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INSIDE JEB

Red-eyed treefrog embryos hatch in seconds

Red-eyed treefrog embryos hatch to escape from a snake. Photo credit: Karen M. Warkentin.

Enclosed in a case of moist jelly, most minute frog embryos take their time emerging from the protective coating; but not the spawn of tree-dwelling *Agalychnis callidryas* (red-eyed treefrogs). When the eggs find themselves under attack by predatory snakes, the tiny embryos can burst out of their eggs in as little as 6 s before dropping to safety in water below. ‘This escape hatching is a mechanism for running away from a really important predator’, says Karen Warkentin from Boston University, USA. But she was unsure how the tiny animals made their escape. Explaining that most frog embryos liberate themselves by releasing enzymes from hatching glands on their heads that slowly degrade the egg membrane, Warkentin thought that it was unlikely that treefrog embryos used the same approach, because the hatching process was so swift and the egg membrane remained largely intact. ‘We had seen them thrashing around and we thought they were somehow breaking out of the egg’, she recalls. So when Mark Seid at the Smithsonian Tropical Research Institute, Panama, got access to a high-speed camera, Warkentin grasped the opportunity to reveal the mysteries of the treefrog embryos’ escape in fine detail.

Warkentin and Seid had no difficulties collecting newly laid frog spawn from a nearby experimental pond overhung with accessible foliage. The duo then allowed the eggs to develop for 5 days before gently prodding them to trigger breakouts. ‘One of the first and most interesting behaviours that we observed was shaking’, recalls Kristina Cohen, who also noticed the embryos repeatedly opened and closed their mouths before the egg membrane ruptured. ‘Following this… you see fluid start to leak from the location in the membrane that is just in front of the embryo’s snout’, says Cohen, who recalls how the embryo then plugged the rupture by lodging its snout against it, before wriggling through in a bid for freedom. The embryos had made a hole in the membrane without even touching it. ‘Once we saw that, we thought it was probably enzymatic’, says Cohen. But where was the enzyme being released from?

‘We came up with this very low-tech experiment’, says Cohen, describing how she spun the embryos around in the egg after they had been trembling and gaping for a few seconds to find out where the rupture formed: ‘If you don’t perform the manipulation at the right time it doesn’t work’, says Cohen. However, when she timed the rotation perfectly, a hole appeared in the membrane next to where the embryo’s snout had been originally located. The embryos must be releasing enzymes from somewhere on their heads to liberate themselves from the egg membrane.

Searching for hatching glands over the surface of the embryos’ heads with a scanning electron microscope, Cohen successfully located tightly packed clusters of the glands on the embryos’ snouts. Then, she painstakingly collected detailed scanning transmission electron microscopy images of the cells from embryos before they hatched and images of tadpoles immediately after they had successfully broken out. ‘Getting those images was indeed a lot of work’, says Warkentin, recalling how Cohen’s patience was eventually rewarded when the images revealed that the hatching gland vesicles had released their contents just seconds before hatching.

So, red-eyed treefrog embryos evade snake predators by rapidly releasing enzymes from hatching glands concentrated on the snout at one specific location to rupture the egg membrane and produce an aperture through which the embryo can wriggle in a matter of seconds in a life-and-death bid for survival.

10.1242/jeb.143693

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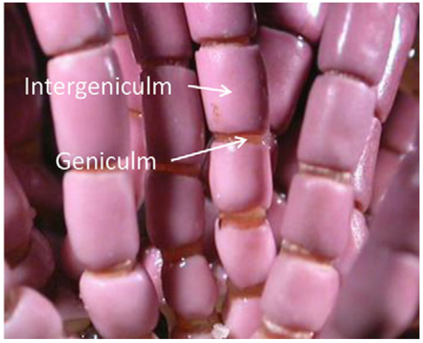
Kathryn Knight

Viscoelastic joints protect coraline algae from disaster



Calliarthron cheilosporioides coralline algae. Photo credit: Patrick Martone.

Tumbled around in the surging surf, many types of seaweed have opted for a flexible approach that allows them to deform and go with the flow. However, some species have armoured themselves with rigid calcium-impregnated cells for protection; ‘Which would seem to pose a real problem’, says Mark Denny from the Hopkins Marine Station of Stanford University, USA. He explains that the otherwise inflexible algae have overcome their paradoxical rigidity by evolving joints – known as genicula – between the calcified segments, which are built from a remarkably resilient material that allows them to bend and sway. Explaining that the joint material is stronger, more extensible and more fatigue resistant than the tissue of other algal fronds and that the joints comprise long hexagonal cells that are anchored solely at each end to the adjoining segments – in much the same way as steel strands in suspension bridge cables – Denny and his colleague Felicia King set about reconciling what they knew about the structure of the joints at the cellular level with the molecular structure of the joints and their movements as they are wrenched around by the water.

