Evolutionary therapies

Can we adapt the chemical defences of termites to combat our own ailments and pests? Graeme O'Neill reports.

Like ants, bees and wasps, termites form large colonies ruled by a single queen, a prolific egg-layer who is mother to all.

This unusually close gaenetic relationship is the source of the colony's complex, cooperative behaviour. But limited genetic diversity is a potential liability: termites inhabit moist environments teeming with potential bacterial and fungal pathogens, parasites such as nematode worms, and predatory arthropods such as ants, beetles, scorpions and centipedes.

They have survived these threats by evolving an arsenal of defensive biochemical weapons. The chemistry varies with the species, and much of it is unexplored, and new to science.

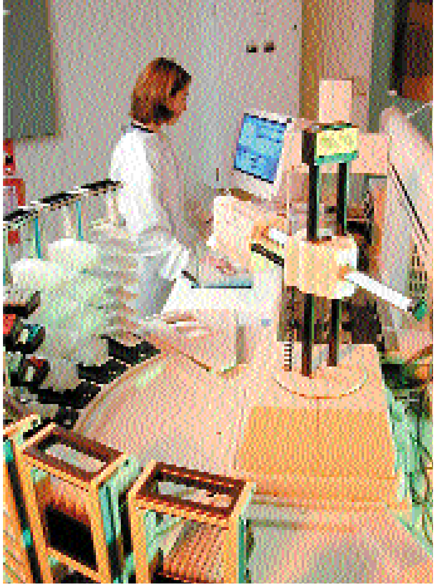
At CSIRO Entomology in Canberra, Dr Stephen Trowell has been bioprospecting among Australia's native arthropods – particularly termites – for natural compounds that might be candidate drugs for human medicine, or 'smart' pesticides for agriculture.

Trowell, a biochemist, says his interest in arthropod biochemistry was kindled by a conference in the United States in the mid-1990s that reviewed progress in identifying new molecular targets for therapeutic drugs.

The pharmaceutical industry was employing high-throughput screening tests on plant extracts to identify prospective compounds for treating cancer, HIV-AIDS and other human ailments. He hoped to apply the same technologies to discovering insecticides and nematicides for agriculture.

Plants figure prominently in traditional pharmacopoeias around the world, and folk remedies have yielded many modern drugs. But Trowell knew that arthropods outnumbered plants at least tenfold; they represent the greatest level of biodiversity available among higher organisms.

In the mid-1990s, combinatorial chemistry, which allows chemists to synthesise hundreds of chemical variants of some basic molecule, was emerging as a



The hunt for new compounds began with a purpose-built laboratory, and field expeditions that, over two years, collected more than 1000 species.

Our interest is in producing new drugs for treating cancer, or novel antibiotics to treat patients with microbial infections that no longer respond to conventional antibiotics.

promising new approach to drug synthesis. But combinatorial chemistry's synthetic diversity is built upon small, novel molecular scaffolds. In some 3.5 billion years of experimentation, nature has been a far more prolific inventor of small molecules than modern human chemists.

'Despite its huge potential, little work has been done on insect biochemistry,' Trowell says.

'Australia is a mega-diverse continent with an advanced scientific infrastructure and skilled taxonomists, and CSIRO Entomology holds the Australian National Insect Collection (ANIC), an invaluable research resource.'

The hunt for new compounds began with a purpose-built laboratory, and field expeditions that, over two years, collected more than 1000 species from promising arthropods groups including termites, millipedes, spiders and scorpions, as well as native worms, slugs and snails.

Voucher specimens of each species, along with details of their environments and Global Positioning System coordinates, were lodged with the ANIC.

To capture all the biochemical diversity, two extracts were taken from each species, and analysed using high-pressure liquid chromatography.

PhD student Chunjiu Zhao purified molecules exhibiting interesting activity and determined their structures, and Trowell reactivated his group's collaboration with Emeritus Professor Rod Rickards, a bio-organic chemist at the Australian National University.

Insectivity

When Dr Trowell's team began screening extracts from its library of samples, it found 'lots of interesting activity'.

'But what we were really looking for was selective activity: something that would kill microbes without killing human cells, or kill cancerous cells without harming healthy cells,' he says. 'So we began to get excited when a surprising number of extracts exhibited selective antibiotic activity.'

Some of the extracts with the most promising antibiotic activity came from termites. They killed several microbes, but were less toxic to mammalian cells.

Nuclear magnetic resonance and mass spectroscopy analyses of the purified compounds identified at least 10 novel molecules based on a trinervitadiene structure.

'Trinervitadiene structures were already known from overseas termites,' Trowell says. 'But nobody had considered testing them for interesting activity first, then identifying the compounds responsible. They were just looking for new chemicals.'

The first trinervitadiene compounds identified came from the cathedral termite, *Nasutitermes triodii*, but then trinervitadiene variants turned up in other species. Their chemistry echoes species diversity: each termite species has its own trinervitadiene variants.

Chemical repellents

'*Nasutitermes* is an advanced genus in an evolutionary sense, and it relies heavily on chemical defences,' Trowell says.

'The soldiers have a prominent snout that exudes a mixture of terpenoids, including trinervitadienes. The workers lack these compounds, so the enzyme machinery varies between castes. That's potentially useful information if we want to perform a partial biosynthesis by cloning some of the genes for these enzymes into bacteria.'

Nasutitermes soldiers use the sticky, irritating exudate from their snouts to repel would-be predators such as ants. As well as being glue-like, this exudate also shows antibiotic activity, raising the intriguing question: which role came first?

