David Sellars' articles for December 2013 LINK

What has Angelina Jolie got to do with genomics? Quite a lot as it happens

Earlier this year American actress Angelina Jolie made headlines when she announced that she had tested positive for a faulty BRCA1 gene, increasing her risk of developing breast and ovarian cancer. She opted to have a preventive double mastectomy to reduce her personal risk.

BRCA1 is a mutated gene, albeit an extreme one. Every living being has around 100 genes which are 'different'. Most will never make their presence known.

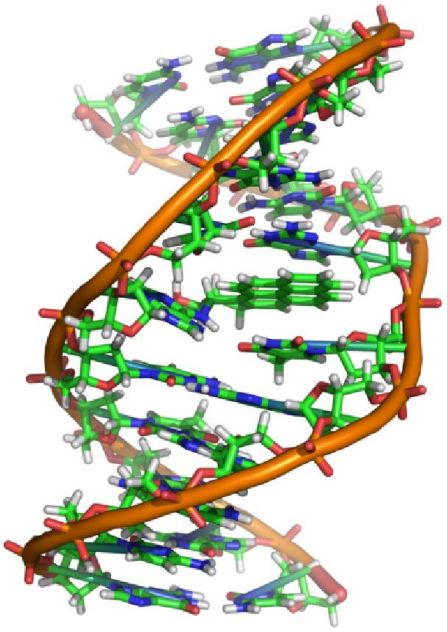
What is a mutation?

A new mutation is where an animal has a small difference in its DNA, and that difference was not present in the DNA of either parent. This was the case with the dairy sire Halcyon; neither of his parents carried the mutation. Once formed, however, mutations can be passed on from one generation to another. This was the case with Halcyon who passed the mutation to his son, Matrix.

Where do mutations come from?

There are many causes of genetic mutations:

- Radiation. There is background radiation everywhere and this can cause mutations. Radiation can be deliberately applied to an animal to cause more mutations than would occur naturally.
- Viruses. The presence of a virus at a critical moment of cell division can cause mutations.
- Errors during the formation of sperm (in the testes of the bull) or eggs (in the ovary of the cow.)
- Errors when DNA is replicated. Think of this in terms of photocopying, the copy is sometimes not quite as good as the original.



When you look at the complexity of DNA, it is not surprising that small errors can be made during the halving process (meiosis) which occurs in the ovaries and testes.

How common are mutations?

Very. Each human has approximately 100 new mutations, ie small differences in their DNA that were not present in their mother or father. This means you are a mutant. Get used to it, it is nothing nasty or abnormal.

A similar number of mutations are likely to be present in every calf. A single mutation can be very small, affecting only one of the 3.2 billion base pairs that make up the DNA of a single cell, or can be larger, affecting a small chunk of DNA.

Are mutations good or bad?

They can be good or bad, but most are neither. Most mutations are neutral, ie they don't have either a negative or positive impact on the animal.

Occasionally the mutation, even though it may be very small, (ie one of the 3.2 billion base pairs is different) will have a large effect. This was the case with Halcyon and his son Matrix. One base pair was different, in a part of the DNA that tells the cell how to make a particular and important protein. So the protein making 'recipe' was slightly wrong.

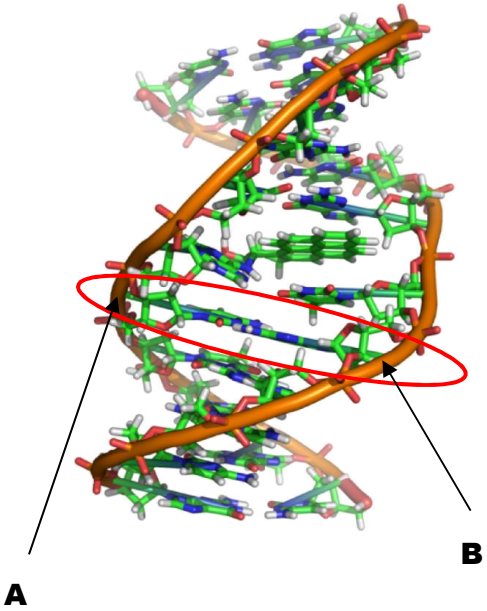
In the case of Halcyon, the particular protein was important in at least three areas of function of the animal, hair regulation, temperature control, and milk synthesis. Because the protein was not of the normal structure in half of the offspring of Halcyon and Matrix, they had unusual phenotypes - hairy, poor temperature regulation and poor milk production.

