BIOLOGIC

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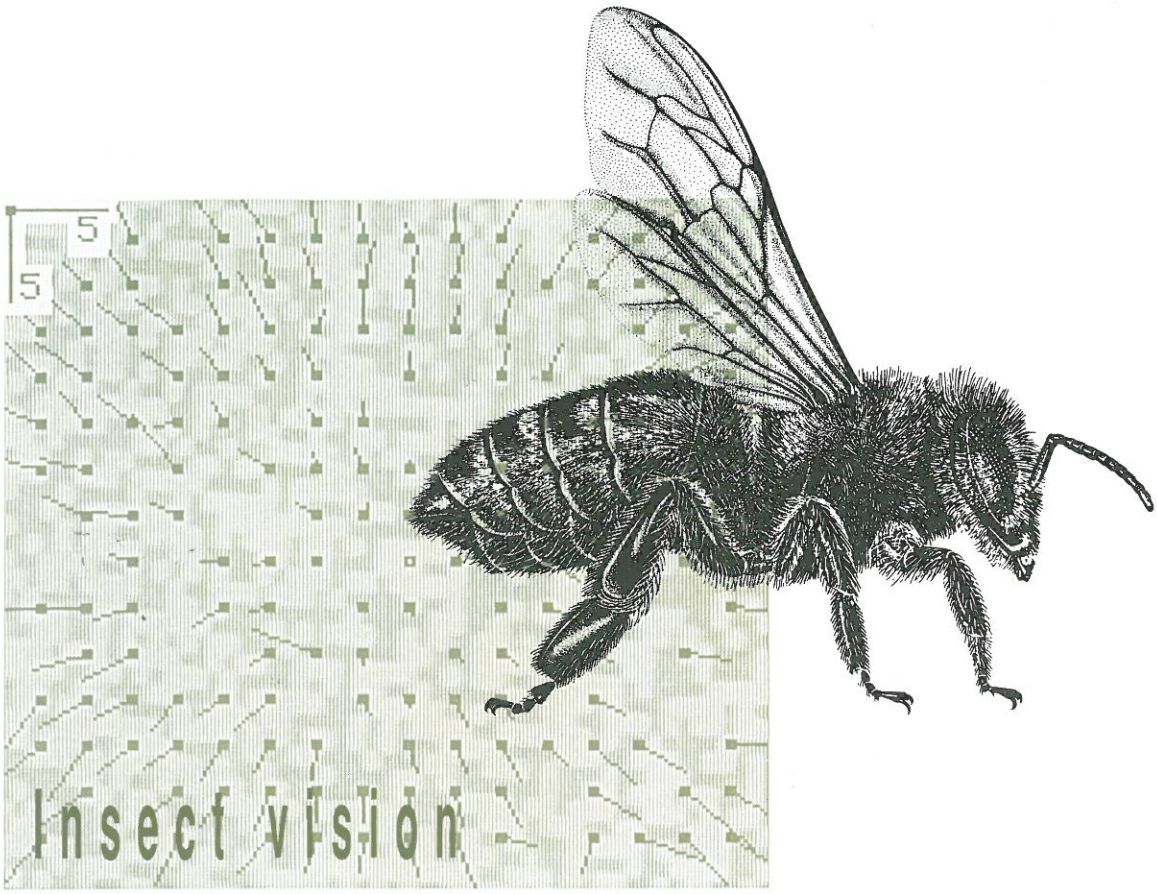
Research School of Biological Sciences
Australian National University



Inside

3. **The world through the eyes of an insect**
Surprisingly, perhaps, insects probably see the world like we do

7. **Victorin toxin — a key to unlocking disease-resistance in all plants?**
It's unexpectedly ubiquitous, extremely potent, and perhaps part of a vital cellular process common to all life

10. **Genetic help for a valuable symbiosis**
A boost for agriculture — subterranean clover engineered for growth in acid soils

12. **The most abundant enzyme: Nature's pinnacle or short-coming?**
A key photosynthetic catalyst appears slow and clumsy. Is it really, and can it be 'improved'?

16. **Using a visual illusion to test for glaucoma**
A set of flickering bars on a video screen could offer fast accurate diagnosis for this insidious eye affliction

20. **Direct from the Director**

The Research School of Biological Sciences at the Australian National University 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 10 research groups, organised as follows.

ECOSYSTEM DYNAMICS seeks to understand the structure and function of plant and animal communities. It develops theories of complex community dynamics, and how communities respond to environmental variations. The aim is to formulate models that can predict how communities will respond to different environments. Group leader: Dr Ian Noble.

DEVELOPMENTAL NEUROBIOLOGY studies the development and activity of nervous systems. Most work involves the sensory systems of hearing, vision and touch, using the sensors of mammals and birds and the eyes of crabs, insects and spiders. Much of the research makes use of an extensive wallaby colony, a facility that allows study of the coordinated development of vision and hearing in pouch young. Group leader: Professor Richard Mark.

DIRECTOR'S RESEARCH UNIT studies fundamental aspects of photosynthesis using novel techniques in biophysical chemistry, biochemistry and cell physiology, and explores their application to ecophysiological problems. Understanding photoinhibition — the process that reduces the efficiency of photosynthesis at high light levels — is pivotal in learning how plants respond to environmental and biological stresses. Group leader: Professor Barry Osmond.

MOLECULAR AND POPULATION GENETICS examines biological problems with genetic and molecular techniques using two organisms — yeast and the fruit fly (*Drosophila*). Because yeasts can be grown and manipulated like bacteria, they are ideal model organisms for experiments in cell biology. The research on *Drosophila* concerns the micro-evolutionary features that affect genetic variation in natural populations. Group leader: Professor John Gibson.

MOLECULAR EVOLUTION AND SYSTEMATICS applies the latest techniques in molecular and information science to study the evolution, systematics, identification and functional biology of selected organisms important to Australia. The work includes study of viruses, their vectors and hosts, as well as the molecular cytology and development of insects and their populations. Group leader: Professor Adrian Gibbs.

MOLECULAR STRUCTURE AND FUNCTION employs molecular genetics to study the development of brain and muscle and the genes that control growth and differentiation of cell types. Group leader: Dr George Miklos.

PLANT CELL BIOLOGY aims to explain the cellular basis of plant development and how it is regulated. The group also investigates cell division in plants using the latest techniques of molecular biology and molecular genetics. Group leader: Dr Richard Williamson.

PLANT ENVIRONMENTAL BIOLOGY conducts research into how physiological, ecological, biochemical and molecular attributes of individual plants contribute to their fitness in agricultural and natural ecosystems. A long-term goal is the identification of genetic elements that could be incorporated into commercially important agricultural species. Group leader: Dr John Andrews.

PLANT MICROBE INTERACTION studies plant pathology, resistance, symbiosis and defence systems. A particular focus of the group is the engineering of nitrogen-fixing bacteria for more effective nodulation of legumes. Group leader: Professor Barry Rolfe.

VISUAL SCIENCES aims to better understand how the optical image captured by the eye is analysed by the visual system to produce perception. To maximise the chances of success in this enterprise, research has concentrated on simple visual systems such as those of insects. Group leader: Dr Srini Srinivasan.

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

Research School of Biological Sciences
GPO Box 475
CANBERRA, ACT 2601

Phone: (06) 249 2999
Fax: (06) 249 4891

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The world
through the eyes of an insect

If you've watched a bee land deftly on a flower, tried to swat a fly, or glimpsed a dragonfly helicoptering over a pond, you will have begun to get a sense of how well these humble insects must see the world.

And yet the brain of an insect is minute: scientists estimate that all that exquisite aerobatics is the result of something like a million neurones packed into a brain fractions of a millimetre across and less than a milligram in weight.

How can the insect's sophisticated flight derive from such apparently small brain power?

This is a question that has intrigued Dr Srini Srinivasan at the Research School of Biological Sciences. Searching for clues, he and his colleagues have devised many ingenious experiments — largely calling for trained bees to fly through a visual maze in search of a reward — and recent results indicate that, surprisingly, insects probably see the world in a way not unlike we do.

> *"The difference between the compound eye of an insect and our eyes is only superficial."*

Complementary electrophysiology experiments on dragonflies by Dr David O'Carroll, also done at the Research School of Biological Sciences, reinforce these findings.

Until scientists were confronted with these results, they generally assumed that insects must view the world in a radically different way to us. It's hard enough to imagine how any other person sees the world — for example, what does a colorblind individual actually see? — but contemplating what it's like to look out at the world through multi-faceted compound eyes (as insects do) is to enter into foreign territory.

