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

LET US LAUGH TO EASE THE PAIN

We all have experienced the positive effects of laughter. It induces a deep state of relaxation and a sense of well-being. It is also an important form of non-verbal communication that allows others to know we agree that something is funny. In this way, laughter strengthens social bonds because when we laugh we lower our guard and do not perceive the other person as a potential threat. Some have proposed that the positive emotions associated with laughter help us learn new things from others and promote cooperation. But what are the mechanisms behind all of this? How does laughter make us feel good? Robin Dunbar from the University of Oxford and his team of collaborators proposed that endorphins might be responsible for many of the beneficial effects of laughter. Endorphins are internally produced opioids that have an important role in social bonding in primates, as well as having an analgesic effect. Dunbar and his colleagues proposed that the physical action of laughing induces the release of these endorphins, just as any form of physical exercise does, causing the positive feelings we are all familiar with.

Because of the analgesic effect of endorphins, it is common practice for scientists to use pain thresholds to assess individual endorphin levels. Using this technique, Dunbar and his colleagues performed a series of experiments in which they evaluated the effect of laughter on endorphin release. During some of the experiments, volunteers were tested in groups whereas other experiments were performed on individuals. The participants were shown either funny videos, such as 'America's Funniest Home Videos' or other comedy shows, or videos with neutral emotional content, such as a documentary. To rule out any effects that positive feelings alone might have on their endorphin levels, the scientists also showed a group of participants non-humorous 'feel-good' videos of beautiful scenery. The researchers recorded the participants' laughter throughout the experiments and tested each participant's pain tolerance before and after they had watched the videos. They did this either by touching a frozen wine cooler sleeve to a participant's skin and measuring the time at which they could not tolerate it anymore or, in a separate set of experiments, by inflating a pressure cuff around the participant's arm until they could no longer stand the pain (ouch!).

Not surprisingly, the people who watched the comedy videos spent much more time laughing than those who saw the documentaries or the videos of nice scenery. Furthermore, those who watched the funny videos in a group laughed much more than those who watched the same videos alone. More interestingly, the participants increased their pain tolerance in a laughter dose-dependent fashion: the more they laughed, the more their pain threshold increased.

The team proposes that the physical exertion of sustained laughter triggers the release of endorphins, in a way similar to other types of exercise. Because humans, in contrast to other laughing apes, are capable of sustaining laughter for several minutes, the opioid effects of a good chuckle might be particularly enhanced in our species, increasing not only our pain thresholds but also strengthening social bonds and promoting collaboration and altruistic behaviour. So it seems that laughter really is the best medicine after all!

10.1242/jeb.064295

Dunbar, R. I. M., Baron, R., Frangou, A., Pearce, E., van Leeuwen, E. J. C., Stow, J., Partridge, G., MacDonald, I., Barra, V. and van Vugt, M. (2012). Social laughter is correlated with an elevated pain threshold. *Proc. R. Soc. B* **279**, 1161-1167.

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SOCIAL SOLDIERS: *CLEAN* MEAN FIGHTING MACHINES

Soldiers are often deemed crucial in the defense of their social insect colonies from foreign invaders. One such insect – the tiny slim winged thrips, *Kladothrips intermedius* – lives and feeds on acacias, forming an abnormal growth of plant tissue that they then control. The enlarged forelimbs of this species' soldiers were thought to aid in defending its turf against its foe, the specialized invader *Kladothrips dyskritus*. However, previous observations of combat between these species yielded conflicting results, casting doubt on the utility of *K. intermedius*' forelimbs for defence and generating speculation about alternative roles for the soldiers. Captivated by these diminutive crusaders, Christine Turnbull from Macquarie University, Australia, and her Canadian and Australian collaborators set out to resolve these inconsistencies. Having previously identified anti-microbial defence tactics in other insects, they opted to evaluate the interaction between *K. intermedius* and micro-organisms, in addition to its contests with its traditional nemesis, *K. dyskritus*.

First, Turnbull and her colleagues assessed the role of *K. intermedius*' fortified forelimbs in defence. The team collected both thrips species from *Acacia* plants in South Australia, tossing one defender (*K. intermedius*) and one attacker (*K. dyskritus*) into tiny tubes to fight to the death, an established technique in studying these warring factions. They then threw sex into the mix, wondering whether male and female soldiers had different odds of victory. The team established the sex of every *K. intermedius* defender and paired each individual in combat with a *K. dyskritus* invader, taking detailed images and measurements of the soldiers' body segments, legs and wings post-battle. They discovered that *K. intermedius* males have shorter forelimbs and are more slender than their female conspecifics, but they did not find a significant difference in combat performance between the sexes. Contrary to the assumption that enhanced forelimbs impart the upper hand in battle, the bulkier builds and heftier limbs of females did not win them any more victories than the more petite males. Forelimb size within a sex also had no bearing on battle outcome, adding to the ambiguity of their value in combat.

