Science & technology

Combating invasive species

Wart wars

TOWNSVILLE, QUEENSLAND

Genetic engineering, among other techniques, could help rid Australia of cane toads

THIS WEEK, between January 18th and 27th, thousands of volunteers in a band of territory stretching across north-eastern Australia from Darwin to Brisbane are venturing into the night with torches and collecting buckets. They are taking part in the Great Cane Toad Bust, an annual attempt to keep a lid on the population of these invasive, toxic amphibians. Toads thus caught will first be chilled in refrigerators and then frozen to death.

Popular though this toad-busting party is, however, it is not very effective. The toad's prolific breeding habits soon replace such losses. To do the job properly, other methods are needed. And one which is gaining ground is tadpole trapping. Toads live in dense populations, and their tadpoles are not above cannibalising the eggs of others, attracted by a chemical signal they release. Scientists at the University of Queensland have isolated this substance to develop lures for tadpole traps. Six thousand of these traps have now been sold.

Cannibalism is one of several weaknesses discovered during years of study into how these Latin American amphibians have adapted to their new home. Combining such knowledge with genetic technologies has brought renewed hope of slowing, or even reversing, the relentless invasion.

Hop it!

The problem began in 1935, when 101 cane toads were released in northern Queensland in a failed attempt to control pesky beetles that were eating the local sugarcane. Tens of thousands of reinforcements were added in subsequent years and, with few natural checks, the animals bred and spread. Well over 200m toads are thought to live in Australia today, hopping determinedly across most of the tropical north and halfway down the east coast.

This population explosion has had serious ecological consequences. Cane toads secrete a substance called bufotoxin from glands in their shoulders. This can be lethal to native wildlife, which has evolved no protection. Predatory marsupials, freshwater crocodiles, monitor lizards (known as goannas) and several of Australia's most venomous snakes suffer as the toads move in. In some places, up to 90% of goannas vanished upon the toads' arrival. The disappearance of these large predators distorts entire ecosystems. Prey species boom. Smaller predators go unchecked. Carrion is left to rot.

Attempts to control the toads have been going on for decades, yet their advance has accelerated. In the tropics, they now travel up to 70km westward every wet season, compared with 10km when they first arrived. They are thus poised to enter some of Western Australia's most treasured ecological areas.

Toad biologists call this acceleration the Olympic Village effect. It is a superb example of evolution in action. Only the most athletic toads make it to the invasion front, where they breed. Over the generations, toads on the front have thus developed larger size, longer legs and even an

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urge to travel in a single direction.

Armed with this knowledge, some propose dropping toads from the core population onto the invasion's front line. These toads are less physically impressive but much more competitive breeders. The hope is to dilute the athleticism of the frontline toads and thus slow the advance, a process called genetic backburn.

Other genetic solutions are in development. Tadpole cannibalism has inspired a team at Macquarie University to engineer "Peter Pan" tadpoles, so called because the genes which allow them to grow up into adults have been disabled. Releasing hungry swarms of these should keep pools clear of toad eggs for years.

These genetic changes are so cautious that Peter Pan tadpoles are not recognised as genetically modified organisms under Australian law. The affected genetic material in them is being deactivated, rather than added to. And the fact that the animals do not mature means changes cannot be passed on to a new generation. "We're very carefully testing reactions of native fauna to our non-metamorphosing tadpoles before we talk about releasing them in the wild," explains Rick Shine, the team's leader. "We're trying not to repeat the folly of 1935."

Turning tadpoles against their own kind is far less labour-intensive than trapping them. However, even Peter Pans die eventually, and must be replaced. So this is not a permanent fix.

Thus far, the new tadpoles have been confined to the laboratory. But New South Wales and the Northern Territory have given permission for them to be tested in the field. The first sites are likely to be small isolated ponds in the Northern Territory, where the team already conducts research, with release happening at the end of this wet season, in March or April. Meanwhile, work continues to scale up the production of tadpoles from a few thousand now to the tens of thousands.

