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Cold adaptation mechanisms in the ghost moth *Hepialus xiaojinensis*: Metabolic regulation and thermal compensation

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ABSTRACT

Ghost moths (Lepidoptera: Hepialidae) are cold-adapted stenothermal species inhabiting alpine meadows on the Tibetan Plateau. They have an optimal developmental temperature of 12–16 °C but can maintain feeding and growth at 0 °C. Their survival strategies have received little attention, but these insects are a promising model for environmental adaptation. Here, biochemical adaptations and energy metabolism in response to cold were investigated in larvae of the ghost moth *Hepialus xiaojinensis*. Metabolic rate and respiratory quotient decreased dramatically with decreasing temperature (15–4 °C), suggesting that the energy metabolism of ghost moths, especially glycometabolism, was sensitive to cold. However, the metabolic rate at 4 °C increased with the duration of cold exposure, indicating thermal compensation to sustain energy budgets under cold conditions. Underlying regulation strategies were studied by analyzing metabolic differences between cold-acclimated (4 °C for 48 h) and control larvae (15 °C). In cold-acclimated larvae, the energy generating pathways of carbohydrates, instead of the overall consumption of carbohydrates, was compensated in the fat body by improving the transcription of related enzymes. The mobilization of lipids was also promoted, with higher diacylglycerol, monoacylglycerol and free fatty acid content in hemolymph. These results indicated that cold acclimation induced a reorganization on metabolic structure to prioritise energy metabolism. Within the aerobic process, flux throughout the tricarboxylic acid (TCA) cycle was encouraged in the fat body, and the activity of α-ketoglutarate dehydrogenase was the likely compensation target. Increased mitochondrial cristae density was observed in the midgut of cold-acclimated larvae. The thermal compensation strategies in this ghost moth span the entire process of energy metabolism, including degration of metabolic substrate, TCA cycle and oxidative phosphorylation, and from an energy budget perspective explains how ghost moths sustain physiological activity in cold environments.

1. Introduction

Ghost moths (Lepidoptera: Hepialidae) are obligate hosts of *Ophiocordyceps sinensis* (Berk.) and the fungal stroma and moth larvae form a very important traditional Chinese medicine called dongchongxiacao (Sung et al., 2007). Ghost moths belong to the earliest lepidopteran lineage and have a primitive appearance (Wiegmann et al., 2002). They are cold-adapted stenothermal animals distributed in alpine meadows on the Tibetan Plateau between altitudes of 3600–5200 m (Jin et al., 2010; Tu et al., 2011). They mainly inhabit the soil layer 5–20 cm below the surface, where temperatures fluctuate between −5 °C and 15 °C throughout the year (Wang et al., 2006). The optimal temperature for development of ghost moths is between 12 °C and 16 °C (Chen et al., 2002; Liu et al., 2007; Zhu et al., 2009), at which their life cycle is completed within one year instead of 3–5 years under natural conditions (Tu et al., 2011). Ghost moths maintain feeding activity and growth at relatively cold temperatures, from 0 °C to a few degrees above zero (Guo et al., 2008; Tu et al., 2011; Wang et al., 2006). This prominent adaptation to cold has received little attention, but revealing the underlying mechanism of this capability is necessary to better understand the principles of environmental adaptation in insects and potentially culturing this economically important species in the future.

In ectotherms, cold exposure depresses the rate of basic metabolic processes and reduces activity such as respiration, locomotion,

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feeding and development, especially when temperatures approach 0 °C (Block, 1990; Lee, 2010). Among the physiological processes influenced by temperature, the reduction in metabolic rate may be the most crucial to the overall physiological status of organisms because it is the energy-producing process sustaining the ATP expenditure of other physiological processes. To maintain energy metabolism homeostasis at low temperatures, ectotherms may either enter into metabolic suppression or sustain activity using thermal compensation strategies (Guderley and St-Pierre, 2002). Metabolic suppression (e.g. diapause) is characterized by delayed development, reduced locomotion and minimal feeding (Schiesari and O'Connor, 2013; Tauber et al., 1986; Vinogradova, 2007) via a coordinated suppression of ATP production and consumption (Storey and Storey, 2004). Thermal compensation makes it possible to maintain energy-intensive activities like locomotion, feeding and development at low temperatures and allows better exploitation of the environment (Cossins and Bowler, 1987), which is presumed to be the case for cold-adapted ghost moth species. Thermal compensation is a regulation strategy amongst many cold-adapted ectothermic species, including lugworms, bivalves, crocodiles and fish (Glanville and Seebacher, 2006; Guderley and St-Pierre, 2002; Sommer and Pörtner, 2002; Tschischka et al., 2000). It is characterized by high metabolic capacity at low temperatures in naturally or artificially cold-acclimated individuals (Cossins and Bowler, 1987; Horwath and Duman, 1983). Thermal compensation is also found as part of seasonal cold adaptation in insects such as *Acheta domesticus* L. (Lachenicht et al., 2010), *Pyrrharctia isabella* (Layne-Jr et al., 1999), *Dendroides canadensis* and *Dendroides concolor* (Horwath and Duman, 1983) and plays a role in rapid environmental adaptation along latitudinal clines in insects (Addo-Bediako et al., 2002; Huey and Pascual, 2009). Though thermal compensation potentially explains environmental adaptation of insects across time and space, subsequent metabolic, physiological and compensation regulation strategies have been poorly studied.

