OUTSIDE JEB

Exercise when young to protect old bones

As you read this article, you're probably sitting in a chair. Your lumbar vertebrae are supporting your weight. The longer you sit, the more your bones will actively remodel – modifying your vertebrae to better support your weight. However, the older you are, the less your bones will remodel in response to a load. This raises the question – to what extent are the benefits of bone remodeling retained with age? Is it possible to future-proof bone?

Stuart Warden and his colleagues at Indiana University, USA, and several other international institutions thought that bone remodeling caused by physical activity during youth might provide some benefits even years later and the team found a great system to test their hypotheses: professional baseball players. These athletes all undergo the same training routines and have similar levels of activity. They start early in childhood, most stop physical activity altogether upon retirement and they come with their own internal control – their non-dominant hand. The researchers took CT scans of baseball players and non-players of all ages, early career to long retired, and compared the humeri of their dominant and non-dominant arms. Then, they used finite element analysis and musculoskeletal models to simulate the strain on each humerus during a fastball. Finally, the researchers compared age cohorts of players to see which, if any, changes were maintained later in life.

In accordance with earlier studies, the humeri in the throwing arms of the baseball players were much larger than those in the non-throwing arms. The throwing arm humerus in the active baseball players had a greater cross-sectional area, greater cortical bone area, greater cortical bone mass and smaller spongy bone area than the non-throwing arm. These changes considerably reduced the torsional and shear strain on the humerus during throwing – protecting the bone from damage.

When the researchers compared the bones of former baseball players, accounting for the number of years since they had last trained, a surprising find emerged: though the cortical bone benefits of training disappeared over time in the absence of training, some benefits remained. The increase in total bone size that resulted from years of throwing was maintained decades later. This extra size meant that even former baseball players had more than one-third of their original benefits in bone torsional strength – even when their throwing muscles had long since diminished. The few players who continued throwing post-retirement lost less cortical bone than their colleagues, resulting in greater retention of their earlier humeral strength.

The authors stress that their work shows the benefit of physical activity, especially during youth. Even 90 year old former baseball players retained some of the benefits of their training, even though they stopped training more than half a century ago. The benefits of physical activity accrue most rapidly when young, and do not completely diminish with time. Perhaps we should get up off the couch when young, so that we can get up off the couch when we're older.

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Warden, S. J., Mantila Roosa, S. M., Kersh, M. E., Hurd, A. L., Fleisig, G. S., Pandy, M. G. and Fuchs, R. K. (2014). Physical activity when young provides lifelong benefits to cortical bone size and strength in men. *Proc. Natl. Acad. Sci. USA* **111**, 5337-5342.

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A novel ant protein for chemical communication

Ants are fascinating insects that distribute tasks among individuals belonging to different castes, including mated and virgin queens, drones and worker ants. To fulfil caste-specific functions, ants have developed a complex system of chemical communication using sensory organs (sensilla) that detect molecules carrying task-specific information (semiochemicals). This system requires carrier proteins to bind the molecules, which are hydrophobic, and transfer them to the chemoreceptors that generate electrical signals for neuronal processing in the brain. However, the number of carrier proteins known is not sufficient to bind the large number of semiochemicals that are recognized by the ants. In a recent study published in *PNAS*, a team of Japanese scientists lead by Yuko Ishida and Toshimasa Yamazaki identified a novel carrier protein involved in chemical communication in worker ants that appears to be capable of delivering various hydrophobic semiochemicals to the nervous system.

The antennae are the major chemosensory organs in ants. They carry numerous olfactory sensors called basiconic sensilla – which are made of different cell types, including sensory neurons – for sensing odours. Once the semiochemicals have entered the sensillum through pores, they reach the aqueous sensillum lymph, which contains carrier proteins that bind the hydrophobic molecules and deliver them to various chemoreceptors residing in the membrane of the neuron's dendrites. As ants can detect a wide

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variety of chemically different hydrophobic compounds, it was hypothesized that some of these carrier proteins must possess a hydrophobic binding pocket that interacts with a variety of these molecules. To identify such carrier proteins, the scientists screened for worker-specific antenna genes in the Japanese carpenter ant, *Camponotus japonicus*. One of the identified genes encodes a protein with high similarity to the Niemann-Pick C2 (Npc2) protein, which is an essential carrier protein for intracellular cholesterol transport in vertebrates and humans and has been suggested to be involved in the regulation of sterol homeostasis in the fruitfly *Drosophila melanogaster*, through the homologous *NPC2* gene. However, in ants this protein appears to serve a different function, as the scientists unexpectedly found that *NPC2* in *C. japonicas* (*CjapNPC2*) is exclusively expressed in the antennae and is specifically detectable in the lymph-filled cavities of the basiconic sensilla.

