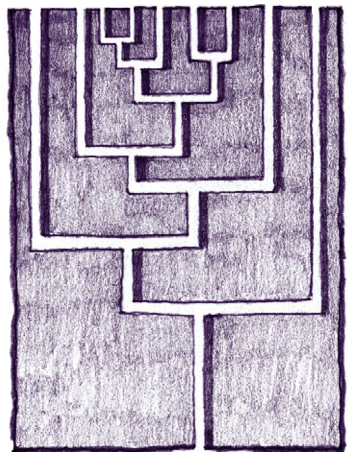
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

VENTILATION



ALLIGATORS, LIKE BIRDS, BREATHE ONE WAY ONLY

It is well established that bird lungs move air in a one-way loop through their gas-exchanging tubes during inhalation and exhalation. In contrast, mammals and presumably all other vertebrates breathe tidally, bringing air into the dead-end gas-exchange structures and back out through the same pathway. One-way airflow is a highly efficient method of breathing, allowing more oxygen to be extracted per breath compared with tidal breathing. Unidirectional airflow is thought to be unique to birds, which have evolved to meet the high oxygen demands of flight. As it turns out, alligators also have a one-way path of breathing, similar to birds.

Conventional wisdom suggests that one-way air flow can only occur with the help of air sacs, bellow-like structures that are found only in birds. C. G. Farmer and Kent Sanders, from the University of Utah, USA, noticed that key features of the alligator lung looked remarkably similar to the bird lung, though alligators lack air sacs. To test the hypothesis that airflow in alligators is unidirectional, the authors performed three separate experiments. First, they artificially ventilated excised lungs from four American alligators and monitored airflow using flowmeters, termed thermistors. Next, they surgically implanted thermistors on six anaesthetized alligators and monitored airflow during normal breathing. Finally, they filled an excised alligator lung with saline fluid containing fluorescent beads and visualized the water flow during artificial ventilation.

All three experiments showed the same remarkable result, that flow occurs in one direction through the lungs of alligators. Notably, air flowed unidirectionally even in excised lungs, suggesting that one-way airflow in alligators is caused by the structural arrangement of the gas exchanging tubes themselves. These results demonstrate that unidirectional airflow can occur in the absence of air sacs, and alternative forms of breathing, such as the hepatic piston method used by alligators, do not preclude unidirectional airflow.

This extraordinary finding has important implications for how scientists think about the evolution of breathing. Farmer and Sanders suggest that unidirectional airflow evolved far earlier than previously thought. They propose that unidirectional airflow was present in basal archosaurs, the common ancestor of crocodilians, birds and dinosaurs. Mammal-like reptiles, termed synapsids, were the dominant land animals during the Permian. However, following the Permian–Triassic extinction, archosaurs emerged as the champions and dominated earth until the Cretaceous–Tertiary extinction, after which synapsids regained supremacy. Farmer and Sanders suggest that unidirectional airflow gave archosaurs a competitive advantage during the low oxygen conditions of the early Triassic by increasing their capacity for exercise. Thus, the alligator's one-way path for breathing could help explain where dinosaurs got the breath to climb to dominance of the earth.

10.1242/jeb.036558

Farmer, C. G. and Sanders, K. (2010). Unidirectional airflow in the lungs of alligators. *Science* **327**, 338-340.

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CLIMATE CHANGE

HOT BUGS SHOW EFFECTS OF CLIMATE CHANGE

Predicting how climate change will affect the survival and development of organisms will be a key challenge for 21st century biology. A recent study of the southern green stink bug, *Nezara viridula*, gives an illustration of how we might go about investigating the effects of global warming.

The stink bug can grow up to 2 cm long and is found in tropical and sub-tropical regions around the world. It is a major agricultural pest on a wide variety of crops, and has been expanding its range towards more temperate zones as a result of global warming.

