

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.



IGUANAS ARE TOO TAME FOR THEIR OWN GOOD

The presence of a predator, in captivity or in the wild, triggers a fight or flight reaction in most animals. As an animal's stress levels rise, so do the levels of stress hormones, or corticosteroids, circulating in the blood. This physiological stress response is caused by an interaction between the brain and the adrenal glands known as the hypothalamo-pituitary-adrenal, or HPA axis. Most animals' behavioural responses to predators are also influenced by their past experiences. If animals are not faced with predators these natural responses might be reduced or even lost during evolution and the animals will appear tame. The central question that Thomas Rödl and co-workers wanted to answer is: when animals' physiological and behavioural responses towards a possible predator have been reduced or even lost over the course of time, are they capable of re-activating them?

An isolated island is the perfect location to look for a predator-free habitat, except that not many islands are totally isolated any more. The researchers set out to investigate the Galápagos marine iguana (*Amblyrhynchus cristatus*), because this animal lived without any land predators for millions of years until dogs and cats were introduced onto the islands a century ago. The authors picked out three different study areas in which the marine iguana lives: one with no predators, one with infrequent predators and finally one near a town with high predation pressure, mostly from dogs.

At each study site, the authors either left the animals undisturbed, restrained them, or chased them and then re-captured them 2–3 weeks later. To assess the animals' stress response the team approached them at a walking speed of 0.5 m s^{-1} and

measured the flight initiation distance, which is how close they could get to the iguanas before they started to move away. This distance was 4 m in animals used to predators compared to only 1 m for the 'predator-free' animals. When the team recaptured iguanas from all three sites, however, the flight initiation distance increased in all the animals, showing that a once-in-a-lifetime capture was enough to change their behaviour significantly.

To further categorise the iguana's stress levels, the team also measured the blood plasma level of corticosteroids. The major finding of the experiment was that isolated iguanas from the predator-free site did not produce stress hormones when chased, while their relatives living in areas with a high predation pressure did. Restraining the iguanas caused a huge increase in corticosteroids in all groups, indicating that their ability to produce corticosteroids is intact even if they live on a predator-free site. This suggests that the predator-induced activation of the HPA axis is dormant in animals isolated from predators but can quickly reactivate when predation resumes. Even isolated animals from the predator-free site started to produce corticosteroids after a few re-captures, showing that they are fast learners.

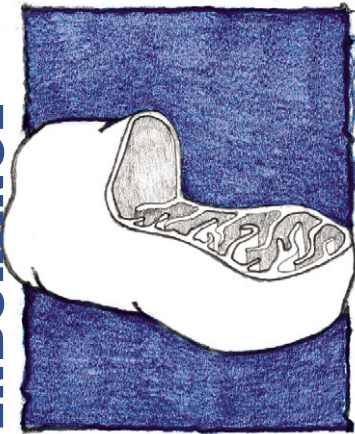
Unfortunately, the changes observed in the stress responses are most likely not enough to actually escape the newly introduced threats, such as dogs or scientists. For this reason, the Galápagos marine iguana has nearly been driven to extinction by dogs in some areas of the Galápagos Islands. The authors speculate that the iguanas' potential for behavioural change is simply not big enough to deal with a mammalian predator, even though they found the physiological stress response to novel predators to be highly flexible.

10.1242/jeb.000828

Rödl, T., Berger, S., Romero, L. M. and Wikelski, M. (2007). Tameness and stress physiology in a predator-naive island species confronted with novel predation threat. *Proc. Roy. Soc. Lond. B* **274**, 577–582.

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ENDURANCE



EVOLUTION OF ENDURANCE ATHLETES

What's the difference between a couch potato and a marathon runner? It's partly that the marathoner actually gets off the couch and runs, training him or herself to become a better runner. However, physiologists know that being a good athlete also depends on genes. Genetics can contribute up to half of the variation seen in human exercise capacity, and some other species have evolved the ability to perform amazing feats of athleticism, often with very little training. It is because exercise capacity has a genetic basis that it can evolve, and can confer a strong advantage to animals in the wild. To investigate further, Norberto Gonzalez from the University of Kansas and his colleagues focus in a recent paper on the evolution of running endurance in rats.

