# PLAYING GOD

**DR. STEVE HARRISON**, Director of Thoroughbred Genetics Ltd (UK) reveals some of the revolutionary 'selection-enhancing' technologies now available to the modern breeder.

As a nation of thoroughbred breeders and owners, Australians have a progressive outlook with regard to the use of new technology to gain a competitive edge. This puts them ahead of many of their more traditional counterparts in Europe. However, recent debates about the roles of artificial insemination (AI) and genetic testing in breeding and racing continue to raise many questions about what is possible, what is needed and what is ethical or even politically acceptable.

There is a common thread in both the AI and the genetic discussions, and that is in the potential for inbreeding to increase within the thoroughbred due to the use of 'selection-enhancing' technologies. The latter, in particular, merits consideration because it is possible to base selection on possession of specific preferred gene versions and so, inadvertently, eliminate less fashionable but equally valuable traits.

Before considering further parallels it might be worth marking out the playing field as far as the thoroughbred gene pool is concerned.

Breeding thoroughbred racehorses is unlike any other process involving different commercial species. Successful animals come in relatively variable shapes and sizes, and more often than not it is difficult to argue a precise genetic reason for their success.

It might be feasible to suggest that, after over three hundred years, useful versions of key genes involved in determining performance will have been fixed through selection and that only small differences exist. However, this may not be so. Unlike strictly agricultural animals, thoroughbreds are not objectively selected for the likes of live weight gain, milk yields or wool quality. They have the opportunity to perform over a range of distances and as a consequence, there is no uniform selection pressure.

Although broad selection is for more speed-orientated animals these days, the term 'selection' is a little tongue in cheek. Naturally, many of the mares bred from are poor quality and have not been 'selected' at all. Many will even have entered breeding careers earlier than anticipated because they have broken down or have physical problems. Most positive gene selection is via the handful of top-class colts entering stud each year. Breeding from lesser mares diminishes selection and fixation of positive gene versions in the breed *per se*. Arguably and ironically this may contribute to breed diversity whilst, at the same time maintaining the presence of undesirable genetic factors in the population. However, there might be some mileage in suggesting that useful race mares are valuable animals and are retained by wealthier individuals with access to proven and more expensive sires. Consequently, there may be some genetic fixation within an elite 'premier division' of top class families which is distinct from the lower divisions. In actuality, although they all belong in the same book, there is certainly already more than one thoroughbred gene pool.

**"Breeding from lesser mares diminishes selection and fixation of positive gene versions in the breed *per se*."**

The 'general' gene pool is in a natural state of flux and adaptation. Breeding trends change and as a whole we are now selecting for stamina capabilities very different from fifty years ago, let alone two hundred. In terms of performance it is arguably futile to consider the prowess or genetic influence of very distant ancestors, as excessive tests of stamina are no longer relevant.

It doesn't take much to divert the flow of the genes in the population. Even via the natural selection methods used over the last hundred years, significant changes to the gene pool occur. Although it has been in a controlled and steady manner, one clear example of this is seen in relation to Mitochondrial DNA (mtDNA), inherited only down the dam line. The genes carried on this type of DNA have

## MITOCHONDRIAL GENE DISTRIBUTION TABLE

| | MITOCHONDRIAL GROUP | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | VIII | IX | X | XI | XIII | XV |
| US 2002 | 18.05 | 6.40 | 13.70 | 1.85 | 3.40 | 8.90 | 2.48 | 0.87 | 4.75 |
| US 1952 | 17.40 | 8.20 | 12.60 | 2.40 | 2.40 | 4.60 | 1.20 | 1.40 | 4.20 |
| US 1902 | 6.20 | 5.40 | 6.60 | 3.00 | 7.40 | 2.20 | 0.60 | 8.20 | 2.20 |
| UK 2002 | 16.58 | 6.42 | 12.90 | 1.77 | 2.29 | 6.44 | 2.93 | 0.73 | 6.89 |
| UK 1952 | 15.60 | 7.80 | 13.60 | 3.80 | 2.20 | 5.00 | 2.20 | 0.80 | 8.20 |
| UK 1902 | 13.20 | 9.80 | 12.80 | 4.60 | 1.00 | 3.60 | 0.40 | 1.00 | 10.20 |

**The table shows the percentage change in mitochondrial groups over three periods in 100 years. Red is a decrease, green an increase and orange is no change.**

a role in determining the stamina potential of a horse. In one of our own studies on horses in the UK and the USA we have seen a shift in the population structure of these genes due to a change in emphasis on fashionable racing distances.

