E-DNA OF PROTECTED SPECIES, AND FORENSIC ECOLOGY

If you've been using our Great Crested Newt eDNA service this season, you'll know that collecting water samples for environmental DNA (eDNA) is much easier than conventional surveying, and offers the client a more cost effective and valuable service they perceive as representing state of the art.

We think the expansion of DNA analysis from the crime lab and into the monitoring of species has the potential to revolutionise the way we care for the planet. Yes, testing eDNA does require meticulous attention to detail, uses expensive reagents and high capital investment in equipment and laboratory facilities, but the process offers huge potential to the ecologist. Hence, these are exciting times to work in environmental and ecological sciences.

SAMPLE CONSIDERATIONS

There are many benefits in embracing eDNA work. First and foremost is the opportunity to identify the presence of certain species from various media, not just the water in which they live. We can extract DNA from faeces, regurgitated pellets, roadkill remnants, and any habitat just as we can when tracking a criminal. The sensitivity of the technique is astonishing. Natural England recommended a sensitivity of 3 picograms per litre (that's a million millionth of a gram per litre) for GCN testing using eDNA. Because we amplify the DNA by a polymerase chain reaction (PCR), if the suspect DNA is present *to virtually any degree* we'll find it. That's where all that Laboratory capital investment comes in useful, to avoid any risk of cross contamination.

eDNA is very specific too. As the Table opposite shows, we can (or in some cases will soon be able to) test for any organism as long as its genome is available. We have a genetic sequencer too, so we could even develop eDNA testing for species which currently have no detection sequence, making us leaders in this field.

A BRIEF DNA LESSON

Explaining DNA on an A4 page sounds daunting, but that's the sort of challenge we face in court routinely as expert witnesses. DNA or deoxy-ribo-nucleic acid is the helical spiral that contains all the genetic code that makes you who you are. Laid out, it would stretch five feet, but yours is crumpled up inside every one of your 70 trillion cells. DNA is made up of 'sugar' saccharide and phosphate ladder legs, with rungs made of just four amino acids; guanine (G), cytosine (C), thymine (T) and adenine (A). The ladder structure spirals into a helix to prevent water penetrating and dissolving the polysaccharide. To form these rungs, G binds to C and A binds to T. Separate them with enzyme, and the two separated legs (now called RNA) have the blueprint to make two new strands of DNA. For example a rung made up of the G-C bond separates to two half rungs of RNA, one ending in G and the other in C. To make a copy of the DNA, the original G molecule finds a new C molecule to attach to, while the original C molecule finds a new G molecule to attach to. The result is a carbon copy of the DNA that is used to make a new cell.

Note that GCN eDNA can be used up to 30th June for planning but can also be used any time if an approval allows yes/no monitoring.

That's exactly how we amplify the DNA in our PCR instrument, but your body does this billions of times a day. In our instrument it is monitored all the time, so it is called real-time PCR, or sometimes quantitative PCR. In time, we will not only tell you what species is present, but also how many, but right now, environmental testing just requires yes/no results.

Common Name	Species
Amphibians	
European Common Spadefoot Toad	Pelobates fuscus
Great Crested Newt (GCN)	Triturus cristatus
(Common) Smooth Newt	Lissotriton vulgaris
(Common EU) Edible Frog	Pelophylax kl. esculentus
Common Brown Frog	Rana temporaria
Moor Frog	Rana arvalis
Fish	
European weather loach	Misgurnus fossilis
Crucian Carp	Carassius carassius
Goldfish	Carassius auratus
Common Carp	Cyprinus carpio
Tench / Doctor fish	Tinca tinca
European perch	Perca fluviatilis
Insects	
Large white-faced darter	Leucorrhinia pectoralis
Crustaceans	
Tadpole Shrimp	Lepidurus apus
Mammals	
European Otter	Lutra lutra
Red Deer	Cervus elaphus
Polecat	Mustela putorius
Birds	
Common wood pigeon	Columba palumbus
Eurasion Coot	Fulica atra
Marsh warbler	Acrocephalus palustris
General Taxonomic Primers species nonspecific	
Amphibians	-
Amphibians / Pelobates	-
Amphibians / Triturus	-
Fish	-
Fish / Tinca	-
Fish / Perca	-

TABLE OF CURRENT e-DNA TARGET SPECIES

WHO ARE YOU?

