Outside JEB

PETAL BUMPS HELP BUMBLEBEES GET A GRIP

Most flowering plants have evolved arrays of tiny cone-shaped cells on their petals. But the adaptive value of these structures for plants has never been clear. Why did they evolve? Heather Whitney, Lars Chittka, Toby Bruce and Beverley Glover recently tested the idea that these conical structures have evolved to act as ‘handles’ for pollinators to grab on to as they approach from the sky.

First the team wanted to see if bumblebees could distinguish between flowers just by the feel of conical cells. To do this, they used mutant white snapdragon plants. These mutants are identical in sight and smell to common white snapdragons but have flattened cells instead of conical cells on their petals. Whitney and her colleagues provided the bees with smooth flowers (from the mutant snapdragons), containing a small tube of sweet nectar at the base of the petal, and bumpy normal snapdragon flowers flavoured with bitter quinine. The team let single bees wander over the flowers and repeatedly sample nectar from both types of flower. Each time an individual landed on a flower, the researchers noted how long the bee lingered and whether or not she made any effort to drink. If the bees really could feel the texture differences between the smooth and bumpy flowers, they would associate landing on a bumpy flower with bad tasting nectar and vote with their wings, taking off to find a better tasting flower. Indeed, after a few trials, individuals started taking off as soon as they came into contact with the bumpy surface. The animals were clearly feeling their way to a decision by gauging the shape of petal bumps.

In the next set of experiments, the researchers tested whether bees could discriminate fine textures without any other biological or chemical cues present. They made epoxy discs with bumpy and flat surfaces closely approximating those found on actual petals. Then they used these artificial flowers in place of actual snapdragons. The researchers tried coupling bitter nectar with bumps and sweetness with flat cells (and *vice versa*). Regardless, the little foragers learned not to waste time on any surface that they had learned to associate with a bitter reward.

So it seems that bees can discriminate between petals on the basis of conical cell texture. But why have plants evolved these structures? After all, there are more efficient ways to attract the attention of pollinators without forcing them to land. The team wondered whether these structures might provide good grip for insects landing on otherwise slippery plant surfaces. To test this, Whitney and her colleagues presented bees with horizontal conical and flat-celled epoxy ‘flowers’, each providing a sugar reward. The bees showed no preference for either surface. Then the team gradually tilted the landing sites. As the steepness of the landing surfaces increased, the bumblebees shifted their foraging to food sources with bumps. The team then went back and did the same experiment with actual snapdragon flowers. Again, when the landing surface was tilted, the bees showed an increasing preference for flowers with conical textures. In both cases, high-speed videography confirmed that the bees were ‘slipping and sliding’ on steep surfaces with flat cells, confirming that the conical cells make it easier for approaching pollinators to grab onto a flower.

The experiments in this study are very simple but also very elegant. This work stands out as a great example of how excellent science can be still done by doing simple manipulations without a lot of fancy equipment. This work is also significant because it effectively tests the question of ‘why?’ in evolution. This is not always an easy question to unambiguously answer. But in the end, it’s one of the most important questions that we can ask as biologists.

10.1242/jeb.021485

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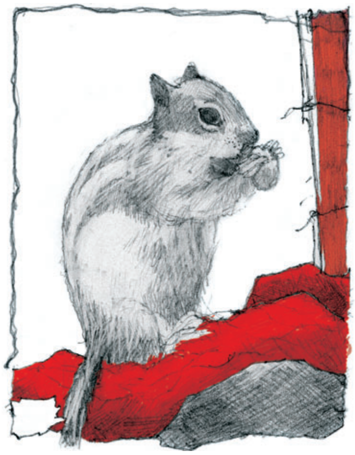
ECHOLOCATION



TWITTER: WHAT ARE SHREWS DOING?

Shrews are famously active predators, constantly on the move and searching for new foraging opportunities. Along with scurrying, they spend a great deal of time 'twittering' – producing brief, high-pitched squeaks. And, for some reason, these sounds become more frequent as shrews investigate novel surroundings. What are these twitters used for?

One theory is that they may be used for 'staying connected in real time' like online human tweeters. Shrews may twitter to determine, connect with or indeed avoid other shrews in the environment. If this is the case, then one might expect more excitement – and twittering – if they had reason to believe that other shrews were at large. In order to test this, Björn Siemers, Grit Schauermann, Hendrik Turni and Sophie von Marten caught common and white-toothed shrews and recorded each animal's squeaks as they wandered around cages, either with or without their own species' scents. As signs of a nearby neighbour did not elicit a significant change in squeak rate, the team concluded that shrews were not using twitters to get in touch with residents.