To test their hypothesis that these warriors may be vital for guarding against micro-organisms, the team collected *K. intermedius* soldiers, as well as members of the docile working class. Washing groups of individuals from each class to obtain the compounds that they secrete onto their body surfaces, the scientists then tested the effects of the soldier and worker rinses on spores of the specialized fungus *Cordyceps bassiana*. Recording the fungal growth using optical density measurements, the team found that the rinses from the soldiers were much more effective at suppressing fungal growth than those from the non-warring workers.

Rather than serving their colony solely with brute force, these researchers believe that *K. intermedius* soldiers also expend considerable resources contending with micro-organisms. It is known that social insects host a diversity of microbes, from pesky parasites that reduce insect reproductive capacity to beneficial bacteria that provide essential nutrients to their insect host, suggesting coevolution in these relationships. The work of Turnbull and colleagues suggests that micro-organisms have played as important a role as macroorganisms in the evolution of social insect soldiers. As these scientists might advise, don't count out the little guy!

10.1242/jeb.064279

Turnbull, C., Caravan, H., Chapman, T., Nipperess, D., Dennison, S., Schwarz, M. and Beattie, A. (2012). Antifungal activity in thrips soldiers suggests a dual role for this caste. *Biol. Lett.* doi:10.1098/rsbl.2012.0184

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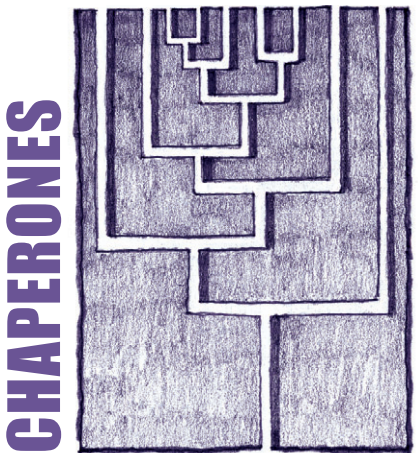

HYDROPHOBICITY COUNTS IN LARGE AMOUNTS

Heat shock proteins are a structurally diverse class of proteins whose gene expression is usually increased in response to heat or other stress. This class includes small heat shock proteins (sHsps), which serve as ATP-independent molecular chaperones. By binding to partially unfolded proteins, they prevent or reverse harmful protein aggregation, which impairs cellular function. Accordingly, sHsp malfunctions have been connected with various diseases such as cataracts and different muscular and neurodegenerative disorders. How they fulfil this important function has been studied extensively, but now a recent paper published by Mason Posner and colleagues from various US institutions provides new insight into the function and evolution of these proteins by comparing sHSPs among bony fishes with different body temperatures.

A key feature of sHsps – as well as vertebrate α-crystallins (α-Cs), closely related eye-lens proteins with chaperone-like activity – is that they bind to exposed hydrophobic regions on the surface of partially unfolded proteins to suppress protein aggregation. This process requires an increase in the hydrophobic surface on the sHsps/α-Cs to facilitate protein binding and chaperone activity. Precisely how this increase in hydrophobicity occurs is uncertain. Posner and his colleagues hypothesized that if they analyzed the amino acid sequences of different α-Cs from teleost (bony) fish adapted to different environmental temperatures, they would be able to learn about the structural requirements for chaperone activity, because it is known that the hydrophobicity of the surface of proteins varies depending on the temperature at which they function. For example, warm-adapted proteins have a smaller fraction of hydrophobic amino acids on the surface than cold-adapted proteins, and *vice versa*.

First, they measured the chaperone function of six different recombinant bony fish α-Cs by testing their ability to protect insulin or lactalbumin from aggregation. They noticed that α-Cs from cooler-bodied fish showed a greater chaperone activity than the α-Cs of warmer-bodied fish at the same assay temperature. This correlation was partially a result of the different thermal stabilities of the chaperones. Next, they carefully analyzed the amino acid composition and found that the α-Cs of warmer-bodied fish had fewer hydrophobic amino acids. Finally, they identified three amino acid positions in the α-Cs, where the local hydrophobicity varied significantly across the six species, potentially affecting the chaperone's stability and activity at different body temperatures.

Predicting that they could reduce the thermal stability of α-C while increasing the chaperone activity by changing the amino acids at these three locations, the team mutated the zebrafish α-C protein accordingly and measured the mutant's stability and chaperone activity. Indeed, one of the three substitutions fitted their hypothesis, as it affected both chaperone activity and protein stability in the predicted directions. However, the other two residues did not perfectly match their prediction; they either only increased activity or reduced thermal stability. Regardless of this deviation from expectation, the results suggested that all three identified amino acids are particularly important for the function of α-C.

Posner and his colleagues have convincingly demonstrated that a comparative approach is extremely useful in the analysis of the structure–function relationship of sHsps/α-Cs. By carefully analysing α-Cs from bony fish adapted to different environmental temperatures, they have not only identified the key amino acid positions that affect the protein's stability and chaperone activity, but also provided evidence for an evolutionary mechanism that has adapted chaperone activity to different environmental temperatures through the alteration of hydrophobicity at crucial locations in the protein structure.

10.1242/jeb.064287

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