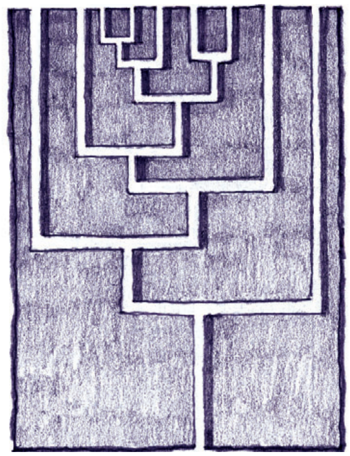
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

EVOLUTION



PLACENTA AS PACEMAKER

Placentas provide nutrients to, and remove waste from, developing offspring. Though straightforward in their purpose, they are surprisingly morphologically diverse. Two key ways that placental morphology can vary are in the amount of surface area available for exchange between mother and young (interdigitation) and the amount of tissue separating their blood supplies (invasiveness). Variation across taxa of both forms is thought to be driven by paternal, maternal and offspring conflicts over resource transfer, as resource transfer is regulated by the mother but can be manipulated by the fetus *via* placental hormones. These conflicts arise because while it is in the best interest of the young and their fathers for them to grow quickly and as large as possible, mothers must often limit the amount of resources that they provide to individual offspring. However, why some mammalian species have one type of placenta while others have another remains unclear. A new study in *PNAS* by Michael Garratt at the University of New South Wales and his colleagues suggests that placental morphological evolution is inextricably tied to life history evolution – the pace of life.

Garratt and his colleagues tested the relationships between placental morphology and life history traits across 155 mammalian species using phylogenetic generalized least squares models. They collected demographic data from the literature, including age of first reproduction, gestation time, yearly reproductive output, maximum lifespan, onset of senescence, rate of senescence and generation time.

The authors found that transitions from the ancestral, large surface area placentation to derived, medium and small area placentation are associated with transitions to traits of 'slower' life history strategies, such as having fewer offspring per year, a longer gestation and a longer lifespan. The authors posit that smaller placental surface areas may be associated with slower paced life history strategies because of the reduced urgency to provision offspring when environmentally driven mortality is low.

Interestingly, the story of transitions in the evolution of invasiveness of placentation shows the opposite trend. The transition from the ancestral, highly and moderately invasive placentation, with direct or relatively direct contact between the blood supplies of mothers and their young, to the less invasive form is associated with a transition to a faster paced life history. Garratt and his colleagues speculate that the wall of tissue associated with less invasive placentation could allow a wresting of control of resources from offspring to mother, and may prevent the manipulation of maternal physiology by fetal hormones, when the need to provision many young is great and the conflict between individual interests is intense.

The results show that evolutionary transitions in placental morphology and life history traits are linked, though which came first is unclear. This chicken and egg dilemma leaves room for future studies of the specific timing of correlated evolution between placentation and life history strategies. Additionally, because some combinations of the two morphological types, such as small surface area and non-invasive placentation, were always found to correspond, the physiological basis of this trend needs to be examined. It appears that the mammalian placenta is a pacemaker of sorts, a watchful little mother of parents and offspring, regulating the dynamics of growth and senescence. And, as widely studied as this mother has been, she still has secrets to reveal.

10.1242/jeb.081687

Garratt, M., Gaillard, J.-M., Brooks, R. C. and Lemaître, J.-F. (2013). Diversification of the eutherian placenta is associated with changes in the pace of life. *Proc. Natl. Acad. Sci. USA* **110**, 7760-7765.

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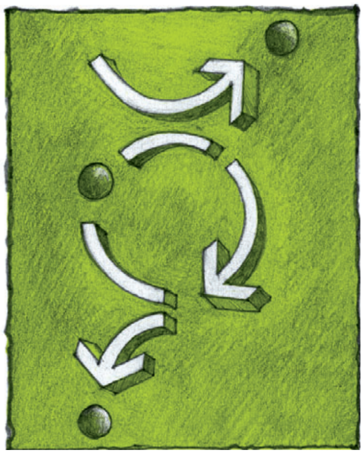
OXYGENATION



ROOT HAEMOGLOBINS BENEFIT FISH ON ACID

Haemoglobin, the oxygen-carrying protein in our red blood cells, binds oxygen at the lungs and releases oxygen at the tissues to sustain metabolic needs and, thus, life. In nearly all vertebrates, carbon dioxide, a product of metabolism, helps haemoglobin release oxygen at the tissues by acidifying red blood cells. Bony fish have ‘Root haemoglobins’ – special haemoglobins that are more pH sensitive than those of other vertebrates. Root haemoglobins help fish deliver oxygen to the retina and gas bladder, two extremely oxygen-demanding tissues. These tissues have specialized acid-producing cells that help them get lots of oxygen from haemoglobin. Other tissues do not have acid-producing cells, but is there a way that these other tissues can take advantage of the sensitivity to pH of Root haemoglobins in order to get more oxygen? Jodie Rummer from the University of British Columbia, Canada, and her colleagues recently discovered the answer to this question and their findings are published in *Science*.