Much of the DNA in an organism is common across the whole species range, It is said that humans share 98.4% of their DNA with Chimpanzees, and anecdotally around 60% with the banana. Here, we need to make a key distinction. In eDNA we are looking for species-specific bits of DNA that are common to all members of that species, and no other. This is very different to crime scene DNA work where we are looking for DNA common only to that one individual. Criminal DNA work looks for unique bits of DNA that has become corrupted in the copying process, to make that individual unique. These coding errors are called polymorphisms, so we look for single-nucleotide polymorphisms (called SNP's and often referred to as 'snips'). These are the bits of DNA that identify an individual in the crowd.

So, looking for criminals, we're searching for those unique *snips* whereas when looking for GCN we look for species DNA that is common to all GCN's. We could even look for DNA specific to the banana within human DNA, and seemingly someone noted above has done that, but we see that as a fruitless exercise.

Once we have amplified the DNA we have to use a probe to link with it and uniquely identify the species we are looking for. Computers search the genome for a sequence of DNA that is unique to that organism, in other words a unique piece of the code that tells the cell it belongs to a Great Crested Newt.

HOW DNA WORK IS COMBINING ECOLOGY AND FORENSICS

Now that eDNA has been widely accepted as a methodology, there are many openings for ecologists to use this technique in a much wider arena.

The 2013 horsemeat scandal is a classic example where forensic ecology gets press attention. Tests revealed that horse and pig commonly ended up in beefburgers, even in products intended for Jewish and Muslim customers for whom the pig is a forbidden animal on religious grounds. Veterinary products given to horses not intended for human consumption also entered the food chain.

The Guardian news paper graph shows the complex supply chain, presumably in an effort to disguise the source.

The scandal reportedly cost £1.2 billion and was probably one of the factors in loss of confidence in some of the biggest supermarket chains.

Just like detecting GCN in pond water, e-DNA can readily identify horsemeat in food products. Real-time PCR can tell you not only whether traces are present or not, but also how much is there. And if you followed the DNA explanation above, you'll appreciate that a combination of *species* DNA and *snip* DNA could not only tell you *what* is present, but *whether it is from the same herd*, and then with a bit of forensic ecology detective work, could go on to pin-down the abattoir responsible.

Using similar analytical techniques, tinned salmon is routinely tested for dolphin DNA. These are just a couple of examples from the many food contamination issues listed by Wikipedia, with case numbers escalating recently.

Using the same techniques for other species, forensic ecology might even identify when a species has been deliberately introduced to a location with the aim of delaying a planning application or rendering it uneconomic.

DNA analysis is not just confined to mammals and amphibians. The parasitic fungus *Batrachochytrium salamandrivorans*, was reputedly introduced from Asia into Holland via imported pets. It is fatal to GCN and the disease is now sweeping across Europe. eDNA can be used to map the progress of such diseases. In time, it seems likely that when we get a positive GCN result, we could test for this fungus too. Fortunately, when sampling for GCN, the meticulous care you take to avoid cross contamination will prevent you inadvertently spreading the virus from one pond to another.