The eye of an insect is made up of a large number of separate light-detecting units called ommatidia, each with its own lens. What then does a fly see as it looks at the world through apparatus so different to a human retina? Does it really see the fly-swat in the kaleidoscopic way the accompanying cartoon suggests? In this connection, remember that we don't see the world upside-down even though the image on our retinas is 'upside-down'.

One widely held hypothesis is that insects recognise patterns by analysing the direction and flicker rate of images as objects pass by the facets of their compound eyes. In other words, it is the temporal properties of the information flowing to the insect's brain that are paramount. A large object would generate a slow flickering; a small one would give a higher flicker rate. Such a scheme would fit in nicely with the well-known finding — discussed later — that insect vision relies strongly on motion cues. Like a frog, an insect is nearly blind to stationary images.

A second view of insect vision is that insects somehow memorise patterns element by element, as if they had photographic memory, but it is hard to see how this squares with the limited size of the insect brain.

Fly's-eye view of a fly-swat (cartoonist's impression). Sorry to disillusion you, but recent research indicates that in fact flies see quite like we do.

An alternative view, suggested by Dr Srinivasan and his colleagues Dr Shao Wu Zhang and Mr Bernie Rolfe in a recent issue of *Nature* (8 April 1993), is that insects analyse the spatial properties of visual information in the same way as higher vertebrates (including ourselves) do. That is, insects extract specific features of a scene — such as a bar or an edge — and analyse its orientation.

"insects even perceive the same visual illusions that we do."

We devote a large fraction of our brains, the visual cortex, to decoding the visual information detected by our retinas. Insects have only a tiny knot of nerve cells, the optic lobe, but apparently they are able to do the same sort of 'cortical' processing. The great anatomist Cajal, when marvelling at the detail, delicacy and precision of the insect's optic lobe, likened it to a miniature watch, and he compared it to the great wall clock of the vertebrate's. They both tell the time, and they operate in the same way, but one is an incredibly scaled-down version of the other.

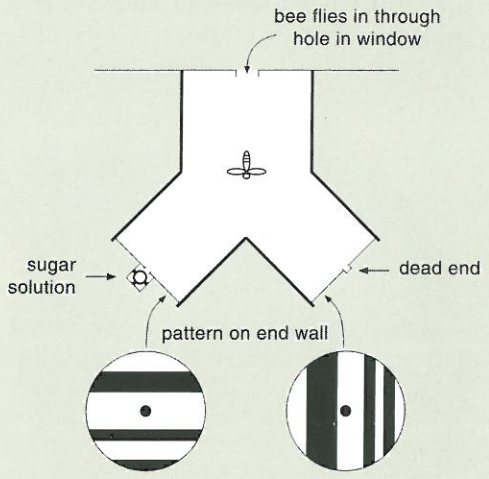
To get inside the mental machinery of an insect, Dr Srinivasan and his colleagues capitalised on the honeybee's fondness for sugar. He keeps a hive of free-flying bees outside his laboratory window, and when an experiment is planned, he lures some of them into his laboratory through a hole in the window. Tracking down the source of a rich sugar solution, the bees are confronted with a fork in the road: one path, say lined with vertical stripes, leads nowhere, whereas the other, lined with a horizontal pattern, leads to 'the good stuff'.

The bees that take the correct arm of the Y are sweetly rewarded, and receive an identifying spot of paint for their trouble. These bees return again and again, and soon learn to 'make a bee-line' towards the horizontal stripes. After a day's training, paint-spotted bees made the correct choice 87% of the time.

The game then gets a bit tricky. The experimenters replaced the stripes with rows of dots. That didn't faze the bees at all: they still preferred the horizontal configuration 85% of the time, suggesting that the bees could discern the over-all orientation of the pattern, not the shape of the constituent elements.

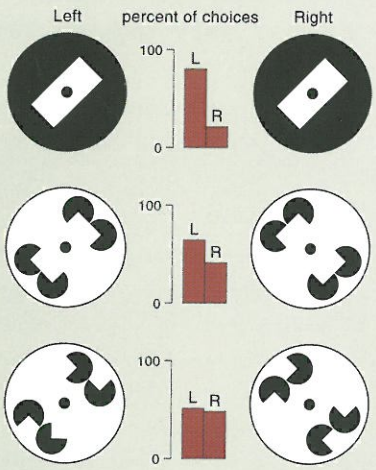
Perhaps, though, the bees were sensing orientation by detecting relative motion between themselves and the pattern? For example, a pattern of horizontal stripes might be discerned by the movement percept it gives in response to the bee's vertical motion.

To turn left or right?

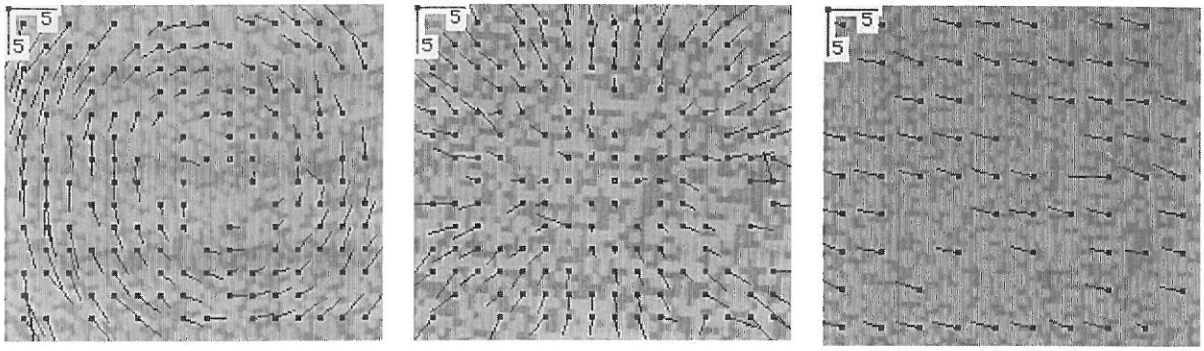


How the scientists tested the bees' visual discrimination. The bee learns to recognise a pattern (for example, horizontal stripes) that is associated with a source of sugar solution. The scientists then record the choices — left or right turns — the bee makes when it returns and is faced with different versions of the learnt pattern.

Bees, too, see an illusory white rectangle



Bees seem to see the same optical illusions we do. After being trained to choose between mirror-image patterns like those at the top, bees ably discriminate the patterns (middle) that show an illusory white rectangle, but fail to differentiate similar ones that don't (bottom).



Using angular speed as a cue to visual depth, an insect in flight will interpret these three patterns as rolling clockwise, zooming in, and moving right. A robot could be programmed to interpret, in the same way, the image from a video camera it carries.

But the experimenters convincingly ruled out that conjecture. First, they began moving the horizontal rows of dots in a horizontal fashion, so if directional motion signals were the key, this should confuse the bees. Not a bit of it; moving or not, the bees homed in on their lure as accurately as before. Secondly, the bee watchers used a video screen so as to give the approaching bees only a momentary glimpse of the horizontal and vertical patterns. The image of the patterns only lasted 2 milliseconds, so all images on the bees' eyes would be still ones. Nevertheless, the bees consistently preferred the horizontal pattern.

Getting even more devious, the experimenters next resorted to a random polka-dot pattern that could be moved either horizontally (in one arm of the Y) or vertically (in the other). If image motion were providing clues, horizontal-seeking bees should prefer the latter. Definitely not: in 82 flyins, 42 went in one direction; 40 went the other.

Finally, if the bees were somehow using image-motion cues to gauge stripe direction, we would expect they could easily distinguish a checkerboard that is moved vertically (in the rewarded arm of the Y) from the same pattern moved horizontally (in the unrewarded arm). However, after two days of training and over 60 trials, the bees couldn't even begin to tell the difference.

Be that as it may, there do seem to be more similarities than differences between the visual perception of insects and ourselves. As the diagram illustrates, insects even perceive the same visual illusions that we do.

The reported findings demonstrate that bees discriminate on the basis of shape, not by flicker from image motion. The bees are able to detect general features of patterns, and they can analyse new patterns and compare what they have in common with previously learned ones. That process is remarkably similar to how higher animals recognise patterns, and indeed Dr Srinivasan suggests that the bee possesses orientation channels just like those in the mammalian visual cortex.

