Secondary Science Review

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Patented petunias Can plants be copyrighted?





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The cover image shows purple 'Surfinia®' petunias. These are trailing petunias, propagated by cuttings. Surfinia® varieties are protected by Plant Breeders' Rights. They belong to the giant multinational corporation Suntory whose other brands include Lucozade, Ribena and Jim Beam. See Sarah Cook's article on page 4.

Food for the future

What we eat has a great impact on our health. The food plants that we rely on have been developed through artificial selection over thousands of years. But plant breeding is changing. Modern biotechnological techniques allow breeders to effect rapid and radical changes to familiar plants, giving them desirable characteristics which may help us to live better and longer.

On pages 1-3 of this issue of CATALYST, Caroline Wood describes one such development, purple tomatoes whose flesh contains high levels of anthocyanins, chemicals which are thought to reduce the incidence of cardiovascular disease and some cancers.

But who owns these newly-bred plants? And is it right that individuals and corporations should own them? Sarah Cook takes a look on pages 4-5.

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Could purple tomatoes help us be healthier?

Key words genetic engineering cancer superfoods

ne of the greatest global health challenges we face is the obesity epidemic. In 2014, the World Health Organisation (WHO) estimated that worldwide 39% of adults over 18 years were overweight (with a BMI of 25+) and 13% were obese (with a BMI of 30+). This has led to a dramatic surge in the levels of non-communicable diseases such as type II diabetes, cardiovascular heart disease (CVD) and certain cancers. We've all heard that certain 'superfoods' contain compounds that can help combat these diseases, but these are often expensive and not accessible to everyone. If only everyday foods could be engineered to have these enhanced health effects... but thanks to genetic engineering, scientists have started to do just that, giving us purple tomatoes!

Our changing diet

When humans lived as hunter-gatherers, we would have consumed a much wider range of fruit and vegetables, whereas today our diets are heavily based on cereal crops (*Figure 1*). In fact, despite there being thousands of species of edible plants, just three crops – rice, wheat and maize – provide an estimated 60% of the world's energy intake. Yet as our dietary repertoire has shrunk, levels of obesity and its associated diseases have rapidly climbed. In fact, a lack of fruit and vegetables is ranked as the second highest risk factor for cancer in men, and the fifth highest in women in the UK (*Figure 2*). This is thought to be because plants produce many products as part of their natural metabolism that are beneficial for our health.

BMI (body mass index) is mass (in kg) / height (in m) squared

Andrew Davis, John Innes Centr





Figure 2 The top six causes of all cancers in men and women in the UK. Source: Cancer Research UK

Of particular interest are anthocyanins, a group of compounds found in red-orange and blue-violet fruits and vegetables. Epidemiological studies (studies of the distribution of diseases across different populations) have indicated that higher anthocyanin consumption is associated with decreased risk of cardiovascular disease, obesity and certain cancers. However, the foods containing high levels of anthocyanins - e.g. cherries, blackcurrants, blueberries and cranberries - tend to be expensive, and you would need to eat a lot of them every day to get a significant benefit. So, a group of researchers at the John Innes Centre in Norwich decided to use genetic engineering technology to see if anthocyanin levels could be increased in a more common crop that is easier to grow.

"We decided to use tomatoes because they are a very versatile food, can be grown in many places and are relatively easy to transform using GM techniques," says Professor Cathie Martin, a plant biotechnologist who led the study at the John Innes Centre for plant science in Norwich. A gene from snapdragon was introduced into the tomato plants that caused them to accumulate anthocyanins in the fruits, making them turn purple (*see Box above*).

How do you make a purple tomato?

Genes are typically divided up into different elements - the coding region, and a promoter which controls how they are turned off/on. The genes the scientists introduced into the purple tomato contained the coding sequences of regulatory proteins from Antirrhinum majus (garden snapdragon) that control anthocyanin biosynthesis, which gives the flowers their purple colour. These genes were driven by a fruit-specific promoter from tomato, so that they would be activated only in the tomato fruits and not in the whole plant, to make sure there were no side effects on the development of the tomato plants. The genes were introduced into the tomato plants using Agrobacterium tumefaciens, a species of bacteria that infects plants by inserting a segment of its DNA into the plant cell which becomes incorporated into the plant's genome. Researchers can use Agrobacterium to introduce desired genes into plants by cloning the gene into the segment of DNA which the bacteria transfer to the plant.

Will it work?

To test the health benefits of the tomatoes, the researchers used a genetically modified strain of mice that is prone to developing cancers. This is because they lack both copies of the *p53* gene which helps to control cell division. "Normally these mice only live for an average of 142 days," says Cathie. "But when we supplemented their diet with 10% purple tomatoes, their average lifespan increased to 182 days." Crucially, the mice didn't live significantly longer if they were fed a diet supplemented with 10% normal red tomatoes, showing that the benefit came from the anthocyanins in the genetically modified fruits (*Figure 3*).