Resistance is useful

But it is not only the toad that is ripe for genetic engineering. A team at the University of Melbourne, led by Andrew Pask, has partnered with Colossal Biosciences, a genetics company in Dallas, Texas, to create gene-edited marsupial cells resistant to bufotoxin. In a preprint last year on *bioRxiv*, the researchers proved they could replace part of a gene in the fat-tailed dunnart, a small marsupial, with a modification found in African and Asian monitor lizards known to be resistant to toad toxins. The results showed a 45-fold increase in resistance to bufotoxin. The team's hope is that they can replicate this in their target species, the endangered northern quoll.

Quolls, which resemble ferrets, are the largest carnivorous marsupials left on the Australian mainland. Northern quolls currently exist in isolated groups either behind or immediately ahead of the toad frontline. Though quolls are also threatened by habitat loss and introduced predators such as feral cats and foxes, studies show the arrival of toads crashes their populations. A toxin-resistant quoll would not only survive the toads' arrival, but might also actively hunt them, thus reducing their numbers. The team hope something similar may also be possible with other predators, such as goannas.

Genetics is already widely used in conservation—for example to monitor elusive species or support breeding programmes. But gene modifications have not been employed in the wild before. "This is really the first demonstration of gene editing for wildlife conservation purposes to target an anthropogenic problem that we've created," says Professor Pask.

His team reckon a toxin-resistant quoll could be ready for release in as little as five years, though the exact schedule will depend on approval by regulators. Peter Pan tadpoles already have the green light. But the gene-edited quoll, the DNA of which would be changed in ways that could (and ideally would) be inherited, is likely to face higher hurdles. More sophisticated forms of genetic engineering, in particular ones that allow for traits to spread rapidly through a population, will be an even tougher sell. But desperate times require desperate measures. ■

Snake bites

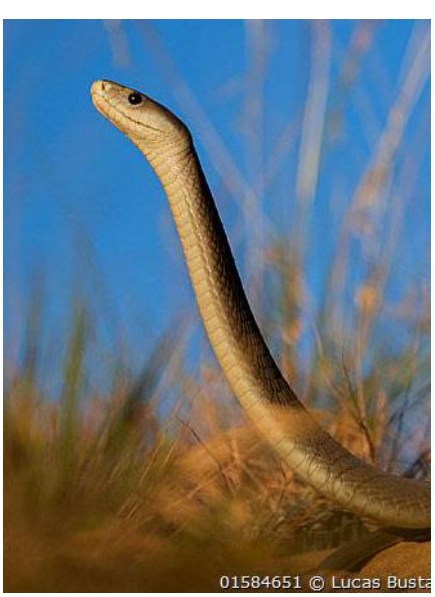
The many and the few

BENGALURU

Genetic engineering and AI are powering the search for antivenins

THE BITE OF a black mamba causes respiratory muscular paralysis. And death. Disturb a Russell's viper and the encounter may lead to kidney damage and excess bleeding. And death. As to the *fer de lance*, well, you get the idea.

Whatever the assailant, though, snakebite treatment has been the same for a century: inject, as quickly as possible, an antivenin made up of antibodies produced in a horse or sheep.



Black Mamba (Dendroaspis polylepis)

Researchers are trying to replace this antiquated solution with something better. The early results of two groups, one working in old-fashioned wet labs and the other using new-fangled artificial intelligence (AI), suggest they are on the right track.

The wet-labbers are based at Scripps Research in San Diego, the Indian Institute of Science in Bengaluru and the Liverpool School of Tropical Medicine. The problem they are trying to overcome, according to Kartik Sunagar of the Indian Institute, is the multiplicity of venom types, both within and between species of snake. To simplify things, they are concentrating initially on a group of molecules called long-chain three-finger alpha-neurotoxins. These are important parts of the armamentaria of the elapids, a group of snakes that includes mambas.

