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

WALKING IN STICKY SITUATIONS

Stick insects can usually be seen doing one of two unexciting things: standing still or walking. During walking, stepping movements are generally considered to be governed by one to several control centres associated with the joints that make up each leg. In order to walk in a coordinated fashion, the activity of these control centres must be synchronised, so that all the joints in one leg are doing the right thing at the right time to produce a 'step.' This is not a simple task because stick insects have to walk in many ways, including backwards and even sideways to cross uneven terrain or avoid obstacles. It would be very complicated to have a different set of rules for each walking direction, yet this is what most previous research has proposed. Setting out to find a sensory signal that stick insects could use to synchronise the activity of their leg-control centres, U. Bässler and colleagues tested the effect that vibration might have on the insect's locomotion control. Although they don't feel vibrations while they walk, so vibration isn't a signal that the insects use in their daily comings and goings, the team have discovered that vibration signals applied by the experimenter are capable of reversing the direction of movement in all leg joints, no matter what the joints are doing. This is an optimistic result; having found a single coordinating stimulus that works in principle (if not in reality), there may be other stimuli that the insect uses in normal behaviour.

To determine how the control of walking behaviour is affected by vibrations, Bässler and colleagues monitored the responses of the nerve cells that control the muscles of the leg joints by using recording electrodes, whilst stimulating the knee's vibration-sensitive organ. This was done in several ingenious ways: either by attaching the organ in restrained animals to a wiggling pole, or by looping it up on a vibrating hook through a hole in the knee of freely moving animals, or by manually vibrating one leg in animals walking on a treadmill.

The results showed that in moving animals, if the knee joint was in the process of extending, vibration would cause the leg to flex, and *vice versa*. Similarly at other joints, if the leg was being raised, vibration would cause it to be lowered and *vice versa*. For walking animals this means that vibration makes it more likely that the leg will be lifted off the ground in a stepping movement.

Since stepping behaviour is determined by leg-control centres, these findings show that vibration signals are capable of directing the activity of leg-control centres in a coordinated way. Because the reversal behaviour is also bidirectional, vibration could work as a synchronising signal for any walking direction.

Although vibration is not a sensory signal that the insect uses in practice, the team have shown that it is possible for a sensory signal to synchronise appropriate movements in more than one joint whilst still being independent of walking direction. Until now, this was not thought to be possible. Perhaps this finding will lead to the discovery of the real coordinating signal, and point the future of locomotor control in a new direction.

10.1242/jeb.00692

Bässler, U., Sauer, A. E. and Büschges, A. (2003). Vibration signals from the FT joint can induce phase transitions in both directions in motorneuron pools of the stick insect walking system. *J. Neurobiol.* **56**, 125-138.

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WINTER WARMING WOOS WINGED WANDERERS

The main impact of climate change on organisms is commonly thought to be increases in thermal stress during the hottest summer months. However, when the geographic range of an organism is limited by cold tolerance, increases in winter temperatures may have a dramatic impact on a population's range. An excellent example of this phenomenon has recently been discovered by Lisa Crozier, who found that the sachem skipper butterfly, *Atalopedes campestris*, which formerly resided to the west of the Cascade mountains in the United States, has recently colonized a part of central Washington State, where the minimum winter temperature has risen from –7°C to –4°C since 1950. Crozier determined that the butterfly's range had expanded to match the –4°C isotherm, thus she wondered if –4°C represented a physiological threshold.

Crozier collected specimens from butterfly populations in Washington State and California. Back in the lab she held the butterflies under cyclical thermal and lighting conditions designed to mimic the natural habitat in central Washington, and developed a set of experiments to analyze cold tolerance of the butterfly at different stages of development.

First, Crozier measured the insect's supercooling point, which tells us the absolute minimum temperature that the insect could survive. By testing acute cold exposure on almost 200 insects, Crozier found that the supercooling point ranged from –17.3°C to –3.4°C with an overall mean of –6.8°C. However, the supercooling point of intermediate-stage caterpillars was significantly higher than larvae or adults, which is relevant, as the intermediate-stage caterpillars are the ones present when the temperature begins to drop in the Fall.

Next she determined the lethal temperature, at which 50% of the insects died, by slowly cooling them to sub-zero temperatures, holding for 12h and then slowly rewarming to room temperature. The lethal temperature ranged from –5°C to –6°C for third instar caterpillars, and the overall mortality ranged from 5% at –4°C to 95% at –7°C!