Figure 3 Graph to show how the lifespan of tumourprone p53-/p53- mice if affected when they are fed diets supplemented with either normal red tomatoes or high-anthocyanin purple tomatoes. Published in Butelli et al Nature Biotechnology 2008 doi:10.1038/ nbt.1506



WT (flavonols)

High delphinidin H

High pelargonidin High

High flavonols

High resveratrol High genistin

High anthocyanin High flavonols

Since then, the researchers have used the same technology to make tomatoes that accumulate high levels of a whole range of beneficial compounds. These include flavonols: antioxidants that are thought to protect against cancer, cardiovascular disease and other age-related illnesses. In this case, the high concentration of flavonols makes the tomatoes turn orange (*Figure 4*). Another type of tomato has high levels of resveratrol – the 'miracle compound' that is supposedly the cause of the antiageing effects associated with moderate red wine consumption. In fact, just one GM tomato contains as much resveratrol as 50 bottles of red wine!

Given the strict regulations on GM foods in Europe, it could be a while before we see fortified tomatoes, or indeed other engineered crops, on our supermarket shelves. At the moment, purple tomato juice is being presented to the Food and Drink Association (FDA) of the United States to establish that it is safe to consume and can be marketed in the USA. Cathie believes that we shouldn't just rely on one or two "super foods" but instead make changes to our diets as a whole. "I personally don't see the future as being "eat all the junk food you like and take a pill or a special tomato at the end" she says. "Improving our whole diets is better for long term health." When plants have so much natural goodness in them, increasing our fruit and vegetable intake is surely one of the easiest ways to start to improve our health.

A fishy story

GM crops are also being explored for their potential to make fortified animal feeds. Omega-3 polyunsaturated fatty acids – particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - are widely known to have a crucial role in protecting against heart disease and also for developing a healthy brain and nervous system. One of the best sources of these is oily fish such as salmon, mackerel and trout. But these fish do not produce omega-3 fatty acids themselves; instead they accumulate up the food chain from marine phytoplankton that do make them – in other words, fish oils are not made by fish, but algae. Consequently, when oily fish are farmed, they are *Figure 4* A range of super-fortified tomatoes produced using GM technology. WT = 'Wild type' (normal)

typically fed on fishmeal and fish oil made from smaller ocean fish, to ensure that the final product contains all the essential fatty acids and proteins. This puts pressure on wild stocks of fish.



Figure 5 False flax (Camelina sativa) has been genetically modified so that it produces omega-3 fatty acids. The unmodified plant is already grown as a source of biofuel.

But scientists at Rothamsted Research have used genetic engineering to alter the metabolism of false flax (*Camelina sativa – Figure 5*) to make EPA and DHA. This plant naturally makes some shorter-chain omega-3 fatty acids: the researchers introduced a set of seven synthetic genes based on those present in marine phytoplankton to convert these into EPA and DHA. It is hoped that these plants could become a sustainable, land-based source of fish meal in the future.

Caroline Wood is a postgraduate research student in the Department of Animal and Plant Sciences, University of Sheffield.

Sarah Cook



The vegetables we eat are the results of thousands of years of selection by growers. Plant breeding is the science of producing new plants with desirable traits such as larger size, better nutrition, more resilience to pests and disease. Plant breeding is practised by all sorts of people worldwide, from individual gardeners to large global companies who produce the seeds that are used to grow the plants you buy at the supermarket.

Why breed plants?

Plants found in the wild are very diverse and possess some traits that are desirable to humans along with others that are not. For thousands of years, people have intelligently selected plants with



Teosinte (left), the ancestor of modern maize (corn) (right). In the centre is an ear of a first generation hybrid of a cross between teosinte and maize.

desirable traits to breed with each other over many generations. This process of selection and breeding by man is called artificial selection.

Today's cultivated plants are easier to eat or digest, taste better and are more nutritious than their wild forebears. An example of this is the maize that is grown and eaten by us today. Over thousands of years we have artificially selected this plant so much so that it is almost unrecognisable compared to its ancestor, teosinte. However, we know that they are the same species because scientists have analysed the DNA of both plants and found they are very alike.

How has plant breeding changed?

Until relatively recently, most plant breeding was carried out by farmers. There wasn't a question of who owned the seeds as the farmers had sole responsibility for sowing, selecting and saving the seeds they grew. However, in most developed countries these days, global industrial companies such as Monsanto, DuPont, Syngenta and Bayer own the seed that farmers grow for food crops. These companies specialise in plant breeding to produce quality seed that they sell to farmers based on the plants' desirable characteristics.

Plant breeders can protect the plants they breed through laws called intellectual property rights, IPR (*see Box opposite*). This means that no one can grow or sell their plants unless they are licensed to do so. They can then make a profit from the plants they have bred to have the desirable traits that farmers want to buy.

In the US it is very common for companies such as Monsanto to hold patents to protect their plant varieties. A patent is a stronger form of IPR that prohibits others from using the variety unless licensed; a licence can be expensive. In Europe, plant varieties themselves cannot be the subject of



a patent as they are specifically exempt and can be protected through PBR.

However, more recently in the UK, companies have been granted patents on naturally occurring traits that they have successfully bred into their varieties. This means that any plant variety bred to possess a particular patented trait will infringe the patent unless a licence is applied for. This is hugely controversial and many people, including organisations such as No-Patents-On-Seeds, strongly believe that naturally occurring traits shouldn't be owned by anyone.

