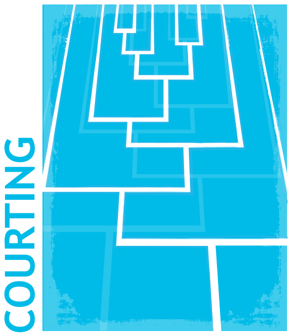
OUTSIDE JEB

Butterflies feeling 'the butterflies'



Biologists have long been captivated by courtship rituals in the animal kingdom, in which (usually) males work tirelessly to advertise their mating desirability to the opposite sex. Some species woo their mates by building elaborate nests for seduction, while others choreograph and execute impressive dance routines. Squinting bush brown butterflies (*Bicyclus anynana*) attract a member of the opposite sex by rapidly opening and closing their wings, flashing gorgeous eyespots on their wings to their potential mate. Interestingly, the frequency at which these tropical male butterflies embark on courtship is strongly dependent on the time of year at which they enter the pupal stage, when they transition from caterpillar to butterfly. Males that transition during the wet season become active courters, whereas males that transition during the dry season have lower courtship rates. Biologists believe that these behavioural differences are mediated by levels of the hormone 20-hydroxyecdysone (20E), produced during the pupal stage, but how 20E acts on the brain to influence courting behaviour was unclear.

In a new study led by Heidi Connahs [National University of Singapore, Singapore (NUS)] and Eunice Jingmei Tan (NUS and Yale-NUS College, Singapore), a team of researchers from NUS and Yale University, USA, questioned whether 20E alters the expression of the *yellow* gene in the brain of male butterflies – a gene that has been linked to courting behaviour in other insects. Wet season pupae tend to have very high levels of 20E in their blood, which the team suspected may 'turn down' the expression of the *yellow* gene, leading to elevated courtship rates in the adult male butterflies. To test this idea, the team reared squinting bush brown butterfly caterpillars under wet season (27°C) or dry season (17°C) conditions until the caterpillars created cocoons and became pupae. After a few days, the team collected the brains of the young pupae so that the expression of the *yellow* gene could be analysed and compared with previous measurements of 20E hormone levels in wet and dry season pupae, as well as with the courtship rates of adult butterflies raised under the same conditions.

The researchers first discovered that the *yellow* gene was expressed at different levels in the brains of wet season and dry season pupae. As expected, wet season males with high 20E levels expressed the *yellow* gene at low levels and courted like love-struck teenagers as butterflies. In contrast, dry season males with low 20E levels expressed the *yellow* gene at high levels and courted half as much. Given this strong correlation, the researchers decided to take their study one step further and experimentally elevated hormone levels in dry season pupae by injecting them with a concentrated dose of 20E. When the team analysed the brains of the injected pupae, the expression of the *yellow* gene appeared to be reduced, leading to high courtship rates and providing further support for the team's idea.

In a final experiment to solidify their findings, the researchers decided to use a cutting-edge gene editing method known as CRISPR-Cas9 to knock-out the *yellow* gene in these butterflies altogether. The team carefully observed the knock-out mutants and found that they courted at very high rates, providing the final compelling piece of evidence that the *yellow* gene regulates courting behaviour in the squinting bush brown butterfly.

Most species in the animal kingdom – including the squinting bush brown butterfly – make their intentions for mating very clear, through goofy dances, thoughtful gift giving or some other wonderfully weird act. Human dating rituals, in contrast, can be confusing and not at all productive. So, the next time you're wondering whether you should go ask that special someone out on a date, simply turn down your metaphorical '*yellow* gene' and maybe you'll get those butterfly feelings too.

