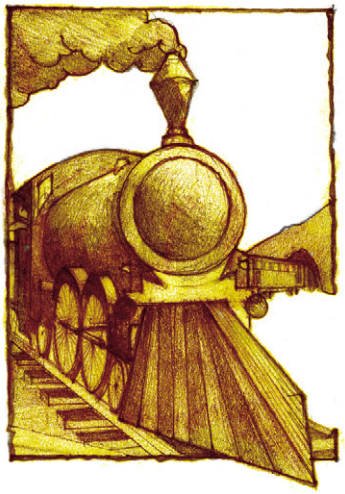
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

ONTOGENY



BIG JUMPERS CAN'T LAST

As animals that require substantial parental care, we cannot fathom the notion of being on our own from day one. Yet as spring arrives, this is the reality faced by many newborns. In such young, the selective pressure to escape predators is probably high from the moment they leave their mother or egg. However, the study of the ontogeny of locomotor performance in animals remains in its infancy; only a few systems have been studied in any detail. In general, vertebrate studies have shown that maximum speeds and levels of endurance increase as animals develop and grow and that these 'improvements' are linked to physiological changes in the underlying musculoskeletal and oxygen transport systems. In a recent paper published in *Animal Behaviour*, Scott Kirkton and Jon Harrison use the grasshopper, *Schistocerca americana*, as a model for studying shifts in locomotor capacity through ontogeny in an insect. It appears that the results from vertebrates don't really apply; instead, an understanding of the grasshopper's life history seems to make better sense of their ontogenetic performance trajectory.

Life history patterns in *S. americana* suggest a locomotor trade-off as animals mature. Specifically, juvenile grasshoppers must evade predators and travel over long distances, but do so using only their legs. In contrast, adults gain the use of wings and fly to cover significant ground. The authors chose to study jumping over a range of developmental stages to test the hypothesis that juveniles exhibit relatively high levels of performance using this locomotor mode, since it is their sole means of transport. Because there is likely stronger selective pressure on jumping performance in juveniles, compared to adults, the authors predicted that both jumping speed and jumping endurance would be higher in younger grasshoppers.

To test their prediction, Kirkton and Harrison studied the jump performance of grasshoppers in four developmental stages: second, fourth and sixth instars and adults. Size across these animals ranged 30-fold from a mean of 0.06 g in the second instar to 1.78 g in the adults. For performance trials, individuals were placed in a large gridded jumping arena and prodded to jump for 20 minutes or until fatigued (defined as 30 s of prodding without a jump). Adults had their wings clipped prior to these trials to prevent them from flying. Jumping frequency and distance, as well as jump energy and power were determined for each minute of a jumping trial.

All results indicated that adults had relatively low endurance. Adult jump frequencies plummeted within 5 minutes of activity and only about one third of adults sustained jumping for the full 20 minutes. For comparison, 2nd and 4th instars exhibited little decrement in frequency over time and approximately 90% continued jumping the entire 20 minutes, agreeing with the team's prediction regarding juvenile endurance. However, despite their poor endurance, adults consistently jumped further and faster early in trials and generated more mass-specific power throughout, suggesting they might rely more heavily on anaerobic metabolism for jumping (older grasshoppers also produce more lactic acid when jumping). So, adult grasshoppers have poor stamina but can jump powerfully, which seems quite reasonable given that they can fly and need only one good jump to get airborne. In contrast, younger instars don't have as much power, but have high endurance, enabling long distance movements over time, for escape or dispersal. In short, neither adults nor juveniles are great in all aspects of jumping performance, but they're good at what they need to do.

10.1242/jeb.02406

Kirkton, S. D. and Harrison, J. F. (2006). Ontogeny of locomotory behaviour in the American locust, *Schistocerca americana*: from marathoner to broad jumper. *Animal Behaviour* **71**, 925-931.

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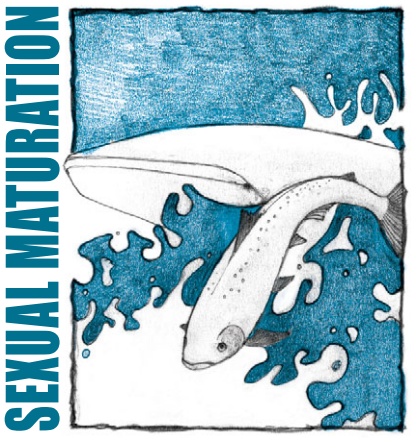

IS SEXUAL MATURATION CONTAGIOUS?

