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Inside JEB

ALASKAN WOOD FROGS STOCK UP ON SOLUTES TO SURVIVE

Outwardly, the tiny wood frog, *Rana sylvatica*, does not look like your regular arctic inhabitant. Yet despite their tiny stature, these little frogs are actually quite hardy and can tolerate freezing of up to two-thirds of their body water. For 25 years, Jon Costanzo and Richard Lee, Jr, both from Miami University, USA, have been studying these wood frogs, which are native to Alaska, Canada and the northern USA, to unravel their secrets. Costanzo explains that one well-known technique in the Ohioan population is to stock up on cryoprotectant solutes: 'When you load your blood with solutes, whether it's glucose, urea, glycerol or whatever, you drive down the [blood's] freezing point. Therefore, at any given temperature, you reduce the amount of ice that forms.' So, even when temperatures drop to –5°C in Ohio, some water remains unfrozen. In Alaska, however, temperatures go down to –30°C during winter and so Costanzo and Lee wondered whether Alaskan frogs have some additional tricks to aid their survival (p. 3461).

To collect the frogs, Costanzo sent two of his graduate students, Clara do Amaral and Andrew Rosendale, out into the Alaskan wilderness during early August. After capturing their amphibian subjects, the duo then made a long 6-hour drive to Anchorage, Alaska, to ship them back to the lab in Ohio. Upon their return the team then set about preparing the frogs for winter; they mimicked the Alaskan fall by gradually shortening day length and lowering the temperature. Once acclimatized, the team then kept the frogs in simulated hibernation in the dark at 4°C for 8 weeks, alongside some hibernating Ohioan frogs.

To begin, the team first tested the Alaskan frogs' freeze tolerance and endurance; some were lowered to an icy –16°C over 2 weeks, while others were kept at chilly –4°C for up to 12 weeks. In both cases, the frogs recovered remarkably quickly, but, as Costanzo recalls: 'If you take the Ohio frogs and freeze them to –4 or –5°C it would take them days to fully revive. These Alaskan frogs were back on their feet within 2 days.'

So, how exactly were the Alaskan frogs able to prevent total body freezing at temperatures that would freeze stiff an Ohioan frog? To find out, the team turned to frogs that they had euthanized at each stage (after the natural summer, and the mimicked fall and winter periods). The team were struck by how large the livers in the Alaskan fall and winter frogs were – they accounted for 22% of the total body mass (compared with just 8% in Ohioan frogs). To fuel such a huge change, the team thinks the Alaskan frogs trim down and use their body fat and some of their muscle protein to make glycogen – an essential precursor of the cryoprotectant solute glucose that is mobilised when the tissues begin to freeze.

The team also found that levels of another cryoprotectant solute, urea, had also shot up by 10-fold. This huge surge in urea concentration, along with a modest increase in glucose levels, caused an increase in plasma osmolality (a measurement of solute amount in liquids) by 100 mOsmol kg^{-1}. However, the team saw that total plasma osmolality had increased by 173 mOsmol kg^{-1}. What solute accounts for this additional 73 mOsmol kg^{-1}? The team have ruled out a few contenders, but they don't know exactly what it is yet. One thing's for certain, it's not a solute found in the Ohioan population. So it seems the Alaskan frogs stockpile the same solutes as their southern relatives (albeit to greater levels), but they also have some unique tricks of their own.

10.1242/jeb.092007

Costanzo, J. P., do Amaral, M. C. F., Rosendale, A. J. and Lee, R. E., Jr (2013). Hibernation physiology, freezing adaptation and extreme freeze tolerance in a northern population of the wood frog. *J. Exp. Biol.* **216**, 3461-3473.

Nicola Stead

THE GENETICS OF SALT TOLERANCE

Although she may not look it, the malaria-transmitting female mosquito *Anopheles gambiae* is a fussy insect; she won't breed in any old water and likes to lay her eggs in clean freshwater. However, some of her relatives are less picky and have evolved the ability to breed in saltwater. This switch is not easy, as Nora Besansky, from the University of Notre Dame, USA explains: 'When the external environment is a lot saltier than they are, [the mosquitoes] have to regulate ion balance by actively pumping out ions or matching the salinity of the environment.' While *A. gambiae* mosquitoes may not breed in saltwater, in recent years they have started to breed in water high in other ions, such as ammonia. As this trait might be related to salt tolerance, Besansky and her team wanted to identify the genetic basis of salt tolerance in close relatives (p. 3433).

