WHAT ARE GENES?

The process of development of the fertilised egg has to be controlled very precisely and each fertilised egg contains a set of plans that is used to control not only the growth and division of the cells, but also to decide which cells become liver cells, kidney cells, retinal cells, and so on. The same plans are also used to orchestrate the intricate cell movements that are required to ensure that each new individual assumes the shape and form characteristic of the species. These plans are stored in the genes and are made of a complex molecule called DNA (more about this later). So, in the dog the fertilised egg contains a set of genes whose role is to ensure that it develops into a dog. Not only do the genes control the development of the fertilised egg, they also control every characteristic displayed by the dog once it has been born.

HOW MANY GENES ARE THERE IN THE DOG?

No one knows precisely how many genes are required to specify a dog, but a fair guess would be that in the region of one hundred thousand different genes are required to ensure the correct development and functioning of a dog. All of these one hundred thousand different genes are present in the canine fertilised egg. In fact, it is more complicated than that because each fertilised egg contains two complete sets of genes, in other words there are two copies of each and every gene. One set, the maternal set, is deposited in the egg as it develops in the dam; the other set, the paternal set, is placed in the sperm as it develops in the sire. When the sperm fertilises the egg it physically injects the paternal set of genes producing a fertilised egg containing two sets of genes, one maternal and one paternal. Prior to each and every cell division that occurs after fertilisation these two sets of genes are faithfully copied so that the daughter cells receive two complete copies of the genes.

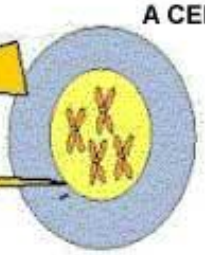
HOW DO GENES WORK?

We have said that the genes store plans, but plans for what? The plans are used by cells to make molecules called protein; essentially each gene contains a plan that enables the cell to make a unique protein. So, if there are one hundred thousand genes in the dog, it stands to reason that you require that number of different proteins to make a dog. It is the activity of these proteins, either working individually or co-operatively in groups, that determines every characteristic, both physical and behavioural, of the dog. Let us give you an example: There is a group of genes that is used by retinal cells in the eye to make their corresponding proteins and these proteins work together in a very precise sequence to convert light that falls on the retina into a nerve impulse that passes down the optic nerve to the brain. In other words, this specialist group of proteins allow dogs to see.

FROM DOG TO DNA.....

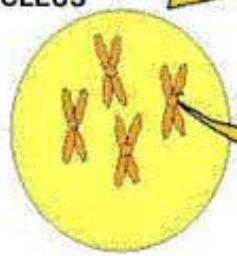
1. A dog is made up of more than a million cells

A CELL



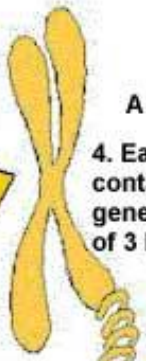
2. Each somatic cell, with the exception of red blood cells, contains a nucleus containing the chromosomes.

THE CELL NUCLEUS



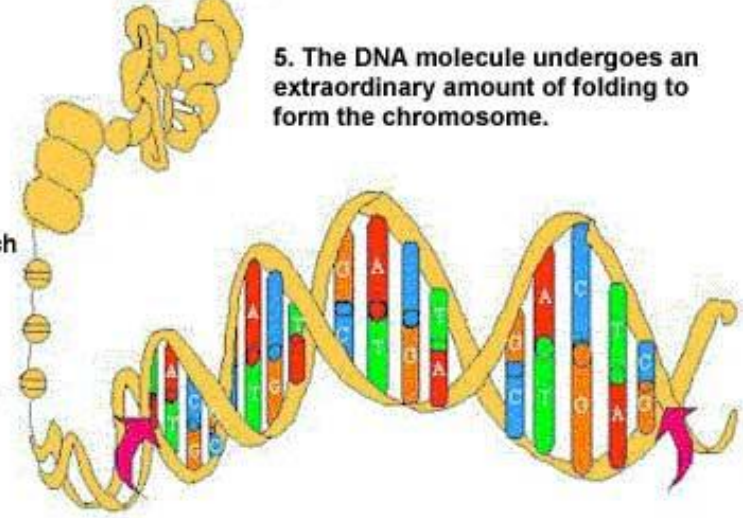
3. Each somatic cell nucleus contains 78 chromosomes - 39 cintributed by the dam (38 autosomes and one sex chromosomes) and 39 by the sire.

