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

COMMUNICATION

## BACK OFF. I'M BIG AND HOT

The question 'Why did this animal evolve to do this?' can often be partly answered by asking 'What tries to eat this animal?' Evolutionary biologists have hypothesized that predator sensory systems in particular guide the evolution of how prey animals sense the world and how prey signals are sent. Aaron Rundus and colleagues at the University of California (Davis) recently tested this hypothesis by studying the kinds of signals California ground squirrels produce during snake attacks.

California ground squirrels are perfect bite-sized meals for many predators. Pacific rattlesnakes in particular appear to relish a squirrel snack. Rattlesnakes have evolved a highly sensitive heat-sensing organ, presumably to help them find 'glowing' mammalian prey. Ground squirrels, in turn, have evolved an impressive repertoire of defenses against the marauding reptiles. For example, when confronted with a snake, squirrels will puff up their tails and vigorously wave them in the snake's face ('tail flagging') while simultaneously kicking up dust and dirt in an attempt to give the snake a good face full. Considering the unique heat-sensory abilities of rattlesnakes, Rundus and coworkers were curious to know whether tail flagging behavior had a thermal as well as a visual component.

In their first set of experiments, the researchers placed hapless ground squirrel volunteers in an enclosure with two different kinds of snake, one with heat-sensing ability (rattlesnake) and one that relies only on visual and olfactory cues while hunting (gopher snake). Understandably, in both situations, the squirrels showed vigorous tail flagging and other close-range snake defense behaviors. But thermal imaging revealed a more complex story. The squirrels significantly heated their tail regions when confronted with rattlesnakes, but not when going tail-to-face with gopher snakes.

In the rattlesnake's world, a hot tail swishing at high frequency might make a small mammal appear much larger and more threatening. To test the adaptive value of such a behavior, Rundus joined forces with a group of mechanical engineers and built a robotic squirrel. The Robo-Squirrel was a stuffed ground squirrel fully equipped with a motorized tail and controllable tail heater. Rattlesnakes are attracted to small baby rodents, so the research team tasked Robo-Squirrel with defending litters of rat pups from rattlesnake attack. When motorized tail flagging was present, rattlesnakes responded by moving less and orienting more towards Robo-Squirrel. These snake behaviors were enhanced when the robotic tail was fired up and began to emit a thermal signature. Furthermore, hot tail flagging by Robo-Squirrel drastically increased the amount of time rattlesnakes spent in two characteristic defensive postures (body coiled, body cocked to strike). These results suggest that hot tail flagging does indeed have adaptive value. The thermal display puts rattlesnakes on the defensive

Rundus and colleagues' simple (if you consider handling rattlesnakes simple) but elegant experiments demonstrate a novel form of animal communication. They show that an animal can use a biological fireworks display to ward off a specific species of heat-sensing predator. These results strongly suggest that the unique sensory abilities of a predator can indeed strongly mold the evolution of signaling in a prey animal. Just as important, this work highlights the limits of our own sensory abilities. As biologists, we have to be careful. If we don't try to see the world through the senses of the animals we work on, we might miss something beautiful and important.

10.1242/jeb.010801

**Rundus, A. S., Owings, D. H., Joshi, S. S., Chinn, E. and Giannini, N.** (2007). Ground squirrels use an infrared signal to deter rattlesnake predation. *Proc. Natl. Acad. Sci. USA* **104**, 14372-14376.

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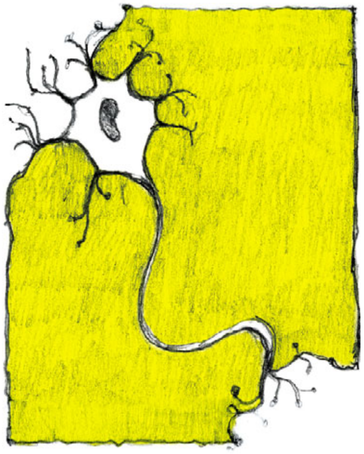
NAVIGATION



## BATS KNOW WHERE NORTH IS

Bats are festooned with super-human abilities. Quite apart from flight and echolocation, remarkable enough in themselves, bats are able to gain useful information from the Earth's magnetic field.

