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

REPRODUCTION

HOT FISH NEED BOXER BRIEFS

It is well known that elevated testicular temperature can lead to low sperm count and other reproductive problems in male mammals. Just ask any father who switched from briefs to boxers when trying to have a baby. It turns out that some fish, such as pejerrey and Florida largemouth bass, also experience heat-induced gonadal damage, except that both males and females are affected. And as fish are unable to regulate their body temperature, increasing global temperatures could have a damaging effect on reproduction and worldwide fish populations.

While the mechanism is poorly understood in fish, in mammals, gonadal degeneration generally occurs through a process called apoptosis. Apoptosis is essentially when a cell commits suicide through a number of characteristic steps. Lauro Satoru Ito from the Tokyo University of Marine Science and Technology and colleagues from Brazil and Japan sought to determine whether apoptosis occurs during warm water-induced gonadal degeneration in male and female pejerrey, whose natural habitat in the shallow lagoons of South America can reach 31–32°C.

To characterize gonadal degeneration and germ cell loss and examine the occurrence of apoptosis, the authors exposed fish to a prolonged heat stress (16 weeks at 29°C) or a short heat stress (36 h at 31°C and then returned to 24°C), or held them at a comfortable 24°C. Then the team sampled the fishes' gonads at various time points (hours, days, weeks) to look for histochemical and biochemical signs of gonadal degeneration, germ cell loss and apoptosis.

The team found clear evidence of apoptosis in the gonadal cells of both male and female pejerrey. Remarkably, all of the fish exposed to a prolonged heat stress survived and only 11% of those exposed to a short heat stress died. Furthermore, all the fish fed normally except for fish during the short 31°C exposure. Thus, this experiment demonstrates that warm temperatures, which may have no effect on survival or even feeding, can nonetheless have negative effects on reproduction.

Not surprisingly, the severity of the gonadal damage was proportional to the magnitude of the heat stress. In addition, males were more susceptible to heat stress than females. Interestingly, Ito and his team observed substantial individual variability in heat sensitivity. For example, some male pejerrey exposed to prolonged heat stress were completely devoid of sperm-producing germ cells at the end of the study while other fish from the same treatment group appeared completely normal. However, the basis of this variability is unknown.

This study provides valuable information in the field of thermal reproductive biology, which is imperative in the face of global warming and its potentially catastrophic consequences for fish populations. The work performed by Ito and colleagues shows that warm temperatures can harm the gonads of pejerrey in a similar manner to mammals. In addition, pejerrey require a lower range of temperatures to successfully reproduce than they do to simply survive. This means that if these fish want to have babies, they need to keep cool. If only fish could wear boxer briefs!

10.1242/jeb.023853

Ito, L. S., Takahashi, C., Yamashita, M. and Strussmann, C. A. (2008). Warm water induces apoptosis, gonadal degeneration, and germ cell loss in subadult *Odontesthes bonaiensis* (Pisces, Atheriniformes). *Physiol. Biochem. Zool.* **81**, 762-774.

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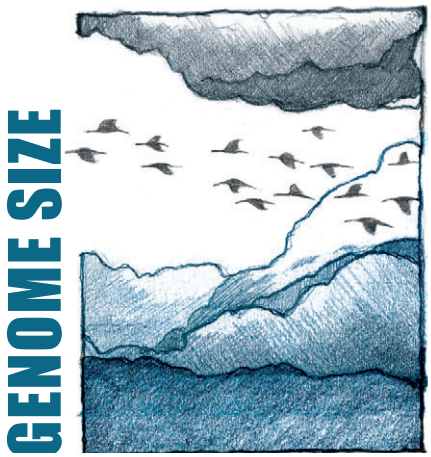

SMALL GENOMES TAKE FLIGHT

Flight evokes awe among us largely earth-bound creatures and the anatomical and physiological adaptations underlying aerial locomotion remain of great interest to scientists and the general public alike. Within extant vertebrates, birds and bats exemplify the notion that there is more than one way to take flight. For example, avian fliers have fused hand bones and use feathers to generate lift while bats construct their wings out of skin spanning extremely elongated fingers. Nevertheless, the physical demands of flight are great enough that vertebrate fliers have converged on various traits, and one that has received much attention is the genome size in these animals. Flying vertebrates have relatively small genomes; recent work suggests that even pterosaurs had a relatively small genome. Why do fliers have small genomes? Less DNA generally means smaller cells, which have a large surface area to volume ratio, facilitating oxygen diffusion to intracellular mitochondria. Flight requires high levels of energy to be sustained and thus improved oxygen transport in flying animals makes good sense.

