BIOTECHNOLOGY IN POULTRY BREEDING I:

From Discovering Heredity To The Use Of Gene Markers

In the October 1995 issue of POULTRY INTERNATIONAL we explained that contrary to incorrect impressions among some people, gene manipulation or 'genetic engineering' plays no part in modern poultry breeding. Now Dr Gerard Albers, head of Euribrid's biotechnology programme reviews the history of DNA research through to the application of DNA technologies. In the first of a series of three articles, he presents a general outline of our knowledge of DNA as the basis of life and the significance of gene markers as new tools for use in breeding programmes.

The concept of heredity (parents passing on certain characteristics to their offspring) must be very old: the ancient Greeks already knew of a variety of chicken breeds with distinct differences.

It took time and a number of false trails before we began to find out just how heredity really works. In the seventeenth century, Antonie van Leeuwenhoek the Dutch inventor of the microscope, for instance, noticed that semen of males consisted of tiny particles, the spermatozoa. This led him to believe that the contribution of the male could only be minor compared to that of the female who produces eggs which are much larger! Only some two hundred years later, was it to become clear that male and female do indeed contribute equally to their offspring in the form of their chromosomes.

Again, to find out exactly how this mechanism operated, took a long time. It was only after DNA had been identified as the main substance in chromosomes, that the truth finally started to unravel. In 1953, Watson and Crick proposed a model for the structure and organization of DNA: a double helix, consisting of four different nucleic acids paired to each other in long and double wound chains. The code, if not the key, was known. (*Figure 1*)

The genetic code

It was now clear that the arrangement of the four nucleic acids in DNA, Thymine (T), Cytosine (C), Adenine (A) and Guanine (G), somehow contained the secret of life. It was a genetic code that was fully deciphered by 1966.

The four nucleic acids in DNA (i.e. the letters of the genetic language) are grouped into codons (three letter words), the codon being transferred by RNA to the cell machinery which produces proteins. Each codon directs the addition of a specific amino acid to a chain of amino acids, which ultimately leads to the production of specific proteins such as enzymes and hormones. In fact the genetic

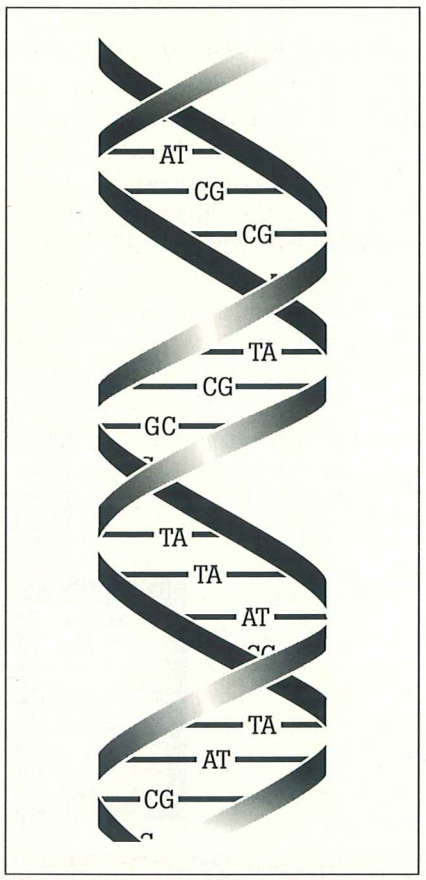

Figure 1. **The genetic code is laid down in DNA as sequences of four different units (A, T, C and G) arranged in the structure of a double helix.**

code is universal with exactly the same system seen in all living beings, from the most simple bacterial species, to the most complicated living beings, like man.

Since 1966 a host of new techniques for the handling and analysis of DNA have provided us with powerful tools to do exactly what a breeding programme is designed for in the first place: to unravel the genetic information carried by selection candidates, in order to ensure that the best sets of genetic information are passed on to the next improved generation of breeding stock.

Traditional breeding and selection

In the complete absence of knowledge about DNA, breeding programmes have so far had to resort to statistical inferences when it came to genetic information carried by selection candidates. Traditional breeding programmes have become extremely sophisticated in combining information on the performance of selection candidates and large numbers of their relatives into reliable predictions of the genetic information they carry. However, estimated breeding values, as they are commonly called, always remain estimates in that they only provide a, poorer or better, guess about the true genetic information carried by the individual selection candidate. The accuracy of these estimations ranges in fact from as low as 15 up to around 75 per cent. This is a large gap with 100 per cent and "reading" the true genetic information would close this gap! Here lies by far the most promising application of DNA technology in breeding programmes.