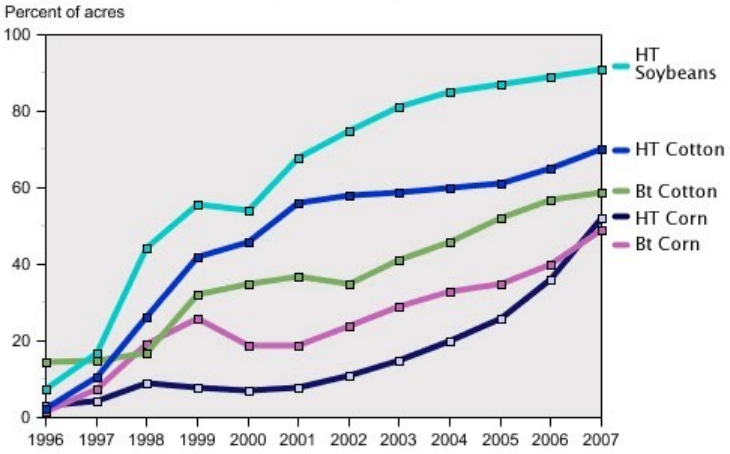
GENETICALLY MODIFIED ORGANISMS

Any discussion about forensic ecology must also include issues of genetic modification, (GM) used to improve crop disease resistance and yield. China is currently expanding GM understanding at whirlwind speed, after years of a GM moratorium. They now claim to lead the world in agricultural biotechnology. They have a justifiable incentive, as many remember Chairman Mao Zedong's "Great Leap Forward" of banning private farming in favour of vast collective farms in1958 which triggered a collapse in food production and distribution. As many as 45 million people died by starvation.

It would take more space to explain genetically modified organisms, or GMO's than we used to explain DNA, but GMO's are set to dominate the future, with strains already being grown that resists virus attack, bacterial decay, drought conditions, herbicide resistance, insect attack, faster maturity and altered nutrition levels.

Several microorganisms are being considered as candidates for future clean fuel producers and biodegraders. One day, GMO's could be used to produce recombinant vaccines. In fact, the concept of an oral vaccine expressed in fruits and vegetables for direct consumption by individuals is being examined as a possible solution to the spread of disease in underdeveloped countries. Work is currently underway to develop plant-derived vaccine candidates in potatoes and lettuce for hepatitis B virus (HBV), enterotoxigenic *Escherichia coli* and Norwalk virus.

Scientists are also looking into the production of other commercially valuable proteins in plants, such as spider silk protein and polymers that are used in surgery or tissue replacement. Genetically modified animals have even been used to grow transplant tissues and human transplant organs, a concept called xenotransplantation.



Graph showing the rise of GMO's grown in USA

The expansion of these organisms might provide ecologists with much to do in monitoring the effects of rolling out these GMO's on existing wildlife. Considering the high cost of developing particular GMO's there might even be a huge market for ecologists in controlling the theft of patented DNA material.

These eDNA projects are very powerful tools. One project we are looking at is the monitoring of coral species in reef structures affected by climate change. Instead of making numerous dives to monitor the death of one coral species and repopulation by others, a water sample will provide a snapshot of the health of the reef, with regular updates on the changes as they unfold.

WHAT OTHER SERVICES ARE ON OFFER?

We have a fully equipped forensic laboratory that serves many industries. We handle water quality, contamination, planning issues, and construction materials as well as component failures and analysis. We're used by many well known companies as their centre of excellence. Here are a few examples of how we can serve you better and impress your clients:

- *Microscopy, including sharp, high definition images of fecal matter, frass, debris, contamination etc up to x10,000*
- *Analysis of debris, particles, contaminants etc.*
- *'Source of origin' or 'who pollutes?' on unknown samples.*
- *Bat species eDNA for bat droppings including photomicrographs*
- *Crayfish, Water Vole, Badger, Otter eDNA; others in test phase*
- *Research partnerships using Scientifics as your lab resource*

Do you have a specific need to test a species? **For more info call or email tom.wood@surescreen.com.**
Check out our other bulletins, or browse our extensive range of information on www.surescreen.com

SureScreen Scientifics Ltd, Morley Retreat, Morley DE7 6DE.
Tel 01332 292003, Fax 01332 292230, email info@ssdlab.com