Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

ACCLIMATION

COOL, CAPTURED FLIES

Coping with temperature variation is part of survival in the wild for an insect whose body temperature closely tracks the ambient environmental temperature. Yet when faced with temperature changes, such as seasonal or daily cycles, animals use a variety of tricks to compensate for these potentially lethal changes. For example, insects can alter their behavioural patterns to thermoregulate more efficiently or they might alter their physiology rapidly within their lifetime to better withstand the impending cold front. But adapting quickly to improve performance in one set of conditions, such as low temperature, might come at the cost of performing poorly at high temperatures. So a hotly debated question in insect physiology is: 'do insects that are cold acclimated perform better than non-acclimated insects under cold conditions in the wild and, if so, does their improved performance come with costs if temperatures rise?'

Torsten Kristensen and colleagues at the University of Aarhus, Denmark, teamed up with Ary Hoffmann and Rebecca Hallas at the University of Melbourne in Australia to tackle this question. Using tens of thousands of *Drosophila melanogaster* fruit flies, the team acclimated the insects to warm or cool conditions, before releasing the insects into cool, warm or hot environments to see how they faired relative to control (non-acclimated) flies. Having dusted the conditioned flies with a coloured fluorescent powder to identify them, the insects were re-captured within a couple of days at banana-baited traps. This essentially provided a proximate measure of field fitness, since flies that are unable to reach food in the wild are far more likely to die than those that can find food. Roughly 100% of the cold-acclimated flies survived and were recaptured at 12°C relative to controls; so flies that had been given the opportunity to experience low temperatures previously were much more likely to acquire food under low temperature conditions in the wild. However, less than 0.1% of the cold-acclimated flies were recaptured at 29°C, showing that flies exposed to low temperatures previously were highly unlikely to obtain food at higher release temperatures in nature. These results clearly show that cold-acclimated flies perform better in the wild under cold temperatures, but that this improvement at low temperatures comes at a cost to high temperature performance.

However, when heat and cold tolerance of the acclimated and control flies were compared in the laboratory, the outcome differed fundamentally. As expected, cold-acclimated flies survived icy conditions better than control flies. For example, roughly 50% of the population of cold-treated flies survived –8°C whereas almost no non-acclimated flies survived these conditions. However, when the team tested the cold-acclimated flies' survival rate at high temperatures in the lab, they were in for a surprise. The cold-acclimated flies survived as well, if not better, than non-acclimated flies at 38°C. There was no apparent cost for cold acclimation at high temperatures in the lab. This result contrasts strongly with the field releases in which few of the cold-acclimated flies made it back to the banana-baited traps at higher temperatures. This finding highlights the fact that laboratory results often do not accurately predict the outcome of physiological adjustments made in the wild.

This exciting study, which hides a massive body of work in its simple elegance, has trenchant results. First, cold acclimation is indeed beneficial in the field under low temperature conditions but this comes at massive costs when flies are tested at warm temperatures. Second, these same costs and benefits were not visible when flies were assayed in the laboratory for hot and cold survival. In conclusion, this research convincingly demonstrates that the ability of flies to acclimate to changing weather is likely to be adaptive, although the extent of the response will be dependent on the relative costs and benefits of adjusting physiologically in a particular environment.

10.1242/jeb.010835

Kristensen, T. N., Hoffmann, A. A., Overgaard, J., Sørensen, J. G., Hallas, R. and Loeschcke, V. (2008). Costs and benefits of cold acclimation in field-released *Drosophila. Proc. Natl. Acad. Sci. USA* **105**, 216-221.

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COMMUNICATION

CHATTING WITH NEAR-FIELD SOUND

The ability to detect air motions with sensory hairs has evolved repeatedly: insects have filiform hairs, whereas spiders and their close relatives use 'trichobothria' – to all intents and purposes sensory hairs, but not homologous to the insect's hairs and, of course, quite different from what we mammals consider as hair. The ability to sense local air motions, or 'near-field sounds', serves a variety of purposes, from locating prey by spiders, to locating mates by mosquitoes. A fascinating, if originally subsidiary, potential function is communication within the species. Roger Santer and Eileen Hebets of the University of Nebraska – Lincoln have been investigating this, with the view that communication using near-field sound might turn out to be very widespread among arthropods.