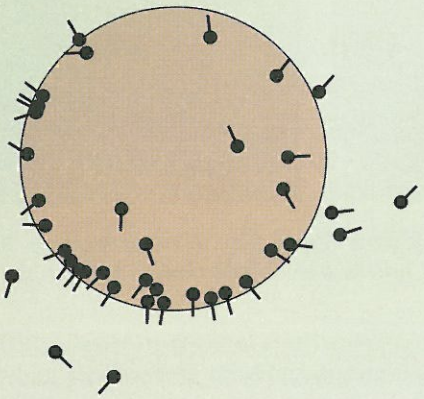
We judge orientation with the help of about 18 orientation-sensitive channels in our cortex, each of which responds to a preferred 10° range in angle. Of course, insects could easily get by with far fewer; in fact three would be enough for an insect to determine unambiguously the orientation of a pattern. Dr Srinivasan suggests that the hexagonal arrangement of the insect eye is well suited to feeding into orientation-tuned channels that can detect three preferred orientations differing by 120°. He also points to the parallel between the insect lobula, the neurones of which are organised in columns, and our own cortex, where the neurones are similarly organised.

The emerging parallel between the pattern-recognition abilities of insects and mammals is underlined by Dr O'Carroll's probings of the electrophysiology of the dragonfly brain. His experiments were done at the Research School of Biological Sciences, but he is now working at the University of Cambridge.

Dr O'Carroll penetrated a restrained dragonfly's brain using a fine electrode and searched for evidence that neurones in its brain were actually responding to spatial features of a presented image, rather than to flicker rate.

The eyes of dragonflies are among the largest of all insects, and the creature's ability to zoom-in on tiny quick-moving prey suggested to him that it must have an exceptionally well-honed facility for visual recognition (and response). His findings, again reported in the same issue of *Nature* as Dr Srinivasan's, showed up two types of cells that

Why a fly lands on the rim of your cup



While in flight, insects are very good at perceiving the distance of an object by observing the angular speed at which it moves by. Because of this, insects usually land near the edge of an object, since an edge provides a good clue to this characteristic. Proving the point, this diagram shows the actual landing points (dots) and direction of approach (tails) when bees landed in the vicinity of a raised disc.

responded to different image elements, just like cells in the cortex of higher animals do.

One type respond only to small moving targets. Objects only 1–2° in size elicited a strong response, while large objects gave little or no response. In contrast, other cells in the dragonfly brain responded best to elongated bars: the longer the bar, the bigger the response. Dr O'Carroll speculates that these orientation-tuned cells may underscore the pattern-recognition ability of bees that Dr Srinivasan detected.

Surprisingly, the responses of the dragonfly's cells as recorded by Dr O'Carroll were essentially the same as those studied in the cortex of the cat. It seems that the way insects and ourselves view the world has far more in common than we previously thought. There appears to be a marked 'evolutionary convergence', as Dr Srinivasan puts it, between the visual systems of insects and vertebrates. "The difference between the compound eye of an insect and our eyes is only superficial," he says, "and the way that the visual information is processed is not that different after all."

One of the implications of that similarity is that we may be able to derive some clues from the way insects see and use them to make robots 'see'. After all, insects must use very efficient image-processing strategies if a tiny brain can generate a comprehensive view of the world. In particular, insects seem to be able to use image motion to build up a three-dimensional picture of the world. Whereas we have two eyes, widely spaced, to do the same, insect eyes are virtually side by side.

Dr Peter Sobey of the Research School of Biological Sciences is therefore writing computer programs that mimic the insect's approach to sensing three dimensions. He is having success in deriving depth information using only one camera that moves — a 'cyclopean eye'. Indeed, a person with one eye tends to adopt a similar strategy and moves his or her head from side to side to get a sense of depth.

The advantage is that it is far simpler to extract depth information from two consecutive frames than it is to do the same from a stereoscopic pair of images. The upshot is that Dr Sobey is now close to succeeding in extracting continuous depth information at video rates: that is, he can just about use a standard work-station computer to derive depth cues at rates of 25 frames per second.

Not only may this be of benefit for supplying robots with vision, but it also could be used to give blind people a sensor that would allow them to freely roam their environment. Instead of a guide dog, they will, in effect, be using a guide 'insect'.

If you want to know more:

•Is pattern vision in insects mediated by 'cortical' processing? M.V. Srinivasan, S.W. Zhang, and B. Rolfe. *Nature* **362** (1993), 539–540.

•Feature-detecting neurons in dragonflies. David O'Carroll. *Nature* **362** (1993), 541–543.

•You are welcome to contact Dr Srinivasan via the address and phone numbers given on page 2.

Dr 'Srini' Srinivasan.

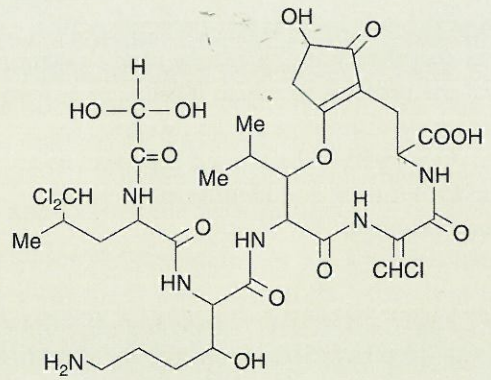
Victorin toxin:

a key to unlocking disease-resistance in all plants?

Isolating and cloning a plant's disease-resistance gene is one of the tantalising goals of plant geneticists. Although many have tried for years, scientists at the Research School of Biological Sciences are undaunted, and they believe they may have a good chance of isolating that elusive gene.

The secret may be to use a 'hook' — victorin toxin — that appears to be involved with a disease-resistance gene in oats. Although this gene is specific to oat crown rust, the scientists hope that the special properties of this system will allow them to follow a trail — at the end of which might be found the resistance genes in other plants and for other plant diseases.

Small, but potent



The chemical structure of victorin C: a partially cyclic pentapeptide. When labelled with iodine-125 or sulfur-35, it acts as a very effective probe for locating the toxin receptor that may be linked to the disease-resistance gene against rust.

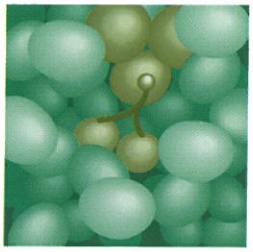
Oats that have succumbed to victorin toxin (left), and a variety (right) that is resistant to the toxin.

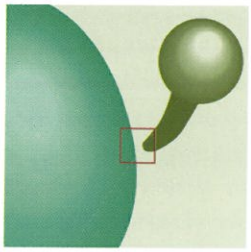
Besides passive resistance (afforded by preformed barriers), plants can actively resist pathogens in two ways. First, they have a general resistance, triggered by most invaders, that is part of the plant's biochemistry and physiology. Second, they have a very specific resistance controlled by a certain gene that somehow engages the genetic machinery of the pathogen. Plant breeders use this 'gene-for-gene' resistance to combat the majority of serious agricultural diseases.

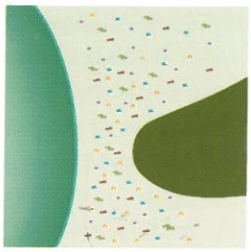
It is this second avenue that attracts the attention of plant molecular biologists. Many of them are working towards cloning a resistance gene and working out the stratagem it uses. The task has proven elusive because of the extreme difficulty of identifying a resistance gene among a million other clones in a gene library. The gene can only be conclusively pinpointed after experiments show that an identified gene confers resistance against the correct pathogen, or we find the expected hypersensitivity to the product of the pathogen's unique triggering gene (called an avirulence gene).

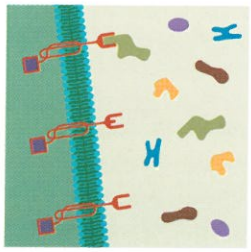
Compounding the problem, the molecular basis of recognition responses in plants is a mystery. However, if we could hook a beacon on to the plant's molecular receptor (where the recognition response takes place), we would be well on the way to isolating the responsible gene. A team of scientists at RSBS, led by Professor Barry Rolfe and Dr David Loschke, believe that a remarkable toxin called victorin C may be the beacon needed to track down the gene in oats. Once they understand the mechanism in the oat plant, they hope they will be able, eventually, to confer strong, durable disease resistance in a very wide range of crops.

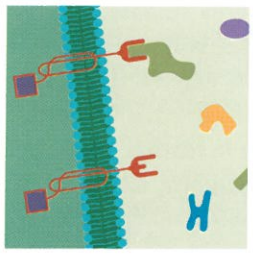
Dr Loschke and his colleagues — Dr Hancai Chen, Dr Luba Tomaska, Dr Ian McKay, Mrs Guanju Xu and Mr Liangcai Peng — are starting with the unique interaction between the oat plant and a pathogenic fungus called *Cochliobolus victoriae*. This fungus produces a toxin called victorin, which is one of the most potent plant toxins known. A quantity of 6 mg is sufficient to kill an entire hectare of oats.











