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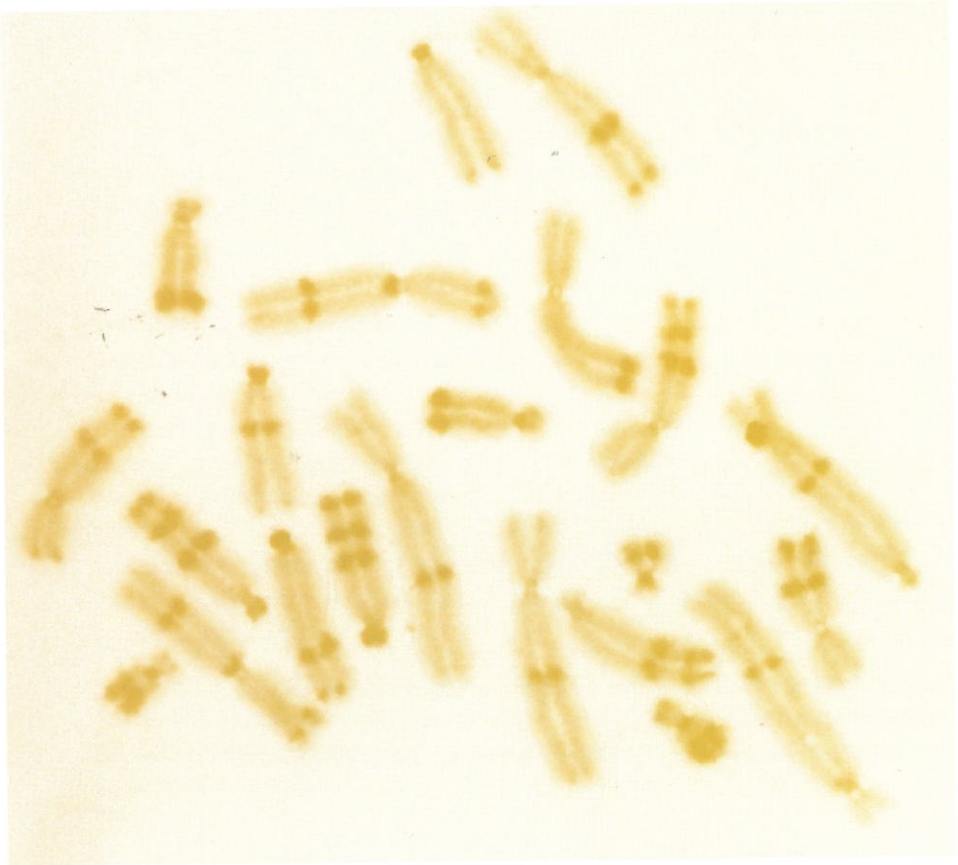

Variation in natural populations

cannabis · yeast · genes · chromosomes · landscapes

Contents . . .

The Research School of Biological Sciences is one of Australia's leading centres for basic biological research and graduate training. Since its inception in 1967, it has focussed on three domains: plant science, genetics, and neuroscience. This work is carried out in 11 research groups, organised as follows:

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Developmental Neurobiology ~ Professor Group leader: Professor Richard Mark

If you would like to know more about any of the research activities at RSBS, you are welcome to contact the principal researchers involved.
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Editorial . . .

– a few words about variation and natural popualtions

Welcome to the eleventh edition of *Biologic* – Variation in Natural Populations. Our theme encompasses two topical issues in biology today. The first is variation, an all encompassing term which has become a catch cry of 90's science. Confusion often arises in regard to variation because of the myriad of circumstances in which it becomes an issue. Where is 'variation' most applicable? It could be, for example, at the level of DNA sequences, or among suits of genes, between individuals or among populations. This edition of *Biologic* examines some of the diverse areas in which 'variation' is important biologically, with each article depicting variation at a different level. We start with 'Picking Cannabis' which examines a species with an extraordinarily high degree of individual variation, then, in 'Who benefits from natural variation in yeast?' and 'Jumping Genes', we cover two examples of variation within genes. In 'Better than Jurassic Park', we have a look at a species of grasshopper with variation in its chromosome morphology and finally, in 'Predicting the dynamics of a landscape', we see how landscapes are important generators of variation in vegetation.

The second component of our theme is 'natural populations'. It may seem redundant to refer to populations as 'natural', however, for our purpose, it is a useful way to define populations outside the laboratory. Many biologists are examining natural populations to enhance their understanding of phenomena discovered in the laboratory. Variation in natural populations facilitates an effective response to natural selection pressures. It is, therefore, important to increase our knowledge and understanding in this area. The article 'Jumping Genes', for example, examines small mobile bits of DNA called transposons in natural populations of vinegar flies. Transposons were originally discovered and researched in laboratory populations, where they are far more common. More recently they have become useful tools for gene transfer experiments in molecular biological research. Before their role in natural populations was examined, it was believed that transposons were simply selfish DNA of unknown evolutionary consequence. Research into natural populations now suggests that transposons may have evolutionary significance.

The topical nature of the theme as well as the diversity of situations where research into variation in natural populations applies is reflected by the number of Departments within the Research School of Biological Sciences which have contributed to this edition, including; Molecular Evolution and Systematics, Molecular and Population Genetics, Eukaryote Chromosome Organisation and Ecosystem Dynamics.

More information about the research presented in *Biologic*, as well as other research conducted at RSBS, may be obtained by visiting our site on the world wide web **http://biology.anu.edu.au/**, or by contacting the scientists.

I hope you enjoy this edition,

Sarah Vandermark

(Editor)

Biologic is published by the Research School of Biological Sciences, Institute of Advanced Studies, Australian National University. Written and edited by Sarah Vandermark. Artwork/design by James Whitehead and Tara Goodsell. Photographs by Jeff Wilson. Printed by National Capital Printing, Fyshwick, A.C.T.

Cannabis – you can: manufacture its fibre for fabric, rope and paper; extract its oils for use in paint, lubricants; take advantage of its therapeutic properties and eat its nourishing seeds but – despite the fact that it will grow just about anywhere – you just can't grow it legally.

Picking Cannabis

It seems ironic that in many circumstances, more is known about the biology and classification of rare plant species than widely cultivated plants. This is true for cannabis, as despite its long association with humans and broad utilisation, comparatively little is known about its biology. This lack of knowledge confounds attempts to manage its contemporary use.

Attempts to learn more about cannabis have been complicated by its great genetic diversity. In fact, cannabis is the most variable of all cultivated plants, one of the reasons why it has had such a successful association with people. It has experienced global travel, been exposed to inhospitable environments, escaped cultivation and become a weed and been altered by artificial selection. In addition to diversity in cultivated plants, wild type cannabis probably has an even greater degree of diversity.

As a result of its genetic diversity, conventional methods of taxonomy which rely upon morphological and or chemical features of plants, are not accurate when attempting to classify cannabis. As a result, considerable controversy exists in relation to its taxonomy, as well as in the more controversial legal and agronomic aspects of the cannabis debate.

A method for 'picking' cannabis and identifying the relatedness of individual plants, has recently been developed by Dr Vidya Jagadish and Prof Adrian Gibbs, at the Research School of Biological Sciences. Their innovative procedure combines advances in molecular biological techniques with designer computer software to produce the first accurate technique for reliably identifying different lines of cannabis. Their results will not only contribute to scientific debate but will have an enormous impact upon forensic science and the rejuvenation of cannabis as a commercial crop.

Cannabis' history

The legendary Chinese emperor Shen Nung, a patron of medicine and agriculture, was the first to introduce the cultivation of hemp in the 28 century BC. During his dynasty, cannabis was also prescribed as a medicine for malaria, beriberi, constipation, rheumatic pains, absent mindedness and other disorders.

It is estimated that for almost 3,000 years, cannabis hemp was the planet's largest agricultural crop, being cultivated to support industries producing a variety of products, including; the majority of global fibre, rope and paper, lighting oil, therapeutics, food oil and protein.

A law passed in the state of Virginia, USA, in 1619, ordered farmers to grow Indian hemp seed. Furthermore, farmers could be jailed for refusing to grow cannabis during times of shortage. More encouragement came from the use of cannabis hemp as legal tender from 1631 until the early 1800s.

Cannabis cultivation never achieved great commercial success in Australia, although it is interesting to note that the British government had plans for reforming Australian convicts by putting them to work tending hemp plantations!

In 1927, Australia agreed to prohibit cannabis, however, for a decade hemp was still grown; wild crops were tolerated and possession was not a crime. It was not until 1938, a year after the introduction of the American Marijuana Tax Act (outlawing cannabis), that real prohibition was introduced in Australia and cannabis was declared a noxious weed.

The present global sentiment for environmental awareness and sustainable development has renewed interest in cultivating cannabis. Cannabis plantations are currently on trial in Tasmania, with another trial being planned in South Australia and Victoria. Such trials are necessary in order to choose which varieties of cannabis will grow vigorously in an Australian environment and produce a high yield of fibre, with a low drug (tetrahydrocannabinol or THC) content.

It is easy to appreciate the difficulty of 'blindly' picking and standardising traits without knowing the genetic profile of stock plants. This handicap is further exacerbated by the outbreeding behaviour of cannabis and its natural genetic diversity. Dr Jagadish's research directly addresses this problem and removes uncertainty from the process.

Assessing Cannabis

Cannabis is native to central Asia, has male and female plants (dioecious) and is a woody, herbaceous, annual herb. It is an excellent renewable natural resource, which commonly grows to 6–8 metres and occasionally grows to a height of twelve metres in one season.

As mentioned earlier, comparatively little is known about cannabis, including genetic information such as the DNA sequence of any of its genes. This makes it difficult to select suitable varieties for plantations, especially as there is no consistent botanical difference between plants grown in distinct geographical locations.

The advent of molecular biology and ready access to organisms' genomes has revolutionised methods of detecting and assessing relatedness. The presence or absence of DNA fragments is one such method for assessing relatedness between samples. This technique, DNA profiling, may be used to test relatedness at any taxonomic level; individuals, varieties, populations or species.

For many years the most commonly used profiling technique was one called restriction fragment length polymorphism (RFLP). While RFLP is still used, it is time consuming, labour intensive and requires a relatively large sample of DNA.

More recently the polymerase chain reaction (PCR) has usurped other DNA profiling techniques, as it is quick, efficient, sensitive and requires only a few molecules of DNA (for more detail see *PCR–a Pretty Cool Reaction*, page 6). PCR was originally designed to amplify a specific region of DNA and required prior knowledge of the DNA sequence.

A myriad of variations based upon the original PCR technique have been developed. One variant amplifies sequences that have, at one end, a randomly chosen short sequence (with which the primer binds) and, at the other, the complement of that sequence. These recur throughout the genome and are targeted instead of targeting a precise DNA sequence. This technique, called random amplified polymorphic DNA analysis or RAPD analysis, produces characteristic patterns of DNA fragments for DNA profiling. Furthermore, these fragments can be generated without any prior knowledge of the DNA sequence.

