INSIDE JEB

Wasp masters manipulate web-building slaves

A parasitised spider on an orb web. Photo credit: Keizo Takasuka.

Some wasps have unpleasant habits. Hijacking an unsuspecting insect or spider, parasitic wasps incapacitate their hapless victims by taking control of their nervous systems and turning them into zombies. Once the wasp has its victim in its clutches, it deposits its egg on or within the victim's body, ready for the next generation to develop. Keizo Takasuka from Kobe University, Japan, explains that one particular wasp, *Reclinervellus nielseni*, turns its spider targets (*Cyclosa argenteoalba*) into drugged navvys whose final act is to construct a tough 'cocoon' web from the original orb web to protect the developing wasp pupa after the spider's death. According to Takasuka, *C. argenteoalba* produce several different styles of web over the course of their lives – 'orb' webs when hunting and 'resting' webs for protection when moulting – each produced by a specific set of behaviours. However, it wasn't clear which of the spider's behavioural patterns and web-types the wasp was adapting to its own ends.

Intrigued, Takasuka headed to shrines in two nearby cities (Tamba and Sasayama) from mid-April to mid-May ready to collect spiders complete with their webs and parasitic larvae. However, Takasuka recalls that keeping the spiders alive in the lab before their zombie state was triggered was quite a challenge. Some refused to build webs in captivity and he occasionally destroyed the delicate structures when he inadvertently snagged supporting frame threads attached to distant objects. However, after weeks of patiently nurturing the spiders, he was rewarded when 10 obediently constructed cocoon webs for their parasite masters.

Analysing the cocoon webs, Takasuka was struck by their similarity to the resting webs, complete with fluffy decorative structures. And when Takasuka analysed the spiders' behaviour as they constructed the cocoon webs over a 10 h period, he saw that the manipulated spiders always constructed the new web on the site of the old orb web, painstakingly removing the sticky spiral first, then reinforcing the radial and frame threads and then adding the fibrous web decorations. And, when the web was complete and the wasp larva done with its spider slave, the larva directed the spider to return to the hub of the web before murdering it.

So, the cocoon webs looked like resting webs, but were they true adaptions of the more conventional web? Takasuka and Kensuke Nakata photographed the webs in UV light and were impressed to see the fibrous decorations shining brightly, to deter other insects from inadvertently blundering into the pupa's nursery. And when Tomoki Yasui, Toru Ishigami and Takasuka investigated the strength of the different types of silk that contribute to the web's structure, they were amazed to see that the cocoon web was significantly tougher than the orb and resting webs: the breaking force of the cocoon radius and frame silks was 2.7–40 times greater than that of the orb and resting webs. However, the breaking stress of the silks was not increased significantly, leading Takasuka to suspect that instead of forcing the spiders to alter silk production, the wasp slave-masters were directing the spiders to lay down more fibres to strengthen the cocoon web, which is essentially a reinforced resting web.

Explaining that parasitised spiders transition into zombie web building even when the parasitic larva has been removed, Takasuka also suggests that resting web construction is triggered by the same hormones that control moulting and he suspects that the larva may inject a substance similar to a moulting hormone into the hapless spider during the later stages of its stay, ready to trigger cocoon web building when the larva is ready to pupate.

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Takasuka, K., Yasui, T., Ishigami, T., Nakata, K., Matsumoto, R., Ikeda K. and Maeto, K. (2015). Host manipulation by an ichneumonid spider ectoparasitoid that takes advantage of preprogrammed web-building behaviour for its cocoon protection. *J. Exp. Biol.* **218**, 2326-2332.

Kathryn Knight

Alligators use resonance for communication

A female Chinese alligator (*Alligator sinensis*). Photo credit: Jim Darlington.

Once humans master verbal communication, we babble incessantly on any topic under the sun. But even animals that are equipped with less sophisticated communication systems depend on the same resonances that we skilfully shape with our vocal tracts for communication. Stephan Reber from the University of Vienna, Austria, explains that vibrations – produced when air is pushed past the vocal folds – force air trapped in the vocal tract to vibrate (resonate) and it is these resonances that shape bird song and human syllables. Animals also use the frequency of these resonances (how high or low the sound appears) to communicate their body size, with the frequency of a resonance honestly reflecting their size – although some species are known to exaggerate by deepening their resonances. However, no one knew whether reptiles, such as crocodiles and alligators, used resonances to communicate with others. 'Anurans – frogs and toads – do not seem to use them', explains Reber, who decided to find out whether modern day reptilian relatives of the dinosaurs, such as crocodiles and alligators, use resonance for communication.

