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

EVOLUTION

BILLFISHES ARE CLOSELY RELATED TO FLATFISH

Scombroids are a collection of primarily predatory fish that include mackerels, tunas, bonitos and billfishes, found worldwide in tropical and temperate waters. Most scombroids are speedy swimmers, with a streamlined body shape as well as muscular, metabolic and cardiovascular features that allow them to swim continuously. They also have enhanced aerobic capabilities to power their athletic swimming. In addition, some scombroids, such as tuna and billfishes (e.g. marlin and swordfish) have a remarkable physiological adaptation known as regional endothermy, where they maintain regions of their body at an elevated temperature relative to their surroundings, allowing them to expand their geographical range. These unique characteristics suggest that tuna and billfishes share a close evolutionary history; however, the phylogenetic relationship between scombroids remains unresolved. Numerous morphological and molecular studies have been conducted to determine the relationships between scombroid family members, producing conflicting results. Chris Moyes and his colleagues from Queen's University in Canada sought to resolve the classification of scombroid fishes using DNA sequencing.

The team collected muscle samples from numerous species of billfishes (e.g. swordfish, striped marlin, blue marlin) and tuna (e.g. bigeye tuna, yellowfin tuna, bullet tuna) near the Hawaiian Islands in the Pacific Ocean. Next, they sequenced the DNA from nine mitochondrial loci and three nuclear loci from each muscle sample. Finally, they used statistical analysis to compare their results with sequences from additional species of fishes available on GenBank (publically available DNA sequences) in order to generate the best pylogeny for scombroid fishes.

Remarkably, the team found that tuna and billfish are only distantly related. This finding suggests that regional endothermy and continuous swimming has arisen independently in these two groups of fishes. What's more, the authors found that billfishes are closely related to flatfishes (Pleuronectiformes) and jacks (Carangidae). This is an astonishing result considering the differences in lifestyle, physiology and morphology observed among these fishes. While billfishes are extremely athletic, and have regional endothermy and elongated bills, flatfish primarily live on the sea floor and are usually asymmetrical with their two eyes located on the same side of their head.

The evolutionary relationships found in this study clearly demonstrate that shared selective pressures can lead to similar adaptations in distantly related species. Equally, it also shows how disparate selective pressures can lead to divergent adaptations in closely related species. These findings have important implications for scientists studying scombroids and their relatives and also provide a dynamic system to study the evolution of endothermy in fishes. At the very least, this study should remind us that you can't judge a book by its cover!

10.1242/jeb.036673

Little, A. G., Lougheed, S. C. and Moyes, C. D. (2010). Evolutionary affinity of billfishes (Xiphiidae and Istiophoridae) and flatfishes (Plueronectiformes): Independent and trans-subordinal origins of endothermy in teleost fishes. *Mol. Phylogenet. Evol.* **56**, 897-904.

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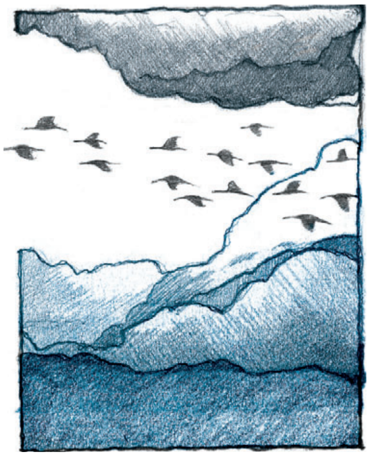
BONE DENSITY



WEIGHING IN ON BIRD BONES

Although the list of things I know about bird biology is short, one thing I felt confident about was that bird skeletons are lightweight. I’ve seen for myself the thin-walled, hollow bones found in many bird species, and this always made intuitive sense: flying is certainly easier if you’re lighter. Betsy Dumont at the University of Massachusetts recently decided to look more deeply into this apparent truism by asking just how lightweight bird bones are.

Using museum specimens, Dumont measured bone density across a wide variety of perching birds, rodents and bats. She chose to focus on the cranium, humerus and femur, which represent the largest components of the feeding and locomotor systems of these animals. Rodents were chosen for comparison as they are of a similar size to birds and also maintain relatively high metabolic rates. Bats were studied because outside birds they are the only vertebrates capable of generating powered flight, and bats too are purported to have lightweight skeletons. To measure bone density, Dumont weighed the bones to find their mass and measured the bone volume using helium displacement, which is like using water displacement, except that helium molecules are considerably smaller than water molecules and so can infiltrate much smaller spaces in bone, leading to better density estimates.

