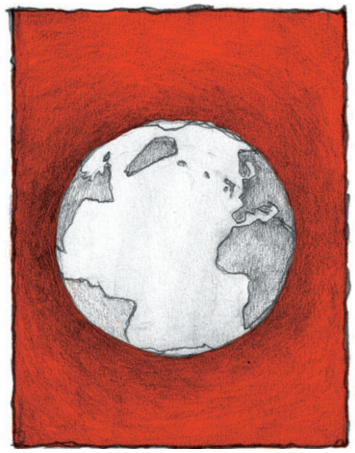
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

GIGANTISM



O_2 LEVELS MAY NOT DETERMINE GIGANTISM

During the Palaeozoic some arthropod species grew to gigantic proportions. The rise of these giants strongly correlates with increased atmospheric oxygen concentrations (~32% *vs* today's 21% O_2). Among present day arthropods marine polar species show remarkable gigantism compared with lower latitude species. The size of these marine arthropods also correlates well with the increased oxygen solubility of the colder polar waters. These positive correlations of body size and oxygen availability form the basis of the 'oxygen hypothesis', which states that increased oxygen concentrations facilitate improved oxygen supply to tissues, thereby allowing the evolution of larger body sizes. This hypothesis is intuitively attractive; however, very little experimental testing has been done to investigate whether the correlation between oxygen availability and size does indeed signify causation. Some data are available for short term developmental studies, mostly for *Drosophila melanogaster*, but stronger experimental tests of this hypothesis only recently started to appear in the peer reviewed literature.

Arthur Woods and colleagues report one such study in *Proceedings of the Royal Society B*, where they tested the performance during forced activity (self righting) of Antarctic pycnogonids from McMurdo Sound. In Antarctic waters pycnogonids (commonly known as sea-spiders) can reach colossal leg spans of 40 cm. Woods worked with 12 species with masses spanning three orders of magnitude. Using a 1000 l temperature controlled aquarium his team presented the specimens with 17% (low), 43% (medium) and 92% (high) air saturation oxygen levels. After brief equilibration they turned the pycnogonids on their backs and noted the time required to right themselves and the number of rightings in 1 h. They also measured body mass and leg diameter as leg diameter is an ideal metric to gauge oxygen diffusion to internal tissues. According to the oxygen hypothesis Woods predicted that larger pycnogonids would perform proportionally worse compared with smaller specimens – especially in lower oxygen levels. Alternatively, should the animals show symmorphosis – when their functional structure is developmentally and/or evolutionarily regulated to match, but not exceed, functional demand – both large and small specimens will perform equally when oxygen stressed.

Woods' team found that Antarctic pycnogonids' body surface areas scaled proportionally with mass, which suggests that the decreased surface area to mass ratios found in larger animals might cause increased oxygen sensitivity. Indeed, oxygen availability had a strong effect, with sea-spiders in high oxygen righting themselves more readily than those in low oxygen. However, it turned out that body size did not have a consistent effect on performance in relation to oxygen availability. Large pycnogonids' righting performance was only slightly worse than that of the smaller ones. Midsize specimens performed best with up to 160 rightings per hour, compared with ~40 rightings for either size extremes. These results contradict the predictions of the oxygen hypothesis and also suggest that pycnogonids do not have wide oxygen safety margins. The team concluded that oxygen availability is unlikely to fully explain the evolution of large size in Antarctic pycnogonids.

One explanation Woods and colleagues offer for their data's consistency with symmorphosis is that millions of years of evolution in cold polar waters could have fine tuned the pycnogonids' oxygen delivery systems to these oxygen rich waters, leaving narrow safety margins. With this experimental approach Woods and colleagues illustrated that oxygen availability is still an important factor but is not the only important factor in attempts to explain the evolution of gigantism.

10.1242/jeb.021428

Woods, H. A., Moran, A. L., Arango, C. P., Mullen, L. and Shields, C. (2008). Oxygen hypothesis of polar gigantism not supported by performance of Antarctic pycnogonids in hypoxia. *Proc. R. Soc. B* **276**, 1069-1075.

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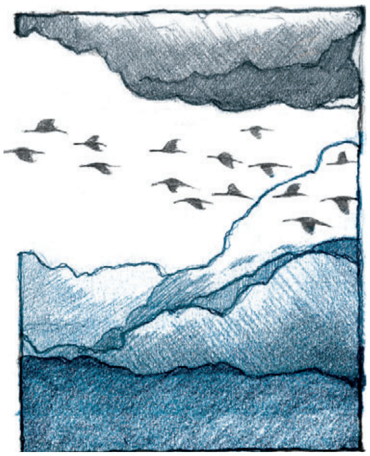
FOOT SHAPE



LONG CLAWS AS SNOWSHOES?

