OCEAN ACIDIFICATION

ACID SEAS BLOCK NEMO'S NOSE

Increased atmospheric CO_2 levels will have a major impact on marine life, in particular on corals and molluscs, through the effects of ocean acidification on calcification. A recent international collaboration by researchers from Australia, Canada and the USA suggests that non-calcifying species, such as fishes, may also be adversely affected, as increased acidity has subtle but important effects.

The researchers, led by Philip Munday of James Cook University in Australia, studied the consequences of increased levels of acidity on the behaviour of larval clownfish – the striking white and orange fish that featured in the film *Finding Nemo*. Clownfish live near the bottom of the sea, but their larvae (which are a dull brown) drift as plankton. As they mature, the young clownfish need to find the adult population and avoid predators. Both these behaviours rely on chemical cues, which may be susceptible to changes in pH.

Munday and his co-workers reared clownfish larvae at normal sea-water pH (8.15), and also at increasing levels of acidity, induced by higher levels of CO_2. They then used a simple flume chamber, in which the fish can choose to position itself in one of two parallel water sources, to test the ability of larvae to respond to various chemical stimuli. Current CO_2 levels are around 390 p.p.m. or 0.039%; in 1960 the figure was around 360 p.p.m. (an 8% increase in 40 years). When reared under 390 p.p.m. CO_2, all ages of larvae were able to avoid predator cues; similar responses were seen in larvae reared under a slightly higher CO_2 level (550 p.p.m.).

However, when larvae were reared at 700 or 850 p.p.m. CO_2 (which some models predict we will see by the end of the 21st century), non-adaptive behaviour began to appear – some older larvae actually preferred the predator-cue water stream, in particular when reared at the highest CO_2 level. To see whether this response was specific to the clownfish, the researchers repeated their experiment with larvae of the bright blue damselfish. The results were essentially the same – the longer larvae were reared at the highest dose of CO_2, the greater the effect on chemosensory behaviour.

In a final test, the damselfish larvae were taken to the ocean and placed on reefs. Their behaviour in the wild mirrored their responses in the laboratory – larvae reared at higher CO_2 levels showed riskier behaviours, venturing further from the reef than control larvae. These behaviours had lethal consequences: the larvae suffered significantly higher predation rates – up to 9-fold those of larvae reared at present-day CO_2 levels.

Exactly how CO_2 is affecting the behaviour of the fish is not clear. It may simply alter chemosensory responses, or it may also be altering the animals' general activity levels. Either way, these results suggest that ocean acidification will have multiple effects on this key part of the planet's ecology. If there were to be a remake of *Finding Nemo* in a few decades, it might not end so happily.

10.1242/jeb.036780

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METABOLIC SCALING

ENDOTHERM METABOLISM TOO HOT TO HANDLE

Energy is presumed to be limited in supply, body warmth is presumed to be a benefit of endothermy, and fractal distribution networks are presumed to constrain the metabolic design of organisms. However, a new *Journal of Animal Ecology* paper by John Speakman and Elzbieta Król from the University of Aberdeen challenges all three premises by proposing that limits to heat dissipation explain the scaling of endotherm metabolism and constrain metabolic performance during periods of intense sustained energy expenditure, such as reproduction.

Speakman and Król review a long series of investigations published in *The Journal of Experimental Biology* seeking to identify factors limiting milk and offspring production in lactating mice. They describe how lactating mice housed in the cold increased food intake, produced more milk and raised faster growing pups than mice held at warmer temperatures. The possibility that cold environments are advantageous to lactating mice because they can produce more milk when it is easier to dissipate heat could explain the experimental evidence but is heretical in the context of prevailing ideas about the warm-body benefits of endothermy. However, a later paper in this series clinched support for this heresy. Shaving fur from the dorsal surface of lactating mice, which increases their capacity to dissipate heat without altering the thermal environment experienced by their offspring, substantially improves their reproductive performance. Body heat is a parental care problem rather than a parental care solution, at least for lactating mice in captivity.

