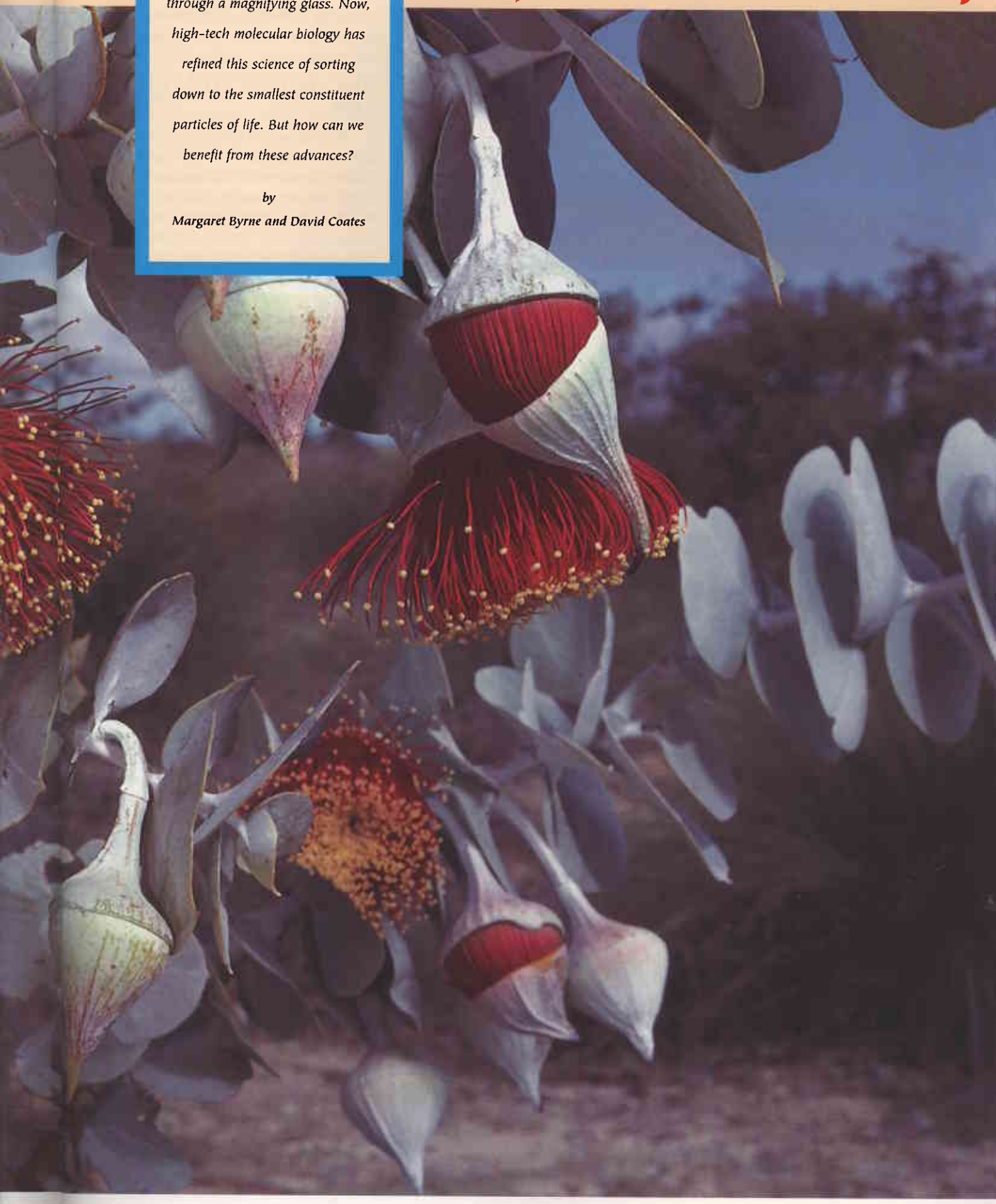
MORE THAN MEETS THE EYE

Our flora's hidden diversity

In Victorian times, differences within and between species were observed with the naked eye or through a magnifying glass. Now, high-tech molecular biology has refined this science of sorting down to the smallest constituent particles of life. But how can we benefit from these advances?

by
Margaret Byrne and David Coates



What makes one species different from another, or one individual of a species different from another individual, is encoded in the tens of thousands of genes contained in the individual's tens of thousands of cells. Taken together, this biodiversity encompasses all the different life forms we know, from animals and plants to micro-organisms.

