Supplementary material

Oxygen and temperature affect cell sizes differently among tissues and between sexes of *Drosophila melanogaster*

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S 1.1 Histological techniques and cell size measurements

S 1.1.1 Wings and epidermal cells

After dissection, wings were preserved at -20°C in Eppendorf tubes. Wings were mounted on microscopy slides using ST Ultra and CV Ultra (Leica Biosystems, Nussloch, Germany) and digitalized under 20× objective magnification. Following Dobzhansky (1929), each trichome on a wing blade represents one epidermal cell. Therefore, we calculated the mean wing epidermal cell size (μm^2) by dividing a defined wing area by the number of corresponding trichomes. Following our previous methods (Czarnoleski et al., 2013; Szlachcic et al., 2023), we considered trichomes on the dorsal wing blade in a 0.031-mm^2 circle placed between the cubital and distal veins. For this purpose, we used two macros embedded in ImageJ software (National Institutes of Health (NIH), Bethesda, MD, USA). Heads were preserved with 1 ml of 20% methanol (POCH, Gliwice, Poland).

S 1.1.2 Eyes and ommatidial cells

Ommatidia, which form compound eyes, are composed of a fixed number of cells (Cagan, 2009). Thus, based on previous studies (Privalova et al., 2023; Schramm et al., 2015; Szlachcic et al., 2023), we used their size as a measure of the size of ommatidial cells. For the measurements, both eyes were covered with a transparent 5SecondFix UV glue, which was dried with a UV lamp (Ontel Products, Fairfield, USA). The eye replicas were removed and flattened on a slide by incising in three places. A drop of glycerine (POCH) was added, and the slide was covered with a coverslip. On the top of each slide, fishing weights (100 g) were placed for 24 h to evenly flatten replicas, before the coverslip edges were sealed with nail polish. The replicas were digitalized under 10× objective magnification. We estimated the mean ommatidium size (μm^2) using automatized methods (Schramm et al., 2015) integrated into ImageJ (NIH).

S 1.1.3 Legs and epidermal cells

Legs were preserved with 1 ml of 70% ethanol (Linegal Chemicals, Warszawa, Poland). For epidermal cell measurements, a leg was placed on a microscopic slide to image the 8th row of the basitarsus under 20× objective magnification. Using ZEN software (Zeiss), we measured the distance (μm) between the first and last bristles of the 8th row (based on the nomenclature of Hannah-Alava (1958)). We counted the total number of bristles along the row (Privalova et al., 2023; Szlachcic et al., 2023). According to Held (1979), the bristle number in a row and

cell number along the basitarsus and the bristle interval and cell diameter are well coordinated. Therefore, to calculate a proxy of epidermal cell size (μm), the measured length of the row was divided by the bristle number in the row. For consistency with other cell types, the linear measures were squared for the final analysis (μm^2).

S 1.1.4 Malpighian tubules and epithelial cells

Malpighian tubules were extracted an abdomen with a help of entomological pins (Paradox, Kraków, Poland). We opened abdomens placed on the microscopic glass in a drop of PBSTX (mixture of phosphate-buffered saline (PBS; Sigma Aldrich, Saint Louis, USA) and Triton X-100 (POCH)). Slides with the extracted Malpighian tubules were covered with a coverslip and digitalized under 20× magnification with a phase contrast objective. We estimated the proxy of cell size in Malpighian tubules (μm^2) from the density of cell nuclei/nucleoli in a defined area of the tubule (Privalova et al., 2023; Schramm et al., 2021; Szlachcic et al., 2023). We outlined the areas using ImageJ software with a LiveWire Plugin (NIH) and counted the number of nuclei/nucleoli in these areas. The measurements were carried out in five to ten images per fly.

S 1.1.5 Thorax and flight muscles

Dissected thoraxes were used to measure dorsal longitudinal muscle cells, a subset of indirect flight muscle cells (Privalova et al., 2023; Szlachcic et al., 2023). According to the modified methodology of Barbosa et al. (2015), we preserved thoraxes for 2 h in Duboscq–Brasil's solution. Over this time, Eppendorf tubes with thoraxes were placed in a beaker with water standing on a hot plate (50 °C) for 10 minutes. Next, thoraxes were washed in PBSTX, dehydrated in 70%, 80%, 90% and 96% ethanol (Linegal Chemicals), butanol (Chempur, Piekary Śląskie, Poland) and isopropanol (Leica), cleared in pure Clearene (Leica), then in a mixture of Clearene (Leica) and Paraplast Plus (Leica) and finally embedded in Paraplast Plus (Leica). Thoraxes were cut with a rotary microtome (Hyrax M55, Zeiss) into 6-μm thick cross-sections. The slides with cuts were deparaffinized in Clearene (Leica), rehydrated in isopropanol (Leica) and 96%, 90%, 80%, 70% and 50% ethanol (Linegal) and distilled water, and stained with Ehrlich haematoxylin (Carl Roth) and then with Gömöri trichrome (BioOptica, Milano, Italy). After these steps, the slides were washed in 0.2% acetic acid (POCH), dehydrated in 96% ethanol (Linegal), cleared in a mixture of phenol (Chempur) and xylene (POCH) and then in pure xylene (POCH), and embedded in CV Mount (Leica). Using 20× objective magnification, we imaged one bundle from each of the six pairs of muscle

bundles. Using ImageJ (NIH), we outlined the cross-section area of individual muscle cells (fibres) (μm^2).

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