Animal reproduction is a complicated process involving the coordination of many systems that act at different levels to control behavior and gonad function. In teleost fish, steroids produced by the gonads are responsible for sexual behavior and sexual differentiation and also have important roles as pheromones, chemical signals released by an animal that trigger specific physiological processes in the recipient. Many fish species use odors and pheromones for gender recognition, synchronization of gamete maturation and spawning. However, most studies to date have focused on the role of pheromones during spawning and not on their role in pre-spawn events, such as reproductive development. In the present study, Mar Huertas, Joan Cerdà and their team have investigated whether the presence of sexually mature European eels was able to stimulate gonadal development and/or maturation in neighboring immature males.

The team first treated groups of male and female eels with hormones to initiate sexual maturation. Then, as these fish were maturing, immature eels were exposed to the water that had been conditioned by either the maturing males or females. This protocol allowed them to determine if the presence of maturing eels would stimulate reproductive development in the immature males. What the team found was that immature male eels exposed to sexually maturing males or females showed a small but significant increase in the size of their gonads. However, sperm production did not occur in the exposed males, despite their increased gonad size. Histological examination of the testis determined that these exposed males had more highly developed testis compared to control fish that had not been exposed to maturing fish, but they were less developed than mature males.

When the team looked at the plasma concentrations of sex steroids in the maturing males and females that had been treated with hormones, they found that the steroid levels changed significantly at the onset of gonadal maturation, with the plasma levels of 11-ketotestosterone and testosterone becoming elevated in maturing males while maturing females showed an increase in plasma testosterone and 17β-estradiol levels. Immature males in contact with water that had been exposed to maturing males or females showed significant changes in plasma 11-ketotestosterone and testosterone concentrations compared to controls. Together with the changes in gonad size and development, these results suggest that water conditioned by sexually maturing males or females stimulates spermatogenesis in immature males.

But which waterborne compounds are involved in triggering the immature male's gonadal development? The team suspected that testosterone, 11-ketotestosterone, 17β-estradiol and other steroids released by the maturing males and females may act as pheromones. However, when the team tested the response of the immature male's olfactory epithelium, which detects pheromones, to these compounds they found no response, ruling them out as potential pheromones. The team went on to assess the olfactory potency of water collected from the maturing males or females and found that it successfully elicited an olfactory response in the immature fish. Moreover, the largest responses were to water collected after the initiation of spermiation or during ovulation, suggesting that a pheromone is released during these times. Whether these odorants are novel compounds, other steroids or steroid mixtures remains to be investigated. However, there appears to be something in the water enhancing maturity in male eels. Now, if only we could bottle that.

10.1242/jeb.02407

Huertas, M., Scott, A. P., Hubbard, P. C., Canário, A. V. M., Cerdà, J. (2006). Sexually mature European eels (*Anguilla anguilla* L.) stimulate gonadal development of neighbouring males: possible involvement of chemical communication. *Gen. Comp. Endocrinol.* doi:10.1016/j.ygcen.2006.01.017

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WATER CHANNELS DRIVE FREEZE TOLERANCE

Ectothermic animals in the temperate and arctic zones of the world are sometimes exposed to severe cold during winter, yet they survive the potentially fatal conditions. Some of these cold tolerant ectotherms deal with very low temperatures by physiological mechanisms that promote supercooling. Others, like the larvae of the rice stem borer (*Chilo suppressalis*) are able to survive ice formation in their extracellular fluids and are able to withstand freezing at temperatures as low as –25ºC. When water freezes in the extracellular fluid, an osmotic gradient is generated across the cell membrane so that water must move out of the cells and/or osmolytes must move in to maintain the osmotic balance. This is true for the rice stem borer where the cells loose water and simultaneously accumulate glycerol during freezing. A recent study by Yohei Izumi and colleagues investigated the importance of water and glycerol transport across the cell membrane for freeze tolerance in rice stem borer larvae.

