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

DIFFUSION

DIFFUSION INFLUENCES CELL DESIGN

In cells, oxygen, small metabolites and macromolecules move around by molecular diffusion. Small cells have shorter diffusion distances than large cells, so the molecules are able to reach their destination more quickly. It is especially important that these various molecules reach their targets rapidly in metabolically active tissues.

Aerobic (dark) muscle fibres are metabolically active tissues that power sustained locomotion. Oxygen must diffuse from the blood through muscle cells to the energy producing mitochondria so that ATP (the energy currency of the cell) can be produced to power contraction. In contrast, anaerobic (light) muscle fibres only require oxygen to recover from short bursts of activity, which are not considered to be as energetically costly. As light muscle fibres are less dependent on oxygen than dark muscle fibres, researchers hypothesize that light muscle fibres may be able to endure larger diffusion distances compared with dark muscle fibres.

Kristin Hardy from the University of North Carolina Wilmington and colleagues from Florida State University sought to determine how diffusion influences muscle cell structure in the blue crab *Callinectes sapidus.* Incredibly, adult blue crab muscle fibres are 7 times larger than the juveniles' muscle fibres, making them an excellent model to examine these questions. Using various microscopy techniques, the authors examined light and dark muscle fibres from juvenile and adult blue crabs. They determined the location of mitochondria and nuclei in the individual fibres and quantified the network of haemolymph vessels (analogous to blood vessels) supplying the light and dark fibres.

The team found that the distribution of mitochondria and nuclei in light muscle fibres changed radically during growth. Juvenile light muscle fibres contained evenly distributed mitochondria, while the mitochondria in adult light muscle fibres were limited to the edge of the fibre, in close proximity to the haemolymph. The distribution of nuclei yielded exactly the opposite pattern: in juveniles, nuclei from the periphery moved to become distributed throughout the adults' light muscle fibres. The authors suggest that as light muscle fibres grow, mitochondria migrate to the periphery of the fibre to be close to the oxygen-rich haemolymph in order to minimize diffusion distances for oxygen. Nuclei relocate throughout the cell since they require the diffusion of large, slow, macromolecules found in the cystol.

Interestingly, the team found no change in the location of mitochondria and nuclei during growth in dark muscle fibres. However, the authors found an intricate network of subdivisions within the dark fibres, allowing haemolymph to circulate freely. Dark muscle fibres primarily limited their mitochondria and nuclei to the edge of the subdivisions, thereby minimizing diffusion distances from the haemolymph.

Next, the researchers used mathematical models to compare how metabolic reaction rates will change depending on whether the mitochondria are located at the edges or throughout a muscle fibre. Clustering mitochondria around the periphery of large light muscle fibres allowed a much higher rate of ATP turnover compared with a simple uniform distribution throughout the cell. Furthermore, they established that dark muscle fibres could not come close to sustaining even a moderate rate of ATP turnover without the network of subdivisions.

This study demonstrates that light and dark muscle fibres in blue crab have completely different strategies to cope with diffusion limitations during extreme fibre growth. Furthermore, the findings suggest that diffusion limitations result in changes to cellular structure during growth. The next step will be to establish whether these patterns hold true in less extraordinary species and cells.

10.1242/jeb.023838

Hardy, K. M., Dillaman, R. M., Locke, B. R. and Kinsey, S. T. (2009). A skeletal muscle model of extreme hypertrophic growth reveals the influence of diffusion on cellular design. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296**, R1855-R1867.

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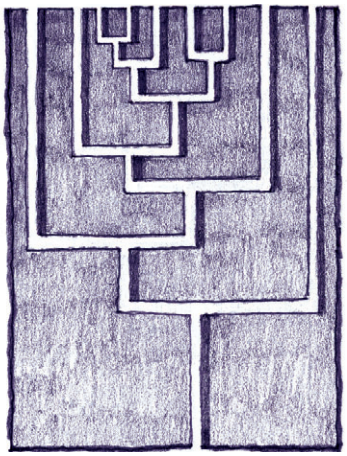
SEXUAL SELECTION



SEXY AND ONLY SLIGHTLY SLOWER

The extravagant and long tails of many male birds are great examples of ornaments that have evolved through sexual selection. Females in such species prefer males with elaborate tails, and hence those that overcome the costs imposed by bearing fancy tail feathers are often reproductively successful. But what are the actual costs, for a bird, of sporting a large, attractive tail? Given that most birds fly, and that long tails might increase the resistance experienced by a flying animal, decreased locomotor performance seems likely. Christopher Clark and Robert Dudley at UC Berkeley wanted to examine this issue more closely and recently quantified how much a long, ornamental tail affected flight performance in Anna's hummingbirds.