Some mutations are beneficial. The easiest example to illustrate this is a butterfly population that relies on camouflage for survival. If a mutation causes a slight change in wing colour that makes that individual more conspicuous, a predator will likely eat it. The mutant DNA is removed from the population. If on the other hand the colour change improves the ability of that butterfly to better disguise itself from predators, it will survive and breed and the DNA change that proved to be beneficial will survive and increase in the population.

These beneficial mutations are part of the natural process of species improvement, or natural selection. In the human timescale, the process can appear slow, but over thousands or millions of years, it is what has powered evolution.

Beneficial mutations tend to be retained within the population due to better survival characteristics. These are the ones LIC seeks to multiply through our bull breeding programme. Mutations that cause undesirable characteristics tend to drift out of the population.

In some places along the DNA molecule, variations or differences may cause no noticeable change in the function of the animal at all. In other places, tiny variations along the DNA molecule can cause very important changes between animals.



A representation of a tiny fragment of DNA. This fragment contains about 15 base pairs. The complete DNA in each cell contains 3.2 billion base pairs.

Recessive

In the diagram above, the red circled link across the DNA molecule has two ends. These ends are called alleles, one labelled A and one B.

Most genetic defects in cattle eg BLAD, CVM and Small Calf Syndrome are recessive. What this means is that both the A and the B end have to be faulty for the defect to show in the phenotype (observable traits) of the animal. If only one end carries the fault, the animal is called a carrier, and will show no symptoms of the condition at all.

Let's assume the A end is the faulty allele. In the ovaries of the female, when the DNA halves to produce the egg, the resultant egg could have the A allele and be a carrier egg, or have the B allele and be completely normal. The same occurs in the testes of the carrier bull; half his sperm will be carriers (have

the A allele) and half will have the B allele and be normal. It is only when the offspring is the result of a carrier egg and a carrier sperm that the offspring will have two of the A alleles, and show the defect.

The mating of two carrier individuals is represented as follows:

		Bull A	Bull B		
Cow	A	AA	AB	25% offspring AA - affected 50% offspring AB - carriers	} normal
	B	AB	BB	25% offspring BB - non carriers	

If only one of the parents is a carrier (in this case the bull) all of the offspring will be phenotypically normal, but half will be carriers.

		Bull A	Bull B		
Cow	B	AB	BB	50% offspring AB - carriers 50% offspring BB - non carriers	} normal
	B	AB	BB		

Small Calf Syndrome is a recessive defect, ie for a calf to show the small phenotype, both parents must be carriers, and even then, only 25% of the offspring will be small. Three quarters of the calves born to carrier parents will be normally sized.

How does LIC protect farmers from carrier to carrier matings of recessive defects?

LIC tests all its bulls for all known defects for which a genetic test is available. One easy way to avoid carrier to carrier matings is to eliminate all carrier bulls from bull teams and this is what we have done in the case of Small Calf Syndrome. This, however, is not practical in all cases. As DNA knowledge improves, many more recessives will be discovered, and there will come a time where, if all carrier bulls were removed, we would not be able to field a team of bulls that are non carriers of all known recessive mutations. There has to be another strategy.

If a bull is a carrier of a known defect and in the top one-third of his year group, he has a greater chance of being selected as an Sire Proving Scheme bull than if he were lower down the ranking.

CVM is a case in point and LIC has marketed some bulls that are CVM carriers provided they have other very desirable characteristics.