By contrast, the insectivores appeared to use their squeaks for some form of echolocation. The rate of squeaks related strongly to the flooring in the cages: the squeak rate was low in cages with only a thin layer of hay and dramatically higher in thick hay. This echoes the phenomenon in bats, where call rates are relatively high in cluttered environments; the shrew results may suggest that echolocation allows them to explore their environment or identify routes. But how effective are shrew squeaks for echolocation?

In order to approach that question, the team made an 'artificial shrew', consisting of a small shrew squeak emitting speaker and a very sensitive microphone to record the squeaks' reflections. And then this pseudo shrew was squeaked in a range of shrew-friendly habitats. The echoes returned were of very different intensities, with moss returning very little sound and leaf litter a great deal. So, echoes from shrew squeaks might carry some information concerning general habitat type.

A couple of issues make bat-like use of echolocation to search for prey very unlikely. One is termed 'forward masking', which is the closest that an object can be and produce a clear reflection without interference from the outgoing squeak. In the case of the shrews, that distance is 1.7 m for a 10 ms squeak. Another is 'backward masking', where the reflections from a prey item's surroundings make picking out a target tricky – the leaf on which the prey is sitting sends back a very similar signal to the prey.

While the properties of the shrew twitter share some of the characteristics of the echolocation squeaks of bats, it appears likely that shrews are unable to 'disentangle echo scenes' and hunt in the same way that bats do.

However, the echolocation abilities of shrews may not be limited to the identification of habitat types; further experiments with the artificial shrew suggested useable information may be available from echoes returning from relatively large and sturdy objects, such as a brick, even when hidden by 'moss' or 'meadow'. While not as impressive as pinpointing a flying moth, this capacity could be most useful. But whether such a signal is ever actually used to determine escape routes or find safe places to hide will take further studies.

10.1242/jeb.021402

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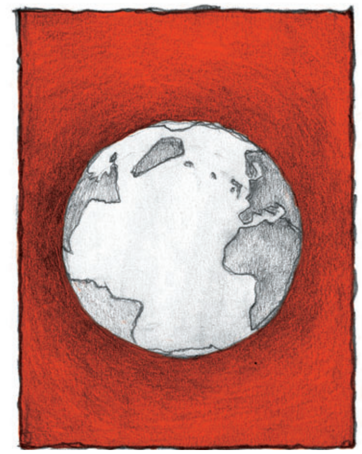
HABITAT



HISSING COCKROACHES ARE VERITABLE MITE OASES

The giant Madagascan hissing cockroach, *Gromphadorhina portentosa*, and the dermanyssid mite, *Gromphadorholaelaps schaeferi*, have evolved a very close partnership. The mites spend their entire life cycle on their host and when the host dies so do its mites. Some female mite foundresses do move to adjacent unoccupied hosts but with this unusually heightened degree of host permanency the mites are entirely dependent on their host for resources. However, they do not parasitise the roaches. Instead they evolved ptyalophagy – feeding on their host's saliva – clustering between the host's legs to feed on saliva and food debris. The mites can also absorb water vapour *via* their oral cavity and cluster around the roaches' spiracles, which release humidified air. In return the mites clean food debris from their hosts, and their predatory nature is seen as an underlying reason for the mite being the only species that lives on *G. portentosa*. Jay Yoder and colleagues set out to investigate the water requirements of this mite that evolved to live exclusively on the hissing cockroach.

In a series of elegant experiments the Ohio-based team recently demonstrated the close correlation between *G. schaeferi*'s water balance strategies and the water levels in the microenvironment provided by *G. portentosa*'s bodies by constructing a water balance profile for each life cycle stage. They determined survival requirements, developmental shifts in water balance and the humidity levels of the environment provided by the hosts.

Measuring desiccation tolerance (minimum amount of body water required) and desiccation resistance (retaining water) the team collected mites at all life stages, weighed them, placed them in 0% humidity at 25°C, weighed the mites at regular intervals and monitored the mites' survival rates. Well-hydrated mites had body water contents ranging from 62% (larvae) to 75% (adults) and could withstand water losses of 17% in adults up to 24% in protonymphs. Mites lost water ranging from 2.8% h^{-1} (larvae) to 0.3% h^{-1} (adults), limiting survival to 7–56 h.

The team also determined the mites' ability to absorb water vapour by exposing them to humidities ranging from 75% to 100%. They found that protonymphs required an atmosphere with 77% and adults 93% humidity to absorb water vapour, while larvae required 100% humidity to prevent water loss.

Finally, assessing whether the mites drink to replenish water loss, the team gave the mites access to free water and roach saliva (both stained with Evans blue so that the team could track whether the mites were drinking). Interestingly, the mites did not drink free water at any life stage; however, all stages, except the larvae, eagerly drank roach saliva.

