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INSIDE JEB

Ticks use sticky pad to hold tight to skin

Castor bean ticks clustered on vegetation. Photo credit: Dagmar Voigt.

Dining opportunities are rare for ticks. The notorious pests spend as much as 90% of their lives clambering around in leaf litter and only embark on epic expeditions to the tips of grass and leaves when they need to feed. Wrapping their limbs around slender smooth stalks of grass when they ascend, ticks must also grasp any passing opportunity to latch onto smooth skin or the hairy pelts of animals. 'We wondered how their feet are designed and how strongly they can hold on to surfaces', says Dagmar Voigt from the Technische Universtität Dresden, Germany, adding that in addition to clinging on to a wide range of different surfaces, the feet of an adult tick may have to bear up to 135 times their unfed weight after ticks have consumed a blood meal. Undeterred by the tick's fearsome disease-spreading reputation, Voigt headed out into the undergrowth to capture castor bean ticks (*Ixodes ricinus*) sheltering under leaves and blades of grass to learn more about their feet.

Back in the lab, Voigt and Stanislav Gorb at Christian-Albrechts-Universitaet zu Kiel, Germany, took a series of increasingly closer looks at the tick's feet. Recalling that Hermann Burmeister had studied tick feet in 1859, Voigt describes how she also saw the two lengthy curved tarsal claws nestled above a pad-like structure – similar to the arolium that many insects use for attachment – that he had described first. However, as the duo scrutinised the structures in greater detail they could see that the pad was packed with springy fibres while the surface was heavily pleated and the long transparent tarsal claws were largely made up of the elastic protein resilin. 'This was a surprise because we have never observed resilin in the claws of other arthropods', says Voigt.

But how would the ticks use this equipment to cling on to surfaces ranging from smooth plant walls to human skin? Filming the arachnids' feet as they secured themselves to a plate of glass, Voigt saw the claws separate and the pleated arolium pad unfold like a fan as it pressed against the smooth surface, generating a large footprint. And when the tick lifted its foot away, it left behind a large patch of tarsal fluid. 'Compared to other arthropods, ticks release a remarkable volume of this fluid', says Voigt. But how tightly would the animals hold on?

Designing a series of increasingly rough resin surfaces, Voigt also made a silicon cast of her own forearm skin in addition to allowing the blood-sucking pests to roam freely on her while measuring how well they clung to each surface when inverted. Impressively, the ticks were able to hold on best to Voigt's own skin, with 90% of the arachnids remaining attached to her and the smooth glass. In contrast, only 52% of the animals managed to cling to the silicon skin cast while only 46% got a grasp on one of the mid-roughness (3 µm) resins. And when Voigt measured the force generated by the ticks as they marched horizontally across various surfaces, the arachnids managed to hold on tightest to the smooth glass surface, with a force that was more than 500 times their ~17 µN weight.

So, ticks are remarkably well adapted to clinging onto smooth skin in addition to snagging themselves in hair with their grappling hook claws, and Voigt is optimistic that her discovery could help in the design of tick-proof surfaces to deter the little pests from hitching a ride when we're out for a ramble.

10.1242/jeb.162610

Voigt, D. and Gorb, S. (2017). Functional morphology of tarsal adhesive pads and attachment ability in ticks *Ixodes ricinus* (Arachnida, Acari, Ixodidae). *J. Exp. Biol.* **220**, 1984-1996.

Kathryn Knight

X*Y super female pygmy mice have larger heads and superior bites

A pygmy mouse from the University of Montpellier colony. Photo credit: Frederic Veyrunes.

Most creatures that carry a Y chromosome turn out to be males: but this is not necessarily the case in African pygmy mice (*Mus minutoides*). Instead of depending on simple combinations of X and Y chromosomes to produce males carrying an X and a Y chromosome and females carrying two X chromosomes, the tiny rodents can carry a third chromosome, X*, with a mysterious sex-changing power. Samuel Ginot and colleagues from the University of Montpellier, France, explain that instead of turning into males, X*Y pygmy mice develop into females, and even though the X*Y females are physically indistinguishable from regular XX females, their behaviour is markedly different. 'X*Y females have higher reproductive success and are much more aggressive', says the team. Could the X* chromosome be contributing to the X*Y female's dominance?

Working with the University of Montpellier's colony of pygmy mice, which were established by Frederic Veyrunes in 2010, Ginot and Julien Claude measured the bite force generated by male and female mice to find out whether the exotic X* feminising chromosome might impact on aspects of the rodents' performance. Offering a small force transducer to the animals to bite on, Ginot explains that the tiny mice were usually keen to cooperate. 'The bite is a reflex defence mechanism, and the

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animals usually bite anything that comes within "teeth's reach"', he says, adding that this included his own finger on some occasions: 'It's the equivalent of being pricked by a needle', he chuckles.