Santer and Hebets used whip spiders – those scary-looking relatives of spiders and scorpions with an enormously extended pair of 'antenniform' legs – collected from Florida to build up a convincing demonstration of the deliberate use of near-field sound in communication. When two male whip spiders meet each other, there is not so much a 'stand-off' as a 'stand very near'. Indeed, each whip spider positions himself so that one of their scary long legs is next to a sensory hair on one of the opposition's regular legs. While, yes, they do occasionally kick each other in the process, this happens sufficiently rarely for direct contact to be unlikely as the means of communication. Once the elongated legs are in position, they vibrate at around 20 Hz with an amplitude of a few millimetres (as observed from high speed video). Such contests can be achieved in complete darkness (so the competitors aren't watching each other), and the vibration display is performed longer by contestant winners than losers, suggesting that the leg-vibrating display conveys information through air movements (though it is not clear what) to resolve the contest.

While these observations alone are highly suggestive, Santer and Hebets took the story a considerable step further. They tuned a 'stimulator', an oscillating 200 μm diameter tungsten wire, to replicate the motions of the vibrating leg. By wiggling this pseudo-leg at frequencies ranging from 1 to 120 Hz, while making electrophysiological recordings of the sensory hairs thought to receive information from near-field sounds, they demonstrated that the observed leg-waggling frequencies (around 20 Hz) caused particularly strong and sustained excitation in the sensory hairs. At lower waggling frequencies, there were fewer sensory cell excitations, as each vibration of the pseudo-leg stimulated a constant number of action potentials. At pseudo-leg-waggling frequencies that were too high, the team found a decrease in the sensory signal through time. At realistic leg-waggling frequencies, the sensory hair produced strong, clear signals from which the duration of a leg-waggling bout could be determined. As this is what the behavioural observations had suggested was related to performance in male–male contests, it appears that the leg-waggling frequency and the sensitivity of the air movement-sensing hairs are well matched.

This combination of behavioural and electrophysiological evidence demonstrates that, yes, whip spiders can communicate using the near-field sounds produced by their shaking legs. Given these air movement-sensing hairs turn up very widely across arthropods, Santer and Hebets suggest that similar signalling using near-field sound might actually be quite prevalent. While this will take further work to validate, Santer and Hebets have convincingly ticked the first box of a potentially very long list.

10.1242/jeb.010827

Santer, R. D. and Hebets, E. A. (2008). Agonistic signals received by an arthropod filiform hair allude to the prevalence of near-field sound communication. *Proc. R. Soc. Lond. B Biol. Sci.* **275**, 363-368.

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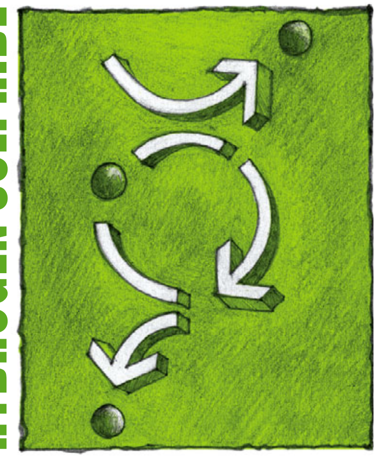
HYDROGEN SULPHIDE



WHAT A GAS!

Hydrogen sulphide (H_2S), better known to some as the 'rotten-egg gas' due to its characteristic pungent odor, is generally thought of as a noxious and toxic gas. Recently though, it was discovered that H_2S is naturally produced in animal cells, that it exists in micromolar amounts in the blood and brain of mammals, and that it plays numerous important physiological roles such as acting as a signalling molecule, neuromodulator and regulator of cardiovascular status. Additionally, other studies have reported the intriguing finding that exposure of mammals and/or their tissues to a low dose of H_2S actually improves the capacity of the animal or tissue to survive otherwise lethal conditions. However, exactly how H_2S exerts these physiological effects remains unknown.

Dana Miller and Mark Roth of the Fred Hutchinson Cancer Research Center in Seattle, Washington were interested in elucidating the molecular mechanisms underlying the beneficial physiological effects of H_2S exposure. Ingeniously, the team recognized that they should approach this problem by utilizing the nematode *Caenorhabditis elegans* as their study species. The genome of *C. elegans* is completely sequenced and there exist numerous, readily available mutant strains (i.e. strains that have been genetically engineered to have specific genes missing or 'knocked-out'). Thus, the team reasoned that by comparing the physiological responses of wild-type (i.e. those with all their genes) and various knock-out strains of nematodes to H_2S exposure, they should be able to determine which essential molecular pathways are associated with the beneficial effects of H_2S.

