One slip, and you're dead…

The lethal toxins produced by cone snails are in hot demand for neuroscience research, and are being developed as potent drugs. Laura Nelson visits a would-be snail 'farmer', for whom milking time is fraught with danger.

Jon-Paul Bingham fumbles around for a condom. Big Bertha is waiting. There's an awkward pause. "It has to be the non-lubricated kind," he says. Bingham rips open the packet and slips the prophylactic over a small plastic test tube.

Big Bertha is one of Bingham's nine tropical marine cone snails. These colourful creatures are some of the most venomous beasts on the planet. But the powerful poisons they produce can, in tiny doses, help to reveal how nerve cells function — and potentially help to treat conditions from chronic pain to epilepsy.

Currently, most neuroscientists obtain their cone snail toxins from dead animals taken from the wild. But Bingham, a biochemist at Clarkson University in upstate New York, believes that the future lies with cone snail farming. Not only might it help conserve wild populations, he says, but it can also yield a wider range of useful toxins.

'Milking' the live snails is a hazardous business. One false move and Bingham could be dead in half an hour. Using forceps, he dangles a dead goldfish, the same length as Big Bertha, in front of her. Behind the bait, the condom is stretched over the mouth of the plastic tube.

"We tried all sorts of membranes, including sausage skin," says Bingham. "But for this species, condoms are the right thickness — and the snails don't like the lubricated ones." Bertha extends her proboscis and, with an abrupt flick, stabs the fish and the condom. She discharges a few microlitres of the toxin into the tube. That's enough to kill ten people.

Violent reaction

There are about 30 recorded instances of people being killed by cone snails — the molluscs are aggressive if provoked and can penetrate wetsuits with their sharp poison-loaded harpoons, which look like transparent needles. Human victims seem to suffer little pain[1], because the venom contains an analgesic component.

Today, the venom's painkilling properties are just one facet of a burgeoning field of research and drug development. Australian scientists first separated cone snail venom into its constituent parts in 1977 (ref. 2). Unlike most venomous animals, which produce one or a few poisons, a single snail can produce up to 100 individual toxins.

When it comes to working out what each component does, Baldomero 'Toto' Olivera, a Filipino based at the University of Utah in Salt Lake City, has led the way. Throughout the 1980s, Olivera's students injected venom into the central nervous system of mice and found that different components had different effects — some would send a mouse to sleep, others would make it shake, run in circles or swing its head back and forth. The researchers showed that the different venom components had specific targets in the nervous system — they blocked different ion channels in cell membranes, which control the transmission of impulses by nerves, or specific receptors for the neurotransmitter chemicals that transmit signals from cell to cell[3].

One snail's poison…

One venom component, called MVIIA and classified as an ω-conotoxin, causes tremors in mice. Olivera's group has found that it blocks a specific type of calcium channel that has been implicated in chronic neuropathic pain, caused by damage to the nervous system[4]. The toxin has since been developed as a drug called ziconotide and is now in advanced clinical trials for patients with cancer and AIDS who are suffering from pain that cannot be relieved by opiates[5].

Different groups of conotoxins — most known by various letters of the Greek alphabet — target other ion channels, such as those for sodium and potassium, or receptors and transporters for neurotransmitters such as glutamate, serotonin, neurotensin and noradrenaline[6,7]. "This is a real pharmaceutical cornucopia waiting to be tapped," says Bruce Livett of the University of Melbourne in Australia, who hopes soon to put an α-conotoxin, known as Vc1.1, into clinical trials for patients with diabetes who are suffering from the neuropathic pain that is a complication of the disease. Another toxin, which blocks a receptor for a specific neurotransmitter, is being investigated as a treatment for epilepsy. The goal is to damp down the hyperactivity that causes epileptic seizures.

In addition to their value as drugs (see Table, below), neuroscientists can use conotoxins as ultraprecise tools to selectively disable particular ion channels, or to block the action of individual neurotransmitters.

Milking time: Jon-Paul Bingham entices his molluscan menagerie to yield a sample of toxins.

