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

DIRECTING TRAFFIC AT THE NEUROMUSCULAR JUNCTION

Motor nerves activate muscles *via* the release of acetylcholine, a neurotransmitter that binds to muscle cells' acetylcholine receptors. The occupied receptors then allow Na^+ ions into the muscle cells, depolarizing them and causing them to contract. This transmission of signals across the neuromuscular junction is most efficient when the receptors are positioned close to the sites of acetylcholine release, so it is not surprising that the receptors form clusters at these locations. But how do they 'know' where to congregate? A recent study of the nematode *Caenorhabditis elegans* by Christelle Gally and colleagues shows that a protein known as LEV-10 enables acetylcholine receptors to cluster properly, at least in this particular organism.

Gally and her co-workers at l'École Normale Supériere (Paris) and the University of Illinois at Chicago studied worms with a mutation in their *lev-10* gene. The researchers initially observed that the mutants' mobility was somewhat impaired. When wild-type and mutant worms were compared in a 'thrashing assay' – a common motor performance test in which worms are induced to thrash by dunking them in a solution of the neurotransmitter serotonin – the frequency of body bends per minute was about 25% lower than normal in the mutants.

Why were the *lev-10* mutants' thrashing abilities impaired? Gally's group suspected a problem with a type of acetylcholine receptor that happens to be sensitive to the drug levamisole. They found that the mutants synthesize normal amounts of intact, functional levamisole-sensitive receptors; however, these receptors fail to cluster near acetylcholine release sites, dampening the muscles' responsiveness to the nervous system. Since the mutants cannot produce LEV-10, the researchers concluded that LEV-10 is required for normal clustering of these receptors.

How does LEV-10, a transmembrane protein, direct the clustering of acetylcholine receptors in wild-type worms? The mechanism must involve LEV-10's extracellular region because this region is sufficient to cause clustering, as Gally and co-workers found when they provided *lev-10* mutants with truncated versions of the protein. The extracellular region contains one LDLa domain and five CUB domains, which are thought to promote binding to other proteins. However, the LEV-10 protein does not appear to bind directly to acetylcholine receptors. Therefore, the researchers speculate, 'LEV-10 might be indirectly involved in the recruitment of signaling molecules that, in turn, cause acetylcholine receptor clustering.' The molecules that initially draw LEV-10 to the cluster sites remain to be identified.

While the importance of LEV-10 itself may be limited to *C. elegans*, there are hints that analogous proteins may control the positioning of receptors within vertebrate nervous systems. Specifically, the mouse protein NETO2 has two extracellular CUB domains with amino acid sequences similar to those of LEV-10, as well as one extracellular LDLa domain. Although the functions of this and related mouse proteins are unknown, it is possible that they too promote the clustering of neurotransmitter receptors *via* extracellular interactions with other proteins.

10.1242/jeb.01465

Gally, C., Simer, S., Richmond, J. E. and Bessereau, J.-L. (2004). A transmembrane protein required for acetylcholine receptor clustering in *Caenorhabditis elegans*. *Nature* **431**, 578-582.

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MOTH SEX PHEROMONES

THE SWEET SMELL OF SUCCESS

…for male moths, that is. Few students of zoology will be unaware of the remarkable mate-finding ability of certain male moths. As this occurs at night, the cues given by a receptive female are necessarily olfactory; she exudes from her abdomen tiny quantities of a volatile messenger, or pheromone. Using their hugely elaborate, sexually dimorphic antennae, males can detect females from (literally) miles downwind, apparently on the basis of receiving a signal from a single pheromone molecule. The male then closes in on the female with a simple strategy; he keeps flying upwind while the pheromone concentration increases, and breaks into zigzag hunting mode whenever he loses the signal. The success of this system is manifest; but how is it achieved at the molecular level? The female sex pheromone of the silk moth *Bombyx mori* is known to be a single molecule, the unsaturated long-chain alcohol bombykol. Sakurai and colleagues have now succeeded in identifying and characterising the male silk moth antennal receptor that responds to this sex pheromone.

Since the receptor responds to a female pheromone, the receptor mRNA was likely to be enriched in male antennae. So Sakurai et al. started with a *B. mori* adult male antennal cDNA library and screened it differentially, using male antennal cDNA as a positive probe and male body cDNA as a negative control. One of the 62 clones that the team sequenced was clearly for a G-protein-coupled receptor that closely resembles other known insect odorant receptors; its closest homologue in *Drosophila* is *Or83b*, an 'orphan' odorant receptor (i.e. one for which no ligand has yet been found). The gene, dubbed *BmOR-1* by the team, was found to be widely expressed only in chemosensory neurones of male silk moth antennae, and nowhere else in males or females, exactly as would be predicted if this receptor detects the female sex pheromone. To demonstrate that *BmOR-1* specifically responds to the female pheromone bombykol, the team expressed the receptor in *Xenopus* oocytes and exposed the oocytes to both bombykol and its natural oxidation product bombykal. They found that the oocytes were sensitive to bombykol, but not bombykal. This ties in well with physiology; although the female co-releases bombykal with bombykol, males only respond behaviourally to bombykol.

