OUTSIDE JEB

Portia (proto-)counts on numerical competency

Portia, a genus of small jumping spiders, has the reputation for being a highly effective hunter that uses superb intellect to take down large, poisonous spiders as prey. As reputations go, it's not a bad one. A Google search reveals the little spider to be a veritable eight-legged Einstein, but just how smart can an animal with a pinhead-sized brain possibly be? Fiona Cross from the University of Canterbury, New Zealand, and Robert Jackson from the International Centre of Insect Physiology and Ecology, Kenya, have spent years investigating the cognitive tricks that *Portia* extracts from that little brain.

One such trick is *Portia*'s ability to observe potential prey from a distance and then pre-plan a complex route from that same vantage point so as to sneak up behind the unsuspecting prey item. *Portia* is remarkably effective at this and has the rare ability to plot routes that move the prey out of visual contact for periods at a time. This behaviour implies that *Portia* creates a mental image of a predatory scene and commits it to a working memory, recalling it when the scene comes back into view. The accuracy with which *Portia* builds and recalls this mental image is of great interest to Cross and Jackson, and this particular study saw them investigate *Portia*'s ability to comprehend numbers, as the little spider regularly encounters numerous prey spiders at a time.

The duo started by building an experimental apparatus that allowed individual *Portia* specimens to view a modifiable prey scene, plot an attack route and then set out on their predatory journey. The investigators maintained the consistency of each *Portia* attack route by exploiting the spider's known aversion to water by surrounding the single route from the initial viewing platform to the prey scene with water. This route removed the prey scene from the spider's field of view for about a minute, during which time the experimenters could modify the number of prey items (lures made from preserved specimens of *Portia*'s favourite food, *Argyrodes* spiders). To interpret the spiders' responses to modified prey scenes, the duo enlisted the expectancy-violation method of behaviour assessment, whereby violations of expected behaviours are interpreted as the test subject's comprehension that something has changed. In this case, the time *Portia* took to re-analyse a prey scene upon its reappearance in their field of view, and the number of *Portia* that followed through with an attack, were analysed for expectancy violation.

The results reveal that *Portia* is surprisingly competent with numbers. When the spiders re-emerged into a prey scene with an altered number of prey items, they spent more time inspecting the scene for further information, and were less likely to mount an attack, than when the prey item number was unaltered. Furthermore, Cross and Jackson observed no significant effects when the original prey number and altered prey number were both $\geq$3, suggesting that *Portia* represents 1 and 2 as discrete number categories, and anything $\geq$3 as the same number category, which the duo called 'many'. The methods cannot reveal just what *Portia* was pondering while investigating a mismatched prey scene, but based on how *Portia*'s principal eyes function, the pair suspect the spiders were individuating the objects in their present field of view and comparing them with those in their working memory, a sluggish but effective process known as 'proto-counting'.

And so *Portia*'s numerical competency will be added to its googleable lore, building on what is already a stellar reputation. That's a good thing because, frankly, few spiders can boast such a claim. But everyone loves a genius, even one with eight legs.

10.1242/jeb.147553

Cross, F. R. and Jackson, R. R. (2017). Representation of different exact numbers of prey by a spider-eating predator. *Interface Focus* **7**, 20160035.

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More haste, less speed for quolls

An animal escaping a predator may be tempted to simply run as fast as it can to escape with its life, but numerous observations suggest that this isn't the case. Indeed, both predator and prey rarely adopt their maximum speeds in the natural world. Quite how and why animals choose their speed remains difficult to understand. However, it is likely that animals have to balance the benefits of high speed with the costs, such as stumbling.

In order to measure how the risk of mistakes affects an animal's speed choice, Ami Nasir and her colleagues from The University of Queensland, Australia, brought wild-caught quolls – agile arboreal marsupials – into the lab. In their neat experiments, the team introduced the timid animals into a Perspex enclosure in which the only means of escape was along a balance beam, the width of which could be manipulated. The researchers videotaped the fleeing quolls, and afterwards analysed how fast they ran and how many mistakes they committed in the process.

Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. 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.

Nasir and colleagues observed that the faster the quolls tried to sprint, the more often they slipped. On average, every mistake halved the speed at which the animals navigated the escape beam. But the nimble marsupials were not headless chickens – they ran more slowly across narrower beams. Furthermore, the calculating quolls were able to fine-tune their getaway and put on the brakes for the final two-thirds of the narrowest beam (they sustained a constant, optimal speed on wider beams, however).

The quolls were also able to learn from their mistakes over time. Each individual was tested 10 times on each beam width, and they got faster and faster on the wide and medium-sized bars. But practice did not make perfect for the narrowest beams, on which the quolls continued to struggle. Even with experience, slips cost the quolls speed during the trickiest task.