Intellectual property rights

Intellectual property refers to creations of the mind, anything from song lyrics to inventions. Intellectual property rights (IPR) allow the creator to benefit from their work or investment in the creation. For plant breeders there are many different types of IPR they can apply for. Two of the most debated are:

Plant Breeders' Rights: Allows plant breeders to protect a plant variety as their own property. Valid for 20 to 30 years depending on the plant species.

Patents: Used to protect many different types of inventions but more recently used to protect plant traits. Many varieties possessing the patented trait can then be covered by one patent. Valid for 20 years.

Using biotechnology

Breeders can use a variety of biotechnology tools to select which plants to breed. In genetic modification, genes from other species are inserted into a plant genome to introduce a physical trait, while in genetic engineering the genome of the plant is edited by molecular probes.

One of the most commonly used tools for breeding is marker-assisted selection (MAS). This allows breeders to enhance conventional breeding methods (sexual and asexual reproduction). Markers are typically DNA sequences, genes, or chromosome attributes that link to a particular gene producing a trait of interest. Using genetic markers, scientists can accurately identify which plants will possess the desirable trait before they are fully grown. This is very different from the days before biotechnology where selection would be done solely on traits that can be observed.

The protection of breeders' rights is a complicated issue, particularly as plant breeding has changed a lot in the last century. Companies invest a huge amount of time, effort and money into breeding programmes which don't always have the desired effect. Therefore they argue that without IPR protection the companies would not be able to sustain themselves as anyone could sell the plants as their own and then plant breeding as an industry would cease.

Sarah Cook is a PhD student in the School of Chemistry, Food & Pharmacy at the University of Reading, UK.

A single species, wild mustard (Brassica oleracea) is the ancestor of many different vegetable varieties.



ou are walking down a street late at night in the dark and you hear footsteps coming up behind you. Your heart starts to race, your breathing accelerates and you begin to sweat. You are experiencing the classic symptoms of the 'fightor-flight' response.

You have purchased a lottery ticket and are listening to the results and your numbers are being read out one after the other. Again your heart starts to race, your breathing accelerates and you start to sweat. It's that 'fight-or-flight' response again.



He can't believe it – all his lottery numbers are coming up.
Figure 1 The fighthere is a fighthere is a figure 1 The fighthere is a fighth

Does it sound strange to experience the same symptoms for two different events, when one is fearful and one is exciting?

This should not surprise you if you think about the purpose of the 'fight-or-flight' response. The aim is to make your body ready for the activity that might be needed to cope with the stress of the event. Whether you need to run away or stand your ground and deal with the event, your body will be admirably ready to expend extra energy.

This is achieved using both the hormonal system and the nervous system working together. *Figure 1* illustrates this complex mechanism.



Figure 1 The fight-or-flight response involves both hormonal and nervous responses.

The brain perceives a stressor and the hypothalamus simultaneously initiates a hormonal and a nervous response. The hormonal response involves a sequence of chemicals released by several glands. The hormone CRH (corticotropin-releasing hormone) is released by the hypothalamus and acts on the pituitary gland to produce ACTH (adrenocorticotropic hormone). ACTH targets the adrenal cortex to release a range of corticosteroids, which act on several organs. One crucial outcome is the mobilisation of glucose as a source of energy.

The nervous response is fast and involves an impulse going to the adrenal medulla causing

You hear footsteps behind you in a dark alleyway – Is it a threat or not?

> Key words stress anxiety memory examinations

adrenalin to be released. This stimulates various responses in the autonomic nervous system, such as an increase in heart, breathing and perspiration rates, along with a decrease in digestive processes.

So after the stress response has been activated the body is 'prepared' for activity.

The positive side

Stress is so often viewed negatively but you can see that it is actually in your interest for the stress response to be initiated at the appropriate time. What can be a problem is for the response to be initiated when you can do nothing about it, such as in a road-rage situation.

A certain amount of stress is actually beneficial and the term 'eustress' is given to this. It 'wakes us up' and makes us alert to our surroundings. However there are problems as stress levels rise.

The stress-response can overpower the ability to think straight and much research has been carried out on people's performance in examinations. There are some people who crumble in the examination room and experience a mental block, which decreases their performance. Whilst other people thrive on the challenge of examinations and, under stress, their performance increases.

Not too much, not too little

Psychologists Robert M. Yerkes and John Dillingham Dodson in 1908 devised a law relating performance to the level of arousal. *Figure 2* shows this relationship and is called the Yerkes–Dodson law. It states that performance increases with physiological or mental arousal, but only up to a point. There is an optimum and if the stress levels exceeds one's own optimum then performance will suffer. One recent study, carried out at the University of Chicago, has shown that the level of mathematics anxiety can interact with the completion of a mathematical challenge in varying ways.

Sian Beilock, author of *Choke: What the secrets of the brain reveal about getting it right when you have to*, and associate professor in psychology at University of Chicago says, "We found that cortisol, a hormone released in response to stress, can either be tied to a student's poor performance on a math test or contribute to success, depending on the frame of mind of the student going into the test."

