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Inside JEB

HUMIDITY SOFTENS SETAE TO TIGHTEN GECKO'S GRIP

Human adhesives are famed for their fallibility. Gooey glues soon lose their grip, are easily contaminated and leave residues behind. But not gecko feet. Geckos can cling on repeatedly to the smoothest surfaces thanks to the self-cleaning microscopic spatula-shaped hairs (setae) that coat the soles of their feet. Back in 2002, Kellar Autumn found that these dry hairs are in such intimate contact with surfaces that the reptiles 'glue' themselves on by van der Waals forces with no need for fluid adhesives. More recent studies had suggested that geckos might benefit from additional adhesion in humid environments through capillary action provided by microscopic droplets of water sandwiched between setae and the surface. But Autumn wasn't so sure, so he and his lab at Lewis and Clark College and the University of Washington, USA, began testing gecko grip to find out how increasing humidity helps them hold tight (p. 3699).

Knowing that geckos replace lost setae when they moult, Autumn, his postdoc Jonathan Puthoff, and Matt Wilkinson collected patches of the 'sticky' hairs from gecko feet and attached them to a mechanical testing device, known as 'Robotoe', that reproduces the way the reptile drags its foot as it contacts a surface. Dragging the setae across two surfaces (one that repelled water and another that attracted water) at different velocities and in environments ranging from 10% to 80% humidity, the team tested whether microscopic water bridges formed in high humidity were helping the geckos hang on. They reasoned that if the reptiles were using microscopic water bridges then the setae would bond more tightly to the surface that attracted water than the surface that repelled water. But when they measured the setae's adhesion and friction it was essentially the same on the two surfaces. And when the team compared the adhesion of setae that were moving too fast to form water bridges with that of slowly moving feet that could possibly form water bridges, there was no difference. The geckos were not supplementing their van der Waals attachment forces with capillary forces from water bridges. So how were they holding on tighter?

Graduate student Michael Prowse decided to take a closer look at the material properties of the reptile's feet. Knowing that setae are composed of keratin and keratin is softened by high humidity, Autumn wondered whether having softer setae could improve the reptiles' contact with surfaces and increase their van der Waals adhesion. The team decided to measure the setae's softness and how it changed as the humidity rose.

Repeatedly stretching and releasing a strip of setae at three different rates (0.5, 5 and 10 Hz) in environments ranging from 10% to 80% humidity, Autumn's team measured the force transmitted through the strip to calculate the strip's elastic modulus – how much elastic energy is stored – to see how it changed. As the humidity rose, the elastic modulus decreased by 75% and the strip of setae became softer. So the strip of setae became more deformable as the humidity rose, but could the increased softness explain the gecko's improved attachment under damp conditions?

Puthoff built a mathematical model to see if softer, more deformable, setae could explain the gecko's improved attachment at high humidity and found that it did. Not only did increased softness strengthen the contact between the setae and the surface but also it made it easier for the reptile to peel its foot off. So instead of improving gecko's attachment through microscopic bridges, higher humidity softens the setae that coat the reptile's feet to help them hold fast and peel free with ease.

10.1242/jeb.052183

Puthoff, J. B., Prowse, M. S., Wilkinson, M. and Autumn, K. (2010). Changes in materials properties explain the effects of humidity on gecko adhesion. *J. Exp. Biol.* **213**, 3699-3704.

COLD FISH REMODEL GILLS TO FLUSH OUT TOXINS

Fish gills are truly remarkable; breathing, salt balance regulation and waste disposal are all in a day's work for this versatile organ. These competing demands present some interesting challenges, says Steve Perry of the University of Ottawa, Canada. Large gills are good for breathing and flushing out toxins like ammonia, but not so good for retaining useful things like salt – a big problem for freshwater fish. 'In cold

water, crucian carp and goldfish reduce salt loss by physically covering their gills with a structure called an interlamellar cell mass,' says Perry. But how can 'cold' fish continue to pump out toxins when their gills are covered up (p. 3656)?

To find out, Perry and his team first had to show that 'cold' fish with covered gills find it harder to expel ammonia than 'warm' fish. But when they measured ammonia levels in the tanks of goldfish housed at 7°C and 25°C, instead of pumping out ammonia at a slower rate, the team were surprised to find that the 'cold' fish pumped out ammonia at a similar rate to the 'warm' fish. However, knowing that acclimation temperature can affect ammonia production, Perry decided to standardise the amount of ammonia that the fish had in their bodies. Sure enough, when the team injected goldfish with large quantities of ammonia, 'cold' fish only managed to pump out 40% of the injected load within 3 hours, while 'warm' fish expelled 90%.