Battlefield strategy. In this schematic sequence, the plant is seen using its genetically programmed membrane receptors to recognise and disable attacking emissaries from an approaching pathogen. Victorin is one fungal toxin that some oat varieties can disable; finding out why has implications way beyond oats.

Importantly, victorin toxin is only toxic to oat plants carrying a specific gene called *Vb*. Furthermore, the dominant *Vb* allele, while engendering sensitivity to victorin, also is genetically inseparable from *Pc-2*, a dominant gene that confers strong resistance to oat crown rust, a disease caused by a completely different fungus, *Puccinia coronata*. It's a good bet, therefore, that the two traits are encoded by the same gene, or at least are members of a common complex locus. This idea has been strengthened by the persistent failure, despite intensive efforts over decades, to separate rust resistance from toxin sensitivity. Like heads and tails of a coin, the two stay together.

The victorin toxin has been extracted from fungal cultures and purified. It separates into five forms: the predominant victorin C (see the diagram), and four other closely related structures. All are chlorinated, and are small partially cyclic peptides.

Victorin C can be radioactively labelled — usually with iodine-125 or sulfur-35 — without losing any of its special properties. Moreover, because it binds to its target proteins with a strong co-valent bond, it can be used to identify any of the proteins to which it binds at any stage of the purification process. In this way, the researchers at RSBS have used the toxin as a probe for locating and identifying victorin-binding proteins.

"every fungus and bacteria seems to possess this victorin-binding protein; it even exists in humans."

It turns out that there are 10 binding proteins, with molecular weights ranging from 12·5 to 98 kilodalton. One of these proteins may be responsible for toxin sensitivity or disease resistance, but no-one can say, as yet, which.

The researchers have evidence that one of the proteins (of 45 kD) is in the outer plasma membrane. The others appear to be members of a larger group, some of which bind to victorin, and some not. The research team seem to be closing in on their quarry, as they have now been able to show, for the first time, a difference in the victorin-binding properties of resistant and susceptible plants.

But the implications go further than just oats. Up until now, victorin has never been thought of as anything except a potent toxin against the *Vb* oat mutant, although considerable interest has been aroused by its unique interaction with the oat's disease-resistance gene.

However, when the ANU researchers tested other species of plants for binding with victorin, they were surprised to discover, in every higher plant examined, both monocots and dicots, that there were similar victorin-binding proteins, similar molecular weights, and similar binding properties. In other words, the highly selective system that is at work in the oats–victorin interaction seems to reflect important biochemical machinery that is universal in the plant kingdom.

But an even bigger surprise was waiting. When the team went further and tested for victorin binding to proteins in other unlikely species — both eukaryotic and prokaryotic organisms — one 12·5-kD protein was always present. That is, every fungus and bacteria seems to possess this protein. It even exists in mammals, including humans.

What is it doing? Its universality suggests that this small protein may be performing a vital physiological function. The ANU team has no idea yet what this may be, but work so far has established that the protein involved, although ubiquitous, is rare among the cell's total protein.

However, the abundance of this small protein exceeds by many times that of the other, larger, victorin-binding proteins. Over 90% of all victorin binding is done by this small protein, and so it is the first one the team is trying to purify and sequence.

Once the vital sequence information is at hand, the team want to use it to design special probes to identify the genes from the large oat DNA library that they have assembled. Better still, they will then be able to examine the molecular biology of simpler plants, such as *Arabidopsis*, and of yeast and bacteria.

A nucleic-acid probe made from the sequence of the 12·5-kD protein may allow the larger victorin-binding proteins to be identified as well, which would avoid the need to separately purify and sequence these proteins.

The team at RSBS is excited by the possibilities. The unexpected ubiquity of victorin-binding proteins, combined with the extreme potency of the toxin, suggests that they have uncovered a very basic and vital cellular process that is present in all living cells.

Although we still don't know what that process is, they have begun a number of experiments to get some clues. They are studying yeasts and bacteria to find out how the protein structure, organisation, and function is modified by victorin binding.

In the end, they not only may get an insight into how plant disease-resistance genes work, but also a privileged look through a new window — an intriguing physiological mechanism common to all life.

If you want to know more:

- A universally conserved vital protein revealed by victorin binding. D.C. Loschke, L. Tomaska, H. Chen, Z. Hong, B. Rolfe and D. Gabriel. In: 'Advances in Molecular Genetics of Plant–Microbe Interactions,' vol. 2, pp. 587–591. Ed. by E. Nester and D. Verma (Kluwer: Dordrecht, 1993)
- Gene-for-gene recognition: the ion channel defense model. D.W. Gabriel, D.C. Loschke and B.G. Rolfe. In: 'Proceedings, 4th International Symposium on Molecular Genetics of Plant–Microbe Interactions,' pp. 3–14. Ed. by R. Palacios and D. Verma. (APS Press: St Paul, Minn., 1988)

- You are welcome to contact Dr Loschke via the address and phone numbers on page 2.

The funding for this program has been provided in part by a private syndicated research and development group organised and administered by ANUTECH Pty Ltd. Other funds came from ANUTECH itself and the ANU.

Dr David Loschke.

GENETIC HELP

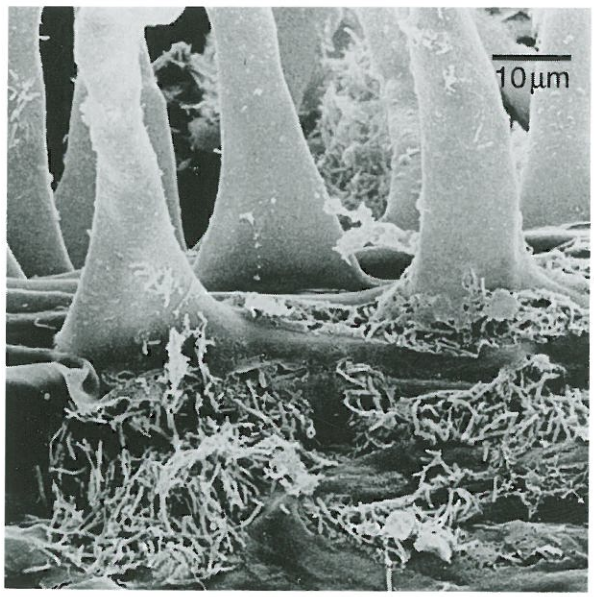
~ for a valuable symbiosis ~

Geneticists at the Research School of Biological Sciences have developed a new strain of acid-tolerant bacteria that allows subterranean clover to flourish in acid soils. The new *Rhizobium* strain, which works in symbiosis with clover to fix atmospheric nitrogen, creates the potential for 10 million hectares or more of our impoverished acid-affected agricultural soils to stage a comeback.

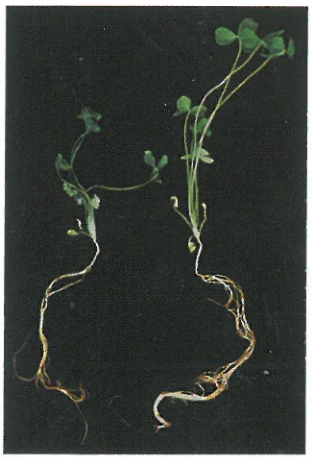
Subterranean clover is sown over thousands of square kilometres of Australia, creating improved pasture that is the backbone of our livestock industry. This Mediterranean legume is valued for its ability to harbour in its roots certain soil bacteria — rhizobia — that can take nitrogen from the air and turn it into ammonia. This process of nitrogen fixation occurs in special root outgrowths called nodules, and the ammonia produced in them is used by the plant as a fertiliser, saving an estimated $2 billion a year in the application of chemically produced nitrogen fertiliser.

Sub clover's benefits are recognised not only by pasturalists. Wheat farmers in marginal agricultural areas of south-western Australia often plant wheat and sub clover in rotation, a long used agricultural practice that makes use of the persistence in the soil of enhanced nitrogen levels.

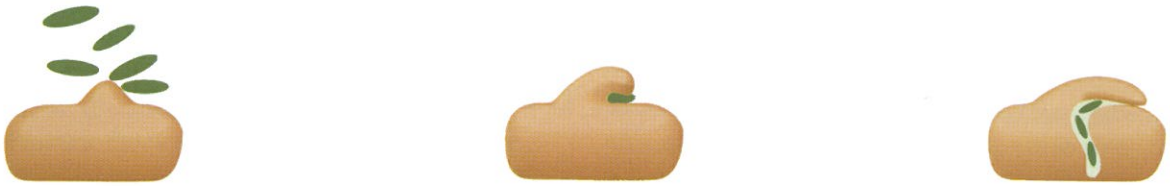
However, one unfortunate side-effect of growing sub clover is that acid levels in the soil gradually increase. This acidity can create nutritional imbalances: deficiencies in calcium and molybdenum, and toxicity from aluminium and manganese. In turn, agricultural production can slump, and even the effectiveness of the clover–*Rhizobium* relationship can suffer. As acid levels rise, the plant continues to grow, but it often ceases to harbour the symbiotic bacteria. In other cases, its root nodules are found to be populated by non-nitrogen fixing rhizobia of little agricultural use. Is the breakdown in this beneficial relationship under acid conditions due to the plant, the bacteria, or both?