The research group looked at cortisol levels before and after attempting a demanding mathematical problem in students with varying working memory abilities. Working memory (WM) is a shortterm system, which is involved in the control and maintenance of a limited amount of information that is needed to complete a task. They categorised students as either possessing low WM or high WM and found that, for higher WM individuals, their performance on a challenging maths task decreased if they suffered from high maths anxiety. In contrast, for higher WM individuals lower in maths-anxiety, the higher their salivary cortisol concentrations, the better their performance – see *Figure 3*.

It appears that higher WM individuals employ strategies during problem solving which are cognitively demanding. This gives them the ability to perform well and achieve a higher level of performance than individuals with low WM capacity. However, when a task is demanding anxiety can lead to distraction away from working memory. This would explain why individuals who are maths anxious *and* have high WM capacity perform less well than low maths anxious people with high WM capacity.



Figure 2 The Yerkes-Dodson law proposes that there is an optimum level of stress which produces the best response.

Figure 3 Scores on a maths test depended on the students' working memory as well as on their degree of anxiety.





Increasing Salivary Cortisol

The message then is this: if you have a high WM capacity and have developed a good skill in mathematical tasks using your WM, then getting stressed during a maths task is detrimental to your performance. If you wish to maintain your high level of mathematical ability you need to conquer the anxiety stimulated by such tasks.

Individuals with lower working memory capacity do not seem to be bothered as much by their level of anxiety. This is probably due to the fact that they employ strategies that do not require as much from their WM and so stress will not hinder them in the same way.

From the description of the stress response you might think that this is impossible since it is an automatic biological response. However you will note that the response (both nervous and hormonal) was initiated by the hypothalamus and this is in communication with the cerebral cortex, which is the 'thinking' brain. In practice, the hypothalamus will not trigger the stress response unless the cortex perceives the stimulus as a stressor. So if the higher brain says you are not stressed you aren't stressed! If you change your attitude to the problem by reducing your perception of it as being anxious then you will allow your working memory to get on and deal with the problem to the best of its ability.

Conquering stress

There are physical methods of reducing your stress level, such as progressive muscle relaxation and controlled breathing.



Some schools include yoga in their curriculum to help students reduce stress levels.

Another way to reduce anxiety is to increase 'selfefficacy', increasing one's thoughts and beliefs about having the personal power or capacity to produce a desired effect. Academic self-efficacy refers to a learner's judgment about higher capability to solve a problem successfully. In other words, learn to believe in your abilities!

Cognitive behavioural therapy (*Figure 4*) challenges people to control their negative thoughts, such as "I'm not good enough," or "I'm going to fail" with more positive ones such as "I can do this," or "I have the ability...".



Figure 4 Cognitive behavioural therapy (CBT) aims to break the vicious circle of negative thoughts and feelings.

Finally then, stop saying you are 'rubbish' at a task and learn to control those negative thoughts by believing in yourself and take positive action to overcome your anxiety.

Ann Skinner is a retired psychology teacher who is now an educational consultant.

The sands of time

In less than a human lifetime, the sands of a beach can become dry land. **Gary Skinner**, Catalyst's Biology editor and resident photographer, explains how.

n nature, everything changes. Mountain ranges which seem to remain unchanged throughout our lifetime are slowly but surely eroding, and over the millions of years of geological time will cease to exist. Even an everyday beach, of the kind where you may have spent holidays, is not a permanent feature. In some parts of the world such beaches are being eroded away. In others, fascinatingly, they are growing and adding to the land area.

In the photograph on pages 10-11 of this issue of CATALYST you can see some of the stages of this growth process.

How dunes start

Fine particles of sand brought down into the sea from eroding mountains are eventually washed onto the beach. Here, onshore winds blow and the sand particles are carried inland. Sometimes, sand may build up around an obstacle on the beach. This may be something non-living, such as a dead branch or even a bit of litter, or it may be a small plant which has managed to take root in the shifting sand – some plant species are adapted to be able to do this. Whichever it is this leads to the formation of a small pile of sand, sometimes referred to as an embryo dune.

An embryo dune can provide habitat for the highly-adapted pioneer plants which first take a hold in the shifting sand beaches. Over time, these plants accumulate organic chemicals through the process of photosynthesis, using carbon dioxide from the atmosphere and water from the sand. Their roots, which are generally very spreading and deep, hold the sand together and provide a habitat for other plants. As these plants die the nutrients locked in their bodies are released by decomposition and add to the quality of the sand as a medium for growth. It is now becoming soil.



The vast root system of a group of marram grass plants

The pioneer plants have now changed the environment where they live so that it is suitable for other plants which, although they do not have the adaptations of the pioneers, are better suited to growing in the changed environment produced by the pioneers. These bigger plants will now compete with the pioneers for light, minerals and water. Slowly but surely, the pioneers are eliminated and the bigger plants take over. Although not so highly adapted as the pioneers this next stage are still very specialised. They include large grasses such as marram and lyme grass. Their root systems go many feet into the sand, leading to the formation of often gigantic dunes.

