

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.

CHEMICAL DECEPTION



HORNETS FOOLED BY ORCHIDS THAT SMELL OF SCARED BEES

Orchids can be highly deceptive. About 10,000 nectarless orchid species deceive insects into visiting them by imitating either the scent of a nectar-bearing flower or, more dramatically, the shape and pheromones of an insect of the opposite sex. A new twist to this story has recently been described in a paper by a Sino-German research team, led by Jennifer Brodmann of the University of Ulm.

The *Dendrobium sinense* nectarless orchid is found only on the Chinese island of Hainan, and its flower looks vaguely like a white daffodil. During field observations in the moss forest of Hainan, Chinese researchers concluded that the orchid's sole pollinator is a hornet, *Vespa bicolor*. But instead of approaching the flower and landing on it gently, the hornets pounced on a red mark in the centre of the flower, as though they were attacking a prey. During this aggressive behaviour, the hornet gets *D. sinense* pollen on its thorax, ready to pass onto the next orchid that it pounces upon.

Laboratory studies using extracts of *D. sinense* flower scent confirmed that the hornets were attracted by a chemical cue released by the flower. To find out exactly what the substance was, the team isolated the compounds from orchid flower scent and injected it into a gas chromatograph to separate out the chemical components of the scent blend. As the flower odours were fractionated by the chromatograph, the researchers simultaneously recorded the electrophysiological activity of sensory neurons in the hornet's antenna, and identified which of the compounds the insects were able to detect. One of the most important responses was induced by a hitherto unknown flower volatile, (*Z*)-11-eicosen-1-ol.

In other orchid–insect deception systems, the substances that flowers mimic to attract male pollinators are often female insect sex pheromones. Here, however, the key compound – (*Z*)-11-eicosen-1-ol – is a component of the alarm pheromone of the Asian honey bee, *Apis cerana*. Behavioural tests confirmed that the hornets were highly attracted to this substance.

The explanation for this complex relation is that *A. cerana* is one of the hornet's main prey species. The orchid appears to be attracting the hornets by mimicking the alarm pheromone of their prey – hence the aggressive pouncing behaviour shown by the pollinating hornets. It is known that hornets use a mixture of chemical and visual cues to track down their bee prey – this study suggests that the bee alarm pheromone may be of decisive importance for them.

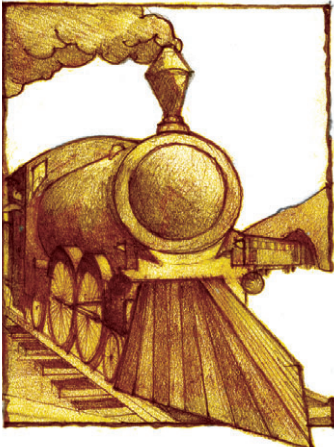
Hornets will raid Asian honey bee nests but can be repulsed by the bees, which wrap the predator up in a ball of their buzzing bodies, heating it to death. This elegant study reveals an extra dimension to the complex arms race between bees and hornets, by showing how a plant can 'eavesdrop' on their relationship in order to ensure pollination. It also demonstrates that chemical deception can be even richer than was previously imagined.

10.1242/jeb.021766

Brodmann, J., Twele, R., Francke, W., Yi-bo, L., Xi-qiang, S. and Ayasse, M. (2009). Orchid mimics honey bee alarm pheromone in order to attract insects for pollination. *Curr. Biol.* **19**, 1368-1372.

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WALKING



GETTING INTO THE SWING OF WALKING

Why do we swing our arms when we walk? This seems like a simple enough question, but if you were to ask two locomotor biomechanists you might get two different answers, both of which sound pretty good. For example, one might answer that arm swinging saves metabolic energy while another might emphasize its importance in stability. Both of these ideas have some empirical support, and if you've ever tried preventing your arms from moving during walking you probably sensed that something about arm swinging made things easier. Recently, Steven Collins, Peter Adamczyk and Arthur Kuo of the University of Michigan combined experimental work and mathematical modeling to more clearly assess the role that active arm swinging plays in the energetics and mechanics of walking.

For their experimental work, Collins and his co-authors instructed 10 subjects to walk using four different types of arm swing: (1) normal, (2) bound, in which subjects' arms were physically restrained from moving, (3) held, in which subjects held their own arms still and (4) anti-normal, where subjects actively swung their arms out of phase relative to normal. By analyzing the exhaled gases of the walkers, the scientists measured metabolic rates of each subject while walking on a treadmill at 1.25 ms^{-1} adopting each mode of arm swing (in random order). Seven subjects also walked over a force plate using the different forms of arm swing so their locomotor mechanics could be compared.

