

# Waler Genetics

## The Genetic Diversity in the Waler Horse

We have supplied over eighty samples of Waler horse tail or mane hair, for genetic analysis in the hope of getting a profile for our Australian horses. We have now benefited from this exercise, however it must be understood that it still very much a work in progress. The purpose initially was to analyse the genetic variability in the Waler in order to determine its conservation status and genetic relationship to other equine breeds. From this and future work we hope to form the Waler genetic profile. The project was the initiative of the Waler Horse Owners and Breeders Association of Australia Inc. (WHOBAA), in conjunction with Dr E G Cothran of the Texas A & M University, Department of Veterinary Science.

### **What is the WHOBAA motto?**

As a breed association we don't breed for overall phenotype such as performance or looks. We breed to keep the historical integrity of the Waler alive, and our genetics are foremost to that. Our constitution states among other principles—*“To wherever possible and practical preserve and utilise the advantageous results of survival in a harsh and selective environment, as may be found in surviving descendants of those horses conforming to the Associations criteria, in remote areas where they have been managed or otherwise.”*

### **Firstly, what is genetic diversity and why is it important?**

Genetic diversity primarily refers to the variation between individuals within a population. It also can be genetic variation between different populations of a species, with some generic variants being specific to a particular population or sub population. The Waler projects looked at these types of diversity, but also the amount of variation that occurs as measured by the degree of heterozygosity (differing pairs of gene alleles).

### **Why is it important?**

Genetic diversity has some advantages for a population. It increases disease resistance, it gives populations the ability to adapt to environmental changes over time, and it buffers

against deleterious recessive genes (lethal/poor outcomes in offspring). It also allows individual animals the ability to respond to different longer term environmental conditions. Overall it is a sign of genetic health.

### **What factors act to change genetic diversity?**

The number one factor is population size - the smaller the size the lower the diversity. When considering these horse populations it is the effective breeding size that is important, i.e. the number of horses that actually breed and contribute to the gene pool. In any one mob, the effective population size is commonly influenced by the number of colts that grow to pass on their genes to the next generation. Inbreeding reduces individual variation, whereas out crossing increases diversity when unrelated individuals are crossed from the same or different populations. In populations where there is or has been, more inbreeding, the effective population size is less as the breeding individuals are genetically similar to each other.

Genetic drift is the *chance* change in allele frequency from generation to generation. As each parent gives fifty percent of their genes then it is true not all gene variants will be passed on. In small populations genetic drift can affect the overall population markedly. Rare genes can be lost and not replaced except by the long term mechanism of mutation. In smaller populations gene flow may be required to maintain a healthy diverse genetic variation. Gene flow will occur if small populations are allowed to intermix particularly with non related mobs. Although indiscriminate mixing can also have adverse consequences. Genetic variation maybe introduced that is inappropriate to the environmental conditions of the recipient population. Co-evolved gene linkages in the recipient population may be broken up.

Natural or human selection usually decreases variation, so that human selection in one 'direction' can cause some variation to be lost forever, including unknown variations associated with the traits being selected for. Variation can also be lost by chance with rare variants being especially vulnerable.

Mutation is the source of variation in populations. Mutation occurs at a slow rate, and although it increases variation over time, it is usually unimportant over a short time period. Already we can see that we as breeders can make a significant influence through our selection on the genetic composition of the Waler, to the benefit or demise of their genetic health.

### **What are we measuring ?**

Some terms that will help. Genes determine genetic characteristics and are found in the double stranded DNA that make up chromosomes. There are two sets of genes, in each cell of an individual, one inherited from each parent (as the chromosomes are in pairs). Each of

these genes can exist in a different forms - these differing gene forms are known as alleles. If both alleles are the same, identical, the gene is said to be homozygous, if one allele is different to its pair then the gene is heterozygous. For some genes there may be only a single allele for the whole population, for others there may be many different alleles. The frequencies of alleles can be used to determine the expected degree of heterozygosity ( $H_e$ ), which is the expected probability that an individual will be heterozygous for a given gene.

'Marker genes' are used to help measure and determine heterozygosity. A marker gene is a gene whose molecular sequence is known and whose chromosomal location has been identified. Marker genes do not code for traits so are not selected for or against by humans or nature. The location of a gene of particular interest can be found by identifying the linked molecular marker. We measure the occurrence of heterozygosity in our samples to find the genetic variation in individuals and the population.

