How fruit flies become winter warriors

Describing the ionoregulatory adjustments that underlie cold acclimation in *Drosophila*

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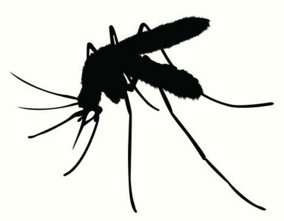

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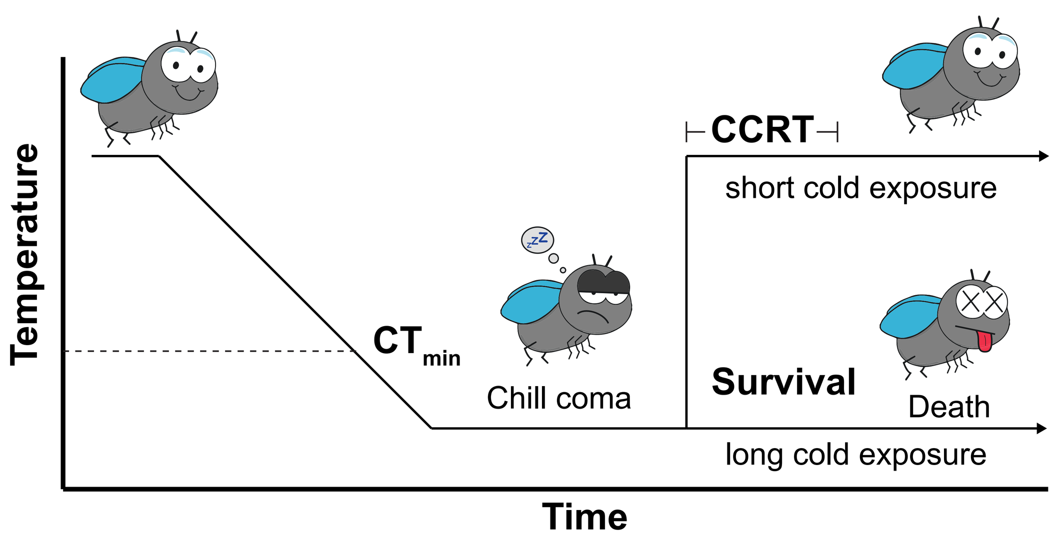
Introduction

- Low temperatures are major determinants of insect distribution and abundance and climate change is rapidly altering winter conditions[1]. Thus, a detailed understanding of insect cold tolerance and plasticity is sorely needed.

- Chill tolerance is primarily assessed in three ways:
  1. **Critical thermal minimum (CT_{min})** – temperature at which insects enter a cold-induced coma (chill coma).
  2. **Chill coma recovery time (CCRT)** – time to recover from chill coma following removal from the cold.
  3. **Survival** – survival rates following severe cold exposure.



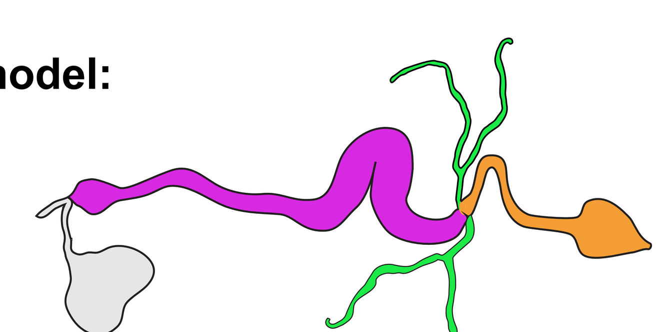
- At low temperatures, insects lose ion homeostasis, presumably because chilling decreases ion-motive ATPase activity in the gut. This causes leak of Na^+ and water into the gut and increased hemolymph [K^+][2].
- High extracellular [K^+] depolarizes cell resting potential, prolonging CCRT, and causing cell death[3].
- However, pre-exposure to mild cold treatments (cold acclimation) mitigates cold-induced hyperkalemia and delays the onset of chilling injuries[4].
- In insects, the Malpighian tubules and the gut are responsible for regulating ion and water balance[5].