Trowell notes that all termites species are social, and live in large colonies in warm, humid environments that are ideal breeding grounds for opportunistic fungi and bacteria.

He wonders whether the trinervitadienes served originally as antibiotics, and were subsequently co-opted into a new role as an insect repellent through evolutionary opportunism.

Despite its huge potential little work has been done to date on insect biochemistry.

'We've now demonstrated interesting and useful biochemical activity in insect extracts, and identified the new molecules responsible for it,' Trowell says

'We have enough evidence, and enough biodiversity resources to work with, and have developed the knowledge and the capability to get a lot more useful molecules out of our extracts.

And the wealth of compounds found in Australian insects is not confined to trinervitadienes.

'We're seeing what appear to be entirely new molecules, some with very strong antimicrobial activity and no measurable mammalian cytotoxicity,' Trowell says. 'As many as three to five new, active compounds are being found in single species. Our next step will be to form a new company to focus on discovering pharmaceuticals from insects, with CSIRO as its major shareholder.

'We've set up a collaboration with associate professor Peter Collignon and Paul Southwell, of the Microbiology Department at Canberra Hospital, to help us test our extracts.

'The commercial imperative is to move towards industrial-scale production of selected compounds. Our interest is in producing new drugs for treating cancer, or novel antibiotics to treat patients with microbial infections that no longer respond to conventional antibiotics.'

Abstract: Termites are vital to the functioning of natural ecosystems, particularly in Australia's tropical north, where they recycle nutrients in a range of ecological niches. Scientists believe that manipulating termite density and activity may help to restore degraded areas faster, or make ecosystems more resilient to disturbance. Termites are also a source of natural compounds that might be candidate drugs for human medicine, or "smart" pesticides for agriculture. CSIRO Entomology has extracted compounds from termites that kill a variety of microbes, but have lower toxicity to mammalian cells. The find has sparked plans to pursue commercially the discovery of pharmaceuticals from insects.

Keywords: termites, land restoration, soil structure, grazing intensity, chemical defences, insect biochemistry.

Derivations of **diversity**

HOW did insects acquire their remarkable species diversity?

According to CSIRO biochemist Dr Stephen Trowell, the answer lies buried in insect history.

'Some orders of insects are recognisable in the fossil record from 400 million years ago, almost 250 million years before the appearance of flowering plants,' Trowell says.

'If you then look at some other invertebrate relatives of insects, say nematode worms, the common ancestor for both groups existed around a billion years ago, so deep evolutionary time underlies their genetic and chemical diversity.'

Trowell says insects inhabit a chemical world. They communicate with chemicals, use sex pheremones to attract mates, lay chemical trails for navigation, and produce a variety of toxins for defence, and venoms as weapons.

'Although very little classical Western research has been done on insect biochemistry, and some find it peculiar that you might exploit insects as a source of pharmaceuticals, there are enough clues to suggest they employ some very sophisticated chemistry,' he says.

'Asian cultures still use insects for food, and the use of insects in both Indian and Chinese traditional medicine is well documented.

'Cantharidin from the "Spanish fly" beetle, used in Europe for it's reputed aphrodisiac properties, is a powerful urogenital irritant.

'It's also cyotoxic and until quite recent times was used in China to treat cancer. It was carefully fried with rice, which causes some of the drug to come off as a vapour, before being ingested.'

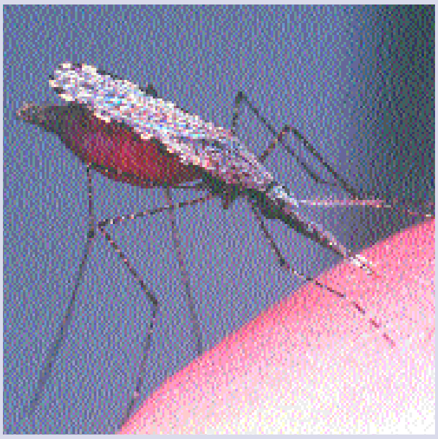
Trowell says some small, brightly-coloured staphylinid beetles, commonly known as whiplash beetles, secrete a potent defensive compound called pederin.

'The females are packed with it,' he says. 'They have caused injuries requiring people to be hospitalised in the Northern Territory and North Africa.'

Pederin is a protein-synthesis inhibitor, and one of the most potent cytotoxins known to science. The only other organisms known to secrete chemicals like pederin are a group of marine sponges of the genus, *Mycale*.

How did a land beetle and a marine sponge come to secrete essentially the same toxin? It turns out that the beetles lack the enzymes required to synthesise pederin. It's made by a symbiotic bacterium that lives in the tissues of both the beetle and, presumably, the sponge.

Trowell says the symbiotic microbes living in the insect gut or other tissues, including protozoans and bacteria that allow termites to digest the lignin and cellulose in wood and other plant materials, are a potentially rich source of pharmaceutical compounds in their own right.



Natural selection has equipped blood-sucking insects with a variety of protein and peptide anticoagulants that disrupt blood clotting.

Many insects, including mosquitoes, ticks and horseflies, feed on the blood of mammals, birds and reptiles.

Trowell says it's a neat trick, given that vertebrate blood tends to clot rapidly. Natural selection has equipped blood-sucking insects with a variety of protein and peptide anticoagulants that collectively disrupt almost every step of the complex biochemical cascade involved in blood clotting.

Insects are also renowned for producing peptides – small bioactive amino acid sequences typically derived from larger protein molecules – to defend themselves against bacterial infections and fungal diseases. Plant geneticists including Dr John Manners, of CSIRO Plant Industry, are exploring the possibility of using anti-fungal peptides as a new source of fungus disease resistance in crops.