Bird feet come in all sorts of shapes and sizes, presumably relating to both evolutionary history and current function. The two-toed feet of ostriches are typical of runners, being relatively small and light; webbed duck feet, while functional on the land, have clearly evolved under pressure to perform well in water; powerful talons characterise the raptors; and jacanas have weirdly long toes, consistent with their lily-trotting habits. But some traits have less clear-cut functional relationships. Many birds – for instance the skylark – have strikingly long claws, and yet are definitely not raptorial.

One idea – the 'snowshoe hypothesis' – is that, somewhat like jacanas, the larger foot spread afforded by long toes and claws might allow birds including 'longspurs', 'longclaws' and some larks to handle life on relatively long, 'rank' (as in thick/luscious, not smelly) grasses. In order to test this, Rhys Green, Keith Barnes and Michael Brooke from Cambridge and Cape Town compared the feet of 'grass-dwelling' and 'bare-ground' bird species. Their hypothesis was that grass-dwellers – typified by the skylark – should have relatively long toes and claws compared with larks from bare ground habitats. And that is exactly what they found. What is more, this was not an oddity of larks: matched pairs of closely related birds from a range of families, one from a stony/bare habitat (e.g. grey wagtail) and one from a grassy habitat (e.g. yellow wagtail), showed the same trend. So, it appears that elongated toes and claws (or, perhaps, relatively small bodies and wings) may be an adaptation to standing in and moving across long grass. While the mechanism behind this benefit is not as clear as in the case of lily-trotters, probably relating to both the unevenness and instability of grass tops, the relationship does tie in with behavioural observations: skylarks prosper in 'untopped' (long) stubble, whereas granivorous birds, including those with toes and claws of ordinary length, do well on cut and shortened crops.

So, what is the down side to having extended toes and claws? One is presumably an increased risk of damage. Indeed, the authors' unpublished observations of Roso larks, a species confined to a barren rocky island, show that around half of older birds have toe or claw damage. A stronger tendency towards lengthening was observed for claws than toes, suggesting that extending the toes may be somewhat more costly. It was suggested that this may be because toes require a blood supply, resulting in heat loss. So, temperate and tropical lark feet were compared, with the idea that heat loss would be less severe in tropical birds. While the direction is as predicted (temperate birds extending claws relatively more than tropical), the effect was not statistically significant. This highlights the problem with such comparative studies: evolution has only occurred a limited number of times, so if the sample size or signal is too small, there is not much that can be done about it, even if the underlying mechanism is perfectly valid.

Overall, however, this study successfully demonstrates a relationship which was not, before, absolutely obvious. And the finding should inspire further study of the physical mechanisms of bird gripping and locomotion, not least because of the growing interest within biomechanics and robotics into movement over complex, unstable substrates.

10.1242/jeb.021386

Green, R. E., Barnes, K. N. and Brooke, M. de L. (2008). How the longspur won its spurs: a study of claw and toe length in ground-dwelling passerine birds. *J. Zool.* **277**, 126-133.

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NEURON GROWTH

SYNAPTIC ACTIVITY MAKES FLY NEURONS SHAPE UP

Brain cells come in a staggering variety of shapes and sizes. So how do neurons grow to be the shapes they are? It has been thought that neural activity at the sites where neurons communicate (synapses) helps mold cell shape. However, experimentally testing this idea *in vivo* has proven tricky. A recent study by Marco Tripoli and co-workers from the University of Cambridge set out to test with a new level of precision and rigor how synaptic activity shapes neuronal growth using the unique and powerful advantages of *Drosophila* as a model organism.

First, the team characterized how known fly motor neuron morphology changes during embryonic life. They wanted to know whether any morphological changes are correlated with the onset of synaptic input to these neurons. Indeed, they found that the cellular arbors of identified fly neurons grow linearly for a time, then suddenly slow their growth. And this slow down happens right around the time when synapses start bombarding the cells with excitatory input.

To test whether there is a causal link between arbor growth and synaptic activity, the team used genetic tools to increase and decrease synaptic input from upstream (pre-) synaptic partners. They found that when pre-synaptic activity was abolished, the neurons grew longer; conversely, when they genetically increased the number of pre-synaptic connections, the neuron trees grew less and stayed closer to home. So the neurons seemed to grow in search of synaptic activity.

The researchers then went in and counted synaptic sites on the growing neurons. Each neuron appeared to match its own growth to the availability of pre-synaptic sites. It's hard not to anthropomorphize: it's almost as if the neurons 'knew' how many synaptic connections they needed to make, and then adjusted their own size until they filled the quota.

The team also found that initially, arbor length was inhibited by the presence of a synapse – regardless of whether the synapse was silent or active. Furthermore, areas farther away from synaptic sites showed more growth. This suggests that early remodeling in this neuron is a ‘locally grown’ operation based on local physical contact, but not activity of the synapse.