A worm's-eye view of rhizobia on the surface of a clover root. The thread-like bacteria can be seen on the root proper and on its root hairs.



How acid-resistant bacteria can improve clover growth. On the left is a clover plants grown at pH 4·4 using normal rhizobia derived from the field. On the right is a clover plant grown at the same pH and using genetically manipulated bacteria.



Since 1985, research at the Plant–Microbe Interaction Group at the Research School of Biological Sciences has been investigating the subtle interplay between the bacterium and its host, and they have gained much insight into what is going on biochemically and genetically. And they have used genetic manipulation techniques to devise an acid-toleratant *Rhizobium* strain that possesses enhanced capacity for nitrogen fixation.

"the mutual signalling is remarkably specific."

Dr Michael Djordjevic and colleagues have studied the complex chemical signalling that occurs in a 'courtship' that eventually leads to the formation of a root nodule populated by rhizobia. He has found that low molecular weight flavonoid compounds are exuded from the plant to the bacterium, which cause it to express specific genes and release specific reciprocating chemicals of the lipo-oligosaccharide family. Concentrations in parts per billion are all that are needed.

Recently, Dr Ian McKay and Dr Djordjevic have shown that, even if rhizobia can grow at low pH, their ability to generate the right lipo-oligosaccharide signals under acidic conditions varies from strain to strain.

If all goes well and the answering chemical signal from the bacterium has sufficient strength and the correct structure, emerging root hairs begin curling in preparation for admission of the colonising rhizobia. The rhizobia are then free to break down the cell wall and infect the plant. The rhizobia proliferate in the root tissues and cause plant cells to grow and divide — producing distinctive root nodules.

The mutual signalling is remarkably specific. For example, *Rhizobium* strains geared to infecting alfalfa plants are incapable of inducing entry to clover plants, and similar recognition between host and colonising bacteria goes on with every other legume.

During the early stages of plant infection, rhizobia can be considered parasites in that they derive nutritional elements from its host and cause many subsequent changes in plant growth and development. However, the bacterial invasion causes minimal disruption to the plant's metabolism, and once nitrogen-fixing nodules are established, the plant benefits by having all its nitrogen requirements met by the bacteria. Legumes and rhizobia have learnt to live together in a beneficial symbiosis.

A small number of companies in Australia actually culture particularly active rhizobia and

continued on page 15

If you want to know more:

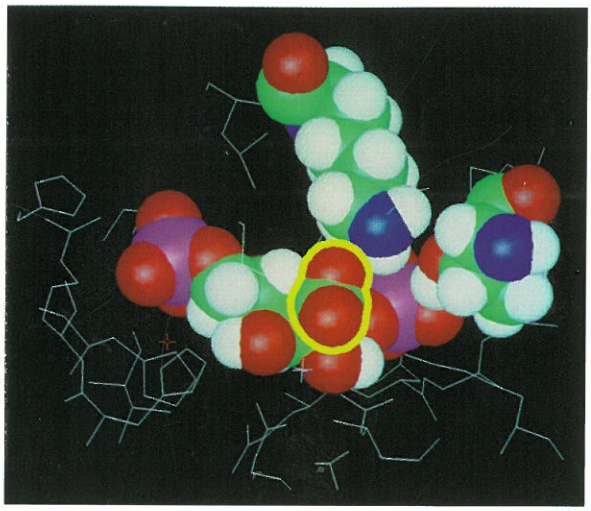
- Factors determining host recognition in the clover–Rhizobium symbiosis. M.A. Djordjevic and J.J. Weinman. *Australian Journal of Plant Physiology*, **18** (1991), 543–557.
- Studies on the physiological and genetic basis of acid tolerance in Rhizobium leguminosarum biovar trifolii. H. Chen, A.E. Richardson and B.G. Rolfe. *Applied and Environmental Microbiology*, **59** (1993), 1798–1804.

- You are welcome to contact Dr Djordjevic via the address and phone numbers on page 2.

Dr Michael Djordjevic.

The most abundant enzyme:

Nature's pinnacle or short-coming?



Caught in the act. A carbon dioxide molecule (outlined) is captured in Rubisco's molecular embrace to become part of a sugar substrate (left). The arms in the middle and right are amino acids crucial for distinguishing carbon dioxide from oxygen, as experiments using different amino acids have shown.

Life on earth depends on a unique plant enzyme called Rubisco. Without this catalyst, plants could not convert atmospheric carbon dioxide into organic carbon. It is the world's most abundant protein, and photosynthetic cells invest about one-quarter of their precious nitrogen budget in it. Having such a key place in the pattern of life, scientists expect Rubisco to have passed, over the aeons, intense selection pressure for speed and accuracy.

And yet they find, surprisingly, that Rubisco is both slow and clumsy. It is abundant only because it's so slow in getting the job done, and it only poorly distinguishes between the starting material of photosynthesis (carbon dioxide) and the product (oxygen).

Plant biologists at the Research School of Biological Sciences are trying to work out why this enzyme appears to be such a grossly inefficient catalyst. Is it really so maladapted, or have we, with our limited insight, misunderstood what is actually going on?

Dr John Andrews and Dr Matthew Morell are trying to answer this question. If indeed Rubisco can be "improved", either by random genetic changes or deliberate genetic engineering, the consequences for crop productivity and global ecology would be profound.

As implied by its full name (D-ribulose-1,5-bisphosphate carboxylase/oxygenase), Rubisco has a split personality. Firstly, and most importantly, it is the enzyme that catalyses the critical initial step in photosynthesis — the reaction (carboxylation) of carbon dioxide into the metabolic machinery of the plant. But, mysteriously, it also acts as an enzyme that catalyses a competing reaction — an oxygenation step that is the initial step in a photorespiration cycle. In the cycle, some of the carbon diverted from photosynthesis by the 'faulty' oxygenation step is retrieved, but it consumes additional energy and some water and carbon dioxide is lost from the plant. "Photorespiration seems to serve no useful purpose," says Dr Morell. "Has Nature made a bad mistake, or has She seen something that we as yet haven't?"

Of course, we can understand that, in ages past, when life began on this planet, the oxygen content of the atmosphere was negligible and so Rubisco could have arisen without consideration of a competing oxygenase reaction. Could it be that when Rubisco evolved under these conditions it failed to accommodate for the long-term result of its activity — an oxygen-abundant atmosphere — which would severely limit its effectiveness so many years later?

That would indeed be ironic, but surely, since the present unfavourably high oxygen levels have existed for at least the last 60 million years, plants should have had time to make suitable evolutionary adjustments?

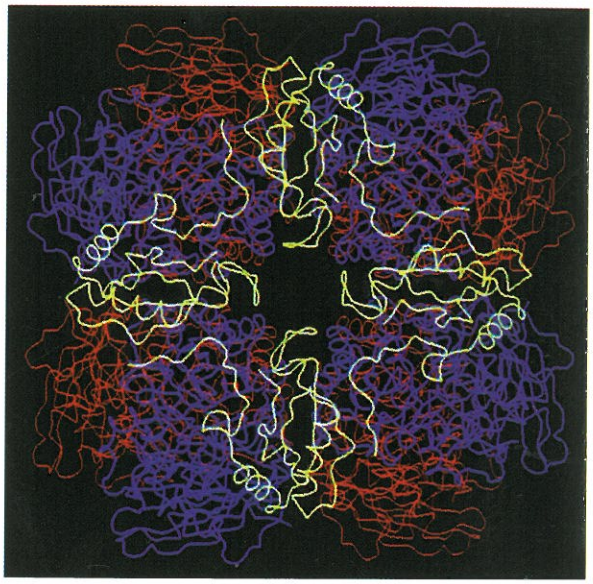
Amino-acid sequencing has shown that all present-day Rubisco is descended from a common ancestral protein that arose some 3500 million years ago. And there is evidence, derived from examining the performance of slightly different Rubisco enzymes from different plants, that some evolutionary progress has been made, although it seems that Rubisco has been remarkably unresponsive to selective pressure to discriminate CO_2 from O_2.