From forensics to agriculture

Dr Jagadish uses RAPD analysis to study the genetic variety and relatedness of cannabis. Using this technique she produces DNA fragments from samples of cannabis DNA. The size patterns of these fragments are then assessed by separating them according to their size, by gel electrophoresis (see Figure 1). Dr Jagadish obtains reproducible fragment patterns from each sample of DNA, signifying that they are indeed reliable indicators of genetic variation. A computer analysis package, called RAPDistance, designed by Prof Gibbs, John Armstrong and other colleagues, uses various algorithms to make pairwise comparisons of each sample's DNA fragment patterns. The results are presented as a dendogram, which, like a family tree, illustrates the linkages or relatedness of samples (see Figure 2a).

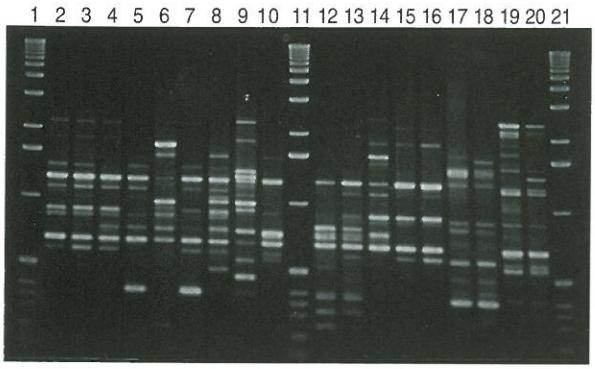


Figure 1. RAPD patterns obtained using random primers with canabis and hops DNA samples.

Tracks 1, 11 and 21 contain molecular weight ladders
- 2 to 10 contain QLD samples
- 12 to 13 contain NSW samples
- 14 to 16 contain Canberra samples
- 17 to 18 contain hops
- 19 to 20 contain PNG samples

While this new technique clearly shows the relatedness of varieties of cannabis, there is one other important factor to consider, in order to give the results meaning. Prof Gibbs notes, 'the production of dendograms is meaningless unless we can correlate the patterns of relatedness with a useful outside variable, such as the locations from whence the sample came.'

As might be expected, forensic scientists and drug intelligence bodies are interested in this method of 'picking' cannabis. In one New Zealand case, investigators resorted to hiring entomologists to conduct a painstaking and laborious investigation of microscopic inhabitants of imported cannabis, to determine its origin. The decision to embark on this project was made during discussions with Dr James Robertson of the Forensic Services Division of the Australian Federal Police Force (AFP). Now that Dr Jagadish's techniques have been shown to be simple and reliable, a complete technology transfer is planned between the RSBS and the AFP, as the latter wish to establish their own facilities for identifying cannabis (see *Links between forensic science and RSBS*, page 7).

Dr Jagadish was supplied with 53 samples of dried cannabis leaves or seeds by the AFP, to assess their relatedness. There were eight samples from Papua New Guinea, ten from the ACT, eight from a NSW crop, twenty-five from two different Queensland crops and two samples of hops, a close relative of cannabis. RAPD analysis with 4 primers produced 102 different fragments of DNA.

RAPDistance analysis found three distinct groups of cannabis, 1) Papua New Guinea, 2) the ACT and 3) NSW and Queensland (see Figure 2b).

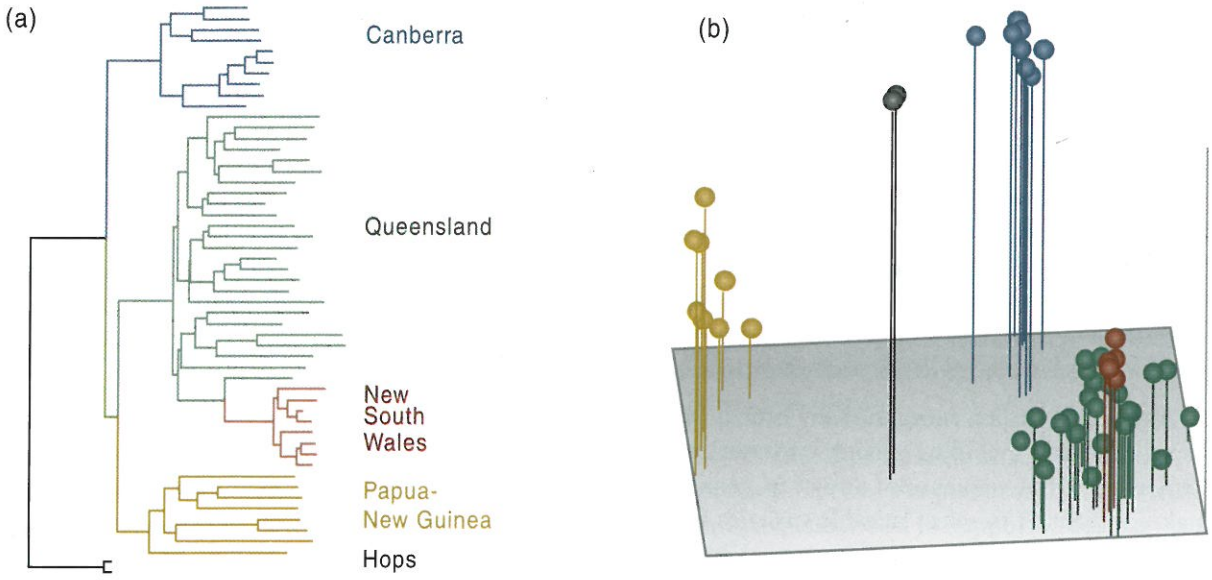


Figure 2. (a) dendrogram and (b) a 3D graphical illustration of the relationships and relatedness of the DNAs extracted from 51 samples of cannabis and 2 hops.

'This result demonstrated that cannabis from differing geographical regions contains genetic differences which are detected by RAPD analysis,' said Dr Jagadish. As the cannabis samples from the NSW and Queensland crops were not resolved, it suggests that these crops were grown from closely related seeds. This information could be useful in conjunction with other supportive evidence. 'It would be very easy to over interpret these results,' warns Prof Gibbs. 'They do not prove that the same person was responsible for cannabis crops in NSW and Queensland, or whether the seeds were derived from the same source. It just shows that the seed stock was closely related and less closely related to those from the ACT and PNG, which may be insightful when considered in collaboration with other evidence.'

Growing cannabis hemp is back on the agenda in at least two States. The Tasmanian Experimental Hemp Trial, is attempting to adapt 6 varieties of hemp, developed in the Netherlands, to the Australian environment. These varieties produce good quality fibre with little THC, however, growth has been poor in Tasmania. Samples from the 6 varieties were collected by Dr Jagadish for DNA profiling. Her results showed that the six varieties were very closely related. 'These samples only represent a small fraction of all the genetic variation which is available in this species,' said Dr Jagadish. 'Agronomic trials of hemp must be monitored by a discriminatory identification technique, such as RAPD analysis, to be of lasting value.'

As the amount of genetic variation in the Netherlands hemp varieties is so small, Dr Jagadish believes that it would be wiser and easier to test a number of different varieties of cannabis. 'The first step should aim to find a variety of cannabis which is suitable to the prevailing environmental conditions and then select for desired traits such as high fibre content and low THC. It makes no sense to limit the amount of genetic vigour available,' she said.

Colouring cannabis

Cannabis plantations may well reappear, however, it is unlikely that they will look and smell the same! Prof Gibbs concedes that it may be desirable to have an immediate way to distinguish cannabis hemp plantations from other similar looking THC-producing varieties. Fibre varieties with small amounts of THC could be given genetically engineered traits, such as an unusual colour or an offensive smell. This would reduce the incidence of theft and limit avenues for controversy.

Prof Gibbs and Dr Jagadish are now planning to develop a database of DNA profiles from a worldwide collection of varieties of cannabis using their RAPD technique. 'A database describing a worldwide collection of cannabis, would be of enormous value for forensic work, as has been demonstrated by the identification of the relatedness of particular samples. This information would also reveal whether particular lineages of cannabis were associated with particular traits, for example, THC production and fibre length, and is essential if humankind wishes to exploit the plant commercially.'

Prof Gibbs' and Dr Jagadish's research will help to keep the controversy surrounding the commercial exploitation of cannabis in control, while allowing the considerable commercial and environmental benefits of this once indispensable crop to be realised in the future.

If you want to know more ...

- RAPD analysis distinguishes *Cannabis sativa* samples from different sources. V. Jagadish, J. Robertson and A. Gibbs. Forensic Science International. (in press)
- 'A molecular methods for assessing the genetic relatedness of plants; A study of specimens of *Cannabis sativa*. L ' in *Forensic Applications of PCR*; Ellis Horwood Series in Forensic Science. (submitted)

Dr Vidyia Jagadish

- You are welcome to contact Dr Jagadish via the address and phone number listed inside the front cover.

PCR
– a Pretty Cool Reaction

Prof Kary Mullis invented PCR – the Polymerase Chain Reaction and made the pertinent point that, 'casual discussions of DNA molecules sometimes make them sound like easily obtained objects. The truth is that in practice it is difficult to get a well-defined molecule of natural DNA from any organism except extremely simple viruses.'

PCR is a process whereby unlimited numbers of copies of a particular gene or DNA sequence may be produced from a small sample of DNA or RNA, thus, potential applications of this technique are enormous. Apart from living sources, a DNA sample may be obtained from, among others, dried blood, mummified humans or ancient pollen.

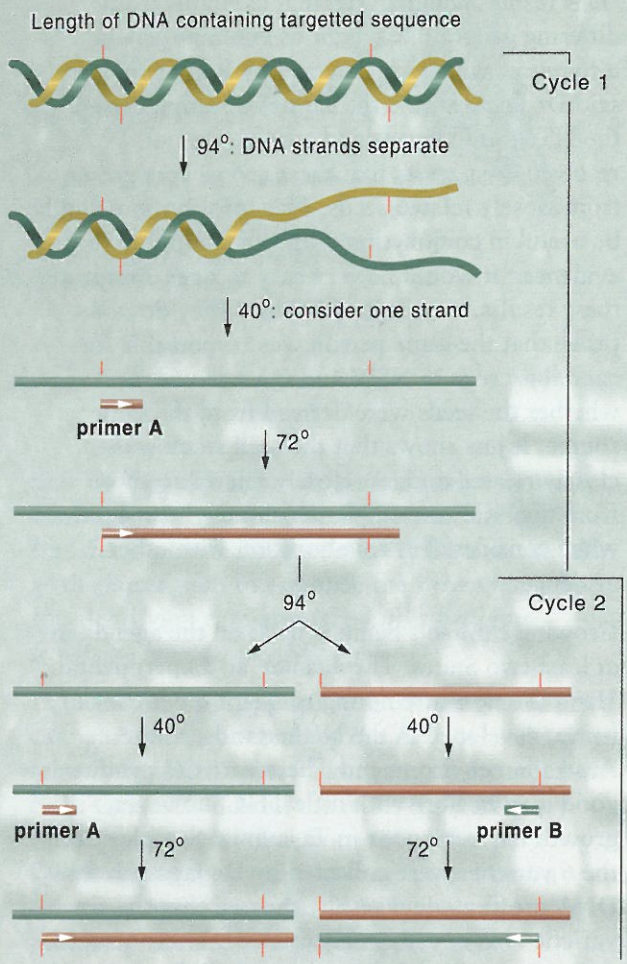
Perhaps the most startling feature of PCR is its simplicity, in both design and procedure. The reaction relies upon a naturally occurring DNA copying enzyme called DNA polymerase. Under normal conditions, this enzyme is located in the nucleus and is responsible for DNA repair.