Unsure how to distinguish between the vibrations produced by the vocal folds and resonance vibrations produced by air in the rest of the vocal tract, Reber asked his thesis advisor – Tecumseh Fitch – for advice, and recalls Fitch's simple response: 'Just put one in heliox'. Explaining that sounds travel much faster in heliox than in normal air, changing the frequency of vocal resonances and producing the Mickey Mouse sound effect that we make after inhaling helium, Reber realised that if the reptiles used resonances in vocal communication, inhaling the modified air should also shift the frequency of sounds produced by resonance. However, he also knew that immersing large alligators in heliox and getting them to serenade on demand would be easier said than done.

Travelling to the St Augustine Alligator Farm Zoological Park, USA, home to all 23 species of crocodilians, Reber and Judith Janisch were fortunate to find a small Chinese alligator in isolation in a tank. 'And when the big alligators in one of the other enclosures were bellowing, she always bellowed', recalls Reber with a smile. Realising that he could convert her enclosure into a rudimentary sound box complete with heliox atmosphere and that the female would happily bellow on demand when played recordings of her own voice, Reber teamed up with the conservation centre's filtration expert, Mark Robertson, to adapt her holding tank. And when he recorded her bellowing grunts in normal air and heliox, Reber was delighted that the grunts sounded different in the heliox atmosphere.

Returning to Austria, Reber then joined Takeshi Nishimura to begin scrutinising the baffling frequency spectra for evidence of shifted resonances. After months of painstaking analysis, he could clearly see that although the bellows sounded deeper in heliox, there were resonances that had shifted to higher frequencies: one increased from 400 to 800 Hz, while the second rose from 1600 to 3200 Hz. Now he could add members of the crocodilian order to the roll-call of animals that use air resonance for communication.

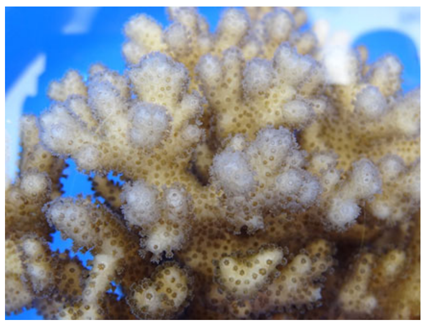
Reber admits that he is pleased that these ancient animals produce calls that are shaped by vocal tract resonance and is keen to find out how body size affects the frequency of an animal's vocal resonances. He is also excited that this is another piece of compelling evidence that dinosaurs may also have used acoustic resonance to communicate size. 'If you see it [resonance] in the last two groups [birds and crocodilians] that share a common ancestor with all extinct dinosaurs, we can infer that dinosaurs probably used formants [resonances] too for communication', says Reber.

10.1242/jeb.128686

Reber, S. A., Nishimura, T., Janisch, J., Robertson, M. and Fitch, W. T. (2015). A Chinese alligator in heliox: formant frequencies in a crocodilian. *J. Exp. Biol.* **218**, 2442-2447.

Kathryn Knight

Coral parents give young a head start in future sea



Pocillopora damicornis. Photo credit: Hollie Putnam.

As the oceans warm and the pH of the water drops, the future looks bleak for corals. Mass bleaching events occur when the conditions are hot and coral hosts lose the algal lodgers that provide their energy and nutrients. Tiny polyps also struggle to lay down their delicate calcified skeletons in more acidic waters. 'The current rates of change in the environment are dangerously rapid in relation to the rate of coral genetic adaptation', says Hollie Putnam from the University of Hawaii, USA, suggesting that corals may not be able to evolve fast enough to keep pace with the damaging effects of climate change. However, Putnam and her colleague Ruth Gates wondered whether there was a glimmer of hope for the apparently doomed animals. Could adult corals pass on advantages to their young that might safeguard their future through alternative mechanisms such as epigenetics? Putnam headed out, into the warm shallow waters of Hawaii's Kaneohe Bay, to collect samples of the cauliflower coral (*Pocillopora damicornis*) to find out whether exposing the adults to future climate conditions might improve their offspring's chances.