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Connahs, H., Tan, E. J., Ter, Y. T., Dion, E., Matsuoka, Y., Bear, A. and Monteiro, A. (2022). The *yellow* gene regulates behavioural plasticity by repressing male courtship in *Bicyclus anynana* butterflies. *Proc. R. Soc. B* **289**, 20212665. doi:10.1098/rspb.2021.2665

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Fish funnel their food with jets

Unlike humans, many fish grab their food by sucking up the water that surrounds it. Once sucked into the fish's mouth, the food then gets swallowed up. What of the water that carried it there? The simplest way for water to exit is across the gills, but if food just followed suit, then fish would be inhaling and spewing it out through their gill slits. Pauline Provini, from Département Adaptations du Vivant, Paris, France, wasn't convinced that this would be the most elegant option and wondered whether fish independently filter their food away from the water. Together with Alexandre Brunet, Andrea Filippo and Sam Van Wassenbergh, they set out to see what goes on inside a fish's mouth while eating.

As if it's not challenging enough to see into a fish's mouth, Provini and colleagues needed to see how food and water swirl around inside; it's hard to persuade a fish to say 'ah' and take a peek. As the next best thing, the team used fluoroscopy – moving x-rays – to see what happens inside the mouths of two kinds of fish, carp and tilapia, from multiple angles. It's hard to see how the food and water swirl about using fluoroscopy alone, so they used metal markers, which show up really well on x-ray images. First, they implanted fish food pellets with small metal beads. Next, to see the flow of water, they crafted an armada of miniscule floats, by encasing 0.4 mm long metal rods in tiny foam life jackets. These drifted about in the water, waiting to be taken in by the hungry fish when they sucked up their food.

The researchers found that the fish used their expandable mouths to create a spout of water, a fast-flowing fluid funnel that shifts water down the centre of the mouth, from the front to the back. Provini and colleagues had wondered before whether this was the case, but they had now seen it first-hand. Pieces of food hitch a ride on this water jet and, instead of being belched out of the fish's gill slits, are taken to the entrance of the oesophagus, the muscular tube that leads to the stomach.

But the story doesn't end there. The fish then synchronised the movements of their jaws and throat to waft the food back and forth a little in their mouth – a form of rhythmical gargling. Provini and colleagues suggest that the fish's ability to extend the consideration they gave to their food was a way to sample and sift the tastiest morsels, before rejecting unpalatable scraps.

In addition, Provini saw that the tiny floats then swirled around the edges of the fish's mouths and out of their gill slits, without a single one ever getting swallowed. Astonishingly, the fish managed to avoid swallowing any water by mistake. Far from being messy eaters, it seems that these fish have impeccable and quite particular table manners.

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Provini, P., Brunet, A., Filippo, A. and Van Wassenbergh, S. (2022). *In vivo* intraoral waterflow quantification reveals hidden mechanisms of suction feeding in fish. *eLife*, **11**, e73621. doi:10.7554/eLife.73621

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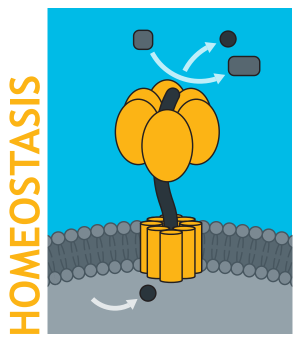
Crustacean stomachs are crabby, but not caustic



There is a bag of hydrochloric acid inside each of us. To digest our food, our stomachs secrete stomach acid, which is powerful enough to dissolve bone and erode teeth. But not all animals are walking bags of caustic acid. Crustaceans' stomachs are literally a thousand times less acidic than ours and are about the same pH as fresh rainwater. Crabs rely far less on stomach acid than we do, so Alex Quijada-Rodriguez, Dirk Weihrauch and colleagues at the University of Manitoba, Canada, studied crabs to find the consequences of using less stomach acid. When our stomach lining pumps in acid, it leaves our blood more alkaline, but if crustaceans use less stomach acid, does eating make them alkaline too?

To investigate, Quijada-Rodriguez and colleagues fed green shore crabs (*Carcinus maenas*) a hearty meal of scallops and sampled the pH within and outside their stomachs. Yet, even while digesting their meal, the acidity of the crabs' stomachs never changed. However, when the team tested samples of the crabs' haemolymph (the crustacean equivalent of blood) after dining, they found something unexpected. Instead of becoming more alkaline, the haemolymph became more acidic.

