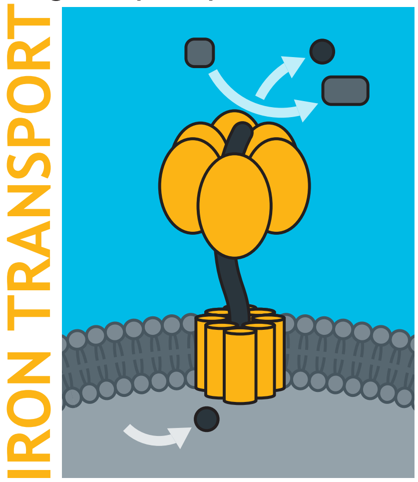
OUTSIDE JEB

Hagfish pump some iron



Along with other nutrients, animals need trace metals to function. Iron, the oxygen-binding component in the blood pigment hemoglobin, is a critical trace metal and is usually absorbed through the gut. Mammals can alter iron metabolism in order to meet oxygen demand during periods of low environmental oxygen – hypoxia. However, it was unknown whether hagfish – a much more primitive group of animals that exhibit unusual tolerance to hypoxia – change their iron dynamics to achieve such resilience and whether they could take up iron from their environment. Knowing that hagfish can absorb some nutrients through their skin, Chris Glover, of Athabasca University, Canada, and colleagues from several other Canadian institutions set out to determine whether hagfish can take up iron across the skin; if so, how it might compare with gut uptake; and whether changes in iron transport during hypoxia contribute to the impressive hypoxia tolerance of these hardy fishes.

To begin to answer their questions, the researchers measured iron transport across the skin and gut tissue of Pacific hagfish. Put simply, they exposed the external surface of each tissue to a solution containing radioactive iron at a range of concentrations and measured how much radioactivity entered a saline solution on the other side of the tissue after 2 h. They also measured how much radioactivity remained within the tissue itself.

Glover and his colleagues found that the hagfish does take up iron through the skin – and at a higher rate than in the gut. Also, over all but the highest iron concentrations, the uptake in both tissue types initially increased with concentration and then reached a plateau. In addition, most iron absorbed by the skin remained in the skin, while iron absorbed by the gut tended to move across the tissue. The initial increase and subsequent uptake plateau indicate that specific transporter molecules are responsible for moving the iron in both tissues (as these molecules at some point reach a maximum transport capacity). However, the discrepancy in transport rates and in the iron's final destination suggests that each tissue handles iron differently.

To find out whether hagfish alter iron transport during hypoxia – when the oxygen-binding metal is particularly essential – the researchers again measured iron transport, but this time on skin and gut from hagfish exposed to hypoxia for 24 h. They also analyzed the oxygen content of the fish blood, the red blood cell count and volume, the proportion of the blood made up of red blood cells and the blood hemoglobin content to determine whether hypoxia increases iron demand in hagfish as it does in mammals.

Unexpectedly, hypoxia exposure did not significantly change iron transport in either the fish's skin or gut. Also, unlike in mammals, hypoxia did not appear to increase iron demand in the blood. These results suggest that changes in iron transport do not contribute to the hagfish's outstanding hypoxia tolerance and that the relative lack of change in blood parameters may preclude the need for any changes in iron transport in the first place.

This study is the first to characterize trace metal transport in hagfish skin or gut and to identify iron transport in hagfish skin. It also paves the way for future research by raising more questions: what is the nature of the transporter; what happens to the iron in hagfish skin; and is skin iron transport ancient or adaptive? Clearly, these primitive hypoxia heroes are not ready to divulge all their secrets just yet; they've got them clenched in an iron fist – er, fin.

10.1242/jeb.147421

Glover, C. M., Niyogi, S., Blewitt, T. A. and Wood, C. M. (2016). Iron transport across the skin and gut epithelia of Pacific hagfish: kinetic characterisation and effect of hypoxia. *Comp. Biochem. Phys. A* **199**, 1-7.

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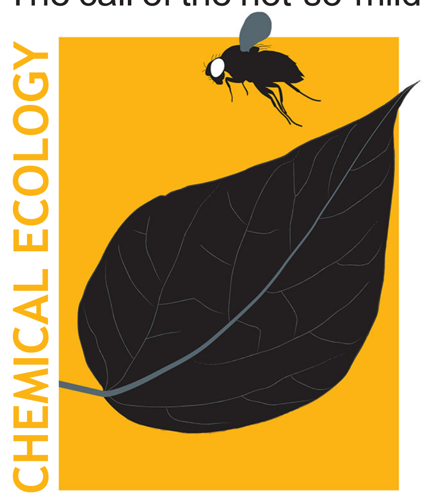
The call of the not-so-mild



Flies and many other insects show an inordinate fondness for feces. They oviposit there and occasionally visit animal excrement to dine. But it turns out that not all poop is created equal, and flies are rather discerning in their choice of fecal targets. While some piles of feces are highly attractive, the fragrant bouquet of displeasing targets is met with extreme prejudice. Now, in a recent paper in *Current Biology*, Suzan Mansourian and her collaborators from the University of Lund in Sweden show why.