The AI track is led by David Baker of the University of Washington, who won a share of last year's Nobel chemistry prize for his work on computational protein design. He and his colleagues also have long-chain three-finger alpha-neurotoxins in their sights. But their search for antidotes is taking place inside computers rather than 96-well test plates.

Both groups are looking for proteins able to neutralise a range of types of the target alpha-neurotoxins—molecules that are, themselves, proteins—by binding to them and thus rendering them ineffective.

As they describe in a paper published

last year in *Science Translational Medicine*, the wet lab team is trying to supercharge antibodies—or immunoglobulins, as they are known to molecular biologists—and also cut out the use of animals. (Existing antivenins are created by the messy process of injecting snake venom into the chosen animal to provoke an immune response, and then extracting the resulting antibodies from the animal's blood serum.)

The amino-acid chains of an immunoglobin include "hypervariable" regions where the sequence of amino acids differs from protein to protein. Different sequences bind to different targets, and a huge number of sequences is possible—theoretically, up to a billion billion. Moreover, it is easy to generate large numbers of different immunoglobins, or fragments thereof, in a laboratory, by inserting the relevant DNA into yeast cells.

To find the right candidate, the team screened billions of antibody fragments, expressed on the surfaces of these genetically modified yeasts, against eight representative alpha-neurotoxins. They then injected groups of mice with the winner and with venom from one of three types of elapid: black mambas, many-banded kraits and monocellate cobras. All survived.

Dr Baker's approach, just published in *Nature*, ignored immunoglobins in favour of entirely new types of protein molecule, designed from scratch. His AI first calculated what shape a protein would need to be to fit snuggly into the toxin's active site (the place that binds to its target). In this he was helped by the fact that, though alpha-neurotoxin molecules vary a lot in their peripheries, their active sites are similar. A second program then worked out which amino acids, and in what order, would be needed to make such optimal proteins, coming up with multiple answers to this question. A third then assessed whether the amino-acid chains thus lit upon really would fold into the desired shape, and thus might do the job.

Only at this point, having picked the most plausible candidates, did the team actually do some experiments. They synthesised pieces of DNA that encoded the most promising designs, inserted them into yeast, churned out the relevant proteins, and tested them against venom samples. They then picked the most successful of these and injected them into mice. Depending on the dose, the toxin and the protein being tested, between 80% and 100% of the mice survived.

How all this will play out in people remains to be seen. Much work remains if these discoveries are to be turned into actual medicines. But if that does happen, human casualties from snake bites, which cause around 100,000 deaths a year and thrice that number of disabilities, may significantly diminish. ■

AI and board games

Your move

Artificial intelligence can improve old-style table-top games

BOARD GAMES have long fascinated artificial-intelligence researchers. They have clear rules, well-defined playing fields and objective winners and losers. This makes them perfect "sandpits" for training AI software. Sometimes, though, their rules contain glitches. Aficionados of Go will be familiar with ko fights—situations in which the basic rule set would permit a game to continue forever, and a special exception has thus had to be invented. Avoiding similar problems in newly invented games is something AI can help with.

That, at least, is the experience of Alan Wallat, a board-game designer from London. He has come up with an offering called Sirius Smugglers, in which interstellar merchants try to make an illicit profit. In olden days, checking its rules would have involved lots of tests by human players, who would need to be enthusiasts and would probably have wanted to be paid—in beer, if not in cash. Instead, Mr Wallat took his brainchild to Tabletop R&D, an AI startup, where a game-playing algorithm allowed him to play thousands of times in the blink of an eye. Once this was over, he was able to scan through the results for irregularities, statistical biases and any features that were under- or over-used.

It was here he discovered a problem. A quirk in the rules meant the decision to end the game rested with the losing players. Whoever was ahead, and therefore had the greatest incentive to bring matters to a close, was unable, alone, to trigger the condition which would finish the game. Like Go without the ko fight exception, Sirius Smugglers could thus go on indefinitely.

