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INSIDE JEB

Young killer whale males learn dialect

A young male killer whale vocalizing. Photo credit: J. Crance, HSWRI.

If you travel through a country and you have an ear for dialect, you might notice odd words pop up that are particular to the location. Dialects are an occupational hazard of travel, and it turns out that killer whales from different geographical regions have them too. Ann Bowles, from the Hubbs-SeaWorld Research Institute, USA, says, 'Some mammals have a repertoire of calls that seem specific to particular geographic areas or particular social groups.' However, why killer whales acquire their dialect is a mystery and discovering the reason is particularly tricky because killer whales form incredibly stable family groups, making it almost impossible to study dialect acquisition. So Bowles turned her attention to six captive killer whales at SeaWorld San Diego, USA. According to Bowles, five of the animals originated from – or had a mother from – the waters surrounding Iceland, while the sixth was from the Pacific Northwest, and all had arrived in San Diego via different routes. Given the animals' different origins and life experiences, Bowles and her colleagues Jessica Crance and Alan Garver were curious to find out how much overlap there was between the animals' use of dialect (p. 1229).

According to Bowles, recording the animals' calls was particularly challenging. Fortunately, SeaWorld had installed eight hydrophones in the walls of one of the pools in the complex that the whales inhabit, so she was able to record them communicating and film them as they interacted. Then Crance identified the calls and assigned them to the individual that was closest to the hydrophone that had made the loudest recording. After months of painstaking analysis, the team clearly identified 17 call types that all of the animals with an Icelandic heritage were using. 'There is enough overlap in their repertoires that we would consider them to have the same dialect', says Bowles.

But then Crance noticed something change in the repertoire of the two juvenile males' dialects after 2005: they adopted some of the call patterns of the older male that shared the pools with them. Bowles admits that she was initially sceptical when Crance showed her the evidence, but as the team investigated further, they realised that the two youngsters had switched their social allegiance – they had hooked up with the older guy – and begun to learn his call patterns.

The switch happened when the mother of one of the youngsters gave birth to a new calf. Until then, her son had spent most of his time with his mother and learned her dialect. But when his mother became occupied with the new arrival, he moved on to associate more with the senior male. And Bowles laughs when she remembers that the other young male in the enclosure followed his friend, spent more time with the older male and learned his dialect too. 'It seemed like a teenage movie', she chuckles. And the youngsters could not have learned the novel sounds from anyone else because the older male had some unusual calls. 'He tends to repeat elements over again, which is not typical, and our guess is that his dialect was shaped to some degree by having lived with bottlenose dolphins for a long time', she says.

So, when the two young males began using the same idiosyncratic collection of sounds to show their new allegiance, Bowles was convinced that the youngsters had learned their mentor's dialect. And she is extremely excited by the possibility that the San Diego killer whales may be able to teach us about how human dialects evolved, saying, 'We have an animal that is starting to look like a pretty good model.'

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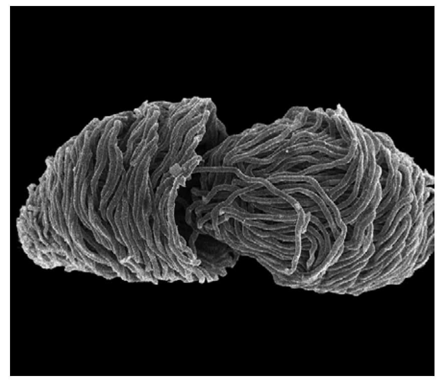
Crance, J. L., Bowles, A. E. and Garver, A. (2014). Evidence for vocal learning in juvenile male killer whales, *Orcinus orca*, from an adventitious cross-socializing experiment. *J. Exp. Biol.* **217**, 1229-1237.

Kathryn Knight

Adhesive constrains hagfish thread skeins



Hagfish slime thread skein. Photo credit: M. Bernards.