The photograph on pages 10-11 shows many of the features of a typical developing dune system. Marram grass Ammophila arenaria and lyme grass, Leymus arenarius are both important dune builders. They out compete the pioneers once the former have improved the soil.

Woodland growing on areas which were sand a few hundred years ago

Sea Rocket (*Cakile maritima*), another pioneer.



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Couch grass; there several related spec important pioneers



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Sea-sandwort (*Honkenya peploides*), a pioneer plant. This plant is adapted to living in the salty environment near the sea. It has fleshy leaves which store water. The roots are extensive and it can produce new shoots when buried in sand.

The sea



Some material on the beach. This is accumulating a mini-dune of sand

Growing on sand

How do pioneer species in an environment where water is a problem? It is salty in the soil which makes it difficult to take up and the constant winds make the atmosphere a very drying one. The pioneers usually have succulent leaves in which they can store water.

And how does marram grass survive when, for example, a storm may bury it in sand? Marram is adapted to grow upwards and out of the top of the dune. In addition, marram and lyme grass have leaves that can curl into a cylinder in order to limit the loss of water in the constant wind. The lower surface of the leaf, where stomata take in carbon dioxide, also loses a lot of water. In a particularly drying atmosphere the leaves curl so that the stomata are protected on the inside, away from the wind.



Cross section of a curled marram grass leaf, showing features which limit water loss in a dry environment.

Soil, getting richer

As these large grasses die they add nutrients to the soil and make the environment suitable for even less demanding plants that will, again, outcompete them. The environment now changes from one dominated by one or two species of large grasses to a much more varied habitat.



Looking towards the sea from a few hundred metres inland, in the varied habitat behind the giant dunes

The soil is now rich enough to support the growth of shrubs and bushes, and even these finally give way in a natural system to trees which will form a woodland.



Woodland growing on dunes which would have been bare sand on a beach a few hundred years ago

In most parts of Britain natural succession has been affected by human activity. Very often what should be woodland growing on old dunes is occupied by a golf course. However, there are still many places around our coasts where you can see sand dune succession.

Gary Skinner is Biology editor of Catalyst. All photographs on pages 9-12 by the author.



In some labs analysis is automated but analysts still put the samples in the machines and interpret the results.

Zoe Ayres

Analytical scientists use a variety of methods and instruments to try to answer two basic questions: what have I got? and how much of it do I have? **Zoe Ayres** of the University of Warwick explains how they set about finding the answers.

Analytical science is essential for many other scientific areas including healthcare, environmental monitoring, forensics, chemical biology, and synthetic chemistry. The development of new analytical techniques is often led by public interest. For example, analytical scientists develop cheap, simple and portable sensors for early diagnosis in disease epidemics. Such sensors were critical in helping to contain the 2015 Ebola outbreak in Africa.



Flame tests can be used to find out which metal is in a sample but not how much – it is a qualitative test. A green flame indicates copper is present.

What do I have?

This is about identification of a substance (the substance to be analysed is called an analyte) and is called **qualitative analysis**. An example at school would be using flame test colours to identify metal ions. An analyst may receive a white powder sample from a crime scene and carry out tests to confirm if any illegal drugs are present.

There are many instruments and methods used to determine the identity of a substance. They use differences in structure and chemical composition to allow the identification. Chromatography is an example, where different inks can be identified based on the different rates that they move up the chromatography paper. The differences rates are caused by differences in structure which affect how the substances interact with the solution and the paper.

How much do I have?

This is about determining how much of an analyte is present and is called **quantitative analysis**. For example, for drink driving convictions it must be determined whether a person's blood alcohol level was above the legal limit when they were driving.

Accuracy is important. If the analytical scientist gives the wrong answer then a drink driver could be exonerated (let off) or an innocent person convicted. An analytical scientist must therefore always consider the reliability of their results, with possible errors arising from many different sources such as during sample preparation and from the actual analytical instruments themselves. To do this, both accuracy (the measure of how close the analytical measurement is to the true value) and precision (how close a set of measurements are to one another under the same experimental conditions) are assessed. Each analytical method must be validated, which means it must achieve a defined accuracy and precision target, usually more than 95%. This way analytical scientists can report their findings with strong confidence in their results.

On the next page we look at five examples of where analytical science is used.







Airport security analysis needs to be accurate but also fast, to prevent large queues from building up

Analytical science plays a large role in ensuring public safety and security. For example, analytical instruments at airports are used to identify illegal drugs and dangerous substances such as explosives. These instruments must be carefully designed to ensure rapid, highly accurate analysis even if only a small (trace) amount of the substances are present.

Pharmaceuticals

Medicines must be analysed to ensure they are safe for human consumption. Tests must ensure that the drug products do not contain toxic heavy metals, which could come from the catalysts used to produce the medicines. It is also really important to confirm that the correct drug molecule has been produced as the wrong structure could have potentially devastating results. For example, in the mid-20th century it is thought that a small change in the structure of the drug thalidomide, given as a morning sickness remedy to pregnant women, caused severe birth defects.