Here, thermal compensation phenomenon in larvae of the ghost moth *Hepialus xiaojinensis* were confirmed at a physiological level. Considering that metabolites are the end products of cellular processes and that variation is expected to reflect the final regulatory effect of molecular signal transduction and explain physiological phenomena intuitively (Bundy et al., 2008; Nicholson and Lindon, 2008; Wishart, 2007), metabolomics based on gas chromatography-mass spectrometry (GC–MS) was chosen to initially explore system-wide metabolic adjustments at cold stress. Then, transcriptional, enzymatic and subcellular parameters were determined to supplement the metabolomic data. Our aim was to describe cold adaptation regulation mechanisms in this insect and identify potentially novel energy metabolism and thermal compensation strategies.

2. Materials and methods

2.1. Insect rearing and cold acclimation

H. xiaojinensis pupae were collected from Xiaojin, Sichuan, China, and the colony was maintained in a 15 °C cold room in Beijing. After molting to the 3rd instar, larvae were reared individually on carrots in 10 cm plastic dishes in the dark. The 8th instar larvae, with average weight of 0.584 ± 0.02 (mean ± SE), were randomly used for all experiments comparing the cold-acclimated and control (unacclimated) groups. The cold-acclimated group was treated at 4 °C for 48 h, and its respiration rate at 4 °C were measured at 24 and 48 h after cold acclimation respectively. The control group was maintained at 15 °C. Other ambient conditions were kept the same between the two groups. It must be mentioned that an acclimation course of 48 h was chosen for its obvious compensation effect on respiration at 4 °C. Besides, a short but effective acclimation course could avoid unexpected outcomes resulting from growth and be able to detect the adaptive adjustments at a transcriptional level, which may not last very long. After temperature regimes, the larvae of both groups were immediately used in subsequent experiments or material preparation.

2.2. Measurement of metabolic rate

We used a Sable Systems respirometry system (Las Vegas, USA) to measure metabolic rate (represented by the production rate of CO_2 and the consumption rate of O_2). The system included an SS-3 subsampler unit, MFC-2 Gas Mixers and Mass Flow Controller, RM8 Gas Flow Multiplexer, CA-10A CO_2 analyzer, FC-10A O_2 analyzer, ExpeData and UI-2 package. For each test, larvae were placed into a respirometry cuvette serially connected to the Sable System, and the cuvette was set in a thermostatic chamber with a designated temperature of 15 °C, 12 °C, 8 °C or 4 °C. After the system was stable, measurement was carried out under a closed-circuit model with a gas flow at 228–236 ml/min for 10 min. Variation in CO_2 and O_2 were recorded using a real-time curve graph.

Respiration data from the Sable System were processed using associated ExpeData software. The CO_2 production rate or the O_2 consumption rate was calculated using the following formula:

$$\text{Metabolic rate} = \frac{\text{Slope} \times \text{Pressure} \times \text{Volume}}{\text{Temperature} \times \text{weight} \times R \times 100000},$$

where, metabolic rate is the production rate of CO_2 or the consumption rate of O_2, mol/s/g; slope is the curve slope of CO_2 production or O_2 consumption, read from ExpeData; pressure is the air pressure in the closed-circuit system, 101.9 kPa; volume is the volume of the closed-circuit system, 140 ml; temperature is the temperature of the closed-circuit system, K; and R is the ideal gas constant.

The Q_{10} coefficient of CO_2 production rate or O_2 consumption rate was calculated using the following formula:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)},$$

where, Q_{10} is the factor by which the reaction rate increases when the temperature is raised by ten degrees; T_1 and T_2 are temperatures (K or °C); R_1 is the measured metabolic rate at temperature T_1 (where $T_1 < T_2$); and R_2 is the measured metabolic rate at T_2 (where $T_2 > T_1$).

2.3. Insect dissection and preparation of material

For each larva, hemolymph (30–60 μl) was collected through incisions made on the prolegs (Oda et al., 1997) and stored at −100 °C. Then, midgut and fat body were dissected from the abdomen and placed into separate Eppendorf tubes. After washing in Ringer's, tissues were stored at −100 °C.

2.4. Thin-layer chromatography (TLC) for total lipids in hemolymph

The method followed the extraction of total lipids by Bligh and Dyer (1959) with appropriate scaling in volume. In brief, 15 μl of hemolymph was mixed with 25 μl Mili-Q water, 50 μl chloroform and 100 μl methanol and pipetted repeatedly for 2 min. After a centrifuging procedure at 12,000*g* for 10 min the supernatant was transferred into a clean tube with 50 μl chloroform and 50 μl Milli-Q water, followed by 15 s of vortex. After being centrifuged at 10,000*g* for 10 min, the chloroform phase was collected with a syringe, and then dried in a vacuum drier. After being dissolved in 30 μl 2:1 chloroform:methanol, the total lipid sample was ready to analyze on the silica gel chromatography plate