The biodiversity we see in our various ecosystems is the result of thousands of millions of years of evolution. Evolution is the selection and adaptation of individuals and species to the environments in which they live. It occurs over long periods of time through changes, or mutations, in their basic genetic material. Mutations that result in useful features, or that do not disadvantage the organism, get passed on in reproduction, generating differences in populations and species over time. The whole of an ecosystem can be seen as a jostling, jockeying crowd of millions of genetic patterns, vying for position in some places, while working together in others.

Until recent years, science's attempts to describe, categorise and understand this diversity have been based on morphology, the study of the organism's visible characteristics. But an organism's outward appearance is only the tip of the iceberg. What can be observed by the eye in a plant's physical appearance represents the tiniest fraction of its differences from other plants—a mere epigram compared to the massive tomes of information contained in its genes.

For nature conservation, advances in molecular biology lend greater depth and possibility to understanding the scope of genetic variation present within a species. Understanding the forces that have shaped a species, and, more specifically, a unique population of a species, can lead to more effective management of that species and the threats it may face. The same understanding can also be applied when selecting particular characteristics for breeding a species for commercial production.

Previous page: The rose mallee (*Eucalyptus rhodantha*) occurs in small, fragmented remnants in the Wheatbelt. Habitat destruction and degradation have resulted in loss of genetic diversity and increased inbreeding in populations.
Photo – Babs & Bert Wells/CALM

Below: The threatened matchstick banksia (*Banksia cuneata*) is known from only two genetically distinct groups of small fragmented populations in the Wheatbelt.
Photos – David Coates

THE BUILDING BLOCKS OF LIFE

The differences between the various plants and animals come from the different pieces of deoxyribonucleic acid (DNA) that they possess. DNA is the basis of life. Built of complex chains of sugars and phosphates, DNA makes up the genes that reside in the nucleus of every cell of every living organism. It contains all the information that dictates the design and creation of the substance, in the form of different proteins, that forms that organism. DNA is the blueprint of the life that carries it, the life which then projects it into the next generation.

DNA is passed on from parents to offspring in different combinations, generated during reproduction. This ability to recombine, inherent in reproduction, leads to a vast array of different combinations, or genotypes. This is the basis of the differences that we can see between individuals of a species, between different populations of the same species, and between one species and another.

GENETIC WEALTH IN WA FLORA

Western Australian flora is very rich and diverse, with nearly 12 000 species found within its land area. Not only is there diversity of species, there is also an impressive range of genetic diversity within each species, between populations of plants and between individuals within populations. For example, many threatened plants, such as grass wattle (*Acacia anomala*), matchstick banksia (*Banksia cuneata*), rose mallee (*Eucalyptus rhodantha*), the round leaf honeysuckle (*Lambertia orbifolia*), and Wongan Hills triggerplant (*Stylidium coronoforme*) show significant genetic differences between populations or groups of populations.

Information on the amount and distribution of genetic diversity plays a vital role in developing strategies for the conservation and management of this wealth of native flora. For example, the development of recovery progress by CALM for threatened species such as the

rose mallee, Wongan Hills triggerplant and matchstick banksia, has relied on genetic information. This information has assisted in the management of existing populations and the re-establishment of critically endangered populations. The same information is also valuable in enabling better understanding of factors affecting genetic diversity, such as size, fragmentation and isolation of populations, and the importance of pollinators.

Conservation strategies aim to maximise genetic diversity and may differ depending on the amount and pattern of the variation. The genetic relationships within the species determine whether it should be managed as a single unit or as two or more discrete groups. For example, in a species where most of the genetic variation occurs as differences between populations, it would be better to conserve a number of populations throughout its range. In some cases, there may be groups of genetically similar populations. A suitable approach may be to concentrate resources and effort on conserving representative populations in each group, while other populations could be targeted for seed collection and storage (see 'Banking for the Future', *LANDSCOPE*, Winter 1996).