Fraud detection



Analytical techniques are often used to fight fraud. For example, in 2013 there was a food scandal where several food products sold as beef actually contained horse meat. To identify the affected products, analytical scientists had to develop new methods of differentiating between horse meat and beef. Test kits have been designed to identify the presence of horse-specific proteins for quick analysis away from the lab, along with conventional DNA tests.



Healthcare diagnostics

Bioanalytical techniques for healthcare are present not only in hospitals and doctors surgeries but also in our homes. The most widely used examples are blood glucose meters for diabetes management and home pregnancy kits. Careful design of at-home healthcare sensors is required to ensure they are simple to use and as non-invasive as possible, yet are still accurate.

Zoe Ayres is a PhD researcher at the University of Warwick developing boron-doped diamond sensors for analytical applications. This article is written on behalf of the Analytical Division of the Royal Society of Chemistry with the aim of promoting the importance of analytical science to the wider community.



How do we determine if an athlete has been using drugs to boost their performance? Analytical science has the answer.

The use of banned substances to enhance athletic performance is known as 'doping'. Historical evidence suggests that doping goes back as far as 776 BC, where ancient Greeks devised a range of different methods to beat their competitors and combat pain. These included drinking herbal teas, mixing concoctions of hallucinogenic mushrooms and even consuming the alkaloid strychnine, a poison that causes muscle spasms in small doses. From the first Olympic games, cheating due to dopant use resulted in athletes receiving lifetime bans from public sport.

In the last century with the advances of modern medicine the range of substances that are used for doping have increased dramatically. These include, but are not limited to: hormones to promote muscle growth; stimulants to improve endurance; and blood transfusions to increase performance. For this reason, the world antidoping association (WADA) exists to fight against doping of all kinds in sport. During the London 2012 Olympics, teams of analytical scientists tested over 6000 samples of blood and urine in the Harlow Olympic testing laboratory for the presence of banned sport enhancing drugs. Analytical techniques can be used to detect these drug molecules directly, or look for traces of other by-products that could only be present due to doping. Professional athletes in all sports are tested regularly to ensure they are not taking performance enhancing drugs.



Athletes have to provide blood and urine samples when requested. These are tested to ensure they contain no evidence of doping.

It's in the blood

One of the main illegal doping techniques is blood doping. This works by having blood transfusions or taking specific drugs to increase the haemoglobin in a person's blood. This results in an increase in the blood's ability to carry oxygen, which increases aerobic capacity and in turn increases physical endurance. When blood is transfused from a donor, specific antigen patterns can be used to identify doping. This is because each person's red blood cells exhibit specific genetic markers.

Identifying if an athlete has re-infused their own blood is more complex. Innovative methods such as searching for metabolites of blood bag plasticisers (broken down by-products of the plastic containers the blood is stored in) and analysing fingernail clippings to assess long term doping have emerged in the last few years. The key analytical technique used is liquid chromatography-mass spectrometry (LC-MS). Recently it has been announced that Olympian Jessica Ennis-Hill is set to be awarded her third World Championship heptathlon gold as the original winner tested positive for blood doping.



A liquid chromatography-mass spectrometry machine being used to test samples.

How much is too much?

For some drugs, a simple positive/negative screening can be enough, with the sheer presence of the substance indicating foul play, as the drug could not have been introduced into the body from natural sources. However, for some drugs, determining the amount of the substance present is extremely important. For example, a contaminant plasticiser from blood bags diethylhexyl phthalate, or DEHP, can be indicative of blood doping. However, the main source of DEHP stems from our diet due to plastic wrappers and storage containers and is thus naturally present in our bodies. An average 'baseline' of how much of the substance is in an athlete's body must therefore be established. Significant spikes in the concentration of DEHP above the baseline are therefore taken to indicate doping, induced by a blood infusion.

Designer Drugs

Historically, techniques have been developed for the detection of specific dopants that are known threats to the integrity of the sporting community. These methods involve the analysis of a 'standard' sample of the drug substance on the selected analytical instrument such as LC-MS, along with the samples provided by the athletes. This means that the signal response or 'spectrum' obtained from the standard can be correlated directly with the athletes' samples, to determine if the restricted substance is present.



Many analytical scientists are involved in on-going research to develop new analytical techniques.

More recently however, the development of 'designer drugs' has emerged. This is where the structure of an illegal drug is altered slightly to evade detection, but still maintains its physiological effects, giving an athlete the edge over their competitors. If the drug is new, with no standard available for confirmation, failure to identify doping may occur. For this reason, the analytical science community must to be ahead of the game, considering the structure of current drugs used for doping, and how they may be altered in an attempt to avoid detection. This highlights the importance of the continuing development of new analytical techniques, in addition to routine targeted screening and laboratory analysis, to stay one step ahead of the cheats.

Zoe Ayres, on behalf of the Royal Society of Chemistry Analytical Division

Chromatography



Chromatography is an analytical process which separates a compound into its constituent chemicals. As the solvent travels up the paper it takes the various chemicals in the ink with it, separating them into a series of coloured bands.

Chromatography is a method of separating a mixture of substances. The substances can then be compared or analysed further. This method uses paper and water but is based on the sample principles as more complex methods of chromatography used in analytical science (see the article on pages 13-14 of this issue of CATALYST).