When the differences in speed and CO_2/O_2 discrimination are measured, we find that some plants — such as spinach — have managed to improve their performance compared to the poorest performer. As you would expect, the wooden spoon in this contest goes to certain anaerobic bacteria that have had no incentive — because of their airless habitat — to lift their game.

Nevertheless, even the spinach Rubisco has only managed to raise its ratio of carboxylation to oxygenation to no more than 2·5 : 1 (for typical oxygen and carbon dioxide concentrations). This means that for every 100 molecules of CO_2 fixed by photosynthesis, some 20 of them will be lost again by photorespiration. From our point of view, that's a regrettable waste, but if all Rubisco had stayed like that of the anaerobic bacteria's, we'd have a far worse situation: the carbon balance would be *negative*! That is, under present-day oxygen levels, this enzyme photorespires 70% more CO_2 than it fixes by photosynthesis.

Although we shouldn't begrudge the improvements that spinach and its cousins have achieved, their performance pales in comparison with that of some other enzymes Nature has created. For example, a certain enzyme used to assemble proteins — tyrosyl-tRNA synthetase — can discriminate tyrosine from phenylalanine (which only differ by a single hydroxyl group) with a precision of 100 000 to 1.

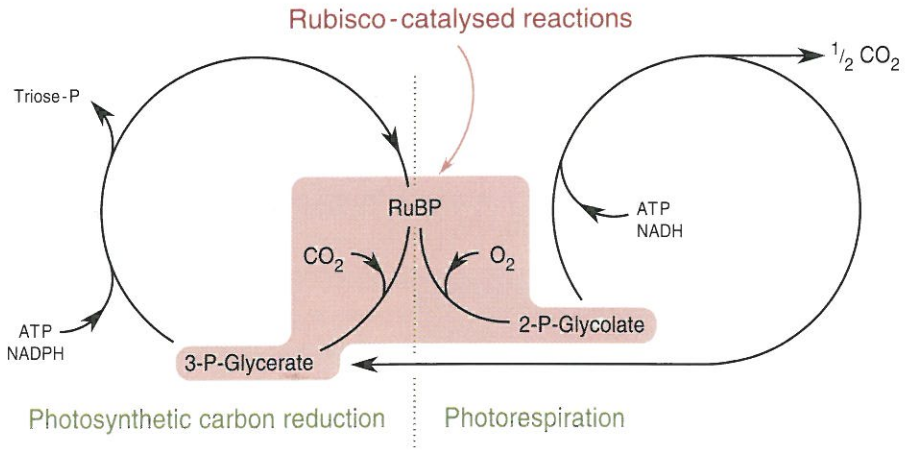
This performance is about 1000 times better than Rubisco's in its discrimination of CO_2 and O_2, and again arouses the dream of biologists to artificially assist Rubisco overcome its limitations. If we could reduce the oxygenation reaction, or even select it out of existence, that would produce untold benefits for agriculture.



The tangled web of a Rubisco molecule. It is made up of eight large sub-units (blue) and eight smaller ones (red). Here viewed from one end, only half its constituents are visible.

Given the size of the enzyme — 500 kilodalton or so — and the subtleties of its genetics and biochemistry, that goal is proving to be a tall order, but, as Dr Morell says, "the prize is so tempting that mere difficulty will not daunt the adventurous from joining the quest for this particular 'Holy Grail'."

Work in the Plant Environmental Biology Group at RSBS has elucidated three benefits of a super Rubisco. First, of course, is the energy efficiency of the plant. At present, plants expend large amounts of ATP in recovering three-quarters of the carbon lost through oxygenation. Some idea of the size of this expenditure can be gauged by growing plants under high CO_2, or low O_2, where this loss is side-stepped. In both cases, the plant utilises 50% more light.



Rubisco stands at the centre of the two major photosynthetic pathways. This vital enzyme catalyses the incorporation of atmospheric CO_2 into the plant (left); but why does it also catalyse the reaction of atmospheric O_2 (right), which serves no apparently useful purpose?

Another benefit would be improved water-use efficiency, and a relevant bench-mark here is the C4 plants, where smaller stomatal apertures and lower CO_2 levels are needed for them to grow as well as their C3 counterparts. Finally, there is the freeing up of nitrogen reserves: the vast quantity of this nutrient presently bound up in Rubisco (about one-quarter of the leaf's soluble nitrogen) could be released for other metabolic activities.

How then can we improve Rubisco? Of course, it could be that, after 3500 million years of Nature's selection experiments, Rubisco is 'perfect' in a sense we do not comprehend, and that no further improvement is possible (without damaging some other photosynthetic process). Nevertheless, Rubisco is now the most studied plant enzyme, and as yet we see no theoretical limit to how efficient Rubisco could be. "Attempts to improve Rubisco are, at the very least, not hopeless," says Dr Morell, "and even small improvements could give tangible benefits for crop growth."

"Rubisco has a split personality."

However, since Rubisco has been under intense selection pressure for so long, it is unlikely that improvements can be got by simple means, such as by a single amino acid change (which would have happened naturally and frequently). Dr Morell looks at it this way. In the 4 billion years of life on earth, there may have been 10^{40} photosynthetic organisms. Although that number has allowed, by random mutation, some evolutionary improvement in the Rubisco enzyme, that protein comprises 20^{600} potential combinations of amino acids, so there must be vast numbers of untried combinations.

In other words, the gulf between present-day Rubiscos and the super varieties that potentially exist might be too wide to have been bridged by natural evolutionary means in the available time-span.

Dr Morell thinks that progress will call for extensive changes involving simultaneous multiple changes in amino acid sequences. There are two ways in which this might be done.

First, Dr Morell and Dr Andrews are developing new biological systems for selecting Rubiscos with enhanced performance. For example, one system involves the use of a bacteria — *Escherichia coli* — instead of a plant. They mutagenise the gene for Rubisco formation and insert it into the bacteria; then they place the mutants in an environment (high oxygen, low carbon dioxide) where growth is only possible if an improved Rubisco is present. In this way, the mutants are put under intense selection pressure and screening is automatic. Because genetic transformation of *E. coli* is so efficient, it is possible to screen as many as 10^{13} different mutant Rubisco genes in a single experiment. In comparison, to screen the same number of plants would take a field the size of New South Wales!

A second approach — site-directed mutagenesis — aims to design a Rubisco with improved performance by putting to use our understanding of the basic principles of organic chemistry, in particular the relationship between the structure of a protein and its catalytic function. The idea here is to use X-ray crystallographic data, obtained in collaboration with a group at the Swedish Agricultural University, to examine the catalytic site and rationally decide how to build a more efficient three-dimensional structure for it.

The ANU researchers have experimented with changing the position of a phosphate group in a cyanobacterium, and have seen clear changes in the ensuing enzymatic efficiency — so far, mostly for the worse, it must be said. Nevertheless, the specific ways in which these enzymes are poorer catalysts is extremely informative. Yet because of the great subtlety of Rubisco's catalysis, Dr Andrews is undecided on the question of whether a super-efficient Rubisco could be captured or designed.

If it could, such a spectacular achievement would mean much for improved crop production. Indeed, if all plants were to employ a theoretically perfect Rubisco, all our present concerns about an excessive level of atmospheric carbon dioxide would vanish — to be replaced by exactly the opposite problem! Carbon dioxide would be scavenged from the atmosphere, and the earth would then be cooler. With a vastly greater biomass, the world might resemble that which prevailed during the Carboniferous era.

We are again reminded how all life is delicately balanced, and that Rubisco, standing at the interface between the organic and the inorganic, occupies a pivotal place in the scheme of things. Understanding it, and modifying it, is within our grasp.

If you want to know more:

- Rubisco: maladapted or misunderstood? M.K. Morell, K. Paul, H.J. Kane, and T.J. Andrews. *Australian Journal of Botany*, **40** (1992), 431–441.
- Rubisco: structure, mechanisms, and prospects for improvement. T.J. Andrews and G.H. Lorimer. In: 'The Biochemistry of Plants,' vol. 10, (New York: 1987).

- You are welcome to contact Dr Morell via the address and telephone numbers on page 2.

Dr Matthew Morell.

continued from page 11

sell the material to farmers, who spread it on their pastures to inoculate clover. The increased nodulation produced by these strains gives the farmer higher soil nitrogen levels.

The group's research has demonstrated that when acid levels increase, the first partner to suffer is the bacteria, not the plant. Clover will grow in soil as acid as pH 4·0, but bacteria fail to thrive when the pH goes below 4·5. Studies by Dr Alan Richardson (then a PhD student with the PMI group) showed that at low pH the clover was still sending out its chemical lure, but not all the bacteria could respond in kind. Only certain acid-resistant strains could switch on their genetic machinery and adequately respond under such demanding conditions.

