Genetic relationships of seven horse breeds in South Africa based on DNA markers

Karen Botha

Dissertation presented in order to qualify for the degree *Magister Scientiae* in the Faculty of Natural and Agricultural Sciences (Department of Botany and Genetics – division Genetics) at the University of the Free State

November 2001

Supervisors:
Prof. J.J. Spies &
Miss A. Strydom

" The one best precept – the golden rule in dealing with a horse – is never to approach him angrily. Anger is so devoid of fare thought that it will often drive a man to do things which in a calmer mood he will regret."

- Xenophan 365 B.C.

Table of Contents

CHAPTER 3: Results

CHAPTER 4: Hardy-Weinberg and heterozygosity

CHAPTER 5: F-statistic

Acknowledgements

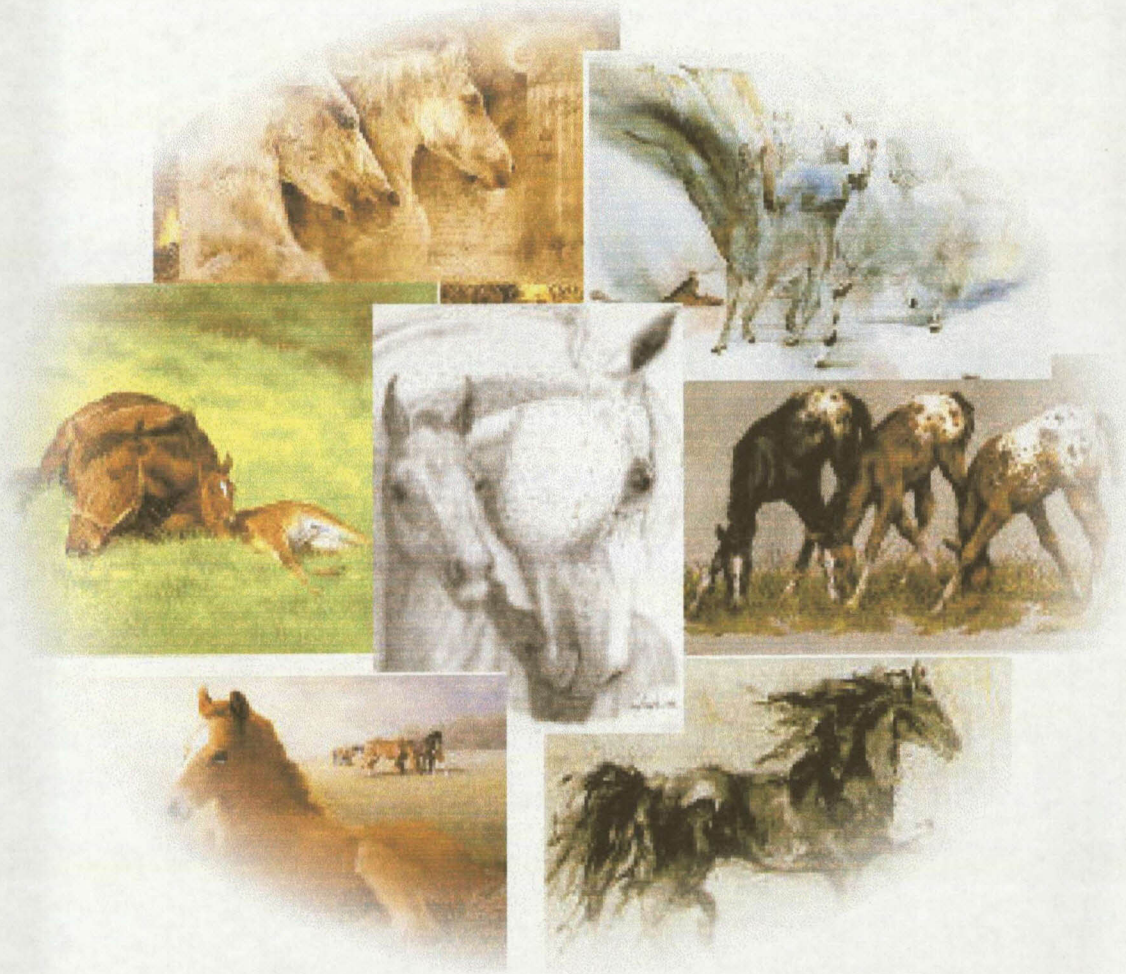
A special word of thanks to my supervisors, Prof Spies, and Adel Strydom, for their constant guidance, helpful tips and their patience.

