OUTSIDE JEB

Poison frog moms and dads use similar brain cells for parenting

Parenting is hard, and everyone takes a different approach. Infant mice are usually reared by mom, whereas songbirds tend to be exemplar co-parents. While scientists know how parenting gets kick-started in feathered and furry vertebrates, other animals, such as poison frogs (*Dendrobatidae*), have been largely under-studied. Despite their alarming name, poison frogs are superb caretakers who travel long distances to safely hide and feed their young. Recently-hatched tadpoles need to be in water to develop, so mom or dad give them a 'piggyback ride' to nearby pools on the forest floor. While some poison frog species do this as a single parent (either just mom or dad), others take after the birds and share the carpooling duty. However, it was not known how these different parenting arrangements are evoked.

Given poison frogs' diverse parenting styles, Eva Fischer in the lab of Lauren O'Connell at Stanford University, USA, along with a team of collaborators in the USA and Ecuador, explored whether the brains of solo parenting versus co-parenting frog species differ. In their paper published in the *Proceedings of the Royal Society B* in July 2019, Fischer and her team focused on three poison frog species with different parenting tactics: single dads (*Dendrobates tinctorius*), single moms (*Oophaga sylvatica*) and co-parents (*Ranitomeya imitator*). To accomplish this, Fischer intercepted parent frogs giving tadpoles a piggyback ride and compared how many brain cells were active in the brain of the caretaker and the uninvolved parent. Two brain regions stood out: the hypothalamus, which controls a range of social behaviours – including parenting – in other animals, and the hippocampus, which is important for navigating and storing memories. Overall, giving a piggyback ride to their kid triggered more activity in both the hippocampus and hypothalamus of mama and papa frogs compared with levels in childless adults or uninvolved partners across species, suggesting these areas are conserved in parenting frogs. But while the boost in activity pinpointed the brain regions excited by parenting, it was unclear which specific cells were active when the frogs dropped off their kids.

Focusing in further, Fischer scrutinized the expression pattern of genes in brain cells that had been activated while the frogs were parenting and how those expression patterns varied between the different parenting styles. Of the many genes in the brain that increased during piggyback rides, one stood out from the crowd: galanin. Galanin is a hormone found in the hypothalamus that is critical for mouse parenting, but its presence outside rodents was unknown. In co-parenting frogs, transport of tadpoles sparked more activity in galanin-expressing brain cells than in frogs without kids. Interestingly, having kids generated more brain activity in galanin brain cells in the uninvolved female partner of solo dads, suggesting that moms are ready to jump in and help if dad fails at childcare. Oddly, dropping the kids at the pool didn't change solo mom frogs much – their galanin-expressing cells were just as active as those in their non-parenting counterpart.

It seems that the hypothalamus – with its galanin-expressing nerve cells – is part of an important and conserved parenting mechanism in many animals, as it exists in amphibians that are evolutionarily more ancient than their avian and mouse relatives. Fischer and her team suggest that this work lays an exciting foundation for exploring future questions about the evolution of parenting in poison frogs and expands our understanding of the complex concoction of genes, brain circuits and hormones that combine to make 'Parents of the year'.

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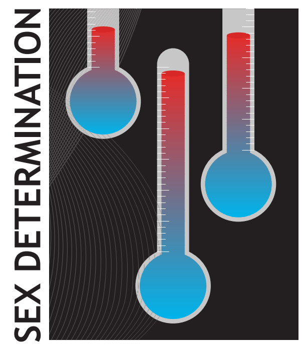
Unhatched turtles know what's hot



Can you imagine if the climate your mother lived in determined your gender *in utero*? Fortunately, for us mammals, this is not the case. However, nest temperature during incubation can influence the gender of many egg-laying reptile species, including turtles, crocodiles and lizards; this phenomenon is called 'temperature-dependent sex determination'. If the nest is too hot, all the young might end up female but if it's too cold, they could all end up male – mothers aim for the perfect middle temperature, just like Goldilocks' preferred porridge. Even though mums choose nesting sites with the best conditions for their eggs, they can't control the weather once the eggs are laid. Sexual differentiation doesn't occur in turtles until after about three weeks of age, which places the vulnerable clutch at risk of producing an uneven ratio of girls to boys, jeopardising their chances of

Outside JEB reports on the most exciting developments in experimental biology. The articles are written by a team of active research scientists highlighting the papers that JEB readers can't afford to miss.

reproducing in the future. As global warming continues and temperatures creep upwards, there is concern that this could spell disaster for species whose biology is influenced by developmental temperature.

But imagine if a reptile embryo could determine its own gender by moving from one side of the egg to the other. Wei-Guo Du from the Chinese Academy of Sciences and his collaborators from the same institution and Macquarie University, Australia, wanted to find out if this was possible for the eggs of an endangered freshwater turtle. They thought that if a turtle embryo could move and find the best temperature inside its egg, it could buffer the effects of unfavourable nest temperature. But is this behaviour possible on such a small scale? To test this, they ran a neat experiment. First, they measured the temperature at each end of eggs inside nests. Then, they gave the eggs a drug that stops embryos from sensing temperature and moving. They also located and marked the exact position of the embryonic turtles by holding up the eggs to a light at the beginning and end of the experiment. To complete the study, they predicted how hatchling sex ratios may differ 50 years into the future under a medium-level greenhouse gas and aerosol emissions climate change scenario.