Prof Mullis had the ingenious idea of employing DNA polymerase to 'repair' or duplicate selected regions of DNA. He realised that he could define a sequence of DNA or a gene to be copied with the use of short sequences of specific DNA called primers. A primer's sequence may, for example, be complementary to the initial sequence of a gene.

When a double stranded DNA sample is heated to 94°C, it dissociates into single strands. Lowering the temperature to 40°C, in the presence of primers, allows primers to bind (or anneal) to complementary regions of the single stranded DNA sample; the small primer strands do this much more quickly than the separated strands. The sample now has short, specific regions of double stranded DNA and this DNA duplex is recognised by DNA polymerase. DNA polymerase is unable to attach to single stranded DNA.

DNA polymerases of most organisms do not maintain their activity above 40°C. Fortunately, organisms that inhabit hot springs, such as the bacterium *Thermophil aquaticus*, have developed heat stable enzymes. The DNA polymerase from *Thermophil aquaticus*, commonly referred to as *Taq*, functions optimally at 72°C but can survive even higher temperatures.

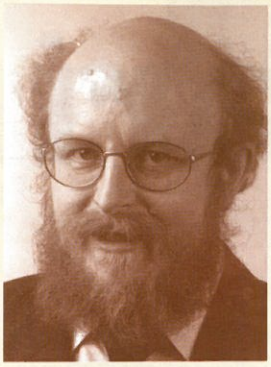
Put simply, one cycle of PCR involves: 1) Heating a sample of double stranded DNA to 94°C, to make single strands of DNA. 2) Lowering the temperature to 40°C to allow the selected primers to anneal to the long single stranded template DNA molecules. 3) Raising the temperature to 72°C, whereupon *Taq* attaches complementary 'copies' of the template DNAs to the primer (see Figure above). This entire cycle takes a few minutes and produces one copy of the targeted sequence, i.e. the DNA sequence between the two primers.



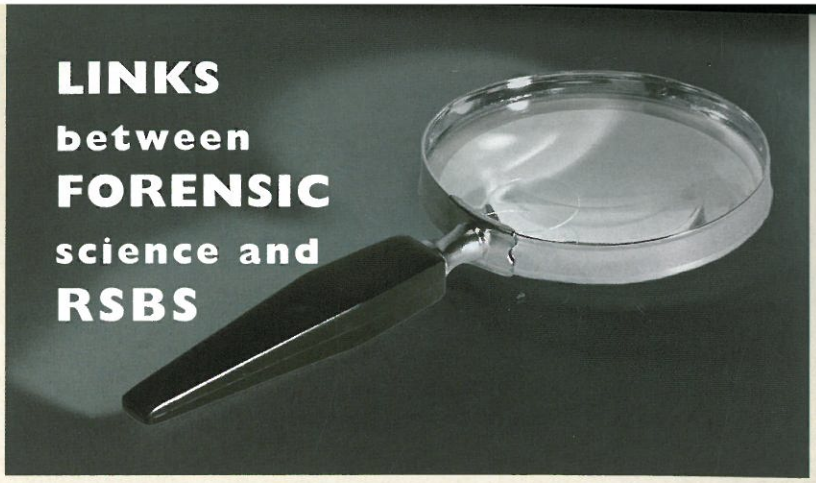
The sum and substance of PCR is that once a DNA sequence has been copied, the PCR cycle is then repeated, so that the copy may be copied. Copies of the targeted DNA increase exponentially by repeating the cycle. Furthermore, all of the reaction components may be contained within a single reaction tube, and the temperature changes provided by a commercially produced, programmable 'thermo-cycler', for as many cycles as required.

More recently PCR has been adapted for a myriad of applications, including RAPD (random amplified polymorphic DNA). The key to RAPD–PCR is that the primers used have an arbitrarily defined sequence, that is, they are not specific for a particular gene or gene sequence and bind to a number of sites in the sample. While the scientist may know the sequence of the primer, they will not know to which gene or repeated sequence the primer will bind.

Once RAPD analysis produces multiple, characteristic fragments of DNA, the fragments are separated according to their size by gel electrophoresis and the results visualised as a series of bands on a gel. This raw data may be used in a comparative analysis in order to determine the 'relatedness' of the various DNA specimens.



LINKS between FORENSIC science and RSBS



Professor Pierre Margot directs the Institute of Police Science and Criminology, at the Lausanne University in Switzerland. The Institute, part of the Law Faculty and located in the Chemistry building, is mainly involved in teaching and research but also participates in legal case work.

Despite the lack of any obvious links between forensic science and the Research School of Biological Sciences, Prof Margot is spending three months of his sabbatical at RSBS. Here, he is partaking in research into the identification of different lines of cannabis (see *Picking Cannabis* page 3). 'Forensic science is always concerned about the problem of identifying links in organised crime. Criminal intelligence is interested in the possibility of linking criminal activities through the geographical distribution of cannabis plants', explaines Prof Margot. In his opinion, knowledge of whether or not two cannabis crops are related, would be most useful during the investigative stages of a case (providing a look at links between cannabis crops and criminal activity), rather than being used later in a prosecution.

It was the multi-disciplinary nature of forensic science and the aspect of generalist rather than specialist, which initially attracted Prof Margot to this field. His transcontinental career began at Strathclyde University in Glasgo, Scotland, where he examined the poisonous and hallucinogenic properties of mushrooms and completed a Masters and a PhD. He then completed a post-doctoral fellowship at the Centre for Human Toxicology, at the University of Utah, in the United States, before returning to Switzerland's Federal Institute of Technology. Having declined an offer to head the Toxicology laboratories at Nestles, he chose instead to pursue forensics in Australia and head an Australian Federal Police Project investigating fingerprinting techniques, based in the Australian National University's Research School of Chemistry. In 1986, he returned to Switzerland as the Director of the Institute of Police Science and Criminology. His current research is a collaborative investigation into the use of forensic evidence in court, with the aim of improving its use.

Forensic science is a growing field with the first Australian School of Forensic Science having recently opened in the Chemistry Department of the University of Technology, Sydney. Prof Margot believes that a good forensic scientist needs to possess the following skills and interests. First and foremost, they must be curious! This healthy curiosity should be coupled with a good grounding in and understanding of the basic sciences; chemistry, physics, biology and maths and a mastery of analytical techniques. It is also very important to have an interest in legal proceedings and a sound knowledge of criminal law. Finally, a fair knowledge of criminology, which Prof Margot calls the 'sociology of deviance', is required. He wishes the best of luck to those wanting to pursue a career in forensic science!

Who benefits from natural variati

When one percent of a population undergoes a seemingly deleterious mutation each generation, it is practically guaranteed that a scientist will take notice. 'One percent of a population is an extremely high rate of mutation,' says Dr Desmond Clark-Walker, of the Research School of Biological Sciences.

Natural populations of baker's yeast, *Saccharomyces cerevisiae*, have been known to carry this high rate of mutation since the phenomenon was first described by a French scientist in 1953. Dr Ephrussi called the mutant yeast 'petite colonie', or small colony, after their diminished size (see Figure 1).

Of the 500 odd species of yeasts, only baker's yeast, brewer's yeasts and a few others possess an unusual capacity to repress their ability to respire and grow by fermentation. Although fermentative metabolism is less efficient, it facilitates the exploitation of a novel niche. Fermentation is of extreme importance and benefit to industries such as brewing and wine making. However, everything comes at a price, and Dr Clark-Walker considers that, 'the price for this ability is the unforeseen and detrimental tendency to generate petite mutants.'

Mitochondria are power stations

All eukaryotic (nucleated) cells, including yeasts, contain organelles (small organs) (see Figure 2). The organelle responsible for the production of energy via respiration is the mitochondrion. Most yeasts and all higher eukaryotes are totally dependant upon their mitochondria for survival.

Mitochondria have some unusual features, for example, they have their own small genome of mitochondrial DNA. Mitochondria cannot, however, function independently, since they require more than 500 proteins which are encoded in the nucleus. It is believed that mitochondria were originally symbiotic bacteria, which invaded primitive eukaryotic cells. As these early precursors of mitochondria were of great benefit to the host, over time they became incorporated as an organelle. During this time, most of their genes were lost or incorporated into the host's genome.

Mitochondria contain two separate membranes, with the inner one folded and stacked into convoluted piles (see Figure 2). The inner membranes house chemical machinery which drive the energy forming reactions of respiration, called the electron transport chain and oxidative phosphorylation. The mitochondria's energy production is dependant upon the maintenance of a positive charge on the outside of the membrane and a negative charge on the inside, called an electro-chemical gradient. If this gradient or membrane potential breaks down, mitochondria can no longer synthesise energy or import proteins and the cell dies.

Mitochondria and petite mutants

Dr Clark-Walker and his colleagues became interested in the mitochondrial DNA (mtDNA) of yeast, while investigating the high spontaneous mutation rate in baker's yeast. They found that baker's yeast mtDNA is very unstable, due to the presence of short repeated sequences spread throughout the mitochondrial genome. 'Over 100 repeats exist and were probably derived from a chance infective agent or something like a transposon, although this is pure speculation,' says Dr Clark-Walker (see *Jumping Genes*, page 11, for more on transposons). These repeat sequences predispose baker's yeast mtDNA to a high rate of mutation because they are sites where deletions can occur.

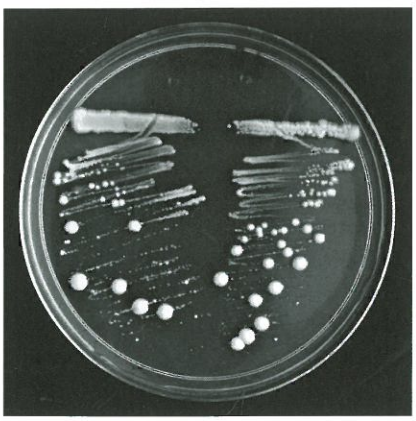


Figure 1. Fermentative colonies of brewer's yeast, highlighting the incidence of 'petite' colonies.

on
in yeast?

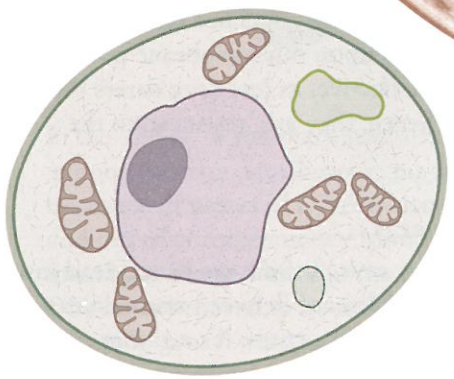


Figure 2. An artistic interpretation of a yeast, illustrating its organelles. The mitochondria are distinguished by their convoluted inner membrane.

The instability of baker's yeast mtDNA can be demonstrated by exposing single cells to a mtDNA mutagen, ethidium bromide. Upon treatment with this mutagen, 100% of these cells form petite colonies, due to deletions in their mtDNA.