A CHROMOSOME



4. Each set of 39 chromosomes contains approximately 100,000 genes, representing a sequence of 3 billion bases.

5. The DNA molecule undergoes an extraordinary amount of folding to form the chromosome.



6. Each DNA molecule contains two strands which are wrapped around each other to form a helix.

Each strand is built up by the side by side joining of the four bases (Adenine, Thymine, Cytosine & Guanine).

A specific sequence of bases (approximately 2000) on one stand of DNA comprises a gene. the base sequence contains a plan that enables the cell to make a unque protein expression of which helps produce a phenotype. Proteins influence the development of phenotype, but often indirectly.

WHAT IS DNA?

Genes are made of deoxyribonucleic acid, more commonly known by its abbreviation, DNA. It is a complex molecule made of four individual chemical structures, known as bases and identified by the initials of their names (A, C, G and T), that are joined end to end. Each gene contains approximately 2000 of these bases in a seemingly random sequence. Within an individual gene the plan that is ultimately deciphered by the cell to make a protein is embedded within this sequence of bases; each gene has a unique sequence of the four bases.

WHAT ARE CHROMOSOMES?

Each cell contains an immense amount of DNA, 2 metres-worth if it was completely teased out. All of this DNA has to fit into a cell which has a diameter of approximately 0.0001cm; clearly the DNA has to undergo extraordinary folding to fit into each cell, giving rise to structures known as chromosomes. Each chromosome has a thread of DNA running along its length and the genes are arranged along this DNA thread, rather like beads on a string. There are 78 chromosomes in the canine cell (actually 39 pairs: one complete chromosome set carrying all the maternal genes and the second set carrying all the paternal genes); 38 pairs of so-called autosomes and two chromosomes involved in specifying sex (X or Y chromosomes). Sex determination in the dog is exactly the same as in humans; bitches have two X chromosomes whilst dogs have one X and one Y chromosome.

WHAT ARE MUTATIONS AND HOW CAN THEY CAUSE INHERITED DISEASE?

So, each gene contains a plan which is embedded in the precise sequence of bases along the DNA molecule that make up the gene and approximately one hundred thousand different genes are required to specify the dog. Sometimes the plan embedded within a gene becomes altered by a process known as mutation. This might involve a change in the sequence of bases, removal of some of the base sequence, or addition of extra base sequence within the gene. The consequences of a mutation will very much depend on the gene in which it has occurred. Some mutations are silent and have no consequences, others can affect the gene to such an extent that the plan can no longer be used to make a functional protein . For example, the mutation of one of those retinal genes that we discussed earlier would mean the retinal cells would be missing one of the proteins that are crucial for sight, leading to a disease like progressive retinal atrophy (PRA) and blindness in affected dogs. The main point is that once a mutation has occurred within a gene, it is fixed forever and cannot be reversed. The animal carrying the mutation will then pass on the mutant gene to some of its offspring; if the consequence of the mutation is a disease state, we now have an inherited disease.

WHAT CAUSES MUTATIONS?

It is fair to say that we do not know all of the causes of mutation, but we certainly know of some. Radiation in its various guises can interfere with the chemical structure of DNA leading to mutations in genes where the alterations have taken place. These radiation-induced changes can occur anywhere within the DNA molecule. Mutations are also probably a natural consequence of life. Remember we said that every time that a cell divides it has to make a complete copy of its DNA so that the two progeny cells each have a full DNA complement. This poses an immense problem because this copying involves the faithful reproduction of a sequence containing three thousand million bases. The machinery that cells have evolved is incredibly efficient, containing systems that attempt to identify and correct any mistakes that might occur, but it is not absolutely perfect and copying errors do occur. Occasionally, these copying errors will lead to mutations within a particular gene.