Crozier then acclimated the butterflies to temperatures simulating several winter conditions, similar to those experienced in their natural environment. She found that the butterflies were able to survive the cold until the daily cycle's low temperature reached –4°C or below, at which point the death rate rose sharply. These results confirm that the butterflies are unable to acclimatize to temperatures below –4°C, the temperature of the isotherm that defines their range expansion.

Because butterflies in areas of higher elevation in the Cascade mountain range live in winter conditions where the temperatures are relatively stable at 0°C, Crozier also included acclimation to constant 0°C in her winter simulations. The butterflies had survivorship levels that were identical to those of specimens held under 4°C to –4°C daily cycles, suggesting that the mean temperature, and not the extreme lows of the treatment, may be responsible for the observed survivorship.

In summary, all of these results suggest that the butterflies are unable to survive habitat temperatures that are cooler than –4°C, and that 20–30% of the population will die at temperatures between 0°C and –4°C. The results of these studies strongly suggest that the range expansion of *A. campestris* into central Washington is directly related to the warming of wintertime low temperatures in this area. Understanding thermal physiology is an essential part of our ability to ascribe a causal relationship between climate change and species range shifts, and this paper provides a beautiful example of how this can be done.

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Crozier, L. (2003). Winter warming facilitates range expansion: cold tolerance of the butterfly Atalopedes campetris. *Oecologia* **135**, 648-656.

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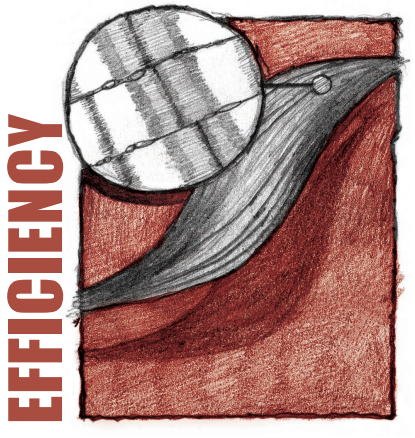

BOUNCING TENDONS KEEP YOU RUNNING WELL

How can the brain ensure that muscles are working efficiently? During running, for example, it seems that it would require exquisitely precise tuning of contraction timing, strength and duration to coordinate all the leg muscles. Does the brain orchestrate the split second coordination or is some other intrinsic property of the leg responsible for our sprinting performance? In his *Journal of Biomechanics* paper A. L. Hof suggests a simple way to ensure efficiency. Hof knew that two muscles in the human calf, the soleus and gastrocnemius, shorten very little during running, even though the ankle moves a lot. This is intriguing, because these muscles are most efficient when they shorten slowly and not very far. He describes his new theoretical model of the lower leg, which indicates that the spring-like Achilles tendon, to which both muscles attach, determines almost all ankle movement. Over a range of running speeds, the muscles can operate near maximum efficiency simply by turning on at the right time as the tendon stretches. So the intrinsic properties of muscles and tendons keep them working efficiently, almost independently of the brain's commands during fast activities, which means that the brain does not need to be very precise at all.

In most previous studies of neuromuscular control, the muscle and tendon properties are seen as secondary effects; they may modulate the commanded force, but the brain is ultimately in control. Surprisingly, Hof's new model implies that the tendon properties are more important than what the brain tells the muscles to do. Based on a linearised Hill model of muscle contraction, the model suggests that the muscle and tendon properties may be primary, and those of the brain only

secondary. The model simplifies into a well-known physical system – a lightly damped spring – which describes the combined muscle–tendon force output at the ankle fairly well. Hof quickly realized that the spring, which represents the tendon, determines most of the force output, while the damper, which represents the muscles and the brain, is less important. In other words, once the tendon starts bouncing, it will keep bouncing for a long time without much external input. The brain doesn't need to do much to keep it bouncing and, as a side benefit, the large tendon length changes mean that the muscles usually contract slowly over a small distance, when they're most efficient.

The portion of the model that represents the muscle reveals another substantial benefit of this bouncy tendon: it prevents eccentric contractions. During an eccentric contraction, a muscle tries to contract against too much weight and ends up being forcibly lengthened, absorbing energy and resulting in negative efficiency. This happens when you slowly lower a heavy weight; your muscles are active to keep the weight from dropping rapidly, but are lengthening as you lower it. However, the model shows that if the commanded muscle force is above a certain level, all the lengthening occurs in the tendon. Even if the total muscle and tendon length increases, the muscles can still shorten slightly, as long as the commanded force is high enough. This reinforces the idea that the tendon keeps the muscles efficient, because they reach peak efficiency when contracting slowly. Ultimately, for efficient running, the brain is relatively unimportant – it's the tendon's bounciness that keeps us running effortlessly.