We therefore hypothesized that:
Functional changes to the Malpighian tubules and gut of *D. melanogaster* preserve ion balance in the cold.

Specifically, we predict that cold acclimation involves:

(1) Altered ion-motive ATPase activity (V-type H^+-ATPase and Na^+/K^+ ATPase) in the gut and Malpighian tubules.
(2) Sustained Malpighian tubule activity at low temperatures
(3) Decreased K^+ absorption in the gut.

Proposed model:

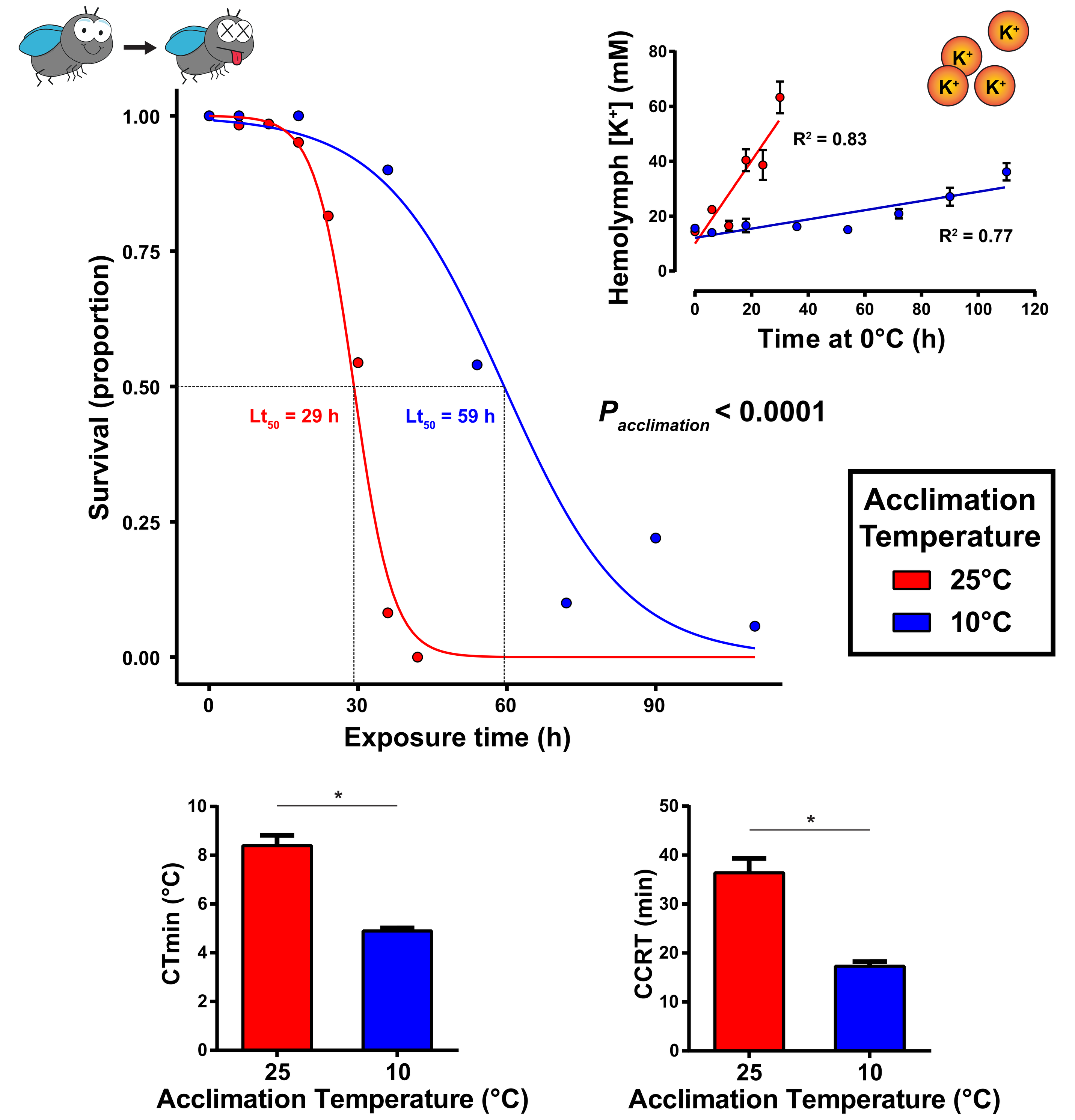


	Midgut	Malpighian tubules	Hindgut
ion-motive ATPase activity	↓	↑	↓
Proposed effect	decreased K^+ absorption	increased K^+ clearance	decreased K^+ absorption

