Rodenticide resistance hots up

At PestEx 2009 there was an entire seminar devoted to rodenticide resistance. Chaired by Adrian Meyer from Acheta, the session painted a worrying picture.

Adrian related the experience of one town in Hampshire where the control of a severe rat infestation was proving totally impossible to achieve. Over the course of 15 months, 210.5 kg of rodenticide bait had been laid with little, or no, effect. With all practical management options explored and the array of available rodenticides exhausted, was this a case of rodenticide resistance?

Up until very recently establishing if a rodent population was resistant or not was both a slow and expensive process. Live rats had to be caught, bred in laboratory conditions and then tested. As a result, wondering if a colony was resistant or not was about as far as pest controllers got.

But all this has recently changed. Researchers in Germany, led by Dr Hans Joachim Pelz made a critical breakthrough. Following the development of new and sophisticated DNA-sequencing technology, they identified which part of the genetic code of rats and mice carried the DNA sequence, or gene, is altered in rodents resistant to anticoagulants. Having established this, it has become possible to look for changes, or mutations, which result in anticoagulant resistance in rodents.

All very interesting – but with your home chemistry set, you are hardly likely to be able to perform these tests for yourself. However, others can. Within the UK there are laboratories willing and able to perform these tests for a modest fee.

Rats from the colony in Hampshire have been tested and resistance clearly established. This fact caused much interest at the seminar. Several questions arose as to how a pest controller can go about organising such a test – what is involved, what do the results look like and show, how much does it cost and who does the tests?

Keen to find this out for our readers, ***Pest*** editor, Frances McKim,

The team at Huddersfield. Left to right: Professor Rob Smith (Dean of Applied Sciences), Ian Johnson (Senior Technician) and Dr Dougie Clarke (Head of Biological Sciences & Nutrition)

went to visit Professor Rob Smith and his team at the University of Huddersfield, the leading UK test centre, to find out more. What follows, is a pictorial record of what's involved.

Resistance testing – it's all in the genes

Testing a rat for resistance is a scientifically sophisticated exercise. For the purposes of this feature, the science has been kept to a minimum and photographs used to illustrate the procedures involved. It is worth pointing out though, that the procedure used is exactly the same as employed for the diagnosis of human inherited diseases such as cystic fibrosis.

But first – a spot of science The characteristics of all living organisms, including humans, are essentially determined by information contained within DNA that they inherit from their parents. DNA (deoxyribonucleic acid) is a chemical structure that forms chromosomes. A piece of a chromosome that dictates a particular trait, such as eye colour, is called a gene.

Structurally, DNA is a double helix: two strands of genetic material spiralled around each other. Each strand contains a sequence of bases that consist of one of four chemicals – adenine (A), guanine (G), cytosine (C) and thymine (T).

The molecular structure of DNA can be imagined as a zip (the two strands) with each tooth represented by one of four letters (A, C, G, or T), and with opposite teeth forming one of two pairs, either A-T or G-C.

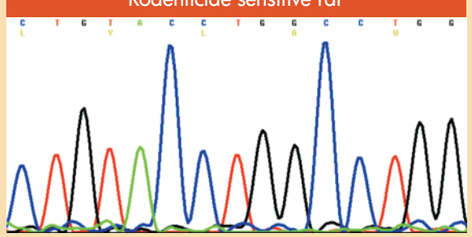
The information contained in DNA is determined primarily by the

Can you spot the difference?

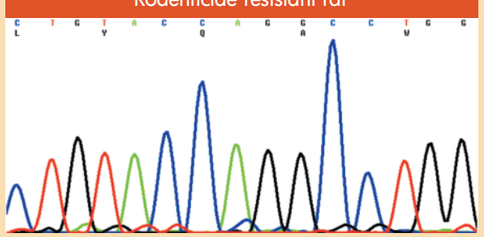
The images below show one of the many mutations the Huddersfield researchers have identified in UK resistant rats and mice. Can you spot the difference in the sequence of the coloured peaks? In the middle of the left-hand image the red peak indicates the chemical 'T' is present in a normal sensitive rat whereas at the corresponding position in the right-hand image the green peak indicates the chemical is an 'A' at this position in a resistant rat. It takes just one change in this chemical sequence to result in resistance.

DNA double helix

Rodenticide sensitive rat



Rodenticide resistant rat



sequence of letters along the zip. For example, the sequence ACGCT represents different information than the sequence AGTCC in the same way that the word "POST" has a different meaning from "STOP" or "POTS," even though they use the same letters.

The traits of a human being are the result of information contained in the DNA code. Living organisms that look different or have different characteristics also have different DNA sequences. It is this change in DNA sequencing that the rat tests reveal.

The magnitude of this search must not be underestimated – you are looking for a change in one sequence out of 3,000,000,000 – or put another way – a spelling mistake in one word in an entire bible.

So, how is it done?

Any pest controller anxious to test for rodenticide resistance (either rats or mice) first needs to contact the laboratory at Huddersfield, which will send a small vial containing preservative suitable packaged for safe arrival. The pest controller then needs to cut approximately 3-5cm off the tail of the dead rat under investigation. Having recorded their details, the vial containing the rat's tail is returned to Huddersfield.

1 *Cut 3-5cm off the dead rat's tail*

2 *Once in Huddersfield the parcel is unpacked*

3 *A small section (5mm) is removed from the tail sample. The remainder of the tail is retained for reference*

4 *The sample is prepared for evaluation, then centrifuged. Here the protein is removed and the DNA released from a purification matrix.*

5 *Then by using a process called PCR (polymerase chain reaction) cloning, the one gene that harbors the resistance mutations is selectively isolated and multiplied.*

6 *This is followed by the DNA sequencing that identifies the sequential order of over 3,000 chemicals in this gene.*

7 *Finally, computer analysis compares this sequence with data from known sensitive and resistant rat DNA sequences held in the Huddersfield database. A report detailing the findings and their significance is prepared and sent back to the pest controller.*

How to arrange for resistance testing of your rats

To take advantage of the service provided by the University of Huddersfield, please contact: Dr Dougie Clarke, Head of Biological Sciences & Nutrition, School of Applied Sciences, University of Huddersfield Email: d.j.clarke@hud.ac.uk
There is a charge, payable in advance, of £60 per rat's tail. Depending on workload, testing can take anything up to six weeks.