OUTSIDE JEB

Green brains kiss(peptin) and tell

Glowing green brains in Petri dishes may sound like science fiction, but in Yoshitaka Oka's lab at the University of Tokyo, Japan, they are more like fine art. In a study recently published in *Endocrinology*, his lab used transgenics – where specific bits of DNA are added to an organism in order to study the function of a gene – to make the brains of medaka fish fluoresce green wherever a protein called kisspeptin is made. Kisspeptin is produced by neurons in discrete regions of the brain and these neurons release the protein to communicate with other neurons in the brain that, in turn, regulate many aspects of reproduction, including sexual maturation. In other words, kisspeptin begins the conversation between the brain and the gonads. While kisspeptin is known to be essential for puberty in mammals, its role in regulating the reproductive cycles of seasonal breeders such as medaka, is not known, so Oka and his colleagues used their transgenic medaka to figure this out.

Knowing that medaka that are raised under 14 h light/10 h dark conditions become sexually mature whereas fish grown in 10 h light/14 h dark conditions do not, the scientists raised their transgenic medaka under the two different lighting conditions to produce a group of sexually mature fish and another group that was not sexually mature. Next, they carefully removed the brains and placed them in dishes. The team could easily see neurons expressing kisspeptin glowing green by looking at the brains under a fluorescent microscope. Then, they recorded the kisspeptin neurons in action using an electrophysiology technique called whole-cell patch-clamping and compared the neuron activity in the two groups of fish. Oka's team found a clear difference between the kisspeptin neurons of mature and immature fish. In mature fish that were ready to breed, kisspeptin neurons glowed brighter, meaning more kisspeptin was being made, and showed higher firing frequencies compared with kisspeptin neurons in immature fish. Since a high firing frequency, or activity level, is required for a neuron to release its peptides and communicate with other neurons in the brain, the team concluded that more kisspeptin is made and released in sexually mature fish.

In a second set of experiments, Oka's team wanted to understand how these kisspeptin neurons are regulated during a breeding cycle. They raised transgenic female medaka to sexual maturity and then surgically removed their ovaries – to effectively end the breeding cycle – and allowed the fish to recover. Since ovaries are the major source of the female sex hormone, estrogen, Oka's team supplemented half of the recovering fish with estrogen while the other half received none. After 3 days of recovery, the team removed the brains and analyzed the kisspeptin neurons as before. They found that the kisspeptin neurons of mature females that had their ovaries removed resembled those of immature fish – they glowed faintly and were mostly inactive, so little kisspeptin was made and released by these neurons. In comparison, giving female fish estrogen after their ovaries were removed returned the kisspeptin neurons to an expression and activity level reminiscent of sexually mature fish.

So, Oka and his crew showed that the production and release of kisspeptin in the medaka brain coincides with sexual maturation and the activity of kisspeptin neurons is sensitive to changes in estrogen. Their results support a conserved role for kisspeptin in regulating sexual maturation among vertebrates with different types of reproductive cycles. Now pucker up and go smerch a perch!

10.1242/jeb.112151

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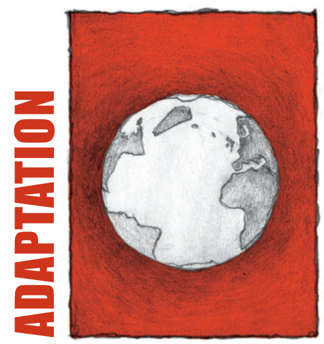
Seasonal swings match latitudinal shifts in shut down



Winter is generally not the best time of year for organisms. Temperatures are low and food is scarce, and for those species that can't fly south for the winter, surviving is a matter of hunkering down and making the best of a bad lot. But some individuals are lucky enough to carry different versions of the same gene (alleles), which make them better at surviving the demands of winter than others; particularly those animals endowed with genes that are able to reduce their metabolic demands, allowing them to survive winter with less food. Not surprisingly, these extra-tough creatures are often those that live at higher latitudes where winters tend to be harsher and longer. Now, a team of researchers from Stony Brook University and the University of Pennsylvania, USA, led by Rodrigo Cogni, have shown that the genetic changes associated with survival at higher latitudes in the fruit fly *Drosophila melanogaster* are also

Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. 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.

associated with seasonal shifts in metabolism.