The nest temperature that produced only females was a mere 5°C warmer than that resulting in only males, but the team found the temperatures at opposite ends of a tiny 34 mm turtle egg could differ by up to 4.7°C. Their main prediction also proved true: embryonic turtles moved to their preferred temperature inside the eggs, influencing their gender. Finally, the researchers' computer modelling revealed that the sex ratios of nests with embryos that couldn't respond to temperature by moving would become more and more uneven with ongoing climate change. This is a big problem, because the survival of future turtle populations relies on there being enough females to reproduce and lay their own clutches of eggs.

Du and colleagues have revealed a fascinating and effective way that turtles can control their temperature during development to avoid the negative effects of less-than-ideal nest temperatures. However, it's worth remembering that turtles have been around for millions of years – more than enough time to have experienced numerous changes in climate – so is this behaviour really important? The team thinks so. The species-specific temperature that produces an even ratio of females to males can evolve and change over time in response to climate shifts, but it doesn't happen overnight. Coping with the swift speed of human-driven global warming needs faster reactions to temperature – such as those behaviours displayed by the turtle embryos in their eggs – to help animals survive in the short term while the evolutionary changes slowly catch up.

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Whirlpools are hotspots for hungry sharks

The open ocean looks like a vast and featureless expanse to land-lovers like us. But to its aquatic inhabitants, the ocean is a dynamic environment, guided by the constant motion of the wind and currents. One key feature of the ever-changing ocean surface are mesoscale eddies: huge areas of swirling water that peel off from larger currents and look like whirlpools from space. Eddies are the ocean's 'internal weather' system, transporting heat, salt and even plankton around the globe. But what happens to animals when they run into these underwater storms? Led by Camrin Braun, a team of researchers from the Massachusetts Institute of Technology, Woods Hole Oceanographic Institute, the University of Washington, and the Massachusetts Division of Marine Fisheries, all based in the USA, used a unique combination of tools to track how a large voyaging predator, the blue shark (*Prionace glauca*), navigated these unstable ocean swirls.

Scientists wishing to simultaneously monitor the eddies – which can stretch across hundreds of kilometres – while tracking the movements of individual animals were hampered until recently by the technical challenges. However, the US-based team overcame this hurdle by using an inventive approach – combining satellite tracking of animal movements, ocean remote sensing and modelling – to record specific interactions between the blue sharks and eddies. The team also explored how ocean weather systems influence the behaviour of sharks living along the northeast coast of North America.

The team tagged 15 individual blue sharks with a pair of electronic tags: one that monitored water temperature and depth, and another that used satellites to precisely track the shark's physical location. By compiling over 2000 days of tracking data and nearly half a million measurements, the team constructed precise 3D models that linked the sharks' movements, the water temperatures they experienced and the positions of eddies in the region. They found that blue sharks frequented warm water eddies during the day and sought out these areas in preference to cold water eddies. Once inside the eddies, the sharks swam in characteristic foraging patterns, diving fast before making leisurely returns to the surface. And these new observations back up earlier reports of cephalopods and other deep-sea fish species found in the stomachs of caught blue sharks, leading the researchers to conclude that the sharks used the warm eddies to tunnel far beneath the surface in search of deep-sea prey.

But why do the sharks bother tunnelling into the ocean at all? Why didn't they just dip and dive as they please, regardless of where the eddies form? The researchers reasoned that it all comes down to the thermal physiology of blue sharks. Unlike some other large open ocean fishes, such as tuna, which selectively heat up parts of their body, blue sharks are true ectotherms; their body temperature is dictated entirely by their environment. By hanging around in eddies, which were frequently 10ºC hotter than the surrounding ocean, blue sharks could dive deeper than the theoretical limits of their cold-blooded biology and make a meal of the most abundant fish community on Earth.

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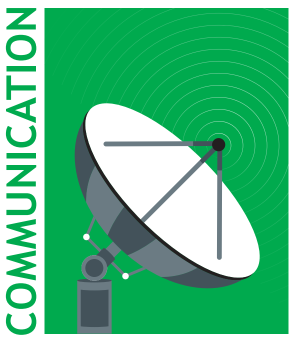
Eggs 'chat' about danger in the nest



In an environment rife with dangerous predators, parents want their offspring to be born ready for life's challenges. Biologists have known for years that parents can affect their offspring's development in the womb or egg by sending signals via their hormones and behaviour. But can developing siblings also share information about the external environment with each other before being born? Exciting new research from Jose Noguera and Alberto Velando from the University of Vigo, Spain, suggests 'yes'. The researchers found that yellow-legged gull eggs may move and vibrate in a way that siblings can detect through their shells to communicate about predator threats.

