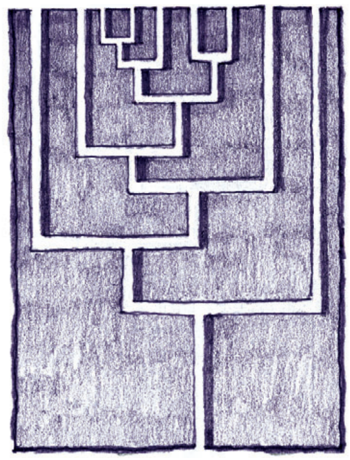
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

SNAIL SPECIATION



LEFT-HANDED SNAILS OUT-TWIST RIGHT-HANDED SNAKES

When genetic differences between two populations of a single species get to the point where the populations cannot interbreed, new species are born. This process (speciation) is a cornerstone of evolutionary theory, but any genetic change that makes it hard to interbreed also makes it hard for an animal to pass on its genes. How can genes for reproductive incompatibility spread from one generation to another? Masaki Hoso at Tokoku University and colleagues recently tested whether a single gene for speciation in land snails could be stabilized by snake predation. They published their work in a recent edition of *Nature Communications*.

The shells of most snails in Southeast Asia coil in a clockwise (right-handed) direction, but a few species have shells twisting the other way. Interestingly, a single gene controls which way shells twist. Mating between the two snail types is almost impossible because the opposite shaped shells get in the way. One group of snakes (Family: Pareatidae) in the region have co-evolved with these snails and have a taste for *escargot*. As right-handed shells are the more common of the two, pareatid snakes have evolved asymmetric tooth arrangements and hunting strategies that are optimized for extracting prey from right-handed shells. Hoso and colleagues postulated that right–left reversal of snail shell chirality could be responsible for instant speciation (it would block interbreeding immediately). Furthermore, they hypothesized that pareatid snake adaptations serve to maintain the genetic change that gives rise to left-handed shells.

First, the team tested how well pareatid snails can eat snails that twist to the left. The team presented rightward and leftward coiled snails to a panel of test snakes. The snakes had no problem eating their usual right-handed fare, but had real difficulty coping with left-handed shells. Size also mattered – the bigger the left-handed shell, the harder it was for snakes to extract their meal. These results suggest that a right–left reversal in shell chirality does indeed confer a survival advantage to snails.

Next, Hoso and co-workers examined the geographical distribution of snails with right- and left-handed shells and compared this with the geographical distribution of pareatid snakes. They found that the diversity of leftward coiled snails was indeed higher within the range of the snail-eating snakes. As a follow-up, the team examined the molecular phylogeny of one snail genus co-existing with pareatid snakes (*Satsuma*). They found that in the presence of snakes, leftward coiling snail species had arisen independently multiple times within the genus. Importantly, the frequency at which this has happened is unusually high, and difficult to explain without invoking snake predation as a stabilizing force.

Overall, the results of Hoso and colleagues support the hypothesis that right-handed snake predation is driving snail speciation by conferring a survival advantage to left-handed snails. This work is important because it shows that changing just a single gene can indeed lead to both adaptation and immediate reproductive isolation. This suggests that speciation could happen much quicker and entail much simpler genetic changes than previously thought.

10.1242/jeb.049734

Hoso, M., Kameda, Y., Wu, S., Asami, T., Kato, M. and Hori, M. (2010). A speciation gene for left–right reversal in snails results in anti-predator adaptation *Nat. Commun.* **1**, 133. doi: 10.1038/ncomms1133.

Stefan Pulver
University of Cambridge
sp553@cam.ac.uk

NANOPHYSIOLOGY

NANOSCALE PHYSIOLOGY ON MILLIMETER SCALE ANIMALS

In the past few decades there has been an explosion of measurement techniques that have made finer and more intricate measurements on smaller invertebrates more commonplace. Developments in electronic and sensor instrumentation have allowed insect physiologists to piece together complex respiratory patterns in insects as small as *Drosophila*, to detect and quantify neurocontrol and muscular control in single neurons and muscle fibres, and to measure rapid changes in body temperature in individual micro-arthropods (mites and springtails – some the size of the period ending this sentence). Now Maxim Dokukin and colleagues, from Clarkson University, have added a new technique to the insect physiologist's tool kit, atomic force microscopy (AFM), allowing them to make finer, more intricate and higher frequency measurements of processes that were previously undetected (or even undetectable) in insects the size of ladybird beetles and smaller.