You will need:

- filter paper
- washable black felt tip pens
- glass or beaker
- water
- cling film (optional)

What you do:

Cut a strip of filter paper about 2 cm wide and long enough to reach to the top of your glass or beaker, plus about another 5 cm.

Put about 1 cm depth of water into the beaker.

Put a dot of black pen about 1.5 cm from the bottom of the strip of filter paper. Allow it to dry and then add another dot on top.

Put a pen over the top of the glass and hang the paper strip on it so that the water is below the black dot. Cover the glass with cling film.

The water will rise up the paper and the different coloured inks present in the black ink will separate.

How it works:

All forms of chromatography work on the same principle. They all have what is called a stationary phase (a solid, or a liquid supported on a solid), which as the name suggests stays still, and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates, like in a race, and this separates them out.

In paper chromatography, the stationary phase is an absorbent paper. The mobile phase is a suitable liquid solvent or mixture of solvents – here we used water.

Different pen inks will be made of different combinations of coloured inks. Although they may all look the same when you write with them, they can be separated out by chromatography which allows you to see the differences.

Vicky Wong is Chemistry editor of CATALYST.

Look here!

More about how chromatography works: http://tinyurl.com/yketr99 Try this

A life in science

Katherine Johnson

Katherine Johnson was not allowed to attend school beyond year 8 in her home town because she was black but she became a mathematician and physicist whose calculations were essential to the success of the US Space Program including the first American in space and the Apollo space missions.

For several years after she was born in 1918, schools in the USA were still segregated (with black and white students taught completely separately) and the education opportunities for young black women were very limited. Katherine's father thought his daughter had potential and so the family moved 120 miles to the next state during the school year so that she could complete her schooling. She had finished high (secondary) school by the age of 14 and graduated with a degree in maths and French by the age of 18 thanks to her extraordinary mathematical talents.

Working for NASA

Katherine became a teacher and a housewife until 1953 when she began working for the National Advisory Committee for Aeronautics, which later became NASA (the National Aeronautics and Space Administration). This was an era before electronic computers and Katherine's job title was 'computer'. She calculated trajectories, launch windows and emergency back-up return paths for





Katherine Johnson at NASA in 1966

many space flights including the trajectory for Alan Shepherd, the first American in space. Some of the astronauts trusted her calculations more than they did those of early electronic computers, asking her to check the calculations the electronic computers had made.

Until 1958 her workplace was segregated so that Katherine and the other African-American women 'computers' had to work, eat and use separate toilets from the white workers.

As electronic computers came into NASA, Katherine combined her talent for mathematics with electronic computer skills to ensure the success of the Apollo space missions and the early flights of the space shuttle programme.

In 2015 President Barack Obama presented Johnson with the Presidential Medal of Freedom, the highest civilian award in the United States. Her story, and those of her black female colleagues, is told in the 2016 film *Hidden Figures*.

Vicky Wong is Chemistry editor of Catalyst.



Katherine Johnson with the US Presidential Medal of Freedom in 2015

Hidden Figures shows the importance of the work of the African-American women who worked as 'computers' in the early days of NASA.

Proxima b Have we discovered our next homeworld?

xtrasolar planets reside in solar systems beyond our own. Almost 3500 have been discovered since the first one in 1992. But on 24 August 2016 scientists excitedly announced the discovery of Proxima b. Because it resides in our nearest neighbouring solar system it is our closest **exoplanet**. Furthermore, it could support life and might even provide a future home for us.

Proxima b orbits a red dwarf star called Proxima Centauri, which is part of the triple star system Alpha Centauri, only 4.2 light years away – that's 40 trillion km, or more than 250 thousand times further away than the Sun. The star, too faint to be seen with the naked eye, is located in the constellation Centaurus, visible from the southern hemisphere.

A sun that never moves

Proxima b is twenty times closer to its star than we are to the Sun, which is part of the reason it only takes 11.2 days to make one orbit of Proxima Centauri. This also explains why the planet is 'tidally locked', always showing the same face to its star, in the same way we always see the same face of the Moon. This would make visiting the planet a strange experience. If you landed on the sunny side of the planet, the red dwarf star would hang motionless in the sky; it would never rise or set.

Most of the radiation from the star is in the infrared region of the electromagnetic spectrum and, because Proxima b receives only 2% of the visible light that Earth intercepts from the Sun, it would be like experiencing permanent twilight. It has a mass 1.3 times that of Earth so we might feel a little heavier until we developed slightly bigger leg muscles.

How was it found?

A star like the Sun is about a billion times brighter than the light reflected by its orbiting planets. This means that it is virtually impossible to see a planet directly and astronomers resort to using indirect methods. Almost 70% of extrasolar planets have been detected by the transit method. Each time an exoplanet orbits between us and its host star some of the starlight is blocked so that there is a periodic dip in the amount of light reaching us – see **Figure 1**. An artist's impression of the surface of Proxima b, and the red dwarf Proxima Centauri on the horizon with stars Alpha Centauri A and stars Alpha Centauri A in the far distance.