Farmers do not normally test their cows for known defects. The DataMate unit used by LIC AB Technicians has all cows of the technician's round loaded into it, and from the ancestry of the cow's sire line, we have worked out the chance of that cow being a CVM carrier. If the cow has a greater than 15% chance of being a CVM carrier, and the bull is a CVM carrier, then a warning shows up on the DataMate and suggests an alternative sire.

We have all got used to bull teams containing bulls who are carriers of something or another. The secret is to have your cow identification as accurate as possible, to ensure DataMate is able to accurately provide its protection in your herd.

How come the LIC Sire Proving Scheme does not identify all recessive defects?

Let's assume that a recessive defect allele is present in 10% of all cows, and one of the Sire Proving Scheme bulls also carries the defect allele. The Scheme uses around 450 inseminations to generate the 80 daughters in a proof, from about 180 calvings. In our example, 18 (10%) of these calvings are from recessive carrier cows. Using Table 1, 25% of the 18 will show the defect, ie around four of the births, two females and two males. This is why it is hugely important that Sire Proving Scheme farmers are meticulous record keepers and why, if the recessive defect allele frequency in the cow population is low, the defect in the bull may not be identified in the Sire Proving Scheme.

Dominant

A dominant defect is one where only one copy of the allele is required for the defect to show its phenotype. Dominant defects are far less common than recessives; LIC has identified only three dominant genetic defects in 60 years of bull breeding. In the simple dominant situation, there are no carriers.

The most recent example of a dominant defect was in the bull Halcyon and his son Matrix. Only one base pair was different in a part of the DNA that tells the cell how to make a particular and important protein. So the protein making 'recipe' was slightly wrong. Even though there was only one copy of the mutation, because it was dominant that was enough to cause the unusual phenotypes – hairy, poor temperature regulation and poor milk production.

When the defect is dominant, and the bull is mated across normal females, 50% of the offspring will show the defect:

Dominant in bull

		Bull (affected) A	Bull (affected) B	
Cow	B (normal)	AB	BB	50% of offspring normal 50% of offspring affected
	B (normal)	AB	BB	

This was exactly the proportions of affected offspring LIC found in the case of Halcyon and Matrix. Unfortunately the half of Halcyon's offspring that were defective were not noticed by their owners as being significantly abnormal until they were around 2 years of age, by which time Halcyon's son Matrix had received wide use.

How should our industry better equip itself to handle the increasing knowledge around mutations?

The position around defects is not getting worse. There will be around the same number as there has always been. It is just that in the past, systems and science didn't pick most of them up. Systems and science are evolving to the point where we are at last getting a much better handle on mutations and becoming better placed to manage them.

From the farmer's perspective, the more accurate your records, the better the position you will be in to avoid the cost of wastage from defective calves.

DataMate has served us very well over the last 15 years in the management of inbreeding and defects. In its current form, it will not be able to cope with the ever increasing complexity of managing mutations. LIC is working on developing a more powerful DataMate platform to manage the ever-increasing amount of knowledge in this area.

Cost of inbreeding to dairy industry – $10M per year

David Sellars, LIC Genetics Consultant

Inbreeding is the term given to a mating between close relatives. The more closely related two parents are, the more DNA they will have in common and the more inbred their progeny will be.

Inbred animals generally produce less, are less fertile, and don't live as long. The converse is also true; the more genetically different two parents are, the less inbred their progeny will be, and the more protein, more milkfat, more volume, better fertility, and longer life they will have. This is called hybrid vigour, outcross, or crossbreeding.

Artificial breeding, as well as natural mating practices, provide plenty of opportunity for inbreeding to occur and it is the role of breeders and AB companies to ensure that systems and tools are in place so that inbreeding (beyond low levels) does not occur.

A good way to view inbreeding is that normal sound herd improvement principles result in genetic gain, and inbreeding is a handbrake that slows that rate of gain. The net benefit of genetic gain is around $300 million per year, and the handbrake effect of inbreeding is currently in the order of $10 million per year.

