

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.

ADAPTATION



EXCEPTIONAL HEAVY METAL TOLERANCE IN FISH

The river Hayle is found in an area of the southwestern United Kingdom with a history of mining since Neolithic times. Drainage from these mines over such long periods of time, particularly during the 1800s when mining reached its peak in this area, has led to exceedingly high levels of metals in this river. Amazingly, this river has a viable population of brown trout (*Salmo trutta*) that can stand these very toxic conditions. But how can the brown trout tolerate such high levels of contamination? Tamsyn Uren Webster, Eduarda Santos and their UK colleagues decided to find out using cutting-edge molecular techniques, and have published their findings in a recent issue of *Environmental Science and Technology*.

First, the team wanted to find out how high the levels of various metals actually were in these fish. They compared zinc, copper, cadmium, arsenic, iron, lead and nickel levels in various tissues from brown trout from the river Hayle with those from fish living in the clean and nearby river Teign. They found that brown trout from the river Hayle had 30–60 times higher levels of metals in their gills, livers and kidneys. Moreover, brown trout from the river Hayle had some of the highest concentrations of copper and zinc ever recorded in fish. These results confirm that these fish are exposed to and absorb these metals and, more importantly, that they are truly tolerant of such high metal contamination.

Next, the team investigated which genes were involved in such high metal tolerance in the Hayle river brown trout. To do this they sequenced RNA transcripts from all expressed genes using cutting-edge technology called high-throughput sequencing. As the genome of brown trout has not been published, the researchers first had to use computer programs to put together the majority of the genome of

brown trout from small sequences of RNA transcripts. This process is an impressive undertaking as it is similar to putting together a puzzle with 70 million pieces without being able to look at a picture of it or knowing what exactly the picture is.

With a large part of the genetic code of brown trout assembled, the researchers compared active genes between the trout from the Hayle and Teign rivers. The researchers predicted that any differences in which genes were active between the populations would be related to surviving in a metal-contaminated river. As suspected, the brown trout from the river Hayle did indeed turn on genes that would help them store and detoxify the toxic metals. Additionally, brown trout from the river Hayle also activated genes that can help them compensate for disturbances to key ions, such as calcium, sodium and chloride, as ion imbalance is a life-threatening problem in this toxic environment.

Brown trout in the river Hayle are champions of metal tolerance perhaps because they have had the chance to adapt to this environment over several generations. Regardless, Uren Webster, Santos and their colleagues have unravelled some of the secrets to surviving in such a polluted environment and they give us hope that some fish can endure large environmental disturbances.

10.1242/jeb.084509

Uren Webster, T. M., Bury, N., van Aerle, R. and Santos, E. M. (2013). Global transcriptome profiling reveals molecular mechanisms of metal tolerance in a chronically exposed wild population of brown trout. *Environ. Sci. Technol.* **47**, 8869–8877.

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SOUND PRODUCTION



HOWL WHEN YOU'RE LONELY

Many species of animal use vocal communication to indicate danger, the availability of food, or other interesting events to their friends. While much attention has been given to the significance of these calls, what the trigger is for these vocalisations remains a mystery. As most animals have a severely limited repertoire of vocal calls, many researchers think that the vocalisations of animals, in contrast to those of humans, are simply a result of involuntary muscle movements. However, few studies have yet been conducted to directly test this hypothesis. Do animals communicate as a reflex, perhaps in response to stress or other internal states? Or is it a more voluntary behaviour, based on and modified by how the animal understands its environment? Now, a team of researchers from Italy, Switzerland and Austria have addressed this question in a study recently published in *Current Biology* by investigating what makes a wolf howl.

When their pack gets fragmented, wolves howl to help them reconnect with stray members. While it is obvious that the howling behaviour is caused by the separation of one or more members of the pack, the immediate trigger is unknown. On the one hand, the physiological stress that separation causes could provoke howling, suggesting the behaviour is more like a reflex, while on the other hand, more flexible social mechanisms could play a role. The team worked with a pack of nine captive wolves to try and understand what mechanisms underlie this behaviour, by firstly focusing on the stress levels of howling animals and secondly on the social relations within the pack.