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Hof, A. L. (2003). Muscle mechanics and neuromuscular control. *J. Biomech.* **36**, 1031-1038.

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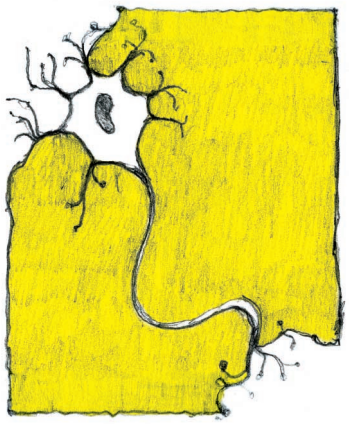
MEMBRANE FUSION



FLIP AND FUSE

Membrane fusion is a pivotal process for eucaryotic cells. It facilitates communication between different cellular compartments and the secretion of diverse molecules such as neurotransmitters and hormones. The underlying cellular machinery mediates the recognition and subsequent attachment of the merging membranes in the course of endocytic and secretory pathways. One of the cellular systems that are thought to be involved in fusion between a vesicle and its target membrane are the SNARE proteins, where vesicle (v-) SNAREs and SNARES in the target membranes (t-SNAREs) interact specifically to form inter-membrane complexes, which subsequently trigger fusion. Although numerous other proteins may be involved in regulating membrane mergers, the SNAREs are currently the best candidates for universal fusion mediators.

In order to fortify the SNARE hypothesis, Jim Rothman and his team tried to obtain the same level of experimental evidence for SNARE-mediated membrane fusion as already exists for viral fusion proteins such as the influenza hemagglutinins. For this purpose they simply asked: Are SNARE proteins, which mediate membrane fusion within cells under natural conditions, sufficient to fuse cells together if they are expressed on the cell's surface?

To answer this question they first had to design a cellular system that allows functional expression of the SNARE proteins on the extracellular surface. SNARE complexes are formed by three subunits that belong to different protein families. The VAMP proteins comprise the v-SNAREs, while syntaxins and SNAP-25s assemble to form the t-SNAREs. During membrane fusion, v- and t-SNAREs associate *via* their cytoplasm-facing interaction domains, so Rothman's team needed to express the SNARE proteins so that the interaction domains were placed on the extracellular side of the plasma membrane, to get cells to fuse.

The team engineered expression vectors to generate SNARE proteins with an *N*-terminal signal sequence that directed the proteins to the plasma membrane and exposed the protein's interacting domains on the cell's external surface. They also introduced some genetic mutations into the SNAREs to prevent unwanted post-transcriptional modifications to the protein. When Rothman and his coworkers expressed the proteins in monkey COS cells and determined the protein's orientation by means of antibodies, they found significant amounts of flipped SNAREs on the extracellular surface.

To test whether the flipped SNAREs could mediate cell–cell fusion, the scientists expressed the v-SNARE (VAMP) in one cell population and the t-SNAREs (syntaxin and SNAP-25) in another. The cytoplasm of the v-SNARE expressing cells was labeled with a red fluorescent protein, and the nuclei of the cells producing the t-SNAREs were labelled with a cyan-coloured fluorescent protein. After combining both cell populations and incubating for a while, the team found numerous merged cells, with multiple cyan nuclei surrounded by red cytoplasm. So the v- and t-SNARE expressing cells had fused within minutes. A series of elaborate control experiments left no doubt that the observed cell–cell fusions were strictly dependent on the presence of functional SNAREs.

Until Rothman and colleagues demonstrated SNARE-dependent cell–cell fusion, evidence for the involvement of SNAREs in membrane fusion was lacking at the cellular level. Now there is strong evidence that SNARE core complex formation is sufficient for physiological membrane fusion. Defining the core of the fusion machinery is also a central issue in neurophysiology and will help us to understand how neurotransmitters are released into the synapse.

10.1242/jeb.00693

Hu, C., Ahmed, M., Melia, T. J., Söllner, T. H., Mayer, T. and Rothman, J. E. (2003). Fusion of cells by flipped SNAREs. *Science* **300**, 1745-1749.

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