A putative hypothesis to explain these observations could be, that baker's yeast has the ability to grow solely by fermentation, while other yeasts may be unable to grow in the absence of functional mitochondria because they do not have the ability to grow by fermentation.

Dr Clark-Walker and colleague Dr Xin-Jie Chen, decided to test this hypothesis by determining whether yeasts with stable mtDNA could grow by fermentation. In order to block respiration, a gene vital for this metabolic pathway was deleted from the genome of a normal yeast, *Kluyveromyces lactis*.

Their expectation, based upon their hypothesis, was that the deletion would be lethal. To their complete surprise, however, this was not the case. The mutant *K. lactis*, although it could no longer respire, was able to grow on fermentable medium.

Hot on the trail

Armed with the knowledge that inability to grow fermentatively could not explain the absence of petite colonies, Drs Clark-Walker and Chen prepared for some more intense detective work. They renewed their efforts to find the difference between normal and baker's yeasts. A procedure was developed to select mtDNA mutants, whereby *K. lactis* was treated with ethidium bromide at the highest non-lethal concentration. This procedure was designed to isolate mutants with deletions in mtDNA that could be recognised as fermentative colonies. From a total of 80 agar plates covered in colonies of yeast, four petite colony mutants were isolated.

To Dr Clark-Walker's surprise, a genetic analysis of these four mutants revealed that the primary change was not in the mitochondrial genome, but was located in one of three nuclear genes. 'At this stage we didn't realise the significance of what we were doing,' explains Dr Clark-Walker. 'By chance, we had obtained mutants and when we did the genetics, found they had changed nuclear genes that somehow allowed the mutants to lose and mutate their mitochondrial DNA.'

In other words, Drs Clark-Walker and Chen had found that there are at least three nuclear genes in normal yeasts, which upon mutation, allow the cell to behave as baker's yeast and form deletion mutations in their mtDNA, generating petite colonies. Dr Clark-Walker named these nuclear genes 'mitochondrial genome integrity' or MGI genes.

Subtle changes can cause a big difference

The title 'genes for mitochondrial genome integrity', suggested that the function of these nuclear genes was to actively protect mtDNA. Yet again, Dr Clark-Walker's expectations did not match his research findings!

The MGI genes were not mtDNA maintenance factors, rather they encoded some of the subunits of an enzyme situated in the inner mitochondrial membrane. This particular enzyme, ATP synthase, has a mix of nuclear and mtDNA encoded subunits (see Figure 3). It plays a very important role of harvesting energy in the form of ATP, while simultaneously maintaining the inner membrane potential.

Mutations in the MGI genes are fairly subtle in terms of the structural damage caused to the ATP synthase enzyme. It is this 'subtle' change, however, which translates into the breakdown of respiration and subsequent recovery of petite mutants. It also provides an explanation for why normal yeasts do not produce petite mutants.

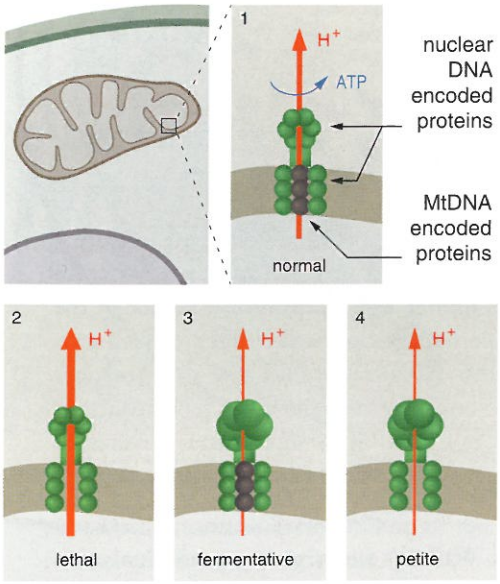


Figure 3.

1) The enzyme ATPsynthase is situated in the inner mitochondrial membrane and consists of two parts. The membrane bound subunits contain nuclear and mitochondrial encoded proteins, whereas the internal subunits contain only nuclear encoded proteins.

2) Normal yeast cannot sustain damage to their mtDNA because loss of the mtDNA encoded subunits would leave a 'hole' in the inner membrane, causing a breakdown in the membrane potential and consequent death of the cell.

3) Fermentative yeasts, with mutated nuclear MGI genes, are viable as although their ATPsynthase is 'leaky', their membrane potential is not totally lost.

4) Fermentative yeasts have a high rate of spontaneous mutation, due to their mtDNA being unstable. When the mtDNA encoced membrane subunits are lost a 'hole' is not created in the membrane. The resulting ATPsynthase, while not active, prevents a total breakdown of the membrane potential. Thus, the mutants survive, although their growth is retarded.

Dr Clark-Walker believes the ability to form petite colonies can be linked with maintenance of the inner mitochondrial membrane potential. In MGI mutants, structural changes to the ATP synthase allows petite mutants to survive because the inner membrane potential, while damaged, is not totally lost.

On the other hand, normal yeasts, with non-mutated MGI genes, cannot form petite mutants because the inner membrane potential collapses upon loss of mtDNA and the encoded ATP-synthase subunits. In other words, viable petites require changed nuclear DNA encoded subunits which allow loss of the mtDNA encoded subunits!

While Dr Clark-Walker has not demonstrated the details of this hypothesis beyond the fact that the MGI genes encode subunits of the mitochondrial inner membrane enzyme, support for this proposal can be found in other species.

There is a mutant variety of the bacterium, *Eschericha coli*, which is resistant to a particular antibiotic. Its method of drug resistance parallels the method proposed by Dr Clark-Walker to explain why petite mutants are resistant to ethidium bromide. The mutant bacteria carry a similar mutation in an ATP synthase subunit, as do the MGI mutants. So, the bacteria, like those yeasts able to produce petite mutants, experience a drop in membrane potential. Since uptake of the antibiotic depends upon this membrane potential, it is rendered ineffective, as less drug enters the bacteria and, hence, they are 'resistant' to the antibiotic.

And the winner is . . .

Dr Clark-Walker says, 'I think we have a lead into discovering the difference between normal yeasts and those able to form petites. It's a rather interesting story because the explanation comes from such an unexpected source. We would certainly never have predicted that the alteration of a mitochondrial enzyme, involved in maintaining the inner membrane potential, would permit baker's yeast to survive after losing mtDNA!'

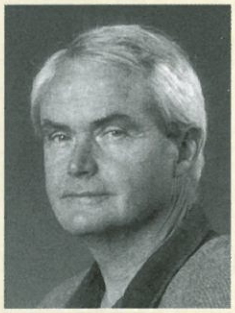
As for the advantage gained from the variation between natural populations of yeasts, there does not yet appear to be a definite answer. There is, however, evidence indicating that the ability to produce petite colonies has arisen on at least two separate occasions. This suggest that a metabolic trait is being selected.

It is tempting to believe that this characteristic is the ability to repress respiration in favour of fermentation and it would appear that the brewing industries are the big winners, since without fermentation, they would be out of business!

If you want to know more . . .

- Mutations in *MGI* genes convert *Kluyveromyces lactis* into a petite-positive yeast. X.J. Chen, and G.D. Clark-Walker, *Genetics*, 1993,**133**, 517-525.

- Specific mutations in the α and γ subunits of F_1-ATPase affect mitochondrial genome integrity in the petite-negative yeast *Kluyveromyces lactis*. X.J. Chen, and G.D. Clark-Walker, *EMBO J*, 1995, **14**, 3277-3286.



Dr Des Clark-Walker

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jumping genes

In the 1940s, a visionary scientist and Nobel prize winner, Barbara McClintock, predicted the existence of pieces of DNA which could jump in and out of chromosomes – 'jumping genes'. This must have seemed incredulous at the time, since DNA was believed to be stable and invariable. 'Jumping genes' were, in fact, isolated from the bacterium *Escheria coli* in the late 1960's and were further defined as specific, small fragments of DNA which were given the name transposons.

Scientific interest in transposons, increased during the 1970's, when it appeared that they assisted in the transfer of bacterial resistance to antibiotics. Furthermore, it soon became evident that they caused most of the spontaneous mutations occurring in laboratory populations of more sophisticated organisms, such as vinegar flies.

We now know that transposons are ubiquitous and may comprise up to 20% of an organism's genome. Prof John Gibson is interested in genetic variation in natural populations and has been investigating transposons in natural populations of the vinegar fly, *Drosophila melanogaster*, at the Research School of Biological Sciences. He has asked the question; Do transposons produce diversity in natural populations? In order to answer this important question, he has monitored genetic variation, by measuring the activity of certain enzymes. When he finds a significant difference in enzyme activity, within a natural population of flies, he searches for transposon DNA within the gene. Prof Gibson has found that transposons do generate genetic variation in natural populations and interestingly, their impact is rarely positive!

What are transposons?

Transposons may sound like something you would buy from a toy shop, however, the most striking feature of transposable elements (TEs) is their mobility. In fact, some have been given esoteric names which are indicative of their mobile nature, for example, the HMS Beagle (Darwin's boat), Stalker (a cartoon character from The Soviet Union), mariner, hobo, Tyrant (a Castilian Knight) first identified by a Spanish geneticist and roo (found in Australia).

Thirty different families of transposons have been isolated from the vinegar fly, often misnamed the fruit fly. They range in size, from 1 to 10 kilo bases of DNA and encode so called 'DNA sites' and enzymes required for their own transposition and maintenance. Many transposons have a unique DNA site (a short, specific sequence of DNA), which acts as a forwarding address, directing the transposon to a complementary DNA site in its host genome. There are usually multiple copies of any given DNA site in the host genome and exactly which site a transposon will attach to is completely random.

The enzymes encoded by transposons provide the physical mechanism for jumping into a host's DNA. Two methods of jumping are known to exist and their characteristic differences have been utilised to classify transposons into two groups.

Transposons in the first group, Class I, appear to jump with an RNA 'parachute', in other words, they change from their initial DNA status into RNA. It is then necessary for this RNA intermediate to change back to DNA. Class I transposons have an enzyme called 'reverse transcriptase' which converts RNA into DNA. After the reverse transcriptase acts on the Class I transposons' RNA they incorporate into the host's DNA.

It could be said that Class II transposons 'free-fall' into their host's genome, as they do not have an RNA intermediate. To accomplish this, they use an enzyme called 'transposase' to incorporate their DNA into their host.

It would appear that transposons are the ultimate example of 'selfish DNA'. After all they are purely parasitic – jumping between different parts of a genome in order to propagate themselves and this is usually to the detriment of their host.

Damage control!

By virtue of their mobility, transposons have a considerable capacity to cause havoc in their host's genome! The arrival of a transposon can have a range of effects including; a mild alteration in gene expression, gene deletion, catalysing a major chromosome rearrangement and in some cases, their action can be lethal to the host or have no effect at all. These various effects are determined by the particular characteristics of the transposon, as well as its site of integration, that is, whether it is within a gene or the gene's regulatory regions. Most commonly, transposons have a negative effect upon their host by inhibiting normal gene action and reducing the normal quantity of a given gene product.

Transposons can sometimes become deleterious to a host when they attempt to leave. They not only display a random pattern of site selection, they also leave random patterns of left-over DNA in the host's genome, when they depart. An 'excision event' may be precise – leaving the host's DNA as it was found, or it may leave certain pieces of DNA behind – catalysing the deletion of genes, chromosomal rearrangements or the translocation of genes within the host's genome. The transposon may even take some of the host's DNA with it to the next insertion site.