MUTATIONS ARE EITHER DOMINANT OR RECESSIVE

There are essentially two types of mutation that can occur in genes and their different effects are determined by the fact that dogs have two copies of every gene. The first type of mutation is known as a dominant mutation which means that if such a mutation occurs in a gene, the consequences will be felt despite the fact that there will also be a perfectly normal copy of the gene present. So, any animal that inherits a dominant mutation will be affected. The second type, a recessive mutation, is in many ways more sinister because if a recessive mutation occurs in a gene its effect is not noticed because the second, normal copy of the gene masks the presence of the recessive mutant gene. A disease caused by a recessive mutation will only be seen if an animal has two copies of the recessive mutation. This means that certain dogs can be carriers of a recessive mutation whilst outwardly appearing normal; you cannot tell a carrier just by looking at it. If two carriers mate, some of their offspring will inherit the recessive mutation from both the dam and the sire and therefore will be affected by the disease

INHERITED DISEASES IN DOGS

More than 350 inherited diseases have been described in dogs and many have been shown to be breed specific. (This sounds alarming, but contrast it with the fact there are over 3000 inherited diseases known in humans.) The precise mode of inheritance of approximately 175 of these diseases is known. 85% of these are known to be caused by a single gene mutation (so-called monogenic disorders); this figure breaks down into 70% being the result of a single recessive mutation and 15% being caused by a single dominant mutation. A further 10% give complex patterns of inheritance which cannot be explained on the basis of a single gene mutation. These diseases are known as polygenic diseases because the disease state results from the mutation of more than one gene. The best known polygenic disease is, of course, hip dysplasia which is present in a number of different breeds. Such diseases are obviously more difficult to interpret at the genetic level because we don’t know precisely how many gene mutations are involved and the precise contribution

each makes to the final disease state. Often, as is the case with hip dysplasia, environmental factors may influence the degree of expression of the mutant genes.

Some of the mutant genes are present on the X chromosome giving rise to so-called sex-linked or X-linked inheritance. Haemophilia is one such sex-linked disease, both in dogs and humans. It can be caused by mutation of a gene which is known to be present on the X chromosome. As many of you will know from your history lessons, Queen Victoria was a carrier of haemophilia. These X-linked mutations give rise to a characteristic pattern of inheritance. The disease gene is passed down the female line, but is only usually expressed in male offspring, the female being carriers. The reason is that males possess a single X chromosome which they always inherit from their mother. If a male, by chance, inherits the maternal X containing the mutant gene, it will be affected. Mothers, on the other hand, are usually protected from the effects of the mutation by the presence of a normal gene on their second X chromosome. Sex-linked inheritance accounts for the final 5% of the diseases where the precise mode of inheritance is known.

IDENTIFYING MUTANT GENES

So, the vast majority of canine inherited disease appears to be caused by a simple recessive mutation. If we could find a way of identifying carriers of these recessive mutations we could use the information to design breeding programmes that will eventually lead to the dilution and possible elimination of the disease gene from an affected breed. The simplest way to identify carriers involves developing tests for the presence of the mutated gene. If a dog can be shown to possess one mutated gene and one normal gene it is clearly a carrier.

Being able to identify a mutated gene and distinguish it from its normal counterpart will allow us to identify carriers of inherited disease. Identifying mutant genes is a real genetic needle in a haystack quest, similar to attempting to find a single mis-spelled word in a copy of the Bible (an analogy coined by Dr Matthew Binns). We clearly need to have some powerful shortcuts if we are to successfully identify individual mutant genes. Fortunately, such shortcuts are now available.

One approach is the CANDIDATE GENE APPROACH

One approach which has proven successful is the so-called candidate gene approach. This requires the study of like diseases in different species. We know a great deal more about the genes of man and mouse, and the mutations that cause diseases in them, than we do of the dog. The use of a candidate gene approach is best exemplified by the research that led to the identification of the mutant gene responsible for PRA in the Irish Setter . PRA in the Irish Setter was known to be very similar to a disease called Retinitis Pigmentosa in man and an inherited disease in mouse caused by a mutation known as rde. Scientists were able to show that mutation of the same gene caused the human and mouse disease. When this gene was studied in the

Irish Setter population, it was also shown to be responsible for PRA. A more recent example is the discovery of the gene responsible for PRA in the Cardigan Welsh Corgi. This newly-discovered mutation is different from the mutation causing PRA in the Setter.

Another approach is to use GENETIC MAPS.