Carefully nurturing the corals in the lab, Putnam kept some in natural seawater (26.5°C and 417 µatm P_{CO_2}) while others were placed in water that mimicked predicted ocean conditions (28.9°C and 805 µatm P_{CO_2}) for 1.5 months. Then, knowing that the corals release their larvae around the time of the full moon, Putnam transferred the adults to collecting bins to capture the youngsters, ready to expose half of them to modern seawater conditions while the others were placed in the future conditions for 5 days before testing to see how they faired.

Measuring the photosynthetic activity of the coral symbionts, and the metabolic and calcification rates of the corals, Putnam was surprised to see that the warmer and more acidic conditions did not seem to affect the corals' ability to grow or their metabolic rate. However, the adult's symbionts suffered: their net photosynthetic rate plummeted by almost 80% while the ratio of algal photosynthesis to the respiration rate of the intact coral also plummeted. But how would the offspring of adults that had already had a dose of the future cope in the tougher conditions?

The larvae of parents that had been incubated in the future ocean conditions were smaller and had a lower metabolic rate than the larvae of parents in normal seawater. However, when the future-conditions larvae were placed in the warmer and more acidic conditions, their size-adjusted metabolic rate increased.

'It is often thought that the early life stages of marine organisms are the most sensitive', says Putnam; however, she and Gates have shown that the larvae performed better when their parents had been preconditioned to the more stressful future conditions. 'This is counter to what would be predicted', she says. Putnam also suspects that the parents that had been prepared in the future climate conditions give their youngsters a head start either by providing them with additional energy reserves or by passing on benefits through epigenetic modifications to the

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DNA (that do not change the molecular code of a gene but instead alter when the gene is activated), which may offer protection to their offspring. In addition, Putnam is keen to continue studying the youngsters through to adulthood and beyond, to find out whether their own young will benefit from the parents' experience: maybe the coral's future is less bleak than we thought.

10.1242/jeb.128694

Putnam, H. M. and Gates, R. D. (2015). Preconditioning in the reef-building coral *Pocillopora damicornis* and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. *J. Exp. Biol.* **218**, 2365-2372.

Kathryn Knight

Snow leopard haemoglobin unprepared for high-altitude life

Prowling through the icy Himalaya mountains, snow leopards seem unfazed by the rarefied atmosphere. Yet, according to an international team of researchers lead by Jay Storz from the University of Nebraska, USA, Jan Janecka from Duquesne University, USA, and Angela Fago from Aarhus University, Denmark, cats of all shapes and sizes are notoriously poor at coping when oxygen is scarce. 'Members of the cat family have haemoglobins [the protein that carries oxygen in the blood] with unusually low oxygen affinities', says Storz – which makes it tough for cats to scavenge oxygen from thin air. Yet, snow leopards, which are content at altitudes greater than 6000 m, buck the trend. Could their haemoglobin be modified to carry more oxygen than the haemoglobins of other cats?

Collaborating with zoos across the USA, the team obtained valuable blood samples from big cats including two African lions, a tiger, one leopard, four snow leopards and a panther, ready for Janecka and Trevor Anderson to clone and sequence the genes for the oxygen-carrying blood protein. However, when Frederico Hoffmann, Janecka and Storz analysed the protein sequence, they were astonished to see that the snow leopards carried exactly the same amino acid swap that compromises the ability of all other cat haemoglobins to carry oxygen. And when Simone Nielsen, Sidsel Andersen, Roy Weber and Fago measured the oxygenation of the cats' haemoglobin, with and without 2-3, diphosphoglycerate (DPG) – which helps haemoglobin to offload oxygen when it is required – the protein showed weak oxygen binding and all of the cat haemoglobins were completely unresponsive to the beneficial effects of DPG.

So, the snow leopards' haemoglobin is equally as inefficient as the haemoglobins of all other big cats and the haemoglobins are structurally and functionally almost identical to those of house cats. However, Storz suspects that the animals compensate for the poor oxygen capacity of their blood by simply breathing harder, and Janecka is keen to compare the performance of tigers and snow leopards at altitude: 'Sounds crazy, but I think it is possible', he chuckles.

10.1242/jeb.128710

Janecka, J. E., Nielsen, S. S. E., Andersen, S. D., Hoffmann, F. G., Weber, R. E., Anderson, T., Storz, J. F. and Fago, A. (2015). Genetically based low oxygen affinities of felid hemoglobins: lack of biochemical adaptation to high-altitude hypoxia in the snow leopard. *J. Exp. Biol.* **218**, 2402-2409.

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