At 5:58 a.m. on the 28th of June 1992, the ground began to tremble in the Mojave Desert, California. John Lighton and Frances Duncan, in the midst of a study on ant locomotion energetics, were sitting near a creosote bush collecting data on their computer when the magnitude 7.4 earthquake struck. It was an impressive event: 'our car was bouncing on its springs', Lighton recalls. The startled researchers had the presence of mind to key a time stamp into their computer and continue their study. Lighton and Duncan realised that they had a perfect opportunity to test the anecdotal reports that animals can predict earthquakes, which have crept into the popular press in recent years (p. 3103).

Lighton and Duncan had been measuring desert harvester ants’ metabolic rate to calculate their cost of transport. Every day at the crack of dawn, as the ants began to forage, the pair laid a compressed-fibre board in the ants’ path to separate the ant trail from the CO\textsubscript{2} coming from the ground. The ants happily marched through a respirometry chamber nestled on the board, so the researchers could measure the ants’ CO\textsubscript{2} production. A video trained on the ant trail allowed the pair to count the number of nest-bound and outbound ants and measure each ant’s size. Finally, they measured air temperature at ‘ant height’ by placing a thermocouple 3 mm above the ground.

With this suite of measurements taken before, during and after the earthquake, Lighton and Duncan could test whether the ants reacted to the multiple quakes, aftershocks and possible precursors on E-day – the day the earthquake struck – relative to other, earthquake-free days. First, they tested anecdotal reports of ant nest evacuations during earthquakes. To detect any mass exodus, they measured the ratio of nest-bound ants to total traffic (nest-bound plus outbound foragers) on E-day and subsequent days. To their surprise, the earthquake and its aftershocks had no effect on ant traffic dynamics. Next, they examined whether ants ran slower or faster during the earthquake than on other days. They found that temperature accounted for 87% of the ants’ running speed, and the relationship between running speed and temperature on E-day was identical to the relationship on subsequent earthquake-free days. Reasoning that seismic activity might cause ants to reallocate tasks and keep ants of certain size inside the nest, Lighton and Duncan looked for differences in foraging ants’ body size. Again, there was no difference between E-day and other days. In a last-ditch attempt to find some sign that the ants weren’t completely oblivious to the shaking earth, they looked for anomalies in metabolic rate on E-day – and failed to find any. ‘We were convinced that the ants would show some abnormal behaviour, so we were amazed to find that the ants kept slogging on as usual’, Lighton says.

Lighton and Duncan concluded that ants can't predict – and apparently don’t even react to – earthquakes. But their study highlights an important issue: what to do if you realise during data collection that you can answer a different hypothesis than the one you set out to test. To test the untestable, like the claim that animals can predict earthquakes, we have little choice but to rely on serendipitous events. After all, how likely is it that you’ll get funding to scrutinize animals while patiently waiting for an earthquake to strike?

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Yfke van Bergen

MOSQUITO SALIVARY GLANDS LURE MALARIA PARASITES

James Dvorak has devoted his career to the study of malaria, ‘one of the major maladies of our time.’ Over the years, Dvorak has probed the intricate details of the malaria parasite’s voyage inside its mosquito host. Now, working with Mayumi Akaki, Dvorak has found that the sporozoite stage of the malaria parasite’s lifecycle finds the chemical lure of mosquito salivary glands irresistible (p. 3211).

The malaria parasite divides its lifecycle between two hosts: humans and mosquitoes. When a mosquito gorges on a
blood meal from a person infected with malaria, it unwittingly sucks the parasite’s gametocytes into its body. Once inside the mosquito, the gametocytes fuse and the resulting ookinete invades the mosquito’s gut wall. Eventually, the developing oocyste bursts, releasing delicate spindle-shaped sporozoites into the insect’s circulatory system. Coursing around the mosquito’s body, the sporozoites are incredibly selective; they only invade the insect’s salivary glands, ready for the mosquito to feast on its next human victim.

Dvorak wanted to know how sporozoites make their way to a mosquito’s salivary glands. ‘This is a tricky question to answer’, he says, explaining that the salivary glands are tucked away in a mosquito’s thorax, which is covered by an opaque cuticle that makes it impossible to study the glands directly under a microscope. Dvorak suspected that sporozoites use a chemical cue to locate the salivary glands as they are swept along by the mosquito’s circulatory system. Teaming up with Akaki, he painstakingly developed a homemade system to discover whether sporozoites are attracted to mosquito salivary glands.

First, Dvorak and Akaki needed to show that sporozoites can steer themselves in a particular direction. If you place the parasites on a glass slide and peer down a microscope, you’ll see that the sporozoites glide around in elegant circles. ‘But that doesn’t get us anywhere, and it certainly doesn’t get the sporozoites anywhere!’ Dvorak remarks. To find out whether the parasites were simply being constrained by their 2D environment, Dvorak and Akaki created 3D surroundings that would allow the parasites to show off their locomotor skills. They developed a tiny chamber that holds a microscopic amount of Matrigel, a semi-solid matrix, and loaded the chamber with green fluorescent protein-expressing sporozoites. Capturing every move of the fluorescent parasites using a video camera and a 3D motion-tracking program, they were delighted to see that the sporozoites adeptly travelled through the Matrigel using a corkscrew-like motion.