Finally, the team wanted to determine what molecules inside the cells could be involved in signaling ‘grow’ or ‘don’t grow’. Protein kinase A (PKA) has been implicated in modulating physiological responses to changing synaptic input. To test whether PKA also plays a role in structural responses, the team went back to their genetic toolbox, pulled out a way to up regulate and down regulate PKA function and found that neurons with reduced PKA function have trouble remodeling themselves, and *vice versa.* All of these results suggest that, through PKA signaling, neurons are able to adjust their shape as they grow to maintain an optimal number of connections, even in the face of varying synaptic input.

One thing that makes this paper powerful is the elegant use of genetic tools. A lot of the experiments are conceptually straightforward, but extremely tough to actually pull off *in vivo*. *Drosophila* genetics makes these experiments possible. This study is also important because it calls out for future work by computational neuroscientists. Clearly, the next step is to try to link structural features to function in the growing neuron arbor. Computer simulations would be the best way to do this, and the detailed 3D reconstructions of neurons in this study are calling out to computer scientists to ‘come model me’. In future years, coupling the *Drosophila* genetic toolbox with the theoretician’s computational toolbox could provide powerful insights into how local activity in the distant branches of cellular arbors shapes how neurons grow.

10.1242/jeb.021469

Tripodi, M., Evers, J. F., Mauss, A., Bate, M. and Landgraf, M. (2008). Structural homeostasis: compensatory adjustments of dendritic arbor geometry in response to variations of synaptic input. *PLoS Biol.* **6**, 2172-2187.

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NICHE EXPLOITATION

MUSSELS DEVELOPING UNDER PRESSURE: COLD IS THE KEY

It is a hard life for a mussel in the deep sea. Temperatures are always a chilly 4°C or cooler, food is scarce or patchy at best, not to mention the hydrostatic pressure of thousands of meters of water constantly pressing on you. If you can’t stand the cold, there is one other option. Hopping from one hydrothermal-vent to another can provide a bit of warmth, at least if you can stand the toxic hydrogen sulfide. Despite these obstacles, mussels of the family Mytilidae are thought to have recently colonized the deep sea from shallow habitats. Most of the Mytilid mussels (subfamily Bathymodiolinae) found in the deep sea harbor symbiotic bacteria that can utilize the chemical energy in compounds like hydrogen sulfide to fuel an autotrophic existence. The microbes provide the food, while the mussels provide a safe habitat for their essential microbial partners. These mussels can be found in cold-seep environments, at hydrothermal-vents, and associated with sunken wood and whale bones.

However, it is presently unclear which path the mussels might have taken to colonize the deep sea. Some speculate that shallow water mussels (which typically tolerate a wide range of temperatures) first colonized wood and whale bone habitats which brought them to the abyss, where they then colonized cold-seeps and hydrothermal-vents. This scenario requires the mussels to first lose their tolerance of warm and fluctuating temperatures in order to thrive in the constant cold of the deep sea and then regain a wider tolerance of temperatures when they subsequently colonized the hydrothermal-vents. Alternately, mussels might have colonized the cold and warm habitats of the deep sea independently. Nélia Mestre and colleagues at the University of Southampton set out to explore the possibility that a shallow water mussel could directly colonize a deep sea hydrothermal-vent environment.

The team chose to study embryonic development of *Mytilus edulis*, a shallow water mussel that thrives in temperate intertidal and subtidal habitats, under a variety of temperature and hydrostatic pressure regimes. They fertilized gametes *in vitro*, at both high and low pressures, and followed their development through time at different combinations of pressure (ranging from 1 to 500 atm) and temperature (ranging from 5 to 25°C). The embryos were allowed to develop under these conditions for a set amount of time, and then were fixed and observed using light and scanning electron microscopy.

Mestre and colleagues found that fertilization can occur at low and high hydrostatic pressures; however, the embryos’ development was adversely affected by the extreme conditions. Higher temperatures and higher pressures led to a greater proportion of abnormally developing embryos. Higher pressures also led to a retardation of developmental rate, independent of temperature. Importantly, they found that at cold temperatures the embryos could develop normally at even the highest hydrostatic pressures tested, although most of the embryos developed abnormally. However, the majority of the embryos developed normally at 200 atm of pressure as long as temperatures were below 20°C.

The ability of *M. edulis* to develop across such a wide range of temperatures and pressures is truly impressive. It is important that embryos are able to make this journey to the deep because they represent the best chance for dispersal in these typically benthic mussels. Few other species can survive this type of stress, and many shallow water species of invertebrates die within minutes of exposure to the hydrostatic pressures employed in this study. This study suggests that shallow water Mytilid mussels could directly colonize deep sea hydrothermal-vents without the requirement of an intermediate evolutionary step *via* wood and whale bone.

10.1242/jeb.021501

Mestre, N. C., Thatje, S. and Tyler, P. A. (2009). The ocean is not deep enough: pressure tolerances during early ontogeny of the blue mussel *Mytilus edulis. Proc. R. Soc. B.* **276**, 717-726.

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