GENES ON THE PRODUCTION LINE

Genetic variation has profound implications for the commercial use of natural resources. Understanding genetic diversity allows for the selection of individuals that exhibit desirable characteristics. This was the basis for the crude 'genetic engineering' humans used in the past to domesticate animals and plants for agricultural use. Molecular biology techniques have now refined this process of managed selection to a highly sophisticated science.

For example, early selection of species of trees for good growth were based on visible morphological features: finding trees that grew taller than others (see 'In Search of the Perfect Pine', *LANDSCOPE*, Autumn 1992). However, features such as salt tolerance, oil production, or disease resistance are not so easily discerned. Study at the molecular level can identify molecular 'markers' that 'tag' the pattern of these genetic variations.

Above: Increased productivity of maritime pine (*Pinus pinaster*) plantations can be gained by using molecular techniques to select variants with improved growth and wood properties.
Photo – Chris Garnett

Left: Natural oil mallee (*E. kochii* subsp. *plenissima*) at Kalannie. Molecular techniques can be used to select variants for improved oil yield and growth of oil mallee trees for farm forestry.
Photo – Wally Edgecombe

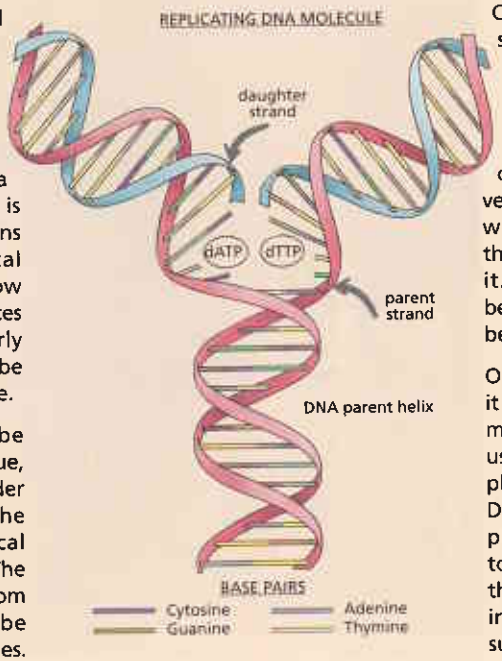
Research around the world is currently being carried out to locate genes controlling characteristics important to commercial production of trees, such as disease resistance, growth and wood properties. This research uses bits of DNA as markers, which can be linked to desirable characteristics. Known as 'marker-aided selection', the technique can be used to select seedlings that are only three or four months old, instead of having to wait the several years it takes for the characteristics to show themselves. Marker-aided selection research is currently being conducted by Western Australia's Department of Conservation and Land Management (CALM), in collaboration with the CSIRO, ALCOA and Western Collieries, to locate genes controlling resistance to jarrah dieback (a disease caused by the fungus *Phytophthora cinnamomi*). This project carries implications for conservation as well as industry.

Projects such as this are only scratching the surface of what might be possible in the future. Genetic variation is the last frontier to understanding the environment and our place within it. New molecular techniques enable environmental science to analyse and understand the most minute genetic differences in increasingly greater detail, relatively quickly and cost effectively. The development of genetic engineering technologies may once have seemed an unnatural act, but now molecular biologists are able to bring the benefits of this technology back to the bush, to protect, preserve and manage the incredible diversity of WA's plant and animal species.

GENETIC ANALYSIS OF A PLANT

Analysis of a plant species' DNA and proteins will show the amount and patterns of its diversity. Analysis of proteins involves taking some seedlings or leaves of a plant and grinding them in a buffer to release the proteins. The mixture is then placed onto a medium, a gel, through which an electric current is passed. The current separates the proteins according to their different electrical charges. The gels are then stained to show different proteins, allowing the differences between individual plants to be clearly seen. These differences in proteins can be directly related back to the coding gene.