Collecting samples of the pink *Calliarthron cheilosporioides* seaweed was simply a matter of scrambling down onto the rocky seashore beneath Denny’s

Inside JEB highlights the key developments in Journal of Experimental Biology. Written by science journalists, the short reports give the inside view of the science in JEB.

lab when the tide was low armed with a kitchen knife to prise the seaweed free from the shoreline. Once back in the lab, Denny and King embarked on a series of laborious experiments where they tested the joint material's responses to the kinds of extreme stresses and strains that they experience routinely as the waves tug at them repeatedly.

Explaining that most biological materials are viscoelastic – that is they are partially elastic, allowing them to snap back into shape after bending and deforming, and partially viscous, allowing them to stretch and permanently deform – Denny and King were surprised to find that instead of becoming stiffer as the joints deformed (as most viscoelastic materials would), the seaweed's joints remained flexible and their breaking strain increased: 'That is unusual', says Denny. King also found that the joints were able to dissipate much of their energy, in addition to deforming and extending, as she continually tugged at them. And when the duo calculated the amount that the joint at the base of the algae deformed over the course of its 6 year life, they found that it extended by only 36%, which is well within its limits – they only break when extended to twice their original length – 'So it's safe', says Denny.

Having discovered how resilient the joints are, Denny and King set about building a mathematical model in a bid to understand their performance. 'Springs and dashpots [shock absorbers] are the basic building blocks when constructing mathematical models of materials', says Denny, who admits that assembling the components in a way that reproduced the joint material's performance was challenging. However, the duo eventually came up with a relatively simple arrangement – a side-by-side spring and shock absorber, attached to a second spring inline with a shock absorber. Estimating the stiffness of the two springs and the viscosity of the two model shock absorbers from their earlier measurements, the duo was impressed when their model effectively mimicked the behaviour of the joints in real life. 'We were quite surprised that such a simple model could do such a good job of predicting the material's behaviour', says Denny, adding, 'If a simple model works well, it's usually a good sign that you've captured the important aspects of a system'.

Interpreting their measurements in terms of the molecular structure of the joint cells, the duo suspects that the cellulose fibres in the genicular cells perform like stretchy springs, while the two shock absorber components of the model simulate the intrinsic viscosity of the long flexible cells and the viscosity as the cellulose fibres slide past each other in the cell wall, shearing the gel-like material in which they are embedded. And Denny is optimistic that his model will help us to better understand how shoreline ecosystems might change in the face of increasing wave activity. 'Our ability to predict when and where joints will break will allow us to predict how the "forest" in the low intertidal zone will change in response to any changes in the wave environment', he says.

10.1242/jeb.143685

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Kathryn Knight

How dog ticks protect themselves from dehydration

A male (right) and female (left) American dog tick. Photo credit: Andrew Rosendale.

While most animals have more than enough blood to spare a drop or two for a famished tick, it's the unpleasant diseases that these small arachnids transmit that engender the disgust that they inspire. 'We are generally interested in understanding tick populations', says Andrew Rosendale from the University of Cincinnati, USA, who is keen to learn more about the tick's vulnerabilities in a bid to reduce the incidence of tick-borne diseases. As ticks spend most of their time lurking in the humid undergrowth and only emerge to perch on the end of a twig or blade of grass when waiting for a passing victim, Rosendale explains that they are mainly at risk of dehydration when on the hunt for a victim. So, in a bid to understand how these pests protect themselves from desiccation, Rosendale, Megan Dunlevy and Joshua Benoit began investigating which metabolic processes ticks mobilise when water is scarce.

Analysing the 497 tick genes that changed expression pattern when the pests experienced dehydration, Rosendale identified protective genes such as glutathione *S*-transferase, which protects cells from oxidative damage; heat shock protein 70, which protects damaged proteins; as well as genes that alter cell structure. 'All three of those gene types would serve to limit damage from dehydration', says Rosendale. He then teamed up with Lindsey Romick-Rosendale and Miki Watanabe to find out whether the ticks were producing any molecules that might protect them from desiccation, although he admits that identifying the metabolites produced by the dehydrated arachnids was tricky. 'Lindsey and Miki primarily work with human samples, so processing the small samples [from the ticks] and calibrating the NMR machine to obtain good data was a challenge', recalls Rosendale. However, their patience was eventually rewarded when the team realised that the ticks were producing GABA and glycerol; although when these molecules were injected into the ticks they did not reduce the animal's water losses in dry conditions. Instead, glycerol and GABA seemed to improve the tick's ability to absorb water from the air when their reserves were low.

'Although ticks change their physiology to try and defend against the negative consequences of dehydration, they are not particularly resistant to dehydration,' says Rosendale, adding, 'Much of their response is likely to limit water loss when possible and to facilitate the reabsorption of water then they can return to a more humid microclimate'. And Rosendale is now keen to find out how the blood-sucking pests fuel the mechanisms that protect them from dehydration to get a better handle on strategies that could help us to avoid their unpleasant attentions.