Transporting oxygen to mitochondria in exercising muscles is extremely important for making energy in the form of ATP. Animals with greater exercise capacity are generally better at transporting O₂ along the pathway from environment to mitochondria, and this pathway has many steps: O₂ is brought into the lungs with each breath, where it diffuses into the blood and is delivered throughout the body, then finally diffuses to mitochondria in the tissues. It was unclear how each step of the O₂ transport pathway evolves in athletic species, which led Gonzalez and his team to find out more by artificially selecting rats for running endurance.

Artificial selection is a way of mimicking natural selection in the lab. Every generation, individual rats with the best running endurance were selected and bred together, generating high endurance populations that could run much further than rats with low running endurance. To understand the basis for these differences, Gonzalez and colleagues first measured the

maximal rate of oxygen consumption ($\dot{V}_{O_{2max}}$) during heavy exercise in rats after 15 generations of selective breeding. They found that the high endurance runners had a higher $\dot{V}_{O_{2max}}$ than rats in earlier generations, so they knew that the oxygen transport pathway was evolving. To find out what was causing the changes in $\dot{V}_{O_{2max}}$, the authors analyzed each individual step in the O₂ pathway. Both the rate of O₂ delivery to the tissues by the blood and rate of O₂ diffusion into the tissues from the blood were enhanced in high endurance runners, which explained their higher $\dot{V}_{O_{2max}}$. All other steps in the O₂ pathway were the same in high and low endurance runners.

Gonzalez and colleagues made a remarkable finding when they compared these results to experiments in the same lines of rats after only seven generations of artificial selection: at this early stage of evolution, only the rates of O₂ diffusion into the tissues were enhanced in high capacity runners. This discovery has important implications for how physiological systems evolve. It implies that when selection is applied to the O₂ transport pathway as a whole, different components of that pathway each evolve at a different pace. The authors conclude that the changes observed in tissue O₂ diffusion at generation seven promoted changes in O₂ delivery at generation 15. In more general terms, evolution of the first physiological trait increased the selective advantage of the second trait, which later evolved as well. Gonzalez and colleagues have therefore shown us that the evolution of endurance capacity involves multiple physiological changes, and that there are many interesting differences between couch potatoes and marathon runners!

10.1242/jeb.000810

Gonzalez, N. C., Kirkton, S. D., Howlett, R. A., Britton, S. L., Koch, L. G., Wagner, H. E. and Wagner, P. D. (2006). Continued divergence in $\dot{V}_{O_{2max}}$ of rats artificially selected for running endurance is mediated by greater convective blood O₂ delivery. *J. Appl. Physiol.* **101**, 1288-1296.

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SALINITY TOLERANCE



NAKED CARP TAKE A SALTY HOLIDAY

Like tourists going on holiday to the Dead Sea, endangered naked carp (*Gymnocypris przewalskii*) migrate annually between freshwater rivers where they spawn, and salty Lake Qinghai, where they take a break to feed and grow. Lake Qinghai, the largest lake in China, is located over 3000 m above sea level, but may soon become a toxic destination for the carp. The lake is continuously drying up and becoming ever more saline. Diversion of water for agriculture as well as climate change have led to a decline of the lake's water levels by about 10–12 cm per year during the past 50 years. With these problems in mind, Chris Wood from McMaster University, Ontario, and his eight collaborators from Canada and China were interested in how naked carp, a very sensitive, slow growing species which feeds on plankton, adapts to living in the saline lake's harsh environment.

To have a closer look how the fish cope with living in such salty waters, the international team measured the fishes' metabolism, water balance and excretion, both on-site in China and in the lab in Canada. To find out how the carps' metabolism was affected, they transferred fish from fresh river water to salty Lake Qinghai water and observed that naked carp also take a metabolic holiday in the salty lake water. In the first 36–48 h after the transfer, Wood and his team saw that naked carps' oxygen consumption was reduced to 60% of the oxygen uptake seen in carp kept in river water. This change reduces the carps' energy demands by 40% while they are in lake water.