Being able to use candidate genes gives a spectacular shortcut to the identification of individual genes involved in canine inherited disease. Unfortunately, for many of the canine inherited diseases, we cannot yet identify similar diseases in man or mouse so it is not possible to come up with candidate genes. An alternative short cut is required which will be generally applicable to all canine disease genes. For a number of years now scientists across the world have been collaborating to produce a genetic map of the canine genes. Just as a road map allows us to successfully navigate round the country and know precisely where we are, a genetic map allows us to wander along the canine chromosomes and know where we are at any particular time. The generation of the map, which is now nearing completion, requires us to lay down unique marker posts along each and every canine chromosome, each marker post identifying a unique position on just one chromosome.

HOW DOES THE GENETIC MAP HELP US TO IDENTIFY MUTANT GENES?

Well, we can use the map to discover which markers are physically close to the mutant gene causing the disease. To go back to the Bible analogy, this would be equivalent to identifying the precise page of the Bible on which the mis-spelled word is to be found, thus narrowing down the field of search. Identifying markers that are very close to the mutant gene, so-called linked markers, will identify a very small region of just one chromosome where the gene in question will be located. Not only will linked markers help narrow down the search field, they may also be diagnostic for the presence of the disease gene; if an individual animal possesses the linked marker it will, in all probability, also possess the mutant, disease gene. Such linked markers form the basis of the test for carriers of the Copper Toxicosis gene in Bedlington Terriers.

ONCE THE MUTANT GENE HAS BEEN IDENTIFIED, A DNA TEST CAN BE DEVELOPED.

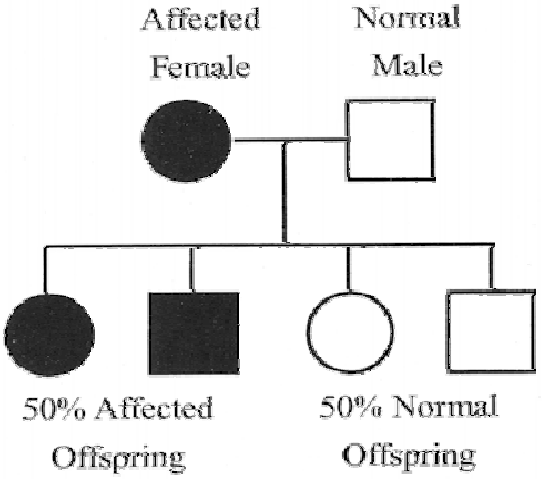
No matter how we have identified the mutant gene, either by the candidate gene approach or the linked marker approach, we now have the basis for a relatively simple test for the presence of the disease gene in individual animals. The test will be based on a small amount of DNA prepared from tissue taken from an individual dog. Ideally blood tissue will be used, but using tissue samples taken with less invasive techniques, for example scraping cells from the inside of the cheek (a buccal cell scrape), have also proved successful. This DNA sample will contain all the chromosomal material, and hence all of the genes, present in that individual. The DNA will then be

screened to see if it contains no copies, one copy or two copies of a particular mutant gene. For a disease known to result from a single recessive mutation, if no mutant copies are present, the animal is clear, if one copy is present the animal is a carrier and if two copies of the mutant gene are present it will be affected.

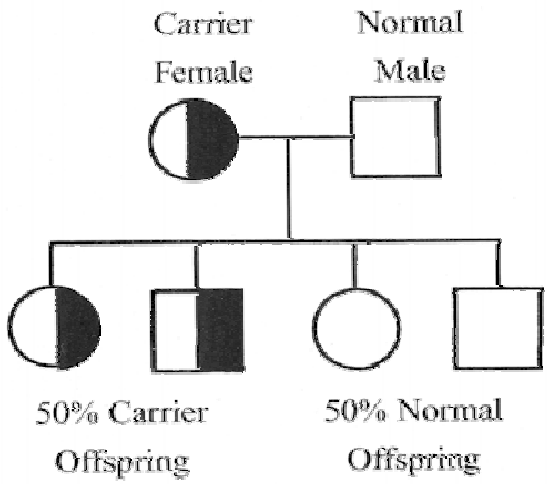
HOW CAN THIS BE APPLIED TO BREEDING PROGRAMMES ?