The need for gene markers

The genome of a bird (the entire collection of DNA on all its chromosomes), although only visible through a microscope, is huge by any standard of DNA analysis. The total genome consists of around 2 billion base pairs and probably contains somewhere between 50,000 and 100,000 genes. To dissect this entire set of genetic information into the separate genes and then to establish the role of each gene is simply impossible. In fact few chicken or turkey genes have been analysed in this way. When it comes to important production or resistance traits, there is no way of pinpointing the genes that really matter, because it is not known which mechanisms are most critical in determining those traits. Our knowledge of the biology of birds is simply too limited.

The use of gene markers is however a way to circumvent this problem by providing a procedure for quick and simple analysis of a limited number of genes which reflect a large share of the variability of all genes in the genome. To explain this, a little bit of genetic theory may be helpful.

About linkage and recombination

Egg cells and sperm cells contain only one set of chromosomes. After fusion of the egg cell with the sperm cell a normal cell with two sets of chromosomes is formed to start the development of the embryo. During the formation of egg and sperm cells all pairs of chromosomes are lined up against each other to allow a fair and equal separation. During this process, however, so called cross-overs occur when chromosomes break and the pieces re-unite. This re-assembly may however be between parts of the two different

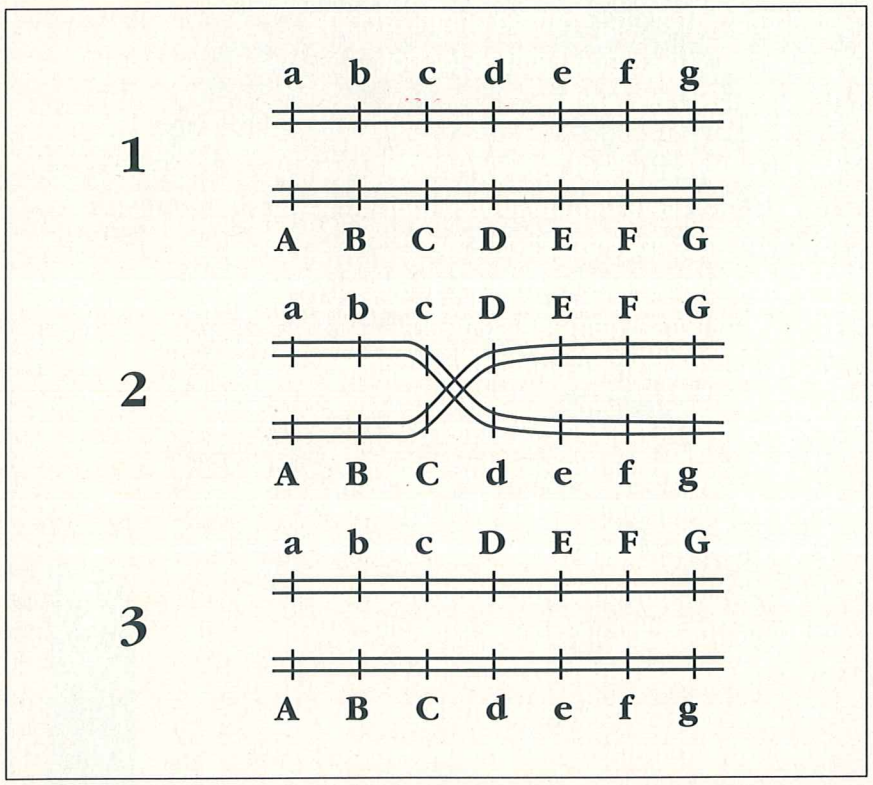

Figure 2. **During the formation of eggs and sperm cells, paired chromosomes (1) may break and re-unite. During the process "cross-overs" occur (2) and we end up with a different arrangement of genes on each chromosome (3).**