The studies were performed on larvae that were overwintering and in diapause (arrested development) or in larvae in a non-diapause state, as only the overwintering larvae are freeze tolerant. The team extracted fat bodies from the larvae and suspended these in a medium. Using these fat bodies they assessed freeze tolerance by monitoring the appearance of freeze damaged or dead cells that could be coloured using trypan blue. The study showed that only the fat bodies from overwintering larvae were able to tolerate freezing but that they could only do so when the medium was supplied with high concentrations of glycerol (0.25 mol l^{-1}). In contrast, fat bodies from non-diapausing larvae became severely damaged after freezing. Even when the medium's glycerol

concentration was increased to 0.75 mol l^{-1} the freeze tolerance of non-diapausing larvae's did not approach that of the overwintering larvae. This shows that the presence of glycerol is essential for freeze tolerance, but that high concentrations of cryoprotectants alone cannot assure freeze tolerance and other mechanisms must also be present.

The authors hypothesised that aquaporins, membrane bound water channel proteins, could play a role in freeze tolerance as these may be capable of rapid transport of water and small neutral solutes such as glycerol across the cell membrane. To investigate this, the group added mercuric chloride, an inhibitor of aquaporins, to the medium containing glycerol and found that this reduced glycerol and water transport in and out of the cells. Moreover this inhibition obliterated freeze tolerance from the fat bodies of the overwintering larvae. Thus, freeze tolerance in overwintering larvae seems reliant on the ability to rapidly transport water and glycerol during freezing. This was further supported by the finding that non-diapausing larvae, that could not endure freezing, were unable to attain the same rates of glycerol transport as the overwintering larvae.

The study by Izumi and colleagues clearly shows that in addition to the presence of cryoprotectants the ability to rapidly move water and cryoprotectants across membranes is essential to ensure freeze tolerance in the rice stem borer. This finding may provide further insight into the synergistic modifications that occur during winter acclimation and/or diapause in many freeze or desiccation resistant ectothermic animals.

10.1242/jeb.02408

Izumi, Y. Sonoda, S. Yoshida, H. Danks, H. V. and Tsumuki, H. (2006). Role of membrane transport of water and glycerol in the freeze tolerance of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). *J. Insect Physiol.* **52**, 215-220.

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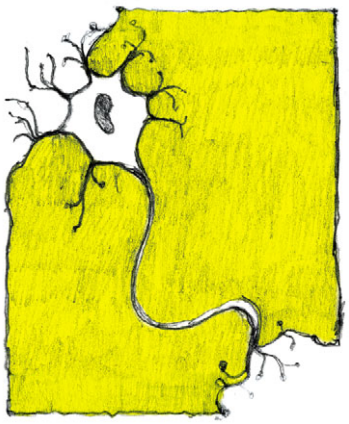
DECISION MAKING



HOPPING FOR WHEATIES

What drives choice? Which conditions do we base our decisions upon: those at the time we learn the information needed to make the choice, or those at the time of choice itself? It might be wiser to base one's decision on the conditions at the time of choice and not be influenced by the past. However, Lorena Pompilio and colleagues from the University of Oxford have recently shown that a grasshopper's choice is dependent upon how much it gained at the time of learning, not at the time of choice. A similar effect has been demonstrated in mammals and birds, but this is the first demonstration in an invertebrate species of remembered gains driving future preferences. This implies that the mechanism of making decisions based on past conditions (state-dependent valuation) was not an early vertebrate acquisition but is a wider phenomenon and perhaps universally present.

The team investigated how a grasshopper's nutritional state at the time they learned to recognize a scent affected their subsequent choices when re-presented with the scent. First the team restricted the grasshoppers' food intake so that they were in a 'poorly fed' state. Next, they trained the 'poorly fed' insects to recognize a scent, lemon grass, while rewarding them with a wheat-seedling treat. Once the insects had learned to recognize the lemon grass odour, the team switched the insect's nutritional state to 'well-fed' by giving them access to unlimited food and retrained them to recognize a peppermint odour associated with a wheat-seedling reward. Next Pompilio and her colleagues tested whether or not the insect's nutritional state at the point that they learned to recognize each scent influenced their choice. The team placed the grasshoppers in a Y-maze; one arm of the Y-maze arms had the peppermint odour and the other was scented with lemon grass, and both scents were accompanied with a wheat-seedling reward. The insects always chose the odour that they associated with the 'poorly fed' state during training, supporting the hypothesis that choice is governed by past gains. Thus, food in the 'poorly fed' state is more rewarding and the odour paired with the 'poorly fed' state becomes linked with this rewarding experience, prompting the grasshopper to choose the odour associated with the 'poorly fed' state since it elicited an expectation of greater reward.

