

## OUTSIDE JEB

### Beauty is in the brain size of the beholder



There are many fish in the sea, but having a large school to choose from doesn't make mate choice decisions any easier. In fact, selecting a mate requires members of the choosing sex to process, integrate and recall a great deal of information related to mate quality across many potential mates. As this choice is directly related to fitness, it stands to reason that the cognitive ability of the choosing sex is under considerable selective pressure. Therefore, Alberto Corral-López, a PhD student in Niclas Kolm's laboratory at Stockholm University, Sweden, along with several colleagues, decided to test the hypothesis that smarter individuals choose better mates. They knew from previous work in the Kolm lab that female guppies with big brains have a higher cognitive ability than female guppies with small brains, so they staged a dating game to test whether or not the big-brained guppies made better mate choice decisions.

To begin the matchmaking experiments, Corral-López presented individual big- or small-brained female guppies with two males to choose from. One male was brightly coloured with a large tail, as these traits are known to represent a high-quality mate in guppies. The other male was dull-coloured and nondescript – a less optimal choice by far. Corral-López tracked how much time a female spent with each male and found that while small-brained females spent just as much time with the ugly male as the attractive male, the big-brained females

preferred to swim with the beautiful beau. While this result supports a connection between female brain size and mate choice decisions, Corral-López wanted to rule out that the preferences he observed weren't caused by inherent differences in the female visual system.

So, for his next experiment, Corral-López tested the ability of females to distinguish and perceive colour. He tracked how well each female oriented herself during an optomotor response test, which involved moving alternating bands of colour (red and green to match the males) along the wall of the fish tank while recording her movements. He also varied the intensity of each colour band to create high- and low-contrast colour stimuli, similar to the attractive and dull male fish. Corral-López found that all females, irrespective of brain size, were better at following high-contrast colour bands compared with low-contrast bands, meaning that brain size does not influence colour perception. Furthermore, small- and big-brained females had similar gene expression profiles in their retinas for the opsins that are essential proteins for vision and colour perception. Therefore, he concluded that the big- and small-brained female guppies were equally capable of distinguishing colourful males from dull males. This means that small- and big-brained females gathered the same visual information from the two males that were presented to them; however, the females with large brains were better at using this information to choose the superior mate. Or, put another way, big-brained guppies are smart enough to judge a fish by its colours.

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### The bee microbiota as a barrier against disease



Poop is all the rage these days. It isn't some new weird trend championed by hipster youth, but rather the enthusiasm of scientists who study our microbiomes, the bacteria that live on and within us. These bacteria, which are often best characterized by studying our poop (hence the rage), provide endless benefits to our health and well-being. They influence metabolism, development, immunity, behavior and much else. But when these beneficial microbes are eliminated by antibiotics, which are crucial life-savers against bacterial pathogens, things can go horribly awry. Most notably, treatment can cause dysbiosis, a microbial imbalance in our guts that increases our susceptibility to opportunistic bacteria like *Clostridium difficile*. But dysbiosis isn't just a human phenomenon. As elegantly shown in a new paper in PLoS Biology by Kasie Raymann and her colleagues from the University of Texas, what's true for humans is also true for bees.

Bees are in global decline and one of the reasons for their troubles is a disease called American foulbrood, caused by the bacterial pathogen *Paenibacillus larvae*. Bee keepers can treat foulbrood by feeding bees or spraying hives with the broad-spectrum antibiotic oxytetracycline, which is effective against *P. larvae*. However, Raymann and her team wondered whether these drugs could also have off-target effects on the normal microbiota of bees and, if so, would the resulting dysbiosis harm bees just like it does humans?

To test this, the team fed bees with oxytetracycline and compared their microbiomes with those of bees fed the same diet without the drug. As expected, antibiotic treatment had a dramatic effect on the core bacterial species of the bee microbiome. Bacterial abundance in oxytetracycline-treated bee guts declined nearly 5-fold as did overall bacterial diversity. However, it wasn't only core bacteria that declined; so too did bee health. Roughly two-thirds of the treated bees died; around twice the mortality of control bees. But why did the treated bees die?

The cause, it turns out, is highly reminiscent of the factors leading to opportunistic *C. difficile* infections in humans: dysbiosis. So, oxytetracycline caused the 'good' bacteria of the bee microbiome to decline and in so doing created a vacuum that allowed other species to thrive, including a well-known insect pathogen called *Serratia*. And when the team fed antibiotic-treated larvae with *Serratia*, these bees died too, providing unambiguous evidence that antibiotic-induced dysbiosis increased bee susceptibility to off-target opportunistic pathogens. More importantly, the results confirmed that the core microbiome is a barrier to disease under normal conditions.