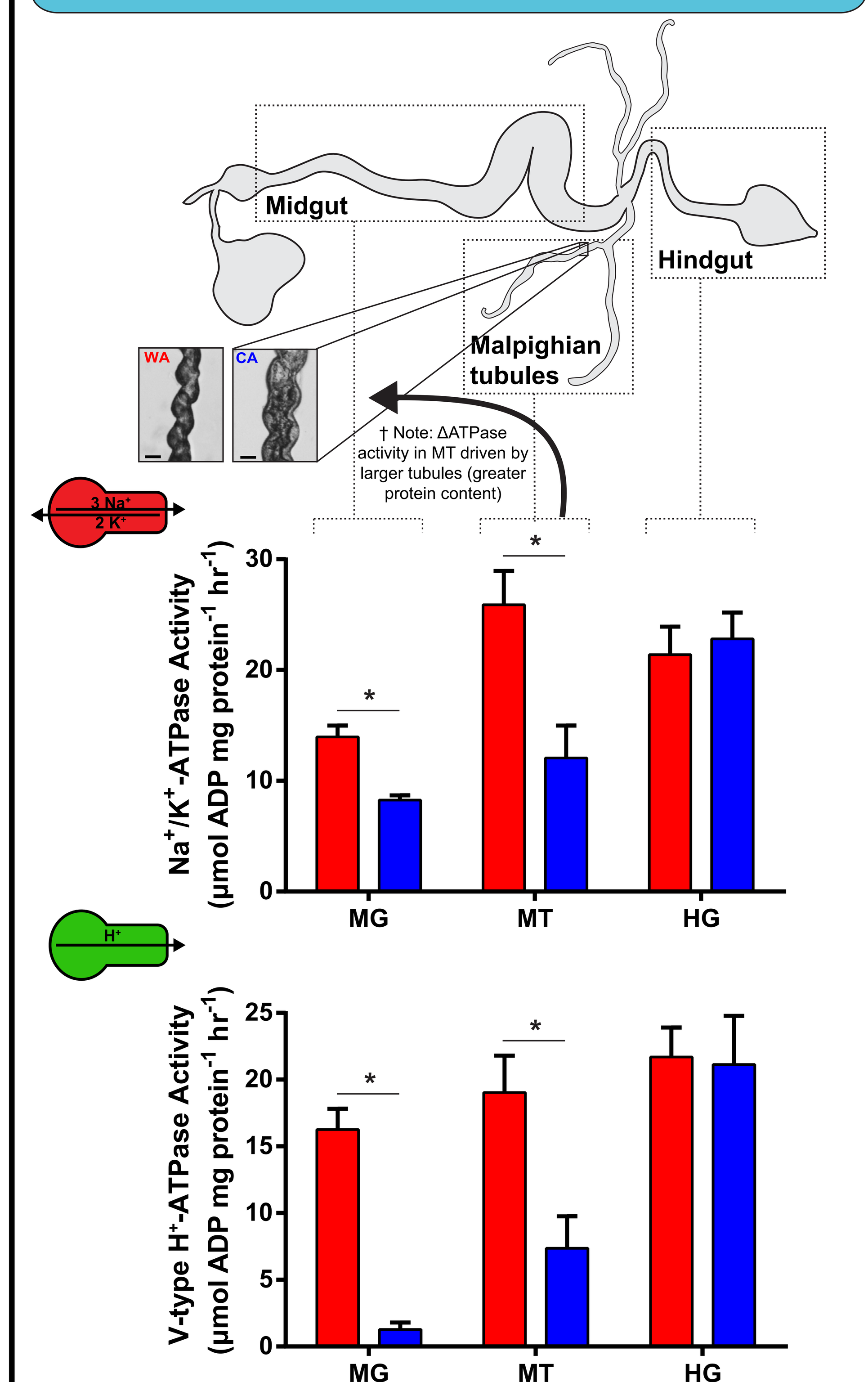
mitigate cold-induced hypekalemia!

