# THE FOUNDATIONS OF STAMINA

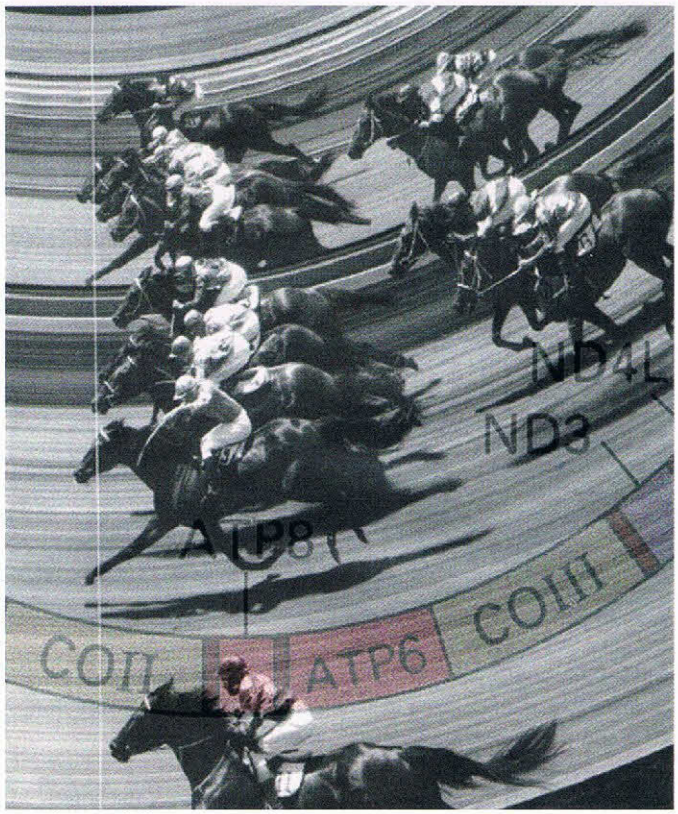
**In April 2006 a paper was published in the peer-reviewed scientific journal, Mitochondrion, entitled 'Mitochondrial DNA: An important female contribution to thoroughbred racehorse performance'. DR. STEVE HARRISON of Thoroughbred Genetics Ltd and JUAN TURRION-GOMEZ of the University of Salamanca describe the research, its findings and the relevance to practical thoroughbred breeding and racing...**

WITHIN the cells of all mammals are enigmatic structures called mitochondria. Often diagrammatically represented in school text books as tiny, sausage-like structures floating within cells, they have a big role to play in a host of physiological processes. They punch well above their weight in providing complications and contributions exceeding their stature to the extent that their full involvement in the lives of higher animals is still not fully understood.

Nobody knows for certain how mitochondria came to exist but it has been suggested that they were once independent microorganisms that, over the course of evolution, became incorporated into the cells of higher organisms.The rationale behind this theory is that mitochondria carry their own set of genes, which are inherited as a circular molecule, independently from 'conventional' genes and almost exclusively via the female line.

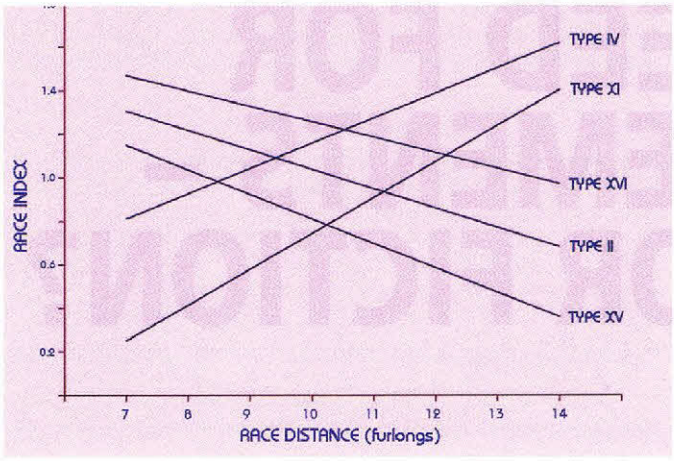
Of greatest note, these genes are mainly involved in energy release in cells and in the production of an energy-rich chemical called ATP. There are an estimated 10,000 mitochondria in each muscle cell and therefore potentially 9,998 more copies of mitochondrial genes per cell than there are copies of genes inherited via the 'standard' chromosomal route. This potentially makes them major players in the determination of exercise and performance characteristics. The role of these genes in the athletic performance of humans has already been demonstrated. The mitochondrial DNA (mtDNA) that makes up the genes is obviously a critically important molecule but it also contributes to the development of a range of medical conditions in humans including Alzheimer's and Parkinson's disease, general myopathy (muscular disorder), cardiomyopathy and importantly, exercise intolerance.

With their bearing on muscular function and potential performance, we were naturally intrigued about the relevance of mitochondrial genes to thoroughbred breeding and racing. The mtDNA molecule carries 13 important genes that contribute directly to processes by which energy is released in the cells, particularly those of the muscles and heart. These genes form part of larger respiratory complex groups, which also include genes carried on the chromosomes. Another region of the mtDNA, called the D-Loop, has a less clear role. It has been studied in the past as a means of assessing female-line diversity but does not provide information about the functional genes. It was our objective to determine if there was variation in the respiratory complex genes carried on the mtDNA by different female lines and whether this resulted in any obvious performance differences.