Sometimes in medicine, the treatment is worse than the disease itself. This is unlikely to be the case here, as the benefits of foulbrood eradication almost certainly exceed the costs of *Serratia*-induced mortality. However, this study nicely demonstrates the unanticipated dangers of perturbing the microbiome. In addition, it perhaps suggests a solution to bee dysbiosis. One of the most promising treatments for *C. difficile* infections is to repopulate the human gut with a so-called fecal transplant. This provides an apparent barrier to *C. difficile* disease and works markedly better than antibiotics. Although I don't envy the bee proctologist administering microbiome enemas to larval bees, perhaps this type of fecal replacement ('bee-cal' transplants) is just what the hive ordered? Such an approach would avoid the dysbiosis that increases the risks of *Serratia* and other pathogens, while maintaining the treatment benefits against foulbrood. Novel solutions are needed to arrest global bee declines. Microbiome

manipulation seems a worthwhile place to look.

10.1242/jeb.147447

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## MantisBot successfully mimics prey tracking



The praying mantis is a notorious insect, known for its calm, motionless stance while waiting for prey to draw near, followed by a swift and powerful strike. Mantises track moving prey using rapid turning head movements to keep their target in the foveal region of the eyes, where the highest resolution images are produced. Though much is known about insect motion and control, exactly how mantises use visual information to produce these sharp head turns remains unclear. What makes this insect especially interesting is that numerous studies have found that once a turn is initiated, it cannot be stopped – meaning that the mantis must predict the future position of its prey. So, what information does the brain send to the nerve clusters controlling locomotion in order to produce these precise movements?

This is the question that an interdisciplinary team of researchers attempted to answer with their mantis-inspired robot, MantisBot. Based on previous studies of praying mantis head movements and insect locomotion in the literature, the team – led by Nicholas Szczecinski from Case Western Reserve University in Ohio, USA – hypothesised that commands to the nerve centres that control locomotion are relatively simple. To test this, they designed a simple nervous network with commands inspired from those biological results, and

incorporated it into MantisBot, a 13.3:1 scale robot model of a praying mantis. Based on information provided by the 'eyes' – five solar cells arranged around the front of the head – a bioinspired brain-like controller gave simple instructions to the unit controlling the robot's movements: either move or stand still, and which direction to orient the body.

To test how well the proposed network directed the robot, the team shone a 1600 lumen LED at it to simulate prey; the difference in voltage of the solar cells told the robot which direction the prey was in. If the prey moved outside a 20 deg cone from the centre of its vision, the controller told the robot to move its head, and if it moved even further (30 deg outside), the robot also used its body and legs too, just like real praying mantises do. But could it track prey accurately?

MantisBot's control network was a success, allowing the robot to track prey to within 30 deg of its centre of vision, even when moving its legs and body. Switching direction mid-step was no problem either, and the robot successfully used its legs to help rotate its body while standing still. The robot's high accuracy in tracking prey demonstrates that simple commands – which direction, and whether or not to move – are capable of producing tracking behaviour comparable to that of the praying mantis, and provides plausible evidence that the information sent to the animal's own locomotion control centre could be just as simple.

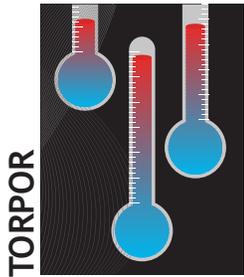
Bioinspired robotic models are not just great precursors to more advanced robots; studies like this demonstrate that they can also supply meaningful feedback, helping us to understand the underlying biology to answer questions that current biological experiments cannot address. Continuing such interdisciplinary work is therefore essential for both fields to progress, and could potentially provide answers to many biological conundrums.

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## Juvenile marsupials need the sun to warm up



Staying warm whatever the weather is energetically costly and many mammals resort to torpor and abandon their stable body temperature during challenging periods in order to save energy. While entering torpor is energetically cheap, coming out is expensive as the body has to warm up, although the costs can be mitigated by basking in the sun. Even though the cost of emerging from torpor may be even more extreme for juveniles – because they are smaller than adults and have a high surface area to volume ratio – they use it frequently, which raises the question: do juveniles attempt to reduce their rewarming costs by basking in the sun?

An Australian team led by Chris Wacker from the University of New England was interested in whether juvenile marsupials – which are the size of a jellybean at birth and spend their first months of life snuggled in their mother's warm pouch – might use the sun to aid rewarming from torpor. Dunnarts (*Sminthopsis crassicaudata*), small nocturnal marsupials that live in desert areas, are known to use torpor throughout the year and often bask in the early morning sun. The team looked at juvenile dunnarts between the ages of 40 and 160 days – the age at which they leave the mother's pouch and switch from depending on her warmth to maintaining their own body temperature. They measured the dunnarts' ability to maintain a stable body temperature, whether and when juveniles used torpor and whether they would use an artificial heat source to reduce the costs of heat production as they aroused from a bout of torpor.