Results

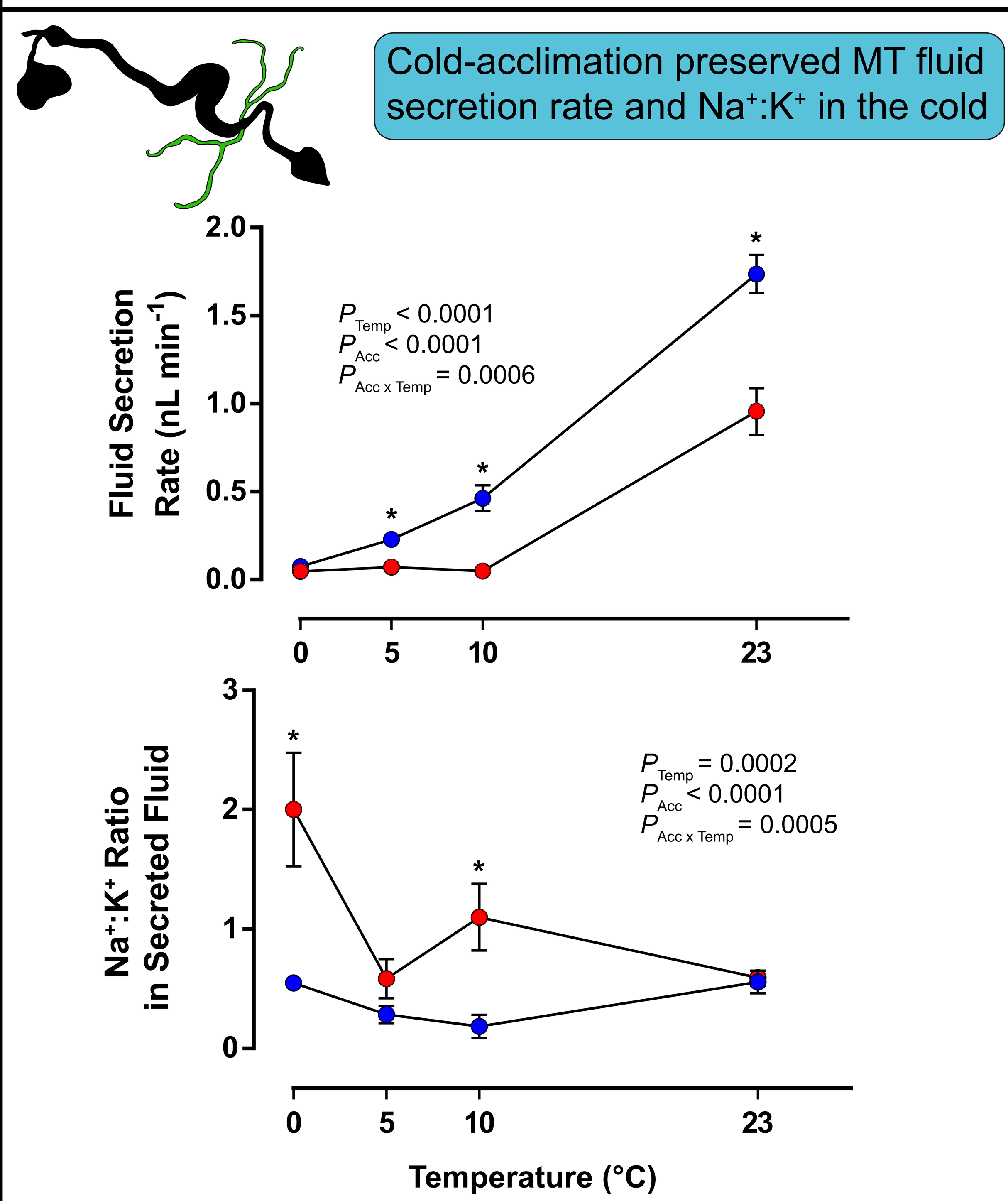
Cold acclimation improved survival, CT_{min}, and CCRT and mitigates hyperkalemia