However, before searching for the molecular mechanisms, the team had to first determine whether and how H_2S exposure is beneficial to nematodes. To accomplish this, the team grew nematodes in atmospheres of room air (the control group) or in the presence of a low concentration of H_2S and compared various indices of the nematodes' health, their lifespan and tolerance to high temperature. The team discovered that nematodes grown in H_2S were as healthy as the control animals, but that they lived 70% longer and could survive 8 times longer at the stressful high temperature of 35°C.

Armed with this knowledge, the team set out to discover whether the benefits of H_2S exposure were linked to any of the known molecular pathways in *C. elegans* responsible for influencing lifespan. Interestingly, the team found that mutant nematode strains grown in H_2S but lacking genes specific to the insulin signalling pathway, mitochondrial dysfunction or caloric dysfunction, were still long lived and thermotolerant, thus excluding the possibility that these molecular pathways are associated with the beneficial effects of H_2S. In contrast, the team discovered that nematodes lacking the gene for *sir-2.1*, an important stress-induced enzyme capable of prolonging life, had the same lifespan and thermotolerance of normal nematodes, despite being grown in H_2S.

The team argues that this finding suggests that one cellular activity of H_2S is to increase the activity of *sir-2.1*, which subsequently leads to increased lifespan and thermotolerance, and wonder whether this mechanism is conserved in vertebrates. Only future studies will tell!

10.1242/jeb.010819

Miller, D. L. and Roth, M. B. (2007). Hydrogen sulfide increases thermotolerance and lifespan in *Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA* **104**, 20618-20622.

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DEHYDRATION TOLERANCE

MOLECULAR SHIELDS PROTECT PROTEINS FROM DEHYDRATION DAMAGE

Life as we know it requires water. Among other things, water hydrates the molecules that make up cells. When water is removed things go terribly wrong: membranes fuse, proteins aggregate and metabolic reactions slow or stop. However, some organisms, including some multicellular plants and animals can survive for long periods of time in the absence of cellular water. The organisms are called the anhydrobiotes.

Very little is known about the genetic requirements needed to survive anhydrobiosis, but there are a handful of genes that seem to be specific to organisms that can survive in the dry state. One family of genes that may be involved in desiccation tolerance codes for proteins known as late embryogenesis abundant or LEA proteins. LEA proteins, which tend to be unstructured in solution and are also extremely hydrophilic, have long been known to accumulate to very high levels in dehydration-tolerant plant seeds. More recently, LEA proteins have been identified and isolated in a number of invertebrates that exhibit tolerance of dehydration, including the nematode *Aphelenchus avenae*. Could LEA proteins be involved in stabilising some aspect of a cell's structure during dehydration? In their recent PNAS report, Sohini Chakrabortee and colleagues at the University of Cambridge and Cambridge Institute for Medical Research set out to test whether a nematode LEA protein could act to protect other proteins from aggregation during dehydration.

To test whether the nematode LEA proteins could prevent protein aggregation during dehydration, the team isolated water soluble proteins from nematode and human cells and subjected them to vacuum dehydration in the presence or absence of purified nematode LEA proteins. They also added the LEA proteins at different times before and during the dehydration–rehydration process. They found that the LEA proteins prevent aggregation of both nematode and human proteins when used at a ratio of 5:1 or greater. This effect was observed in all size classes of proteins, and thus it appears that LEA proteins act in a general manner, and not on a specific subset of proteins. The greatest protection from aggregation was gained when the LEA proteins were added before dehydration, but addition of LEA proteins after dehydration and within 5 min of rehydration did afford some protection.

The team also tested the ability of the nematode LEA protein to prevent aggregation of proteins under hydrated conditions. To do this, they created human cell lines that co-expressed a huntingtin-derived protein (that is prone to aggregation) and the nematode LEA protein. They found that the LEA protein significantly reduced the number of cells that accumulated huntingtin-derived protein aggregates.

Chakrabortee and colleagues have illustrated that the nematode LEA protein functions in general to prevent protein aggregation, but is it acting as a molecular chaperone or a shield? The fact that the LEA proteins are functional while remaining highly unstructured seems to suggest that the LEA proteins act as molecular shields by coating proteins and preventing them from aggregating, rather than through a conformation-dependent mechanism as would be expected for a molecular chaperone. However, a definitive answer to this question remains to be tested.