It has been known for some time that many animals – the prime example being homing pigeons – are magneto-sensitive. The situation with bats is only now being revealed because they are far less convenient to work with: they tend to bite, preen vigorously, fly in the dark, and there is no bat-racing industry. In 2006, a team led by Martin Wikelski from Princeton University [Holland et al. (2006) *Nature* vol. 444, p. 702] followed displaced big brown bats as they struggled to find their way home during sunset, after exposing them to rotated magnetic fields. These field measurements, involving pursuing bats with light aircraft, suggest that the animals use a sunset-calibrated magnetic compass.

In order to sort out how bats use the Earth's magnetic field, a lab-based measure in which the bats can be kept in man-made magnetic fields is required. This is what Yinan Wang and colleagues in China and New Zealand have developed and recently published online in the *Proceedings of the Royal Society* B, allowing them to determine whether bats use the same compass mechanism as birds. They found the roosting behaviour of the Chinese noctule bat provided a very simple method for spotting exactly what about the magnetic field is being sensed. The bats that Wang and co-workers were studying chose to roost at the north end of an upturned bucket. The team placed the roosting bucket in a set of Helmholtz coils, effectively electromagnets, which allowed the scientists to manipulate the bucket's magnetic field. Knowing that the bats preferred to sleep at the north end of the roost-bucket, the team switched the bucket's magnetic polarity and found that the bats were 'fooled' into roosting at the south end of the bucket: they detected the polarity of the bucket's magnetic field and roosted accordingly.

This is completely different from the way birds sense magnetic fields: birds are insensitive to polarity, but can recognise the inclination of the field (i.e. the Earth's field lines are inclined vertical at the poles, horizontal at the equator and slowly change their angle relative to the earth as you move between the poles). Birds can calculate the direction towards the nearest pole by measuring the magnetic field's inclination. Testing whether the bats also measure inclination, the team experimentally switched the inclination, but not the polarity, of the magnetic field: yet the bats continued roosting at the north end of the bucket. Unlike birds, bats did not measure the field's inclination.

It is tempting to suggest an adaptive significance of this difference between bats and birds. As birds only detect field inclination, they cannot use magnetism to determine north when they are at the equator: after all, which way is 'down' on a horizontal magnetic slope? They must use other factors to navigate at the equator. Bats, on the other hand, are able to tell the difference between north and south perfectly well from the magnetic polarity, allowing them to migrate and forage large distances near to the equator.

What is perhaps more interesting is what this means in terms of evolution. In all non-mammalian vertebrates, such as birds and lizards, tested so far, direction sensing is based on inclination, whereas mammals, such as naked mole rats and some bats, are receptive to polarity. These findings raise the possibility that compasses have evolved more than once in vertebrates.

10.1242/jeb.010769

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CIRCADIAN CLOCK

## STOPPING THE CLOCK

Hibernation is a fascinating, yet enigmatic, physiological phenomenon utilized by some mammals to successfully cope with the extreme conditions of a harsh season such as winter. During the inhospitable season, hibernating animals repeatedly alternate between brief periods at normal body temperature (Tb) of 37°C (known as euthermia) and a state of torpor when Tb drops to as low as 5–10°C and biological processes such as heart rate, respiration rate, immune and renal functions and neural activity are slowed to a minimum. This strategy allows for substantial energy savings, enabling the organism to survive the severe conditions.

Normally, an animal's circadian system serves to coordinate internal biological processes with each other and the environment to ensure health and survival. However, it has long been debated whether the circadian clock continues to function in hibernating mammals. Numerous studies have indirectly investigated this question by examining several different markers of a functioning circadian clock in hibernating animals. But, results have been conflicting; with some studies contending the clock continues to function, whereas others claim it stops.