Switching their attention to the effect of the transfer between river and lake water on water balance and excretion, they measured the function of the gill and kidneys, by looking at the activity levels of

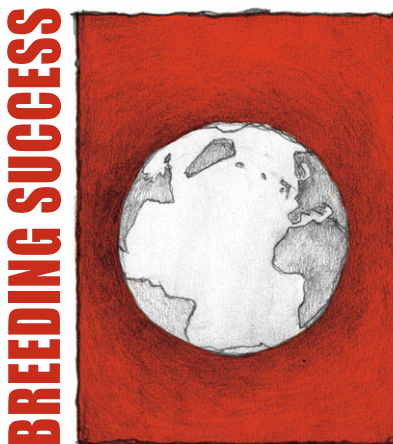
the sodium-potassium pump, an enzyme called the Na^+, K^+ -ATPase. This enzyme regulates ion transport into and out of the cell and therefore plays an important role in water balance. They found that enzyme activity in salty fish was reduced by 50–70%, which would also lower the carp's metabolic costs further since this enzyme uses a lot of ATP to function. This reduction in enzyme activity affected the level of electrolytes, such as salts, in the blood: the researchers found that lake-water-acclimated fish had blood concentration levels of electrolytes very close to the ion concentrations of the saline lake. This suggests that naked carp equilibrate their blood plasma ionic concentration to match the salty waters in Lake Qinghai, by reducing the activity of ion pumps in the gills and kidneys.

But how would such salty blood affect the fishes' kidneys? Wood and his colleagues looked at the work done by the kidneys in response to the ionic challenge caused by the lake water. They found that in river water the kidney excreted water more efficiently than salt. However the kidneys of fish kept in the salty Lake Qinghai water conserved water better, excreting more salt. This observation indicates that carp adapt their metabolism as well as gill and kidney function to cope with the dangers of excessive water loss and a surplus of ions in their cells caused by salty water. Wood and his team show that naked carp can survive in Lake Qinghai, despite its saltiness. However, they warn that as the lake is getting ever more saline, all the carps' adaptations might not be enough to help them cope with the salty water, with lethal results.

10.1242/jeb.000802

Wood, C. M., Du, J., Rogers, J., Brauner, C. J., Richards, J. G., Semple, J. W., Murray B. W., Chen, X. Q. and Wang, Y. (2007). Przewalski's naked carp (*Gymnocypris przewalskii*): An endangered species taking a metabolic holiday in Lake Qinghai, China. *Physiol. Biochem. Zool.* **80**, 59-77.

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FAST FATHERS FATHER MORE

It is widely assumed that in the animal kingdom faster means better. Speed often implies that an animal is better at escaping from predators, and capturing prey. Indeed, a growing number of studies are linking whole-organism performance traits, such as locomotor speed, to an animal's probability of survival. This suggests that natural selection acts on such traits if they are heritable, which they often seem to be. Recent work by Jerry Husak and colleagues at Oklahoma State University uses collared lizards in a novel approach to understanding the evolution of locomotor performance by addressing whether sexual selection might also shape traits such as running speed. In particular, the research team examined whether reproductive success was positively related to sprint speed in these lizards. If so, this would provide evidence that sexual selection might play a critical, if underappreciated, role in the evolution of locomotor performance in these animals.

To understand the relationship between locomotion and reproductive success, adult and yearling lizards were captured in a small region of Pawnee County, Oklahoma, USA, during the animals' breeding seasons in 2003 and 2004. Captured lizards were first brought into the lab and carefully measured for traits that could impact running speed such as body size and limb length. On days 2 and 3 of captivity, the researchers removed the lizards from their heated chambers and chased them six times along a 3-m trackway. Using video footage of the chases the team calculated the fastest one-meter split from each of the six runs for every lizard, calculating each animal's maximum sprint speed. They also collected blood samples and toe clippings from each lizard, so that they could use the DNA for paternity analyses. They targeted a number of sections, or loci, in the DNA samples to

determine which adult males were most likely to be the fathers of the yearling lizards that they had also collected.