The ARC Irene is thanked for the use of their facilities and their financial support.

The following people are thankfully acknowledged for contributions made to this study, through technical and other assistance.

Mrs. A. Kotze (ARC)
Mr. J.P. van Zyl (ARC)
Mrs. E.J. Harris (ARC)
Mrs. E. Weiermans (ARC)
Prof. H. van der Bank (RAU)

Chapter 1



CHAPTER 1

INTRODUCTION

1.1 Introduction

The story of the horse begins nearly 60 million years ago. Even before the emergence of *Homo erectus*, *Equus caballus* emerged from its small mammalian beginnings. Herds of horses were initially a source of food for humans, their importance being recognized in the cave art of Cro-Magnon Man (15 000 and 20 000 BC). Between 5 000 - 6 000 years ago on the Eurasian steppes, nomadic Aryan people began the process of domesticating the horse (Edwards 1994).

Domestication resulted in different breeds, which served a variety of purposes. During the last century, several hundred different breeding societies throughout the world defined salient characteristics and established stud books in an attempt to preserve the unique combinations of traits. The types and extent of variation present in these breeding groups are essential for science, breeders and breeding societies. Among traits that vary, external form, colour and disposition are relatively difficult to measure (Bowling & Clark 1985).

Horses are now mainly used for pleasure and sport of which there are many different varieties (Meade *et al.* 1984).

Hunting: Fox hunting, as we now know it, started in Britain but in the Middle Ages, horsemen of Eurasia would ride out with hounds or trained hawks in pursuit of ducks, birds, hares or deer. The boldest rode after big game: wild bear, lion and leopard, which they stalked and pursued over rough hilly country or through forests. The early huntsmen expected their horses to be no more than functional – even expendable in some cases. Through the ages, paintings have shown horrific falls over 'unjumpable' obstacles, demonstrating that there have always been sportsmen with more courage than skill (Meade *et al.* 1984).

Western riding: The original western riders were workers, not skilled horsemen. They were the cowboys who recognized that, though they might understand the need to move cattle, only the horse could get the job done. A rider who tried to dominate or direct his horse fell behind, lost his cow and frustrated his mount. So the basis of western riding is that the horse works freely rather than under the control of a rider. The rider requests, the horse responds. Western horses must be trained to understand the communication system so that they can respond to the request. The riders recognize and understand the anatomy of the horse. They know which aids will elicit a particular response from nerves and muscles. They make the decisions, but the horse carries out the task (Meade *et al.* 1984).

Long-distance riding: Long-distance riding must have the oldest origin of all equestrian sports. In America and Australia, long-distance riding has been a thriving and popular sport for over thirty years. The competitions are organized under various associations with strict rules to safeguard the welfare of the horse. Both countries run competitions nationwide for all categories of riders, including the top prestigious 100-mile (161.9 km) day rides of the Tevis in California and the Quilty in Australia. South Africa also has an active long-distance group with an annual 100-mile ride (Meade *et al.* 1984).

Showing: Showing is not undertaken for financial gain, as the prize money is often small. Shows, however, are valuable market places for breeders and professionals to advertise their wares. This category is divided into two main categories – showing in-hand and showing under-saddle. All breeds, ages and types of horse are catered for, from Shetlands to Shires (Meade *et al.* 1984).

Dressage: The ranks of dressage riders are growing rapidly. More and more people are awakening to the challenge of establishing a partnership with their horses whereby they can teach them to use their bodies with increasing grace, power and suppleness – to become equine gymnasts (Meade *et al.* 1984).

Driving: International driving trials are perhaps the most exciting modern development in harness sports. Their universal appeal has spread from Europe to Britain and is now emerging in America, Australia and South Africa. They are based

on ridden horse trials and following their format closely, consisting of three separate competitions which, together, test all the qualities and abilities of horse and driver. Competition A contains two elements, presentation and dressage. Competition B, the marathon, is the equivalent of the speed and endurance phase of a ridden three-day event. Competition C is the obstacle competition (Meade *et al.* 1984).