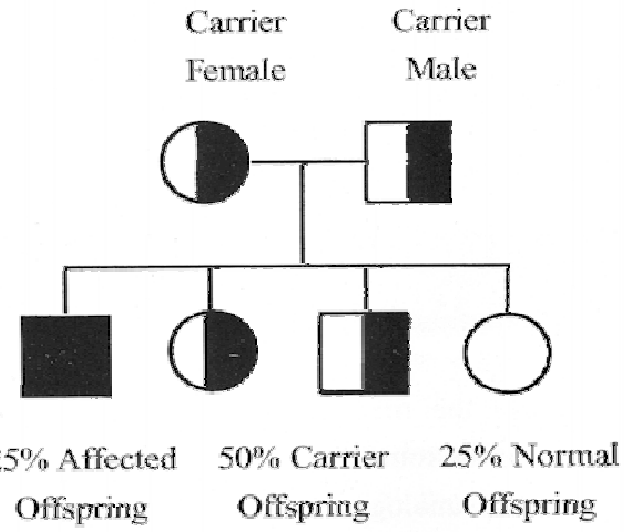
Being able to identify carriers will have an immense impact on breeding programmes which will be able to be designed to reduce the frequency of the mutant gene in the breed. If possible, carriers can be removed from the breeding stock within a breed, thereby preventing them passing on their mutation to further generations. However, DNA technology offers far more than this. (In fact, carriers can be identified from traditional pedigree analysis without sophisticated DNA tests and removed from the breeding stock.) Removal of carriers from breeding stock may not be the ideal solution because, although you are minimising the spread of the mutant gene, you may also be removing positive qualities that the breed requires in order to maintain its overall health; in other words, you could be throwing the baby out with the bath water. The availability of a DNA test allows much more subtle manipulation of breeding programmes to reduce the frequency of a particular mutation whilst retaining some of the positive features present in affected lines. For example, let's say that we have been able to identify a carrier bitch using a DNA test. Rather than simply removing this bitch from the breed's pool of breeding bitches, we can screen potential mates and identify a dog that is clear of the mutation. If the clear dog and carrier bitch are now mated, approximately half of their offspring will be carriers and the other half will be totally clear of the disease gene, so there will be no clinically affected dogs in the litter. However, we can go one step further, by DNA testing the Reality can be different. In principle, if you flip a coin it has 50% chance of coming down 'heads' and 50% chance of coming down 'tails'; in reality you often get runs of either 'heads' or 'tails'. So, the proportion of offspring in individual litters could differ from the expected outcomes given above.progeny we can identify the carriers and the normals. Once identified, the normals can then be bred on thereby removing the mutant gene from the population, but at the same time retaining many of the positive features that the line has to offer.

A. Dominant Mutation



B. Recessive Mutation





The DNA based test is a ‘once-only’ test which can be performed early in the life of the dog and the result will not change during its life.

The early identification of dogs affected with a late onset disease, one which often appears only after the natural reproductive life of the dog, will also be invaluable. Often the diagnosis of affected dogs is not made until after the natural reproductive life of the dog which has thus passed on its mutant gene before you have realised it was affected. Early identification of such dogs will allow their removal from breeding programmes and prevent them passing on the mutation to their offspring, but again, if necessary, they can be used for breeding in exactly the same way as we have already outlined.

For further information, contact the Kennel Club Canine Genetics Co-ordinator, Dr Jeff Sampson or Diana Nicolson at the Kennel Club, 1 – 5 Clarges Street, Piccadilly, London W1J 8AB. Telephone 020 7518 1023

GLOSSARY

(brief explanations of some terms that dog breeders may encounter)

Alleles: Alternative versions of the same gene, for example different alleles of genes are responsible for different eye colours.

Autosome: The general name given to all chromosomes other than the two involved in determining the sex of an individual (the X and Y chromosomes). The dog has 38 pairs of autosomes and one pair of sex chromosomes.

Bases: There are four bases which join together to form DNA: Adenine, Guanine, Thymine and Cytosine, commonly identified by their initials A,G,T and C. The bases join end to end to give a molecule of DNA. The bases join in a specific sequence, and it is this base sequence that represents the genetic plan.