To complicate matters further, incomplete transposons, which cannot move by themselves, may be reactivated by an 'active' transposon located elsewhere in the host's genome. For example, an active transposon can share its 'transposase' with a locally situated incomplete transposon, restoring its mobility, including the ability to leave the host's genome!

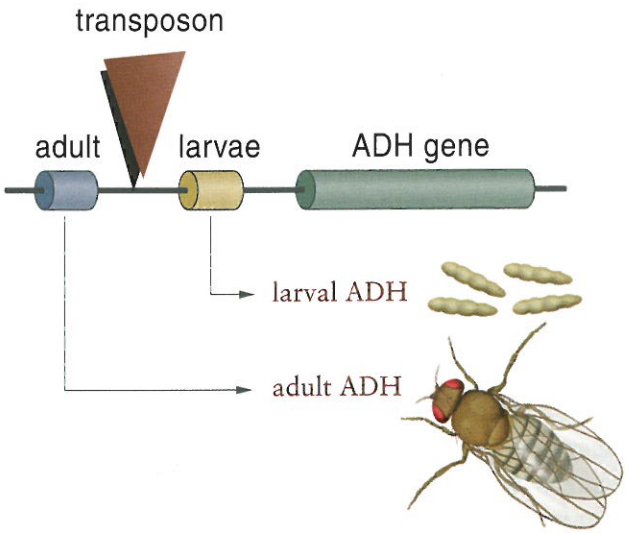

Figure 1. A simplified illustration of the vinegar fly's ADH gene, showing the insertion site of the P-element transposon, between the adult and larval promoters.

Not surprisingly, the rate at which transposons jump into a genome is very low. The higher the frequency of insertion the higher the probability of a lethal insertion. In the vinegar fly, under normal conditions, an average of 10^{-4} insertions occur per generation. This does not mean that the entire genome has the same affinity for transposons, rather some genes appear to be particularly attractive to some transposons.

Transposons in natural populations

Prof Gibson and his post-graduate student Yan-Hong Wu, chose to study the enzyme alcohol dehydrogenase (ADH) as part of their investigation into variation in natural populations. 'We had collected samples of flies from wineries in South Australia and Tasmania and from orchards in Queensland. When we assayed these samples for ADH activity, we found that some adult flies that had extremely low enzyme activity,' said Prof Gibson.

Previously, transposon-induced changes had only been identified in laboratory strains of vinegar flies. To link transposons to their sample of flies with low ADH activity, Prof Gibson and his colleagues had to find evidence for the presence of a transposon in the affected ADH gene.

The regulation of gene expression in cells with a nucleus (eukaryotes), involves various specialised regulatory sequences of DNA in and around the gene. One important region is called the 'promoter', the point where gene expression is initiated.

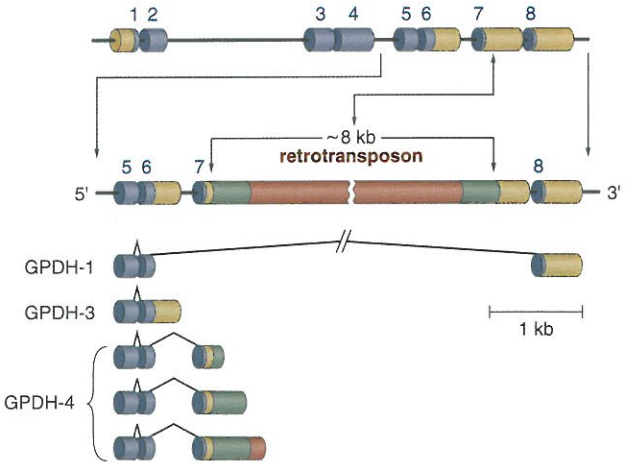

Figure 2. Top: an illustration of the normal GPDH gene, with the insertion of the retrotransposon into exon 7 shown directly below. The normal GPDH transcripts are shown underneath ; GPDH1 contains exons 1-6 and 8 and GPDH3 contains exons 1-6. There are three transcripts which give rise to the new protein, GPDH4, and they contain exons 1-6 with differing amounts of exon 7.

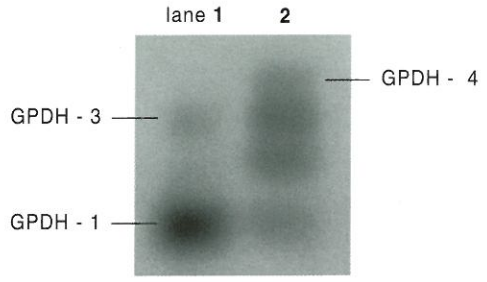

Figure 3. An electrophoretic separation of vinegar fly proteins stained for GPDH. Lane 1 contains protein from a normal fly and shows GPDH1 and GPDH3. Lane 2 contains protein extracted from a fly containing the retrotransposon and shows the presence of the new protein GPDH4.

The ADH gene is a little unusual as it contains two promoters; one used in the larval stages and the other in mature flies. Prof Gibson and Ms Wu found that larvae had normal levels of ADH activity until they reached maturity. A low level of ADH activity correlated with the switch over to the adult promoter. This clue directed Ms Wu to examine the promoter region of the ADH gene.

Sure enough, Ms Wu found a transposon, in the ADH gene, situated between the larvae and adult promoters (see Figure 1). They hypothesised that the presence of the transposon, a particular type called a P-element, was inhibiting the quantity of ADH being synthesised in adult flies.

In order to prove their hypothesis, they removed the transposon to see whether normal ADH activity was restored in adults. 'There are methods to make some transposons jump out of a gene,' says Prof Gibson. 'Upon removal of the P-element transposon, we found an increase in ADH activity and adult levels returned to normal.'

'This was a very exciting finding, as it was an example of transposons affecting phenotypic variation in natural populations,' says Prof Gibson.

A good jump

It is possible that incorporation of a transposon could be beneficial to a host. The first example of such a beneficial effect induced by a transposon was found by Prof Gibson and colleague Dr Tom Wilanowski. They studied the enzyme GPDH (glycerol-phosphate dehydrogenase), which is involved in the generation of energy required for flight. The *Gpdh* gene has eight protein coding regions (exons), interspersed with seven non-coding regions (introns). When they looked at the molecular landscape of an unusual *Gpdh* gene in a natural population of vinegar flies, they found an enormous 8 kilo base insertion, towards the end of the gene. 'The entire gene is only supposed to be 1.8 kilo bases in total,' exclaimed Dr Wilanowski (see Figure 2). He noted that they had found a form of GPDH which had never been seen before.

'In essence, this insertion changed the expression of the gene, which led to the production of a novel form of the enzyme of possible benefit to the host,' said Dr Wilanowski. By its chance insertion into a regulatory domain of the GPDH gene, the retrotransposon modified the production of the enzyme without modifying its function. It did this by causing three new transcripts of *Gpdh* to be produced, each containing exon 7 (see Figure 3). 'This is the first example of the formation of a novel protein by the insertion of a transposable element,' said Dr Wilanowski.

Perhaps what's more important than the finding itself, are the possible evolutionary ramifications of this discovery. 'This novel method of making a new protein highlights the potential significance of transposons in natural populations. It may be that in the past, some transposon-induced mutations have proven advantageous and survived in other natural populations. Indeed, there is evidence in some human gene sequences of vestiges of old transposons' said Prof Gibson.

Jumping into the future

Some transposons, such as P-elements, can be manipulated in laboratory experiments to introduce a modified gene into a vinegar fly. Other species, such as the fruit fly, which is of more economic significance than the vinegar fly, contain transposons and scientists are trying to learn how to manipulate them for the purpose of biological control.

Transposable elements have thus been transformed from a biological oddity to a valuable tool that can be used for genetic engineering of higher organisms.

If you want to know more ...

- Retrotransposon insertion induces an isozyme of sn-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster*. T.M. Wilanowski, J.B. Gibson and J.E. Symonds. *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 12065-12069.
- Eukaryotic transpoable elements and genome evolution. D.J. Finnegan. *Trends in Genetics*, 1989, **5**, 103-107.

Prof John Gibson

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Better than Jurassic park

'Better than Jurassic Park!' is how Dr Dave Shaw describes the possible implications of his research. Dr Shaw is a geneticist at the Research School of Biological Science and studies grasshoppers – which are hardly comparable with the awesome grandeur of dinosaurs. However, it is the grand genetic feat outlined in Jurassic Park, of recreating, or cloning the dinosaurs which Dr Shaw is challenging. In terms of genetic manipulation, Dr Shaw believes we can go one better than Jurassic Park. To him, the dream of using genetic technology to recreate something from the past, is not nearly so interesting or powerful as creating something completely novel – a new species. Furthermore, he advocates that modern genetics may well determine the next major episode of evolutionary change.

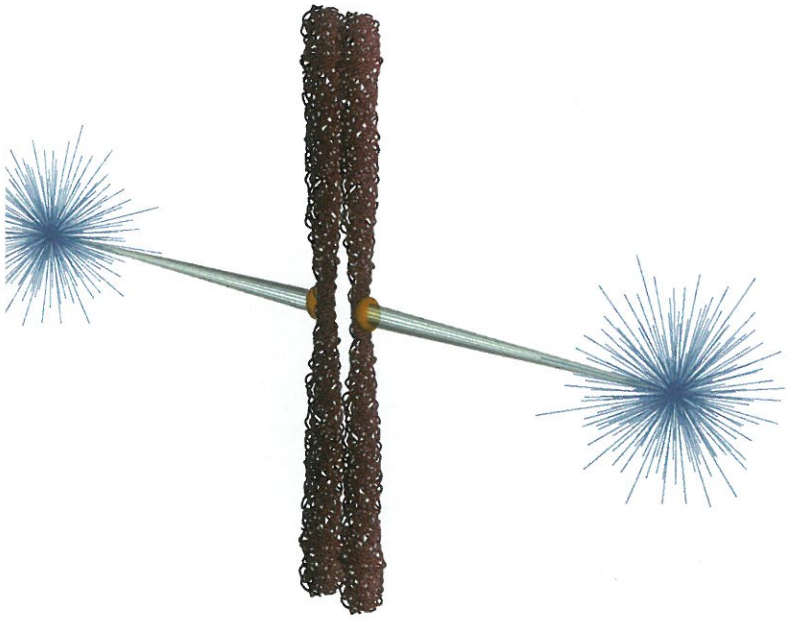

Figure 1. Centromeres provide attachment for metaphase chromosomes to their respective bunch of spindles (microtubules) during cell division.

Surprisingly, it is not a gene or gene sequence that is the key to Dr Shaw's claim but a small region of the chromosome called the centromere (see Figure 1). This structural component is comprised of a complex of DNA and associated proteins, often flanked by vast stretches of apparently functionless or 'junk' DNA. The centromere's function is to attach each chromosome to spindle fibres (microtubules) during cell division and facilitate their separation during cell division (mitosis and meiosis).