First, Rummer and colleagues wanted to determine whether they could make fish muscles pick up more oxygen by simply making their blood more acidic. They monitored the oxygen level in trout muscle in real-time by inserting a very small probe into the muscle. Even though they applied only a moderate acid stress, the oxygen in the muscle increased by 65% and there was an approximate doubling in the delivery of oxygen to that muscle. The team calculated that Root haemoglobins are 20 times more efficient at delivering oxygen to a fish’s muscle than human haemoglobins are at delivering oxygen to our own working muscles!

Fish red blood cells contain an acid pump that can pump out acid from the red blood cell, increasing pH inside the red blood cell, and making haemoglobin better able to pick up oxygen at the gills during stress. Rummer and her colleagues hypothesized that the red muscle capillaries of fish have high levels of carbonic anhydrase – an enzyme that produces carbon dioxide. As one of the fastest acting enzymes known, it could quickly acidify the blood, which would undermine the action of the acid pump, as carbon dioxide readily diffuses into the red blood cells. As a consequence, the pH in the red blood cells would be lower when they entered the capillaries near the muscle and the Root haemoglobins would release more oxygen and therefore increase oxygen delivery to the muscle. Rummer and colleagues used the same experimental setup as before, but this time added a carbonic anhydrase inhibitor to the fish’s blood. This time the oxygen in the muscle did not increase. This showed that carbonic anhydrase found in the capillaries of muscles was necessary for the enhanced oxygen delivery to that tissue.

Rummer and colleagues have shown for the first time that Root haemoglobins help fish deliver oxygen to all tissues, not just the retina and the gas bladder. More importantly, Root haemoglobins are responsible for the extraordinary capacity of bony fish for oxygen delivery, allowing this vertebrate group to successfully conquer almost every body of water on the planet.

10.1242/jeb.081653

Rummer, J. L., McKenzie, D. J., Innocenti, A., Supuran, C. T. and Brauner, C. J. (2013). Root effect haemoglobin may have evolved to enhance general tissue oxygen delivery. *Science* **340**, 1327-1329.

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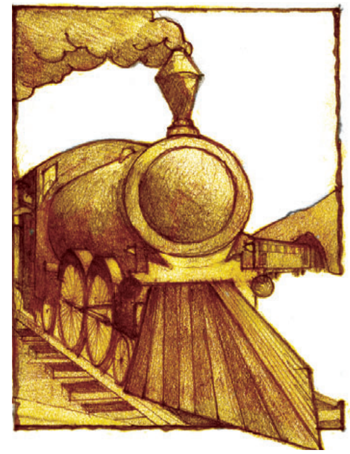
CHEMOTAXIS



CO_2 FOR MATING ON THE SLY

Mating is a secretive affair for small male *Loligo* squid. Whereas their larger brethren try to woo females by showing off their changing colours and beating each other into submission, the smaller males do not stand a chance in this courtship game. Instead, they have to rely on stealth tactics. After a large ‘consort’ male successfully courts a female, he deposits sperm into her oviduct. A small ‘sneaker’ male then rushes towards the mating pair and quickly deposits his sperm onto the female’s skin, aiming for a small receptacle near her mouth. The female expels the eggs from the oviduct, where most have been fertilised by the consort sperm, but as she deposits the eggs onto the seabed using the arm crown around the mouth, they are exposed to the sneaker sperm, resulting in external fertilisation of the remaining unfertilised eggs. How can this form of fertilisation be effective, given that there is little preventing the sneaker sperm from drifting off?

A team of researchers from various institutions in Japan, Germany and Italy have found at least part of the answer to this question, in a study recently published in *Current Biology*. When the team put sperm of consort and sneaker males into chambers and labelled each population with a different dye, they found that the sneaker sperm, but not the consort sperm, formed swarms by independently swimming towards each other. This behaviour has been seen in other animals, and will help the sperm to stay in the same spot. When the researchers then introduced a filter into the chamber that only allows small molecules to pass, they noticed that the sneaker sperm swarmed on either side of this barrier. As the cells cannot touch each other across the membrane, this suggests that the sperm are attracted by a molecule that they themselves produce. What molecule could this be?