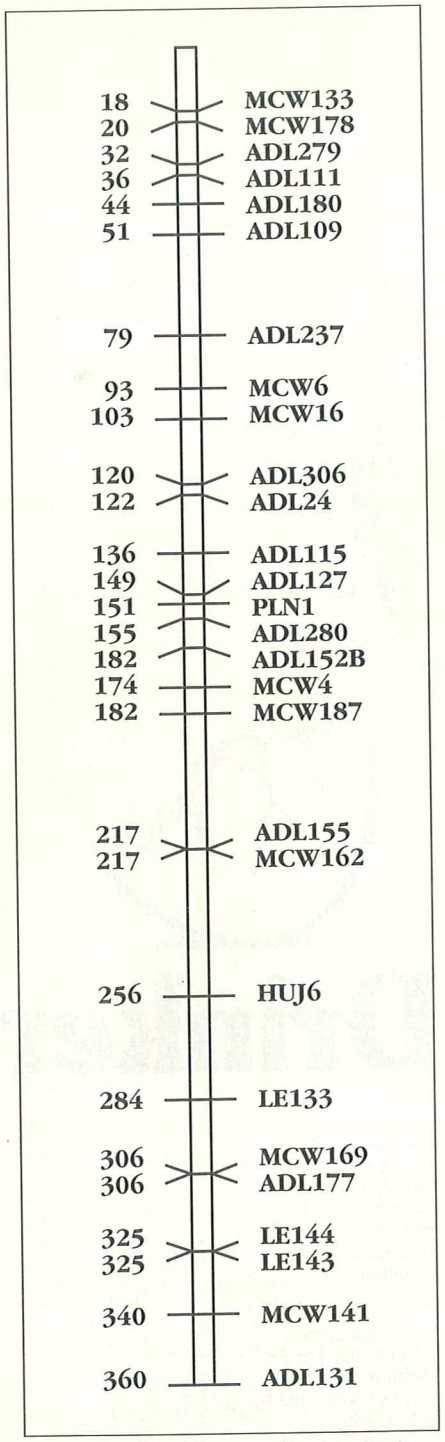

Figure 3. **A genetic map of a chicken chromosome with a large number of gene markers. On the right are the code names of the gene markers. On the left are the distances between them. One unit of distance equals one percent chance of being separated in a recombination event during formation of egg or sperm cells.**

chromosomes of a pair. This means that the resulting chromosomes may be reconstituted from parts of both original chromosomes. This biological process is called recombination. (*Figure 2*).

In other words, recombination causes genes that were previously together on one chromosome to become separated on two (homologous) chromosomes. The chances of genes of one chromosome to become separated are lower when these genes are located closer to one another on the chromosome. The closeness of location of two genes is termed their "linkage".

Gene markers are locations on a chromosome which are being used to identify the segment of the chromosome which is "linked" to that location. Because gene markers thus represent a chromosome segment rather than a single gene, far fewer gene markers are needed than there are genes present. Gene markers are used therefore as an "index" to the total set of genes on all chromosomes.

Gene maps

Gene maps are simply schematic drawings of all chromosomes: the distance between genes or gene markers on such a map reflecting the degree of linkage between them. The ideal map of gene markers contains evenly spaced gene markers at distances which are small enough to guarantee that unmapped genes between them are sufficiently linked to one or other of the markers. (*Figure 3*).

In this way the total set of gene markers can be used to reflect the variability of all genes at the chromosomes, both known and unknown.

Microsatellite markers

Important characteristics to note of gene markers are:

1. they should display a large degree of variability because this maximises the chances of them being linked to variable genes in the vicinity;
2. they should be available in large numbers and be evenly spread over the chromosomes;
3. they should be easy and cheap to identify.

So-called microsatellite markers fulfill all these requirements: they are highly variable, there are thousands of them, they are randomly spread over the genome and their analysis can be done semi-automatically by using the PCR(Polymerase Chain Reaction) technique and automated DNA fragment analyzers.

Literally thousands of assays can be done in one day with these advanced technologies. Microsatellite markers are therefore the markers of choice and they are now widely used around the world especially in human medical research into predisposition towards numerous genetically determined diseases and defects.

Applications

Gene markers are a powerful tool to "read " genetic information and such a tool can be used in a variety of ways in a breeding programme. There are applications in genetic quality control for instance, in the area of parentage testing and the testing of genetic purity of reproduction flocks and breeding products. The most exciting application is however, in their use for reading genetic information for production and disease resistance characteristics of selection candidates.

These applications will be the subject of the forthcoming articles in this series on biotechnology in poultry breeding. — *Dr Gerard Albers, Euribrid BV.* PI