Show-jumping: Show-jumping is competitive precision riding over a set course of obstacles, which, although solid in appearance, are easily knocked over. Each knock or refusal incurs faults, which are marked against the competitor. The winner is the rider who can jump the course clear, and then jump at least part of it again, this second time racing against the clock, to be clear and faster than any other rider (Meade *et al.* 1984).

Eventing: This is the complete test of all-round horsemanship; it combines dressage, cross-country and show-jumping. An event may be held over one, two or three days, with a cross-country course of 2.4 - 6.4 km and 20 or 30 solid fences varying in the degree of difficulty to suit the standard of the event and the grade of horses competing (Meade *et al.* 1984).

1.2 History of horses in South Africa

Van Teylingen brought the first horses into South Africa in 1652, but the horses had to be left in St. Helena to run wild because of a storm. Two of these horses were recaptured and brought to the Cape in 1655. In 1653 four Javanese ponies were imported, but a lion killed the stallion just after their arrival (Van der Merwe 1981).

In 1659, 12 stallions and four mares were imported from the East. These horses were of a very poor quality. Two horses from Europe were imported in 1676 and in 1683 two Persian horses arrived from Mauritius (Van der Merwe 1981).

Until this time, all the horses belonged to the Dutch East India Company but, in 1665, the free burghers were allowed to breed horses of their own. By 1744 the Colony owned 5749 horses. Andalusian horses from South America were imported in 1778 and, in 1782, eight English stallions and five Berber horses were imported from

Boston in the U.S.A. Roadsters, Norfolk Trotters, Cleveland Bays and Friesian horses have been imported since 1880 (Van der Merwe 1981).

1.3 Breed characteristics

A breed is a group of animals within a species that has a specific phenotype that differentiates them from any other group (breed) from the same species. Their siblings must inherit the characteristic phenotype of the breed (Van der Merwe 1981).

The major division in the modern species is between saddle (light horses), and the heavy agricultural breeds. The former is by far the most numerous and is sub-divided into horses and ponies. Ponies are considered to be breeds below 1.52 m (Edwards 1994).

A further distinction is made between horses that are "hot-bloods", "cold-bloods" and "warm-bloods". The Arabian and Barb horses are termed "hot-bloods" along with their direct derivative, the Thoroughbred. The title indicates a unique purity of bloodline possessed by no other breed of horses in the world. "Cold-blood" is the term given to the heavy draught horses of Europe. Horses combining cold and hot blood in various percentages are called "warm-bloods". Hot- and warm-bloods are, for the most part, long in the limbs and narrow in the body. The "cold-bloods" have a vertically deeper chest and shorter, thicker legs than the warm blood horses of the same shoulder height. Their feet, in relation to the length of their faces, are very broad (Edwards 1994).

In a modern context, a breed refers to horses registered in a studbook. These are horses that have been bred selectively over a sufficiently long period of time to ensure a consistent production of common stock sharing and defining characteristics in respect of size, conformation action, and also colour (Edwards 1994).

Genetics enables breeders to predict the coat colour of an unborn foal. It is known that grey is dominant to all other colours. The order of dominance for the other colours is bay, brown, black, and chestnut (Figure 1.1). Accepting simple genetic principles, it follows that it may be possible for physical and mental characteristics,

such as jumping or racing ability, for example, to be transmitted within a carefully formulated bloodline. Breeding on the basis of documented pedigrees and performance records as well as taking into account the proven ability of the progeny of both parents, represents the practical application of genetic theory (Edwards 1994).

Figure 1.1: Differences in coat colours (Edwards 1994).

It is, therefore, the goal of the breeders and breeding societies to keep the bloodline pure by keeping records of all the registered horses of each horse breed (Van der Merwe 1981). There are a number of horse breeds in South Africa. In this study, seven of these breeds will be compared to determine the genetic variability within and between different breeds.