To begin with, they removed one wolf at a time from the pack, taking it for a stroll. As expected, this physical separation induced howling in the remaining wolves. All howls were recorded, after which the concentration of a stress hormone, cortisol,

was measured in the saliva of each of the wolves. They found that the remaining wolves indeed have higher levels of cortisol, suggesting that the physical separation is a stressful event. However, crucially, the authors found no correlation between the stress levels of an individual and the number of times it howled. This suggests that howling is not necessarily simply a consequence of the levels of stress an animal experiences.

Before doing the experiment, the team had determined the social dynamics of the pack: which wolves are more social towards each other, and what the hierarchical structure is within the pack. When they looked at which wolves were howling in response to the separation of a given individual, they found that those wolves that had displayed the greatest affection towards it were howling most frequently. These results suggest that there is a clear link between the social interactions within wolf packs and their vocal communication, perhaps allowing individuals to promote contact with those animals that are important to them.

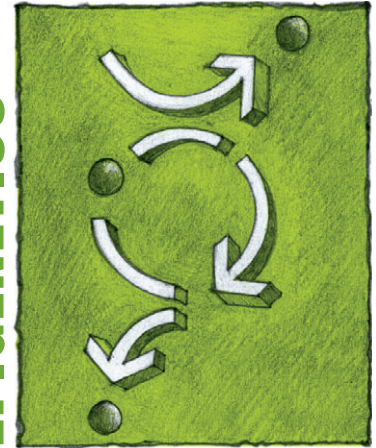
This study sheds light on how animals use vocal communication in their social interactions, and suggests that, contrary to previous suggestions, animal vocalisations are not just involuntary muscle movements. Furthermore, it is good news for lost travellers in dark forests around the world: those wolves they're hearing are just looking for their friends.

10.1242/jeb.084517

Mazzini, F., Townsend, S. W., Virányi, Z. and Range, F. (2013). Wolf howling is mediated by relationship quality rather than underlying emotional stress. *Curr. Biol.* 23, 1677-1680.

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EPIGENETICS



FOREVER IN LOVE, THANKS TO EPIGENETICS

If you want to get hitched for life, you have to make the right moves from the start. For example, prairie voles (*Microtus ochrogaster*) form lasting pair-bonds that are initiated when a female displays special interest – known as partner preference – in a male. For the female to form her partner preference she releases two hormones, oxytocin and vasopressin, that bind to their receptors in the pleasure and reward centre of the brain – the nucleus accumbens. An increase in the expression of oxytocin and vasopressin receptors in the nucleus accumbens helps to kindle a female's affection toward a male, but how their expression is regulated is not known. One method of increasing gene expression is to change the way in which DNA is packaged around the region of the gene that regulates gene expression – a process known as epigenetics. Histone acetylation is a type of epigenetics that unlocks the tight DNA spiral to help activate key genes. This effect can be reversed by deacetylating the histones to deactivate genes. Postdoctoral researcher Hui Wang and a team of neuroscientists at Florida State University, USA, set about testing whether epigenetics are involved in partner preference formation in female prairie voles.

Using a drug that promotes gene activation by preventing histone deacetylation – called trichostatin A (TSA) – Wang and colleagues injected the drug into the brains of female voles and then housed each female with a male for 6 h to find out how TSA affected the female's partner preference. Normally, a female will not develop a preference for a male over such a short period of time. However, Wang and colleagues found that injected females did favour their partner male over a stranger – indicating that blocking histone deacetylation and increasing histone acetylation permitted the females to form a partner preference.

Wang and colleagues then measured changes in gene and protein expression of the oxytocin and vasopressin receptors, as well as the amount of histone acetylation in the regulatory regions of these two genes. They found that the TSA-injected females had an increase in oxytocin and vasopressin receptor expression in the nucleus accumbens, likely driven by a measured increase in histone acetylation in the regions of those genes that regulate their expression.

