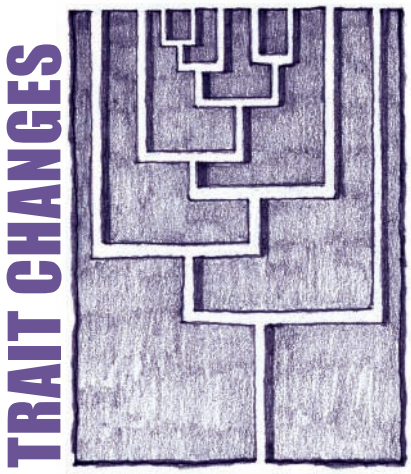
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB



CAPTIVE'S SUPER SNAP

The behavioural impact of a life 'behind bars' has long been recognised in captive animals. As a result, many modern zoos have gone to considerable lengths to provide stimulating and familiar environments for their animals. But, whilst improved conditions have successfully reduced abnormal behaviour, the anatomical impact of a captive life is often inescapable – particularly in animals that are born and reared in artificial conditions – resulting in a range of shapes and sizes across animals of the same species. For example, captive alligators are typically heavier and have shorter jaws and broader heads than their wild counterparts. But do captivity-induced anatomical differences matter? A new study by Gregory Erickson and his colleagues at the universities of Florida, Florida State and Northern Arizona tackled this question by investigating differences in bite-force performance between long-term captive and wild American alligators. They set out to answer two fundamental questions: (1) do captivity-induced changes in head shape affect biting ability and (2) if present, can these differences be linked to measurable changes in parameters such as jaw length, snout–vent length or body mass?

Erickson and his team bravely set about testing bite force in 47 alligators spanning a fourfold range in snout–vent length and nearly a 150-fold range in body mass. Safely secured to a platform, the animals were encouraged to 'open wide' with gentle taps on their snouts before a precision transducer was gently placed on the most prominent tooth at the back of the jaw; the 11th maxillary tooth. Unsurprisingly, this interference triggered extremely aggressive snapping reactions from the animals.

Amazingly, the team measured the highest bite force ever measured in an animal (13172 N), with other animals registering forces ranging from 217 N upwards. The team also discovered that bite force differed significantly between captive-reared and wild alligators when normalised to jaw length; captive alligators bite more forcefully than their wild counterparts.

Physical differences generated by captive conditions therefore can, and do, affect performance. And although the exact mechanism for altered bite performance has yet to be pinpointed, the authors suggest two possible explanations. Firstly, the captive alligators' shortened jaws bring the 11th maxillary teeth closer to the fulcrum of the jaws and may therefore provide greater mechanical advantage. Secondly, the broader heads of captive alligators may give more space for jaw muscles compared with their wild counterparts.

Erickson's study also highlights the importance of normalising to the right parameters when comparing performance measurements between groups. In this study, the team have shown that captive alligators have bites that are either the same force or harder than wild alligators, when the data are normalised to the animal's jaw length or to body mass. If meaningful ecological ties are to be made between studies on wild and captive-reared animals, researchers must be aware that normalising to different parameters can reveal conflicting results. Erickson's work has also shown that differences in biomechanical performance between animals in their natural environment and zoos must also be investigated, as we can't always assume that captive populations have retained their wild forebears' physiological characteristics. And if this feature has triggered your imagination to investigate the effects of captivity physiology, have no fear; less dangerous animals are available too.

10.1242/jeb.01030

Erickson, G. M., Lappin, A. K., Parker, T. and Vliet, K. A. (2004). Comparison of bite-force performance between long-term captive and wild American alligators (*Alligator mississippiensis*). *J. Zool. Lond.* **262**, 21-28.

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RUSH HOUR

It's a familiar scene for thousands of commuters every morning; bumper to bumper, going nowhere! Traffic control is a major issue for human societies with large numbers of people wanting to get to, and from, the same place at the same time. But it's not a problem we often associate with other creatures. Animals such as wildebeests, herrings or locusts often converge in large numbers, but their traffic tends to be along a one-way street – all the individuals move in the same direction at the same time. Foraging ants, on the other hand, have to cope with traffic control problems that closely parallel our own. An ant trail is not an unfamiliar sight, one ant following another to a food source along a specific path whilst other ants, already laden with food, return using the same route. These trails are formed when a scout ant finds food and returns to the nest, leaving an odour trail to direct other ants to the source of food. Each ant that returns to the nest adds to the odour trail, reinforcing the signal for subsequent ants to follow. This system leads to ants travelling to and fro, along the same route. Normally this system works well, but Audrey Dussutour and co-workers from Toulouse, Dresden and Brussels asked how ants cope when faced with congestion.