To examine the protein's binding properties and structure, the team expressed *CjapNPC2* in *E. coli* cells and purified it to homogeneity. Binding studies revealed that the protein indeed interacts with a broad spectrum of different hydrophobic compounds including long-chained fatty acids, alcohols and acetates. They also found that some of the tested compounds can provoke electrophysiological signals in the worker ant's antenna. Next, the team went on to solve the 3D structure by X-ray crystallography when bound and unbound to oleic acid. They found that the structure of CjapNPC2 consists primarily of antiparallel β-sheets forming a larger hydrophobic cavity for binding of the ligand in a U-shaped manner. Most strikingly, CjapNPC2 exhibits some intrinsic flexibility (independent of ligand binding), particularly at the entry to the binding cavity, which may contribute to its moderate selectivity and thus facilitate entry and binding of a range of semiochemicals.

Ishida and Yamazaki's team has identified a new carrier protein involved in ant chemical communication. Most interestingly, the protein can bind and deliver various hydrophobic compounds. As the protein's function is different from that known in vertebrates and other insects, it seems that ants have recruited and modified this protein during evolution to comply with their need for a carrier protein of moderate ligand specificity. This study may also help in our age-old battle with these tenacious insects, as knowing the mode of ligand binding may help us to develop new tools for pest control.

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Ishida, Y., Tsuchiya, W., Fujii, T., Fujimoto, Z., Miyazawa, M., Ishibashi, J., Matsuyama, S., Ishikawa, Y. and Yamazaki, T. (2014). Niemann-Pick type C2 protein mediating chemical communication in the worker ant. *Proc. Natl. Acad. Sci. USA* **111**, 3847-3852.

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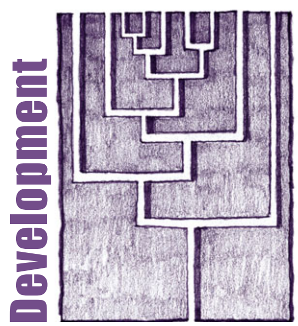
Deep appreciation for a freakish crab



Many important insights in biology have been gained through the study of anomalous, or so-called 'freak', animals. An early example of the quest to reconcile such mutants is William Bateson's 1894 book *Materials for the Study of Variation*. In this imposing 600 page tome, Bateson argues that biological variation is not necessarily continuous, but can sometimes occur through sudden leaps. This argument was in contrast to Darwin's theory of gradualism, which posits that evolution acts through the incremental accumulation of minor random changes.

Bateson points out that small variations within development are more likely to have a drastic impact on adult traits, and thus large changes can occur even within a single generation. He includes hand-drawn illustrations and dense descriptions of many anatomical abnormalities, from a human female with a pair of ears on her neck to a trout with a 'bull-dog head'. In addition to providing tattoo designs for future generations of hipsters, Bateson's extensive catalogue of eccentric creatures laid the foundation for the concept of the 'hopeful monster' – that very rarely, genetic mutations will produce freaks with a unique competitive advantage. The hypothetical image of a web-footed frog out-swimming its conventionally toed siblings provides an evocative, though perhaps improbable, example of Bateson's discontinuous evolution.

In the anecdotal tradition of William Bateson, a recent paper from Gerhard Scholtz and colleagues describes an anomalous crab with an immoderate number of eyes and antennae. Co-author Stephen Moore discovered the freak crab specimen, a member of the species *Amarinus lacustris*, in the Hoteo River of New Zealand's North Island. *Amarinus lacustris* are adorable little crabs, each about the size of a raisin, that live in freshwater and estuarine waters throughout New Zealand and in parts of Australia. This particular mutant crab possessed three eyes positioned in a horizontal row, a doubling of the carapace region in front of the eyes (the rostrum), and an aberrant antenna on top of its head.

The authors studied their unusual specimen through histology and 3D reconstruction of the crab's nervous system. They found that axons from the supernumerary eye successfully innervated the brain, suggesting that the third eye was functional. However, the extra antenna did not appear to connect with the brain, indicating that it was not operational. Finally, the neuroanatomical reconstruction revealed that the crab's brain was abnormally shaped, perhaps due to its juvenile state or possibly as a result of the same factors that produced its bizarre external morphology.

