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

MOTOR SYSTEMS

LEECH BRAIN ADDS FLEXIBILITY TO COMMAND NEURON OUTPUT

Spinal cords and equivalent structures in invertebrates (nerve cords) often generate rhythmic motor patterns without inputs from sensory systems or the brain. Preparations that generate such 'fictive rhythms' have been used to uncover properties of motor circuits in many animals. The (largely untested) assumption has always been that circuits in these preparations are fundamentally the same with or without the brain. However, Olivia Mullins and Otto Friesen at the University of Virginia recently decided to see whether this assumption is true in the leech *Hirudo verbena*.

In previous work, Mullins and Friesen identified a neuron (E21) that, in the absence of the brain, appears to be a 'command-like' neuron. When stimulated, it initiates and enhances fictive swimming exclusively. The team wanted to see how E21's ability to drive swimming would be modulated when the brain was intact. To do this, they dissected out the brain and nerve cord and put them in a dish. Then they evoked fictive swim episodes by shocking a motor nerve. During swim bouts, they stimulated E21 and measured any resulting effects on fictive motor patterns. Surprisingly, the duo found that with the brain attached, E21's actions within the swim circuit were wildly variable. In some preparations, stimulating the cell enhanced fictive swimming, but in others, stimulation had no effect or exactly the opposite effect. The presence of the brain clearly added flexibility to what was thought at first to be a very rigid command circuit.

Leeches swim in water but crawl on land and the motor programs underlying each behavior are very different. The team wanted to see whether E21 activation can drive locomotor rhythms tailored for these two behavioral states. To this end, they kept the brain and anterior regions of the leech intact, while simultaneously recording from E21 and motor nerves involved in swimming. The front part of the leech was placed in sensory environments ranging from deep water (to enhance swimming) to solid substrate (to stimulate crawling). In the watery environment, E21 went back to enhancing swim episodes exclusively. But when the animal was faced with solid ground, E21 activation initiated and enhanced crawling. This suggests that instead of rigidly commanding downstream circuits to do a single behavior, E21 cells are commanding motor circuits to do an appropriate behavior based on sensory cues. This effect was completely masked in isolated preparations lacking brains.

How does the presence of the brain add flexibility to the circuits called into play by E21? The team measured how descending inputs from the brain affect the strength of a synapse between E21 and a downstream cell type known to 'gate' swimming. Gating cell activity is required to maintain swim episodes, so when the E21-to-gating cell synapse is weak, triggered swim episodes are shortened. In the absence of the brain, E21 strongly excites gating cells; however, when the brain is present, the connecting synapses are weaker and show increased variability. The mechanism remains mysterious, but it is possible that the modulation of this synapse by descending inputs accounts for much of the variability seen when E21 is stimulated in brain-attached preparations.

The work of Mullins and Friesen is important because it shows that a long-held assumption in motor systems research is not always true. Motor circuits can indeed be fundamentally different in the absence of the brain, and so we need to be careful how we interpret data from 'brain-less' preparations. Paradoxically, the team's work also highlights how useful it is to begin with such isolated preparations and then progressively add complexity back into them. At the end of the day, this simple approach may be the best way to really grasp how flexibility is generated in motor systems.

10.1242/jeb.064253

Mullins, O. J. and Friesen, W. O. (2012) The brain matters: effects of descending signals on motor control. *J. Neurophysiol.* doi: 10.1152/jn.00107.2012.

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COMMUNICATION

WHEN THE TIMES GET TOUGH, THE LIZARDS START LYING

For the most part, it seems that animals communicate honestly with one another. Individuals use various signals such as elaborate colours or ornaments to convey information to others about their fighting ability or potential value as a mate. Signals are useful, and communication can save time and energy. For example, mismatched rivals can avoid a risky dual if they can communicate their fighting ability, and the weaker opponent cede to the stronger.

The lingering puzzle of animal communication is why animals are honest with one another. On the surface, it would seem beneficial to embellish communication signals. What animal would not want to appear stronger and more attractive? The classic explanation is the 'handicap hypothesis', or the hypothesis that the cost of communication signals maintains the honesty of these signals. One common example is the tail of the peacock. This lavish adornment is so cumbersome that only strong males can haul around an impressive tail and still escape from predators.