We can divide all thoroughbreds into groups based on their mitochondrial genes. From this we can work out the percentages of these groups in the general population in different time periods. In the results of the unpublished study summarised in the table above we can see how with many mitochondrial groups, there has been significant change in their percentage representation in the populations in the USA and the UK over a 100-year period.

**"Horses in the UK and the USA have seen a shift in the population structure of these genes due to a change in emphasis on fashionable racing distances."**

So, it is possible to effect quite a change via normal selection practices. Use of artificial selection practices based on a narrow selection criterion does have the potential to speed up population change significantly. However the degree to which they are employed or regulated will affect the impact.

The results of the Arrowfield Standardbred population study carried out by Peter Jenkins indicate that use of relatively unrestricted AI can result in an increase in inbreeding and diminution of the gene pool with little contribution to enhanced stallion fertility. However, the benefits of AI are broader than just potentially increasing fecundity. Similarly, it should be easy to regulate by limiting the number of artificial coverings using certification. There are many benefits, but really only one major downside and that is controllability. AI is a very attractive proposition that can make life easier for everybody, horses and humans alike.

The legal challenge to the sport's governing bodies about the use of AI, planned by Bruce McHugh, may find some support in the lack of consensus between the sport's global influential associations and some international legal precedents may soon be set. Whereas the Australian authorities may take a dim view of artificial methodology, in Ireland, the Irish Thoroughbred Breeders Association is already lending some support to selection of racehorses based on the use of single-gene selection methods. Whilst they are probably cost prohibitive to many and are likely to be used on an irregular basis, they also have potential to result in the loss of genetic material and diversity. In his struggle to present the case for AI to the authorities, Mr. McHugh may seek some solace in this and see some light at the end of the AI tunnel.

Our own area is in the field of genetic testing and assessment of thoroughbreds. It is always interesting to look at the parallels of DNA-based tools with AI in terms of gene pool effects. The most effective and responsible use of DNA analysis in managing the thoroughbred gene pool and breeding structure requires a balance of maintaining diversity whilst being realistic about the types and number of genes that will affect physical performance.

What exactly is the current state of play regarding the number of potential genes involved in athletic performance in racehorses? The best indication comes from the field of human performance genetics, where better insights have been gained as a result of greater financial support from the world of medicine. On a regular basis, workers in the human field supply updates on the number of genes ratified for implication in affecting athletic performance. In 2009 this number was estimated to be around 250 compared with 140 in 2004. The number is therefore rising consistently.

It is obvious from this that athletic performance is controlled by a multi-factorial genetic complex. All contributing genes are just components. We are a long way off the pace in racehorse breeding but the principles and mechanisms are the same, i.e. many genes are involved.

At Thoroughbred Genetics in 2006 we reported the first instance of an association between specific genes and thoroughbred performance. Further work has been carried out since and this year, the University of Dublin furnished details of the *MSTN* gene that affects muscle mass. However, we are still talking about components.

It is good to know the effects of single genes and to take note of their action but because many genes are involved in performance, single gene approaches for the selection and breeding of horses are not appropriate. If we considered selection based on any single gene, two possible pitfalls readily come to mind and these are more likely to affect the 'premier division' breeders and horses.

**"Mitochondrial DNA (mtDNA), inherited only down the dam line. The genes carried on this type of DNA have a role in determining the stamina potential of a horse."**

Firstly, a heterozygous horse will not 'breed true' and knowing it is heterozygous for any performance gene has diminished value in helping a breeding decision because it could be capable of producing numerous genotypes in the offspring. As a result, breeders could then shy away from heterozygous breeding stock and avoid using heterozygous stallions. If any test is used to select against heterozygous animals, this could increase inbreeding in the population. Additionally, selection based on a single gene that correlates with performance could lead to other correlating gene versions being overlooked.

Another effect will be that stallion owners will not want it publicised that a potential sire is heterozygous, as it is less likely to be pre-potent. This would be unfortunate as heterozygous animals are likely to be the most genetically sound. Importantly, from a commercial viewpoint, they could miss out on two to three years of nomination fees that they normally take before people realise the horse doesn't 'breed true'. In this respect, although more upfront, it wouldn't make much commercial sense for large stallion owners to