Having identified heat dissipation as a limiting factor, Speakman and Król proceed by developing a mechanistic model of endotherm heat dissipation, which predicts that the maximum capacity to dissipate heat increases with body size with an exponent of about 0.63. This provides a much better empirical match to the scaling of field metabolic rate than the 0.75 exponent predicted by the metabolic theory of ecology. Often the residuals around these allometric patterns are as informative as the slope of the overall relationship, and Speakman and Król speculate that marine mammals have higher rates of energy expenditure than other similar-sized mammals because they occupy aquatic environments that are highly conducive to heat dissipation. The authors invert the observation that many animals increase insulation in winter to ask why animals reduce insulation in summer if heat and energy conservation is a year-round priority. Speakman and Król also suggest that latitudinal patterns of body size variation – first described by Carl Bergmann in 1847 – are driven by more intense selection for the reproductive advantages of small body size and heat dissipation in warm environments.

Is body warmth generally a cost rather than a benefit of endothermy? Does heat dissipation capacity offer a better explanation for the allometric scaling of endotherm metabolism than fractal distribution networks? Are major trade-offs in endotherm energy allocation governed by constraints associated with energy demand rather than energy supply? These are revolutionary and readily testable hypotheses proposed by Speakman and Król reflecting major uncertainties and research opportunities in endotherm energetics.

10.1242/jeb.051300

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ECHOLOCATION

WHEN BATS LISTEN CLOSELY THEY ALSO LISTEN WIDELY

Several animals such as bats and whales use echolocation to aid them during navigation and foraging. These animals emit calls that produce echoes when the sound waves bounce off an object in their environment. The different characteristics of these echoes, such as the amplitude or the time it takes for the sound wave to reflect, provide the animal with valuable information about the characteristics of the surrounding objects.

The biosonar of bats consists of short high-frequency sound pulses. When hunting, bats emit faster and shorter pulses as they get closer to their prey, increasing feedback frequency during the final approach. The directionality of the sonar should also be important; a directional sonar would be effective in locating insects in front of it, while filtering 'noise echoes' from peripheral objects. However, as the bat nears its prey, a broader beam of sound would be more beneficial, as the field of view covered by a directional beam is reduced at short distances.

A recent study by Lasse Jakobsen and Annmarie Surlykke from the University of Southern Denmark investigated whether insectivorous bats have the ability to adjust the sound beam's width according to the proximity of their prey. To do this, they trained six vespertolionid bats to feed off tethered mealworms inside a large room. They then recorded their calls as they hunted for the mealworms using 12 microphones arranged in a cross. The multimicrophone array was located at the back of the room, behind the mealworm. This configuration allowed the scientists to determine the bat's position using the differences between the time of arrival of the sound waves at each microphone, and the direction of the sound beam by calculating the vertical and horizontal angles from the bat's position to each microphone.

During the initial hunting phase, the bats emitted highly directional echolocation beams with a half-amplitude angle of approximately 40 deg horizontally and 45 deg vertically. As the bats closed in on the mealworms, the beam broadened dramatically, and the half-amplitude angle of the beam more than doubled. The bats were able to do this by decreasing the sound frequency by about an octave, from 55 to 27.5 kHz. In 1989 Elisabeth Kalko and H. U. Schnitzler from the Universität Tübingen in Germany noted that *Myotid daubentonii* lowers the frequency of its echolocation pulses during the moments preceding the catch. They speculated that this change in frequency represented a physiological constraint and that the bats were physically unable to produce high frequency pulses at the high repetition rates emitted during the final approach; Jakobsen and Surlykke's study dismisses this hypothesis by pointing out that many other bats are capable of producing pulses at these high repetition rates without a concomitant decrease in frequency, and proposes a functional significance to the phenomenon.