As centromeres are crucial for cell division, it would seem likely that they would be highly conserved between species and across kingdoms. Microtubules from yeasts and humans are highly conserved and one might, therefore, assume that their centromeres would show a similar degree of conservation. Due to genetisists' preoccupation with the gene, little is known about centromeres. Initial investigations by Dr Shaw and his colleagues have, however, revealed that centromeres posses some unexpected properties. . .

The evolution of centromeres

'Genetic diversity' is a frequently encountered phrase of 1990's science and is also the focus of attention in political and economic arenas. In scientific terms, genetic diversity refers to the degree of heritable variation within or among species. Genetically speaking, organisms as we now know them have not always been so diverse (see Figure 2). The expansion of genetic diversity was a significant evolutionary event, occurring approximately 1.5 billion years ago, when the potential for exploiting ecological niches was suddenly advanced. It is surprising that this evolution in diversity was catalysed by the acquisition of two types of highly specialised DNA sequences; centromeres and telomeres.

Eukaryotes, are cells with a nucleus and evolved from prokaryotes, cells which do not contain a nucleus. Initially, they were both simple, single-celled organisms (see Figure 2). Prokaryotes have remained basically the same, whereas, eukaryotes have evolved into a myriad of sexually dimorphic, complex and diverse organisms, comprising the plant and animal kingdoms.

Centromeres and telomeres facilitated the organisation of eukaryote genomes into a set of stable, linearised chromosomes, which could replicate and divide precisely during mitosis and meiosis and through evolutionary time. This favoured an increase in genetic diversity due to the potential for exchanging genetic information between paired chromosomes during meiosis (recombination) and sexual reproduction.

Telomeric DNA has been isolated from a wide range of eukaryotes. The isolation of centromeric DNA, however, has eluded scientists for a number of reasons. In some organisms, including humans, it is enclosed by vast stretches of highly repeated DNA sequences (satellite DNA), it is not transcribed or translated into a detectable product, there is no functional assay to test putative sequences for centromeric activity and the physical structure of centromeric DNA differs from the rest of the chromosome. Now that the evolutionary significance of the centromere has been recognised, scientists such as Dr Shaw, aim to rectify this situation.

What's so big about grasshoppers?

There are a number of factors contributing to Dr Shaw's decision to study grasshoppers. Firstly, he says, 'the good thing about grasshoppers is that their chromosomes are absolutely huge.' Secondly, the species he chose to study, *Caledia captiva*, is distinguished by variation in centromere position which correlates with the geographical location in which it is found (see Figure 3).

Joseph Banks was the first to collect *Caledia captiva* on the Cook expedition of 1770. It is distributed along the entire northern and eastern seaboards of Australia and into Papua New Guinea. This morphologically identical species is comprised of three different 'taxa'. These taxa are distinguished by individual differences to their chromosomal organisation (karyotype). The three taxa are called: Torresian, Daintree, and Moreton (see Figure 3 for taxa location).

Dr Shaw's research has focused upon the two most broadly distributed taxa, the Torresian and Moreton. He found that these two display dramatically different patterns of genome organisation (see Figure 3). The centromeres of

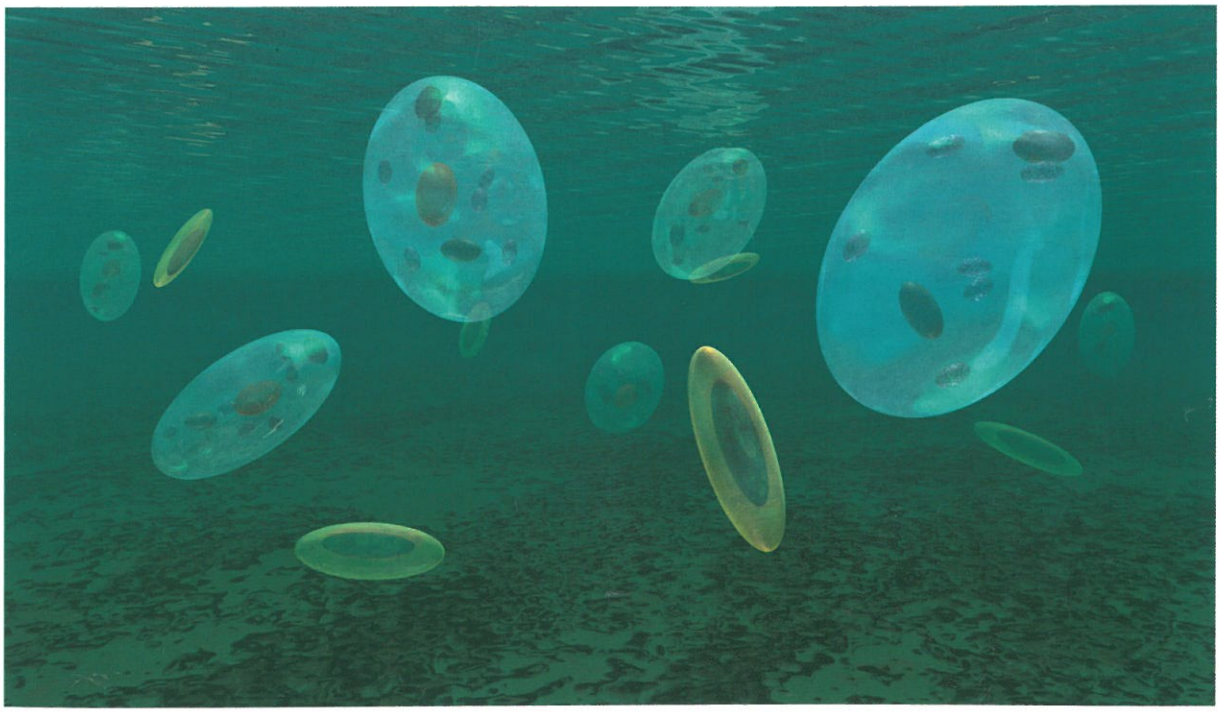

Figure 2. An artistic impression of the 'primordial soup', 1.5 billion years ago, illustrating primitive prokaryotic and eukaryotic cells.

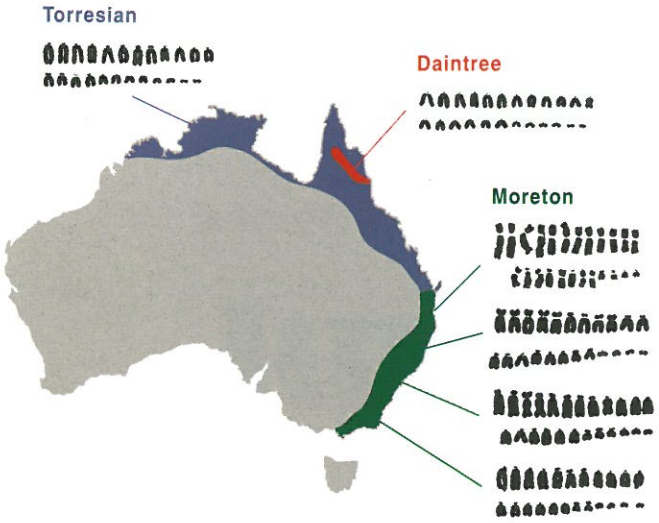

Figure 3. Geographical distribution of *Caledia captiva* showing the location of the three taxa; Daintree–red, Torresian–blue, Moreton–green and their karyotypes. The Torresian and Daintree taxa have telocentric centromeres. The position of the Moreton's centromere correlates with latitude, with northern populations having metacentric centromeres and southern populations having telocentric centromeres.

Torresian grasshoppers are always located at the ends of their chromosomes. In contrast, centromeres from Moreton grasshoppers occupied distinctive positions along each chromosome, depending upon the latitude at which they were collected. Grasshoppers from the northern limit of the Moreton taxa had centromeres located in the middle of their chromosomes. At the southern limit the Moreton grasshoppers (like the Torresians) have their centromeres located at the ends of their chromosomes. In the regions between these geographical limits, the position of the Moreton centromeres were found to exist in a gradient between the middle and end of their chromosomes.

Dr Shaw was perplexed as to the evolutionary significance of this unique pattern of chromosomal change. 'This type of concerted change, involving the entire genome, cannot be explained by either chance events or prevailing genetic theory and seems to indicate an adaptive role for chromosome organisation,' says Dr Shaw.

Analysing genomic variation in natural populations

A wide range of genetic markers are commonly used when investigating the evolution of chromosomal morphology. 'C-banding' is a process whereby highly repeated sequences of DNA are visualised as distinctive chromosomal bands (see Figure 4). 'Mitochondrial DNA restriction enzyme fragment length polymorphism' (rflp), involves taking mitochondrial DNA (mtDNA) extracted from different taxa and digesting it with a specific set of restriction enzymes (enzymes that chop up DNA at specific locations). The resulting fragments of DNA are compared, across taxa, to illustrate change. Similar tests can be used to examine the variation in ribosomal DNA and in soluble enzymes. Collectively, these data can be used to construct evolutionary relationships between taxa.

PhD student, Adam Marchant, conducted a study of mitochondrial DNA rflp variation among Torresian and Moreton grasshoppers and compared it with their centromere's position. His results conflicted with the genomic organisations of the two taxa. The Torresians, with conservatively located centromeres, had a considerable amount of mtDNA variation, whereas within the Moreton taxa, with changing centromere positions, there was little mtDNA variation.

His results complemented previous research investigating enzymatic differences and C-banding between the two taxa. These results clearly showed that while Moreton populations had dramatic changes in their centromere position according to their geographic location, they retained the same profile of genetic markers.

The interpretation of these results has generated an exciting, novel hypothesis that challenges concepts concerning the evolutionary significance of chromosomal rearrangements in natural populations. The rapid evolution of the Moreton's genome and its correlation with latitude and local environment, suggests that selection is acting directly upon genome structure. This suggests that chromosome structure *per se* might possess important evolutionary properties quite independent of the information content of its genes.

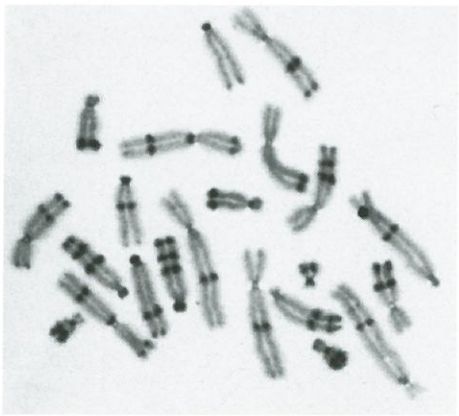

Figure 4. *Caledia captiva* chromosomes with characteristic chromosomal bands or c-bands.

