

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.

## MANIPULATION



### PARASITES AND ZOMBIE GAMMARIDS

Richard Dawkins famously suggested that animals are little more than 'robot vehicles blindly programmed to preserve' their selfish genes. However, the true pilots of animal vehicles may not be their own genes, but rather those of their parasites. Whereas some parasites consume the host they colonize from within, others change the behaviour of the host to ensure a meal for the host's predator. A foolish strategy? According to new research led by Thierry Rigaud from the Université de Bourgogne, France, the answer is yes and no, depending upon the particular stage in the life of the parasite.

Some parasites spend their lives exploiting a single host. Others, by contrast, do a bit of travelling, spending time in one or more intermediate hosts before finally settling into a definitive host where the parasite reaches maturity. For these parasites, reaching the final host requires that their intermediate hosts are eaten by their definitive host. And thus the parasite takes over the strings of its puppet host to make it suicidal.

Examples of parasitic manipulation of behaviour are widespread: parasites can reduce the fear of predators, e.g. rats that become fatally attracted to cats. Others change the appearance of their hosts to make them look tasty, e.g. by turning an ant's abdomen bright red to make it look like a berry, or by making the tentacles of snails pulsate with light to make them look like caterpillars. Perhaps because scientists have spent so much time marvelling at these bizarre changes that increase predation, they have failed to recognize the flip-side, that there are key moments of a parasite's development where they wouldn't want their host to be consumed. Can parasites also make their hosts less susceptible to predation?

To examine this possibility, Rigaud's team infected freshwater crustaceans called gammarids with an acanthocephalan parasite that reaches maturity in a trout. This parasite passes through two developmental stages in the gammarid. The first, called an acanthella, is not infective to trout, while the second, called a cystacanth, is. To analyze behavioural effects of parasitism, the researchers infected gammarids with parasite eggs and allowed them to develop into acanthella or cystacanths. They then measured the time gammarids spent hiding in a refuge. As expected, gammarids infected with acanthella were the models of caution. By contrast, gammarids infected with cystacanths were downright reckless.

But are these manipulations adaptive for the parasite? In a word, yes. Gammarids infected with acanthella are less likely to be eaten than uninfected animals, while those infected with cystacanths are more likely to be preyed upon. It thus appears that the parasites in this system play both sides of the game. As immature acanthella they keep their hosts safe. But when they become ready to see the world, cystacanths rapidly change tack and send their hosts into the eye of the storm.

What better example for Dawkins' idea that animals are little more than 'lumbering robots' than zombie gammarids being sustained and then summarily marched to their death by their parasites?

10.1242/jeb.049932

**Dianne, L., Perrot-Minnot, M.-J., Bauer, A., Gaillard, M., Léger, E. and Rigaud, T.** (2010). Protection first then facilitation: a manipulative parasite modulates the vulnerability to predation of its intermediate host according to its own developmental stage. *Evolution* doi: 10.1111/j.1558-5646.2011.01330.x

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TRACHEOLE STRUCTURE



TRACHEAE & TRACHEOLES: A FORTUITOUS DISCOVERY REFINES A KEY DEFINITION

It is always interesting when a seemingly minor study actually contributes significantly to our understanding of a key concept in animal biology. A key concept in the field of insect respiratory biology is understanding the structure, function and formation of the insect tracheal system. Not using lungs (for gas exchange) nor blood circulation (for oxygen and carbon dioxide transport and exchange) is a characteristic that sets insects apart from vertebrates and even some invertebrates. The insect tracheal system consists of a ramifying system of successively finer tubes – entering the insect body at pairs of openings (spiracles) on certain body segments, infiltrating tissues and terminating in blind-ending tubes between or even within cells. Air moves down these tubes, called tracheae, transporting respiratory gasses toward the very fine blind ends, the tracheoles, where aerobic gas exchange occurs. Thus, the tracheal system dually transports and exchanges respiratory gasses directly between tissues and air.

A uniquely curious thing about the tracheal system is that it is part of the insect cuticle, or exoskeleton. Consequently, developmentally during each moult, not only is the external skeleton being replaced but also the respiratory system. Prior to moulting, the existing tracheal cuticle layers (also the exoskeleton) detach from the epithelial layer, a moulting fluid – containing enzymes that partially break down old cuticle – is secreted between the old cuticle and epithelial cells, new cuticle lines the epithelium, and the old cuticle is discarded along with old tracheal cuticular tubes withdrawing from the newer larger tubular system.