This smart study has clear relevance for the perilous, arboreal world of snakes and birds of prey in which the quolls live. But it also serves as a metaphor for the balancing act of speed and safety that may dictate how fast animals in general choose to move.

10.1242/jeb.147561

Nasir, A. F. A. A., Clemente, C. J., Wynn, M. L. and Wilson, R. S. (2017). Optimal running speeds when there is a trade-off between speed and the probability of mistakes. *Funct. Ecol.*

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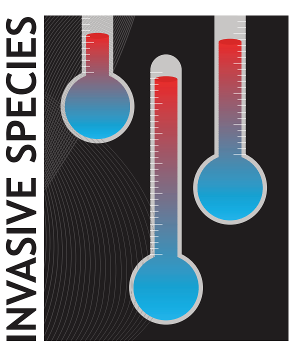
How a global invader gains the upper edge



Upper thermal and hypoxia stresses affect the metabolism of fishes and these limits are likely, in turn, to affect how species re-distribute across the globe in response to climate change. One key question is how invasive species gain the upper hand in novel environments over the indigenous residents that have evolved in harmony with the environment. Perhaps the answer is hidden in how animals cope with extreme climate events that induce rapid changes in temperature and oxygenation. Rick Stoffels and his team at La Trobe University, Australia, used an experimental physiology approach to understand the physiological limits of the global invader – the mosquitofish (*Gambusia holbrooki*) – and a threatened native freshwater species – the pygmy perch (*Nannoperca australis*) – when they both experienced high temperatures and hypoxic stress.

The team used respirometry to measure the routine and the standard metabolic rate – required to maintain essential bodily functions – of mosquitofish and pygmy perch. The fishes then experienced a period of hypoxia, before the team reaerated the water and measured the metabolic cost of recovery from hypoxia, to learn more about the fishes' capacity to supress metabolism when oxygen is scarce. Repeating the procedures at low and high temperatures, the team was able to build a profile of the fishes' responses to hypoxia under different thermal regimes.

They discovered that the invasive mosquitofish exhibited a greater tolerance of hypoxia than the native pygmy perch. More importantly, the hypoxia tolerance of the mosquitofish was not as compromised as that of the perch at higher temperatures. The team reasoned that the mosquitofish's greater hypoxia tolerance was due to its four times greater ability to suppress metabolism than the perch and this capacity was also maintained at the higher temperatures. Additionally, the standard metabolic rate of the mosquito fish increased less at higher temperatures than it did in the perch. In other words, the perch had a harder time meeting their metabolic demands at higher temperatures during hypoxia.

Having found that the mosquitofish appear to be more robust than the indigenous perch when oxygen is scarce, the team wondered how this strength gives mosquitofish the edge in the same habitats. Stoffels and his colleagues monitored the dissolved oxygen and temperature in habitats shared by the two species. They discovered that the dissolved oxygen throughout the water column drops below the threshold where both species can support their standard metabolic rate when heat waves occur. However, the mosquitofish's ability to suppress its metabolism allows it to thrive in conditions where the perch would suffocate. In addition, high water temperatures throughout the summer will further favour the mosquitofish.

Thus, the invading mosquitofish will have an advantage over the pygmy perch as the frequency of heat waves and hypoxic episodes increases. However, this is merely one case of a species re-distribution where global climate change may tip the balance in favour of an imposter and the reality for entire ecosystems will be far more complex. Will invasive species still have the edge if the indigenous species are already well adapted for naturally occurring, physiologically harsh extremes? It's hard to say, but currently the march of the mosquitofish through Australia's freshwater habitats seems inexorable.

10.1242/jeb.164699

Stoffels, R. J., Weatherman, K. E. and Allen-Ankins, S. (2017). Heat and hypoxia give a global invader, *Gambusia holbrooki*, the edge over a threatened endemic fish on Australian floodplains. *Biol. Invasions* **19**, 2477-2489.

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Mating stimulates fighting in female fruit flies

When fruit flies mate, males do not transfer just sperm to females. Instead, male ejaculate fluid contains a cocktail of sperm and sex peptides that have striking effects on female behaviour. For example, sex peptides make females ramp up egg production, while reducing their willingness to mate again. This means that

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sex peptides help males manipulate females into investing more in their current offspring, even if it carries long-term fitness costs to females. Eleanor Bath and colleagues at the University of Oxford, UK, suspected that male ejaculate might affect another important aspect of female behaviour – aggression.