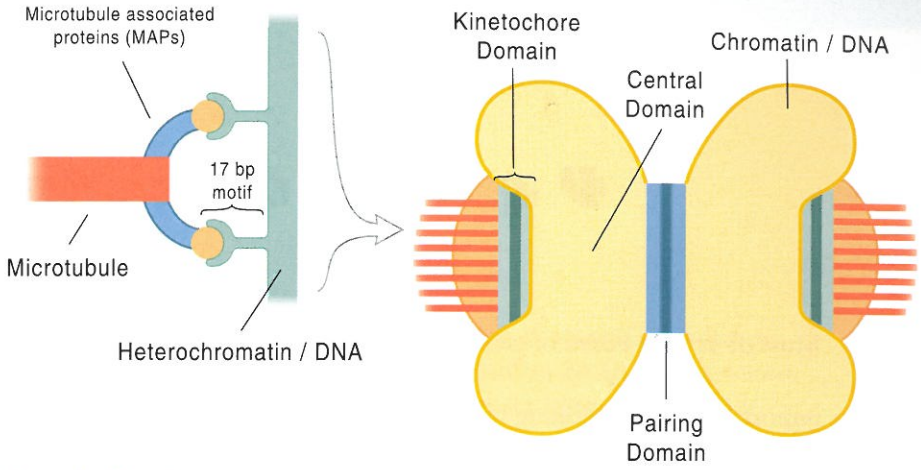

Figure 5. The hypothesised structure of a human centromere showing three domains; the pairing domain – where two centromeres join holding a chromosome together, the central domain – comprised of DNA or chromatin, the kinetochore – the site where a centromere binds to spindle fibres (microtubules). Dr Shaw is particularly interested in the DNA sequences in the kinetochore and associated proteins (MAPS).

DNA structure – can it change the individual?

To find evidence in support of the hypothesis that chromosomal change represents an adaptive response, Dr Shaw looked for any relationship between Moreton karyotype change and phenotypic variation (physical changes to the individual). 'We wanted to know if there was any selection pressure generated by the prevailing environmental conditions which would favour a different genomic structure,' says Dr Shaw.

The major environmental factor covering *Caledia captiva's* extensive habitat, is a gradual increase in 'seasonality' when moving south. As a consequence, the amount of time available for successful reproduction decreases until, at its southern limit, there is barely enough time to complete the grasshopper's life cycle.

Torresian grasshoppers, which have distal centromeres, reproduce once a year in spring. All northern coastal populations of Moreton grasshoppers, which have central centromeres, reproduce twice each year – once in spring and again in winter. In Moreton populations sampled further south, however, the winter generation progressively wanes. At its southern most limit, where the distally located centromeres appeared, the winter generation completely disappears.

Dr Shaw and postdoctoral fellow, Dr Fran Groeters, also timed embryo development (embryogenesis) of grasshoppers collected from different locations. They found development was slower when centromeres occupied a central location in the chromosome, whereas, distally positioned centromeres always coincided with faster development. Northern Moreton populations took 10% longer to develop than populations at its southern limit.

With good evidence of chromosome structure influencing phenotype, Dr Shaw says, 'it appears that the changes in centromere position may lead to adaptive changes in cellular parameters, which modulate growth and development. These structural changes may allow the grasshopper to adapt to its immediate environment.'

A new evolutionary paradigm – biophysical evolution

The new paradigm 'biophysical evolution', encompasses the idea that chromosomal structure possesses important evolutionary functions. 'We are only just beginning to appreciate the precision and complexity of events within the nucleus and the dynamics of cell division,' says Dr Shaw. While this field is still highly contentious, research into the genetics of *Caledia captiva* have cemented Dr Shaw's beliefs in the existence of biophysical evolution.

Dr Shaw's research clearly indicates that centromeres are far more complex than originally believed. Despite the conserved function of centromeres in cell division in all eukaryotes, their molecular structure has only been isolated from baker's yeast, *Saccharomyces cerevisiae*. Surprisingly, these centromeres do not function in other yeasts and their DNA sequence is not present in the chromosomes of other organisms. Thus, it now appears that variation within centromeres themselves is of a perplexingly high order.

Paradoxically, instead of being highly conserved (as suggested at the start of this article), Dr Shaw says, 'centromeres can be expected to show a high degree of variation to the point of being species specific. Furthermore, if it transpires that centromeres are rapidly evolving regions of the chromosome, then they may play an important role in speciation.'

continued on page 21

Predicting the dyna

Most of us have heard of genetic diversity and the importance of maintaining variation and diversity in all organisms. This issue is complicated by the different levels to which the amount of diversity or variation is measured. Reductionists will refer to the four nucleotides – their order and combination – which generate diversity within individual genes. Beyond the single gene is another level of genetic diversity, the extraordinarily complex suites of genes and gene combinations which have been chosen by natural selection and are represented in individual organisms. Then there is a more holistic level, 'biodiversity' and most of us are aware that natural populations, such as those living in tropical rainforests, are a rich source of biodiversity.

The issue of diversity and variation in landscapes is not so well known. Landscape diversity describes the patterns of change in vegetation from place to place and through time. Dr Ian Noble has had a long term interest in changing vegetation, beginning with research he carried out toward his PhD degree, which explored the changes in a paddock being used by sheep. He now believes that landscapes are another level of variation which have previously rarely been given enough attention.

The ability to model and predict landscape dynamics is being developed by Dr Noble at the Research School of Biological Science (RSBS). 'Knowledge of landscape variation could be applied to current issues in land management such as fire, grazing and flood-water management. In the context of global climate change, such knowledge could help to determine how changes in community dynamics will translate into changes in landscapes and even wider regions,' explains Dr Noble.

As with genetic diversity, there are various levels of complexity present within 'landscape variation'. Dr Noble has addressed this problem by developing a suite of models of varying complexity. 'It is more economical to have a number of models, each one specifically designed to assess a particular level of variation, than to try and develop one all-encompassing model,' he said.

Figure 1. The different patterns generated in these three simulations illustrate Dr Noble's hypothesis, that landscapes generate variation. Each colour represents the two identical species and maps their pattern of establishment across each grid, from left to right.

mics of a landscape

Back to basics

'Landscapes generate their own variability,' says Dr Noble. This comment may seem strange, however, it is easily understood by examining his simplest model of landscape variation.

Dr Noble has designed computer software for determining the dynamics of vegetation within a particular landscape. In his simplest model, he allows two identical species to invade a uniform landscape. Imagine it as a large flat plane recently cleared by a fire or mud flow and a strip of surviving vegetation along one edge.

Under such uniform conditions, it may seem unlikely that the landscape would generate any variation between simulations. As seen in Figure 1, however, not only do the two species form complex patterns of colonisation, these patterns are not repeatable. Out of 3 simulations, shown in Figure 1, each example has a unique distribution pattern generated by the two identical progenitor species. Thus, as Dr Noble states, 'even on this most basic level, landscapes can generate variation.'

Introducing a disturbance

Prior to Dr Noble's research, most attempts to examine variation in landscapes only described a static state (i.e., the patterns of vegetation at a given time) rather than the dynamics of a system. When designing his models, Dr Noble believes that it is important to attain a balance between simplifying the diversity of a natural landscape, while retaining information about the important interactions and processes occurring within it.

This more complex model, called the Vital Attributes Simulating Landscapes or VASL, was designed in collaboration with Dr Sandra Lavorel and Dr Ralph Slayter. It aims to predict the dynamics of communities of vegetation which are regularly perturbed. In the example shown in Figure 2, Dr Noble modelled the vegetation of south-west Tasmania, which is subjected to regular disturbance from fires.

The first step in the modelling process is to reduce the large number of species present in a landscape into groups of species with similar behaviour; i.e., similar responses to fire, growth rate and so on. The model then predicts all the various ways these 'functional groups' interact with each other and with fires to form different vegetation types.

In the Tasmanian ecosystem, the interaction between functional groups can be represented by a simple successional replacement sequence, as shown in Figure 3. The model actually predicts a more complex replacement sequence than the one shown in Figure 3 and reality is even more complex, however, this simplification is sufficient for exploring landscape changes.

The model predicts that button grass will eventually be invaded by shrubby species, for example acacias, then by eucalypts and finally by

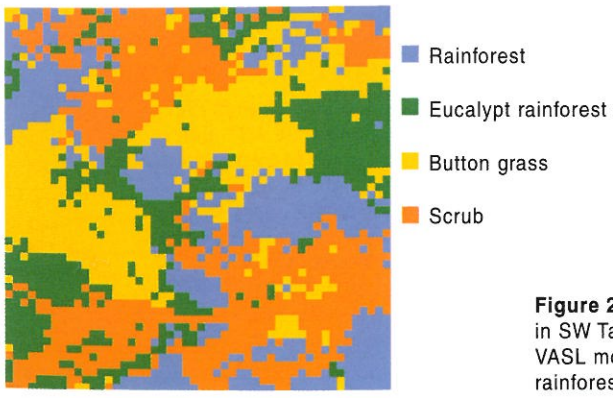

Figure 2. A simulation of the succession of vegetation in SW Tasmania, after disruption by fire, using the VASL model. Note the patches of button grass next to rainforest; a sight often found in SW Tasmania.

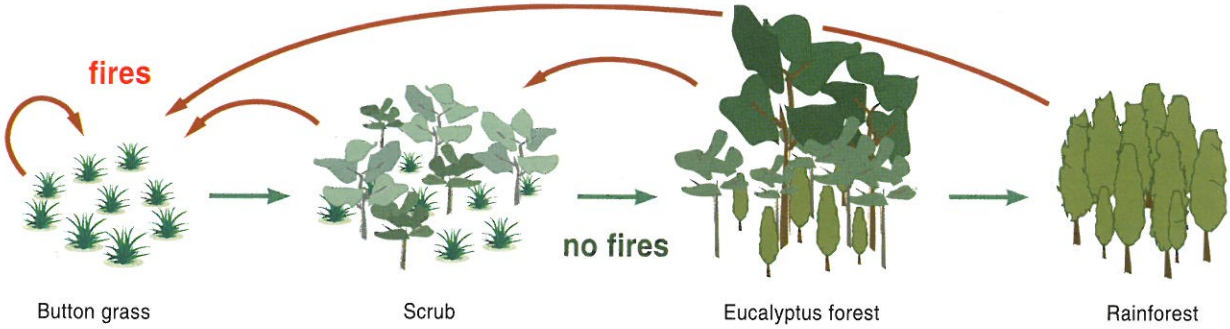

Figure 3. The succession of the four functional groups of vegetation, from Tasmania, used in the VASL model.

rainforest species. Eventually all but the rainforest species die leaving a pure rainforest community. Rainforests burn only rarely (only once in 300 to 400 years), but when they do they are replaced by a button grass community. Button grass burns quite frequently (every decade or so). Thus, once an area is covered by button grass, it may remain that way for many centuries, kept there by frequent fires. Scrub and eucalypt forests each burn less frequently than button grass.

The model begins with all functional groups present (i.e., a eucalypt forest). The VASL software generates random fires that spread through the vegetation in a realistic pattern. It then simulates the succession through the different vegetation types until another fire occurs and so on. Very quickly a pattern develops. Some patches are by chance burnt by two fires within a few decades and become button grass; others are missed by all the fires and become rainforest.

'We were really surprised to find that this model was reproducing landscapes very similar to the ones we see in Tasmania,' says Dr Noble. One peculiar pattern in the resulting landscape is rainforest surrounded by a field of button grass (see Figure 2). 'This pattern of vegetation occurs in Tasmania, where you can traverse a field of grass and come upon a small patch of rainforest,' he said.

'It is interesting and curious that applying sensible best estimates and simplifying a landscape, captured many of the patterns and dynamics of this complex community,' he says. The next step is to add topography and make more elaborate fire models . . .