Dr Paul Pévet's group at Université Louis Pasteur, Paris, reasoned that the best way to resolve whether or not the circadian clock continues to operate during hibernation would be to directly examine whether the 'core clockwork machinery' (i.e. the molecular mechanisms underlying the ticking of the clock) still functioned as normal during hibernation. The team explains that circadian oscillations result from the recurrent expression of so-called clock genes in a region of the brain known as the suprachiasmatic nucleus, or SCN for short. These clock genes interact in complex, interlocked transcription/

translation feedback loops, resulting in significant day/night differences in gene expression. Therefore, the team surmised that a stopped clock during hibernation would be reflected by a loss of the rhythmic expression of the clock genes.

Employing the European hamster (*Cricetus cricetus*), a well-defined hibernator, as a model species, the researchers examined the expression levels of three clock genes and another clock-controlled gene in euthermic and hibernating animals. As expected, the researchers observed that in non-hibernating hamsters, significant day/night changes occurred in the expression of the clock genes. In contrast, in hibernating hamsters exhibiting torpor, the day/night differences in gene expression disappeared. Rather, the expression of the clock genes in the brain's SCN remained constant. Most importantly, the team observed that the oscillations in clock gene expression reoccurred during the inter-torpor periods of euthermia.

Overall, the authors argue that their novel data provide strong evidence that the molecular circadian clock stops 'ticking', at least in the European hamster, during the torpor periods of hibernation. The teams' next steps will be to elucidate the mechanisms by which the stopping of the clock occurs and whether the phenomenon is species and/or temperature specific.

10.1242/jeb.010751

**Revel, F. G., Herwig, A., Garidou, M.-L., Dardente, H., Menet, J. S., Masson-Pévet, M., Simonneaux, V., Saboureau, M. and Pévet, P.** (2007). The circadian clock stops ticking during deep hibernation in the European hamster. *Proc. Natl. Acad. Sci. USA* **104**, 13816-13820.

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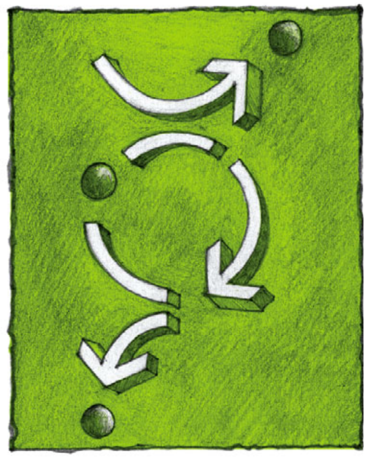
ENERGETIC COSTS



## FLASHDANCE

To humans, fireflies are rather eye-catching little creatures. This is a consequence of their bioluminescent signals, which are mainly used for signalling mating availability and, thus, attracting mates. Once the interest of another firefly has been sparked, the flash pattern changes and a new pattern is used to convince the potential mate that they have made a wise decision. But to another firefly, not all flashing signals are equally attractive and fireflies that are overly lascivious in their signalling might not be gaining much in the long term. Putting on a great visual performance could, for example, increase the risk of predation or, alternatively, decrease valuable energetic resources with later repercussions for survival. So how important are these various costs to the soap-opera lifestyle of fireflies and what factors keep a check on a firefly's visual display?

William Woods and co-workers at Tufts University set out to explore these questions using two complementary experiments. In the first experiment, using four species of fireflies, they compared the relative energetic costs of flashing by estimating resting metabolic rates under controlled laboratory conditions in several *Photinus* species differing in their bioluminescence characteristics. The team found no significant differences in metabolic rate between the nocturnal *Photinus* species that use flashing to communicate and day-active *Photinus* species, which do not signal by flashing.

In addition, the team monitored flash rate and metabolic rate within one of the nocturnal species that uses bioluminescent signalling, *Photinus greeni*, as the fireflies signalled, rested or wandered around. Here they found two important and novel discoveries. First, when individual fireflies were flashing, metabolic rates increased by roughly 37%, although this was approximately 20% less than the cost of walking. Second, they saw a positive relationship between signalling rate and metabolic rate: individuals of *P. greeni* that flashed more quickly incurred a higher energetic cost. Therefore, from an energetics perspective, flashy performances were indeed more costly within a firefly species.