Chandler Andrews and Stuart Mackenzie, along with T. Ryan Gregory, from the University of Guelph, who has a strong interest in genome diversity, wanted to investigate more closely the relationships between DNA, cell size and flight ability in birds while carefully controlling for phylogeny. To do so, they collected data on genome, nucleus, cell and body size from animals spanning 74 species, 51 genera and 18 families within the largest avian order, Passeriformes. In addition, from these same species they also made measurements of wing shape (aspect ratio) and wing loading (ratio of wing area to body mass) to look for connections between cellular features and macroscopic flight morphology.

Genome size ranged between 1.15 and 1.62 pg (mean, 1.32 pg) among the 74 species. To give you some sense of scale, most non-flying mammals have genomes at least twice this size, and salamanders, known for their large genomes, can reach values of over 100 pg. Even within this relatively small range among passerines, however, genome size was found to be positively related to nucleus and cell size in phylogenetically independent contrasts. Moreover, nucleus size increased disproportionately such that cells with larger genomes also had less cytoplasm. As bird erythrocytes have retained their nuclei, having a relatively large genome reduces the volume of cytoplasm in the cell and may consequently reduce their hemoglobin levels and oxygen carrying capacity. However, whether erythrocytes with larger genomes carry relatively lower levels of hemoglobin remains to be tested.

With respect to wing attributes, aspect ratio and genome size were largely unrelated. This is intriguing because it suggests that flight 'style', at least among the limited range sampled in Passerines, doesn't impact on genome size, i.e. a bird with wings more suited to gliding will have a similarly small genome compared with an animal with wings designed for maneuverability. However, wing-loading index was positively related to genome size. The authors point out that as there was no correlation between body size and genome size, this relationship occurs because birds with larger genomes also tend to have relatively small wings. Although they make it clear that this is not due to some causal effect, they emphasize that even at a relatively fine scale, animals likely to be considered good fliers (i.e. those with low wing-loading index) also have small genomes and cell sizes.

As the database of genome sizes gets ever larger and more phylogenetically diverse, the window into evolutionary patterns and physiological consequences of a cell's DNA content will open wider. But even today it is becoming ever clearer that genome size and flight ability are both tightly and inversely linked.

10.1242/jeb.021659

Andrews, C. B., Mackenzie, S. A. and Gregory, T. R. (2009). Genome size and wing parameters in passerine birds. *Proc. R. Soc. B* **276**, 55-61.

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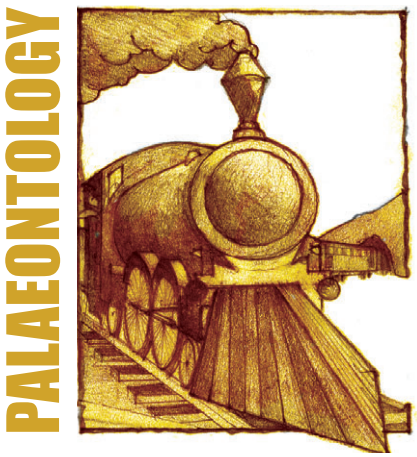

WHAT MADE THE TRACKS?

It is not often that a single, non-experimental observation can undermine our view of what happened hundreds of millions of years ago, but that is what happened 780 m below the surface of the Atlantic, around the Bahamas.

A group of US and Australian marine biologists, led by Mihkail Matz from the University of Texas, were surveying the seabed in a research submarine, when they noticed grooved tracks snaking across the seafloor. The apparent creators of the tracks were dark-green grape-like objects, around 30 mm in diameter. Sequencing of small-subunit ribosomal RNA from these objects showed they were massive single-celled organisms – mega-protists of the genus *Gromia*, previously known only from the Arabian Sea. These amoeboid organisms consist of a thin spherical layer of protoplasm under a membranous 'test' (a kind of shell), with the bulk of the organism's volume being made up of inorganic matter.

For most of us who are unfamiliar with deep sea gromiid testates, the very existence of these blobs is a cause for amazement – they are one of the largest single-celled organisms. But the truly dramatic part of the observation comes from the fact that these organisms leave tracks. Could their ancient ancestors be responsible for some of the oldest trails in the fossil record?

Fossilised bilaterally symmetrical animals (Bilateria), which appear in the fossil record around 542 million years ago and may have appeared 80 million years earlier according to molecular data, are thought to have left sinuous groove shaped traces on the pre-Cambrian seabed that eventually became preserved in the fossil record. However, some of the marks can be found in rocks up to 1.5 billion years old, long before the evolution of Bilateria. These tracks have

never been explained, and have led some paleontologists to suggest that Bilaterians, which had been believed to have produced the tracks, appeared far earlier than the Cambrian era.

The discovery of similar traces associated with modern non-Bilaterians raises the possibility that the ancient grooves were made by giant protists instead. If the fossil tracks cannot be unequivocally assigned to Bilaterians, then the earliest date at which we can be confident our distant ancestors appeared will be brought forward to their first appearance in the fossil record, rather than the first appearance of the tracks that had been attributed to them. It might also suggest that the Ediacaran biota, the earliest known complex organisms that precede the animals of the Cambrian explosion, were giant, turgid protists, like *Gromia*.