Another view is that inbreeding is the opposite of crossbreeding. The adverse effects of inbreeding (production loss, reduced fertility and reduced longevity) are the opposite of the beneficial effects of crossbreeding (hybrid vigour).

You may be familiar with the term line-breeding. This describes deliberate closely related matings in the hope of doubling up the genes that contribute to some desirable trait to further improve that trait. Unfortunately it is just as likely to double-up on genes for traits that are undesirable.

Why should inbreeding be avoided?

As stated, inbred animals will simply not be as productive as animals which are not inbred – and they'll be likely to suffer from the impact of recessive genetic defects. There are, of course, examples of inbred animals which perform at acceptable levels, perhaps contributing to the old adage that if the cow born from an inbred mating performs well it is 'line-bred', if it disappoints, it is 'inbred'. In reality there is no difference between these two terms.

How is inbreeding measured?

Inbreeding is expressed as a percentage – an estimate of the proportion of genes in the two parents that are common. The percent inbreeding figure is technically called the inbreeding coefficient. It can be calculated by a computer programme that analyses the ancestry of both prospective parents and spits out the answer. For a father-daughter mating it is 25%. For a half-sib mating (son over father's daughter) it is 12.5%. The charts in this article will help you understand how these levels are arrived at.

What is the cost of the inbreeding disadvantages?

The measured loss that results from inbreeding is known as 'inbreeding depression'. Estimates calculated in New Zealand for the average losses per 1 % inbreeding per year amounts to around 0.1% production loss per 1% of inbreeding. The fitness traits of fertility and longevity also suffer with inbreeding, around 0.5% drop each per 1% of inbreeding eg –

	NZ Estimate
Milk	4.1 litres
Milksolids	0.36 kg
Longevity	9.1 days

These figures don't sound very large, but soon add up when multiplied by 25 in the case of a father-daughter mating, and by another 5 to account for the 5 lactations of the average New Zealand cow.

The average lifetime loss of production from a father-daughter mating is:

512 litres
45 kg milksolids @ $8.00 = $360
225 days less longevity

The production loss is from poorer performance, and does not include the production loss from the animal living around one lactation less. The shorter life simply makes the lifetime production numbers much worse.

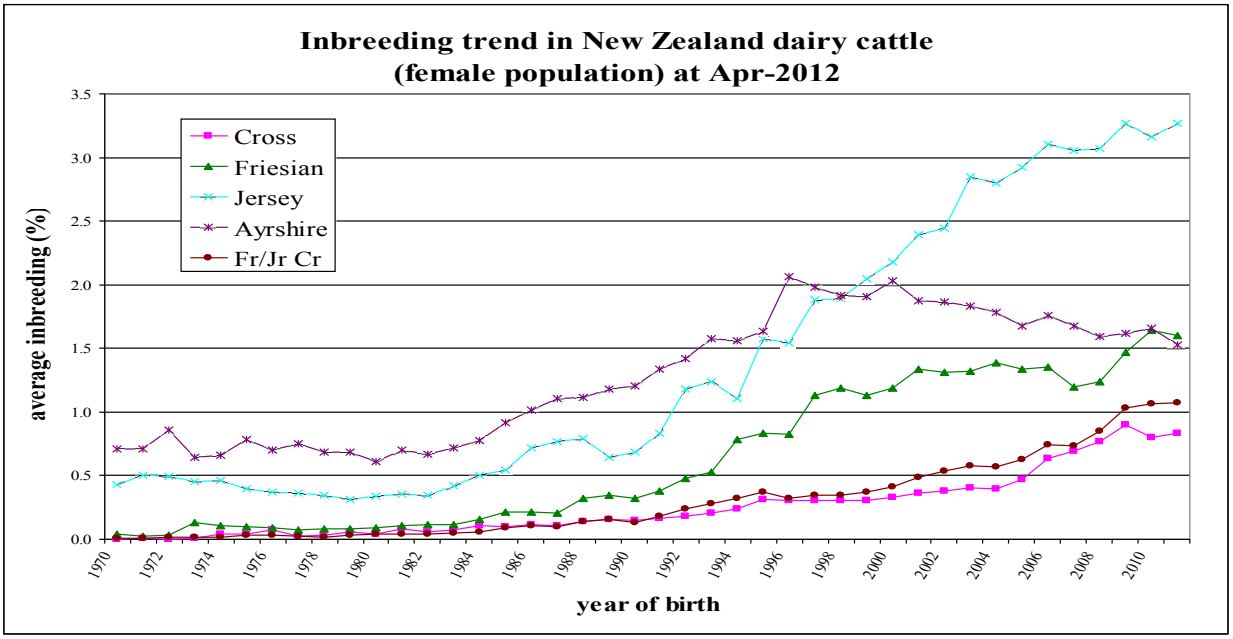
The lifetime loss of production from a half-sib mating (son over father's daughter) is half these figures ie $180 and 112 days shorter life.

