

Wheat's ancestry has been investigated using repeating DNA.

Drosophila has several long repeated base sequences.

DNA tells of species links ~ by repeating itself

Which modern research tool has caused zoologists to rethink their classification of wallabies, is helping farmers find out which wheat-rye hybrids come under the jurisdiction of the Wheat Board, and has resolved the mystery surrounding the origins of an Australian all-female grasshopper?

The answer is: highly repeated DNA. Deoxyribonucleic acid, to give DNA its full name, has attracted enormous attention since it was discovered less than 40 years ago to be the carrier of genetic information in all organisms except some viruses.

The science of molecular biology is founded on the study of DNA: its composition, its shape (especially the well-known double-helix form), its behaviour, and the genetic code by which the molecule's constituent units direct a cell's chemistry.

Because it plays a central role in the genetics and development of animals and

plants, DNA seems an obvious subject for studies of taxonomy and evolution. The more closely related two species are, the more similarities their DNA molecules may be expected to show.

All DNA molecules have an extremely long 'backbone', attached to which are the bases that constitute the elements of the 'genetic code'. There are four bases, known as A, C, G, and T, and sets of any three of these make up units of genetic information. Molecular biologists can often 'read' series of bases, 'translating' the triplets into the string of amino acids whose code they hold.

Much DNA departs drastically from this pattern, however; the bases form series that are repeated thousands of times, such as ATATATAT . . . , which occurs in several crustaceans. Sometimes the repeating units contain hundreds of bases. In that geneticists' workhorse, the tiny fly *Drosophila*, one class of repeating DNA with a unit of more than 300 bases recurs hundreds of thousands of times, and one of the repeating units of rye contains about 500 bases.

Nobody yet knows precisely what part highly repeated DNA plays in the organization of a cell. Presumably no genetic message is encoded in the base sequence.

Researchers are finding evidence that this DNA exerts some influence on genes, and this is not surprising, as at least 5% of an organism's DNA is generally of the highly repeated form. The proportion is much greater in some species: 20% or more in *Drosophila*, for example.

One sequence of more than 300 bases in Drosophila's DNA recurs hundreds of thousands of times.

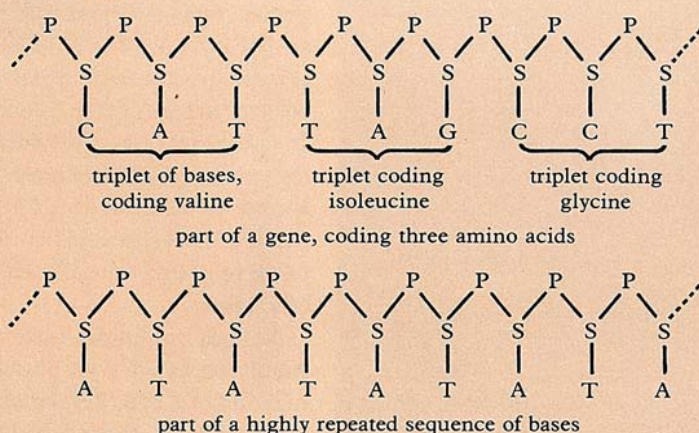
Taxonomic tool

But, whatever its function, this DNA offers biologists a novel means of sorting out relations between species. The tales of affinity that repeated DNA has to tell could constitute a check on the stories told by the other structures, habits, and functions employed in biological classification. Corroboration will strengthen existing ideas; contradiction will demand a fresh examination of all the evidence.

A group of geneticists in the CSIRO Division of Plant Industry, including Dr Jim Peacock, Dr Rudi Appels, Dr Wayne Gerlach, and Dr Liz Dennis, has made several comparative studies of highly repeated DNA. In all species so far examined, the arrangement of repeated DNA is constant within any one species, and characteristic of the species; other species have different arrangements. But the arrangements in related species show similarities.

In the laboratory, the researchers begin by isolating portions of repeated DNA from one species and making it radioactive. Then they take some intact DNA from another species, ease apart the two strands of the double helix — still in their chromosomes — using heat or acid, and let

Two types of DNA



the strands come together again in the presence of the radioactive DNA, which they call a probe.

Occasionally probe DNA will take the place of part of one of the original strands when the double helix re-forms. For this to occur, the probe must have the same sequence of bases as the portion of DNA that it replaces. Using film sensitive to radioactivity, the scientists can find out whether any probe DNA has been taken up and, if it has, identify the sites that it occupies. Then they can draw conclusions about relations between the species.

The procedure is called *in situ* hybridization, because the DNA strands remain within their chromosomes throughout the exercise.