1.3.1 Arabian horses

The Bedu tribes of Arabia kept few written records, but preserved their horses' pedigrees by word of mouth. One of the first written accounts of Arabian horses was produced by the Arab historian, El Kelbi. He attempted to record the history and pedigrees of the Arabian horse, starting around 3 000 BC. His work, although sometimes more allegorical than factual, underlines the antiquity of this equine race (Edwards 1994).

Figure 1.2: A photograph of an Arabian mare and foul (Anonymous 1998a).

Later, the Emir Abd-el-Kader (1808-83), in his correspondence with a Frenchman, General Daumas (1803-71), divided the history of the breed into four eras: Adam to Ishmael, Ishmael to Solomon, Solomon to Mohammed, and then from the Prophet onwards. The four eras proposed by Abd-el-Kader are more firmly rooted in fact. The first may not be entirely relevant, although Ishmael, the outcast son of Abraham and the first ancestor of the desert Bedu tribes was an actual person who made use of horses. After the break-up of the Bedu tribes, which followed the death of Ishmael, the story of this desert horse continues in King Solomon's reign. With total disregard for the Israelite law that forbade the keeping of horses on the grounds of idolatry, Solomon encouraged horse-breeding by keeping no fewer than 1 200 ring horses and 40 000 chariot horses in his royal stables. Finally there is the influence of

the Prophet Mohammed and Islam, which ensured the spread of the breed throughout the Old World (Edwards 1994).

The Arabian horse is a specialized desert product and a close descendant of the primitive stock of Arabia. The Arabian horse is typically gentle, affectionate and familiar to the point of being troublesome. The Arabian horse is renowned for its soundness of limb and ability to withstand hardship (Anonymous 1998a).

The appearance of the Arabian horse is unique among the equine races (Figure 1.2). However, it would be wrong to accept the assertion put forward by some breed enthusiasts that there is only one true type of pure Arabian – there are differences of detail, nuances within an overall type. The most distinctive feature, apart from the outline, is the short, very fine, "dry" head, which has clearly visible veining. In profile, the face is notably concave, and the forehead is convex. Arabian horses have 17 ribs, 5 lumbar vertebrae, and 16 tail bones, whereas other horses have an 18-6-18 pattern. This bone formation accounts for the distinctive shape of the back and quarters and the high tail carriage (Edwards 1994).

The colours of Arabian horses are grey, chestnut, bay and roan and occasionally black. Although individuals vary, most are between 14.2 (1.14 m) and 15.2 (1.22 m) hands in height and weigh between 360 and 450 kg (Anonymous 1998a).

The South African breeders of Arabian horses, geographically isolated from other Arabian breeding nations due to the country's location on the southern tip of Africa, have developed their own unique Arabian population based mainly on old English lines, especially Crabbet and Hamstead, with an admixture of modern Egyptian and a small amount of Russian and Polish blood (Anonymous 1998a).

Today about 11 000 pure and partbred Anglo-Arabians are registered or recorded in South Africa. The breeding and showing classes of part Anglo-Arabians are very popular in South Africa and many studs breed only partbreds and Anglos or Welsh/Arabian/English Thoroughbred crosses for riding and performance (Anonymous 1998a).

The first purebred Arabian horse arrived in South Africa at the beginning of the last century. It was the stallion *Azrek*, imported by the South African mine magnate, Cecil John Rhodes, from Wilfred and Lady Anne Blunt's stud in England. A few years later, Captain Gomer Williams imported a few pure Arabian mares and stallions and started the first Arabian stud in the country. Sir De Villiers Graaff, who imported Arabians from Argentina, and Mr. W. Lovemore, who imported Crabbet Arabians, soon followed suit. However, the offspring of these first three studs were mixed with other breeds and thus lost to the Arabian breed. Betty Arnold, of Bedford in the Great Karoo, founded the oldest existing Arabian stud in South Africa in 1951, starting her Olford Arabian Stud with the importation of three pure Crabbet mares from England. After 1960, many new studs were started all over South Africa and many horses were imported from England, Europe and the United States. The Arab Horse Breeders' Society of South Africa was established in 1961 and presently has about 500 members, most of them active breeders (Anonymous 1998a).