Large mammals can be partitioned in many different ways, and one of the larger divisions concerns diet and the traits that go with it. Beyond the obvious differences between carnivores and herbivores (teeth, claws, etc.), these animals also differ in the length and structure of their guts.

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Deeper still, they maintain dramatically different microbiomes to aid in digestion. And it is this latter difference, according to Mansourian and colleagues, that matters most to flies.

When the research team offered flies a choice between carnivore and herbivore feces for oviposition, they found marked differences. While flies were attracted or indifferent to herbivore feces, they were positively repulsed by carnivore waste. Moreover, their aversion disappeared when the assays were repeated with flies lacking a specific type of olfactory neuron, making it clear that their choice was caused by an odor preference.

To determine why one animal's waste smelled better than the other, the team subjected the fecal samples to detailed chemical analysis. It was little surprise that the feces contained a complex brew of smells. However, while many chemicals distinguished feces in general from, say, a bottle of lager or a ripe banana, only very few were directly tied to the animal's diet. Phenol, in particular, was abundant in carnivore feces but entirely absent from the excrement of herbivores. More important, the team determined that this chemical alone was sufficient to drive fly behaviors. When normally acceptable giraffe feces was spiked with phenol, the flies turned up their noses and oviposited elsewhere. And when flies were forced to oviposit on phenol-laced food, they layed nearly five times fewer eggs.

But why should flies avoid phenol? To determine this, the team analyzed the bacteria found in herbivore and carnivore guts. The most interesting differences, it turned out, were for bacterial species that are pathogenic for insects. And these bacterial species, not coincidentally, make phenol. But don't trust just flies. When the team offered dung beetles, bonafide poo experts, a choice between herbivore and carnivore feces, these too chose the former. Thus, aversion to phenol cues appears to be widespread.

The neatest aspects of these results are the diverse links between chemical ecology, neurobiology and animal behavior. But these same aspects also highlight the loose threads that offer some promising avenues for future research. Among these, why are frugivorous fruit flies so discriminating about feces? How reliable a cue is phenol for the risk of fly disease? Does phenol concentration scale with the true risk to flies? Are the phenol producers really pathogenic? I hope the authors can address at least some of these intriguing questions in the future.

10.1242/jeb.147173

Mansourian, S., Corcoran, J., Enjin, A., Lofstedt, C., Dacke, M. and Stensmyr, M. C. (2016). Fecal-derived phenol induces egg-laying aversion in *Drosophila*. *Curr. Biol.* **26**, 2762-2769.

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Basking behavior reveals vulnerability to climate change

Rising surface temperatures are threatening biodiversity worldwide, and the impacts of climate change on organisms are hard to predict. Ectothermic species, such as lizards, rely to a large degree on environmental temperatures to maintain their body temperatures and might therefore be especially impacted by the predicted changes. A team of international researchers led by Martha Muñoz, from Duke University, USA, has compared the thermal behavior and physiological characteristics of seven lizard species living in eight different locations and elevations of an Australian rainforest region in order to examine how basking behavior and thermal habitat influence the evolution of thermal physiology such as heat tolerance. This extensive study, published in *Evolution*, suggests that the ability of a lizard species to tolerate hot environments is dependent not so much on the climate in their habitat, but on their basking behavior, and highlights the high vulnerability of ectothermic species to a warming climate.

The team first identified the thermal microhabitats and basking tendencies of seven lizard species by observing the animals in their natural environments and measuring the temperatures of the surface where the lizards were perching. They then collected lizards from the field and transferred them to the laboratory to conduct a series of tests to classify the physiological traits of each species. First, they flipped the lizards on their back at different temperatures and determined the lowest and highest temperature at which an individual was able to right itself. Following these tolerance tests, Muñoz and colleagues determined the thermal sensitivity of sprinting speeds to establish the temperatures at which sprinting was quickest for each species.

When the team analyzed the data, including information on the phylogenetic relationship of the seven species, they found that the basking species were considerably more heat tolerant than the shade-seeking skinks. Based on this observation, Muñoz and her colleagues concluded that the evolution of heat tolerance likely occurred when the lizards specialized and selected either basking or shade-dwelling as their preferred lifestyle. They also suspect that this led to the differences that they found in heat tolerances even between lizards that live in the same location. Unfortunately, their finding that habitat temperature had no influence on heat tolerance also indicates that these species may not be able to adapt quickly enough to match the pace of climate warming.