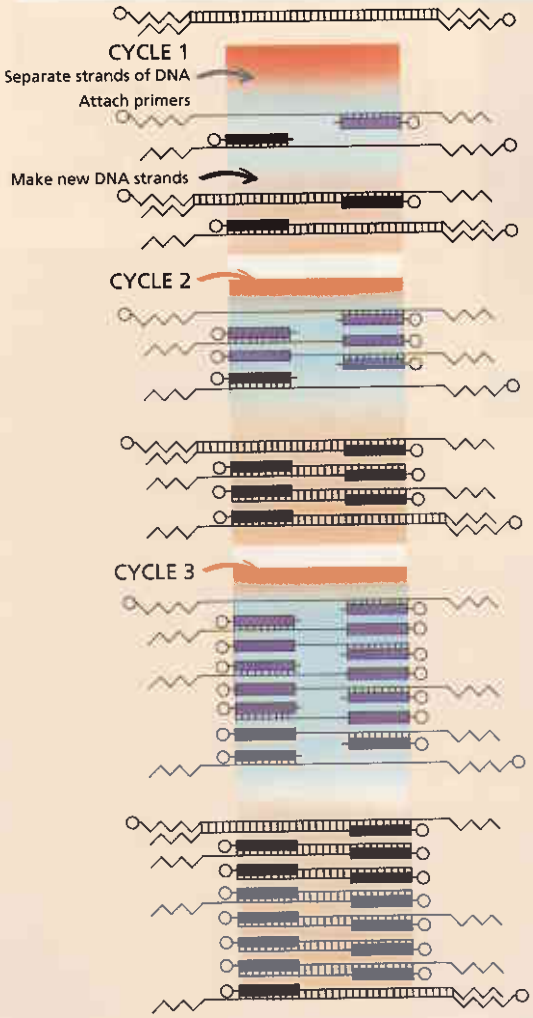
Analysis of DNA requires DNA to be extracted from the plant. Some plant tissue, usually leaves, is ground into a fine powder and mixed in a buffer. The cells in the mixture are broken down by chemical action, and the contents are released. The DNA is then separated and purified from other cell contents. The DNA can be analysed by one of a number of techniques.



One method is to use a 'probe', which is a small piece of DNA, to investigate what that bit of DNA is like in different individuals. Using a lot of probes gives information about lots of bits of the DNA. Another method, known as 'polymerase chain reaction' (PCR), involves using two very small pieces of DNA, called 'primers', which bind to the DNA, sandwiching it and, through the action of an enzyme, amplifying it. After amplification, the differences between the sandwiched bits of DNA can be seen on a gel.

One advantage of the PCR method is that it uses only a very small amount of DNA (five millionths of one gram), so that it can be used on small herbarium specimens or on plants that only have a few small leaves. DNA analysis can be more extensive than protein analysis, because the DNA available to be analysed is virtually unlimited, while there are a limited number of proteins, and in some plants only a few proteins can be sufficiently resolved.

POLYMERASE CHAIN REACTION ANALYSIS



Just as the analysis of DNA has revolutionised genetic research, the PCR method has revolutionised DNA analysis. It is now possible to get analysable DNA from fossils, museum specimens, and dried plant specimens which may be hundreds of years old.

PCR is a technique which is used to amplify (make lots of copies of) a piece of DNA. DNA is made of two strands. These are held together by bonds which can be broken by heating to 94°C. When cooled to 55°C the separated strands bind to the 'primers', two small bits of DNA which were used to flank the bit of DNA to be amplified. The temperature is then raised to 72°C, allowing an enzyme called 'Taq polymerase' to make a new strand of DNA to match the original one. At the end of one cycle of heating to 94°C, cooling, and heating to 72°C, there are four strands of DNA, two sets of two, instead of the original two strands. During the next cycle of heating the primers attach again and the process is repeated. A single piece of DNA would not be visible, but after 30 or 40 of these heating cycles, the numerous copies of the piece of DNA are clearly seen when run on a gel and stained.

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The threat from below . . . How can we defeat our greatest environmental enemy? Read about salinity and what we can do about it on p. 10.

One of the best aids to plant conservation is completely invisible. See our plant DNA story on p. 18.

LANDSCOPE

VOLUME THIRTEEN NUMBER 1, SPRING 1997

Dryandra, one of the last refuges of the native wildlife. Now you can experience this woodland wonderland for yourself. Find out how on p. 36.

Europeans brought alien plants and animals to WA's rangelands, which have since become degraded. What can be done? See p. 42.

How old is the Stirling Range? Read about this stunning area in our story on p. 48.

FEATURES

REGULARS

COVER

The Fitzgerald River National Park boasts a startling array of habitats, mammals, birds and other species. Its wildflowers in spring are often spectacular. Our story on p. 28 is a fascinating tale of variety, beauty, and threat in this aged land.

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