Drosophila melanogaster is a short-lived fly that can produce a new generation every few weeks in warm weather, so the researchers were curious whether there could be adaptive changes in the frequency at which these tougher alleles are found over the span of seasons, as well as in geographic space. Previously, they had shown that the version of a gene known as *couch potato*, which is associated with the seasonal shutdown of metabolism, was found more commonly at higher latitudes in spring than at lower latitudes in summer. In their most recent study, they broadened their scope to genes responsible for the major branching points in metabolism to see whether the way that versions of *couch potato* varied over time and space could be generalized across the major metabolic pathways.

The team began by focusing on single nucleotide polymorphisms (SNPs) in 46 genes that code for central metabolic enzymes identified from the 37 different genome sequences for *Drosophila melanogaster* published in the genetic database Flybase. To study populations along a latitudinal gradient, they collected flies from 20 different populations in eastern North America, from the city of Sudbury in Northern Ontario through to southern Florida. They also collected flies from a single orchard in the southern part of the range several times to study the population over the course of a year.

From these collections, Cogni and his team sequenced copies of each of the genes that they were interested in, then estimated how often different base pairs were found at each SNP in each population, then correlated the frequency of each allele to latitude and time of year of collection. They found that 31 out of 46 genes had allele frequencies that changed significantly with latitude, but only two had frequencies that changed with time of year. But those genes whose allele frequencies tended to be highly correlated with latitude also tended to have allele frequencies that were highly correlated with the time of year. And this pattern became stronger when the team looked only at those SNPs that were correlated with latitude, which the authors suggest means that these areas are under particular selection for adaptation to cold weather whether due to seasonal shifts or latitudinal.

For a fly out in the cold the challenges are best handled by a well-adapted set of genes. And it turns out that the exact same pathways that adapt flies to a northern climate also help with seasonal shifts too.

10.1242/jeb.112128

Cogni, R., Kuczynski, K., Lavington, E., Koury, S., Behrman, E. L., O'Brien, K. R., Schmidt, P. S. and Eanes, W. F. (2015). Variation in *Drosophila melanogaster* central metabolic genes appears driven by natural selection both within and between populations. *Proc. R. Soc. B* **282**, 20142688.

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Building the social brain, one task at a time

Is it easier to live in a small town than in a big city? Living in a big city requires frequent interactions with many different individuals and responding appropriately to a huge variety of social situations, while living in a small town requires fewer types of interactions, with fewer individuals. The complexity of living in big groups has led to the 'social brain hypothesis', which suggests that as group size increases, brain size should increase to meet these social demands. However, the 'task specialization hypothesis' is a counter theory, which suggests that as social group size increases individuals start to perform increasingly specific tasks. According to this theory, someone living in a small town is more likely to be a jack-of-all-trades, while a big-city dweller will be more likely to have a highly specialized job. Thus, the 'task specialization hypothesis' suggests that as social group size increases, the brain should become increasingly specialized, rather than becoming larger overall.

Sabrina Amador-Vargas, from the University of Texas at Austin and the Smithsonian Tropical Research Institute, with colleagues from both institutions and the University of Arizona, decided to investigate the 'social brain hypothesis' versus the 'task specialization hypothesis' in acacia ants (*Pseudomyrmex spinicola*). These ants live in social groups with a single queen and a host of workers, and have a symbiotic relationship with acacia trees. Each social group lives within a tree and workers chase off animals that try to feed on the acacia leaves. In return, the trees provide the ants with 'Beltian bodies' – small packets of food that the ants find irresistible.