There appears to be little or no effect on conformation as a result of inbreeding. There is a negative effect on fertility, but this negative effect is already accounted for in the loss in longevity days.

The other big downside that results from inbreeding is the effect of recessive genetic defects. These defective genes have to be carried by the cow and the bull for the defect to show in the calf. The more closely related the cow and the bull are, the more likely it is that the calf will receive the defective gene from both parents, and show the defect's condition. Fortunately there are not many known in the New Zealand Jersey population but in the Holstein-Friesian breed there are several eg BLAD, CVM and Citrullinemia.

What level of Inbreeding is considered acceptable?

Scientists recommend that the level of inbreeding should not rise above 6.25%. In New Zealand the average level of inbreeding for cows in the national herd is in the 1% to 3% percent range as shown in the following chart:

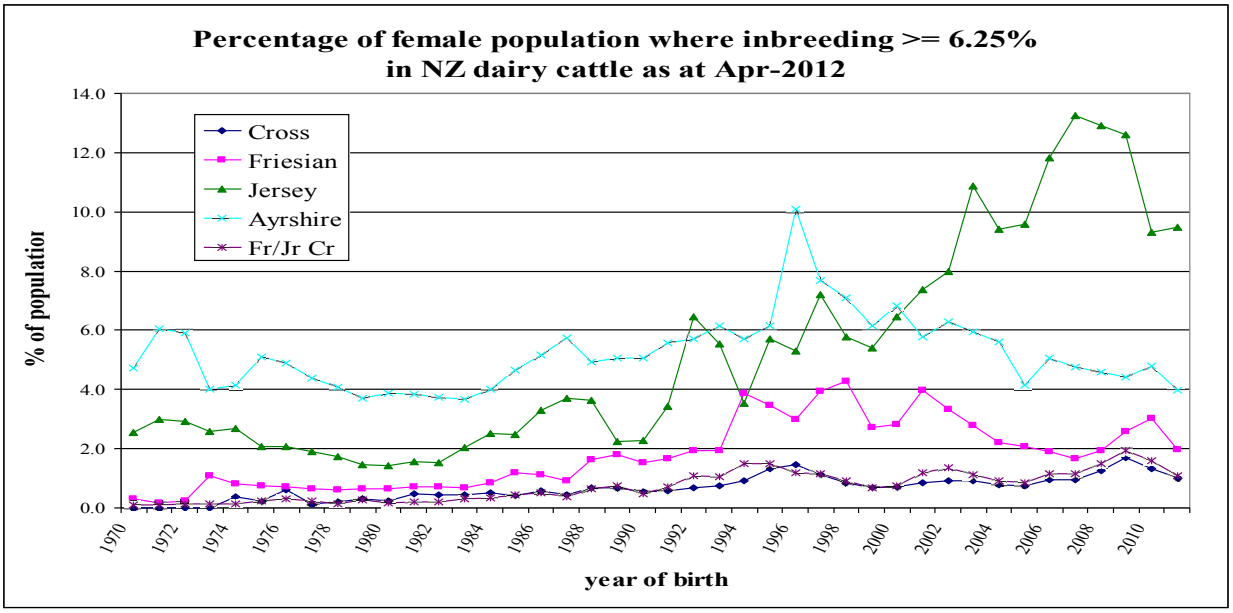


The trend for crossbred cows is, understandably, low at less than 1% because they generally originate from the two genetically different breeds of Holstein Friesian and Jersey.

The Holstein Friesian is also at acceptable levels, around 1.5%.

The Jersey population, at around 3.5%, is at levels we would not want to see increase much further. The breed is small and is struggling to identify outcross genetics. The USA estimates the inbreeding in its Jersey population to be around 6.5% - levels which would be unacceptable in New Zealand.

The Ayrshire breed was trending up, like the Jerseys, until the late 1990s when the Finnish Ayrshires began to be imported, and they were sufficiently genetically different to arrest and reverse the Ayrshire upward trend.



Another trend worth monitoring is the percentage of the cow population that has inbreeding levels above the 6.25% threshold:

As can be seen, DataMate's ability to alert the technician to possible inbreeding by issuing a warning in advance of a mating, is playing an important part in limiting the number of inbred cows.

Whose responsibility is it to avoid inbreeding?

In selecting young bulls to prove, it is the AB companies' responsibility to ensure that excessively inbred animals are not purchased for progeny testing.

The mating programme LIC uses to produce the next generation of bulls, takes future inbreeding into account by generating bulls that are not themselves inbred or closely related to the cow population that will be around when the bulls are widely used.