The minds behind Tabletop R&D, Diego Perez-Liebana and Raluca Gaina, are computer scientists at Queen Mary, a college of the University of London, who wanted to build a general games-playing AI platform on the cheap. The approach which built the chess AIs that defeated grand masters and the Go AIs that beat world champions involved a system playing itself, over and over again, and learning from its victories and defeats until it reached superhuman potential.

But that requires a lot of computing time. Instead, they chose to use a less resource-intensives approach called a Monte Carlo tree search, to look forward to possible future games positions and choose appropriate play from among them. It was intended as an academic exercise but, in the process of doing it, says Dr Perez-Liebana, they realised they had accidentally developed a tool that had value in its own right as an aid for game designers seeking to perfect their creations.

For this to happen, the AI must be taught to play like a human. Unless told otherwise, AIs are liable to chase victory single-mindedly but without strategic vision, like a chess player who refuses to sacrifice pieces for a stronger long-term position. This training can be subtle. In games where players are assigned information hidden from their opponents (for example, in card games like bridge or poker, where others cannot see a player's hand), designers must decide whether to give the AI the ability to memorise play so far and to card-count the pack perfectly, or else to act in a sloppier—and more humanlike—manner.

Giving the AI more time to think, and thus plan for a wider range of outcomes, is equivalent to adjusting the skill with which it plays. To simulate beginners, it can be set to act as if on instinct, after less than a tenth of a second. To mimic competence it is allowed to think for as long as five seconds per move, and is thus able to plan many moves ahead.

When they're good, they're good, says Dr Gaina of the resulting models. Testing the approach with a copy of Terraforming Mars, a famously weighty strategy title, she says, they found the system was more than capable of defeating that game's creator.

The data a game-run provides are detailed enough to let designers tweak the parameters they care about, from ensuring a game is fair to avoiding long periods of dull gameplay. At least, that is the plan. Mr Wallat is Tabletop's first customer. More may soon be tempted. Fun is hard to measure, says Dr Gaina, but things that make a game bad, never-endingness among them, are easier to spot. ■

Wasp evolution

Viral load-up

Wasps stole some of their genes from viruses

PEOPLE DOMESTICATED sheep and cattle, wheat and maize. Wasps domesticated viruses. And, just as domesticating other species helped human populations to explode, so viral domestication assisted an explosion of wasps. That, at least, is the conclusion of Benjamin Guinet, an evolutionary biologist at Lyon University, in France. As he writes in the *Proceedings of the Royal Society*, he thinks an ancestor of a group of wasps called the Cynipoidea, which parasitise flies, corralled 18 viral genes into it genome in an act of domestication that happened 75m years ago, and that this helped the group to flourish.

The large, black-and-yellow picnic-disrupting terrors that generally come to mind when the word "wasp" is mentioned are actually unrepresentative of the group. Most wasps are small, solitary and reproduce by laying their eggs in or on other arthropods, particularly insects and spiders. Cynipoidea specialise on flies. As with other parasitoid wasps, when their eggs hatch, the hatchling larvae then eat their hosts alive.

To assist their offspring in this endeavour, mother Cynipoidea wasps also squirt into the flies a mix of venom, viruses and other materials that sabotage the host's immune system. Some of this material consists of proteins that look remarkably like ones which viruses themselves produce to attack other organisms.

These virus-like proteins are, nevertheless, encoded not in viral genes but in genes which are now part of the wasps' genomes. Dr Guinet therefore presumed that ancestral cynipoids had swiped them from viruses at various times in the past. He wondered when. To find out, he and his colleagues analysed the genomes of 41 Cynipoidea wasps from six subfamilies using molecular-clock techniques that estimate how fast genes in different lineages have diverged from each other. That let them work out when each gene had arrived in the ancestral genome.