By having a narrow but highly directional beam of sound aimed directly at the prey during the beginning of the pursuit, the sound beam is optimized to locate insects positioned in front of the bat, while filtering noise resulting from surrounding objects. As the bat closes in on its prey, a wider sound beam translates into a wider detection angle, preventing prey from escaping by quickly flying outside the bat's field of view. Future research will likely reveal that the biosonar of other echolocating species also exhibit dynamic control of the width of the sound beam in response to changes in their surroundings.

10.1242/jeb.036764

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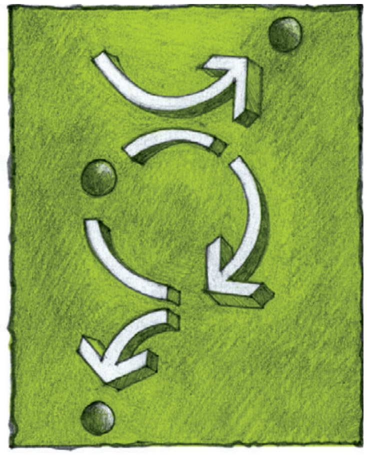
NON-CODING mRNA



Pri PEPTIDES SWITCH ON TRANSCRIPTION FACTOR

For decades scientists believed that large regions within metazoan genomes are non-functional relicts from evolution because they do not encode proteins. However, we also knew that significant parts of these regions are transcribed into non-coding RNA (ncRNA). The view that ncRNAs have no function changed dramatically with the discovery of regulatory RNAs, such as microRNAs. However, most ncRNAs discovered so far lack any obvious function. Among them are polyA-containing RNAs that have only short open reading frames, some of which encode small peptides. However, the function of these peptides remains largely elusive. In a recent *Science* paper, French and Japanese scientists led by François Payre and Yuji Kageyama report the function of peptides derived from the *polished rice* (*pri*) gene in *Drosophila* development.

The *pri* gene was originally identified in the red flour beetle (*Tribolium castaneum*) and shown to encode multiple peptides rather than one single protein. When *pri* expression is silenced in *Tribolium*, the abdominal segments of the embryos turn into thoracic segments, providing the embryos with additional pairs of legs. Likewise, *pri* mutations display prominent defects in *Drosophila* embryogenesis, including defects in leg formation and epidermis differentiation. But how can the lack of small peptides produce such complex developmental phenotypes?

In *Drosophila*, epidermal cells form a distinct pattern of bristles known as trichomes on the body surface of the embryo. Mutants that are unable to form trichomes helped to identify some of the genes controlling this process and the team used one – known as *shavenbaby* (*svb*) due to the embryo's lack of trichomes and smooth appearance when the *svb* gene is defective – to identify the function of *pri* encoded peptides.

The *svb* gene is a master regulator and encodes a transcription factor, which controls the expression of other genes required for trichome formation. To examine the function of *pri* in epidermis differentiation, the French/Japanese team analyzed mutant embryos defective in *pri*, and observed that similar to *svb* mutants they lack trichomes. Knowing that the expression of *svb* target genes was lost while *svb* expression itself was unaffected, the team concluded that the *pri* encoded peptides must somehow act on the Svb transcription factor.

The key to understanding *pri* function was the multiple domain structure of the Svb transcription factor which contains an amino-terminal repression domain, a central activation domain and a carboxy-terminal DNA binding domain. The team carefully analyzed the ability of *svb* and *ovoB* (an activated version of Svb that lacks the repressor domain) to induce trichome formation in epidermal cells that usually lack trichomes. As expected, when *svb* was expressed in smooth epidermal cells, they formed trichomes, but not when these cells lacked *pri*. In contrast, *ovoB* (lacking the repressor domain) induced trichome formation even in cells lacking *pri*. These findings indicate that the *pri* encoded peptides act by triggering the removal of Svb's repressor domain to activate the transcription factor. Finally, immune blots and micro-sequencing showed that the mobility of Svb on electrophoretic gels varied depending on whether or not *pri* was present, and the repressor domain gets lost when the Pri peptides trigger the switch of Svb's transcriptional activity.