Where frozen Alpha Nominated semen is used, it is clearly the farmer's responsibility to ensure inbred matings are avoided. LIC assists customers who use CustoMate Plus, because this programme takes both inbreeding and recessive gene management into account thereby protecting the farmer from these 'evils'. Alpha Nominated customers who use LIC's AB Technician service gain protection through DataMate. DIY Alpha clients gain protection too and farmers are able to get an Actuate Inbreeding Report, on request, from their LIC Customer Relationship Manager.

In the case of random matings using liquid semen such as Premier Sires, LIC believes it is a shared responsibility of both the AB company and the farmer to avoid inbreeding. The farmer needs to take a responsible attitude to the issue, and the AB Technician must enter cow numbers into DataMate prior to insemination, to enable warnings to be generated.

Many farmers purchase Premier Sires for the ease and convenience of the product, and inbreeding control and deleterious gene management are part of that package.

How does DataMate control inbreeding?

DataMate contains a file of the farmer's cows, plus three levels of ancestry (if available) for every cow. It also contains a file of bull AB codes plus three levels of ancestry.

DataMate checks the inbreeding percentage of the proposed mating, and a warning is issued if the inbreeding level is greater or equal to 6.3%. DataMate also contains a record of the Long Last Liquid semen

the AB Technician has been issued for the day, and the frozen semen in the bank, so if an inbreeding warning comes up and the Technician selects the 'better semen available' option, DataMate will display bull codes of bulls more suitable for use over the cow.

What are the alternatives when the warning comes up?

During the AB Technician pre-run. the farmer will be asked what action should be taken if the warning comes up. Options include -

- Use the next bull of the same breed in the team.
- Use current bull from other breed.
- Use farmer's Nominated straw.
- Use Premier Sires backup.
- Ignore warning and inseminate anyway.

The farmer's preference will be written down on the AB certificate book for reference and followed when the warning comes up. LIC estimates that around 80,000 inbred matings are avoided annually – that's around two matings per Technician per day.

Semen allocation from LIC Newstead is managed to ensure that wherever possible, in a three day dispatch, the issued bulls will be by two or more sires. This way the next bull on the issue is unlikely to trigger a warning.