Explaining her ultimate plan, Besansky says, ‘The way you genetically dissect a trait is by crossing organisms that differ in that trait and then follow the segregation of genetic markers associated with the trait in extended pedigrees.’ However, the team first needed to determine what concentration of salt would kill intolerant, but not tolerant, mosquitoes. ‘We exposed colonies of the freshwater- and saltwater-[tolerant] species to different doses of salt and then monitored their survival’, explains Besansky. ‘In acute assays we limited exposure to 24 h at a late developmental stage of the larvae, whereas in chronic exposures the different doses were kept constant from hatching up to the emergence of the adult.’

Besansky admits that the experiment took a lot of patience; her post doc Bradley White, and undergraduate student Peter Kundert, spent hours gently prodding over 50,000 larvae from three different mosquito species to see whether they reacted and were alive. The work paid off, and the team found that 15.85 g NaCl l^{-1} was enough salt to kill off all larvae from two freshwater species, *A. gambiae* and *Anopheles coluzzii*, in both chronic and acute exposures.

However, although the salt-tolerant *Anopheles merus* larvae survived without problems during the chronic exposure, the team noticed they weren’t tolerant during acute exposures. Prior to acute exposure all the larvae were kept in freshwater, and the team wondered whether *A. merus* larvae need earlier exposure to salt to gain tolerance. Sure enough, the team found that larvae only became tolerant if they had been exposed to salt within 24 h of hatching. Besansky knew that in a very distant relative, early exposure to saltwater was crucial for altering the tissue location of a Na^{+}/K^{+}-ATPase (an ion pump, which may help when dealing with salty water). Teaming up with Paul Linser’s group at University of Florida, USA, the team found a similar switch occurring in *A. merus*, although what controls this location switch is unknown.

Next, the team decided to see how offspring with *A. merus* and *A. coluzzii* parents would fare, and found that their tolerance was in-between that of their parents. ‘We think this means that there are multiple factors that control the genetics of this trait, and it tells us that the factors conferring salt tolerance are not dominant, otherwise the offspring would have looked like the saltwater-tolerant parent’, says Besansky. The team also found that offspring whose mothers were saltwater tolerant did better than those with a freshwater-loving mum. Besansky suspects that this means that some of the tolerance may be inherited through the female X chromosome. An extensive breeding program now lies ahead of Besansky and her team to pinpoint the exact genetic basis of salt tolerance, but the hunt has begun.

10.1242/jeb.092692

White, B. J., Kundert, P. N., Turissini, D. A., Van Ekeris, L., Linser, P. J. and Besansky, N. J. (2013). Dose and developmental responses of *Anopheles merus* larvae to salinity. *J. Exp. Biol.* **216**, 3433-3441.

Nicola Stead

MUSCLING IN ON THYROID’S ROLE WHEN IT’S CHILLY

As endotherms, we mammals don’t have to worry too much about fluctuating temperatures – we’re pretty good at maintaining a constant body temperature no matter what the weather is outside. Ectotherms, however, aren’t so lucky. For example, as temperatures drop, so do their body temperatures, with detrimental consequences, as Alexander Little explains: ‘Enzyme rates that support all physiological processes slow down and this can cause problems specifically in the muscle, where it can affect contraction strength and frequency of contraction.’ However, ectotherms, such as zebrafish, don’t stop moving just because it’s cold. In fact, Little, a PhD student in Frank Seebacher’s group at the University of Sydney, Australia, found that cold-acclimated zebrafish actually perform better than their warmer friends. Thyroid hormone is well known for its central role in temperature regulation in mammals, therefore, ‘we were interested in whether or not it also changed muscle function in ways that could enhance swimming capacity [in zebrafish]’, says Little (p. 3514).