Candidate gene: A gene potentially involved in a particular inherited disease in the dog which has been identified because the same gene is known to be involved in a similar disease in either man or mouse.

Carrier: In the context of inherited disease, is a dog which carries a recessive, mutant allele that is matched by the presence of a normal allele. On average, it will pass on this mutant allele to half of its offspring.

Chromosome: The body that carries the DNA or hereditary material within the nucleus of cells. A thread of DNA runs along the length of each chromosome carrying individual genes. The cells of the dog contain 78 chromosomes in total (38 pairs of autosomes and two sex chromosomes).

Cloning: (A gene) The technique used to isolate a specific gene and make multiple copies of it.

Congenital: Present at birth. May be inherited, but not necessarily.

DNA: (Deoxyribonucleic Acid), the major constituent of genes. DNA consists of two chemical strands wrapped around each other in the form of a helix. Each strand is made up by the joining together of chemical units called bases. The base sequence of one of the two strands within a gene stores the genetic message that allows a cell to make a particular protein.

DNA Sample: There are different ways that samples of cells containing DNA can be collected from an individual. A blood sample, a scraping of cheek cells (known as a buccal sample), or hair roots are all sources commonly used.

Dominant mutation: A mutation that can express itself when present only as a single copy, even in the presence of a normal allele.

Gamete: A reproductive cell. In the male this is the sperm and in the female the egg. Gametes carry only half the genetic material needed to form an individual. At fertilisation, a male and female gamete unite and the genetic material combines. The process of gamete formation is known as gametogenesis.

Gene: The basic unit of inheritance; a region of DNA which controls the hereditary characteristics of an organism. Individual genes consist of a unique sequence of about 2000 bases which permits the cell to make a particular protein. Each individual has two sets of genes and passes on a copy of one set to each of its offspring.

Gene Pool: All of the genes that exist within an interbreeding population.

Genetic Map: Built up by discovering and recording the precise location of genes and other regions of DNA along the chromosomes. The locations of those different sequences of DNA represents the genetic map.

Genome: The name given to one complete set of chromosomes, and hence genes, within an organism.

Genotype: All of the genes found in the cells of an individual. The genetic make-up of an individual will influence the appearance or phenotype of that individual.

Heritability: The transmission of characteristics, or proportions of characteristics, from parent to offspring via the sex cells. Some characteristics or diseases have a higher heritability than others.

Heterozygous: Individuals that have two different alleles of a gene for a particular characteristic. If one allele is recessive and one is dominant, the effect caused by the dominant allele will be apparent.

Homozygous: Individuals that have identical alleles for a particular characteristic. Recessive characteristics will only show if an individual is homozygous for that characteristic.

Inbreeding: The mating of first degree relatives such as mother to son.

Line-breeding: Breeding between closely related individuals.

Linkage: Describes the co-inheritance of alleles of different genes. Genes that are linked are physically close to each other on the DNA molecule that makes up a chromosome.

Locus: Each gene has a unique position or locus on a DNA molecule.

Marker: A component of a genetic map which uniquely identifies a locus.

Microsatellite: A special region of DNA which possesses an unusual base sequence where two, three or four bases are repeated over and over again, for example CACACACA etc or GCGCGCGC etc. these microsatellites have proved to be very useful markers in developing the canine genetic map.

Monogenic: A characteristic controlled by a single gene.

Mutation: A permanent change in the base sequence of DNA. This may be the result of changing a single base to another one, the removal of part of the base sequence or the addition of extra bases in the sequence. When a mutation occurs within a gene, it may alter the genetic plan that is embedded within that gene.

Nucleus: A structure present within most cells which contains the DNA in the form of chromosomes.

Phenotype: The overall appearance of an individual; looks and behaviour. The phenotype is the physical expression of an individual's genotype.

Polygenic: A characteristic controlled by more than one gene.

Recessive mutation: A mutation that is masked by the presence of a normal counterpart. Recessive mutations are only expressed when there are two copies of the mutation.

Sex chromosomes: Special chromosomes involved in determining the sex of an animal. In the dog, females possess two X chromosomes and males possess one X and one Y chromosome.

Sex-linked inheritance: Inheritance of characteristics that are determined by genes present on either the X or Y chromosome.

Somatic: All cells in a body apart from the reproductive cells (gametes).