First, the team allowed black garden ants (*Lasius niger*) to forage for a tasty sugar solution food source, but to reach their goal they had to cross a diamond-shaped bridge, which gave them a choice of routes once they'd begun to cross. The team expected the ants to use only one branch for both their outward and return journeys, thanks to the odour trails left by previous foragers. And this is exactly what happened when the branches of the bridge were wide. However, as the team replaced the bridge with narrower and narrower branches, the ants began to use both branches of the bridge simultaneously, ensuring that the number of ants getting to and from the food remained about the same, despite the constriction.

How do the ants achieve this traffic control? Odour laid down by ants returning to the nest with food serves as a guide for ants leaving the nest to find food, so when there's no congestion and the odour trail is strong, garden ants stick to that path when travelling to and from the food source. However, when monitoring the movements of ants on a congested bridge, Dussutour and co-workers found that two factors play important roles in optimizing ant traffic: the concentration of odour on a trail and interactions between ants moving in opposite directions on the same trail. An ant chooses a particular branch of the bridge because of the odour concentration on that branch. This ensures that only one branch is used, but as traffic becomes more congested, it takes longer for ants to battle their way through the crowd so the number of ants returning along the branch declines and the odour concentration drops. This causes ants to start making random choices between the two possible branches, so that they begin using both branches. Interactions between ants travelling in opposite directions along a narrow path also encourage the ants to explore alternative routes, by redirecting one of the ants down the other branch.

There may be lessons to be learnt from the strategies adopted by the ants to optimize their traffic flow but some of these strategies are already familiar. For example, traffic updates on the radio (just like the interactions between ants) warn commuters about congested routes and encourage them to find alternatives to avoid the rush hour.

10.1242/jeb.01027

Dussutour, A., Fourcasslé, V., Helbing, D. and Deneubourg, J.-L. (2004). Optimal traffic organization in ants under crowded conditions. *Nature* **428**, 70-73.

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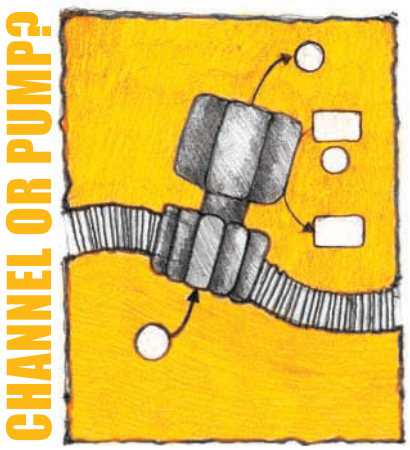

WHEN IS A CHANNEL NOT A CHANNEL?

Most physiologists would not have too much difficulty describing the difference between a channel and a transporter. A channel is a hydrophilic pore through a membrane, which can let through many thousands of ions each time it opens. It can be selective for particular ions, but ions can only move 'downhill' – that is, they cannot move up their electrochemical gradient. By contrast, transporters move far fewer ions, but some have the potential to move them up their electrochemical gradient – what is called 'active transport'. This distinction is fundamental to physiology. It is thus surprising to find that the archetype of a major class of ion channels, the chloride channels, or ClCs, actually moves chloride up its electrochemical gradient in exchange for hydrogen ions: that is, it behaves as an exchanger.

Alessio Accardi and Christopher Miller work on a chloride channel from *Escherichia coli*, the bacterium that we carry around (in kilogram quantities) in our own guts. ClC-ec1 is an archetypal chloride channel, and its sequence is close enough to the chloride channels of animals to be a clear member of the family. Like other chloride channels, it lets through chloride ions and is activated, not by membrane voltage, but by low pH. It has thus been seen as a proton-activated chloride 'leak' channel that may help *E. coli* live in the acid environment of our guts. However, the simple story does not stand up to detailed investigation. When studied in isolation, the reversal potential of the channel (the potential at which no current flows through the channel when it's open) is 30 mV, rather than the 45 mV that would be predicted for a pure chloride channel from the transmembrane chloride distribution. This must mean that the

channel is permeable to more than just chloride. In addition, the channel has relatively low conductance, letting through only 10^4–10^5 ions per second, compared with 10^6 ions per second through the classical sodium channel. In their paper, Accardi and Miller reach the stunning conclusion that ClC-ec1 is actually not a channel at all, but an exchanger that moves two chloride ions in one direction for one proton in the other. Thus, the activation by low pH is not a gating effect but simply reflects increased activity of the exchanger when extra protons are available. The low conductance is thus a property of the 'channel' not being a channel at all, but an exchanger. And critically, by imposing an appropriate proton gradient, it is possible to drive a chloride flux against its electrochemical gradient – a clear example of 'secondary active transport'.