To investigate, Little first took his zebrafish and split them into two groups, acclimating one group to a pleasantly warm 28°C and the others to a cooler 18°C. After 3 weeks at their new temperatures, Little put his fish to a fitness test: he placed them in a swimming flume, gradually raising the rate of flow every 10 min until the fish became exhausted and were no longer able to carry on swimming. ‘It’s almost like a beep test for fish’, explains Little. ‘So, it’s a combination of [testing] speed and endurance and we saw that fish that are cold acclimated, compared to the warm-acclimated fish, can swim longer and at faster speeds.’ Little and Seebacher also saw that fish accustomed to cooler water beat their tails more frequently.

Next, having confirmed that his cold-acclimated fish did indeed perform better than their warmer kin, Little focused on characterising changes to the muscle. He began by looking at expression levels of several muscle-associated genes and found that a specific isoform of the sarco/endoplasmic reticulum Ca^{2+} ATPase, SERCA 1, was upregulated in cold-acclimated fish. Little explains that contraction of the muscle is induced when calcium ions are released from the sarcoplasmic reticulum, which indirectly allows the contractile myosin fibres to undertake their power stroke. To reset the stage for another round of contraction, the calcium needs to be pumped back into the sarcoplasmic reticulum *via* the ATPase SERCA. SERCA1 is the fastest of the three SERCA isoforms and Little speculates, ‘Increased SERCA1 could allow the calcium to be cleared from the muscle after contraction faster and restore the calcium to the sarcoplasmic reticulum so that the next contraction can happen more quickly.’

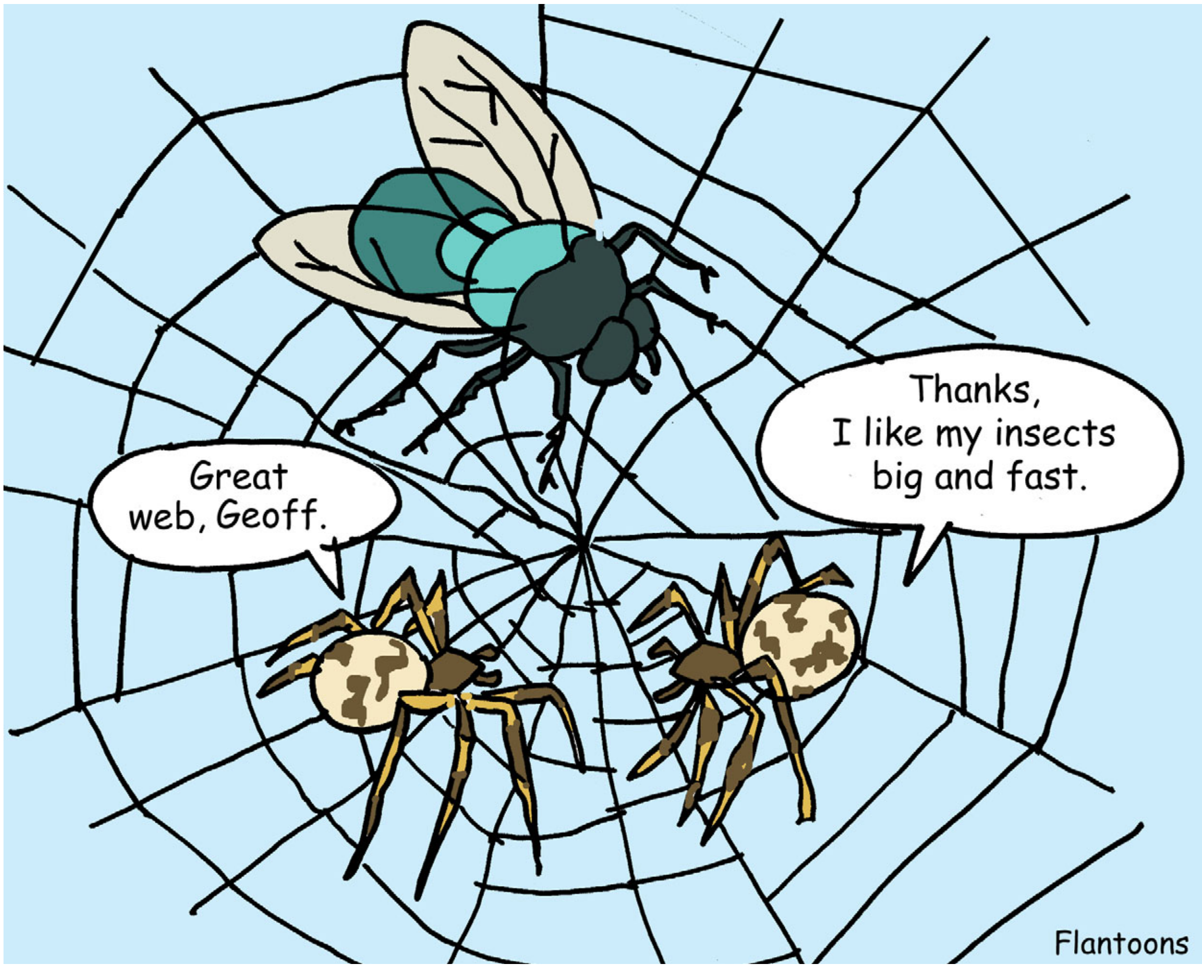
But was the upregulation of SERCA1 controlled by the thyroid hormone? Little treated his cold-acclimated fish with two drugs that inhibit both the production and the breakdown of the thyroid hormone into its active derivatives. Sure enough, in the hypothyroid fish, SERCA1 levels decreased, alongside a drop in their swimming performance in the beep test. What’s more, when Little measured the activity of SERCA by its ability to use ATP (which it uses to power calcium pumping), he found that its activity decreased in cold-acclimated hypothyroid fish. Little was able to reinstate the SERCA1 expression levels and the superior sporting performance by providing the cold-acclimated hypothyroid fish with synthetic thyroid hormone or an active derivative of the hormone. While Little doesn’t yet know exactly how the thyroid hormone upregulates SERCA1 activity, his work has shown, for the first time, that during cold acclimation the thyroid hormone can significantly alter muscles in ways that impact how fish can swim.