Hagfish are pretty repellent by most standards: reputed to consume prey from the inside out, the animals look ghastly and, worst of all, they release gallons of disgusting slime in less than 0.1 s when under attack. 'The slime lodges in and clogs the gills of any fish that tries to eat a hagfish', says Doug Fudge from the University of Guelph, Canada. But that hasn't deterred Fudge from investing 16 years of his life in studying the revolting gunge. He explains that irritated Atlantic hagfish release minute volumes of concentrated mucous and microscopic skeins of protein filaments that rapidly unravel when mixed with seawater and the mucous to engulf their victims. 'If either of these [the mucous or the mixing] is missing, skein unravelling is compromised,' says Fudge. And he had no reason to think that the same would not be true for Pacific hagfish until his undergraduate student, Isdin Oke, decided to test their skein unravelling, only to discover that these protein filaments unravelled spontaneously in seawater. Fudge had another hagfish mystery on his hands (p. 1263).

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Thinking about possible mechanisms that could rapidly liberate the tightly bound skeins, Fudge came up with two alternatives: that the threads swell in contact with water to burst the skeins open; or that the stiff protein fibres are coiled into skeins that are secured by an adhesive that dissolves in contact with water, releasing the skeins to spring open.

Curious to find out which theory held water, Oke and Mark Bernards gently anaesthetised Pacific hagfish to collect minute samples of the unexploded mucous and carefully extracted the coiled skeins of slime fibre. Then they tested different strengths of simulated seawater to find out which stabilised the skeins and which burst them open. Monitoring the proportion of ruptured bundles as they increased the temperature and salt concentration, the team was reassured to see that the skeins rapidly unravelled in conditions that simulated the animal's natural aquatic environment but were reluctant to unravel in dilute conditions and at high temperatures. And when the team took a close look at the skeins before and after unravelling with a scanning electron microscope, they could clearly see fluffy clumps coating the tightly coiled skeins that vanished after the skeins burst apart. Could they be some sort of protein adhesive?

Stabilising the skeins in dilute seawater, the team added a protein-digesting enzyme, trypsin, and waited to see whether the skeins sprung apart – which they did. And when the team scrutinised the surface of the trypsin-treated skeins, the enzyme had removed the clumpy coating that had restrained the coiled skeins.

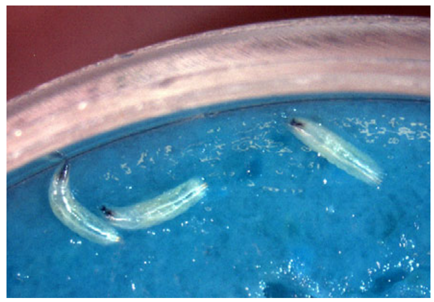
So, the Pacific hagfish slime fibres are coiled into tight skeins that are restrained by a water-soluble protein adhesive that dissolves on contact with seawater to release the strain energy stored in the stiff fibre coils. Having confirmed that the Pacific hagfish deploy slime fibres in a completely different way from their Atlantic cousins, Fudge is keen to learn more about the adhesive that keeps the skeins intact. He also hopes that hagfish slime fibres will eventually adorn the catwalks of the fashion world. 'We are currently working on a project whose aim is to produce protein fibres that are as strong and tough as hagfish slime threads in the hope that we could one day replace petroleum-based polymers like Nylon with more eco-friendly protein-based materials,' he says.

doi:10.1242/jeb.105841

Bernards, M. A., Oke, I., Heyland, A. and Fudge, D. S. (2014). Spontaneous unraveling of hagfish slime thread skeins is mediated by a seawater-soluble protein adhesive. *J. Exp. Biol.* **217**, 1263-1268.

Kathryn Knight

Gut flora odours attract *Drosophila* to best squidgy fruit



Fruit fly larvae spontaneously join each other in a test dish. Photo credit: R. Dukas.

Humans aren't the only animals that like to hang out; birds and sheep flock, cattle herd and even *Drosophila* enjoy swarming around a juicy piece of fruit. Reuven Dukas and colleagues from McMaster University, Canada, explain that fruit flies are particularly partial to fruit that is already infested with larvae. However, Dukas and his team had already shown that the odour that attracts the flies to feast was not produced by the fruit, the yeast residing on it, or fly and larval waste. Could bacteria associated with the flies and their larvae be responsible for the irresistible scent? Dukas and his colleagues, Isvarya Venu, Zachary Durisko and Jianping Xu, began investigating the source of the tantalising aroma (p. 1346).