The authors go further. By studying the recently elucidated crystal structure of their channel, they identified a glutamate residue that they predicted might be critical for Cl^-/H^+ coupling. When this amino acid was changed to alanine, ClC-ec1 became a 'classical' chloride channel with no sensitivity to pH.

The authors wryly remark that the distinction between a channel and a pump may be exceedingly fine. Given that many chloride channels are found near big pH differences – they've been argued to be the classic 'partner' for the proton-pumping V-ATPase – it may thus be pertinent to re-examine our understanding of these 'channels' in animals, too.

10.1242/jeb.01028

Accardi, A. and Miller, C. (2004). Secondary active transport mediated by a prokaryotic homologue of ClC Cl- channels. *Nature* **427**, 803-807.

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MALE OR FEMALE? CAN THE ENVIRONMENT DECIDE?

Sexual differentiation in zebrafish occurs after hatching and is a labile process. During early life, gonads are undifferentiated and become either testes or ovaries during sexual differentiation. The balance between circulating levels of male (androgen) and female (oestrogen) hormones is crucial to the process of sexual differentiation, determining whether a fish becomes male or female. Therefore, zebrafish are particularly susceptible to factors in the environment that may modify or interfere with this process.

Endocrine-disrupting chemicals are natural or man-made compounds that can mimic endogenous hormones and cause physiological disturbances. Current research has focused mainly on chemicals that mimic the effect of natural oestrogens, but endocrine disruptors can also interfere with the synthesis of endogenous hormones. So, can endocrine disruptors influence the sexual differentiation of zebrafish?

The enzyme aromatase converts androgens to oestrogens, playing a vital role in maintaining a balance between male and female hormones. Two forms of the aromatase-encoding gene have previously been identified and are mainly expressed in the gonad (*CYP19A*) and brain (*CYP19B*). The important nature of this enzyme made it an appealing focus for this study. Exposing fish to aromatase-inhibiting chemicals, Martina Fenske and Helmut Segner measured both the formation of gonads and the expression of the aromatase genes to determine the effects of these chemicals on fish sexual differentiation. The team chose to work with a compound called fadrozole, as it is a competitive inhibitor of aromatase activity. Taking two sets of fish, the team kept one lot in water to monitor normal development, while they exposed the other zebrafish to fadrozole during the period of sexual differentiation. After fadrozole exposure, they sampled some fish directly, while others were returned to control water and raised to adulthood to see if exposure-related effects were reversible.

In control fish, 44% developed testes and 56% ovaries, and aromatase gene expression varied depending on the developmental stage. During sexual differentiation there was no detectable difference in aromatase gene expression between males and females, but in reproductively active adult zebrafish *CYP19A* expression was higher in females than in males.

Amazingly, complete gonadal 'masculinisation' occurred in all the fish exposed to fadrozole, and the effects persisted through to adulthood. Fadrozole also reduced gonadal *CYP19A* expression during sexual differentiation. Interestingly, while these fish possessed male gonads, 36% of fadrozole-treated adult fish showed female-like expression of *CYP19A*, whereas 64% had male-like expression. So while 100% of fish would be identified as males based on their gonad morphology, sexing of fish based upon expression of *CYP19A* might suggest that some of the fish were female.

This study highlights that zebrafish sexual differentiation is susceptible to interference by chemicals. Manipulation of the aromatase system completely and irreversibly altered the formation of gonads. Despite this, gene expression still displayed a dimorphic expression in adult fish, suggesting a partial recovery from fadrozole treatment. However, the genetic gender of these fish could not be identified due to the lack of sex-linked markers for zebrafish. The likelihood of masculinisation of female organisms by aromatase inhibitors is likely to depend on species, developmental stage and exposure concentrations. Nevertheless, it appears that endocrine-disrupting chemicals provide potential for the environment to decide: male or female?

10.1242/jeb.01029

Fenske M. and Segner, H. (2004). Aromatase modulation alters gonadal differentiation in developing zebrafish (*Danio rerio*). *Aquat. Toxicol.* **67**, 105-126.

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