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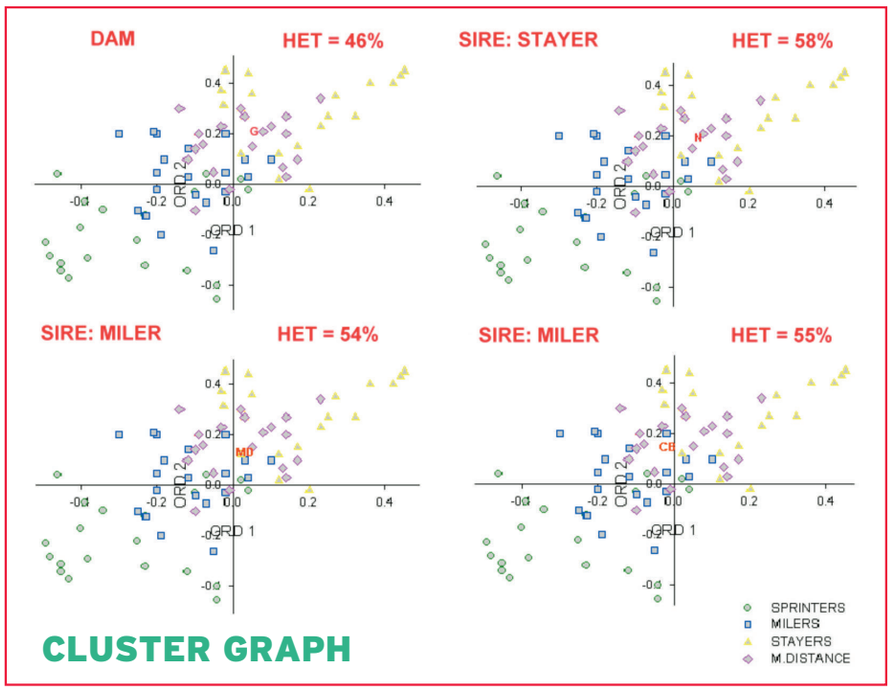
Are we limiting the gene pool?




CLUSTER GRAPH

publicise heterozygous stallions as they will not be pre-potent.

Certainly, as many geneticists and practical breeders agree, finding or breeding the desired speed/stamina attributes of a horse is a key to success. Because performance is genetically multi-factorial and setting aside commercial concerns, much use can be made of analyses that are not so specific but give a broader picture of a horse's stamina or performance potential. Importantly, these are not quite so damaging in terms of losing genetic diversity in the population.

As an example, this Cluster Graph chart illustrates such an approach to selecting or scoring horses on a multiple gene marker approach. Using a large number of non-specific DNA markers it is possible to group horses into clusters based on similarity. Each symbol in the chart refers to a DNA sample from a specific horse and tends to group them in line with stamina range. The letters on each of the charts represent a specific horse whose profile we are trying to assess. Horse 'G' is the dam of the other horses. 'N' is a Melbourne Cup placed horse by a stayer, and MD and CB are half-brothers by milers. There are general stamina groups but with some overlap. Although the dam and the Melbourne Cup horse are stayers, it can be seen that by using miler sires, we are able to move the stamina of the progeny more towards the shorter-range group. Gaining an idea of a horse's stamina cluster can assist us in knowing how far we need to shift the stamina profile of the progeny, which sires might be best to achieve this and even, what the potential stamina range an eventual foal may have.

**"Selection based on a single gene has pitfalls."**

On top of this, further use can be made of multiple gene markers to provide an added safety net for preserving genetic diversity by monitoring heterozygosity levels. There is an optimal level that is desirable in a horse. Below this, an animal is too inbred and there is a danger it may be subject to harmful inbreeding depression and less likely to perform well. Too far above this level will make the animal too out-bred, and an unreliable breeding prospect also means that fewer useful genes affecting performance might be 'fixed'. This is important and cannot necessarily be determined by pedigree.

Taking account of a horse's heterozygosity enables prediction of the potential status of resulting foals through calculations based on the mate's pedigree and *vice versa*. This allows us to aim for the optimum level in the foal, giving the best chances of performance and breeding success. From the graphs it can be seen that the dam's heterozygosity is quite low at 46%. As the three progeny all have higher values, we can also see that choice of sire can be used to increase genetic variation in the progeny whilst shifting the stamina profile to a desirable position.

In essence, we are able to more accurately manage stamina potential without the need to discard horses on the basis of single gene determinants, thereby preserving genetic variability and health.

At this stage, the main criticism levelled at the use of artificial insemination is the potential to limit the thoroughbred gene pool. We must always ensure that this is where comparisons between gene testing and AI end and that it is clear that new genetic technologies can be used to responsibly manage the breed.

For more information contact
Dr. Steve Harrison +44 1227 732 472
Website: www.thoroughbredgenetics.com