By comparing adult sprint speed with the results from the paternity tests, the team found that faster sprinters fathered more offspring. But was this caused by body size or leg length? They found that while larger lizards didn't run faster, longer limbs led to faster lizards. To try and determine what other factors might explain the fast lizards' reproductive success, the team observed the animals in their natural habitat and found that faster runners had larger territories, which could lead to more mating opportunities. They also examined blood testosterone levels to make sure that higher sprint speeds weren't caused by higher levels of this hormone. They found that there was no relationship between blood testosterone and sprint speed, offspring numbers or territory size. In fact no measured trait except maximum sprint speed predicted both reproductive success and territory size in these lizards, suggesting a direct link between running performance and fitness.

Why might faster sprint speeds lead to higher numbers of offspring in these lizards? The link between higher speeds and larger territory size hints that faster males may defend their territories better. Males with larger, better defended territories can attract more females. Faster is better indeed! As more biologists begin to consider links between performance traits and reproductive success, the relative importance of sexual *versus* natural selection in shaping animal form and function will become clearer.

10.1242/jeb.000794

Husak, J. F., Fox, S. F., Lovern, M. B. and Van Den Bussche, R. A. (2006). Faster lizards sire more offspring: sexual selection on whole-animal performance. *Evolution* **60**, 2122-2130.

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MEMORY STABILITY



KEEPING THE MEMORY ALIVE

During memory formation, proteins are synthesized that transform newly learned information into stable synaptic changes, such as the insertion of new receptors at the synapse. This stability, or consolidation, generally occurs in a time window which covers the first few hours after learning. But, proteins have a constant turnover; they are always being made and broken down, and thus have a limited life span. One puzzle that memory researchers have been trying to solve is how the synaptic changes that occur during memory formation are maintained despite this molecular turnover. Pedro Bekinschtein and colleagues from the Instituto de Biología Celular y Neurociencias in Argentina have shown in a recent *Neuron* article that a memory trace likely undergoes several rounds of

consolidation-like events in order for it to persist over time.

To find out how a memory is maintained in rats' brains, the authors inhibited protein synthesis at numerous time points before and after learning by injecting the drug anisomycin into the hippocampus, which is a brain area essential in memory formation in a variety of tasks. One such task is called inhibitory avoidance, in which a rat is placed on a platform over a wire grid. When the rat steps down onto the grid it receives a painful foot shock. Not surprisingly, the rat learns to avoid the foot shock by remaining on the platform. This type of training only requires one trial and the memory is long-lasting. When the authors inhibited protein synthesis in the hippocampus immediately before training or 3 h post-training, the rats did not remember to remain on the platform 2 days later. Inhibiting protein synthesis 9, 12 or 24 h post-training did not affect the 2-day memory: these rats remembered to remain on the platform. These results represent the classical consolidation effect, where memory is impaired if protein synthesis is inhibited within the first few hours after training.

However, when the authors looked to see if the memory was maintained 7 days after learning the avoidance task, a slightly different story emerged. Again, when they inhibited protein synthesis in rats immediately before training or 3 h post-training, the animals formed no memories. And, again, rats remembered their training when the researchers inhibited protein synthesis 9 or 24 h after training. The

difference occurred when the team inhibited protein synthesis 12 h after training. The rats remembered their training 2 days later, but not 7 days later. This implies that there is a second time point 12 h after training, in addition to the time period immediately after training, in which another round of protein synthesis is required to stabilize memories and make them persist.

These results challenge the classical view that a memory undergoes consolidation only once, immediately after learning, by demonstrating that there is at least one other critical time window 12 h after learning. The authors replicated this effect using a second hippocampal-dependent memory task, suggesting that this process of repeated consolidation events may occur after different types of learning tasks. Having recurrent consolidation windows not only helps the memory to persist during protein turnover, but also allows a time window where the memory can either be strengthened, resulting in persistence, or weakened, resulting in forgetting.

10.1242/jeb.000778

Bekinschtein, P., Cammarota, M., Igaz, L. M., Bevilacqua, L. R. M., Izquierdo, I. and Medina, J. H. (2007). Persistence of long-term memory storage requires a late protein synthesis- and BDNF-dependent phase in the hippocampus. *Neuron* **53**, 261-277.

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