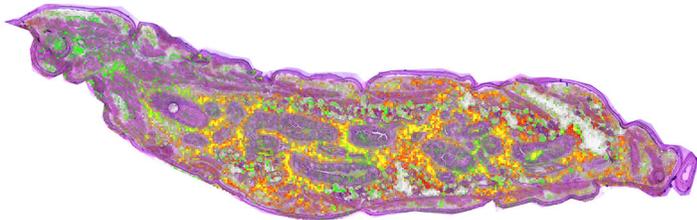


INSIDE JEB

Malt fly antifreeze cocktail more than the sum of its parts



Cross-section through a malt fly (*Chymomyza costata*) larva showing where proline (green), trehalose (red) and both (yellow) occur within the insect.

Even though water is a prerequisite for life, ice is a killer. Forming crystals in frozen tissue, ice ruptures cells and causes irreversible damage. But a select band of creatures have evolved strategies for avoiding the perils of freezing, and the larvae of the malt fly, *Chymomyza costata*, are possibly some of the most resilient creatures on the planet.

‘Overwintering cold-acclimated larvae in diapause can survive freezing to -75°C and even survive long-term (18 months) cryopreservation in liquid nitrogen’, says Vladimír Košťál from the Czech Academy of Sciences, Czech Republic. Knowing that the robust insects flood their bodies with an amino acid antifreeze, proline, for protection as temperatures plummet, Košťál and colleagues from several Czech institutions – including the Czech Academy of Sciences, the University of South Bohemia and the Crop Research Institute in Praha – set out to identify additional compounds which could offer the hardy larvae protection from a bitter winter.

Simulating winter conditions in the lab by cutting the larvae’s day length from 16 h at 18°C to 12 h, the team then dropped the temperature to 11°C , while plunging the insects into complete darkness for a week before chilling the

larvae further to 4°C to trigger an extreme form of suspended animation, known as diapause. Then the team collected haemolymph – insect blood – from the dormant insects, as well as portions of various tissues, including muscle, fat and the midgut, to find out which protective components accumulated in the insects’ bodies, and where, as they prepared for winter.

In addition to a colossal (~ 10 -fold) increase in the amount of proline circulating in the insect’s haemolymph (from 35 mmol l^{-1} to 313.2 mmol l^{-1}), the larvae also accumulated two other amino acids (asparagine more than doubling to 25.8 mmol l^{-1} and glutamine rising from 33.6 to 55.2 mmol l^{-1}). The antifreeze sugar trehalose also increased more than twice, up to 107.5 mmol l^{-1} , while several other trace compounds that are known to protect cells from freezing and dehydration increased by more than 50%. But how well does this antifreeze cocktail protect the dormant insects from frost damage?

The team investigated the impact of the five most abundant components (proline, trehalose, glutamine, asparagine and betaine) on the insects’ haemolymph as the temperature dropped and realised that

instead of forming ice crystals, the fluid became like a solid glass. And when the team tracked how the different components of the cocktail protected the insects as the temperature fell, it was clear that the sugar trehalose was a key player, driving the transformation of body fluids to form glass. Meanwhile, the amino acid proline allowed the haemolymph to remain fluid at temperatures down to -30°C , accumulating between delicate tissues and areas packed with ice crystals forming a protective bumper.

But could the antifreeze cocktail protect unprepared malt fly larvae from the damaging effects of ice formation at low temperatures? Košťál and colleagues fed 17-day-old larvae a diet supplemented with the antifreeze cocktail’s main components (proline, trehalose, glutamine, asparagine and betaine, mixed in the correct proportions) for 3 days and were amazed that 58% of the larvae survived freezing in liquid nitrogen for an hour, with almost 20% going on to develop into adults. However, when the team tested the survival of larvae fed individual components alone, only proline seemed to provide a little protection from freezing. ‘Simple addition of the individual effects of the components was not sufficient to explain the effect of the complete cryoprotective cocktail, suggesting a synergy and division of labour between the components’, says Košťál.

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