But are the sporozoites attracted to mosquito salivary glands? To find out, Dvorak and Akaki added salivary gland extract to one end of the chamber. Sure enough, the sporozoites navigated towards the extract. After carefully ruling out other explanations for the sporozoites’ movements, the pair concluded that the parasites use chemotactic gradients to locate a mosquito’s salivary glands.

Dvorak and Akaki’s findings offer a tantalising new approach in the fight against malaria. Treating a mosquito with an anti-chemotactic substance to counteract the sporozoites’ chemical attraction to the salivary glands could leave the parasites stranded without a map on their long journey through the mosquito body.

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**FISH SING, FISH HEAR**

Vocal communication is important for the survival and reproductive success of all animals. This is especially true for the nocturnally breeding teleost fish, the plainfin midshipman – fondly nicknamed the ‘Californian singing fish’ because their humming mating calls are familiar nocturnes to Californian locals. Reproductive females must recognize the high-frequency components of those ‘hums’, which is absent in other types of vocal signal, to locate the love nests carefully prepared by the calling males. One question that has been puzzling fish biologists is how auditory function in fish changes during development. Joseph Sisneros of the University of Washington and Andrew Bass of Cornell University studied auditory encoding in the plainfin midshipman, and report significant differences in the auditory function of adults and juveniles of this species (p. 3121).

Sisneros and Bass conducted an analysis of age- and size-related changes in the encoding properties of individual auditory neurons in the plainfin midshipman. Recording individual neurons in very small juvenile fish is no mean feat. These are very delicate creatures, but the midshipman has now proven to be a useful model system for fish physiologists that want to study changes in hearing as an animal transitions from young life history stages to an older, reproductively mature state. Sisneros and Bass opened midshipman’s skulls and inserted electrodes into auditory neurons in the inner ear’s sacculus – the main hearing organ in midshipman. They then lowered the fish just below the water surface, ~10 cm above an underwater loudspeaker in a tank that was housed inside an acoustic isolation chamber on a vibration isolation table.

In this sophisticated recording setting, Sisneros and Bass were able to test whether juveniles hear as well as adults, and whether they can discern the vocal signals that adults can hear. The pair first studied the basal firing rate of auditory neurons in adult fish and in small and large juveniles – 130–160 and 160–370 days post-fertilisation age, respectively – in the absence of acoustic stimulation. They found that basal neuronal activity, a reflection of the neuron’s sampling rate and excitability, increases with fish age and body length. The researchers then determined the lowest intensity of a fixed-frequency acoustic stimulus that the fish could detect, as judged by the presence of neuronal firing. Interestingly, they discovered that large juveniles and adults hear five times better than small juveniles. But can juvenile fish differentiate various types of vocal signal with different frequency composition? Animals preserve a sound’s frequency information by adjusting neuronal firing patterns to the time-varying structure of the sound wave – a process known as synchronisation or ‘phase-locking’. Sisneros and Bass found that, like their non-reproductive adult counterparts, both small and large juveniles are best adapted to low frequency ‘grunts’, which are important for agonistic encounters. But juveniles do not phase-lock to the higher frequency components in ‘growls’ and ‘hums’ used as mating calls by midshipman males, presumably because these signals are not yet relevant to them. ‘It isn’t clear if the juveniles are sonic. They may make only ‘grunts’ – to warn and to avoid predators may be the most important thing for them at this stage’, Sisneros concludes.

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The world is filled with polarized light, but unfortunately we’re oblivious to it. While we have to accept the limitations of our visual capacities, some birds and insects eagerly exploit their ability to see polarized light. But can any animals actually discriminate between different orientations of polarized light? Martina Mussi, Theodore Haimberger and Craig Hawryshyn set out to test whether damselfish can detect differences in the angle, or e-vector, of UV polarized light (p. 3037).

Hawryshyn and his colleagues trained damselfish to swim towards a particular e-vector orientation (0° or 90°) of UV polarized light. When the team gave the trained fish a choice between two light beams with 0° and 90° e-vector orientations, they found that damselfish chose the ‘correct’ beam over 80% of the time. What’s more, fish made the right choice regardless of brightness differences between the two beams, so they don’t simply rely on differences in light intensity. The team concludes that damselfish can clearly discriminate between the horizontal and the vertical plane of UV polarized light. But when the team filtered out the light beams’ UV wavelengths, the fish chose randomly, suggesting that damselfish need UV light to distinguish between different e-vectors.

How might damselfish use this curious ability? It could give them an edge when they’re hunting transparent plankton in their underwater homes; plankton exoskeletons scatter polarized light, so hungry damselfish with an eye for different e-vectors may find it easier to spot their bite-sized meals.

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