### **What have we found?**

The Waler's observed heterozygosity is above the domestic horse mean, and among the highest of the known horse breeds, indicating a healthy genetic variation as a breed. The observed heterozygosity ( $H_o$ ) is nevertheless lower than the *expected* heterozygosity ( $H_e$ ) for a randomly mating population. This could infer some inbreeding but this is discounted as the Waler population is split and made from sub groups—sub group populations having less diversity than the overall population. The central Australian Walers also had some gene flow into the populations through mixing with other sub populations in the wild, or by some human intervention, of which is supported by research into the breeding stations history. The exact degree of gene flow into the Waler sub populations is not clear however.

The interesting find is the allelic variation, i.e the variation of the different alleles present in the population. The allelic variation is high, again a good measure of genetic health. This high occurrence is due to the high number of rare alleles that have been found in our Walers, which however occur in low frequencies. Nature mutates our genes over time which is a feature of natural selection. Rare alleles are those found that nature has kept in the genetic store house sometimes over the centuries, or as recent mutations over a few generations. They may come in handy one day, *but* the downside is they have such a low frequency of occurrence they are at risk of being lost—once a (rare) gene is lost it is gone forever. This may happen naturally but again careless human selection and breeding may caused this loss.

So, our Walers have some rare genes, rare genes that may well indicate that the populations have over time been kept by nature as a free living genetically healthy mix, or as a direct

result of adaptation to a particular environment. As breeders we need to think genetic diversity and health.

The Waler DNA was also compared to other equine breeds to try and determine a relationship and ancestry. Several analyses were done. Comparing allele frequencies in breeds gives a rough measure of ancestry. Genetic scientists (and I hastily add I am not one), illustrate genetic similarities in a RML diagram which is a tree type diagram that measures ancestral distances between breeds. Breeds that are genetically similar, group more closely together than those which are more different. So, amongst the eighty two breeds the Waler finds itself next to the Irish Draught—whats going on there?

Remember the RML tree doesn't designate the same ancestry but *linked* ancestry, i.e.—the Irish Draught (ID) has a similar *ancestral* genetic background.

Other close breeds are the Silesian , Quarter Horse, Welpokski, Sella Francais, Trakhehner, Don, Holstein and Hanoverian - again whats going on there?

The Waler was never influenced by these breeds but shares a similar background and that answer lies in the influence of the (English) Thoroughbred (TB). The TB has influenced many breeds and this comes out strongly in our Waler genetically, and backs up our historical knowledge. It is not that the Waler is directly descended from the TB but also from the other (mainly British) breeds with TB ancestral influence. The next test done was using sixty three breeds without the Irish draught, and this was found that there was no significant pairing with the other breeds, the Waler placement was between the Holstien and the Quarter Horse. All placement analysis pointed to the TB as a key ancestor. The ancestry of the Waler is closely tied to the TB, *but* not just the TB. The closeness of the Irish draught does not mean that it was a direct ancestor but implies the Waler has TB genetics *plus* genetics from other British horses.

It is timely to remind ourselves we are talking Old World TB, not modern TB, which has changed over time due to line breeding.

Dr Cothran and his team continued on with more tests. This time using fifteen equine breeds representing the diversity of breeds for this analysis. Five of those breed influences were found in the Waler. One particular test, (K2 test), compared and revealed that the Waler, Irish Draught (ID), Quarter horse (QH), Andalusian, (AN), Turkoman (TU), and Argentina Criollo (AC), all had similar clusters that indicated Thoroughbred (TB) and Syrian (Persian) Arab (SY) ancestry.

The Syrian Arab?

Firstly the samples used for analysis were from the Syrian Arabs (Persian horses), and again it is timely to remind us that the Arabian horse of today does not immediately equate to the Persian Arabian of days long gone. The term Arab was generalised in the 16th and 17th Century to include the Turkoman and Barb horses. The Persian horses were well sort after by the Indian Army. Some of those Persian horses made their way out to Australia in the early 1800's. Again the genetic analysis of the Waler bears some credence to our historical research. Whilst the Waler shows marked Syrian Arab influence, it can be summarised that this was from earlier Persian horse breeding, however it also showed that the TB itself had a very high degree of Syrian Arab in its genetic ancestry.