Next, the team injected new females with TSA but this time they also injected some of these females with additional drugs, one of which blocked the oxytocin receptor and the other the vasopressin receptor, to find out whether this would prevent partner preference formation induced by TSA. They found that females given TSA in combination with either of the receptor blockers did not favour their partner male over a stranger, meaning that partner preference was not formed.

Having used TSA and other drugs to show that the expression of the oxytocin and vasopressin receptors in the brain could be epigenetically regulated, Wang and colleagues decided to find out whether the animals use this epigenetic mechanism in practice. To do this, the team housed a new set of uninjected females individually with a male for 24 h and then measured the gene and protein expression of oxytocin and vasopressin receptors in the females' brains, and the amount of histone acetylation in the genes' regulatory regions. They found that the expression of oxytocin and vasopressin receptors in the nucleus accumbens increased, and that the activation of these two genes is achieved by histone acetylation. So, Wang and colleagues have shown that partner preference is epigenetically driven in the pleasure and reward center of the brain in female prairie voles, improving our understanding of the neurobiology of social affiliation as well as possibly unearthing the secret behind Cupid's magic arrows!

10.1242/jeb.084525

Wang, H., Duclot, F., Wang, Z. and Kabbaj, M. (2013). Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles. *Nat. Neurosci.* **16**, 919-924.

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PASSIVE-AGGRESSIVE JOINT FORCES

Most animal movements are actively controlled by muscle contractions. However, passive forces, such as those due to the elastic properties of muscles and tendons, have been shown to be invaluable to locomotion. But what about passive forces originating in the joints themselves? What role do they play in movement, and how do they relate to the active forces being generated at those joints? Although passive forces originating in the joints of insects have been documented, little else is known about their role in locomotion or their specific importance in different modes of locomotion such as walking and jumping. In a recent study in *Current Biology*, Jan M. Ache and Tom Matheson from the Universities of Leicester, UK and Cologne, Germany, sought to understand whether passive joint forces are adapted to the requirements of insects with legs specialized for different types of locomotion.

To understand the extent of passive joint forces in generating leg movement, Ache and Matheson measured flexion and extension of the tibia of two insects, the locust *Schistocerca gregaria* and the false stick insect *Pseudoprosopopia scabra*. In the locust, the authors stimulated muscle contractions of the large and powerful extensor tibiae muscle and recorded the velocity and angle of the tibia during the resulting forced extensions and subsequent passive flexions back to a resting position. In the false stick insect, the authors held dissected legs in fully extended or flexed positions and recorded passive movement back to resting position.

The authors found that tibial flexion from an extended angle occurred without contraction of the flexor muscle in both insects. This passive flexion occurred even

after all associated tissues (with the exception of the essential cuticular structure of the joint) were removed, showing that passive forces were originating from the joint itself. In addition, Ache and Matheson discovered that these passive joint forces counteracted active extension, likely an adaptation to help stabilize the joint during powerful kicks. In contrast, the authors found almost no passive extension in the hindlegs of either insect. When they examined passive joint forces in the middle legs of the false stick insect, however, they found that passive flexion and extension were evenly matched. In these middle legs, the passive forces help to support the relatively weaker flexor and extensor muscles. The authors then compared their results with published data on the stick insect *Carausius morosus* and found that in the stick insect, which has a contrastingly weak extensor muscle and stronger flexor muscle, the joint provides passive extension, not flexion.

Data from all three insects suggest that passive joint forces have evolved to individually match the strengths and requirements of the muscles of each type of leg. While the hindlegs of the locust and false stick insect are adapted for jumping, the false stick insect's middle legs and the stick insect's hindlegs are designed for walking. This finding has important implications for robotics, which have long used insect morphological and locomotor designs as inspiration. As robotics focus more on incorporating passive forces, it will be essential to consider the functions of each limb. In animals where a strong muscle is needed for a specialized movement, strong passive forces could provide a balance for, and may allow a more rapid recovery from, that movement, without the need for neural input. These balancing forces are the key to the passive-aggressive dynamics of limb movement.

10.1242/jeb.084533

Ache, J. M. and Matheson, T. (2013). Passive joint forces are tuned to limb use in insects and drive movements without motor activity. *Curr. Biol.* **23**, 1418-1426.

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