The authors suspect that nutrient levels in the hemolymph of underfed insects drop and the taste receptors become increasingly sensitive to key depleted nutrients resulting in greater feedback when the insect contacts a food item. The team explains that this probably alters the insect's memory. Instead of remembering the attributes of each option and weighing them against the current nutritional state, state-dependent valuation, situations where the insect makes a decision based on its previous experience may be computationally more efficient. It may reduce errors and make decision-making quicker by limiting the amount of information that is processed during decision-making. In the event of competition for food resources it could aid in initiating a faster and more successful dash to the prize.

10.1242/jeb.02409

Pompilio, L., Kacelnik, A. and Behmer, S. T. (2006). State-dependent learned valuation drives choice in an invertebrate. *Science* **311**, 1613-1615.

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HIBERNATION

PROTEIN COMPLEX IS CANDIDATE HORMONE FOR HIBERNATION

Scientists have long been intrigued by the question of which factors drive seasonal hibernation in mammals. In 1992, a team of Japanese researchers lead by Noriaki Kondo offered the first hint of an explanation. They found that the expression of specific protein complexes in the blood of hibernators was seasonally regulated and decreased significantly before the onset of hibernation. They proposed a seasonally regulated molecular network influenced by the so-called 'hibernation specific protein complex', which they suggested could modulate hibernation in mammals. However, it was not clear how the complex regulated hibernation. Following his initial breakthrough, Kondo combined forces with colleagues from several Japanese research institutions to try to identify the complex's function, and in a recent issue of *Cell* they describe how they have shown that hibernation specific protein complex is a hormone that carries chemical signals essential for hibernation to the brain.

The team chose to work with a champion hibernator, the Asiatic chipmunk. Inducing torpor in animals (by exposing them to the cold and dark conditions of winter), while stimulating summer levels of activity in other chipmunks (by exposing them to long warm days), the team measured the complex's gene expression levels in the liver and protein levels in the blood of both groups of animals. They observed that chipmunks from both groups had very different protein complex level patterns, which appeared to be correlated with their hibernation state; animals exposed to perpetual summer retained annually fluctuating and seasonally dependent levels of the complex. The team concluded that the hibernation specific protein complex production was endogenously regulated according to the animals precisely ticking circannual clock.

Curious to know where the newly found factor takes effect, the team directed their attention to the chipmunks' brains and analysed hibernation specific protein complex levels in the cerebrospinal fluid. Kondo and his collaborators found that hibernation specific protein complex levels in the blood and the brain were inversely regulated. They found that before hibernation, complex levels were high in the brain but low in the blood. They explained that this could indicate that hibernation specific protein complex becomes activated when it enters the chipmunk's brain. From the similarity of the complex with other hormones that are produced in the liver before transportation to, and activation in, the brain, the team concluded that they have isolated a hormone for hibernation.

Suspecting that hibernation specific protein complex regulates hibernation in the brain, the team tested whether disrupting the hormone with antibodies targeted to the complex may block the process. By doing so, Kondo and his colleagues were able to prevent some of the animals from going into hibernation, while reducing the length of other animals' torpor. Thus, they demonstrated, that the 'hibernation hormone' develops its function in the brain.

Of course, the holy grail of hibernation research is to eventually apply the mechanisms to non-hibernators, such as ourselves; hibernation specific protein complex could send us into hibernation too. Perhaps, the dream of sending torpid astronauts to Mars will soon come true.

10.1242/jeb.02410

Kondo, N., Sekijima, T., Kondo, J., Takamatsu, N., Tohya K., Ohtsu, T. (2006). Circannual control of hibernation by HP Complex in the brain. *Cell* **125**, 161-172.

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