

Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

NO TWO WAXES ARE THE SAME



Distinguishing friend from foe is as important for insects as it is for any other creature. You need to know when you're on someone else's territory and when an impostor is threatening. Many insects depend on their sense of smell to identify nest mates, and bees are no different; they recognise wax scents picked up by bees from their own nest, explains Robert Buchwald. However, wax's role in communication was probably a secondary evolutionary factor after its other main purpose: construction, where bees sculpt wax into nests and exquisite hexagonal combs to store honey and nurture larvae. Knowing that bees can distinguish wax scents carried by bees from other nests, Buchwald and Michael Breed wondered whether these subtle differences in composition also impacted on the material's structural properties. They decided to investigate waxes from several species to find out whether they were mechanically indistinguishable, or each had been honed to suit the structural needs of each species' nests (p. 3984). The pair teamed up with mechanical engineer Alan Greenberg to measure several waxes' mechanical properties, but first they needed nests to test.

Buchwald explains that getting hold of *Apis mellifera* nests was straightforward; he simply visited the apiary at the University of Colorado at Boulder. However, tracking down the more exotic *Apis* species was much trickier. Fortunately, the team established a strong collaboration with Canadian scientist Gard Otis, who supplied them with nests during his field work in Asia, despite running the constant gauntlet of bee stings.

Melting down the nests, Buchwald and Greenberg cast each species' wax into a cylinder shape to remove the nests' architectural differences, before compressing the wax to test its structural properties. But working with the soft wax samples was very different from the construction materials that Greenberg usually studied; the team had to

find the most sensitive stress detector for the compression system that they used to calculate each wax's mechanical strength and stiffness.

Analysing the results, the team realised that *Apis dorsata*'s wax was by far the strongest and stiffest, while *Apis andreniformis*'s was the weakest and softest. Each species' wax was mechanically unique and unlike the other three's.

Buchwald suspects that the bees' nesting habits could account for the mechanical differences. He explains that *Apis dorsata*'s colossal nests not only have to support the greatest weight, but also must withstand knocks and high winds in their exposed locations, suspended from tree branches high above the forest canopy. Meanwhile, *Apis cerana* and *Apis mellifera* build their nest combs in protected cavities, such as dead trees, which seems to have resulted in the insects evolving intermediate-strength waxes. However, Buchwald was most surprised by *Apis andreniformis*'s wax. He explains that, like *Apis dorsata*, *andreniformis* hangs its nests from tree branches. But *andreniformis* nests are much smaller than hefty *dorsata*'s and are located in the relative protection of the forest's lower reaches, hanging from springy branches that protect the nests from mechanical shocks. Buchwald suspects that these differences in lifestyle have allowed *andreniformis* to evolve softer wax than *dorsata*'s robust blend.

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Buchwald, R., Breed, M. D., Greenberg, A. R. and Otis, G. (2006). Interspecific variation in beeswax as a biological construction material. *J. Exp. Biol.* **209**, 3984-3989.

IS HYDROGEN SULPHIDE AN OXYGEN SENSOR?

Hypoxia is a potential killer. Prolonged exposure over decades can lead to hypertension and cardiac damage. While peripheral blood vessels tend to dilate during hypoxia, pulmonary blood vessels usually contract to minimise the hypoxic exposure. However, even though the physiology of the mammalian pulmonary response is relatively well characterised, the mechanism was unknown and had intrigued scientists for years. So when Ken Olson realised that hydrogen sulphide could turn out to be the key player, it set him off on the most exciting scientific odyssey of his career (p. 4011).

Olson explains that he became interested in the effects of hydrogen sulphide on smooth muscle when he heard that the gas triggered relaxation in the rat aorta. Intrigued, he suggested that Ryan Dombkowski characterise the effects of hydrogen sulphide on blood vessels from various creatures, but he only spotted the potential link between hydrogen sulphide and the hypoxic response

when Dombkowski appeared in his office with a trace of the rat pulmonary artery's response to hydrogen sulphide. Olson instantly recognised the plot; it was identical to a plot Michael Russell had just shown him of the aorta's response to hypoxia shortly before. He realised that hydrogen sulphide could regulate the hypoxic response.