AFM employs a sharp, cantilevered probe that detects small variations in the sample surface, which necessitates very secure mounting to restrict animal motion. Most commonly the small cantilever deflections are detected optically with a laser reflected onto a photodiode. The reflections are then translated into force measurements with picometer precision. Recording the reflections with a high frequency (50,000 Hz) system, Dokunin's team measured the surface vibrations of adult ladybird beetles, *Hippodamia convergens*.

Once a sample is securely mounted, surface vibrations are recorded at high frequencies and very small amplitudes. To distinguish animal vibrations from background noise, the team made additional measurements on dead specimens and of room sounds. They then quantified patterns in these vibration recordings and interpreted them mathematically using Fourier spectral peak analyses – where peak height indicates amplitude and peak position frequency. Dokukin's team then used three approaches to identify physiological processes from spectral peaks: they placed the probe close to a target organ, induced a drinking stimulus, and measured the insects' responses to increasing air CO_2 levels.

With the probe tip close to the heart the AFM physical approach showed previously documented heartbeat patterns at 0.6 Hz, and also a new high frequency peak at 293 Hz related to heart activity. Giving the beetles sugar water to drink the team identified a reduction in certain low frequency spectral peaks (15–17 Hz), shifts in higher frequency peaks (from 280 to 273 Hz), and new peaks at 18–20 and 350 Hz. The peak reductions and/or shifts may imply movement of fluids affecting tissue elasticity, while the new peaks may indicate muscle activities. Finally, when the team increased air CO_2 levels they found that low frequency (<5 Hz) activities were suppressed and the 280 Hz peak decreased by 40% and was shifted to 273 Hz. These responses were similar to those recorded when the insects were drinking and may be a general response to an external stimulus. But there were also new peaks observed, strong at 133 Hz and weaker at 170, 350 and 480 Hz, which are likely associated with spiracular closer muscle activities.

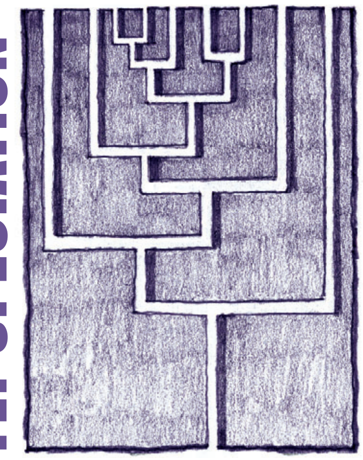
These measurements are based only on minute picometer scale vibrations off the elytral surface and will require several extensive studies to understand the full physiological significance of the observations. Nevertheless, the authors' novel AFM approach has shown that selective positioning, application of external stimuli and mathematical interpretation of spectral patterns have identified several previously undetected physiological processes and this, as the authors said, 'could lead to the emergence of "insect nanophysiology"'.

10.1242/jeb.049700

Dokukin, M. E., Guz, N. V. and Sokolov, I. (2011). Towards nano-physiology of insects with atomic force microscopy. *J. Insect Physiol.* **57**, 260-264.

C. Jaco Klok
Arizona State University
cjklok@asu.edu

FLY SPECIATION



BACTERIAL DRIVERS OF NEW SPECIES

We are often cautioned that 'we are what we eat'; eat poorly and pay with your health. In an interesting twist, new research in *Proceedings of the National Academy of Sciences* shows that diet can influence far more than your waistline. It can also determine mate choice, with potentially important consequences for the evolution of new species.

Scientists led by Gil Sharon in Tel Aviv established two populations of fruit flies that differed only in the diet they were provided. To their surprise, when flies from the two diets were mixed they preferred to mate with like-diet flies, while shunning those from the other diet. The most striking aspect of this shift in mate preference was the fact that it appeared within a single generation. This implied that the altered behaviour was not caused by genetic changes in the flies, but rather by something that evolved on a more rapid time scale.