To discover how *N. viridula* responds to increased temperatures, a group of scientists in Kyoto, led by Dmitry Musolin, kept a population of the bugs outside; half of them were reared in transparent incubators that were 2.5°C warmer than the ambient temperature. The advantage of having glass-walled incubators was that the experimental insects were subject to the same daylight conditions as a group of insects that were kept alongside them in transparent plastic containers with no additional heating.

Every 2 weeks in summer 2006, new clutches of *N. viridula* eggs were added to the experiment, until the insects began to over-winter from November to March. In spring 2007, two further egg clutches were added and allowed to grow. Carried out over 15 months, the study examined how many eggs hatched, and the rate at which the young insects reached maturity and mated, and also measured various physical characters such as size and colouration.

Higher temperatures affected every aspect of the bugs' biology. The insects in the incubators became active earlier in the year, grew more rapidly and laid more eggs. However, in the hot summer months the experimental group showed several responses that suggested that they could not cope with the higher temperatures: the bugs found difficulty shedding their skin as they grew, and tended to die earlier and show physical abnormalities.

The authors point out that their study was somewhat artificial – the temperature increase was constant throughout the year, no predators could get at the bugs, and the experiment was carried out on only one experimental group. Nevertheless, this is one of the first attempts to understand how climate change could affect the future of life. In particular, it suggests that species' responses to increased temperatures may be complex. At some points in the year the warmed bugs prospered, but they did not produce an extra generation because of the damaging effects of the increased summer temperature. The exact reason for these effects is unknown, but it is possible that the bacteria that live in the gut of the bugs were unable to cope with the high temperatures.

In the real world, increased temperatures would affect not only the bugs and their bacteria but also their host plants and their predators. Each of these components of the bugs' ecosystem would probably respond differently to climate change, making the overall outcome hard to predict. However complex this might seem on paper, the reality will soon become apparent to everyone on the planet. This article gives us a glimpse of what the future might hold.

10.1242/jeb.036541

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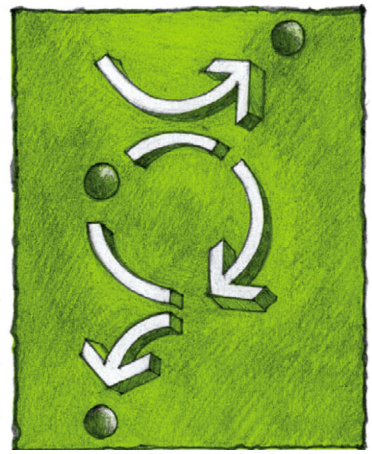
FUNCTIONAL DIVERSITY



THE FLY SAYS LESS IS MORE!

Throughout animal evolution, several innovations allowed organisms to generate genetic 'novelty'. In vertebrates, repeated genetic duplication events fostered the expansion of several gene families. For example, a family that has three or 4 distinct genes in mammals may only have one equivalent gene in insects. So a recurrent question is: how do insects or other animals that didn't experience these large duplication events generate functional diversity? Nigel Price and colleagues examined this question by looking at the metabolic enzyme carnitine palmitoyltransferase 1 (CPT1) in *Drosophila*, as most insects only possess one copy of this gene. This enzyme is essential to import long-chain fatty acids into the mitochondria to break them down for energy production. In comparison to flies, mammals express three distinct isoforms in a tissue specific manner, each isoform differing in its affinity for its substrate (carnitine), and its response to its inhibitor (malonyl-CoA). Price and his team specifically hypothesized that *Drosophila* (and other insects) generated a similar kinetic diversity of this enzyme through alternative splice variants of their single gene. That is, this single 'insect CPT1 gene' could give rise to distinct CPT1 proteins through alternative expression of different parts of the gene.

Initially, the team searched genomic databases to identify possible alternative versions of the CPT1 gene in insects. They discovered that most insects possessed a region of their CPT1 gene that could generate two versions of the protein *via* usage of two interchangeable expressed sequences (exons).