Kangaroos

Perhaps the most interesting taxonomic results to come from these experiments so far arose out of work on the family Macropodidae, the wallabies and kangaroos.

Some of their findings — obtained with the help of three Ph.D. students from the Australian National University and Dr John Calaby of the CSIRO Division of Wildlife Research — confirmed conventional taxonomy. For example, all the kangaroos and wallabies studied shared the three probes used, suggesting that they do indeed form one related group descended from a common ancestor. And the eastern and western grey kangaroos, on the evidence of their repeated DNA, belong where taxonomists have put them, close together but in separate species (*Macropus giganteus* and *M. fuliginosus* respectively).

But, unexpectedly, all three probes pointed to the red-necked wallaby (*M. rufogriseus*) being a close relative of the red kangaroo (*M. rufus*) and euro (*M. robustus*). No previous evidence had suggested this.

Highly repeated DNA does not carry the genetic code.

No detectable changes have occurred in the repeated DNA of two wallabies separated 12 000 years ago.

Two probes also linked the agile wallaby (*M. agilis*) with the tammar (*M. eugenii*) — an intriguing result, as the traditional taxonomic criteria of body morphology, skull measurements, and behaviour reveal no close affinity, but their chromosome shapes and blood proteins are similar.

The kangaroo study also supplied some reassuring evidence of the stability of highly repeated DNA over many generations. The researchers could find no difference between the DNA of the mainland red-necked wallaby and that of the Tasmanian form, Bennett's wallaby, which is conventionally regarded as another subspecies of the same species.

In other words, no detectable changes have occurred in the highly repeated DNA of the two wallabies since Tasmania became separated from mainland Australia by a rise in sea level about 12 000 years ago. This implies that repeated DNA has the stability needed in a reliable tool for evolutionary studies.

Hybrid cereals

Propelled by plant breeders, evolution has occurred far more swiftly in cereals than among wallabies, and highly repeated DNA is proving valuable in studies of hybrid plants formed from two or more species.

One hybrid cereal attracting particular interest among Australian farmers is trit-

icale, which, like its name, is derived from wheat (*Triticum*) and rye (*Secale*). Professor Colin Driscoll of the University of Adelaide's Waite Agricultural Research Institute has set up Australia-wide trials of 20 forms of triticales in a quest to produce types suitable for different regions. Other scientists are carrying out similar research, looking for such qualities as rust-resistance, salinity-tolerance, and a low demand for water.

The nuclei of triticales cells are essentially wheat nuclei with a number of rye chromosomes added. In Canberra, Dr Rudi Appels has developed a technique that shows clearly which rye chromosomes each variety of triticales possesses. This technique involves cloning highly repeated DNA to produce a purer probe than can be obtained by the usual isolation procedure.

Scientists could eventually relate agronomically desirable qualities in particular plant lines to the combinations of chromosomes that the plants contain.

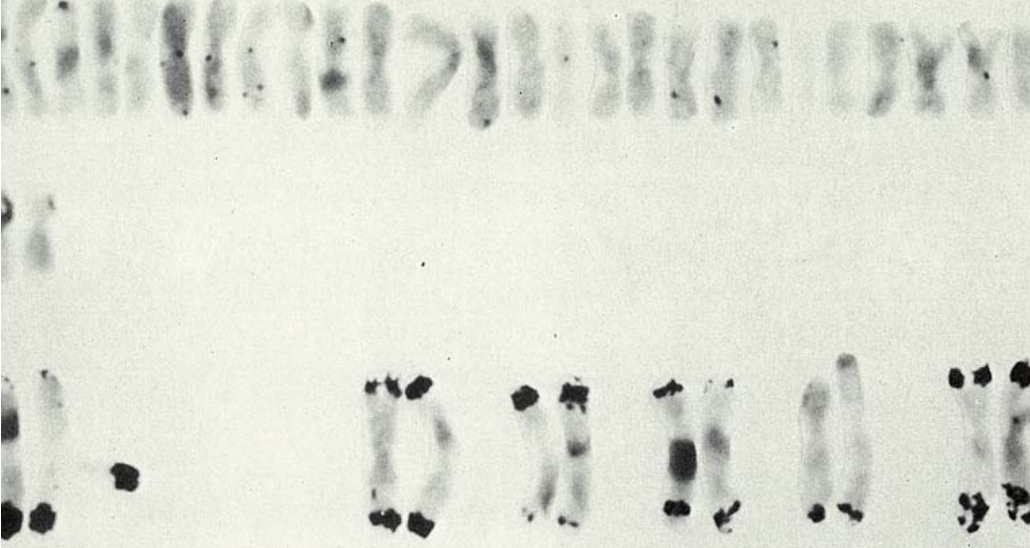
Rye cells contain seven pairs of chromosomes, but few triticales varieties possess the full set. Hybrids with fewer than four pairs are officially deemed to come under the jurisdiction of the Australian Wheat Board. Highly repeated DNA is helping determine to which bureaucratic category each variety must be assigned.