Three common ‘understandings’ distinguished tracheae from tracheoles: (1) tracheae, similar to exoskeletal cuticle,

consist of an epicuticle layer and a chitin–protein matrix layer while the smallest blind-ending tracheoles lack the chitin–protein layer; (2) the tracheolar epicuticle remains intact during moulting (no detachment); and (3) the tracheoles do not have taenidia (the structural support rings similar to vacuum cleaner tubes and human windpipes). Earlier studies on true bugs reinforced the idea that the tracheolar epicuticle is not shed during moulting. However, subsequent studies challenged these ‘understandings’. Studies on moths showed tracheolar epicuticle being shed after the third larval instar whereas in flies tracheolar epicuticle is shed during early moults but remains intact later on. These data introduced ambivalence into how to define tracheoles, at least in a developmental context.

Edward Snelling and colleagues, from the University of Adelaide, prepared several juvenile third-instar locusts, *Locusta migratoria*, for light and electron microscopy. Unbeknown to them, one individual, giving no external indications, entered the early stages of moulting. This was clear in the way the cuticle layers of the tracheal system in the jumping leg detached and retracted from newly laid-down layers. What was most significant in this serendipitous observation was that the epicuticular layer of the terminal ends – the tracheoles – detached and withdrew from the newly formed layers and showed micro-taenidia analogous to tracheae. This discovery, in effect a fortuitous study with an  $N=1$ , provided confirmatory evidence, along with supporting information from recent studies on tracheole structure and development, and it improves our understanding of the insect respiratory system by allowing for a more functional definition of tracheoles: tracheoles are the blind-ending terminal tubes, with a  $<2\mu\text{m}$  diameter, arising along cytoplasmic extensions from terminal tracheolar cells, differing structurally from tracheae, and provide the primary sites of gas exchange in the insect tracheal system.

10.1242/jeb.049940

Snelling, E. P., Seymour, R. S. and Runciman, S. (2011). Moulting of insect tracheae captured by light and electron-microscopy in the metathoracic femur of a third instar locust *Locusta migratoria*. *J. Insect Physiol.* doi: 10.1016/j.jinsphys.2011.06.006

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AUDIBILITY



CHAOTIC GRUNTS FROM TWO-VOICED TOADFISH

In many vertebrate species, evolution has favored morphological innovations that allow an animal to simultaneously speak with more than one voice. By independently controlling multiple sound-producing organs, frogs, songbirds and even chimpanzees can belt out ‘two-voiced’ calls that contain chaotic non-linear properties. Such features are thought to make vocalizations more noticeable for animals on the receiving end of a song or call. Among vertebrates, this ability has previously only been documented in tetrapods (limbed vertebrates); however, a new study by Aaron Rice, Bruce Land and Andrew Bass at Cornell University shows that the ability to produce acoustic chaos has also evolved in the largest group of vertebrates, the fishes.

Rice and colleagues chose to work on the three-spined toadfish (*Batrachomoeus trispinosus*), an intensively studied vocal fish. These whiskered, lumpy swimmers produce a range of ‘grunts’ and ‘hoots’ for communicating with rivals and mates in muddy-bottomed estuaries. Unique among fish, this species makes sounds with a swim bladder that is separated into two parts – each with its own independently innervated vocal muscle.

First the researchers housed multiple toadfish together and recorded grunts and hoots. Spectrograms of toadfish calls showed linear harmonic (bands of power at multiples of a dominant frequency) components. But about one-third of all calls also showed non-linearities. There were frequency bands at a consistent fraction of an original harmonic series (subharmonics), quick jumps in all frequency components, harmonics plus ‘noisy’ energy across many frequencies (deterministic chaos), splits in harmonic bands (bifurcation) and, in some cases, overlap of two harmonically independent ‘voices’ (biphonation). The

team then used a set of analytical tools derived from chaos theory to show that these toadfish non-linearities are indeed very similar to those observed in tetrapods. These results show that toadfish can generate highly complex vocalizations comparable to those seen in frogs, songbirds and mammals.

Rice and colleagues then wanted to know whether toadfish generate chaotic calls by independently controlling the two parts of their swim bladders. To test this, the team recorded grunts in individual toadfish before and after transecting the vocal motor nerve leading to one side of their bladder. After surgery, the animals could only produce calls with linear components. These experiments suggest that independent neural control of a bilateral swim bladder is ultimately what gives toadfish their two voices.