However, as the females carrying Y and X* chromosomes are physically indistinguishable from the XX and XX* females, Julie Perez had to search the genes of every female for evidence of the *Sry* (sex determining) gene, which provides incontrovertible evidence of the presence of a Y chromosome. Having identified which of the biters were double-X females (XX and X*X) and which were feminised males (X*Y), and then recorded their bite forces – which ranged from ∼2.5 to 16 N – Ginot and his colleagues were impressed to see that the X*Y females out-bit all of the other mice, males included. And, when Ginot and Claude analysed the head structures of the mice to identify the cause of the X*Y females' ferocious bites, they discovered that they had larger skulls.

But why is a harder bite advantageous for the X*Y females? Explaining that it should allow the X*Y females to eat harder food than other pygmy mice while allowing the tougher females to mate with more males, Veyrunes says, 'The effect of the X* chromosome goes well beyond feminisation'. The team adds, 'These X*Y females are characterised as "super females"', and they hope that the animals' enhanced aggression, larger skulls and stronger bite forces will help us to learn more about the impact of sex chromosomes.

10.1242/jeb.162636

Ginot, S., Claude, J., Perez, J. and Veyrunes, F. (2017). Sex reversal induces size and performance differences among females of the African pygmy mouse, *Mus minutoides*. *J. Exp. Biol.* **220**, 1947-1951.

Kathryn Knight

Snails prioritise protein stability at sizzling temperatures

Echinolittorina snails frequently encounter extreme high temperatures. Photo credit: Ming-ling Liao.

No matter how warm it gets outside, most endotherms maintain a reasonably stable internal temperature; if they don't, there's usually a very good reason for their temperature to rocket. However, the body temperatures of ectothermic species that cling to life on the rocky tidal shore are not so dependable. When the tide is out and some molluscs are left high and dry on the sea shore, their body temperatures can soar to levels where the delicate protein structures within cells could begin to unravel. Yet, the remarkable *Echinolittorina* family of periwinkle molluscs routinely experience perilously high body temperatures in excess of 50°C as they bake in the sun. Intrigued by the extraordinary resilience of these diminutive creatures, George Somero from Stanford University, USA, and Yun-wei Dong from Xiamen University, China, decided to find out how members of the robust family reinforce delicate protein structures so that they remain intact at temperatures where other proteins would disintegrate.

Choosing the ubiquitous cytosolic enzyme malate dehydrogenase, which produces malate for use by mitochondria in the production of ATP, Ming-ling Liao and Shu Zhang collected two members of the *Echinolittorina* family – *E. malaccana*, which can tolerate temperatures in excess of 55°C, and the less robust *E. radiata* – to find out what gives *E. malaccana* proteins the thermal edge. After isolating the enzyme from both molluscs, Liao and S. Zhang provided the enzymes with oxaloacetic acid at temperatures ranging from 20 to 40°C and measured the rate of conversion of the acid to malate. They found that *E. malaccana* malate dehydrogenase functioned better at high temperatures than the *E. radiata* enzyme. And when the duo measured the proteins' stability by holding them at temperatures up to 57.5°C and recording how their activity altered over an hour, the *E. radiata* malate dehydrogenase was completely inactive by the end of the experiment, whereas the more resilient *E. malaccana* protein was still functional, although at a much lower rate.

Curious to find out why the *E. malaccana* enzyme was so much tougher than the *E. radiata* malate dehydrogenase, Liao and S. Zhang analysed the sequence of amino acids in both proteins and identified two key locations – position 48 and 114 in the peptide chain – where the serine amino acids in the *E. radiata* protein were replaced by glycine amino acids, which are much smaller, in *E. malaccana* malate dehydrogenase. Wondering how these subtle differences might affect the proteins, Liao, Guang-ya Zhang and Yun-meng Chu calculated how increasing heat would affect the proteins' function by effectively boiling them up to 42 and 57°C in computer simulations that replicated the effects of different temperatures on the delicate protein structures. Analysing the calculations, the team found that the core of the *E. malaccana* enzyme was much more stable at 57°C than that of the *E. radiata* enzyme. However, the simulations also showed that regions of the more heat-resistant protein that were involved in the conversion of oxaloacetic acid into malate became more flexible. The team suggests that these local increases in flexibility could permit the enzyme to continue functioning at lower temperatures while allowing the protein to remain stable at higher temperatures where other proteins would collapse.