Females typically fight to protect their offspring and to secure resources to provide for them. This means that fighting should be a flexible trait in females and that females should be most aggressive when they have offspring. Given this, Bath and colleagues predicted that mating might act as a cue to trigger female aggression. To test this idea, the team paired virgin and mated females in a location where food was scarce and recorded how often females butted heads, fenced, shoved or retreated from one another. The result was clear: mated females spent twice as long fighting as virgin flies.

The team then began teasing apart the elements of mating responsible for elevating female aggression. They suspected that the costs of egg laying might drive this elevated aggression, prompting females to fight to secure enough resources to lay eggs. To test this idea, they compared aggression in mated wild-type females and in mutant females that cannot produce any eggs. Surprisingly, levels of aggression were similar in wild-type and mutant females, showing that mating elevates female fighting regardless of whether they produce eggs.

If the costs of egg laying do not trigger elevated aggression, maybe a component of the ejaculate itself is responsible? To find out, the team compared aggression in females mated to wild-type males and male mutants that can transfer seminal fluid proteins but no sperm. Females mated to these spermless males were much less aggressive than control females, showing clearly that sperm stimulates female fighting. To see whether sex peptides also play a role, wild-type females were mated to mutant males that produce sperm but not sex peptides: the females mated to these mutants were more aggressive than virgins, but not as aggressive as females mated to wild-type males. Both sex peptides and sperm are needed to trigger the full increase in female fighting after mating.

These results show that mating triggers fighting in female fruit flies and does so independently of egg laying. However, they can't tell us why male ejaculate triggers fighting in females. Perhaps this is another example of conflict between the sexes, where males try to manipulate female behaviour to elevate their own fitness, even if it carries long-term costs for the females, such as injury? Additionally, the neuronal mechanisms that underpin female responses to seminal peptides are unclear. Understanding the mechanistic basis of how sperm and sex peptides stimulate female aggression and the fitness consequences of female fighting are exciting avenues of future research.

10.1242/jeb.147538

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Polar regions and mountaintops are safe havens for caterpillars

Worldwide biological studies are rare; however, they are vital to understanding how biotic interactions vary in different regions. We know that biodiversity is highest in tropical regions close to the equator and decreases as you travel towards either the North or South Poles. But how does this influence how species interact with each other? Tomas Roslin from the Swedish University of Agricultural Sciences and his large team of international colleagues undertook an enormous task to begin to make headway into the vast topic of species interactions.

Specifically, Roslin and his colleagues wanted to quantify the risk of predation on insects in a variety of habitats around the world, that all differed in latitude and elevation. The 31 sites encompassed in the study spanned six continents, a latitudinal range from Newholme in Australia in the southern hemisphere (30.4°S) all the way up to Zackenberg in Greenland in the northern hemisphere (74.3°N) and an elevational range of 2100 m. The ecosystems in which the experiments were executed included forests, tundras/shrublands and wetlands.

Before undertaking the experiment, each group of scientists received a package containing superglue and little green caterpillars made from Plasticine – the exact same stuff you may have played with and eaten as child. As the team wanted to avoid any bias from predators for a particular design of caterpillar – to ensure that the recorded predation rate differences were solely attributed to variation among the sites and not the fake caterpillars – they had one researcher at the University of Helsinki, Finland, create all of the thousands of plasticine caterpillars to ensure that they were identical. The bogus caterpillars were then deployed at each of the study sites by Roslin's colleagues, who superglued them to leaves and left them there for up to 18 days. During this period, the caterpillars were observed and removed if there were signs of predation, such as beak or teeth marks. These caterpillar carcasses were then shipped back to the University of Helsinki for analysis to determine which animals had been duped into trying to eat the phony prey.

Roslin and colleagues' initial predictions were supported by the enormous amount of information collected from all over the planet: the chance of a caterpillar being preyed upon was much greater near the equator and also at sea level than at the poles or high altitude. This trend is largely attributed to arthropod predators rather than the presence of birds or mammals. As arthropod abundance and diversity are often greater in the tropics and they are generally more active at warmer temperatures, it is not surprising that the caterpillars were more heavily preyed upon at lower latitudes and elevations –

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both of which are warmer than high latitudes and elevations. However, the likelihood of the caterpillar model being attacked by a bird or a mammal was similar across all study sites. This result perhaps could also have been expected, as birds and mammals can be active regardless of the temperature and many species have evolved to function in the very cold conditions experienced at high latitudes and altitudes.

This research has shown that using relatively simple, but standardised, methodology can produce exciting and vital results in terms of biotic interactions around the world. The findings are an important contribution to this field as they have shown that the increased biodiversity observed in tropical regions does indeed affect how species interact. Further, this study will undoubtedly pave the way for future research endeavours to uncover global ecological trends.

10.1242/jeb.147546

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