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INSIDE JEB

Repeated freezing costs flies offspring

An opened Canada goldenrod gall containing a gall fly (*Eurosta solidaginis*) maggot. Photo credit: Brent Sinclair.

In the search for inhabitable planets beyond our solar system, water is top of the list of requirements for life. However, when temperatures fall below 0°C, water becomes our enemy, shattering delicate cell structures and destroying life. Brent Sinclair, from the University of Western Ontario, Canada, explains that overwintering insects in Canada frequently experience temperatures well below −40°C as maggots, yet emerge unscathed as adults in spring; and the survivors have usually endured multiple icy encounters. He suspects that goldenrod gall flies (*Eurosta solidaginis*) – which spend the winter entombed in a dead plant gall – can freeze as many as 20 times before spring. ‘But most of the experiments have been done by simply exposing animals to one cycle of cold’, says Sinclair. Wondering how the robust maggots respond as the mercury repeatedly plunges below zero, Sinclair and Katie Marshall designed an extensive series of simulated winters to test the insects’ survival strategies.

Fortunately, 2009 was a bumper year for the flies, with more than half of the 5000 goldenrod galls harvested by Marshall and an army of undergraduates yielding juicy fat maggots. Marshall then repeatedly froze the undeveloped insects to temperatures of −10, −15 or −20°C for 12 h intervals, interspersed with recovery periods at 0.2°C of 1, 5 and 10 days, before repeating the chilly experience 2, 5 or 9 more times. However, 3 days in, disaster stuck. Marshall seriously fractured her leg slipping on ice. Fortunately, the lab rallied round. ‘Katie knew this was going to be an epic experiment, so had made a detailed protocol for what treatments needed to happen and when’, recalls Sinclair, adding, ‘Even so, Katie did a lot of this from a wheelchair’.

Eventually, after months of patiently collecting maggots at the end of their personal chilling protocols, Marshall meticulously measured the amount of antifreeze in their bodies – including sweet-tasting sorbitol – in addition to how the types of lipids varied and recording how many maggots survived, going on to develop into adults.

Impressively, the maggots survived repeated freezing remarkably well, with the insects that had the longest recovery period (10 days) recording a survival rate of 80% at the lowest temperature (−20°C). Also, the maggots that had experienced ten 12 h long freezing sessions contained ∼50% more sorbitol than the maggots that had been frozen for a single 120 h period. In addition, Marshall discovered that the repeatedly frozen insects had converted more of their high-energy lipids into acetylated triacylglycerols, which are less energy rich but remain fluid at low temperatures.

Most intriguing was the effect that repeated freezing had on the amount of energy that each female could invest in her own reproduction. Knowing that the adult female flies emerge complete with a lifetime’s supply of eggs ready for their brief existence, Marshall wondered how repeated freezing might impact the number of eggs carried by the new adults. After allowing some of the maggots to develop, she counted the eggs carried by the female adults; the winter experience had taken a toll, reducing egg production by almost 10%.

‘Repeated freezing matters’, says Sinclair, adding that extrapolations based on single freezing events could significantly underestimate what happens in the real world. He also hopes to continue his long-term field study of overwintering maggots in order to identify factors that will allow him to predict how well insects will tolerate future winters. ‘We’ve been going for a decade … maybe another 15 years or so will do the trick’, he smiles.

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