> Key words exoplanet transit red shift space travel



Figure 1 A planet passing in front of a star blocks some of its light so that there is a brief dip in the star's brightness.

Proxima b was detected by the radial velocity or 'Doppler wobble' method. Any star exerts a gravitational pull on an orbiting planet and, according to Newton's Third Law of Motion, the planet exerts an equal and opposite force on the star. So, as the planet orbits its star, the star also moves in a circle although, because the star is much more massive than the planet, its orbit is much smaller than the planet's.

Light waves from a star moving towards us are compressed. This means that their wavelength decreases and shifts towards the blue end of the visible spectrum. When a star is moving away from us, light waves are stretched – the light is red-shifted. This is the Doppler effect – see *Figure 2*. Astronomers can use the shift in the wavelength of starlight to work out the speed of the star as it moves towards or away from us – see the box below.

Amazingly this can be used to work out the mass of the planet and its distance from the star.

Temperature, brightness and mass

It is easy to work out the surface temperature of a star using its black body spectrum (see *Thermometry* ... *a hot topic* in CATALYST volume 23, issue 3, February 2013). Knowing a star's apparent brightness and how far away it is we can work out its luminosity (or power output).

So many stars have been catalogued and they are so well understood that astronomers can use the Hertzsprung-Russell diagram to find the mass of a star (see *Figure 3*). On this diagram, temperature is plotted along the horizontal axis while luminosity (or the power output of the star) is plotted along the vertical axis. Knowing the temperature and luminosity of a star, an astronomer can plot its position on the Hertzsprung-Russell diagram and infer its mass.



Figure 2 At A, the star is moving towards the Earth and its light is blue-shifted. At B, it is moving away and its light is red-shifted.



Figure 3 Alpha Centauri is a triple star system. Each star is plotted on the Hertzsprung-Russell diagram according to its surface temperature and luminosity – notice Proxima Centauri at the bottom right among the coolest, dimmest stars.



The spectrum of light from a star – this is an absorption spectrum because some wavelengths are missing and appear as black lines. These wavelengths have been absorbed by atoms of different elements in the star's atmosphere. If the star is moving towards or away from the Earth, the wavelengths of these lines are shifted towards the blue or red ends of the spectrum. The faster the movement, the greater the shift, and so astronomers can deduce the star's speed.

b to make a complete circuit of its star.

Living on Proxima b

Could we make Proxima b our next homeworld? Obviously it would need water and a source of energy. Astronomers already knew the luminosity of Proxima Centauri and now the distance to the planet so can work out the temperature on its surface. Assuming that Proxima b reflects as much starlight as Earth does, it would have a global mean surface temperature (GMST) of 233 K. That's minus 40 °C, too cold for liquid water and possibly for life. But without the natural greenhouse effect provided by our atmosphere, Earth would have a GMST of minus 18 °C. Thankfully our atmosphere warms Earth by 33 °C to make life possible. So the presence of an atmosphere on Proxima b is key.

Like the planet Mercury, Proxima b is 'tidally locked' so there is a danger that the side permanently facing the Star will be unbearably hot while the 'dark' side could be freezing cold. But research suggests that, if Proxima b has an atmosphere with a pressure at least 30% of what it is here on Earth, winds could transfer sufficient thermal energy to the dark side of the planet, which would be sheltered from dangerous UV radiation emitted by the red dwarf. Any people who went to colonise Proxima b might have to live on the dark side and create day and night artificially.

In order to determine whether Proxima b is habitable (or even inhabited), scientists need to study its atmosphere to look for gases like methane, water vapour or oxygen. And the best hope of this is a **transit** – when the planet (and its atmosphere) passes directly in front of the star across our line of sight. The wavelengths of light absorbed by the atmosphere will betray its composition. But there is only a 0.02% chance of this happening. Astronomers might be able to observe the atmosphere directly using the James Webb Space Telescope or powerful ground-based telescopes currently under construction in Chile and Hawaii (with mirrors 20 to 40 meters in diameter) or perhaps we could send a spacecraft.

Mike Follows teaches Physics.

Turn to the back page where Mike describes a project to send miniature spacecraft to explore Proxima b.

Breakthrough Starshot

Physicist and venture capitalist Yuri Milner is planning to send a fleet of over 1000 nanobots to visit Proxima b, the nearest exoplanet to Earth.

What's a nanobot? Each StarChip is a tiny spacecraft about one cubic centimetre in size with a mass of a few grams. It will take pictures as it flies by the exoplanet and transmit them back.

Why send so many? Collisions with dust particles as well as other mishaps mean that a single one is unlikely to reach its target, but in 20 years they will be cheap to make in large quantities.

A StarChip with its sail deployed

Yuri Milner shows a mock-ip of a StarChip at the project launch.

How fast will they travel? At 20% of the speed of light; their journey will take about 20 years.

How will they be powered? Once released from their mothership, each StarChip will unfurl a 4 metre square solar sail. An array of ground-based lasers will focus their beams for 10 minutes on each sail in turn in order to transfer 1 TJ of energy and accelerate them at about 100 km/s² – 10 000 times the acceleration of free-fall.

Ground-based lasers will accelerate the StarChips.