As a final flourish, the group took the work back to the organism, to prove that this was a bombykol receptor *in vivo*. They made a baculovirus (a natural virus for lepidoptera) encoding the receptor, and infected female antennae with it. Expression of the transgene was detectable by RT-PCR, suggesting that the receptor was expressed in female antennae, and these female antennae showed an electrophysiological response to bombykol – which of course they would not normally show, since females do not normally respond to their own pheromone. These results convinced the team that the receptor responds to bombykol *in vivo*.

The team then searched the emerging silkworm genome data, and identified 29 putative odorant receptor gene sequences. They expressed every one of these in *Xenopus* oocytes, and showed that none of them responded to bombykol. It thus seems clear that *BmOR-1* is likely to be the only such sex pheromone receptor in *Bombyx*.

10.1242/jeb.01466

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INSECT ADHESION

STICKY FEATS

Have you ever wondered how a fly can walk upside-down on a ceiling without falling off? This question has puzzled biologists and physicists for years, and now a study by Langer and colleagues has brought us one step closer to understanding this remarkable feat. Geckos can cling to walls and ceilings using millions of tiny hairs on their toes that adhere to surfaces *via* molecular attractive forces called van der Waals interactions. The adhesive pads on fly feet are similarly endowed with tiny hairs (or 'setae') that end in flat structures called terminal plates, but the mechanism of insect adhesion is not well-understood. There is some evidence that flies' feet secrete a sticky fluid that might mediate foot adhesion, so Langer and colleagues decided to investigate the adhesive properties of both terminal plates and the fluid secretion.

The team reasoned that if fluid secreted from the terminal plates of individual setae is responsible for adhesion, then the footprints left behind when a fly walks on glass should be stickier than the glass alone. Furthermore, if the stickiness is really caused by the terminal plates' secretion, the footprints' stickiness should be similar to the stickiness of the terminal plates. To measure these tiny adhesion forces, they used an atomic force microscope, which allowed them to measure attractive forces between an extremely sharp triangular probe and individual footprints as well as between the probe and the terminal plates of the flies' feet.

The sensitivity and resolution of the atomic force microscope is such that they could measure forces in the nanoNewton range and map the adhesive properties of the entire surface of a single terminal plate, which is only 2 μm long and 1 μm wide. When they measured the adhesive forces between the microscope tip and micro-

drops of fluid left by flies on glass, they found that these values were very similar to the forces from the centre of the terminal plate, suggesting that the terminal plates' secretion is responsible for the adhesive properties of the fly footprints. They also found that the adhesive forces between the microscope tip and the footprints left on the glass were considerably more than the attraction between the microscope tip and a clean glass surface. This suggests that the secretion creates capillary forces that probably assist the fly in its sticky endeavours. The team noticed that the adhesive forces between the microscope tip and the footprints decreased considerably over time, and corresponded to a decrease in the volume of the micro-drops due to evaporation. So as the fluid evaporates, its sticky benefits disappear.

If foot fluid secretion is important for adhesion, the team reasoned that it should be possible to make a fly's feet less sticky by simply washing off the secretion. Sure enough, they found that rinsing flies' feet with a buffer capable of dissolving the sticky footprints caused a dramatic ninefold decrease in terminal plate adhesion, suggesting that the sticky substance really does help the insects scale walls and ceilings.

Langer and colleagues conclude that flies don't fall off the ceiling because the terminal plates on their feet secrete tiny fluid droplets that create capillary forces and help the insects stick. We'll have to stay tuned for the answer to the next obvious question – how do they become unstuck when they want to fly or walk away?

10.1242/jeb.01467

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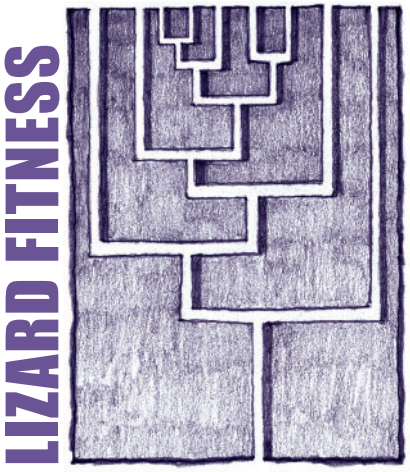

BORN TO RUN?