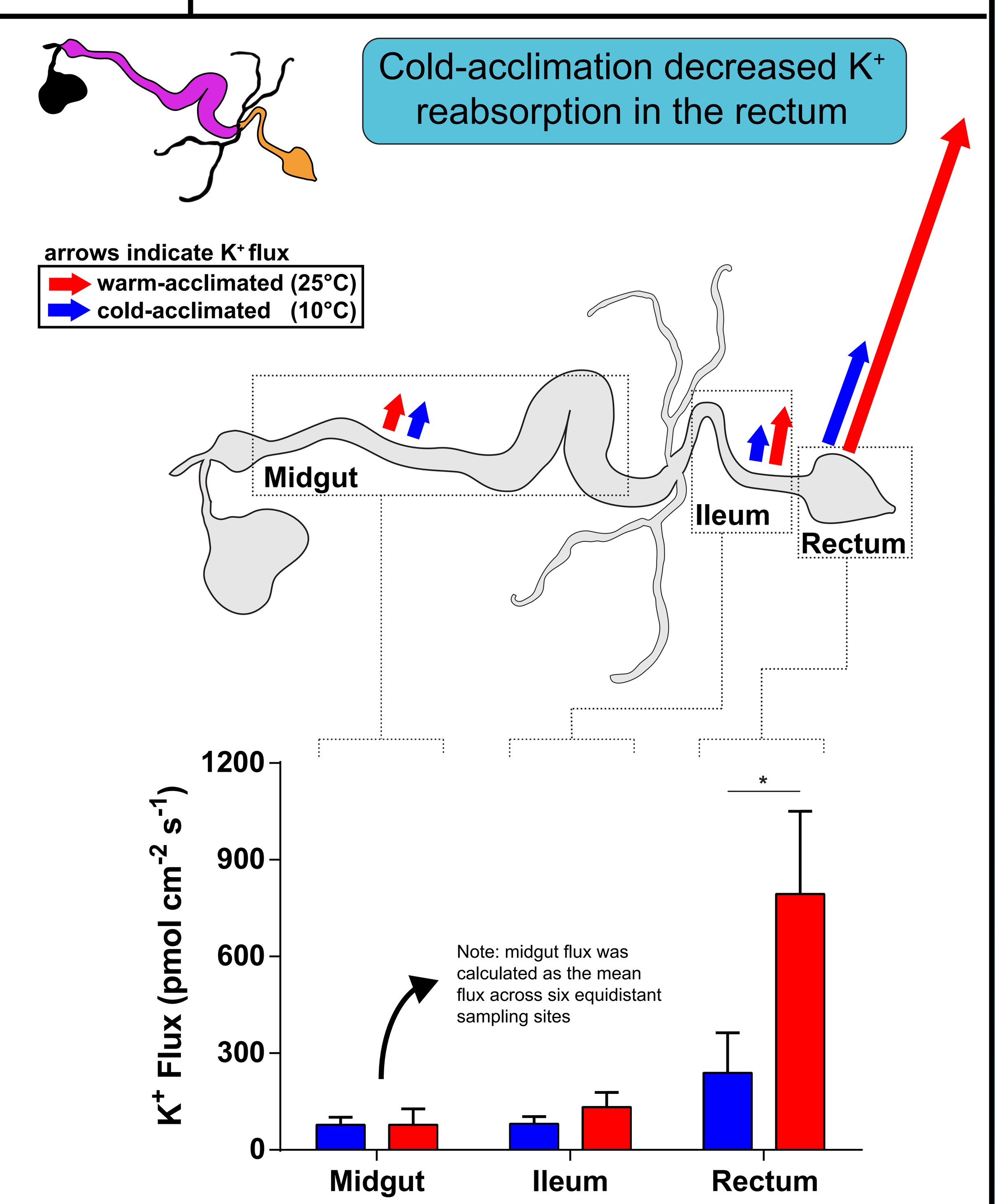
Cold-acclimation reduced ion-motive ATPase activity in the midgut and Malpighian tubules†