Being acidic is dangerous, whether you're a human or a crab. Acid impairs our hearts and brains, so animals keep a tight rein on their pH. Normally, crustaceans get rid of extra acid in the same way we do: they breathe it out. Our blood converts excess acid into carbon dioxide, which we exhale to reset our pH, and crabs do too. So Quijada-Rodriguez and colleagues sampled the crabs' haemolymph after a meal to measure carbon dioxide, but things looked dire. Even 6 h after eating, carbon dioxide was ~50% above normal. This means the crabs couldn't breathe out the carbon dioxide fast enough to reset their pH. Left unchecked, this build-up of acid could prove fatal. But, of course, crabs don't die after every meal. Surely, something else was expelling the acid.

Acid is not the only by-product of digestion. Protein in the crabs' diet produces ammonia, a toxic chemical used in household cleaners. And when the team checked ammonia concentrations in the crabs' bodies after dining, the toxin doubled, and peaked at the same time as acid in their haemolymph. Yet, instead of compounding each other's toxicity, acid and ammonia may cancel each other out. The University of Manitoba team recorded how quickly acid and ammonia left the animals. Although the crabs secreted only a small amount of acid, they cleared ammonia at a much higher rate. However, ammonia appeared to carry acid away with it. Weihrauch's lab had previously shown that crustaceans secrete ammonia as a weak acid called ammonium. In essence, ammonia soaks up the crabs' acidity before it's quickly thrown out. Just like us, after a messy meal, crabs use ammonia to clean house.

We humans go through all the trouble of producing stomach acid – an acid so corrosive that laboratories like Quijada-Rodriguez's label it with 'Danger!' and lock it away in specially made safety cabinets. Perhaps in time, the team will clarify how crustaceans can digest without such extreme digestive systems, given that crabs' stomachs are less acidic than the coffee I'm drinking now.

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Quijada-Rodriguez, A. R., Allen, G. J. P., Nash, M. T. and Weihrauch, D. (2022). Postprandial nitrogen and acid-base regulation in the seawater acclimated green crab, *Carcinus maenas*. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **267**, 1-11. doi:10.1016/j.cbpa.2022.111171

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How distinct killifish populations respond differently to stress

Down the east coast of North America, populations of Atlantic killifish, *Fundulus heteroclitus*, experience wildly different thermal environments. Southern populations experience water that is, on average, 12°C hotter than northern populations annually. These northern and southern killifish populations cope with stress differently. When fish are stressed, such as when predators are nearby, they activate a suite of mechanisms that regulate the release of the stress hormone cortisol, allowing the fish to remain in good condition. Researchers found previously that killifish from southern populations had higher plasma cortisol levels than killifish from northern populations after they experienced the same stress. To figure out why some fish respond strongly to stress while others do not, Madison Earhart and co-workers from the University of British Columbia, Canada, with colleagues from the University of Manitoba, Canada, and the University of Glasgow, UK, set out to determine what genes are responsible for the differences in killifish stress responses by investigating different mechanisms that trigger cortisol release after experiencing stress.

Earhart and colleagues travelled to the USA and collected adult killifish from northern New Hampshire and southern Georgia populations. Then, they brought the fish back to the lab in British Columbia. First, the team wanted to see whether the differences in population stress response changed when the fish experienced stress briefly or were exposed to the same stress repeatedly over the period of a week by either putting the fish in buckets and shaking them for half an hour or repeatedly shaking them every day for a week. After stressing the fish, the team collected samples of the fish's blood to measure their cortisol plasma levels, in addition to collecting samples of the fish's brain, head kidney – the organ that produces cortisol – and liver – the organ that produces and breaks down glucose – to measure the expression of genes important for the production and release of cortisol due to stress.