For many people in Western societies running is an activity that is confined to the gym or watched on television! But running may be a matter of life or death for many terrestrial animals. Running performance is thought to influence an animal's ability to capture prey or to avoid becoming prey itself. The ability to run for longer or faster than others is therefore likely to be extremely favourable, and natural selection would be assumed to produce animals with increasing endurance and speed. A day out at the races watching artificially selected thoroughbred horses reveals the potential improvements that can be made to running performance. Why, then, do large amounts of variation in running ability persist in natural populations of many animals?

To answer this question, Jean-François Le Galliard and his co-workers chose to examine running performance in the common lizard, *Lacerta vivipara*. They expected endurance running to affect common lizards' survival because running ability is thought to influence social status and competition for basking sites or prey. The team tested the lizards' initial endurance one day after birth by making the youngsters run on a treadmill until they were exhausted. Most lizards could only manage about 200 seconds of running, although some showed exceptional endurance (over 1600 seconds) that even Olympic marathon runners would be proud of. This endurance (or lack of it) was highly heritable. But does this endurance actually affect lizards' survival? To find out, the team released the lizards into enclosures and recaptured them to see how many had survived after one month and after one year. The recaptured lizards revealed that initial endurance did affect survival; low initial endurance lizards were less likely than high initial endurance lizards to be alive a month or a year later. So the weaker lizards had been eliminated by natural selection, but there appeared to be only weak selection for high endurance.

It is surprising that stronger lizards did not have a clear survival advantage over weaker lizards, as we might expect the superior athletes to outperform the lower endurance lizards. Le Galliard and co-workers hypothesised that perhaps the initial differences in endurance measured one day after birth were not maintained as the lizards matured. One possible reason for this could be the availability of food – with plenty of food available, lizards with low initial endurance might improve their endurance by investing in more muscle, enabling them to overcome their disadvantage at birth. But when food is scarce, lizards with low initial endurance may be unable to invest in more muscle and the difference in initial endurance will be maintained. To see if this was the case, the team tested common lizards' initial endurance and then divided the lizards into two groups: they fed one group fully but restricted the other group's diet. As the team had expected, under dietary restriction the initial differences in endurance were maintained, whereas fully fed lizards with high initial endurance lost their original advantage, presumably because the abundance of food allowed the weaker lizards to catch up with the stronger lizards. This suggests that the effect of initial endurance upon survival in the common lizard is dependent upon early environmental conditions.

These results are clearly at odds with the idea that performance measured at birth is a good indicator of lifetime performance. It is likely that these findings are not limited to lizards but that they occur throughout the animal kingdom and may, at least partly, explain some of the variation observed in animal running performance.

10.1242/jeb.01468

Le Galliard, J.-F., Clobert, J. and Ferrière, R. (2004). Physical performance and Darwinian fitness in lizards. *Nature* **432**, 502-505.

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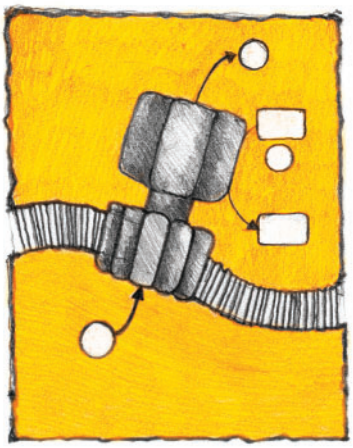
COPING WITH ANOXIA



NO ARREST NEEDED TO PREVENT CARDIAC ARREST

Most vertebrates die within a few minutes when deprived of molecular oxygen (anoxia) because the heart and brain depend on a continuous supply of oxygen. However, some cold-blooded vertebrates are remarkably anoxia-tolerant and can survive for months without oxygen. One of the keys to surviving prolonged anoxia is being able to balance energy supply and demand. One way to achieve this is to reduce energy-consuming processes of cells to a level that can be supported by the decreased amount of energy available from anaerobic respiration. Since ion pumping is energetically costly, energy can potentially be conserved by reducing the density of cellular membrane ion transport channels in excitable tissues such as the heart. Matti Vornanen and Vesa Paajanen of the University of Joensuu, Finland, set out to test this 'channel arrest' hypothesis in the crucian carp (*Carassius carassius*), an extremely anoxia-tolerant fish that spends a third of its life in anoxic water under the ice of shallow Finno-Scandinavian ponds.