Because of the known role of bacterial symbionts in manipulating insect reproduction, the team asked whether these were responsible for the shift in fly mating behaviour. Consistent with this possibility, when flies were fed antibiotics that rid them of their bacterial flora, they became less choosy. Reinfecting antibiotic-treated flies with the bacteria isolated from their food caused them to become more discriminating. Most surprisingly, they found a single bacterial species, *Lactotoccus plantarum*, was sufficient to cause flies to mate selectively. But how do bacteria within the gut modify a behaviour as apparently complex as fly mate choice?

Flies make mating decisions based upon sex pheromones. They smell and taste one another before deciding whether to initiate courtship and accept or reject propositions. As the final piece to this puzzle, Sharon and his collaborators found that flies fed different diets produce different pheromone cocktails and that antibiotic treatment reduces these differences. This provided the direct link between diet and mate choice. In short, diet, through its effect on gut bacteria, can cause flies to either accept or reject one another.

New species arise when reproductive barriers form between groups of individuals. This research shows that these barriers can form within a single generation and be caused by factors outside of an animal's genome. Apparently, because of the influence of bacteria on fly pheromones, the route to a fly's heart is indeed through its stomach.

10.1242/jeb.049692

Sharon, G., Segal, D., Ringo, J. M., Hefetz, A., Zilber-Rosenberg, I. and Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **10**, 20051-20056.

Daniel Rozen
University of Manchester
daniel.rozen@manchester.ac.uk

COMMUNICATION

MEMORY PROBLEMS? BLAME YOUR GUT AND STRESS LEVEL

Our guts are industrious factories, supplying our bodies with nutrients and energy. Much of the work is completed by our own cells; however, a large portion of the labour is outsourced to the millions of bacteria that colonise our intestines. Regardless of who does the job, communication between the gut's bacterial lodgers and the brain must be excellent, yet the links between these two systems are unclear. Mélanie Gareau and colleagues from the Hospital for Sick Children in Canada have looked at the effect of stress on memory formation in mice with and without normal gut flora to find new links between the two.

First, the team altered the animals' gut flora by infecting them with a pathogen and looked for changes in the animals' intestines. They found changes in the size of colonic cells – an indicator of bowel inflammation – changes in gene expression of several factors involved with the immune response of the intestine, and they identified the effects of these changes on gut bacterial inhabitants and their population size.

Then the team used a stress test, where the mice were suspended over a shallow bowl of water, followed by a series of memory tests on mice with altered intestinal bacterial communities and 'germ-free' mice that were raised without exposure to bacteria. Memory formation was shown by the recognition of objects the mice had previously been introduced to, as well as recognition of paths previously taken through a maze. Interestingly, the unstressed mice with altered gut flora showed normal memory formation but the mice with altered gut flora that were stressed had trouble forming new memories. What is more, the germ-free mice had memory problems even without being stressed. This suggests that the intestine and its many inhabitants influence the ability to form memories, implying that there is a novel line of communication between the two organs.

Next, the team looked at new memory formation in the brain using fluorescent labels that bind to cell markers indicative of the formation of new memories. They found a decreased expression of cell markers in the brain that are associated with novel memory formation in stressed mice with altered gut flora 30 days following infection. This suggests that there are long-lasting consequences to changes in the intestine, which are reflected by changes in the brain.

Finally, the team investigated whether daily treatment with probiotics – living micro-organisms such as *Lactobacillus helveticus*, which is commonly used to make some cheeses – would protect against these memory abnormalities. Not only did probiotics appear to reverse the effects of stress on memory in infected mice, they also blunted the increase in cell swelling as well as the increase in the immune response. The decrease in cell markers associated with memory formation seen in mice carrying the gut flora-damaging infection was erased as well. Probiotics seem to be able to fill in for native bacteria found in the gut.

So a healthy gut helps prevent memory problems in stressed mice. Probiotics appear to prevent these memory abnormalities when the gut isn't so healthy. When all gut bacteria are lost, memory formation is compromised even without stress. This unexpected and exciting connection between the brain and the belly illustrates just how interconnected biological systems are. Employing a multi-disciplinary attack has revealed a hitherto unrecognised link between two apparently unrelated systems.

10.1242/jeb.049726

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., MacQueen, G. and Sherman, P. M. (2010). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* **60**, 307-317.

Carol Bucking
University of Ottawa
carolbucking@gmail.com