10.1242/jeb.010843

Chakrabortee, S., Boschetti, C., Walton, L. J., Sarkar, S., Rubinsztein, D. C. and Tunnacliffe, A. (2007). Hydrophilic protein associated with desiccation tolerance exhibits broad protein stabilization function. *Proc. Natl. Acad. Sci. USA* **104**, 18073-18078.

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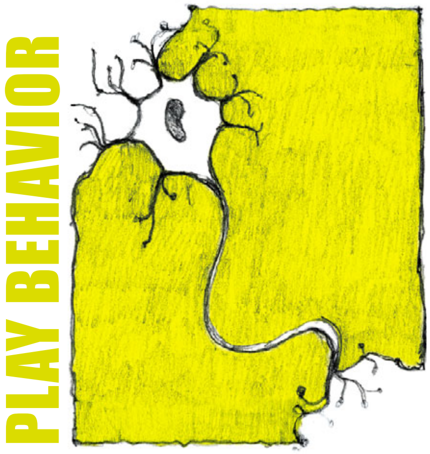

TO CATCH A THIEF

Most humans don't consider bird brains to be particularly impressive. Witness the fact that 'bird brain' is an insult used to denote stupidity in humans. Perhaps some birds are not particularly intelligent by human standards (especially the ones that we have bred for eating). However, there are avian species with cognitive powers on a par with those observed in many primates. In particular, birds in the genus *Corvus* (crows, ravens, jackdaws and rooks) are good examples of just how intelligent birds can evolve to be.

That said, not all corvid behavior makes sense at first glance. For example, ravens (*Corvus corax*) commonly bury brightly colored objects in the presence of other ravens and human handlers. The birds do this in plain view, and the objects they hide have no obvious value. This behavior, termed 'play caching', has puzzled researchers for years. After all, it's not as if ravens don't know how to hide things. Food items, for one, are buried out of sight, with the utmost care and secrecy. So why would a raven waste energy doing this seemingly silly public behavior? Thomas Bugnyar and colleagues at the University of Vienna recently asked this question, then tested the idea that ravens use the experience as a way to gauge whether or not other individuals have a tendency to steal.

In their first set of experiments, Bugnyar and colleagues gave brightly colored baubles to hand-raised ravens, then gave the birds a chance to bury their treasures on a snowy forest floor. Two new researchers then stepped in and played good cop–bad cop. One researcher (O) simply inspected every hiding spot; the second individual (P) inspected, then blatantly pilfered every cache.

How did the ravens react? In both situations, ravens tended to inspect both sites after the humans had left the scene. The birds were clearly curious to see what O and P had been up to. The birds responded by changing the location of subsequent caches relative to both observers. Overall, they buried items progressively farther and farther away from the humans. However, despite having been robbed the first time, the ravens reacted by shifting the cache closer to P than to O. They behaved as if they were testing P. Notably, the ravens made no attempt to modify other aspects of their caching strategy across training sessions. In particular, they continued to cache in plain site of P, and did not attempt to camouflage their stashes more carefully in the presence of the pilferer. P kept stealing their baubles and the ravens just stood by and watched.

But does this mean that the ravens weren't learning anything about P? In the second set of experiments, the team tested how ravens cached items of real value (i.e. food items) given to them by O, P and a neutral observer (N). In the presence of P, the ravens started caching earlier than in the presence of O and N. Furthermore, they placed their food stashes behind obstacles, and took a longer time covering the items than in the presence of non-pilferers. These data suggest that the human researchers had become the researched. Ravens had learned from their play caching experiments that P was prone to thieving.

The experiments of Bugnyar and coworkers provide evidence that ravens can actually be pretty good scientists; they can use play behavior as a tool to probe the minds of competitors. Humans and some non-human mammals can also do this but, so far, this ability has not been widely documented in other animal phyla. This work adds to the growing body of evidence suggesting that the complexity of corvid social behavior can rival that seen in many mammals.

Just as importantly, Bugnyar and colleagues' work gives neurobiologists a taste of what else is out there ready to be worked on. The neural mechanisms underlying raven behavior are largely unknown. Compared with other bird species, we know relatively little about neuroanatomy and neurophysiology in the raven, crow and brethren. The richness and complexity of behavior in these species suggests that the corvid bird brain is actually a wondrous place, a place just waiting to be explored by neurobiologists.

10.1242/jeb.010850

Bugnyar, T., Schwab, C., Schloegl, C., Kotrschal, K. and Heinrich, B. (2007). Ravens judge competitors through experience with play caching. *Curr. Biol.* **17**, 1804-1808.

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