However, the lizards' cold tolerance seems to be shaped mainly by their habitat conditions. The researchers found that all lizards, regardless of their basking behavior, were more cold tolerant when they inhabited cooler habitats. In the cold, animals have no other option than to adjust their physiology to cope better in chilly conditions. Interestingly, lizards from lower elevations, and therefore warmer environments, sprinted optimally at lower temperatures than lizards from cooler areas. The authors concluded that at lower elevations, lizards have a greater

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risk of overheating and that the lower optimal sprinting temperatures could be caused by an alteration of the lizards' activity patterns to avoid heat stress.

This study emphasizes the high vulnerability of shade-specialist lizards with low heat tolerance to climate change, and suggests that even closely related species may differ substantially in their ability to tolerate increasing temperatures.

10.1242/jeb.147181

Muñoz, M. M., Langham G. M., Brandley, M. C., Rosauer, D. F., Williams, S. E. and Moritz, C. (2016). Basking behavior predicts the evolution of heat tolerance in Australian rainforest lizards. *Evolution* **70**, 2537-2549.

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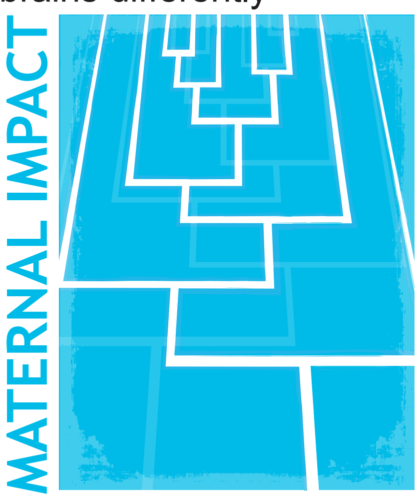
Mom shapes daughters' brains differently



It's no secret that who we are has a lot to do with our parents (genes) and how we were raised (environment). Add on a layer of biological gender, and you're sure to have a unique individual: it's fascinating to consider that much of what defines us is determined long before we're born. That's because environment and genes can interact to influence critical processes such as brain development, and this shapes individuals through neuroplasticity. In humans, for example, if a mother is extremely stressed during pregnancy, her baby is more likely to suffer from mental health issues as an adult, and the nature of these effects are often sex-specific. But does the same hold true for egg-laying animals, such as fish, where the eggs develop outside of the mother's 'environment'? David Metzger and Patricia Schulte, from the University of British Columbia in Canada, asked this question in their recent study published in *Proceedings of the Royal Society B*. They wanted to know what effect a mother fish's experiences have on her offspring's brains, and whether these effects are different in sons and daughters.

Metzger began his experiments by stressing threespine sticklebacks. Over a 2-week period, he chased and air-exposed these fish for 1 min every day, which increased their stress hormone levels. He also left some fish undisturbed during these 2 weeks for his unstressed group. Metzger then made several half-sibling crosses, where he used sperm from the same unstressed father fish to fertilize eggs from one stressed and one unstressed mother fish. These half-siblings differed only in their mother's genes and her experiences. Metzger spent a full year carefully raising the offspring under identical conditions before checking to see how their brains differed.

Using a technique called RNA-seq, Metzger identified and quantified essentially all of the genes being expressed in the brains of the offspring. He compared the gene expression data of male and female half-siblings against the backdrop of 'Mom' and found that maternal stress had a big effect on neuroplasticity. Even more striking, Metzger also found that sons and daughters of stressed mothers tended to have opposite changes in gene expression, resulting in profoundly different neural phenotypes. For example, the expression of genes involved in cellular metabolism and protein synthesis increased in daughters and decreased in sons of stressed mothers; whereas the expression of genes for neural development and synapse formation increased in sons and decreased in daughters. And don't forget that the main factor driving these changes – maternal stress – happened while these fish were merely un-spawned eggs, and a whole year had passed since then. This study by Metzger and Schulte offers compelling evidence that even in egg-laying animals, environmental factors that occur at the earliest stages of development have a dramatic and unique effect on sons and daughters. So neuroplasticity might just be one word made up of six syllables, but it adds up to some really exciting science!

10.1242/jeb.147207

Metzger, D. C. H. and Schulte, P. M. (2016). Maternal stress has divergent effects on gene expression patterns in the brains of male and female threespine stickleback. *Proc. R. Soc. B* **283**, 20161734.

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