Yoder's results show that species that thrive in dry environments have low water loss rates whereas species with high water loss rates require moist habitats. *Gromphadorholaelaps schaeferi* are extremely vulnerable to desiccation and have high rates of water loss, especially the larvae. Lacking functional mouthparts, the larvae neither drink nor absorb water vapour but avoid desiccation by progressing rapidly to the protonymph stage 6–8 hours after birth. To remain in balance with their environment, protonymphs absorb water vapour at lower humidities whereas adult mites reduce their water loss rates to survive. Throughout all life stages *G. schaeferi* require large amounts of fluid and their refusal of free water for roach saliva clinches their dependence on *G. portentosa*. By providing an ideal and stable moisture-rich microhabitat, meeting all of the mites' water and nutritional requirements, each individual hissing cockroach thus effectively becomes a self-contained mite ecosystem.

10.1242/jeb.021444

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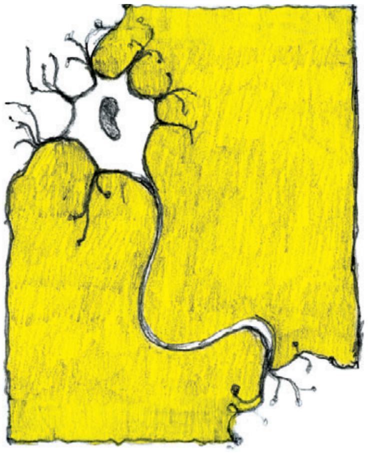
HYPOXIA TOLERANCE



PROGRAMMED CELL DEATH THWARTED IN ANOXIC TURTLE BRAIN

The brains of most vertebrates, mammals especially, are extremely sensitive to a lack of oxygen. Within a few minutes of oxygen deprivation induced by lack of blood flow (ischemia, stroke) mammalian neurons experience a massive loss of ATP, loss of mitochondrial integrity, depolarization of cellular membranes, and the release of calcium stores and a number of compounds that induce cell death. Cell death can be immediate due to massive failure of the cell to maintain homeostasis (necrosis) or delayed due to conditions that activate a variety of programmed cell death (apoptosis) pathways that take several hours to initiate and execute. However, a handful of vertebrates, including the freshwater turtle, *Trachemys scripta elegans*, have evolved the ability to survive without oxygen for extended periods of time with no apparent loss of neurons. Shailaja Kesaraju and colleagues at Florida Atlantic University set out to explore how the neurons of this turtle avoid cell death in the face of a complete lack of oxygen (anoxia).

Sampling the brains of turtles after 24 h of anoxia at room temperature (22–23°C) and after 1 and 3 days of recovery from anoxia, the team examined sections of the turtles' brains to see how they faired after oxygen deprivation. The team measured levels of overall cell survival and organization, and monitored changes in neurons and astrocytes in the brain sections and found no major changes in cell number or morphology in the turtles' brains 3 days after a 24 h exposure to anoxia.

In a separate set of experiments, the team examined the expression of a suite of proteins in turtle brains 1, 4 and 24 h after anoxia. They also assessed how the turtles recovered from anoxia by measuring the expression of these proteins after the turtles experienced 4 h of anoxia followed by a 4 h period of recovery in air. Proteins monitored included a number of heat shock proteins (Hsp), such as Hsp72, Hsp27, and proteins involved in programmed cell death such as Bcl-2, Bax, apoptosis-inducing factor (AIF) and caspase-3.

The levels of the stress proteins Hsp72, Hsp60, heme oxygenase-1, Hsp27 and the glucose-regulated protein Grp94 rose significantly after 1 h of anoxia and remained elevated after 24 h of anoxia. The increases in stress response proteins are likely to reduce cell death by stabilizing components of the cell (e.g. macromolecular complexes, cytoskeletal elements) that may be disrupted by changes in cell physiology associated with anoxia and by blocking induction of apoptosis. This is especially true of Hsp72 and Hsp27, both of which are potent molecular chaperones that act to stabilize proteins, membranes and DNA to protect the cell from stress-induced damage. They have also been shown to block apoptosis in mammalian cells. The team also found that the turtle brains block multiple pathways that induce apoptosis, including AIF, Bax and caspase-3 proteins.

The wealth of information on stress response and apoptosis-regulating proteins presented in this paper paint a very complex molecular response to anoxia that blocks the induction of apoptosis in turtle neurons and prevents tissue death. These data indicate that turtle brains are probably pre-adapted to block the molecular events that induce apoptosis in mammalian neurons during anoxia, in order to protect them from brain damage during oxygen deprivation.

10.1242/jeb.021527

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