To confirm these predictions, the team undertook gene expression analysis to see whether and how these two putative CPT1 versions were expressed in *Drosophila* tissues. First, through searches in available collections of expressed genes, they identified two separate CPT1 gene transcripts in *Drosophila*. These two transcripts only differed by one small portion of the gene where only one or the other functional alternative exon was expressed, the remainder of the gene transcript being identical. The team confirmed the presence of these splice variants in most *Drosophila* tissues and quantified their relative levels in flight muscle and fat body. Their results established that these two variants were differentially expressed in these tissues indicating possible regulatory and functional differences between the alternative *Drosophila* CPT1s.

Finally, the team characterized the enzymatic properties of the two splice variants in yeast. They determined that the isoform predominantly expressed in flight muscle (a tissue with high levels of fat oxidation) had overall greater enzymatic activity than the other CPT1 variant, suggesting fine-tuning of the CPT1 enzyme profile according to the metabolic demands of the tissue.

The results of the current study clearly show that flies can still create functional diversity in their metabolic processes despite having comparatively fewer genes than some other animals. In addition, the unique kinetic characterization of a non-mammalian CPT1 enzyme generates interesting hypotheses on the functional diversity in CPT1 across animals. Furthermore, this work begs the question of how functionally different the various CPT1 would be in an animal that experienced repeated duplication events and as a result possesses many additional CPT1 copies.

10.1242/jeb.036533

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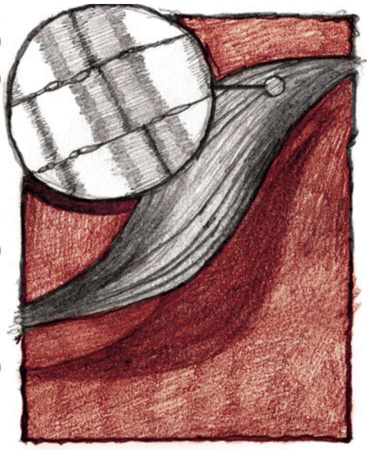
BIOMECHANICS



FROG MUSCLES START STRETCHED

The limb muscles of a frog produce high levels of power to propel the animal into the air during a jump. To do this, they generate large forces quickly, and contract over relatively long distances (up to 30% of their resting length). However, the notion of muscles generating high forces while contracting over a large distance presents a bit of a conundrum because muscles have a relatively narrow range of lengths over which they generate their highest forces. It seems unlikely that muscles that shorten over great distances, like those in frog legs, will spend much time at lengths where forces can be maximized. Manny Azizi and Tom Roberts of Brown University recently addressed this conundrum and, in the process, revealed some surprising results.

Using bullfrogs, Azizi and Roberts chose to measure *in vivo* activation patterns and length changes in the major ankle extending plantaris muscle during jumping. Following these jumping trials, they used an *in vitro* preparation to determine a force–length curve for each muscle they measured *in vivo*. Force–length curves describe how a muscle's length affects its capacity for generating force. They consist of three parts: (1) an 'ascending limb' where force capacity increases as muscles go from short to intermediate lengths, (2) a plateau at intermediate lengths where muscles can maximize force production and (3) a 'descending limb', where further stretching leads to decreased forces. Azizi and Roberts wanted to know where the plantaris was operating in relation to its force–length properties as it contracted during a jump. They predicted that the muscle would start near its optimal length and move down its ascending limb as it shortened during contraction. But they were wrong.

Prior to a jump, it turns out the plantaris is resting at a relatively long length, positioned on the descending limb of its force–length curve, well beyond its optimum length for generating force. During the jump, the muscle contracts enough to put it onto the plateau of its force–length curve – in short, the plantaris ascends its descending limb. The authors were also able to show that this particular shortening pattern, where the muscle starts stretched and ends up at intermediate, optimal lengths, generates higher forces than the alternative strategy of starting at optimal lengths and contracting from there.