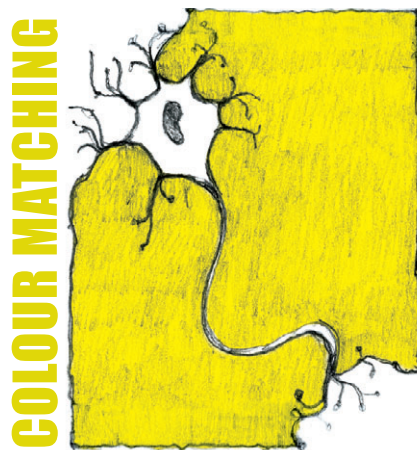
The experiments in this paper are elegant and the writing is clear. But one frustrating thing about this study is that the reader never gets to actually hear a toadfish grunt and hoot. While it may not have been necessary from an analytical point of view, presenting audible examples of the chaotic calls themselves would have been a very simple way to add richness to this paper and get more people excited about the work.

Lack of audible grunting aside, the work of Rice and co-workers is important because it suggests that strong selection pressures favor innovations that enable non-linear signalling across all major lineages of vocal vertebrates. It is now apparent that this is a powerful force shaping the evolution of acoustic communication across vertebrate taxa. Finally, this work is beautiful because it shows that even something as simple as a fish grunt can contain a stunning level of complexity.

10.1242/jeb.049965

Rice, A. N., Land, B. R. and Bass, A. H. (2011). Non-linear acoustic complexity in a fish 'two-voice' system. *Proc. R. Soc. B* doi: 0.1098/rspb.2011.0656

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## COLOUR-MATCHING WHEN COLOUR-BLIND

When your strategy to elude predators is to camouflage yourself to blend in with your surroundings, how do you accomplish this when you are colour-blind? It seems that cuttlefish, which are among the world's foremost artists in the medium of body camouflage, are unable to see colours. Despite this, they are able to colour-match their surroundings precisely enough to fool predators. This apparent paradox has been vexing scientists for years, so Chuan-Chin Chiao and his colleagues at the Marine Biological Laboratory in Woods Hole, USA, set out to use a novel imaging system in the hope that it would reveal potential clues as to how cuttlefish colour-match their surroundings. They also used the image data to create images of the cuttlefish as viewed through the eyes of potential predators.

Setting up cuttlefish in tanks with one of three natural substrates on the floor in order to get them to produce one of three distinct camouflage patterns, Chiao and his colleagues then used a hyperspectral imaging system to take pictures of the animal's bodies and the surrounding substrates. Hyperspectral imaging is a unique technique that provides much more spectral and spatial data than a regular digital photograph and produces a 3-D cube of data representing both the colours and their reflection spectra. Once a picture of the camouflaged cuttlefish had been taken, the team used known visual properties of several potential cuttlefish predators to convert the spectral data into a predator's eye view, which allowed them to analyse the colour-matching abilities of the cuttlefish from the predator's perspective.

The hyperspectral images of the cuttlefish revealed that the cuttlefish displayed colour

and reflectance spectra similar to the background, with one notable exception: the majority of the reflectance spectra from the cuttlefish were in the infrared range, which was surprising as the natural substrates did not extend into the infrared spectrum. The biological importance of this curious finding is unknown at this time. Regardless, analysis of the spectral data revealed that the spectral properties of the skin of cuttlefish and those of three natural substrates were similar.

Modelling of the hyperspectral data to create images of the cuttlefish through the eyes of predators revealed that cuttlefish not only accurately match the background colour but also the surrounding pattern, rendering them invisible to predators. The question of how exactly they do this is still unclear, but the hyperspectral system may allow scientists to more accurately test the abilities of cuttlefish and analyse their responses to various backgrounds.

So, how do you successfully blend in with your surroundings when you are colour-blind? Previous work has shown that variables such as brightness, contrast and edging are essential to induce camouflage in animals. However, scientists have been limited by technology in their analysis of the minute details of the camouflage created by colour-blind animals. Hyperspectral imaging appears to allow detailed examination of the colour, the reflectance and the pattern of camouflage, bringing us one step closer to solving this vexing problem. Additionally, the system creates data that can generate pictures of the animals through the eyes of other animals. This allows scientists to potentially see prey as predators see them.

10.1242/jeb.049957

Chiao, C. C., Wickiser, J. K., Allen, J. J., Genter, B. and Hanlon, R. T. (2011). Hyperspectral imaging of cuttlefish camouflage indicates good colour match in the eyes of predators. *Proc. Natl. Acad. Sci. USA* **108**, 9148-9153.

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