Toxin	Class	Species	Condition	Stage in development	Company website
Vc1.1	α	*Conus victoriae*	Neuropathic pain	Preclinical	www.metabolic.com.au
CVID	ω	*C. catus*	Neuropathic pain	Phase II clinical trials	www.amrad.com.au
MVIIA	ω	*C. magus*	Cancer pain	Phase III	www.elan.com
MrIA/B	χ	*C. marmoreus*	Neuropathic pain	Preclinical	www.xenome.com
Contulakin-G	Contulakin	*C. geographus*	Chronic pain	Phase II	www.cognetix.com
Conantokin-G	Conantokin	*C. geographus*	Epilepsy	Preclinical	www.cognetix.com

In addition, researchers can use fluorescently labelled toxins to find the pattern of distribution of ion channels in cell membranes. "We can home in on them," says Bingham.

Fulfilling this research potential depends on a steady supply of cone snails. Which is why, once a year, Bingham joins a gang of 'coneheads' — amateur enthusiasts who collect the snails' shells, heading out at night on to the beaches of the tropical Pacific, weighed down with backpacks carrying the batteries used to power their flashlights. "It's difficult to find the snails," says Bingham. But jumping up and down in the sandy shallows can help draw the creatures to the surface.

Unlike his companions, Bingham is intent on keeping his quarries alive. Each time he finds one, he pops it into a football sock so the snail draws it body into its shell and keeps its harpoon out of harm's way — cone snails don't like sock material. Bingham then takes the creatures back to the United States in a plastic container — "like a bucket you keep your beers in" — after filling out copious customs paperwork.

The animals are difficult to keep in captivity. Bingham's cone snail farm currently consists of just one tank of snails from a single species, *Conus purpurascens*. Although it bears the label 'Danger — venomous snails', at first glance, the tank looks as if it contains nothing but dirty brown sand. But Bingham calls it "cone snail Hilton". He sprinkles a little fish-flavoured water into the tank. "Where else do you get waited on hand and foot?" he asks. The buried molluscs smell the hint of a meal and emerge over the next couple of minutes, until all nine are visible.

Cream of the crop

Once milking time is over, the next job is to put the samples through a high-pressure liquid chromatography machine, which separates the toxins within the venom and produces a printout with peaks that correspond to individual compounds.

The conotoxins have very similar amino-acid sequences, and most are fewer than 30 amino acids in length. Using techniques including mass spectrometry and sequence analysis, Bingham is trying to work out the exact differences between the toxins.

Bingham has also found that his milked toxins are subtly different from those that are extracted from the venom glands of dead snails. The toxins are processed by enzymes as they move down the venom duct, he believes.

Perhaps his most fascinating finding, not yet published, is that the profiles of toxins produced by an individual snail are not uniform over time. Instead, the snails produce different toxins at different times — possibly influenced by external conditions such as temperature. If these conditions could be understood and controlled, says Bingham, neuroscientists could reap more valuable material.

For Bingham, these findings illustrate the superiority of cone snail farming over the traditional approach of extracting the toxins from dead snails. Conservationists, meanwhile, are worried that collection of the animals to provide material for neuroscience research and drug development could have a detrimental effect on wild populations. In October last year, Eric Chivian, director of the Center for Health and the Global Environment at Harvard Medical School in Boston, wrote a letter to *Science*[8] expressing concern about the overuse of cone snails for scientific research. "No one really knows the number of cone snails that are killed," says Chivian.

Neuroscientists working in the field think that the problem isn't as bad as Chivian fears, but agree that cone snail farming could help reduce the toll on wild populations. "The milking technology could have a nice impact in preserving biodiversity," says Olivera.

But achieving real sustainability will require the animals to breed in the lab, and this is more difficult than anyone anticipated. Bingham sheepishly admits that he is unable even to verify whether Big Bertha is a female. And, so far, he has not observed his snails mating. In the wild, cone snail orgies allegedly occur, but no one knows what triggers these erotic extravaganzas. For now, sex in the cone snail Hilton remains the stuff of Bingham's dreams. ■

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