These are surprising results! Operating at such long lengths is, or I should say was, considered an anathema for muscles. But this view may be mammal-centric. In mammals, passive tension in stretched muscles increases very rapidly, and it is almost absurd to think of mammalian muscle starting to contract at the sorts of lengths these frog muscles are using regularly (1.33 resting length). However, as Azizi and Roberts show, passive tension in frog muscles stretched to this degree is quite low, allowing them to operate safely at such long lengths. The next time you find a frog, look closely at how the thigh, lower leg and foot are folded on top of one another, and remember that this seemingly awkward limb positioning is likely critical for stretching the underlying muscles so that they can contract over large distances without losing too much force.

10.1242/jeb.036566

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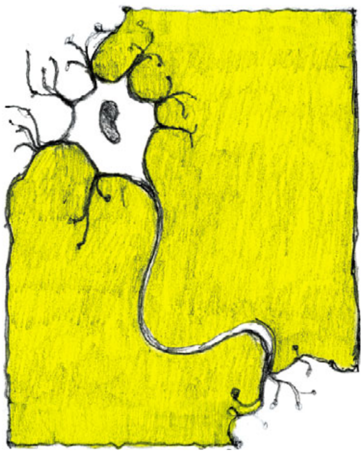
MIMICRY



MALE MOTHS HIJACK BATS' CALLS TO CATCH A MATE

Vision is often almost useless at night so many nocturnal species, such as gleaning bats, resort to acoustic methods to perceive their environment. They broadcast ultrasonic echolocation calls to locate insect prey. Yet many prey species have evolved ultrasonic hearing in a bid to outsmart their hunters, and some prey species have even gone a stage further. They have appropriated bat calls into their own repertoire for sexual communication. Borrowing from their predator, male corn borer moths use ultrasonic songs resembling bat calls to increase their copulation success. Wondering how mimicking bats helps the courting males, Ryo Nakano and colleagues decided to find out precisely how female moths respond to the males' advances and publish their results in *Physiological Entomology*. It seems likely that male calls induce female moths to behave as if they are under attack, rendering them motionless and suppressing female interference during the act of mating.

The team used an experimental approach to listen in to the inner workings of the private world of moth sexual relations. First they silenced the males by surgically removing the sound-producing scales located on their forewings, while puncturing both of the females' tympanic membranes so that they could not hear males' calls. Next they observed how deafened *versus* intact females reacted to male advances, and how frequently mute or intact males convinced females to copulate with them.

Nakano and his team confirmed that when both partners were intact the females readily accepted the males as partners and they copulated successfully 98% of the time. However, when either partner was acoustically impaired (through losing either their hearing or the ability to call), their success fell to roughly 60%: courtship songs are instrumental in corn borer mating. And when the team analyzed the female's responses to the male's ultrasonic courtship calls they found that the females reacted as if they were under attack and froze, making it easier for the males to mount them.

Focusing on call function, the team recorded male songs to see whether they increased the sound level of subsequent mating attempts if the initial attempt failed. Nakano and his colleagues found that the moths did get louder during subsequent mating attempts, suggesting that males can pump up the volume as desperation kicks in. Finally, the team played synthesized calls to intact females at various sound levels and found that sound level impacted strongly on whether the males were accepted as mates. They found that the louder males were more successful, so increasing the volume after a jilting makes perfect sense.

In summary, females' responses to predators and amorous males are identical. They are immobilized by the males' calls, which trigger their anti-predator freezing behaviour. In effect, the male exploits a female sensory bias against calls heralding dangerous predators, causing her to act in a way amenable to mating attempts. However, the calls do not really suit classic notions of chivalrous males wooing females. Here, courtship seems instead to carry decidedly manipulative undertones. After all, males are pulling the wool over their mates' ears by profiting from an existing anti-predator response in order to mate. As males seem to be selected to immobilize females effectively to ensure successful copulation, it would be interesting to investigate existing variation across males in call quality or ability to vary sound levels. Equally, it would be exciting to see whether females can resist manipulation by reliably recognizing differences between bat sonar and male calls.

10.1242/jeb.044529

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