After experiencing the single stressful situation, the southern killifish population had higher cortisol levels than the northern killifish population. However, when the fish were repeatedly stressed over the period of a week, there was no difference in cortisol levels between the northern and southern populations. The southern population of killifish, which had a stronger response to the individual stressful situation, expressed more of the genes involved in cortisol production in the brain and head kidney. In addition, the livers of fish from the southern population were more responsive to the cortisol stress hormone as they expressed larger amounts of the gene for the protein that triggers the protective mechanisms that are activated by cortisol when a fish is stressed.

The team also measured the condition of the fish after repeated stress. They found that the southern killifish, which were more stress responsive than the northern killifish, were in better condition after experiencing repeated stress than were northern populations. This showed that responding to stress by increasing cortisol was beneficial for the southern population of fish.

Earhart and colleagues noted that the answer as to why some fish respond more strongly to stress than others is more complex than simply increasing cortisol levels in the body. They showed that there are differences in the expression of genes all along the pathways that make cortisol, respond to cortisol and cease cortisol production in these two different populations of fish. Therefore, it is important to continue to study these differences in populations of the same species adapted to different environments.

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Hot eggs make fast mitochondria

Mitochondria are fascinating organelles that have achieved fame on the internet as the 'powerhouse of the cell', producing 90% of the cell's energy in the form of ATP. Based on this essential role, it is thought that differences in mitochondria determine the strengths and weaknesses of individuals and even entire species. As a result, there's interest in trying to understand how the environment and genetics shape mitochondrial function. In their recent study, Antoine Stier from the University of Turku, Finland, and colleagues from the University of Glasgow, UK, incubated Japanese quail eggs (*Coturnix japonica*) at different temperatures and studied changes in how their mitochondria performed from early-life into adulthood. They wanted to know whether animals can adjust how their mitochondria perform, depending on the temperature they experience before birth, and whether these changes are maintained as they develop into adults.

The scientists incubated Japanese quail eggs at high (38.4°C), medium (37.7°C) and low (37.0°C) temperatures – a temperature range that maximizes

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differences in how quickly embryos grow. They also included an unstable temperature treatment (dipping briefly, several times, to 29.5°C from 37.7°C) to mimic when the mother leaves the nest to forage. When the chicks hatched, the researchers transferred the animals to 21°C as they grew, collecting blood samples at 20 days of age and at 60 days to investigate how the mitochondria in the blood cells were affected by the temperature at which the chick embryos developed and how they changed after the chicks hatched and grew into adults. The team measured the oxygen consumption rate of the mitochondria, which is tightly linked with the amount of ATP these structures can produce, the amount of DNA damage they had incurred and the length of the telomere structures that cap chromosomes – which shorten with age.

The team found that the oxygen consumption rates of the mitochondria of the chicks incubated at 38.4°C were higher than those of the chicks from colder eggs, probably because the embryos in the warmer eggs developed faster and needed more energy to support their rapid growth. In addition, the mitochondria of the adult quails that had developed in hot eggs had higher oxygen consumption rates, meaning that this increase was not temporary and that the thermal environment that these birds experience as an egg shapes their ability to produce ATP throughout their lives. But the blood cells with mitochondria that consumed oxygen faster only had a small increase in the amount of DNA damage and no change in telomere length – which is surprising because overactive mitochondria that consume more oxygen can produce toxins that damage DNA and cause aging.

As the adult birds aged, their mitochondrial oxygen consumption decreased, probably reflecting the extreme energy demands they experienced as they grew rapidly during the first 20 days after hatching. When the team checked the oxygen consumption rates of the mitochondria of the birds that were incubated at unstable temperatures – as if their mothers kept wandering off – they found they were similar to those of birds incubated at a constant 37.7°C. This tells us that when the environmental temperature is unstable, these birds use the temperature that they experience the most as the cue to program their mitochondria throughout life.

The role that the environment a developing embryo experiences has on programming mitochondrial function after birth is a research area with major implications for human health and conservation efforts. Stier and colleagues have shown that something as simple as increasing incubation temperature can cause a change in mitochondrial function that persists into adulthood. Understanding how these changes in mitochondrial function come about and whether they are important for improving whole-organism performance and ultimately survival will be critical areas of future research.

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