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

SEEING WHAT FISH THINK

Have you ever wondered about what goes through a fish's head? Recent advances in molecular biology and genetics are now making it possible to look at the activity of single neurons in free-swimming fish. Akira Muto from the National Institute of Genetics in Shizuoka, Japan, and his Japanese colleagues have taken advantage of the transparency of zebrafish larvae and recorded neuronal activity in the brain while the fish were swimming and searching for their prey. Their research was recently published in *Current Biology* and is an important step in understanding how animals perceive their environment, one of the fundamental questions in neurobiology.

First, Muto and colleagues took a strain of zebrafish that was able to emit fluorescent light inside cells when these cells had a rapid increase in calcium concentration, such as when a neuron in the brain is active. They then introduced a mutation to make this strain of fish emit an even brighter light that could be easily detected under a fluorescence microscope. Once they succeeded, they screened and selected zebrafish that emitted light only in the optic tectum, the part of the brain thought to be involved in perception.

Muto and colleagues then wanted to determine how these fish perceive a moving object. The researchers immobilized the zebrafish and placed a screen to one side of them. A fluorescence microscope was used to then visualize changes in the brightness of the neurons that occurred as a dot of light was turned on and off on the screen. The team saw a cluster of neurons 'light up' in the neuropil area, where neurons from the retina connect with neurons from the optic tectum. When the dot on the screen was moved up and down, neurons in the optic tectum became active in the same direction, showing that the activation of these neurons was related to the perception of the visual stimulus.

Next, the researchers wanted to see how the fish would respond to a natural object, whilst still immobilized. This time they used a free-moving paramecium, a natural prey of zebrafish larvae. The neurons in the tectal area moved as predicted, tracking the movement of the paramecium, but these neurons were not active when the paramecium was motionless. This showed that a moving object can activate tectal neurons but a stationary object cannot.

Finally, the researchers wanted to study neuronal activity during prey capture in free-swimming larvae. In this experiment, the zebrafish larvae and its prey, the paramecium, were swimming freely under the microscope. Muto and collegues found that the anterior tectum of the zebrafish was more active just before the paramecium was captured than when the zebrafish was locating and swimming towards the prey. These findings indicate that the anterior tectum is responsible for linking visual and motor pathways during the prey capture behaviours.

This is the first study to visualize neuronal activity in free-moving animals. This method is a powerful tool in the future understanding of how animals perceive their world and which areas of the brain are involved in these behavioural responses: one step closer to reading a fish's mind!

10.1242/jeb.077867

Muto, A., Ohkura, M., Abe, G., Nakai, J. and Kawakami, K. (2013). Real-time visualization of neuronal activity during perception. *Curr. Biol.* **23**, 307-311.

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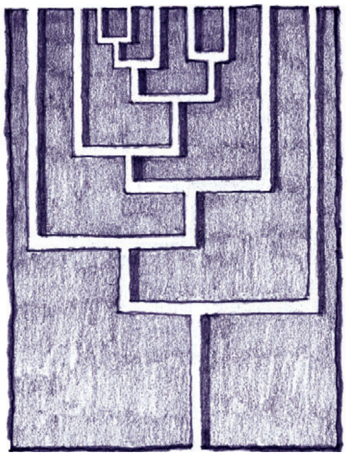
BRAIN EVOLUTION



BEING SMART COMES AT A PRICE

We humans like having big brains. After all, our noggin has helped us become one of the most successful species of animal on this planet. So why haven't all animals developed big brains?

Scientists have pondered this question for decades. One of the most highly favoured theories suggests that the size of brains is limited because of its inherent metabolic cost: for instance, the human brain, accounting for only 2% of our body mass, takes up 20% of our total energy expenditure. This theory, the 'expensive-tissue hypothesis', suggests that the size of the brain is part of a trade-off between the advantages of greater cognitive ability and the energy demands that having a bigger brain entails.

A key prediction from this model is that developing a bigger brain should come at the expense of other tissues, most notably the gut, the other most energetically expensive tissue in our bodies. While a number of studies have shown that for many animals brain size and gut size are negatively correlated, no one has done experiments to test whether evolving a bigger brain affects the size of the gut. Now, a group of scientists at Uppsala University have taken a direct approach to address this question, recently published in *Current Biology*. Using the guppy, they determined the effects of artificial selection on brain size in order to put the theory to the test.