Simon Lailvaux, Rebecca Gilbert and Jessica Edwards from the University of New Orleans put the handicap hypothesis to the test in a unique way using green anole lizards (*Anolis carolinensis*). The green anole is an excellent subject for such a study, as male green anoles signal to one another with a brightly coloured dewlap – a flap of skin beneath the jaw that they can extend and retract – and the size of this dewlap is an honest signal of their bite force, an essential trait for fighting. Importantly for the study, the dewlap is developmentally unrelated to the muscles surrounding the jaw that generate bite force, so it is possible for the two traits to vary independently of one another. Lailvaux and his colleagues investigated whether resource limitation would change the honest relationship between dewlap size and bite force. They predicted that if the handicap hypothesis is correct, and the cost of maintaining the bright dewlap maintains the honesty of the signal, then food restriction should limit both dewlap size and bite force. The question is, when the times are tough, do the lizards stay honest?

To answer this question, the authors captured juvenile male green anoles and raised them to sexual maturity in the laboratory, under either food-restricted or plentiful diets. At the end of the study, both dewlap size and bite force were measured. The authors discovered that while lizards with plenty of food developed the typically honest relationship between dewlap size and bite force, the food-restricted males developed the bright dewlaps without developing the corresponding bite force. Therefore, food-deprived lizards were not as honest as their resource-rich counterparts. Moreover, it appears that for green anoles, bite force itself is more costly than the bright dewlap signal.

As the cost of a bright dewlap doesn't keep the lizards honest, what factors maintain dewlap size? The authors suggest several explanations, including the possibility that social feedback might play an important role. In this study, lizards were raised in individual cages, and did not interact with one another. However, in the wild, exaggerating about bite force with a bright dewlap might land a lizard in a fight where he is overmatched and could be injured. While more research is necessary to fully understand the factors that maintain honest communication signals, it seems that, for lizards, honesty is still the best policy.

10.1242/jeb.064246

Lailvaux, S. P., Gilbert, R. L. and Edwards, J. R. (2012). A performance-based cost to honest signalling in male green anole lizards (*Anolis carolinensis*). *Proc. R. Soc. B* doi: 10.1098/rspb.2011.2577.

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CRYOPROTECTION

FRUIT FLIES ON ICE

The ability to survive freezing comes naturally to a select group of insects. These cold-adapted insect species live in areas where they might experience sub-zero temperatures for at least some of the year. But *Drosophila melanogaster*, the favourite fly of researchers from several life sciences disciplines, is better known for its preference for the warmth of human kitchens and is injured by cold – even at temperatures above freezing. Scientists would love to know how to successfully put these flies into suspended animation using cryopreservation to maintain their valuable stocks of laboratory-modified *D. melanogaster* lines. In recent work published in *Proceedings of the National Academy of Sciences*, Vladimir Koštál and colleagues from the Czech Republic show that with a few simple tricks picked up from a freeze-tolerant cousin, it is possible to convert the chill-susceptible *D. melanogaster* into a fly that can survive freezing.

Earlier work by Koštál and his colleagues showed that *Chymomyza costata*, a drosophilid fly closely related to *D. melanogaster*, has two requirements to survive freezing: (1) it must be in developmental arrest (called diapause) during an overwintering stage, and (2) it must accumulate large quantities of the free amino acid proline. The authors thought a similar protocol might work for *D. melanogaster*. First, the team reared larvae at either room temperature or a relatively low temperature for *D. melanogaster* (15°C) until they reached the final larval stage. Then, they subjected the larvae to temperatures that fluctuated between 6°C and 11°C for 3 days to induce a type of diapause. In addition, some of the insects were fed diets rich in known cryoprotectants: glycerol, proline or trehalose. Finally, the team slowly cooled the flies to –5°C and held them there for over an hour before allowing the insects to resume development.

The researchers found that feeding the larvae diets rich in cryoprotectants or subjecting them to fluctuating temperatures both increased the larvae's survival of freezing, but only for a short time after the stress. However, the combination of the proline-rich diet in particular with the fluctuating temperatures had a synergistic effect, producing larvae with almost a 10% chance of surviving to reproduce successfully after being frozen at –5°C for over an hour – which is long enough to convert half of their body water to ice.