It turns out that humeral density is not distinguishable among the groups, averaging just above 2.0 $g\,cm^{-3}$ in birds, rodents and bats. Femoral density is lowest in birds, near 1.8 $g\,cm^{-3}$; however, cranium density is highest in birds, averaging nearly 2.2 $g\,cm^{-3}$ (for comparison, rodent crania have densities averaging below 1.8 $g\,cm^{-3}$). In short, different bones within and across major vertebrate clades vary with respect to density, a fact that may not raise many eyebrows. What is surprising though is that average bone density, which accounts for femora, humeri and crania and is weighted to reflect the relative contribution each bone makes to the total mass of its respective skeleton, is highest in birds and lowest in rodents. Flying animals appear to have denser bones than terrestrial counterparts of similar size.

In bones, differences in density generally mean differences in mineral content. Higher mineral contents are linked to greater stiffness and strength. Dumont thinks that such material properties may be central to understanding why bone density is relatively high in birds and bats. Along with flight, birds and bats have also evolved small body size, and one way to design a small skeleton while preserving its strength is to make its bones more dense. Investigating links between the material properties of bone and the evolution of flight and small body size in birds and bats is going to require much more work – if only we could get bone density measurements from transitional forms in the fossil record like Archaeopteryx. In any case, the next time you happen to handle a bird skull, don’t worry so much about it breaking. What may appear to be a fragile, lightweight structure, is likely both heavier and stronger than you think!

10.1242/jeb.036681

Dumont, E. R. (2010). Bone density and the lightweight skeletons of birds. *Proc. R. Soc. B* **277**, 2193-2198.

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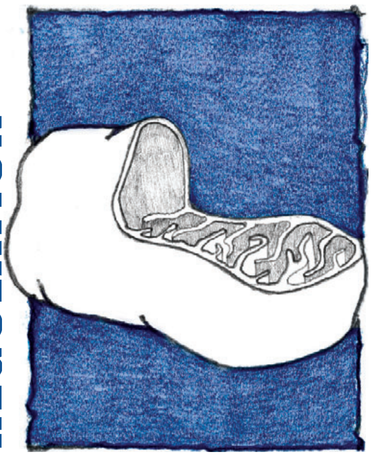
REGULATION



MITOCHONDRIAL REGULATION: AN INSIDE JOB?

Mitochondria are the powerhouses of the cells, producing energy in the form of ATP under aerobic conditions. Although these organelles contain their own genetic material, over the course of evolution they surrendered most of their genetic makeup to the nuclear genome. Out of the thousands of genes necessary for proper mitochondrial function, only 13 are encoded by the mitochondrial genome. Therefore, anytime a cell needs to produce more mitochondria or enzymes required for oxidative processes, an intricate coordination of both nuclear and mitochondrial gene expression is necessary.

Over the last decades, several regulators of mitochondrial and nuclear gene expression have been identified. Some of these regulators are capable of inducing expression of nuclear genes that are targeted for mitochondrial function. For example, the peroxisome proliferator activated receptor gamma coactivator-1α (PGC-1α) and the NAD+ dependent protein deacetylase sirtuin 1 (SIRT1) promote the expression of a suite of nuclear encoded genes, including the mitochondrial transcription factor A (TFAM), which promotes the replication, transcription and maintenance of mitochondrial DNA. Although until now both SIRT1 and PGC-1α have only been found in the nucleus acting on nuclear gene expression, a team led by Katia Aquilano from the University of Rome and IRCCS San Raffaele in Rome postulated that these regulators could also regulate gene expression directly within the mitochondrion.

Initially, Aquilano and colleagues used microscopic staining to localize these two factors in the cell, and for the first time discovered that PGC-1α and SIRT1 colocalized with mitochondrial proteins. To confirm these results, the team further identified these proteins in purified mitochondria from both tissue cultures and mouse tissues. The group also used human platelets, cells with mitochondria that are devoid of nuclear DNA, to definitely show that both these factors were directly associated with the organelles.

Given this newly found localization, the team tackled more mechanistic questions; notably, whether these factors were associated in the mitochondrial nucleoids, submitochondrial regions packed with mitochondrial DNA and regulatory proteins. They purified nucleoids and were able to detect both SIRT1 and PGC-1α in these purified fractions. Knowing that a region of the mitochondrial DNA, the D-loop region, is where mitochondrial DNA replication and transcription originate, the team postulated that both SIRT1 and PGC-1α were associated with this region through interactions with TFAM (which specifically binds to this region). Using antibodies directed against TFAM, SIRT1 or PGC-1α, they confirmed that these factors were associated with this regulatory region of mitochondrial DNA. Further, the team unveiled the presence of large protein complexes containing these factors in purified mitochondrial extracts, suggesting a functional relationship between these regulators.