The oxygen content of the water in the ponds where crucian carp live is too low for the fish to produce energy aerobically for five and a half months throughout the winter, so carp need to conserve energy during winter. Vornanen and Paajenen reasoned that since L-type Ca^{2+} channels regulate the force of heart muscle contraction through changes in the amount of free intracellular Ca^{2+}, these channels are good targets for channel arrest. They hypothesized that, if crucian carp use channel arrest to conserve energy during anoxia in winter, the number of dihydropyridine receptors (subunit of the L-type Ca^{2+} cardiac channel that triggers channel opening) and density of L-type Ca^{2+} current should both be decreased in winter-captured, anoxic carp compared with summer-captured, normoxic animals.

Vornanen and Paajanen captured wild carp monthly throughout an entire year and measured the number of dihydropyridine receptors and the density of L-type Ca^{2+} current in the ventricles of the carp's hearts to see how these changed with the seasons. Unexpectedly, they found that the number of dihydropyridine receptors in winter fish was the same as in summer fish. Thus, cardiac L-type Ca^{2+} channels were not downregulated by seasonal anoxia in the natural environment, suggesting that carp do not use differential expression of the Ca^{2+} channel protein to save energy in ion pumping or in reduced cardiac contractility during anoxia. This finding, in conjunction with the team's earlier finding that inward rectifier K^+ current density of crucian carps' heart cells is also unaffected by prolonged anoxia, led Vornanen and Paajanen to conclude that crucian carp do not use channel arrest as an energy-conserving mechanism in the heart during prolonged anoxia.

However, Vornanen and Paajanen did observe a 6.1-fold reduction in Ca^{2+} current in winter compared with summer fish, indicating that low temperatures depress Ca^{2+} current. They suggest that when temperatures drop, the resulting decreased Ca^{2+} current may allow for sufficient savings in ATP-dependent ion pumping and reduced cardiac contractility to attain a balance between energy supply and demand, providing protection against anoxia.

10.1242/jeb.01469

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RAPID COLD HARDENING

FLIES TOUGHEN UP IN BITS AND PIECES

Like other cold-blooded animals, insects constantly adjust their behaviour and physiology to cope with diurnal and seasonal temperature changes. Many studies have focused on the acclimatization that insects undergo to deal with seasonal temperature changes, but some rapid acclimatory responses have also been shown to enhance insects' cold tolerance following acute drops in temperature. One of these rapid responses is termed rapid cold hardening (RCH), where a short pre-exposure to cold markedly improves insects' tolerance during a subsequent severe cold insult. The RCH response occurs in many insect orders and, in addition to enhanced survival after cold shock, it enables them to remain active at low but sub-lethal temperatures (typically above 0°C). Even though RCH has been found in many insects, little is known about the physiological basis of this response. To improve our understanding of this phenomenon, Yi and Lee investigated the effects of RCH on *in vivo* and *in vitro* cellular viability following cold shock in flesh flies (*Sarcophaga crassipalpis*).

To see if RCH protects fly tissues from cold shock, Yi and Lee decided to compare cellular survival in cold-hardened flies (pre-exposed to cold for two hours) and non-hardened flies after exposure to a cold shock. If RCH offers cold protection, they expected the tissues of cold-hardened flies to fare better than the non-hardened flies. The authors exposed both groups to a cold shock, and dissected the insects immediately after the shock. To see which tissues are protected by RCH, they assessed the cellular survival of four different tissue types (fat body, gut tissue, salivary gland, and Malpighian tubules) using a modified live/dead sperm assay that allowed them to distinguish live cells from

dead with fluorescent dyes. As hypothesised, they found that the cellular viability of the hardened flies was markedly improved compared with the unhardened flies. This effect was significant in all four tissue types examined, demonstrating that RCH offers protection to several different tissue types, rather than being exclusive to a particular tissue type.

So just two hours of cold-hardening can provide protection from subsequent cold. But how is the RCH response activated so quickly? The team wondered if the RCH response can function *in vitro*, independently of central nervous or hormonal regulation, which could explain how the RCH response is transmitted so quickly. To test this, the authors used the same fluorescent dyes to investigate cellular viability, but this time the four tissues were dissected from the flies before the RCH and cold shock treatment. Sure enough, they found that *in vitro* cold-hardened tissues had a higher cellular survival rate after a cold shock than unhardened tissues, so RCH does protect cells *in vitro*. In fact, *in vitro* cold-hardened tissues responded much like tissues that had been hardened and cold shocked *in vivo*. These results are the first to demonstrate that RCH occurs independently of central regulation.

Even though this study does not resolve the basic physiological mechanisms underlying RCH, it provides guidance for future studies on RCH by emphasising that the nature of RCH is likely to be found among the basic cellular responses to lowered temperature.

10.1242/jeb.01494

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