Further testing, (K3-K5 tests) narrowed down the Waler's (and the Irish Draught), genetic similarities to the Thoroughbred, Syrian Arab, some Exmoor (Mongolian) pony, Coaching horse (Cleveland Bay), and some cold blood breeds ( Welsh pony, Timor pony, Belgian draught, Suffolk Punch Dales pony, and Shire horse). The cold blood representation at the time could not be split further but our historical research points to the Timor pony as the likely major source and heavy draughts also as a source of genetic influence. That is why the continuation of DNA research with Timor samples is important to WHOBAA, further research is necessary to try and answer that particular question.

As to the Exmoor pony?

The Exmoor, the Portuguese Garrano, Fjord pony, and the Timor are all 'related' primitive breeds linked directly -we think most likely- to the now extinct Tarpan. In 1292 the Mongols invaded the Indonesian island chain with their Mongol ponies. These ponies were a mix of Tarpan and Asiatic horse (Przewalski horses), so maybe that is the link with the Exmoor even though we know that some Exmoor ponies were bought into Australia. What we don't know is what cross breeding occurred, if any.

This area needs more work, with increased sampling. It is being carried out as a part of the research to establish a world wide equine genetic database. Genetics, as yet, cannot distinguish Old World 'blood' from the new, and that is a problem that may be solved in the future. That in turn means we must rely on the best research possible, to determine exactly what outside influences are present with any horse presenting as a Waler. Genetics will only presently assist in determining acceptability as a Waler.

So far we now know our Walers have a genetic association with the five groups mentioned, and importantly the population samples indicate a varying mix of those horse breeds. Something our founding researchers knew and recorded.

The last tests run were to determine the relationship of Waler populations to one another. Our samples represented seven major horse breeding stations that we had been able to source samples from. Of those stations some were poorly represented with few samples, (other historic stations were not represented because we had no samples from them).

None of the Walers had a strong association with the area they came from, (with the exception of Mt Weld). The Waler from central Australia was found not to be unique to any of the stations or areas nearby. Mt Welds had unique genetics identifying them, where the central Australian horses were not identified with individual stations, the Mt Weld's are specific to their area.

This can be explained by the migration of sub-populations and the import and export of horses. Stations moved horses around, all the mares were bushed after mating, so were allowed to wander vast areas and only mustered infrequently. Traders such as Kidman bought in stock on a rotational basis.

The Central Australian Walers did not associate with the Mt Weld Walers. The Mt Weld horses were very much isolated and didn't/couldn't mix with outside station horses as the Gibson Desert provided a natural barrier.

The Waler genetics project is not static. Dr Cothran and his Lab need further samples to test and analyse toward a complete genetic definition of the Waler, but the pity is we just cannot supply any more samples from our original foundation stations. There is research work however, toward investigating Granite Downs, Todd River Downs and Indulkana stations as acceptable Waler stations.

## **Conclusions**

We know what we have in our Walers and this knowledge will be increased with more testing and scientific advances in genetics, although there is more work to do.

We cannot recreate the Waler with modern blood, using Thoroughbred, Arab, Timor, Cleveland Bay, or a cold blood breed. As previously explained these breeds themselves may well have undergone some changes as to what they were one hundred years ago, mainly because of human selection and breeding. We know Stations that try to reproduce the Waler or Waler type but that clearly does not constitute a Waler as we know from our founding horses.

The Waler shows levels of healthy genetic diversity that are near the average for the domestic horse breeds and there is no immediate concern for loss of that variation if we continue properly breeding to maintain this, even though the recognised Waler population is

small as a breed size. It is our responsibility as breeders, through the studbook, to ensure the Waler breed program takes into account the need to keep the genetic diversity of our old foundation bloodlines.

The Waler as we know it, is an endangered breed. The origins of the breed are as we have seen less clear cut, but we have strong (old world) TB influence—not just directly but from other British breeds. This means WHOBAA is right to define the breed on a historical base rather than a genomic one for the present time. In the future however we should have a more defined profile that will assist our classification of the Waler. Further it was found we had good evidence of genetic input from the ‘Cold Blood’ horses, such as heavy draught and true ponies, especially the Timor pony. I am confident that a clearer picture will emerge in time, but Cothran warns, “*However because horses are all related and there has been so much mixing of horse populations over time, it may be impossible to get the conclusive answer, for that we must wait*”.

I acknowledge Dr Cothran and his staff in preparing the Genetic Analysis of the Waler, for WHOBAA, and assisting our efforts. This paper was prepared from that Analysis.

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Reference:

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