10.1242/jeb.162461

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Kathryn Knight

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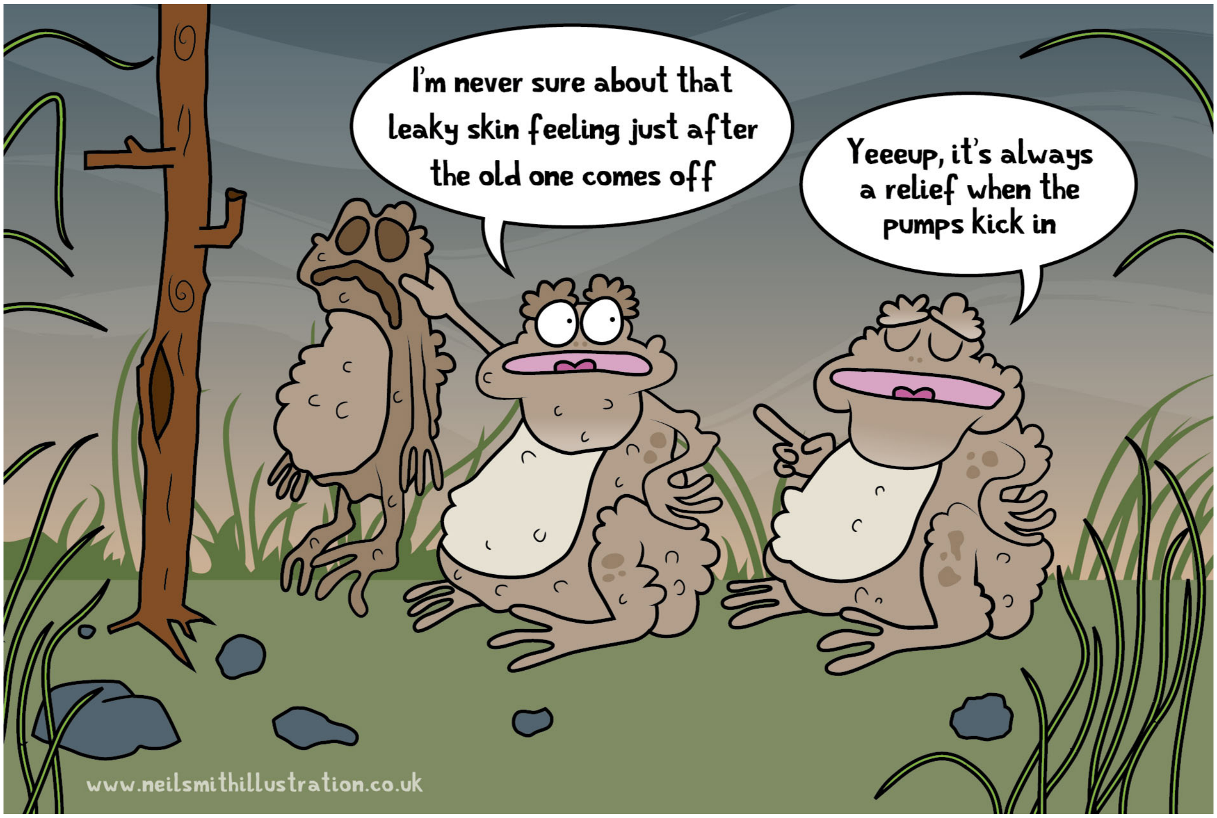
Salt pumps protect leaky toads when replacing skin



Animals shed their skins for various reasons – from disposing of tough outer coatings that have grown too small to replacing worn out tissue that no longer serves its purpose. During these transitions, many animals are vulnerable to predators, while amphibians – which depend on their leaky skins to maintain a healthy internal balance of salts and water – could be at risk if their skins become even leakier, allowing salt to seep out while water soaks in. Intrigued by the challenges faced by skin-sloughing cane toads during their not-so-quick transition, Craig Franklin, Nicholas Wu and Rebecca Cramp from The University of Queensland, Australia, measured salt loss across the amphibian's skin and how the salt-transporting properties of the skin change when a toad replaces the old with the new.

Bathing cane toads in fresh water and measuring the water conductivity during the period between moults, in the 12 h preparation period before the moult, during the moult and after, Wu found that the salt loss rocketed almost 200-fold from 0.5 μS h^{-1} to 90 μS h^{-1} when the old skin had been sloughed off. However, when he checked the toads' blood stats, the animals appeared to be unperturbed and had not lost essential salts, despite the increased skin leakiness. Puzzled, Wu measured the amount of salt flowing into the toad's body through specialised protein channels that pump salt against the natural gradient, and was impressed to see that the inward flow had doubled after the animal had shed its skin. And when he investigated the quantity and location of the pump proteins through the different stages of the moult, it was clear that the animals were producing more of the essential pumps and locating them deep in the skin after the old skin had been shed to maintain a healthy internal salt balance.

So, leaky cane toads install protein pumps to protect themselves from salt loss while they replace their skin. However, the team warns that other skin-sloughing amphibians may suffer if they begin replacing their skins more frequently in a bid to rid themselves of harmful skin infections, such as the deadly skin-attacking chytrid fungus, which threatens amphibian populations world-wide.

10.1242/jeb.162628

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Kathryn Knight
kathryn.knight@biologists.com