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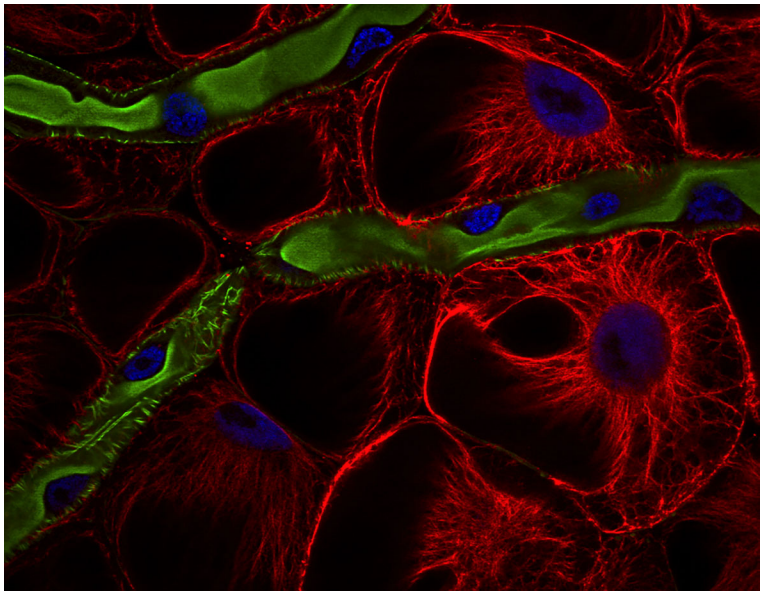
INSIDE JEB

Super freezing larvae survive despite incurred damage



Fat body cells (red) from a super freeze-tolerant larva frozen to –30°C. Photo credit: Lauren Des Marteaux.

We're a long way off cracking the problem of surviving subzero temperatures, but some creatures just take getting icy in their stride. Lauren Des Marteaux, Tomáš Štětina and Vladimír Koštál from the Academy of Sciences of the Czech Republic explain that the larvae of the humble malt fly (*Chymomyza costata*) are some of the most cold-tolerant animals known. It is possible to produce larvae with tolerances ranging from super freezers that survive immersion in liquid nitrogen (–196°C) to less robust animals that expire at the first hint of frost by simply adjusting the temperature or day length, or including proline in the larvae's diet. Taking advantage of the mini-beasts' versatility, Des Marteaux set about creating larvae with dramatically different freeze tolerances to learn how low temperatures 'do for' some of the creatures while others emerge from ice apparently unscathed.

After preparing extreme freezers, which survive immersion in liquid nitrogen for several months, moderately robust larvae, which cope down to –20°C, and normal larvae, which struggle to survive below –5°C, Des Marteaux then chilled some of each type of larvae to –5°C, while cooling others to –20 and –30°C, in addition to plunging some into liquid nitrogen (–196°C) while others were maintained at 18°C. She then removed the animals' internal organs – the gut, Malpighian tubules (the insect equivalent of the kidney) and a storage organ called the fat body – and scrutinised the tissues for damage. 'The greatest challenge was capturing and analysing 2700 microscope images', says Des Marteaux, admitting, 'Processing the sheer volume of visual data across 15 malt fly variants and three tissues was extremely time-consuming'.

However, after months of painstaking analysis, the team was surprised to find that the larvae's guts and Malpighian tubules were remarkably unscathed, even at the lowest temperatures. In contrast, the treatment that enabled the larvae to survive subsequent immersion in liquid nitrogen clearly affected the structure of the fat body cells, which shrivelled, while the cells from the flies that struggled to survive at –5°C remained plump. 'We think this [shrivelling] could help protect the cells from freezing damage', says Des Marteaux.

And the effects of freezing on the minute droplets of lipid that pack each fat body cell were equally impressive. The droplets merged when the least hardy flies were cooled to a lethal –20°C, while the hardiest flies experienced significantly less droplet fusion at the same temperature. However, when the hardiest flies recovered after thawing, Des Marteaux was surprised to see that instead of repairing the destruction, the cells appeared even more damaged; the droplets had merged entirely to form super droplets. 'We had expected that this "damage" would kill the insects if unrepaired', says Des Marteaux, yet it appears to cause no harm, 'illustrating how little we know about freeze injury at the cellular level', she says.

She also noticed that spots of one cytoskeleton protein (known as F-actin) formed at the periphery of fat body cells when the least cold-resistant larvae froze. However, the larvae that could survive immersion in liquid nitrogen seemed better protected, with no evidence of the damage forming until –30°C. And when she checked the fat bodies of the larvae after they had recovered, the damage had disappeared, suggesting that the larvae can somehow repair the damaged cytoskeleton, which Des Marteaux suspects is crucial for their survival. 'It seems likely that survival of freezing requires protection of protein structure', she says, adding that Štětina is continuing to search for evidence of the injuries that explain why freezing is fatal and how survivors recover or avoid damage.

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