good insight to insect life support system. Additionally, by leveraging modified oxygen levels, pest management becomes a more sustainable and effective practice, providing long-term protection for stored products and reducing reliance on chemical pesticides.

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