The other triticales ancestor, wheat, itself boasts a complex history of hybridization between wild grasses. Since the genetic crosses have probably been occurring over at least 10 000 years, with man's intervention limited to the selection of desirable hybrids, wheat's chromosomal heritage is shrouded in some mystery.

Traditional techniques of cell study showed that some wheat cells contained the chromosomes from two ancestral species, and some had those from three. Using highly repeated DNA, the CSIRO researchers have confirmed this, and have narrowed the list of suspects in the hunt for the donor of the most mysterious set of chromosomes, known to geneticists as the B genome.

Any information on the ancestry of wheat holds potential value to plant breeders, as it could help them understand the processes by which qualities such as high yield and disease resistance become established in a species. The same considerations apply to other cultivated species such as maize, which the CSIRO scientists are currently investigating.

The study of triticales holds similar interest, emphasizing as it does the speed



Triticale chromosomes lined up for inspection: this variety has six pairs of rye chromosomes (bottom row), labelled with a radioactive probe. The wheat chromosomes (top two rows) have not taken up the probe.

with which changes can occur and the complexity of some of the new chromosomal combinations. Sometimes the chromosomes from different ancestral species actually swap sections, so not only the nucleus, but even an individual chromosome, may be a hybrid.

In triticale, such hybrid chromosomes appear more stable and better equipped to go successfully through cell division than pure rye chromosomes. The use of highly repeated DNA for the detailed characterization of triticale chromosomes may prove important in helping scientists and plant breeders to understand just what makes some varieties succeed.

Virgin birth

The problem of the all-female grasshopper was brought to the Plant Industry group's attention by Professor Michael White of the Australian National University.

Like some other insects, the grasshopper, *Warramaba virgo*, reproduces parthenogenetically — that is, by virgin birth. As a result, the individuals are reproductively isolated from one another, and taxonomists find it hard to say exactly what constitutes a species. Professor White thought highly repeated DNA techniques might be able to throw some light on the origins of the different populations of this grasshopper.

Most parthenogenetic species keep their ancestry a secret from evolutionists, but some, including *W. virgo*, show enough similarities to other species to have suggested plausible theories. The CSIRO group's work has now clarified the situation somewhat: it shows *W. virgo* must have evolved on at least two separate occasions by hybridization between sexually reproducing species of grasshopper.



The wheat-rye hybrid, triticale.

One cross occurred a relatively long time ago, and the resulting grasshopper subsequently spread over much of eastern and western Australia. Another hybridization took place more recently and gave rise to the parthenogenetic grasshoppers in a comparatively small area north of Kalgoorlie. In the light of this discovery, biologists have re-examined the morphology and other aspects of the grasshoppers, and have found supporting evidence for the theory.

Chromosome 'fingerprints'

Research into highly repeated DNA at the Division of Plant Industry began in 1971, when Dr Jim Peacock, now Chief of the Division, was investigating the chromosomes of *Drosophila*.

Traditional nuclear-staining techniques had distinguished two types of chromosomal material: euchromatin, which was known to contain genes, and heterochromatin, whose composition remained a mystery. The CSIRO team, with Dr Doug Brutlag, now at Stanford University, California, discovered that *Drosophila* heterochromatin included long stretches of highly repeated DNA, and that each chromosome possessed a unique arrangement of the different forms of repeated DNA, which could therefore be used

Repeated DNA helps determine to which bureaucratic category each variety of triticale belongs.

like fingerprints to identify individual chromosomes.

Drosophila still features among the species studied in the Plant Industry laboratories. Although its genes have been more thoroughly studied than those of any other animal except perhaps the laboratory mouse, the study of its highly repeated DNA has emphasized that important new discoveries can still be made. For example, recent experiments involving deletions of various proportions of a chromosome's heterochromatin have shown that this region influences the extent to which a gene on that chromosome expresses itself.

Current research is also extending knowledge of the genes occurring within heterochromatin — that is, the relatively few genes studding the long sequences of highly repeated DNA.

Although still in its infancy as a research tool, highly repeated DNA has already proved its worth in several diverse fields, and looks set to make further important contributions to both fundamental genetics and applied biology.

John Seymour

More about the topic

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