Introducing topography

The 'Firescape' computer program simulates fire frequency and intensity in a 'real' landscape, or a landscape which includes detailed topological information. PhD student Geoff Cary developed Firescape when he noticed that landscapes within similar climatic regions and with similar vegetation, showed quite different patterns of perturbation by fire. This observation showed the importance of terrain upon fire frequency and intensity.

To develop Firescape, a digital terrain model of the ACT and surrounding Brindabella Ranges was combined with a daily weather model, to generate the 'landscape'. Each cell within the landscape grid is 100 metres square and the model contains 980

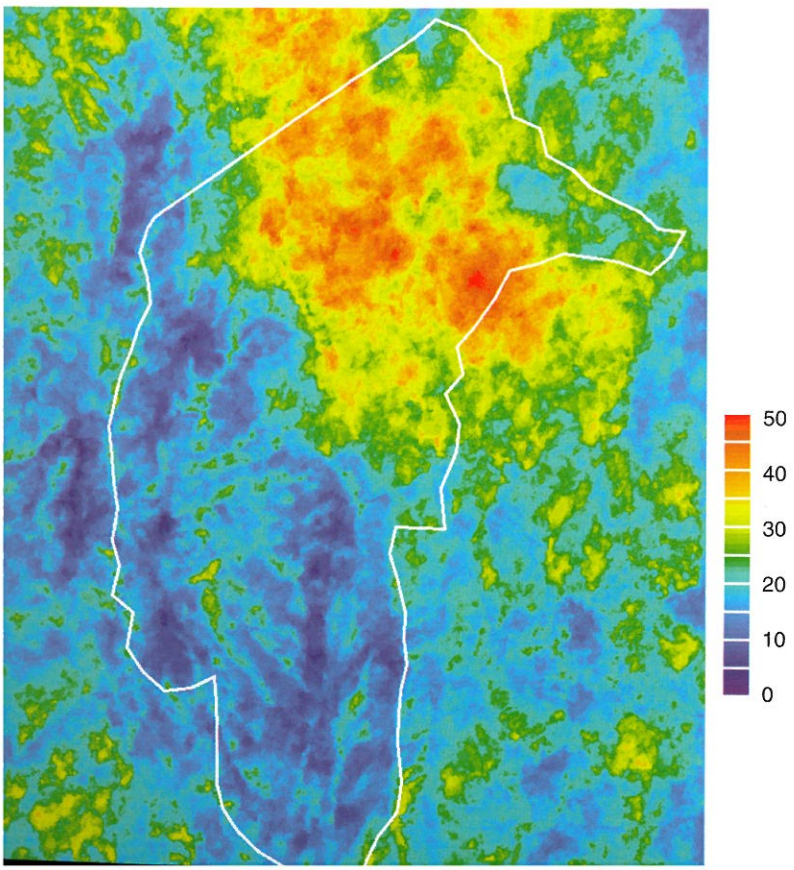

Figure 4. A Firescape model showing the long-term fire frequency for the ACT and surrounding region, generated by a 1000 year simulation. Ignition probability, fuel moisture content, fuel load, fire spread, daily climate patterns and terrain are parameters included in the model and it assumes continuous vegetation cover. Mr John Gallant (Centre for Resource and Environmental Sciences) assisted with the preparation of this figure.

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thousand cells! The model is, however, independent of scale as it can simulate fire patterns for a single rock platform or for the whole area. Records of lightning strikes from the region were used to generate a 'natural ignition' model, to predict where fire starts and lastly, fire spread algorithms were added.

A one thousand year simulation is shown in Figure 4. In the warmer, less humid areas, fires occurred around once every twenty years. Fires occurred most frequently outside the mountainous terrain. 'Scientists used to look at ecological and vegetative effects to explain variation within a community's vegetation and any residual variation was usually blamed upon historical disturbance regimes,' says Mr Cary. 'I believe that this residual variation may be landscape induced variation in fire.'

Mr Cary's research has illustrated Dr Noble's observation that landscapes create variation. In the Firescape model, terrain affects the diversity of fire regimes, which in turn has an impact upon species diversity.

Future R&D

'There is still a lot of work to be done before we can understand how landscape patterns are formed,' says Dr Noble. 'Some patterns arise mostly from the topography, such as ridge and valley vegetation types; others arise from interactions among topography, disturbances and chance.' The next challenge is to understand how these patterns will change if humans change fire regimes, either by lighting fewer or more fires, and what will happen if climate changes. Landscape diversity is an important feature of our environment and the better we understand it the better we will be able to manage our landscapes to sustain the fullest range of benefits.

If you want to know more ...

• The use of vital attributes to predict successional changes in plant communities subject to recurrent disturbance. I.R. Noble, R.O. Slatyer. *Vegetatio,* 1980, **43**, 5-21.

• A model of the responses of ecotones to climate change. I.R. Noble. *Ecological Applications,* 1993, **3**, 396-403.

Dr Ian Noble

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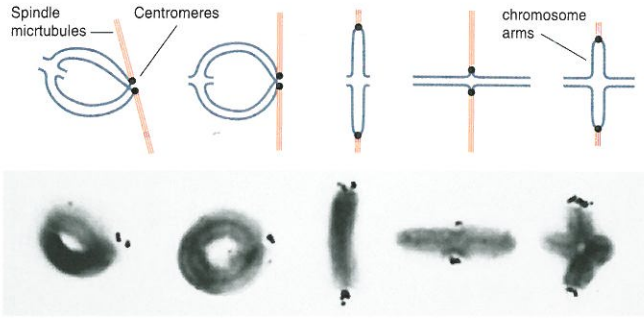


Figure 6. The black dots represent the location of radioactively lablled DNA sequences at the centromeres. These sequences have been isolated, cloned and sequenced. Their interaction with centromeric proteins is currently being investigated. Top: A schematic representation of the paired chromosomes.

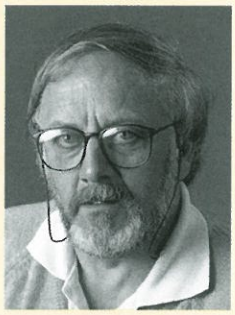
Another component of Dr Shaw's research is concerned with the molecular structure of the centromere and associated proteins in *Caledia captiva* (see Figure 5). As these grasshoppers show so much variation in the location of their centromeres, Dr Shaw and his group are attempting to define those DNA sequences and proteins that interact to form the centromere during cell division. 'We have already isolated, cloned and sequenced several putative centromeric DNA sequences from *Caledia* and have also identified some of the proteins that bind specifically to these sequences during cell division (see Figure 6).'

'I believe that within the next decade we will be able to construct synthetic chromosomes, with their individual centromeres, that can then be incorporated into recipient genomes.' With this prediction in mind, Dr Shaw foresees that the next episode of evolution may be manifested by humans, using synthetic chromosomes to create novel genomes and with these tools, create new species!

If you want to know more ...

• The genomic and molecular organization of centromeres in the genus *Caledia*. D.D. Shaw, N. Contreras and V. Maclean. In: *Kew Chromosome Conference IV* (ed P.E. Brandham & M.D. Bennett), pp 199 -132, Royal Botanic Gardens, Kew.

• Centromeres: moving chromosomes through space and time. D.D. Shaw. *Trends in Ecology and Evolution,* 1994, **9**, 170-175.



Dr Dave Shaw

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. . . AND THEN, TO CHANGE THE WAY WE THINK ABOUT THINGS

You may remember my last headline for the back page was "first...to know the nature of things", a translation of the motto of the Australian National University. It is a good summary of the Institution's dedication to basic research. Basic research tends to have the effect of changing the way we think about things. It is self-evident that if we change the way we think about things, there is a good chance we may, in fact, go about things differently, that ideas, innovations and applications will follow.

The phrase, to change the way we think, also sums up much of what needs to be said about the process and practice of basic research. It implies that we have to search, that there is a lot we don't know, and even that we may presently misconstrue what we do know. It implies approximation to truth in terms of our present vocabulary and insight, and that this approximation is subject to continual revision.

What drives people to engage in research? Almost certainly, not the money! One of the most pure of mathematicians, G.H. Hardy, spoke of "*three highly respectable motives*", namely intellectual curiosity, professional pride, and a desire for reputation. One of the most pragmatic of biologists, Peter Medawar, looked instead to the values given by scientists to their work. In his view, respect for achievement was not to be found in the purity or the applicability of research.

Rather, Medawar considered that scientists valued research findings "*Foremost... (for) their explanatory value... second (for) their clarifying power, the degree to which they resolve what has hitherto been perplexing... third, (for) the feat of originality involved in the research, the surprisingness of the solution to which it led, and so on*". Medawar might have accepted that motivation in research and evaluation of its achievements could be summarised by whether it might, or indeed had, changed the way we think.

The ability of Australian science to keep its competitive edge, to change the way we think, and to meet the challenge of tomorrow, begins in the classrooms of today. We hope *BIOLOGIC* provides a small stimulus. As has been said at the National Science and Technology Centre, the hope is that the next generation will find "*science in every think*"!

Whether or not an individual or a body of work has changed the way we think can be measured by citation impact, i.e. by what others think about our research. For example, my own field of research in plant sciences happens to have been second top in the charts for citation impact of Australian research through the 80s and early 90s, after agricultural research in soils and water. Amongst other things, I think achievements in plant science have been influenced by the fact that there has been an easy interaction, and mutual respect in the past, among researchers driven by curiosity and those driven by utility.

But things are changing. Almost every government in the world seems to be pushing for short-term utilitarian objectives at the expense of basic research. Australian plant sciences research is showing strong utilitarian polarisation in CSIRO, in primary industry funded research, and in the new wave of Cooperative Research Centres. I believe the same may apply in other disciplines, and that it is time to strengthen basic research and to restore balance. For example, it is already being said, and evident to me first-hand, that we may have run out of good ideas to sustain new Cooperative Research Centres. Getting the balance of research right is a matter of urgency.

Strengthening basic research was the theme of a major international conference held in Canberra at the end of November. Sponsored by the premier scientific journal *Nature*, the Institute of Advanced Studies at ANU and all four Australian learned academies, the conference theme was "Nurturing creativity in research: ideas as the foundation of innovation" The papers presented are being published on the Internet:. (http://biology.anu.edu.au/Pages/Pubs/NatConf/Nathome.html.)

Over 130 participants from around the world and throughout Australia, drawn from researchers of all persuasions, and from those responsible for public expenditure on research, heard Senator Peter Cook (Minister for Science) open the conference reinforcing his earlier statement that Australia must move now "*to build a cultural shift towards thinking of ourselves as a science-competent nation*" and "*to nurture an ideas culture in Australia - one that recognises the primary importance of knowledge, creative skills and innovation in every aspect of our future*".

We also heard how the Parliament of Japan has published a new law, described by Tania Ewing in the Melbourne Age as a "Bill of Rights for Researchers", that guarantees support for basic science. It is generally acknowledged that the economic miracle of Japan has been based on application of research findings from elsewhere. Japan's recognition of the need for its own basic research, to secure its future knowledge base, is a sobering signal to the rest of the world.

Professor Eguene Wong from the Hong Kong University of Science and Technology had the last word at the conference. "*It has been said that to foretell the future, one has to invent it. To be able to invent the future is the dividend that basic research pays.*"