Several variables were clearly affected by arm swinging mode. Not surprisingly, energy expenditures were lowest in the normal condition and increased 7% for bound, 12% for held and 26% for anti-normal. Vertical ground reaction moments were even more substantially affected. Peak values were lowest during normal arm

swinging, approximately 60% higher during the bound and held modes and nearly 3-fold greater when arms were swung out of phase relative to normal. Experiments also revealed that joint torques and power required at the shoulder and elbow joints were very small during arm swinging.

In combination with the modeling work, these experimental data support a number of intriguing ideas. For example, the small torques and work rates in the upper arm suggest that arm swinging during walking requires little effort and that the small amount of muscular energy that is required is more than made up for by the energetic savings it provides (relative to not swinging the arms). Further, the energetic benefits likely stem from reduced ground reaction moments during normal walking. Because forces from the ground are transmitted up through the leg, limb muscle actions must be used to counteract them, and the smaller these forces, the smaller the amount of muscle energy required. The authors point out that speed likely impacts the role of arm swinging during walking, but determining the details will require more work.

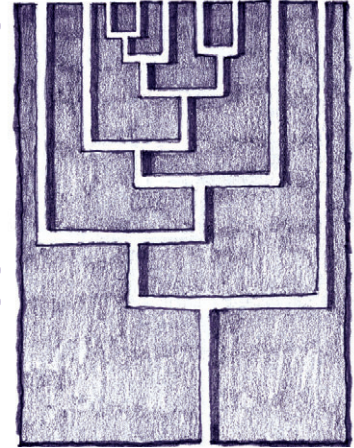
As the authors conclude, arm swinging is essential to energy savings during human walking. And while our two hypothetical biomechanists might still favor different notions for why we swing our arms during walking, I know I'm now going to have a lot more to say if anyone ever asks me that question.

10.1242/jeb.021683

Collins, S., Adamczyk, P. and Kuo, A. (2009). Dynamic arm swinging in human walking. *Proc. R. Soc. Lond., B* 276, 3679-3688.

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FITNESS DEMANDS



SPERM FIND BIG EGGS BEST

There is widespread evidence that egg size has evolved through offspring and maternal fitness demands. Larger eggs typically bestow more nutrients, a larger birth weight and increased chance of survival on the offspring. Egg size is balanced with egg number to maximize maternal reproductive success. However, a new player has recently been added to the roster: sperm limitation, which occurs when there is an insufficient quantity of sperm to fertilize all of the available eggs. It has been suggested that in externally fertilizing animals, sperm limitation could also act as a selective force influencing egg size.

Externally fertilizing animals, such as sea urchins or fish, release their sperm and eggs into the surrounding environment with the hope that a sperm will bump into an egg and successfully fertilize it. It has recently been shown in three species of sea urchin that larger eggs need lower sperm concentrations in order to be successfully fertilized. Christopher MacFarlane from the University of East Anglia in the UK along with colleagues from Brandon University and the University of British Columbia in Canada sought to determine whether the same could be said for sockeye salmon, a fish with substantial natural variation in egg size where males and females spawn in much closer association compared with broadcast spawning marine invertebrates like sea urchins.

In order to test whether larger sockeye salmon eggs were preferentially fertilized under conditions of sperm limitation, the authors collected sperm from 20 males and pooled eggs from 15 females. Dividing the pooled eggs into groups, the team added just enough sperm to each group of eggs to ensure fertilization success rates ranging from 20% to 80%. After allowing the eggs to incubate for 10 days, they measured the surface area of the fertilized and unfertilized eggs. Finally, the authors also

included two treatments where all the eggs or none of the eggs were fertilized to account for possible changes in the size of the egg over the course of time or in response to fertilization. This also allowed the team to test whether there is a relationship between egg size and fertility when a surfeit of sperm is available.

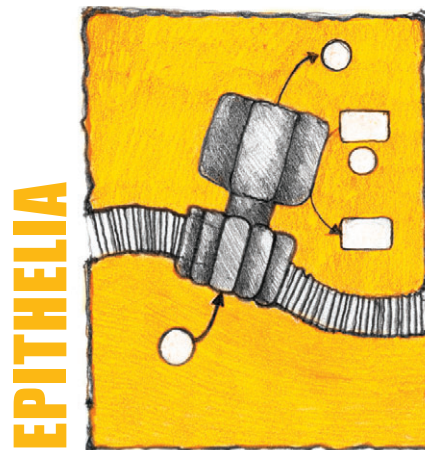
The team found that under sperm-limitation conditions, the eggs that were fertilized successfully had a significantly larger surface area (by 7%) than the unfertilized eggs, suggesting that increased egg size could evolve under conditions of sperm limitation.