Interestingly, the team found that the juveniles are already capable of entering torpor even before they can regulate their body temperature fully and they were able to achieve this by basking. When

the juveniles emerged from the pouch at around 60 days, they were able to maintain a more or less stable body temperature for a few hours, although they entered torpor during the later part of the night. However, they were unable to restore a warm body temperature after torpor unless they had the opportunity to crawl under the warmth of a heat lamp.

To find out whether the juveniles were just losing too much heat, causing them to cool down involuntarily, or had entered a genuine state of torpor, the team compared the juvenile dunnarts' heat loss rate with the heat loss of dead animals that had been warmed to the same initial body temperature. This comparison showed that the live dunnarts cooled faster than the dead animals, indicating that they controlled the decrease in body temperature and entered torpor. And when the team monitored the juveniles' use of torpor, they found that animals were still entering torpor during the night at around 90 days of age when they were already able to maintain a stable warm body temperature during the course of the entire night, although the older youngsters were able to rewarm and emerge from torpor with greater ease. Nonetheless, dunnarts of all ages still basked under a heat source whenever they had the chance.

Wacker and colleagues conclude that juvenile dunnarts are able to enter torpor even before they are capable of producing sufficient heat to maintain a stable temperature, and they need to bask in the sun to resume a warm body temperature after torpor. This observation also raises the possibility that basking may be a crucial step in the development of heterothermy – where an animal does not always maintain a single stable body temperature – in small animals that live in warm habitats. Importantly, the team has revealed that basking is not an option for juvenile marsupials wishing to capitalize on energy savings during torpor; it is essential.

10.1242/jeb.147454

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## Unearthing HIFs in a hardy marsh fish



When it comes to surviving hypoxia, or low oxygen, animals get by with a little help from their factors – hypoxia-inducible factors (HIFs), that is. HIFs are useful molecules that, when activated by hypoxia, help form switches to crank gene expression up or down as needed, thereby regulating cellular processes that help animals cope with the stress. Because of the low capacity of water for dissolved oxygen, fish are especially likely to encounter this stress, particularly in shallow, productive environments like estuaries and salt marshes; therefore, estuarine fish, particularly the hypoxia-hardy mummichog (*Fundulus heteroclitus*), are useful models for studying HIFs. In general, HIF molecules are made of two subunits: the  $\beta$ -subunit, which is always expressed and available, and one of multiple varieties of the  $\alpha$ -subunit. One variety, HIF2 $\alpha$ , has already been described in the mummichog, but Ian Townley and his colleagues at the University of New Orleans, USA, as well as researchers from Xavier University of New Orleans, USA, and Woods Hole Oceanographic Institution, USA, sought to discover what other forms of HIF $\alpha$  exist in this fish.

To hunt for HIF $\alpha$ s, Townley and the other researchers used a small piece of a HIF1 $\alpha$  gene from rainbow trout and a piece of what they suspected to be a HIF3 $\alpha$  gene from the mummichog to probe mummichog liver DNA for matching sequences. This enabled them to determine the full mummichog HIF $\alpha$  sequences and compare them with HIF $\alpha$  genes in other vertebrates. They also investigated in which of the fish's organs the different forms of HIF $\alpha$  were expressed. Finally, they used cultured cells *in vitro* to translate the HIF $\alpha$  coding sequences into proteins and looked at how well the proteins performed their jobs, such as binding to areas of DNA called hypoxia response elements (HREs) in

order to influence gene expression. In this case, the researchers analyzed expression of a specially engineered reporter gene containing mummichog HREs that produces light when activated; this made it easy to detect and quantify HIF $\alpha$ -induced activation by measuring light production.

The team discovered DNA sequences for HIF1 $\alpha$  and HIF3 $\alpha$  genes in the mummichog that were similar to those of other fish and encoded proteins that functioned as HIF $\alpha$ s should, confirming that mummichog HIF $\alpha$ s operate under the same basic framework as those in other species. They also found a shorter version of HIF2 $\alpha$ , HIF2 $\alpha$ b, whose sequence suggests it is unable to detect hypoxia or activate genes; alternatively, it may be involved in regulating other HIF $\alpha$ s,

similar to one variety of HIF3 $\alpha$  found in mammals. The group further noticed that the three full-length forms of HIF $\alpha$  were distributed among tissues in patterns similar to those in other fish. Surprisingly, however, HIF $\alpha$  expression was very low in the brain and heart compared with reported values for other fish species, with unclear implications for HIF $\alpha$ -mediated processes. Furthermore, there were several areas where the sequence was variable in the newly identified HIF $\alpha$  genes as well as the previously identified HIF2 $\alpha$ a gene, which could allow for variation in hypoxia responses among individuals or populations.

Many questions remain about exactly how the expression patterns and sequence variations might translate

into protein abundance and function, as well as which HIF $\alpha$  characteristics might influence the mummichog's hardiness. Nevertheless, this characterization of mummichog HIF $\alpha$  genes lays important groundwork for further research into why these water warriors are so good at surviving in low-oxygen environments.

10.1242/jeb.147488

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