Clark and Dudley captured birds in Berkeley CA, and trained them to fly over a range of speeds in a wind tunnel. To alter tail length they either glued long feathers from a different species (red-billed streamertail) onto the tails of their hummingbirds, or removed some or all of the hummingbirds' tail feathers. They also had two controls, an unmanipulated condition and a sham condition, in which hummingbird tail feathers were first removed and subsequently glued back on. To quantify locomotor performance the researchers examined two parameters: metabolic cost and maximum speed.

In respirometry experiments, birds exhibited the now familiar 'U-shaped' curve relating speed and metabolism in which minimum costs are achieved at low to moderate flight speeds and costs increase at slower and faster speeds. Animals with augmented tail length incurred greater metabolic costs, on average, than controls, and this was most evident at high speeds where long tails led to an 11% increase in metabolism. Maximum flight speeds ranged between 13 and 16 $m\,s^{-1}$ across all treatments, but animals consistently showed a small decrease in maximum speed after tail elongation (average decrease of 3.4%).

The main conclusion appears to be that the cost of bearing a long tail is pretty minimal in hummingbirds, at least in relation to steady flight performance (maneuverability might be another story). This point is even more striking when you consider that the tails used to augment length in these experiments are among the longest found in any species of hummingbird – more modest tail elongation presumably is even less costly. Moreover, the greatest increases in metabolism were found at the highest flight speeds, which animals use only about 5% of the time in the wild. If these ornamental tails are in some sense 'handicapping' the males, they certainly don't seem to be doing so very severely when it comes to steady locomotion. And if females are using variation in tail length as a cue for interpreting male 'quality', the signal to noise ratio doesn't seem likely to be very good. Subtle, intraspecific differences in tail length among males are likely meaningless in terms of their effects on flight costs.

More quantitative work evaluating the costs of sexually selected ornaments is required before large, sweeping generalizations can be made. However, this work on the tails of hummingbirds, as with work on the horns of dung beetles, suggests that evolving elaborate structures to attract females or combat other males may not necessarily always come at as much of a cost as we once imagined.

10.1242/jeb.021675

Clark, C. J. and Dudley, R. (2009). Flight costs of long, sexually selected tails in hummingbirds. *Pro. R. Soc. B* **276**, 2109-2115.

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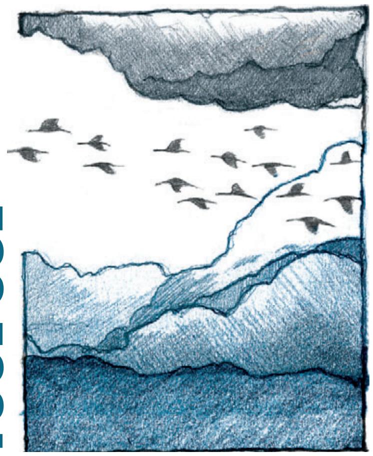
TOOL USE



SMART ROOKS USE TOOLS

Birds of the crow family are widely considered to be intelligent animals and the New Caledonian crow has been shown to be as proficient in its use of tools as most of the great apes. In a recent article in *Proceedings of the National Academy of Sciences of the USA*, the aptly named Christopher Bird, together with Nathan Emery, has revealed that in the laboratory, rooks can show a level of tool use that is amongst the highest in the animal kingdom. Even more intriguingly, rooks in the wild have never been reported to use tools.

Bird and Emery presented hand-reared rooks with a series of experimental challenges that were carefully designed to explore the exact nature of the birds' abilities. First, the rooks were presented with a vertical tube in which a tasty but inaccessible worm could be retrieved by tipping the platform on which it was placed, by dropping stones down the tube.

The rooks were able to select stones of the right size and shape to fit into the tube, and would also go and get appropriate stones if none were provided near the apparatus. These behaviours indicate that the birds were able to select tools that were functionally relevant to the task. If the rooks were not given access to stones, but instead to a novel stick, all the birds immediately picked up the stick and solved the problem using this new tool. When provided with a combination of a functional stick and a non-functional stone (or *vice versa*), the birds chose the tool that would enable them to get the worm, reinforcing the interpretation that the functional aspect of the tool was fundamental for their choice.

Amazingly, the rooks were also able to show 'metatool use', in which a tool was used to get access to a tool that would, in turn, gain access to the worm. This reveals that the rooks could recognise that a tool can be used on a non-food item, and that they were capable of hierarchically organizing their behaviour, resisting the temptation to try and get the food immediately. This is something that monkeys find extremely difficult. Success rates in this test were very high – nearly 97% and all birds solved it on the very first trial.