'This was consistent with our idea that fish with covered gills find it harder to expel ammonia than fish with uncovered gills,' says Perry, 'but the better performance of the "warm" fish could also be explained by their faster breathing and higher blood flow compared with fish in cold water.' To test this, the team chased 'cold' fish around the tank with a net until they were breathing as fast as 'warm' fish, and then measured ammonia levels in the tanks of exercised and non-exercised fish. 'Lo and behold, we were able to turn a 7°C fish into a 25°C fish!' says Perry. So 'cold' fish can avoid becoming poisoned just by breathing faster – but was this the complete story?

The team were still troubled by the fact that they were comparing fish at different temperatures, as 'warm' fish eat more, breathe faster and have higher blood flow, all of which can affect ammonia excretion. So the team devised a way to take temperature out of the equation; they compared 'cold' fish with covered and uncovered gills. Starving some fish of oxygen so that they uncovered their gills, the team were delighted to discover that these fish pumped out ammonia much more quickly than fish with covered gills. 'This showed clearly that the interlamellar cell mass does impede ammonia excretion,' says Perry. 'We had now come full circle. When "cold" fish have covered gills, how do they manage to excrete ammonia as well as "warm" fish?'

The team knew that ammonia moves through channels called Rhesus proteins, so they decided to take a closer look at Rhesus protein expression and distribution in 'cold' and 'warm' fish. While Rhesus protein expression didn't change with temperature, the team found that Rhesus proteins were redistributed to gill edges in 'cold' fish, allowing them to shuttle out ammonia even when their gills are covered. Perry concludes that, when the temperature drops, fish can remodel their gills to cut salt loss and still manage to avoid poisoning simply by relocating their ammonia transporting proteins.

10.1242/jeb.052191

Perry, S. F., Schwaiger, T., Kumai, Y., Tzaneva, V. and Braun, M. H. (2010). The consequences of reversible gill remodelling on ammonia excretion in goldfish (*Carassius auratus*). *J. Exp. Biol.* **213**, 3656-3665.

Yfke Hager

FIRST STUDY OF NITRIC OXIDE METABOLITES IN FISH

For such a small molecule, nitric oxide (NO) punches well above its weight. As a key signalling molecule in cardiovascular regulation, it plays a major role in protecting organisms from hypoxia by triggering vasodilatation when oxygen levels fall too low. Although many fish experience hypoxia on a daily basis, Frank Jensen from the University of Southern Denmark explains that little was known about nitric oxide levels in fish and how the molecule responds to hypoxia. However, measuring nitric oxide levels is notoriously difficult. Nitric oxide is extremely unstable, surviving for as little as 2 ms in some circumstances, so Jensen and his colleague Marie Hansen decided to measure nitric oxide metabolite levels in a champion of hypoxia tolerance – the goldfish – to find out how nitric oxide levels respond to normal and hypoxic conditions (p. 3593).

Measuring nitrite, nitrate, *S*- and *N*-nitroso compound and Fe-nitrosyl compound levels in seven different goldfish tissues in response to extended periods of normoxia and hypoxia by chemiluminescence, Hansen and Jensen found that the goldfish's nitric oxide metabolite levels were essentially the same as those of normoxic mammals, with blood plasma nitrite levels of $0.75\,\mu mol\,l^{-1}$. Jensen explains that this suggests that the production of nitric oxide by the enzyme nitric oxide synthase is similar in fish and mammals. However, after 2 days of hypoxia things were very different. The goldfish's blood plasma nitrate and nitrite levels had dropped dramatically.

Jensen and Hansen explain that this large drop in nitrate and nitrite levels could be due to two causes. One possibility is that the fish are recycling nitrate and nitrite to produce nitric oxide when oxygen is scarce. Alternatively, nitrate and nitrite levels could be low because the low oxygen levels limit nitric oxide synthesis by nitric oxide synthase.

However, despite the spectacular reductions in nitrate and nitrite levels that Jensen and Hansen found in blood plasma, when they examined these and other nitric oxide metabolites in liver, kidney, heart, brain, skeletal muscle and gill after hypoxia, they found that they were essentially the same in normoxic and hypoxic tissues. Jensen and Hansen suspect that although goldfish net nitrite levels fall during hypoxia, the fish transfers nitrite from extracellular compartments to intracellular compartments to ensure that sufficient nitric oxide is available to regulate cellular function when oxygen is scarce.

10.1242/jeb.052209

Hansen, M. N. and Jensen, F. B. (2010). Nitric oxide metabolites in goldfish under normoxic and hypoxic conditions. *J. Exp. Biol.* **213**, 3593-3602.

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