There is a catch, however. The researchers did not actually see the mega-protists making the grooves, although the grape-shaped organisms were always seen with their axes perpendicular to the trace, and they had extended pseudopods that may, as in other gromiids, be used to pull the protist along. The authors hypothesise that *Gromia* creates the grooves by picking up sediment in front of the test and excreting it behind it. They suggest that it is this process, rather than movement itself (the protists have nearly neutral buoyancy), that produces the grooves.

The final factor that facilitates the appearance of the traces is the persistence of the sediments and the extremely weak currents in the area. The authors suggest that the observed tracks – none more than 50 cm long – may have taken months to produce. Catching the gromiids in the act may prove extremely difficult, unless they can be brought to the surface and persuaded to roll in the laboratory. Deciding whether pre-Cambrian trace fossils were left by mega-protists or Bilaterians will be even trickier, but these deep-sea observations have raised an important doubt over what were thought to be the earliest traces of animal life.

10.1242/jeb.021733

Matz, M. V., Frank, T. M., Marshall, N. J., Widder, E. A. and Johnsen, S. (2008). Giant deep-sea protist produces bilaterian-like traces. *Curr. Biol.* **18**, 1849-1854.

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ECDYSIS CONTROL

BEETLE'S MOULT IS DIFFERENT FROM FLY'S

An insect's exoskeletal cuticle protects it from injuries and desiccation, as well as serving as an attachment site for skeletal muscles. As the cuticle is an incredibly hard-wearing structure, growth and development are only possible when it is shed and replaced by a larger one, a vital process known as ecdysis. Ecdysis is tightly regulated by various hormones that control the biosynthesis of essential proteins and initiate stereotyped behavioural patterns to remove the old cuticle. Although these hormones are conserved among moulting animals, their use varies across different taxa. In a recent paper published in *Mechanisms of Development*, a team of scientists led by Yoonseong Park from the Kansas State University report striking differences between the ways that beetles and flies moult.

20-Hydroxyecdysone triggers the onset of ecdysis, which is then orchestrated by various peptide hormones that regulate the physiological responses and behavioural patterns associated with ecdysis. Some of the most important ones are the eclosion hormone, ecdysis-triggering hormone (ETH), crustacean cardioactive peptide (CCAP) and bursicon. Working with the red flour beetle, *Tribolium castanaeum*, the team analysed the hormone's function during pupal-to-adult ecdysis. First they carefully determined the developmental expression pattern of the genes encoding the hormones as well as the hormones' cognate receptors. Then they went on to analyse the precise ecdysis behaviour, which consists of three phases.

Next the team took advantage of the insect's fully sequenced and annotated genome. Most importantly, the expression of particular target genes can be blocked very efficiently in *Tribolium* by RNA interference. This requires the injection of appropriate double-stranded RNAs into the small beetles, which needs a very steady hand. Fortunately Yasuyuki Arakane was the right man for the task, injecting dsRNAs for each of the target genes to identify the hormone's function.

The team found that almost all of the tested genes were absolutely essential for survival. The beetles either stopped developing at pre-ecdysis when the expression of the genes encoding eclosion hormone, ETH and the ETHR-A receptor were knocked-down, or at ecdysis when expression of the CCAP and CCAPR-2 receptor genes was impaired. Furthermore, they observed deficiencies after ecdysis when expression of the bursicon and bursicon receptor (*rickets*) genes was switched off. Only the insects that were kept from expressing the CCAPR-1 and ETHR-B receptors successfully completed ecdysis.

Comparing their results with data published for the fruit fly *Drosophila* the team concluded that ETH and eclosion hormone are necessary in *Tribolium* for pre-ecdysis and ecdysis behaviour, while eclosion hormone is not essential in the flies. Also, CCAP function seems to differ between the beetles and flies. While CCAP is necessary for ecdysis behaviour in *Tribolium*, *Drosophila* flies that lack CCAP show normal ecdysis. Possibly the most striking finding was that bursicon has a role in *Tribolium* postecdysial behaviour, but it is not required to tan the beetle's cuticle even though it is essential for tanning in *Drosophila*.

Studying the function of peptide hormones controlling ecdysis in *Tribolium* has revealed that conserved peptide hormones control ecdysis in different ways in different insect orders, yielding exciting insights into the evolution of this vital process. When it comes to ecdysis, it appears that nature is able to play different melodies using the same set of instruments.

10.1242/jeb.021691

Arakane, Y., Li, B., Muthukrishnan, S., Beeman, R. W., Kramer, K. J. and Park, Y. (2008). Functional analysis of four neuropeptides, EH, ETH, CCAP and bursicon, and their receptors in adult ecdysis behaviour of the red flour beetle, *Tribolium castanaeum*. *Mech. Dev.* **125**, 984-995.

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