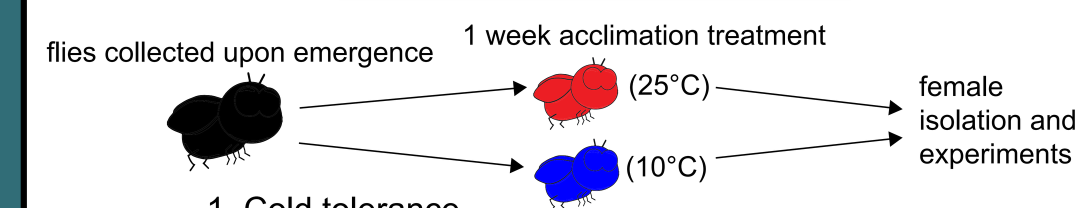
Cold-acclimation preserved MT fluid secretion rate and $Na^+:K^+$ in the cold

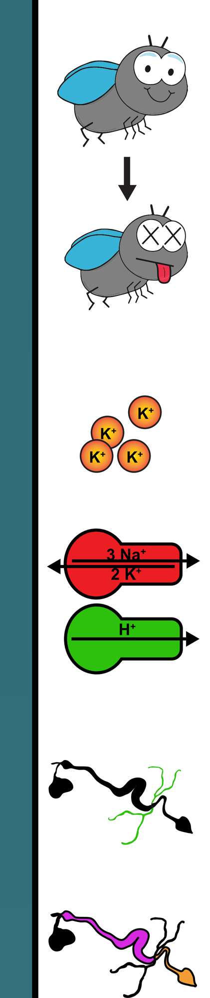


Cold-acclimation decreased K^+ reabsorption in the rectum



Methods





1. Cold tolerance
   - **CT_{min}** was measured by placing individual flies into 4 mL vials and cooling them (0.15°C min^{-1}) under contant observation.
   - **CCRT** was measured by careful observation of flies in 4 mL vials following a 6 h exposure at 0°C.
   - **Chill survival** was assessed visually 24 h after the removal of flies from cold exposures (0°C) of varying lengths.

2. **Hemolymph [K^+]** was measured using the ion-selective microelectrode technique (ISME)[6].

3. Tissue-specific enzymatic **activities of Na^+/K^+-ATPase and V-type H^+ ATPase** were assessed using an enzyme-linked spectrophotometric assay on dissected midguts, Malpighian tubules, and hindguts[6].

4. **Malpighian tubule fluid secretion rates** were determined using ramsay assays[7]. **K^+ and Na^+ concentrations** in the primary urine were determined using the ion-selective microelectrode technique (ISME)

5. **Gut K^+ flux** was measurd using the scanning ion-selective electrode technique (SIET)[8].

Conclusions

- Cold acclimation mitigated cold-induced hyperkalemia and improved the cold tolerance (Chill survival, CCRT, CT_{min}) of *D. melanogaster.*
- Na^+/K^+- and V-type H^+-ATPases, the main drivers of epithellial transport in insects, decreased in activity in the Malpighian tubules and hindguts of cold-acclimated flies.
- Malpighian tubule fluid secretion and $Na^+:K^+$ secretion ratio were better conserved at low temperatures.
- Rectum K^+ flux was reduced in cold-acclimated flies.

Overall, cold-acclimation increases Malpighian tubule K^+ secretion in the cold and limits rectal K^+ reabsorption in *D. melanogaster*. This mitigates the otherwise lethal accumulation of K^+ in the cold.

These functional changes, however, are not modulated by alterations of ion-motive ATPase activity and may instead result from:

(1) Changes to paracellular permeability.
(2) Altered ion channel expression.
(3) Altered plasma membrane composition.

References
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