In the second experiment, using field experiments designed to assess the potential predation costs associated with courtship signals, the researchers built tiny light traps that mimicked firefly courtship patterns in the wild and monitored how many predatory *Photuris* fireflies they caught. Predatory *Photuris* fireflies were attracted significantly more frequently to the light traps emitting courtship signals that simulated *P. greeni* signal patterns. Quite literally, hundreds of predators flocked to this bevy of potential meals during these trials. In addition, the more closely the pattern resembled *P. greeni*'s true mating signal, the more predatory *Photuris* fireflies were trapped at the miniature lights. Eavesdroppers were indeed keeping close track of their food source.

This study by Woods and co-workers represents an exciting glimpse into the world of energetic costs of courtship signalling in fireflies. Furthermore, the team has clearly demonstrated that natural variation in signalling alters the risk of predation and can incur a metabolic cost. In consequence, the flashdance of fireflies probably represents an evolutionary compromise between attracting the right mates without using too much energy, and simultaneously minimizing the risk of becoming another firefly's dinner.

10.1242/jeb.010777

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## ON THE LEGS OF GIANTS

Judging by the number and variety of 'giant insect' horror movies, there is something primal about the human fear of bugs, especially really big ones. Imagine having to dodge the attack of an ancient dragonfly with a wingspan of nearly a meter! Giant insects were common during the late Paleozoic era. Luckily, there appears to be a size limit on modern insects that keeps them from becoming the stuff of science fiction, but the nature of this limitation is as of yet unknown. One major difference between the late Paleozoic and modern times is the amount of oxygen in the Earth's atmosphere. Back in the time of giant insects, oxygen comprised about 30% of the Earth's atmosphere, compared with about 20% today. Some believe that the higher levels of atmospheric oxygen in the Paleozoic period allowed insects and other creatures to grow to sizes much larger than currently possible by allowing higher rates of oxygen delivery to the tissues. In their recent PNAS paper, Alexander Kaiser and coworkers from Midwestern University and Arizona State University set out to test the validity of this 'oxygen limitation hypothesis' by measuring the relationship between insect body size and the tracheal system to see if the oxygen delivery system could limit insect size.

The team used synchrotron x-ray phase contrast imaging to non-invasively measure the volume of the tracheal system in live, but immobilized beetles. They investigated four species of darkling beetles that differed in their body mass by three orders of magnitude.

The team found that in the largest beetles about 4.8% of their total body volume is composed of the tracheal system, while in the smallest beetles the tracheal system accounts for only about 0.5%. They also noted that different body compartments appeared to be under different constraints. Tracheal volume increases in a roughly equal relationship to body mass in the head region, and they concluded from this that oxygen delivery to the head is not likely to limit body size. However, oxygen delivery to the legs is a different story. In smaller beetles the tracheal tube occupies only 2% of the leg orifice, while in larger beetles about 18% of the opening is occupied by the tracheal tube, leaving precious little space for all the other parts to operate.

These results suggest that larger beetles must devote a greater proportion of their body volume than small beetles to the tracheal system for gas exchange purposes. This relationship appears to be especially important in the supply of oxygen to distant and isolated parts of the body such as the legs. The team suggests that the increased proportion of space occupied by the tracheal system in large beetles will likely impose tradeoffs in other physiological systems, and may eventually lead to a constraint on maximal body size. Based on the data presented for oxygen supply to the legs, the authors predict a maximal beetle body length of around 16 cm, which closely matches the 17 cm size of the largest living beetle *Titanus giganteus*. An increase in atmospheric oxygen concentration such as that experienced in the late Paleozoic era would certainly help to alleviate constraints on body size imposed by the tracheal system in modern insects, by simply delivering more oxygen to the tissues per unit of air exchanged.

Maximal body size in insects may very well be limited by oxygen concentrations in the atmosphere. So for now we can all breathe easy knowing that giant insects are a thing of the past. However, don't be surprised if the opening scene of the next big horror movie spotlights an ordinary cockroach scurrying into an oxygen bar!

10.1242/jeb.010785

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