After ruling out a number of chemoattractants, the authors found that a bubble of CO_2 strongly attracted sneaker, but not consort, sperm. This was very interesting, as CO_2 is a product of cellular respiration, and therefore it fits with the hypothesis that the sperm, itself, produces the attractant. In fact, when the team added inhibitors of a common sensor for CO_2, carbonic anhydrase, to the chamber, the swarming behaviour of sneaker sperm was reduced.

However, both sneaker and consort sperm have this sensor, so why are only sneaker sperm sensitive to CO_2? The authors noticed that when sneaker sperm came into contact with CO_2 their intracellular pH dropped. In a series of molecular experiments they showed that it is precisely this acidification that underlies the swarming: when the intracellular pH drops, the sperm keep swimming in the same direction, but when it shoots up, they make a turn. This process results in a net movement towards the source of CO_2. The consort sperm simply lack the molecular machinery that acidifies the intracellular milieu in response to CO_2, and are therefore not sensitive to it.

This study elucidates how sperm has adapted to different mating behaviours through the process of evolution. It tells us that physiology adapts even within the same sex of the same species to ensure individuals can reproduce, so even the little guy has got a chance.

10.1242/jeb.081661

Hirohashi, N., Alvarez, L., Shiba, K., Fujiwara, E., Iwata, Y., Mohri, T., Inaba, K., Chiba, K., Ochi, H., Supuran, C.T. et al. (2013). Sperm from sneaker male squids exhibit chemotactic swarming to CO_2. *Curr. Biol.* **23**, 775-781.

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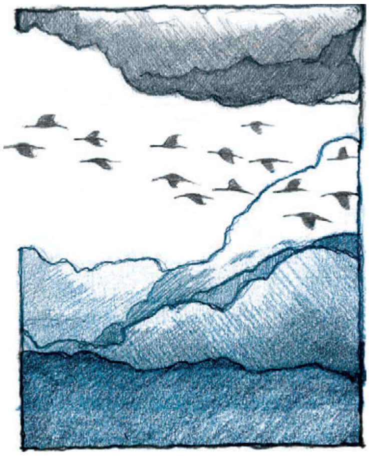
FLIGHTLESSNESS



BIOMECHANICS OF FLIGHTLESSNESS IN PENGUINS

For anyone who has sat through March of the Penguins, a documentary film following the arduous journey that Emperor penguins make between the ocean and their ancestral breeding ground, and wondered, ‘Why can’t penguins just make life easier for themselves and fly?’, a recent paper published in *PNAS* should help to answer the gnawing question. An international team of researchers set out to understand why certain seabird species may have evolved in such a way that a journey through the air (taking hours) would be deemed less advantageous than a journey on land (lasting several days).

The team hypothesized that certain seabirds may have lost the ability to fly as a trade-off with another activity. For example, moving between air and water, flying and diving, requires optimized wing designs – perhaps a wing good for diving is a terrible wing for flight, and *vice versa*. The scientists wondered whether, as flightlessness evolved in certain lineages of seabirds, such as in penguins, their wings became more biomechanically adapted to swimming and diving and less and less able to sustain flight. Using the thick-billed murre (*Uria lomvia*) and the foot-propelled pelagic cormorant (*Phalacrocorax pelagicus*), two diving seabird species that are also able to fly and are considered counterparts to ancient ancestors of penguins, the scientists were able to test this biomechanical hypothesis and to compare flying seabirds with flightless seabirds.

The murre and pelagic cormorant are similar to penguins in their diving and swimming behavior, so the research team decided to measure the energy cost of flight as well as the energy cost of diving in these birds. During the summer of 2006 and 2012, the researchers captured 41 thick-billed murres in Canada and 22 pelagic cormorants in Alaska, respectively, and fitted them with specialized sensors that measure the birds’ energy expenditure at the same time as following their diving and flight behavior using data loggers. Both species dive underwater to hunt for prey – the thick-billed murre are wing-propelled divers, while the pelagic cormorant are foot-propelled drivers – yet both are considered only adequate fliers. The team was able to measure how much energy the birds need to expend during flight and during swimming dives and found that the birds used more energy to fly compared with any other known bird and used more energy to swim compared with penguins of similar size. Furthermore, the authors found that the ‘flight cost’ for the thick-billed murre and pelagic cormorant is the highest recorded so far for vertebrates.

Just as flight has been shown in many instances to have evolved independently, so too has flightlessness. The authors suggest that flightlessness in penguins evolved as a result of biomechanical pressures on the wings. In order to become expert divers, they argue, penguins gave up the ability to fly in favor of increasing dive endurance. As seals, sharks, sea lions and killer whales all pose a risk to diving penguins, a wing designed for aquatic expertise becomes a must. On land, penguins have less predation to fear – a wing designed for flight is less beneficial. Thick-billed murres and pelagic cormorants, in contrast, have many land-based predators, including foxes and wolves, leading researchers to suggest that the pressure to maintain wings optimized for flight in these species is strong. The authors conclude that there must be a compromise in wing design – a wing cannot excel equally well in both air and water – and that trade-offs are inevitable.