So why not culture these acid-resistant strains and spread them round the country-side? Indeed, a similar strategy has been used with another *Rhizobium* that induces and colonises nodules on the roots of the pasture legume, medic. Acid-resistant strains of the bacterium now enable medic to thrive over 500 000 ha of Western Australia in soils that had previously been considered too acidic to support medic growth.

Unfortunately, however, the same procedure can't be used on sub clover. The big stumbling block here has been that all the acid-resistant strains isolated by the researchers have, inexplicably, been very poor at nitrogen fixation.

At this point, a little genetic manipulation seemed called for.

Dr Hancai Chen took an acid-tolerant strain and used recombinant DNA techniques to remove its nitrogen-fixating genes; he then replaced them with genes from a strain with good levels of nitrogen fixation. The result is a hybrid strain that, in the laboratory, has been shown to grow on media as acidic as pH 4·5, while retaining the capacity to fix agronomically useful amounts of nitrogen.

Encouraged by this success, the researchers have progressed to plot trials, and plans are being made for field trials.

Using a visual illusion to test for glaucoma

Glaucoma is an insidious affliction in which visual cells in the retina progressively degenerate; the sufferer first, imperceptibly, loses peripheral vision, is soon left with 'tunnel vision', and if untreated, finally ends up blind. The disease affects about one in every 50 Australians over the age of 40, and is one of the three leading causes of blindness in those over 55.

A real problem is diagnosing the disease before the retinal cells are irretrievably damaged. By the time sufferers notice that their vision is impaired, a lot of damage has often been done. Yet to confirm a diagnosis of glaucoma using conventional tests typically requires three sequential visits to an ophthalmologist, a process that can take many weeks, even a year, a time-span that can permit deterioration in vision.

Dr Ted Maddess of the Research School of Biological Sciences has developed a test for glaucoma that simply requires a person to look at a specially arranged set of flickering bars on a video screen. If he or she can readily perceive a visual illusion — that the number of bars on the screen is double the number actually displayed — then their retinal cells are fine. But if the illusion is difficult to see, then they have a high chance of suffering glaucoma.

Tests currently in train with a practising ophthalmologist are showing good results. Dr Maddess is hopeful that the test could be offered commercially to ophthalmologists throughout Australia and overseas in the near future.

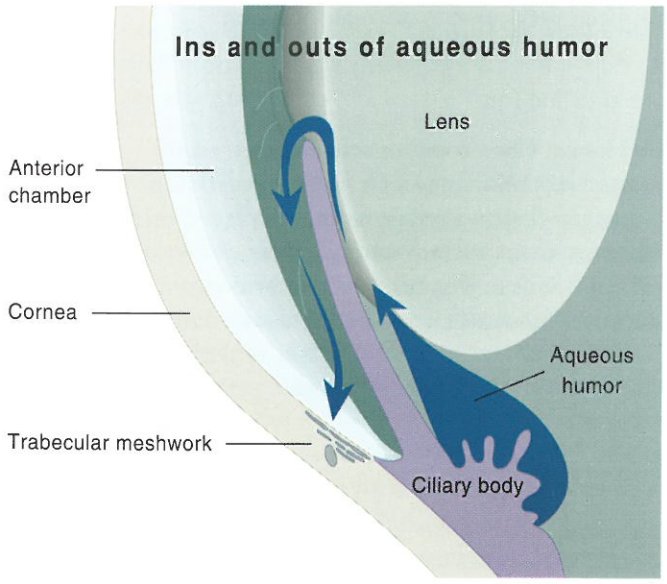
"by the time sufferers notice that their vision is impaired, a lot of damage can be done."

Like most diseases, glaucoma has been afflicting mankind since the beginning of recorded history. In the Aphorisms of Hippocrates, the term 'glaucoma' was used to describe blindness appearing in advancing years. The name derives from the bluish-green cast that a blind pupil assumes.

A common cause of glaucoma is high pressure within the eyeball. When suffering from excessive pressure, an eyeball will sometimes feel hard, like an over-inflated basket-ball. This hardness to the touch used to be one of the signs doctors last century used to diagnose glaucoma.

The eye has its own pressure-regulating system which, when operating normally, keeps the eyeball in its correct shape. As the diagram shows, the fluid involved — aqueous humor — is constantly produced by the ciliary body and flows out via the trabecular meshwork to the veins. Overproduction of aqueous humor, or a blockage in the drainage system, can give rise to excess pressure.

Commonly, the raised pressure is a chronic condition, which can be alleviated by certain drugs, usually beta blockers. However, a sudden rise in pressure can sometimes occur, and this acute condition calls for swift surgical intervention to save sight.



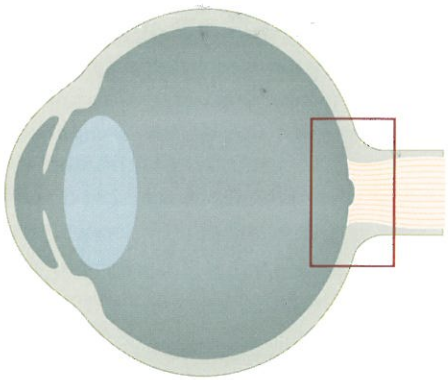
Aqueous humor is produced in the ciliary body and drains, via the trabecular meshwork, to the veins. Overproduction of fluid, or blockage in the exit route, can cause increased intraocular pressure, and glaucoma.

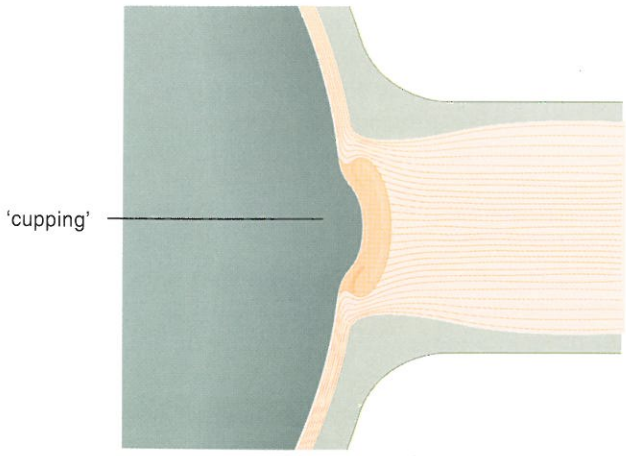
Specific diagnostic instruments have been developed in which the pressure in the eye can be directly measured. These days, an ophthalmologist or optometrist will suspect glaucoma if intraocular pressure exceeds 21 mm of mercury.

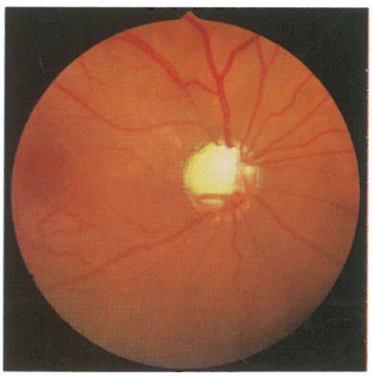
However, scientists have discovered recently that pressure is not the only agent that can destroy retinal cells. Whereas a pressure of 50 mm can rapidly cause blindness, some people can have persistent high pressure with no accompanying visual deficits. In contrast, others develop impaired vision while experiencing no rise in pressure.

As Dr Maddess says, "In the past, glaucoma used to be seen as a condition caused by high pressure in the eyeball. But we now know that about 40% of glaucoma sufferers have normal pressure levels. And 8% of people over 50 have elevated pressure but no glaucoma."

A sure sign of glaucoma







Although the causes of glaucoma are not at all clear, it seems that a deficit in blood supply to the retina — which can be aggravated by increased intraocular tension — underlies most cases. Given that the affliction has a combination of somewhat elusive precipitating factors, how can an early diagnosis be made?

"it relies on the fact that large retinal cells are more easily damaged than smaller ones."

The most accurate indicator to date is for the ophthalmologist to perform a test called perimetry, in which a person is asked to detect a small spot of light in various parts of their peripheral vision. If, at certain areas, the spot needs to be relatively bright to be seen, glaucoma is indicated. The ophthalmologist may prescribe drugs to reduce intraocular pressure, and ask the patient to come back in a few weeks. Only if the deficit remains over three sequential visits will glaucoma be confirmed.

"The problem with perimetry," says Dr Maddess, "is that it may take about a year for a diagnosis of glaucoma to be confirmed. Moreover, because of limited precision of the test, it is difficult to gauge if vision is deteriorating, and if medication is having the sought-for effects. We hope that our new test can allow an ophthalmologist to get a definite yes or no in a single visit ... it could be a reliable front-line screening device for glaucoma."