This study by MacFarlane and colleagues demonstrates that in addition to offspring and maternal fitness demands, egg size could also be influenced by sperm limitation in salmon. The next step in this line of research is to assess the importance of sperm limitation in fish in a natural setting. Though salmonids are generally considered to have high fertility in the environment, other species may not be so lucky. One thing seems to emerge clearly from this study: it's easier to hit a bigger target!

10.1242/jeb.023820

MacFarlane, C. P., Hoysak, D. J., Liley, N. R. and Gage, M. J. G. (2009). In vitro fertilization experiments using sockeye salmon reveal that bigger eggs are more fertilizable under sperm limitation. *Proc. R. Soc. Lond., B* **276**, 2503-2507.

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EPITHELIAL POLARITY: A COMPLEX PICTURE

Epithelia are layers of adherent cells that line the inner and outer surfaces of diverse tissues and organs throughout the body. Their main function is to separate body compartments, and to mediate transport of different substances between them. This is only possible because epithelial cells are 'polar' in the sense that their apical membranes (on the cell's outer surface) differ in protein composition from their basolateral membranes (on the inner surface). Many proteins of the apical or basolateral membranes have been recognized as important players in establishing cellular polarity, but the mechanisms that regulate epithelial polarization are still poorly understood. In a recent *Nature* paper a US/Canadian team of scientists led by Ulrich Tepass have genetically dissected some components of the underlying machinery which regulates epithelial polarity.

The organization of epithelial cell membranes has been intensively studied in *Drosophila* embryos and several genes that function in epithelial polarity have been discovered. These genes include *yrt*, *cora*, *Nrx-IV* and *ATPa*, which encode the basolateral proteins Yurt, Coracle, Neurexin IV and the α -subunit of the Na^+, K^+ -ATPase, respectively. To gain more insight into their functions in epithelial polarity, the scientists first generated transgenic flies, which were defective in one or two of the above genes. In a second step they assessed the effects on epithelial polarity by using antibodies to identify the locations of two proteins, 'Crumbs' and 'Discs large', which served as marker proteins for apical and basolateral membranes, respectively.

When the team examined early *Drosophila* embryos that lacked the *yrt* gene, they

observed that in addition to being found in the apical membrane, 'Crumbs' was mislocalized in the basolateral membrane, indicating that the Yurt protein is required for correct positioning of 'Crumbs' and epithelial polarity. In another type of mutant embryo, which lacked a functional *yrt* gene, but was provided with *yrt* mRNA to produce Yurt protein by the mother's follicle cells (surrounding the egg), mislocalization of 'Crumbs' was far less pronounced. Because the *yrt* gene was still expressed to some extent, the team could study genetic interactions between *yrt* and polarity genes that did not show mislocalization of 'Crumbs' when they were mutated individually.

The scientists hypothesized that if they observe polarity defects in a double mutant, the deleted genes must be acting synergistically, suggesting that they function in the same pathway of epithelial cell differentiation. From six genes that they investigated in combination with *yrt*, they observed mislocalization of the 'Crumbs' protein in the *yrt/ATPa*, *yrt/Nrx-IV* and *yrt/cora* double mutants, suggesting functions for the Na^+, K^+ -ATPase and Neurexin-IV in establishing epithelial polarity.

Next they carefully analyzed the polarity phenotypes of the various mutants at different developmental stages. In doing so, the team provided genetic evidence that Yurt, Coracle, Neurexin-IV and the Na^+, K^+ -ATPase are a new group of functionally cooperating proteins that are involved in establishing an epithelial cell's polarity, and the proteins seem to act in two partially overlapping pathways.

Together, Tepass's team have provided evidence that epithelial polarity is controlled on multiple levels involving different protein complexes. Yurt seems to play a major role in this process. It appears that Yurt is a general core regulator of epithelial membrane organization, as this function is conserved even in mammalian cells.

10.1242/jeb.021725

Laprise, P., Lau, K. M., Harris, K. P., Silva-Gagliardi, N. F., Paul, S. M., Beronja, S., Beitel, G. J., McGlade, C. J. and Tepass, U. (2009). Yurt, Coracle, Neurexin IV and the Na^+, K^+ -ATPase form a novel group of epithelial polarity proteins. *Nature* **459**, 1141-1145.

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