Developing eggs don't sit idly by, waiting to hatch. They jiggle when the embryos move in response to external sound or contact from their parents and siblings, and these good vibrations have been shown to help nests hatch more synchronously. But whether eggs can warn their siblings about lurking predators and what effects these communications might have on chick development was unknown. Noguera and Velando collected wild yellow-legged gull eggs and brought them to a field lab to answer this question. Raising the eggs in nests of three inside cosy incubation chambers – no parents needed – the researchers assigned the nests to one of two groups. The first group was exposed to recordings of the shrill alarm calls of their parents when threatened by predators, while the other group listened to white noise only. Then, the researchers added another level of complexity to their experiment to be sure that they were recording genuine cross-talk between the nestmates. Only two eggs from each nest heard the alarm calls or white noise inside a sound-proof box for 12 min each day, while the third egg was excluded.

The researchers discovered that the eggs were certainly affected by the parents' alarm calls, as they developed in ways that might enhance their survival. Compared with the white noise nests, the chicks that had heard alarm calls as eggs were quieter and quicker to hide when they experienced alarm calls after hatching. They also had increased levels of stress hormones and more of their DNA was methylated, which suggests that they have altered gene expression patterns to help them cope with danger. However, these survival benefits also came at a cost. The chicks exposed to scary sounds inside the egg grew more slowly, possibly because of excessive stress hormones or because they had fewer mitochondria in their blood cells (an indicator of energy production ability). Most excitingly, the chick inside the third egg developed exactly like its nestmates, despite not hearing the frantic parental alarm calls while developing inside its egg.

The eggs were clearly sharing information before hatching, so the third egg was equally well prepared to deal with predator threats as its nestmates. Noguera and Velando also filmed the eggs before they hatched and scored their vibrations from the video footage. The eggs that had been exposed to their parents' alarm calls vibrated more than the eggs that had only heard white noise and the third egg, which had not heard the alarm calls, mimicked the other eggs' vibrations; the vibrations are a likely means of egg-to-egg communication.

More research is now needed to understand exactly how external cues like shell vibrations cause such major changes in an animal's development, but it is clear is that nearby siblings can eavesdrop on each other and gather important information that helps programme their biology in preparation for life on the outside.

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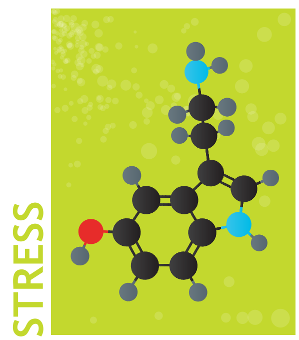
Tackling obesity with zebrafish mutants



Obesity is a worldwide epidemic that is influenced by a combination of factors, such as lifestyle choices, stress levels and genetics. Each of these individual aspects, or a combination, can significantly alter a person's development, starting early in the womb and continuing into adulthood. But which of these components, or their interactions, are key to understanding the obesity epidemic and its detrimental effects on longevity and quality of life?

Erin Faught and Matt Vijayan, two researchers at the University of Calgary, Canada decided to tackle this monumental question by examining how the interaction between stress and genes during early development impacts fat accumulation and breakdown in the body. For their work, the team turned to zebrafish, small unassuming tropical fish that are quickly becoming a superhero of medical research because their genes can be mutated easily, allowing researchers to learn more about the roles of these genetic elements during growth and development. Faught created two groups of zebrafish, each with a mutation in the genes encoding one of two receptors – the glucocorticoid receptor and the mineralocorticoid receptor – for the stress hormone cortisol. The researchers made these mutations so that they could compare the mutated fish with normal fish to figure out which of these receptors is activated during stress and how that activation affects fat accumulation and breakdown in the body.

Working with zebrafish embryos, the duo exposed both mutated and normal youngsters to either cortisol (i.e. stressed fish) or no cortisol (i.e. non-stressed fish) conditions from 3 days post-fertilization (dpf) up to 15 dpf. The researchers did this to simulate a stressful environment during development and compare the response with that of the non-stressed animals. By the end of the 15 days, the non-stressed fish with an intact mineralocorticoid receptor gene accumulated more fat than those with this gene mutated. The authors also found that mineralocorticoid and glucocorticoid receptors work together: the mineralocorticoid receptor allows the embryos to accumulate fat when there is no stress during development, while the glucocorticoid receptor is activated during stress, to mobilize energy and allow the organism to overcome the challenge. In addition, the receptors work together to increase lipid breakdown, allowing the fish to use the fat they accumulated from food as an energy source and cope with the stress. Faught and Vijayan concluded that the mineralocorticoid receptor is essential during development to allow lipid accumulation. They also suggest that exposure to stress during development, particularly in children, may lead to deficiencies in how the organism is using those lipids and how efficient it is at breaking them down. The authors conclude that these findings may help us to understand the influence of stress on gene expression and how exposure to a stressful environment early in development may lead to genetic disorders and obesity.

While this study has shown a clear interaction between genetics and stress during early development, and how one can influence the other, it raises more questions. Can we predict the effects of stress on adults based on their experiences during development and early life? What effects does early exposure to chronic stress have on the way adults cope with stress and fat storage/breakdown later in life? Could genetic manipulation of genes such as the mineralocorticoid receptor be the answer to human metabolic diseases and offer the hope of personalized medicine for all? Only more research and time will tell.

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