10.1242/jeb.081679

Elliott, K. H., Ricklefs, R. E., Gaston, A. J., Hatch, S. A., Speakman, J. R. and Davoren, G. K. (2013). High flight costs, but low dive costs, in auks support the biomechanical hypothesis for flightlessness in penguins. *Proc. Natl. Acad. Sci. USA* **110**, 9380-9384.

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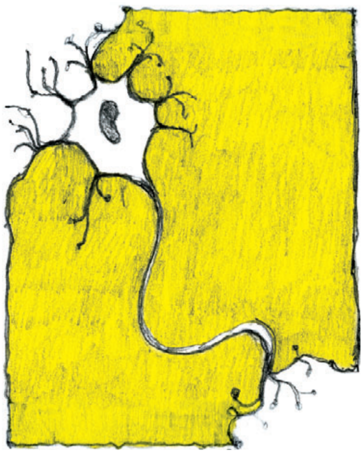
NOCICEPTION



HOW IS NOCICEPTIVE ‘PAIN’ PROCESSED BY SQUID?

The question of whether invertebrates feel pain is difficult to assess as it is a subjective experience perceived by sentient beings (neurobiologists generally exclude all invertebrates from this group). As such, it is not surprising that pain-like states have received little to no attention in the invertebrate scientific literature. In contrast, nociception, which in mammals is responsible for the long-lasting pain sensation following injury, has been investigated in several species of invertebrates including *Drosophila*, *Caeonorhabditis*, *Aplysia* and now recently a cephalopod.

New research published in the *Journal of Neuroscience* by Robyn Crook and colleagues at The Woods Hole Marine Biological lab, USA, has presented convincing evidence that squid (*Doryteuthis pealeii*) possess nociceptors. They report for the first time the presence of nociceptor activity in the fins of squid. They showed that when the fin was crushed, neuronal afferents (nerves that relay sensory information to the brain) displayed long-lasting sensitization and spontaneous activity after injury, which resembles the activity associated with painful stimuli in mammals. More interesting was their finding that spontaneous activity recorded in neuronal afferents relaying stimuli information was not just restricted to the site of injury but also observed on the un-injured fin on the opposite side of the body. This suggests that however the squid interprets this sensory information, the response to it is not localized to the site of injury, but spreads across the body. In mammals, such an increase in spontaneous afferent activity at the site of injury promotes defensive limb withdrawal and directs the individual’s attention specifically to the site of injury.

Their findings do not directly address the question of whether pain-like states and pain-induced suffering are experienced by cephalopods. As it is understood in humans, pain is a highly variable, subjective experience incorporating many kinds of sensory input (mostly nociceptors) that culminates in a negative feeling and subsequent aversive reaction to avoid the unpleasant sensation. It is strongly influenced by previous experiences, including conditioning from past personal experiences and witnessing painful responses to stimuli on others, suggesting that a portion of its perception is learned. Physiological and behavioural changes that occur in response to pain may include the following: reflexive limb withdrawal, increased heart and breathing rates, vasoconstriction/blood pressure increase, vocalization, aggression and subsequent inflammation. When we observe these behavioural correlates in other mammals we conclude that the animal is experiencing pain and suffering. However, this presumption becomes harder to defend as one examines animals from phylogenetically more removed species such as invertebrates.

An unfortunate mistake made by many people is the personification of invertebrate behaviour. When one witnesses an insect recoil in response to a poke by a stick or observes the tail-flips of a lobster as it is placed in boiling water, one may erroneously think the animal is experiencing pain. The scientific basis for this assumption is questionable. For the lobster tail-flip example (caridoid escape response), a very simple (just a few neurons) neuronal circuit coordinates this behaviour whereas perhaps many hundreds of thousands of neurons participate in pain cognition in mammals. However, the question of nociceptive sensation in complex invertebrate species such as cephalopods is worthy of further scientific inquiry as these animals possess the most complex nervous systems of invertebrates, which rivals those of similarly sized vertebrates and allows them to interact socially, possess an extensive repertoire of behaviours and have an impressive capacity to learn (unlike most other invertebrate species). Clearly, with this evidence in hand one should be mindful and err on the side of caution when interacting with these animals.

10.1242/jeb.093047

Crook, R. J., Hanlon, R. T. and Walters, E. T. (2013). Squid have nociceptors that display widespread long-term sensitization and spontaneous activity after bodily injury. *J. Neurosci.* **33**, 10021-10026.

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