DNA samples from a group of 1,000 thoroughbreds were taken for study. This selection covered the majority of current European, U.S. and Australian female lines. We applied a laboratory procedure known as PCR to the samples, which effectively multiplies the genes of interest millions of times to provide enough genetic material for analysis. Then, using DNA sequencing and a scoring process called SSCP, we were able to check whether different versions of the genes are present in the various female lines.

Though five of the genes showed no variation in either the different female lines or a diverse group of non-thoroughbreds, there were eight genes for which a number of different variants were identified. By scoring the variant of each of these genes in each horse and by combining the information, we could assign all horses to one of 17




genetic groups or 'haplotypes'. Haplotypes were assigned Roman numerals.

As the mitochondrial DNA is maternally inherited, we would expect that all members of the same family should have the same haplotype. However, in our analysis of DNA from horses from 33 different female families we identified 28 'incorrect' sub-branches carrying mtDNA that would not have been expected had we been relying on pedigree or studbook information. These anomalies were spread over 19 of the 33 lines (58%) examined.

It is likely that 100% of thoroughbreds have numerous pedigree anomalies. Unfortunately, for the purposes of extended pedigree analysis and for a variety of reasons, it could only ever be possible to identify a minority percentage of all errors and an even smaller percentage would be correctable. Anomalies will be spread throughout pedigrees, including the patri-lines and not restricted to the bottom line. Ironically, it is testimony to the excellent record keeping of the studbooks that any errors can be identified at all and it was inevitable that past irregularities, out of the hands of the record keepers, were bound to happen. The keepers of the great books should not feel responsible for this. Parentage testing, introduced in the eighties, will eventually 'equalise' the anomalies over the years as the chromosomal influence of anomalous ancestors becomes more distant.

From our own position, the main point to absorb was that it is not possible to predict common performance trends amongst thoroughbred 'families'. On top of pedigree anomalies, families share haplotypes and haplotypes share families. The term 'haplotype' is not synonomous with 'family'. As an example, Haplotype I contained members of nine different families, five of which also occurred in other haplotypes. Similarly, a number of families had representatives in four or five different haplotypes. Therefore, as genetic similarities are based on haplotype and not families, it is with the former where our interest lay.

Bearing this in mind, we could determine the likely mtDNA haplotype of members of the current thoroughbred populations, making corrections when necessary. Using similar methods, it was also possible to assign haplotypes to winners of major horse races run between 1954 and 2003. The paper in the journal specifically refers to UK three-year-old races and this is also the example we will concentrate on here, but this was also carried out for two-year-old and Weight for Age races and to cover French, Irish, U.S. and Australian premier races. In the paper, the shortest British race was the 7f Greenham and the longest was the 1m 6f St Leger.

We could calculate the percentage winning success of each haplotype for each race during this period. This was done by dividing the percentage of wins of each haplotype by the percentage of its occurrence in the general population. This provided a Race Index (RI) for each race.

At three years old, there were significant correlations between RI and race distance for five haplotypes, accounting for 51.6% of the total three-year-old population. In particular, Haplotypes II, XV and XVI leaned towards shorter distances and Type XI and Type IV were better over longer. All of the others showed stamina optima distributed in between the extremes. The results confirmed that the order of racing merit amongst these haplotypes changes depending on the distance of race under consideration. This shows that there is a place for all haplotypes in racing. It does not mean that breeders should all go and breed from mares of a specific, preferred haplotype, discarding all others. As haplotypes and families are not the same thing, what it does mean is that it is possible to manage mares of particular mitochondrial haplotype more effectively to make the most of basic stamina attributes affected by the genes studied.

Naturally, mtDNA is not the 'be all and end all' of genetic influence on thoroughbred performance; many other genes also play a role. The title of the paper specifies the 'contributory' role of mtDNA to performance and needs to be considered in relation to other, complementary genetic factors.

However, identification of any single significant genetic factor contributing to stamina/speed determination can translate to better management of that variable. Even a small percentage improvement over more traditional breeding approaches could convert to noticeably better track performances.

Although the 'official' thoroughbred is over 200 years old, it is a funny old breed. It is obviously not a 'wild' population but neither is it a truly selected one. It is unlikely that preferred gene versions for performance will have already been 'fixed' in the population for two main reasons: Firstly, there is the opportunity for horses to race over different distances, which require variable physiological and genetic attributes; and secondly, there is actually little selection for performance (or anything else) on the distaff side of things. The widespread policy of crossing horses from different stamina optima also contributes to the 'mongrel' nature of the thoroughbred.

The upshot is that most horses probably carry a mixture of genetic 'components' not targeted to any specific stamina range and fixation of performance genes has not occurred. This is likely to end in failure and costs most breeders and owners a lot of money. Improved success requires more co-ordinated breeding approaches where horses are bred with specific stamina targets in mind and a mare's limitations or strengths are recognised and utilised.

The existence of this thoroughbred genetic soup means that identification of contributing genetic factors has particular relevance for the direction of breeding programmes. We hope that the information from our study and databases will help breeders target their schedules more effectively by supporting co-ordination of similar stamina features in potential offspring. We believe that the mitochondrial genes provide appropriate foundations on which to build. Pacemaker

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