10.1242/jeb.143677

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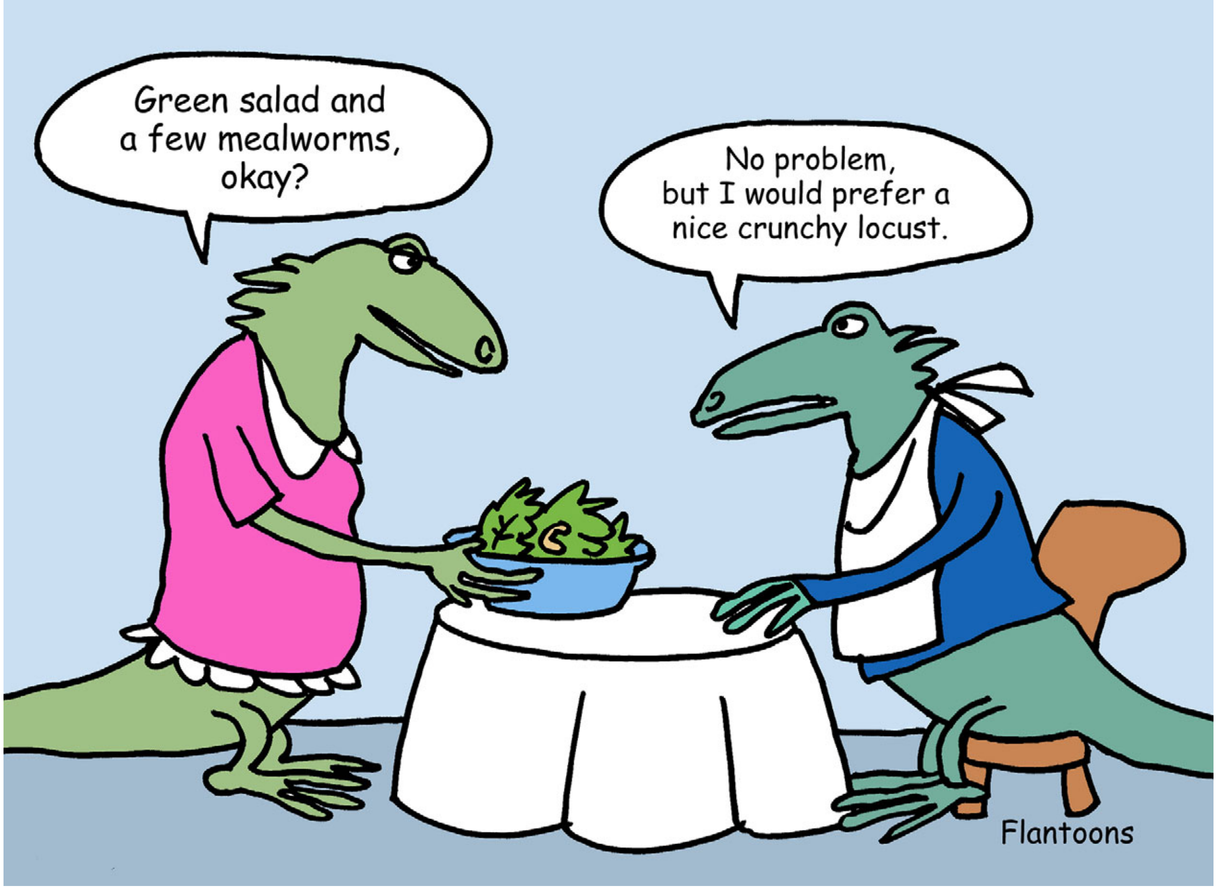
Kathryn Knight

Iguana guts can adapt to being veggie



Fruit and vegetables are an essential part of many mammals' diets; however, few reptiles ever tuck into their greens. 'Herbivory is extremely rare in squamate reptiles', say Kevin Kohl and colleagues from Vanderbilt University, USA, and Universidad Nacional de San Luis, Argentina, adding that most reptiles prefer to feast on insects. Although the lack of vegetation in the reptile diet is understandable – it often lacks protein and can be tough to digest – the team wondered if reptiles were simply incapable of extracting enough nutrients to survive on a diet of greens alone because their guts could not adapt to the higher fibre diet. Intrigued by the possibility, Kohl and his collaborators set about feeding Ruibal's tree iguanas on an (almost) vegetarian diet of rabbit food supplemented with a few mealworms and compared how they fared with animals fed on a mixed diet of 50:50 rabbit food and mealworms for 40 days.

Monitoring the animals' body mass and collecting their faeces, the team found that all of the animals successfully maintained their mass and were supplied with enough protein in the diet, although the veggie lizards excreted less protein waste than the animals on the mixed diet. And when they investigated the animals' digestive tracts, they saw that the small intestine of the veggie iguanas was almost 20% longer than that in the omnivorous iguanas and the hindgut was significantly larger, which means that the iguanas can adapt their guts to a vegetarian lifestyle. They also found more bacteria that ferment plant material in the gut flora of the animals on the veggie diet. So lizards are not prevented from adopting a diet of greens by their digestive tracts and Kohl and colleagues say, 'We hypothesize that ecological contexts and the likely fitness benefits of feeding on energy-dense insects may be more critical in constraining the evolution of herbivory in liolaemid lizards'.

10.1242/jeb.143701

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