Finally, the authors tested the ability of rooks to make their own tools, either by removing side-branches from a stick, so that it would go into the tube, or by making a hook that would enable them to get the worm directly (the birds had already shown they could use an existing hook to this end). In both cases the birds showed spontaneous tool creation, manufacturing a hook or changing the shape of the stick. Their behaviour in this respect rivals that of the New Caledonian crow, and is superior to that of virtually every other animal that has been tested, with the exception of the great apes.

The particularly intriguing part of this study is that while rooks may be clever, there is no previous evidence of them using tools, either in the laboratory or in the wild. Similar findings have been reported in capuchin monkeys, which very rarely use tools in the wild but will do so readily in the laboratory. So what do rooks normally use their brainpower for? Why did the mental structures that underlie this behaviour evolve?

Either rooks do indeed show forms of tool use in the wild, and these have simply not been recognised, or their highly developed social foraging is the selective basis for their intelligence, and this can be applied to a variety of problems, including solving cunning experiments. By showing that natural tool use and advanced intelligence can apparently be separated, this study raises important questions about where, how and why animal intelligence evolved.

10.1242/jeb.021758

Bird, C. D. and Emery, N. J. (2009). Insightful problem solving and creative tool modification by captive nontool-using rooks. *Proc. Natl. Acad. Sci. USA* **106**, 10370-10375.

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FERTILITY REGULATION

GnRH EVOLUTIONARILY CONSERVED IN REPRODUCTION

Neuropeptides are tiny messenger molecules used by neurons to control physiological functions and behaviour. Some of these neuropeptides are hormones that play a key role in the control of reproduction. In vertebrates, these neuropeptides include gonadotropin-releasing hormones (GnRHs), which regulate fertility through follicular growth and ovulation. The GnHRs bind to special receptors, which transmit the hormone's signal from the surface of the target cell to the interior, where the cell responds according to the message carried by the hormone. Genes encoding GnRH-like peptides and their receptors have also been identified in various invertebrates, including nematodes and insects, but their role in invertebrate reproduction was unclear. In a recent *Proceedings of the National Academy of Sciences of the USA* paper an international team of scientist led by Liliane Schoofs provides the first direct evidence that GnRH-mediated signalling has a role in invertebrate reproduction, and shows that this pathway is evolutionarily conserved from worms to humans.

Several years ago molecular biologists identified the first ligand of the insect GnRH receptor, the adipokinetic hormone (AKH), which is an invertebrate neuropeptide related to vertebrate GnRHs and is known to control energy metabolism. Although a role for AKH in invertebrate reproduction has been suggested, experimental proof was missing. It needed the help of a little worm, the nematode *Caenorhabditis elegans*, to confirm this assumption. In *C. elegans*, a GnRH receptor homologue had been reported previously, but it was thought to be an orphan receptor because no one had identified an AKH/GnRH-like neuropeptide that could bind to the receptor in the worm's genome.

To identify the hormone that binds the worm's GnRH receptor, Schoofs' team first tested whether the receptor is capable of binding AKH from another invertebrate, the fruit fly *Drosophila*. They cloned the worm receptor and expressed it in human cells. To demonstrate that the fly neuropeptide binds and activates the worm receptor, they measured the calcium concentration in the human cell's cytoplasm, as the calcium level is known to increase when the receptor is activated by binding its hormone. The *Drosophila* AKH peptide triggered an increase of the calcium concentration suggesting that an AKH/GnRH-related peptide is the natural ligand of the worm GnRH receptor.

Motivated by this finding, the scientists tried to identify an AKH/GnRH gene in the *C. elegans* genome. By improving their search scheme, they finally discovered a gene encoding a neuropeptide that was strikingly similar to arthropod AKHs and vertebrate GnRHs. The next step was to figure out whether the newly discovered AKH/GnRH-related peptide also activates the worm's GnRH receptor. Synthesizing the peptide, the team tested it at different concentrations to see whether it binds to the worm receptor and triggers the calcium response, and found that it did, even at low concentrations.

But the scientists still did not know the biological function of the newly discovered neuropeptide. To get some clues to its putative role in reproduction, the team knocked out expression of the genes encoding the AKH/GnRH-related peptide and its receptor, and analysed the worm's ability to produce offspring. The worm's fertility was affected. The scientists observed a delay in egg laying and a decrease in the number of progeny.

Liliane Schoofs' team has provided the first clear evidence that an AKH/GnRH-related peptide and its receptor have a role in the control of reproduction in invertebrates. It seems that the fundamental concepts in the regulation of reproduction are highly conserved throughout the animal kingdom. Obviously, the underlying signalling machinery evolved very early in metazoan evolution.

10.1242/jeb.021717

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