The answer was the same for all 18. It seems, therefore, that the domestication of these genes was a single event. Intriguingly, this corresponds to the moment in the Cretaceous period when the group of flies that cynipoids parasitise began itself to diversify. Dr Guinet reckons that viral domestication helped facilitate the wasps' diversification in response to the multiplication of the number of host species. ■

Well informed

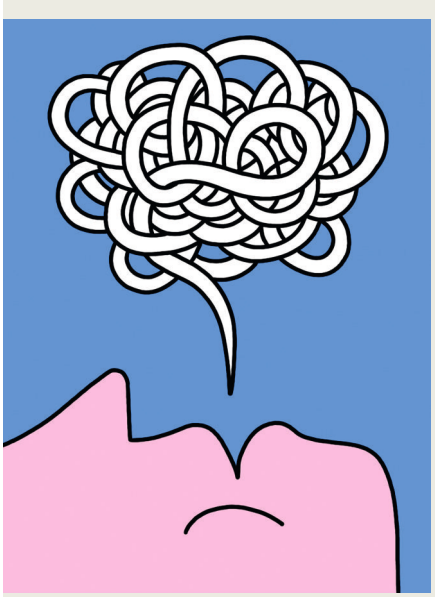
Can you breathe stress away?

It won't hurt to try. But scientists are only beginning to understand the links between the breath and the mind

AT 7BREATHS, a meditation studio in central London, groups of young-professional types gather several times a day simply to breathe. The studio offers yoga and meditation sessions but their signature class is focused on "breathwork". Those attending sit cross-legged atop small cushions in the warm, minimalist space, as an instructor gently guides them first to pay attention to their breath and then to gradually lengthen the inhales, the exhales and the pauses in between. The goal: to de-stress.

The Bhagavad Gita, a Hindu scripture from 1st or 2nd century BC, talks about "pranayama"—a yoga practice of controlling the breath—and yoga texts from a few centuries later describe its benefits for steadying the mind. For modern breathwork-enthusiasts who say that guided breathing helps them feel better, it undoubtedly does. But to test whether such exercises can reduce stress in the as-yet-unconverted, you need randomised-controlled trials (RCTs).

A meta-analysis published in *Scientific Reports* in 2023 compiled the results of 12 RCTs, including some 785 participants, to examine the effect of slow-breathing on stress. The studies used a mixture of in-person coaching, online classes and self-guided breathing. Participants who took part in the breathwork sessions reported greater stress-reduction than those in the control group. The effect was small but significant, roughly in line with the benefit from online cognitive behaviour therapy.



These findings come with caveats, however. Several studies, for example, recruited participants who were seeking help for stress and compared a subset who took part in breathwork classes with others who remained on a waiting list for care. This is a problem, as waiting for mental-health treatment can create a "nocebo effect", where well-being gets worse. Comparing the people who receive treatment with a deteriorating control group can make interventions look better than they really are.

In 2023 researchers at Stanford University published a study in *Cell Reports Medicine.* Participants performed either mindfulness, "cyclic sighing" (two short inhales, one long exhale), "box-breathing" (inhale, pause, exhale, pause), or "cyclic hyperventilation" (30 short inhales and exhales, followed by a 15 second pause), for five minutes a day, for a month. Everyone got an initial mood boost at the start, but only those who were doing breathwork reported that their mood continued to improve as the study progressed. The best results were in the cyclic-sighing group.

How might breathing control mood? One idea is that it forces attention away from negative or stressful thoughts. Researchers have also found that voluntarily slowing breathing can increase heart-rate variability—the fluctuations in the timing between heart beats. This is often low in people with psychiatric disorders like depression, bipolar and ADHD. Increasing it, the theory goes, should therefore be a good thing. There is also evidence that slow breathing and stress regulation might share brain circuits, at least in rodents. A study published in *Nature Neuroscience* in November 2024 found that stimulating a pathway which causes slow breathing in mice also suppressed their anxiety behaviours.

The evidence on breathwork might still be unclear, but the practice appears to have no real downsides. Everything from gut health to infection is now understood to influence mental health. Slow, controlled breathing may soon be added to the list.