First, they bred multiple generations of guppies, and for each generation selected the individuals with the largest and those with the smallest brains, creating an 'up' selected and a 'down' selected strain of fish. They found that after only two generations, brain size within the 'up' selected group had already increased by 9%, showing that brain size is a trait that can rapidly change during evolution.

Next, they determined whether the fish with larger brains have greater cognitive abilities. As guppies have a crude sense of numbers, these fish can be put to the test using simple numerical learning tasks. The authors found that females with larger brains are indeed better at this task than their 'down' selected counterparts, showing that the increase in brain size gave them a cognitive advantage.

Furthermore, when the team weighed the guts of the fish from the two different groups, they found that the larger brained females had 8% smaller guts, while the larger brained males' guts were 20% smaller. This demonstrates that acquiring a bigger brain leads to a smaller gut, and shows that the increase in brain size is the result of a trade-off between cognition and metabolic cost.

Interestingly, when the authors looked at the offspring of larger brained animals, they saw that, although the offspring were of the same size, there were fewer of them per generation. This suggests that increases in brain size come at the expense of the reproductive process, as well as gut size.

This study has provided the first direct evidence of the expensive-tissue hypothesis by showing that evolving a bigger brain comes at the expense of gut size and reproductive performance. This gives us clues as to how our own brains might have evolved: the availability of more highly nutritious foods is thought to have allowed us to grow a bigger brain, with the reduction in gut size that that would cause.

10.1242/jeb.077875

Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., Immler, S., Maklakov, A. A. and Kolm, N. (2013). Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Curr. Biol.* **23,** 168-171.

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PARASITISM

ANT-EATING SPIDER IS MEAL FOR PREDATORY WASP

A common predatory method among spiders is aggressive mimicry. The ant-eating *Zodarion* spider species performs this trick too: anatomically disguised as an ant, they are able to stealthily hunt and capture their preferred prey. However, this crafty technique by no means puts the ant-eating spider out of harm's way. Several members of the insect order Hymenoptera, which includes wasps, have been found to feed on spider hosts. The wasp larvae subsist by consuming the spider or the spider's eggs. In a study published in *ZooKeys*, lead author Stanislav Korenko from the Czech University of Life Sciences, Czech Republic, describes two wasp species, *Calymmochilus dispar* and *Gelis apterus*, and newly identifies them as parasitoids of the ant-eating spider *Zodarion styliferum*.

Korenko and colleagues set out on 31 field expeditions throughout Central and Southern Portugal during springtime with the aim of cataloging parasitoids of the ant-eating spider. The scientists investigated several diverse spider habitats including sand beaches, river banks, olive groves, woodland habitats and arid meadows, and collected larvae and pupae of hymenopteran parasitoids found within spider dens – small igloo-shaped stone hideaways constructed by spiders to protect them from enemies and the environment. Unfortunately for the spiders, their igloos do not prevail to be enemy proof. Scientists found that predatory wasps were able to attack the host inside the shelter by penetrating the stone walls with their distinctively long ovipositors (a specialized piercing organ used by the wasp to lay its eggs within a host's body) – depositing wasp eggs into the abdomen of juvenile spiders, and in turn providing the wasp's offspring with nutrients for their development.

The research team observed spiders being attacked during the daylight when they are typically inactive and hidden within their igloo retreat. In order to identify the diversity of spider and wasp species, the scientists collected whole igloos if there was a wasp pupa or parasitized spiders inside and transferred them to containers for host and parasite species verification. While researchers identified three different species of *Zodarion* spiders overall during their fieldwork, they found *Z. styliferum* to be most abundant. To their surprise, the team found wasp larvae of *C. dispar* and *G. apterus* specifically within the igloos of *Z. styliferum* – these wasps had never been found before in Portugal nor had they been previously known to attack *Z. styliferum*.