Overall, these results highlight new roles for two important regulators of mitochondrial function. Their localization within the mitochondrion and their association with TFAM, a major mitochondrial transcription factor, suggest that SIRT1 and PGC-1α have similar functions within the nucleus and the mitochondrion in promoting gene expression. Thus, although the abundance of these 'master controllers' has long been considered important for mitochondrial function, we now have to also consider the localization of these proteins and the ever expanding number of regulators that they can interact with.

10.1242/jeb.036657

Aquilano, K., Vigilanza, P., Baldelli, S., Pagliei, B., Rotilio, G. and Ciriolo, M. R. (2010). Peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis. *J. Biol. Chem.* (epub ahead of print). doi: 10.1074 /jbc.M109.070169.

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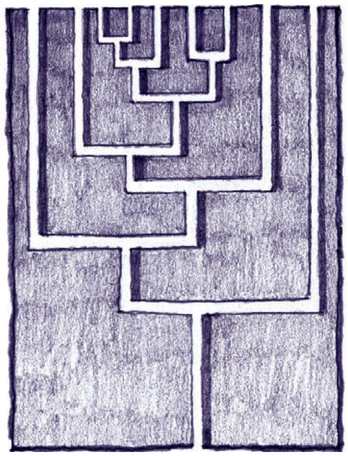
MATING STRATEGIES



THE DAMSELFLY ENIGMA: BETTER BIGGER OR SMALLER?

Damselflies show abrupt, darting flight, which is the envy of aero-engineers. This amazing ability is used both to capture prey and, by males, to establish territories that can attract females. Insects are ectothermic, so maintaining this flying ability in the face of fluctuating environmental changes is a major challenge. Furthermore, body size has both a direct effect on manoeuvrability and an indirect effect, through its impact on heat retention. Two Japanese researchers from Kyoto University, Yuka Samejima and Yoshitaka Tsubaki, have studied how body size and temperature affect flight ability in this stunning insect.

The damselfly they chose to study – *Mnais costalis* – lives by fast-flowing mountain streams and shows male polymorphism: orange-winged larger males tend to have territories while clear-winged smaller males do not. These morphs reflect different mating strategies, with smaller males 'sneaking' mating opportunities.

The authors used an infrared thermographic camera to measure the surface temperature of males, which they manipulated in the laboratory by using a halogen lamp, and studied the flight performance of each male. They estimated maximum lifting force and size-corrected lifting force, which they measured by attaching weights to the insects' wings with fishing line. Size-corrected lifting force is an index of acceleration that is linked to the damselfly's superb aerial acrobatics.

The authors found that both measures of flight performance were positively correlated with body temperature. This is not particularly surprising, as it is well known that insect flight muscle activity increases with temperature. However, although body size led to higher maximum lifting force, it was negatively correlated with size-corrected lifting force. Simply put, larger males were less agile.

When the authors took their thermographic camera into the field, they discovered that the story was even more complex: larger, territorial, males showed substantial variation in body temperature, as their territory showed varying patches of light and shade. Smaller, non-territorial males, however, generally had higher body temperatures, as they tended to bask in sunlit areas, as part of their 'sneaky' mating strategy.

This combination of behavioural ecology and physiology enriches our understanding of the maintenance of polymorphic mating strategies in this species. Due to their smaller body size and their more constant, higher body temperature, smaller males are apparently more agile, and therefore gain an advantage in terms of 'sneaky' mating and avoiding predation. However, their smaller size means that they are less able to lift females – essential during mating – or to combat larger males.

The best strategy, it would appear, would be to be a large male with a perpetually sunlit territory. Indeed, the authors' unpublished data suggest that such males have higher reproductive success. However, such territories are rare and may be temporally or physically fragile; natural selection has led to the current polymorphism of alternative male strategies, with underlying alternative physiologies. Who would have thought that the beautiful flight of the damselfly concealed such complexity?

10.1242/jeb.036665

Samejima, Y. and Tsubaki, Y. (2010). Body temperature and body size affect flight performance in a damselfly. *Behav. Ecol. Sociobiol.* **64**, 685-692.

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