In wild ant colonies in Panamá, the researchers estimated colony size by marking and recapturing worker ants, and counting the number of entrance holes on the host tree. The scientists then assessed task specialization by counting how many of the worker ants performed the same task day after day. They also observed how workers responded to simulated foraging or defensive tasks. The researchers predicted that as colony size increased, worker ants would be less likely to switch jobs and more likely to exclusively perform either foraging tasks on the leaves or defensive tasks on the trunks. They further predicted that as social group size increased, the foraging ants would be more likely to ignore an intruder, while the defensive ants would be more likely to ignore Beltian bodies. After conducting behavioural observations, the researchers collected worker ants and measured the volumes of different brain areas.

They found that as colony size increased, task specialization also increased. In larger social groups, worker ants were more likely to perform exclusively either defensive or foraging tasks, rather than switching between jobs. Importantly, the scientists showed that specific brain regions related to learning and memory became larger in foraging workers as social group increased, but smaller in defensive workers as social group increased.

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Thus, these results support the 'task specialization hypothesis', where ants living in small groups are more general jacks-of-all-trades and ants from big groups become more specialized for a specific job. The social brain isn't necessarily a bigger brain, but it is a unique brain that is built one increasingly specialized task at a time.

10.1242/jeb.112144

Amador-Vargas S., Gronenberg W., Wcislo W. T. and Mueller U. (2015). Specialization and group size: brain and behavioural correlates of colony size in ants lacking morphological castes. *Proc. R. Soc. B* **282**, 20142502.

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The invisibility cloak of filefish

Few sensations beat the smell of fresh coffee in the morning, beckoning you from bed and directing you to the kitchen. But in a diner, all is lost. The sharp recognizable coffee odour is swallowed in a sea of sensations from dozens of coffee cups at dozens of tables, and I am distracted by other smells (bacon mainly). By blending in with their surroundings, prey animals camouflage themselves in much the same way. They hide in plain sight, and for these species, not being seen, heard or smelled is a matter of life and death. But how do prey animals ensure a convincing match with their surroundings? An exciting new paper by an international team of researchers led by Rohan Brooker in *Proceedings of the Royal Society* found that for some fish the answer lies in their diet.

Harlequin filefish live in coral reefs where they feed on coral polyps of one particular genus. The problem is, they are not alone. The reef is a busy place and while the filefish focus on eating coral, other bigger fish focus on finding and eating them. Previous work from Brooker and his colleagues found that filefish visually mimic their coral prey to escape predation, but here they posited that filefish also use another form of crypsis mediated by chemicals and olfaction. Chemicals travel easily in water and predatory fish are known to sniff out prey.

To determine whether filefish avoid detection using chemical crypsis, the researchers established two groups of fish and fed each one an exclusive diet of one of two corals. They then gave coral-eating crabs a choice, based on odour alone, between their favourite coral and a fish from either of the two treatment groups. When the crabs were given their favourite coral and a fish reared on their less-preferred coral, they unambiguously chose the coral. However, when the crab could choose between coral and a filefish fed on the same coral, they found that the crabs couldn't always tell the two apart. Incredibly, a filefish that had eaten coral smelled enough like the real thing to trick a true connoisseur!

But what about the fish that matter most: predators? They too failed to take the bait. When predatory cod were placed in a tank with coral and filefish fed on the same coral, the cod swam about lazily. But when faced with filefish fed on the alternative coral, the predator picked up pace and went on the prowl. And finally, when cod were confronted with two fish, one odour-matching the background coral and the other not, the cod made a bee-line for the mismatched filefish expecting a meal. Meanwhile, the matching filefish basked in safety.

Although the mechanisms of filefish chemical mimicry remain elusive, the benefits of doing so are immediately evident. Equally, they suggest an impressive flexibility in multimodal camouflage. First, filefish hide by matching the colours and shape of their coral diet. Second, they coat themselves in compounds that render them effectively invisible to their predators. If filefish can engineer this trick with other food items, it would permit safe passage across the reef. Furthermore, like a chef preparing the perfect curry, filefish could potentially mix and match the food they eat to maximize the efficacy of their protective cloak.

10.1242/jeb.112110

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