10.1242/jeb.092684

Little, A. and Seebacher, F. (2013). Thyroid hormone regulates muscle function during cold acclimation in zebrafish (*Danio rerio*). *J. Exp. Biol.* **216**, 3514-3521.

Nicola Stead

SPIDER WEBS ARE DESIGNED FOR BIG APPETITES



Most animals risk their own lives when hunting for prey. Many spiders reduce this danger by spinning orb webs out of sticky silk, to net unsuspecting insects. However, this doesn't mean that capturing their next meal is problem-free. Spiders face a choice – do they stick to a web design that will capture small insects with little nutritional value and allow bigger, more satisfying prey to escape with minimal damage to the web, or do they try their hardest to capture the more enticing, tastier, larger insects, even though it could mean frequently suffering damage if the gamble doesn't pay off? Andrew Sensenig from Tabor College, USA, decided to investigate (p. 3388).

Sensenig and his colleagues collected spiders from 10 different species of the orb weaver clade, Araneoidea, and allowed them to build webs back in the lab. Under the watchful gaze of a high-speed camera the team then launched a series of tiny balsa wooden blocks at the webs. To mimic different insects found in the wild, they varied the mass (30, 100 and 300 mg) and the speed (1.3–5.5 m s^{-1}) of the projectiles.

For all projectiles, the team found that as speed increased, the probability of catching and retaining the balsa wood blocks decreased. This was not surprising: the bigger and faster the insect or block is, the more energy it has to break through the silky net. But Sensenig explains that even during breakages, the web will absorb some of the projectile's energy. To find out how much, the team measured the speed of the balsa blocks before and after passing through the web. Knowing the mass and speed, the team could then work out the kinetic energy of the block before and after its collision. They found that the faster the projectile had been travelling, the more energy the web absorbed. In fact, the energy-absorbing ability of the web increased sixfold over the range of speeds they investigated. The results suggest that spiders will invest in a web that maximises its chances of catching a big meal.

10.1242/jeb.091959

Sensenig, A. T., Kelly, S. P., Lorentz, K. A., Lesher, B. and Blackledge, T. A. (2013). Mechanical performance of spider orb webs is tuned for high-speed prey. *J. Exp. Biol.* **216**, 3388-3394.

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