Death of retinal cells can cause depression of the optic disk — where the optic nerve leads away from the retina — in a process known as 'cupping' (left). This can be seen in an eye examination through the pupil (right).

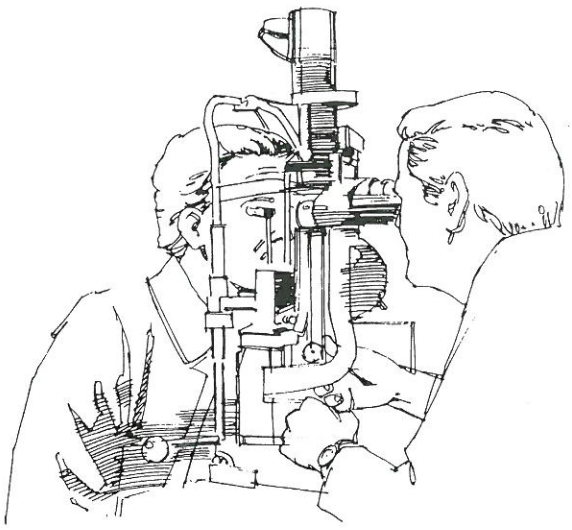
How glaucoma progresses

Peripheral vision deteriorates first, often imperceptibly. With further loss, tunnel vision develops, and if uncontrolled, total blindness can result.

Even if the test turns out not to be as conclusive as that, it would appear to be much more rapid and easy than existing tests, and would seem to be ideal for monitoring the efficacy of medication. "The ANU glaucoma test gives a precise numerical read-out of the threshold contrast needed for the patient to see the illusion, and if this threshold stays constant," says Dr Maddess, "then we can be confident that the glaucoma is under control."

One conclusive test for glaucoma — although only for advanced cases — is for the ophthalmologist to photograph that part of the retina where the optic nerve begins (the region responsible for the 'blind spot') and examine it for evidence of 'cupping'; death of retinal cells causes the optic nerve to be depressed and resemble a cup. The problem here is that clear photographs are not always easy to come by. Drugs can cause contracted pupils, and cataracts and other causes of cloudiness can obstruct the view. Gauging the extent of cupping is an inexact technique.

The ANU glaucoma test relies on the fact that large retinal cells are more easily damaged than smaller ones, and so these cells are the first to be lost at the onset of glaucoma. The largest cell in the retina is a ganglion cell called the M_Y cell, the function of which is to regulate the perceived contrast of retinal images. M_Y cells represent about 1% of all ganglion cells and respond best to flickering spread-out stimuli. When you glimpse a TV set in the corner of your eye, the ill-defined but flickering image you perceive is due in large measure to M_Y cells.



"the new test could be a reliable front-line screening device for glaucoma."

An interesting and, as it turns out, important side-effect of the way M_Y cells act is that they respond non-linearly. That is, they unevenly pool responses from different areas of the retina. This property makes them susceptible to a particular visual illusion. If the contrast of a number of broad bars is continuously changed at a sufficiently fast rate (above about 15 times a second), an observer will report that there appear to be twice as many bars as are actually present.

Dr Maddess was the first to explain this illusion in terms of M_Y cells, and in later experiments he went on to see if he could use the illusion as a test for missing M_Y cells. He surmised that if glaucoma had begun to destroy someone's retinal cells, these cells should be the first to go, and hence that person's susceptibility to the illusion should be impaired.

He conducted a number of experiments that have confirmed this thinking. Firstly, he found that various parts of the retina differed in their sensitivity to the illusion, and this difference matched the known variation in density of M_Y cells over the retina. In fact, the density is so low that even the loss of a single cell will show up as a distinct loss in illusion susceptibility.

Secondly, he did experiments with two groups of people: one with high intraocular pressure and one without. The illusion test was reliably able to distinguish the two groups.

Encouraged by these results, Dr Maddess has now arranged for an ophthalmologist to use the test on glaucoma patients and compare the results with conventional techniques. The latest results with the new test have been good, with the number of false positive and false negative diagnoses falling below the numbers falsely diagnosed by perimetry.

Moreover, a high proportion of people diagnosed by the new test as having incipient glaucoma have evidence of optic disc cupping. This suggests that the new test may be better at detecting glaucoma than the conventional perimetry test.

Dr Maddess is continuing with more experiments designed to better understand the doubling illusion. He has been able to show that the subjective experience of doubling has an objective counterpart in the electrical signal picked up by electrodes placed on the eyeball. The implications here are that it may even be possible to diagnose glaucoma in people who are unable to describe what they see, in babies for instance.

If you want to know more:

- Performance of nonlinear visual units in ocular hypertension and glaucoma. T. Maddess and G.H. Henry. *Clinical Vision Science* **7** (1992), 371–383.
- Information on glaucoma is available from the Glaucoma Foundation of Australia, P.O. Box 420, Crowsnest, N.S.W. 2065.
- You are welcome to contact Dr Maddess via the address and phone numbers given on page 2.

Dr Ted Maddess.

Research Scholarships

FOURTH YEAR (HONOURS) SCHOLARSHIPS IN BIOLOGICAL SCIENCES

The Research School of Biological Sciences is offering three scholarships for suitably qualified students wishing to undertake their fourth (honours) year at the Australian National University in 1994.

Scholarships are for one year and are valued at $6000 plus limited travel expenses (where applicable).

Applicants should be completing, or have recently completed, the third year of a relevant Bachelor's degree course with majors in the fields relevant to the biological sciences.

CLOSING DATE FOR APPLICATIONS: 30 NOVEMBER 1993.

Application forms and further information may be obtained from:

Ms Anna Weidemann
School Secretary
Research School of Biological Sciences
GPO Box 475
CANBERRA, ACT 2601

Phone (06) 249 4138

CHANCE AND UNCERTAINTY: INGREDIENTS OF TOP SCIENCE

Late in 1991, the former Director of the Institute of Advanced Studies, Professor Max Neutze, called together a small committee to 'crystal ball' some of the main issues facing the Institute, of which the Research School of Biological Sciences forms a major slice. One of the concerns of the committee, of which I was a member, was the issue of public communication.

Another member of the committee was Professor Ted Ringwood, a distinguished geochemist and former Director of the Research School of Earth Sciences. He has gone on record as saying that "the Institute of Advanced Studies has failed to win awareness and respect for its achievements, not only among the general public, but also among the decision-makers of this country..."

"This failure must be laid squarely at our own door," he said, and "... it is an unfortunate fact that the majority of the academic staff have not recognised the importance of the public communication of their research activities."

In acknowledging these deficiencies and endeavouring to discover avenues for their rectification, I helped convene a conference on 'Communicating Research' earlier this year. In attendance were a wide cross-section of the university and the national media, including top professionals from *The Australian*, Prime Television, CSIRO, and the Public Relations Institute of Australia. A special guest was former science editor for *The New York Times*, Cornelia Dean.

One of the points I tried to put across was that in communicating research we must work to explain two complementary aspects: the process of science, and the progress of science. Understanding the *process* that underlies creative research is necessary before one can fully appreciate the factors that permit *progress*.

The process of research depends critically on chance and uncertainty, a concept that managers and bureaucrats hate. You can plan until the cows come home, but if one doesn't leave room for serendipity — for being able to pick up and follow through on a chance paradoxical finding — then scientific understanding will not progress. As someone said, paradox is but truth standing on its head in order to call attention to itself. We have to leave ourselves free to follow that call.

Curiosity is the cornerstone of research, and we must allow the best researchers the latitude to pursue that leading. I hope you will discern that vital element in the reports of our progress described in this issue of *Biologic*. Indeed, there would be very little to talk about at all if we didn't strive to create an environment here at RSBS in which we are frequently surprised, and often perplexed, by the unexpected.

I believe a unique curiosity-oriented environment has driven this Research School, and the Institute of Advanced Studies in general, to paramount positions in world science.

With all the recent posturing from the 'magnificent seven' research universities, the achievement of the Institute of Advanced Studies remains the best kept secret of the last half-century of research in Australia. This achievement must now be held prominently in public view. You should know that the science-based Research Schools of the Institute of Advanced Studies do from 13 – 47% of the research in Australia in those fields in which they engage. This accomplishment comes at a cost of about 5% of the nation's public expenditure on research — a bargain by any reckoning.

In this School, our research finds its way into preeminent high-impact journals where it fashions the international research agenda in biology. The Institute's performance in those areas where it really matters, such as those reported in these pages, has been ranked at 5 – 10 times better than the world average.

We are proud of this Research School's contribution and hope you can share this pride in Australian achievement as you peruse this issue of *Biologic*.

Barry Osmond