To investigate how these treatments protected the diapausing larvae from freezing to death, the authors measured the concentration of several of the larvae's metabolites after consuming their cryoprotectant-supplemented diet, using mass spectrometry. They found that the larvae fed on the supplemented diets all accumulated extra cryoprotectant, although the proline-supplemented diet had the largest effect on cryoprotectant concentration. The authors thought that perhaps the larvae do not control their proline levels as tightly as they do other metabolites, which might have contributed to the success of proline in producing freeze tolerance.

While Koštál and colleagues plan further studies into the mechanisms of proline's effects, this first report of inducing freeze tolerance in a tropical insect like *D. melanogaster* contributes to the elucidation of the mechanisms of natural freeze tolerance, and may lead to an end of the labour-intensive work of maintaining laboratory fly stocks.

10.1242/jeb.064238

Koštál, V., Šimek, P., Zahradníčková, H., Cimlová, J. and Štětina, T. (2012). Conversion of the chill susceptible fruit fly larva (*Drosophila melanogaster*) to a freeze tolerant organism. *Proc. Natl. Acad. Sci. USA* doi: 10.1073/pnas.1119986109.

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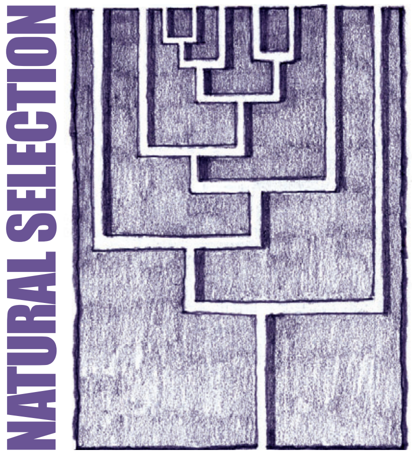

PEPPERED MOTHS IN BLACK AND WHITE

The peppered moth represents one of the most iconic examples of natural selection in action. The popular account of its evolution is both simple and intuitive. Nineteenth century industrialization in Britain dramatically altered the landscape. Pale lichen covering tree branches was killed and trees blackened with soot. On this background, white peppered moths that sheltered on the trees became conspicuous targets to visual predators and were eaten, causing their decline. Simultaneously, because they escaped detection, mutant black moths increased in frequency. The predation explanation was plausible and well tested. It was a classic textbook case.

In 1998, however, the story took an unexpected turn. Cambridge geneticist and lepidopterist Michael Majerus published a book that raised legitimate questions about Bernard Kettlewell's predation experiments, the foundation of the moth story. Were Kettlewell's experiments natural? Was it appropriate to study the responses of birds to dead moths arranged on trees? Did moths even rest on tree trunks where Kettlewell had put them? This questioning triggered a firestorm of controversy that raged.

A book review in *Nature* implied that the moth story was as disappointingly uncertain as Santa Claus, while another book accused Kettlewell of fraud. Despite the defence from scientists that it was not the predation hypothesis itself that was in doubt, creationists rejoiced. The curiously named Institute of Creation Research railed that 'the supposed best proof of evolution in action is so flimsy that it cannot stand the test of truth'. However, this controversy has finally been put to rest in a *Biology Letters* paper based on Majerus's experiments by Laurence Cook from the University of Manchester and an international team of collaborators.

Working in his Cambridge backyard, Majerus overcame the flaws in Kettlewell's design and validated the predation hypothesis. Black and white moths indeed rest on tree trunks and branches. More importantly, predatory birds do feed preferentially on the less camouflaged morph. In today's cleaned-up post-industrial landscape, the black morph is more conspicuous and its survival level is about 10% lower than that of the white morph. However valid the doubts were about Kettlewell's experiments, Majerus's results should now silence the critics.

Science, unlike creationism, is self-correcting. Cherished ideas can be tested and amended or overturned. The strategy of creationists, in contrast, is to target single examples for scorn while offering neither ideas nor credible tests to support their challenges. Majerus was not the only person in Britain with moths in his backyard, but he was the only one carrying out the hard work to test predictions of the peppered moth story. Unfortunately, he died before he could prepare his work for publication. Now freely available, his data are both interesting and important. They also serve as a clear demonstration of how science works by building an ever more refined understanding of the natural world. Creationists, with their unscientific agenda, working at so-called 'research' institutes, can offer nothing of substance in response.

10.1242/jeb.064220

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