First, the team tested the allure of fruit infested with normal larvae (complete with their usual gut flora) and sterilised larvae for larvae and adult flies. They found that both the adults and larvae found the infested fruit most attractive when the resident larvae retained their usual gut flora, so the bacteria were responsible for the attractive odour. Next, the team investigated the larvae to discover which bacterium produced the essential scent by isolating the gut flora, which they identified as *Lactobacillus brevis*. And when the team tested the effect of odours produced by another bacterium – *Lactobacillus plantarum*, which is also harboured in the insect's intestines – on foraging larvae, they confirmed that the odours were as attractive as the odours produced by larvae that had normal gut flora. Finally, the team offered *L. brevis* to the fly larvae on several different food sources and found that the odours produced by the isolated bacteria were sufficient to attract the larvae.

Having confirmed that *Drosophila* larvae are attracted by odours produced by gut bacteria, the team was curious to find out how the insects benefit from being lured to areas that the flies have already infested. However, when the team tested the larvae's preferences for food that had previously been occupied either by larvae that produced the attractive odours or by larvae that did not, they were surprised to find that the larvae didn't seem particularly attracted to fruit that had been infested with larvae producing an odour. 'That led us to search for another factor that may lead to larval preference for used over fresh food', says Dukas. So the team tested the larvae's preferences for food that had been churned up by burrowing larvae and food that they had poked with needles to simulate a larval infestation. This time they found that compared with intact fresh food, the larvae were much keener on the food that had already been used.

So fruit fly larvae use the odours produced by the gut flora of other larvae to direct them to the best squidgy fruit. The team also suggests that a patch of food infested with hoards of munching larvae is the best recommendation of a meal's quality and point out that the insect gut flora suppress harmful microbes in the vicinity, encouraging the proliferation of microbes that are beneficial to the larvae at the expense of unsafe species, making it favourable for foraging fruit fly larvae to congregate rather than go it alone.

doi:10.1242/jeb.105866

Venu, I., Durisko, Z., Xu J. and Dukas, R. (2014). Social attraction mediated by fruit flies' microbiome. *J. Exp. Biol.* **217**, 1346-1352.

Kathryn Knight

Cold incapacitates chill coma insects

Most of us don't particularly enjoy getting cold, but we can keep going. Insects, however, simply keel over when temperatures fall too low: 'they enter a coma-like state,' explains Anders Findsen and colleagues from Aarhus University, Denmark. However, the mechanisms behind this loss of control are not clear. The team explains that insects were thought to enter a chill coma when extracellular potassium levels rise in muscle, causing the muscle to depolarize and become incapacitated. So the team decided to measure the responses of locusts to cooling, the impact of chilling on the extracellular potassium levels in the insect's tibial muscles and the effect of these changes on the muscles' force production to learn more about how low temperatures send insects into a coma (p. 1297).

Cooling the animals gradually or rapidly until they stopped moving but did not freeze, the team found that most of the insects entered a chill coma at temperatures around freezing. However, instead of disrupting the insects' delicate ionic balance, the low temperatures barely disturbed the balance of salts in their bodies. Next, the team measured the force produced by the insect's tibial muscle as they electrically stimulated it to contract while maintaining normal levels of extracellular potassium, and measured a colossal 80% fall in force production at low temperatures. And when the team raised the extracellular potassium levels from 10 to 30 mmol l^{-1}, the force fell by 40%. 'Combining these two stressors almost abolished force production', the team says. So ionic disturbance and low temperature disrupt muscle function in cold insects, and the team suggests that both factors could also contribute to insects' recovery from chill coma.

doi:10.1242/jeb.105858

Findsen, A., Pedersen, T. H., Petersen, A. G., N. Ole B. and Overgaard, J. (2014). Why do insects enter and recover from chill coma? Low temperature and high extracellular potassium compromise muscle function in *Locusta migratoria*. *J. Exp. Biol.* **217**, 1297-1306.

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