Payre, Kageyama and their colleagues have provided exciting evidence for a novel mode of gene regulation mediated by small peptides that trigger cleavage of their target proteins. Possibly, they function as small adaptors guiding proteases to their specific cleavage sites. If so, these peptides create fascinating possibilities to control gene expression and hence facilitate new therapeutic approaches.

10.1242/jeb.036756

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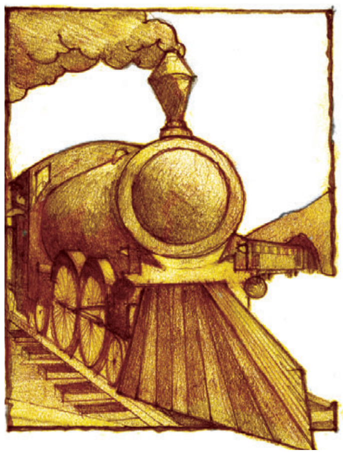
NEURAL CONTROL



FLUCTUATIONS IN WALKING REVEAL NEURAL CONTROL

When people walk, they unconsciously choose a step length and frequency that minimizes the energy required – on average. But every step is actually a bit different. Sometimes the ground is uneven; sometimes the upper body posture is different; and besides, no one can swing their legs exactly the same way every time.

So how do people manage the variability in their steps? The variability isn't purely random, because it is correlated over time: one quick step is more likely to be followed by another quick step than by a slow step. These correlations could be important, because they tend to be highest in healthy people and lower in the elderly and in people with Huntington's disease.

To probe the neural control of cycle-by-cycle changes in walking, Jonathan Dingwell at the University of Texas, Austin, and his colleagues Joby John and Joseph Cusumano at Penn State University, looked at healthy humans walking on a treadmill at a variety of different speeds. The subjects had a clear – if artificial – goal: don't drift off the ends of the treadmill. The authors hypothesized that the subjects used a simple strategy to achieve the goal – match the treadmill speed at each step.

The researchers looked at fluctuations in stride length, stride duration and instantaneous walking speed. Variations in length and duration tended to persist – one short or slow step was more likely to be followed by another short or slow one – a sign that the subjects' nervous systems weren't controlling stride length or duration very tightly. Walking speed had the opposite property, technically termed 'anti-persistence' – high speed steps tended to follow low speed steps and *vice versa* – which the researchers interpreted as a sign of tight neural control.

Dingwell and colleagues propose that the subjects were optimizing performance on what they called a 'goal equivalent manifold' or GEM. On the GEM are all the ways the subjects could walk so that their speed matched that of the treadmill. Variation along the GEM doesn't affect the strategy of matching the treadmill's speed. For example, short quick steps and long slow steps may match the speed equally well. But variation perpendicular to the GEM – short slow steps, for instance – leads rapidly to failure, like falling off the end of the treadmill. The nervous system aims to eliminate that sort of variation. Indeed, when they looked at variation perpendicular to the GEM, they saw statistically significant anti-persistence. In other words, the subjects were correcting themselves to stay on the GEM.

The strategy seems obvious but, in fact, there are ways to stay on a treadmill that don't require any information about the GEM. For example, the subjects could have simply tried to minimize variation in step length and duration independently of one another. But, in that case, the researchers wouldn't have seen any anti-persistence in speed and, indeed, statistical tests indicate that the subjects were not using that strategy.

But what about energy minimization? Short quick steps and long slow steps are energetically costly, even if they do meet the goal of staying on the treadmill. The researchers saw few of these extreme steps. So it appears that the subjects were striking a balance between low energy costs and the strategy of matching the treadmill speed.

The researchers conclude that the nervous system controls tasks in a smart way. It doesn't try to reduce variation that doesn't affect the goal – in this case, staying on the treadmill and simultaneously keeping energy costs low.

10.1242/jeb.036731

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