In order to calculate the parasitism rate at each recovery site, the number of parasitized spiders was compared with the number of all spiders observed at that location. The rate of parasitism was recorded for only localities where *C. dispar* and *G. apterus* parasitized spiders: one location for *C. dispar* and six sites for *G. apterus*. For example, in one southernmost locality, described as an arid slope next to a roadway, the team found two out of 10 spiders were parasitized by *C. dispar* wasps. From six other diverse field sites peppered along the south-eastern boarder with Spain, the scientists collected between 12 and 44 spiders at each site and found between one and two of these spiders to be infected by *G. apterus*.

This study represents the first documentation of these two particular wasp species being associated with *Z. styliferum*, but also gives scientists interested in these host–parasite relationships a good hunch of where to look next. Members belonging to the largest genus of ant-eating spiders have been found to be widely distributed across Europe, Asia and North America – and where hosts are found, predator parasites are sure to strike.

10.1242/jeb.077883

Korenko, S., Schmidt, S., Schwarz, M., Gibson, G. A. P. and Pekar, S. (2013). Hymenopteran parasitoids of the ant-eating spider *Zodarion styliferum* (Simon) (Araneae, Zodariidae). *ZooKeys* **262**, 1-15.

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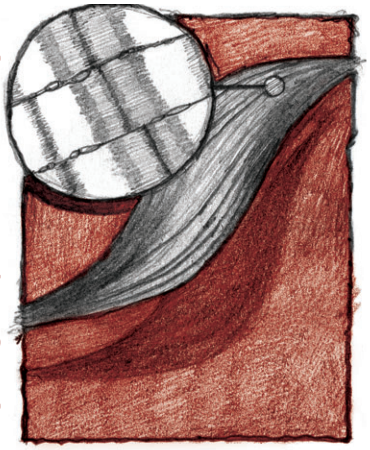
FORCE GENERATION



OPEN WIDE (AND CLOSE MORE SOFTLY)

The force with which a muscle can contract depends on its initial length. The so-called length–tension curve of muscle characterizes this dependence and demonstrates that muscles intrinsically have some optimal length for generating force, and longer or shorter lengths lead to decreased force production. Despite our understanding of this general relationship, we remain blissfully unaware of actual muscle operating lengths during most behaviors, and thus don't know where on their length–tension curves muscles are working. Recent work on a fish pharyngeal jaw adductor muscle by Nick Gidmark, Beth Brainerd and colleagues at Brown University highlights how a better understanding of operating lengths and force–length characteristics can reveal constraints on behavior and performance.

Many fish have two sets of jaws, one oral like the rest of us, and one farther back in the throat known as the pharyngeal jaws. Black carp (*Mylopharyngodon piceus*) use their pharyngeal jaws to crush snails of varying size for food. Prey size of course determines how wide apart the jaws must be at the start of a bite, which in turn stretches and influences the length of the jaw-closing muscles before they begin to contract to generate bite forces. Gidmark and colleagues took advantage of this and used prey size to manipulate the operating range of a jaw adductor muscle and ask whether force–length properties alter bite performance. The researchers first created artificial prey to feed three fish and assess how bite force was affected by prey size. They then used X-ray video to record multiple feeding trials from each fish feeding on prey of varying size. These videos were later used to measure the operating lengths of the jaw-closing levator arcus branchialis muscle during each feeding event. Finally, fish were killed and a preparation of a single pharyngeal jaw with its closing muscle attached was developed to allow stimulation and force measurement as the muscle's initial length was systematically varied so as to develop an *in situ* force–length curve for the muscle.

X-ray data combined with the *in situ* force–length results revealed that prey size indeed altered the operating length of the jaw-closing muscle and thus its potential for developing force. The larger the prey item, the further the jaw adductor was stretched and moved onto regions of the force–length curve where force-generating capacity was reduced. Indeed, all three fish had trouble crushing large prey, even when they were of comparable strength to smaller prey that could be crushed readily. In short, it pays to be big if you're a snail living with black carp. Such results not only help us better appreciate how prey size can impact bite performance in this species, but also remind us more generally of how intrinsic muscle properties can constrain function in behaviors where muscles operate over different length ranges.

10.1242/jeb.077891

Gidmark, N., Konow, N., LoPresti, E. and Brainerd, E. L. (2013). Bite force is limited by the force-length relationship of skeletal muscle in black carp, *Mylopharyngodon piceus*. *Biol. Lett.* **9**, 20121181.

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