Anatomical malformations can be produced by environmental conditions that influence developmental processes, or through mutations in the genes that control development (or some combination of environmental and genetic factors). Scholtz and colleagues conjecture that the extra eye and antenna may have arisen through a duplication of the embryonic head, as occurs in conjoined twins. Crabs regenerate their eyes if they are damaged, and, under some circumstances, this regeneration can erroneously produce an antenna instead of an eye. Thus, the antenna may have originated from a regeneration event gone awry.

Was this three-eyed crab a hopeful monster? Because the crab was dissected before it could procreate in the lab, we will likely never know whether its unusual traits were heritable. But regardless of whether or not the three-eyed body plan had a promising future, such anomalous individuals continue to provide an important substrate for imaginative speculation about an animal's life history, development and evolution.

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Scholtz, G., Ng, P. K. and Moore, S. (2014). A crab with three eyes, two rostra, and a dorsal antenna-like structure. *Arthropod Struct. Dev.* **43**, 163-173.

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Hidden capacity for water preservation in mammals

Nearly 65 years after Per Scholander and his colleagues published their pioneering work on mammalian thermoregulation (Scholander et al., 1950, *Biol. Bull.* **99**, 237-258) the general consensus is that we have a good understanding of the relationships between body temperature, ambient temperature and metabolic rate. However, more recently the model has been expanded to incorporate patterns of water loss. This is important because losing water via evaporative cooling is one of the primary means of maintaining a lower body temperature at high ambient temperatures. In general, evaporative water loss is relatively constant at and below thermoneutrality (where organisms maintain a stable temperature with little metabolic investment), and increases dramatically above the upper critical limit (where costly cooling measures result in increased metabolism) as water-dependent processes such as panting and sweating are recruited. However, the effects of relative humidity on these patterns have received little attention. At temperatures above the upper critical limit, high humidity reduces the animal's ability to dissipate heat via evaporation, often resulting in increases in body temperature. Below this temperature, however, water loss was generally believed to be dictated purely by the laws of physics and therefore dependent entirely on the humidity gradient between the animal's surface and the air. However, in a recent study on a small arid-zone Australian marsupial (the little red kaluta, *Dasykaluta rosamondae*) published in *Proceedings of the Royal Society B*, Phil Withers and Christine Cooper, of the University of Western Australia, have demonstrated that instead of increasing with decreasing humidity, evaporative water loss below the thermal neutral zone is remarkably constant.

In a previous study the authors had found that kalutas exhibited a number of physiological characteristics that made them well suited to their arid habitats, including low metabolic rates and a high degree of flexibility in body temperature regulation. They also observed that these tiny animals managed to remain cool at high temperatures using lower rates of evaporative water loss relative to other species. Kalutas therefore presented a good candidate to test for potentially beneficial water-conserving adaptations to changes in humidity at lower temperatures. The authors evaluated this by exposing the animals to varying levels of humidity at temperatures in and below the thermoneutral zone and measuring metabolic rate, body temperature and evaporative water loss. Somewhat surprisingly, rather than finding that evaporative water loss increases with decreasing humidity, they found no change.

Their results mean that even in low humidity environments, where the humidity gradient and the laws of physics would favour increased water loss, minimal rates of evaporation were maintained, allowing the small marsupials to save significant amounts of water (nearly 40–50% at a relative humidity of 20%). The authors concluded that this must be indicative of a level of physiological control over evaporative water loss previously unrecorded in mammals. Although they initially believed this means of water conservation to be an adaptation to the arid habitat of the kalutas, they proceeded to find similar patterns in a number of species from a range of habitats. These findings indicate that mammals may have more control over rates of evaporative water loss at lower temperatures than was previously believed. This has important implications for water conservation, as most mammals live at temperatures within or below the thermoneutral zone where additional means of water conservation were not believed to be available. It remains to be seen what mechanisms species such as the kaluta employ to achieve control over evaporative water loss at these temperatures, how common this is among endotherms, and what this means for water and energy budgets in free-ranging animals.

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Withers, P. C. and Cooper, C. E. (2014). Physiological regulation of evaporative water loss in endotherms: is the little red kaluta (*Dasykaluta rosamondae*) an exception or the rule? *Proc. R. Soc. B* **281**, 20140149.

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