But Olson needed a good model system to test his theory, and fortunately he had stumbled across the ideal system shortly before, during a visit to Malcolm Forster's lab in New Zealand. Olson had made the unexpected discovery that the hagfish aorta contracts during hypoxia, a function that had been thought to be restricted to pulmonary vessels. Back in his Indiana University laboratory, Olson pursued his discovery and found that the sea-lamprey's aorta also contracted spectacularly during hypoxia, so he decided to test out the gas transmitter in his new lamprey model to see if hydrogen sulphide gas fitted the bill.

First, Olson and Dombkowski compared the lamprey aorta's responses to hypoxia and hydrogen sulphide and they were identical. But that wasn't enough to show that hydrogen sulphide mediated the response. Olson realised that he needed to show that exposing the aorta to hypoxia would block its response to a subsequent dose of hydrogen sulphide, only then could he be sure that both responses functioned through the same mechanism. Dombkowski put Olson's theory to the test: the lamprey aorta didn't contract further after systematic exposure to hypoxia and hydrogen sulphide, or *vice versa*. The lamprey aorta's responses to hydrogen sulphide and hypoxia seemed to follow the same mechanism.

Next, Olson and his team needed to show that the aorta generated hydrogen sulphide intrinsically, so Nathan Whitfield built a hydrogen sulphide sensitive electrode and he, Sally Head and Meredith Doellman used it to test for the gas in the aorta tissue. Having found it, the trio went on to test the effects of hydrogen sulphide precursors on tissue gas levels and found that they rose. Finally, knowing which enzymes are responsible for hydrogen sulphide synthesis in mammals, the team wondered whether hydrogen sulphide inhibitors could inhibit the hypoxia response in lamprey aorta. Head exposed the aorta to various inhibitors and the vessel's hypoxia response either vanished or was reduced. All of the results pointed to hydrogen sulphide mediating the hypoxia response.

But lampreys are fairly distant relatives of higher vertebrates, so Olson needed to test out his theories on mammalian pulmonary vessels. Turning to Holstein cows, the team ran the mammalian blood vessels through the same battery of tests, and found that exposure to hydrogen sulphide and hypoxia not only produced the same effects in the pulmonary

artery, but hydrogen sulphide seemed to be essential for mediating the response.

Olson is very excited about his discovery that hydrogen sulphide could be the mechanism that mediates the hypoxic response and suspects that this enigmatic gas could eventually turn out to be a universal oxygen sensor, but admits that only time will tell.

10.1242/jeb.02543

Olson, K. R., Dombkowski, R. A., Russell, M. J., Doellman, M. M., Head, S. K., Whitfield, N. L. and Madden, J. A. (2006). Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J. Exp. Biol.* **209**, 4011-4023.

DROPPING LIKE FLIES



Picture by Donna Folk

When the going gets hot, fruit flies turn on heat shock proteins (HSPs) to protect themselves. But when physiologist Donna Folk teamed up with evolutionary biologist George Gilchrist to study heat tolerance in the humble fruit fly, she found that HSP protection during a heatwave comes at a price. Curious about the protective roles of HSPs in insects and the different methods used to assess insects' heat tolerance, Folk joined Gilchrist at the College of William and Mary in Virginia to study fruit fly populations that can tolerate different temperatures. High tolerance flies had been artificially selected to cope with high temperatures. Flies selected for low tolerance, on the other hand, collapsed at much milder temperatures. But what role did the insects' heat shock responses play in their physiological responses to a hot blast (p. 3964)?

Folk explains that animals that have experienced a previous heatwave have already churned out HSPs, so they have them onboard for protection when the mercury rises later; the insects are said to have 'hardened'. Knowing that high tolerance flies usually trigger HSP production at high temperatures, Folk expected that a mild heatwave would only trigger the low

tolerance insects' heat shock systems, leaving the high tolerance flies more vulnerable to heat shock than their low tolerance counterparts. To find out, Folk first hardened the flies by giving them a mild heat shock to build up the insects' HSP reserves. She then gave the flies a serious heat shock, and counted how many flies were alive a day later. To her surprise, the high tolerance flies survived better after hardening; their heat shock response had unexpectedly been triggered by the previous mild heatwave.

