QIRT'2022
10.21611/qirt.2022.3004
16th Quantitative InfraRed Thermography Conference

Monitoring *Drosophila melanogaster* thermogenesis by Infrared Thermography

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Abstract

Larvae of the poikilotherm insect *Drosophila melanogaster* reared at 23°C and switched to lower temperatures (i.e. 10°C) for over an hour are warmer than the surrounding medium. This is likely to be related to uncoupled respiration, resulting in thermogenesis. Indeed, silencing of the uncoupling protein UCP4C by suitable genetic manipulation significantly reduces larval thermogenesis. Measurement of temperature in wild-type, knock-down and knock-out larvae (positioned on a Thermal Controller maintaining surrounding average temperature constant) is performed by Infrared Thermography.

1. Introduction

Heat is produced in warm-blooded animals by *thermogenesis*. Apart from that induced by voluntary muscles movement, thermogenesis can be classified as shivering and non-shivering. The shivering is due to the transformation of Adenosine Triphosphate (ATP) in kinetic energy, that eventually becomes heat. The non-shivering one occurs in brown fat, and is mediated by Uncoupling Proteins (UCP). UCPs are capable of dissipating the proton gradient generated by pumping of protons from the mitochondrial matrix to the mitochondrial intermembrane space. The energy used in dissipating the proton gradient is lost and the protons diffusion dissipated as heat [1]. Such non-shivering thermogenesis is essential in the regulation of body temperature of hibernators in winter months, small rodents and human babies, i.e. when the ratio between body surface and volume is high. While UCPs are assumed to mediate mitochondrial non-shivering thermogenesis in mammals, whether they work in a similar fashion in arthropods remains debated [2].
The fruit fly *Drosophila melanogaster* originated in tropical Africa and colonized Europe only about 15,000 years ago (end of the last glaciation) eventually spreading to Australia and the Americas over the past few centuries [3]. This insect can be classified as poikilotherm (i.e. its body temperature essentially reflecting that of the surrounding environment).

It is a human commensal species that adapts to a wide range of environmental thermal conditions, even though the physiology of its climatic adaptation is not fully understood. Based on sequence homology with mammalian UCPs, four UCPs have been identified in *Drosophila melanogaster*, i.e. UCPs 4A, 4B, 4C and 5 [4]. While Drosophila UCP5 does not seem to be a *bona fide* uncoupling protein, the involvement of UCPs 4A, 4B and 4C (4C being the most interesting candidate) is currently under investigation. In a previous study, larvae of *Drosophila melanogaster* reared at 23°C and switched to 14°C for 1 h were shown to be 0.5°C warmer than the surrounding medium. Silencing of *Ucp4C* conferred sensitivity of respiration to oligomycin and uncoupler, and prevented larva-to-adult progression at 15°C but not at 23°C. Uncoupled respiration of larval mitochondria required palmitate and was dependent on UCP4C. Thus, UCP4C seems to be required for development through the prepupal stages at low temperatures and may be a *bona fide* uncoupling protein [5].

2. Materials and methods

Flies used for the experiment were maintained on standard cornmeal medium and at 23°C (70% relative humidity) on a 12 h light, 12 h dark cycle. After suitable preparation the Oxygen consumption rate (OCR) was measured at 25°C either with a Seahorse XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Billerica, MA) (BWPs and intact cells) or with a Clark-type oxygen electrode (isolated mitochondria and permeabilized cells). The XF24 Extracellular Flux Analyzer measures the OCR through a sensor cartridge embedded with a fluorescent sensor for oxygen coupled to a fibre-optic waveguide. The waveguide delivers light at 532 nm and transmits a fluorescent signal through optical filters (650 nm for oxygen) to highly sensitive photodetectors.

To assess whether uncoupled respiration is linked to heat production, we used infrared thermography to study body temperature of wild-type, knocked-down and knock-out larvae, as well as of a clock-mutant in which *Ucp4C* is expressed constitutively (as opposed to the wild-type, in which *Ucp4C* shows a circadian fluctuation in transcription abundance). Larvae were reared at 23°C and switched to 10°C for 2 h before thermographic recording. They were positioned over a Petri dish containing an agar bed and positioned over a PTC-100 Programmable Thermal Controller, as shown in Fig. 1.

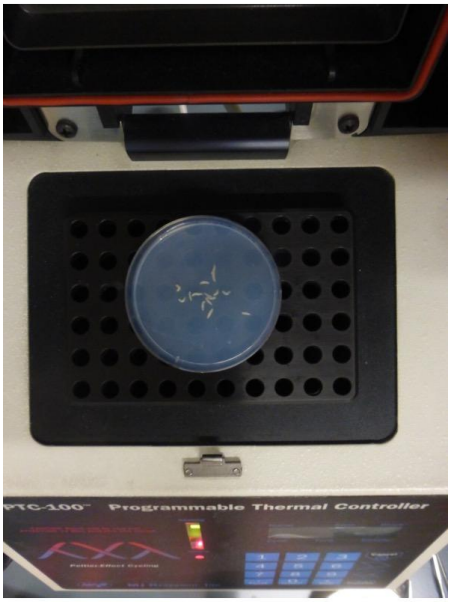

Fig. 1. *Larvae in a Petri dish containing an agar layer and positioned over the PTC-100 Programmable Thermal Controller and maintained at 10°C.*

Measurements were carried out by a FLIR SC660 camera, which is characterized by a 640 X 480 pixels bolometric array and FLIR THERMACAM SC3000, 320 X 240 pixels, with close-up lens for improved spatial resolution (100 μm pixel resolution) and sensitivity (NETD < 30 mK). Figure 2 shows a typical IR image of the larvae, appearing warmer than the background. These preliminary results indicate of a clear effect of thermogenesis in wild-type larvae. The levels of thermogenesis observed in *Ucp4C* knock-down and knock-out larvae were less consistent, most likely in relation to off-target effects in the case of the knock-down and compensatory effects in both knock-down and knock-out. As expected, higher levels of thermogenesis compared to the wild type were observed in the clock-mutant larvae due to constitutive expression of *Ucp4C*.

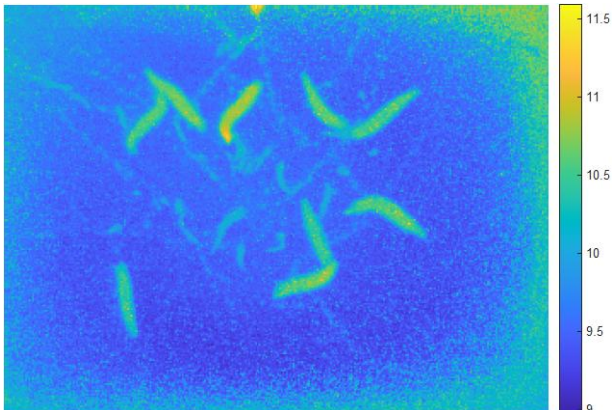

Fig. 2. *IR image of larvae which clearly appear to be warmer than the surrounding medium (temperature scale on the right hand side).*

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