Knowing that flies lose the ability to move around at very high temperatures, Folk wondered whether hardened high temperature tolerant flies would retain their ability to move at higher temperatures compared with their non-hardened counterparts. Using a 5 foot long column, surrounded by a heated water jacket to control the column's air temperature, Folk allowed flies inside the column to move freely as she slowly raised the temperature, counting the numbers of flies that became incapacitated as the air temperature rose in 0.5°C increments. Pouring hardened and non-hardened flies into the column, Folk was startled to see that the hardened high tolerance flies fell out of the column at a cooler temperature than their non-hardened counterparts. Despite their improved survival, the high tolerance flies' ability to move in hot air suffered more from the hardening process than unhardened insects from the same population.

This was a big surprise, Folk admits, 'because people assume that hardening improves heat tolerance. This really made us stop and think about what could be going on.' Like all good things, it seems HSP production comes with a cost; Folk suspects that high tolerance flies struggle with energetic challenges when the mercury rises. 'Their metabolic rate can shoot up by 70%,' says Folk, 'and HSPs also use up lots of energy to work.' This may cause the flies to 'overheat' more quickly, she concludes.

Folk also noticed that high tolerance females survived better than low tolerance females after a heat shock. To explain this, Folk turned to HSC70, an HSP that is not produced in response to heat stress but is found naturally in flies. Folk found that HSC70 levels drop off sharply in low tolerance females during the first minutes of a heat shock. If high tolerance females are somehow slowing their HSC70 degradation rate, Folk says, this might explain their better survival.

10.1242/jeb.02542

Folk, D. G., Zwollo, P., Rand, D. M. and Gilchrist, G. W. (2006). Selection on knockdown performance in *Drosophila melanogaster* impacts thermotolerance and heat-shock response differently in females and males. *J. Exp. Biol.* **209**, 3964-3973.

Yfke Hager

TIME IS OF THE ESSENCE



Axel Innis is a postdoctoral fellow at Yale University

Watch an ant scurrying around searching for food, and you're looking at the tip of an ant iceberg; each forager is just one individual in a well-oiled foraging network that fuels their entire community. Species that survive on nectar make repeated foraging trips to flowers and other nectar sources, but many return with their crops only partially filled. Pablo Schilman and Flavio Roces explain that this apparently inefficient nectar-delivery has long puzzled scientists, and while the nectar's concentration seems to influence the ants' decision about how much to fill up, it wasn't clear whether the semi-loaded foragers were minimising their energetic foraging costs, or limiting the amount of time spent supping to maximise the return on their nutritious load. Schilman and Roces decided to measure the metabolic rates of freely feeding *Camponotus rufipes* as they fuelled

up on sugar solutions over a range of concentrations delivered at various flow rates (p. 4091).

Surprisingly there was no correlation between the insect's metabolic rate and their final load; no matter how heavy their nectar burden, the insect's metabolic rate was relatively unaffected. Instead the team found an 'increase in individual metabolic rate with increased sugar flow rate' explains Schilman, which he suspects is caused by the insect's increased activity levels as they become more motivated upon finding an abundant nectar supply.

The team also compared the amount of energy carried back by a forager with the energy expended during the trip to find out if the ants cut their foraging trip short to conserve energy, but even the weakest sugar

solution was enough to sustain the intrepid insects over distances far exceeding the average foraging trip. The ants weren't limiting their loads to conserve energy, it seems that 'decreasing their foraging time is more important than increasing individual energetic efficiency', says Schilman. He suspects that the insects cut time filling up to give themselves more time to spread the good news when they return to the nest.

10.1242/jeb.02541

Schilman, P. E. and Roces, F. (2006). Foraging energetics of a nectar-feeding ant: metabolic expenditure as a function of food-source profitability. *J. Exp. Biol.* **209**, 4091-4101.

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