1.3.2 Quarter horses

In spite of being associated in many people's minds with the American West, the Quarter horse had its origins in the Eastern American states and the early European colonization of that area. The Spaniards brought Barbs (and possible Arabian horses), whereas the later settlers from England brought in Galloways from Scotland and Thoroughbreds. The American Indians stole horses from the Spaniards and, in due course, the English obtained some horses from the Indians and crossed their Thoroughbreds with these 'Chickasaw' ponies to produce a tough, strong animal, which developed (Figure 1.3) into what is known as the Quarter horse (Meade *et al* 1984).

The most prized characteristic of this horse, created by the sport-loving English settlers, was the ability to sprint over short distances from an explosive standing start. The horses were raced over 400 m (quarter-mile) stretches and for this reason these horses became known as the "Quarter horse" or "Quarter miler" (Edwards 1994).

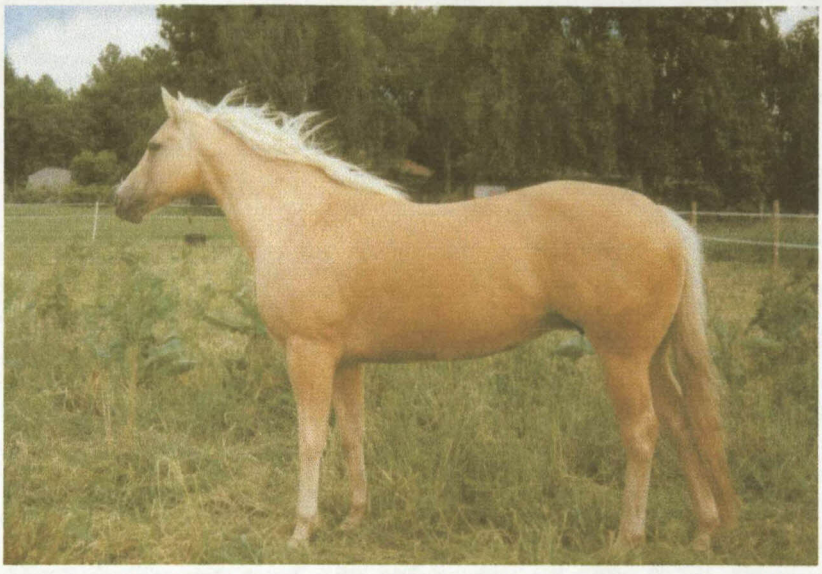


Figure 1.3: A photograph of a Quarter horse (Anonymous 1998a).

There are 12 principal Quarter horse families, at the root of which are the breed's two most notable foundation sires, *Janus* and *Sir Archy*. *Janus*, an imported English horse who died in 1780, was responsible, through his son of the same name, for the great Printer line, which is one of the most influential. *Sir Archy*, the son of the first English Derby winner, *Diomed*, was also involved with the beginning of the American Saddlebred (Edwards 1994).

The American Quarter Horse Association (AQHA) is the world's largest breed registry and equine recreational organization, with more than 3.1 million American Quarter horses registered worldwide and AQHA membership numbering more than 200 000. The AQHA international headquarters in Amarillo, Texas, maintains the pedigrees and registration records of all American Quarter Horses and oversees various programmes and incentives, including races, shows and recreational activities (Anonymous 1996a).[1]

The AQHA was founded in March, 1940, in Fort Worth, Texas, by a group of horsemen and ranchers from the South Western United States. They were dedicated to preserving the pedigrees of their ranch horses, many of which could be traced back to colonial America and the year 1611. This distinct strain of horses was respected for

[1] Anonymous 1996a. Quarter horse. American Quarter horse Association. Oklahoma State university . http://www.ansi.okstate.edu/breeds/horses/

unique conformation, athleticism and disposition, all results of selective breeding for both speed in quarter mile races and cow sense on the open range (Anonymous 1996a)[1].

The purpose of AQHA is to collect, record and preserve the pedigrees of Quarter horses in America. They also publish a stud book and registry and are involved in all other matters such as the history, breeding, exhibiting, sale or improvements of this breed in America (Anonymous 1996a)[1].

The first fully registered American Quarter horses arrived in South Africa in December 1988. The world-famous golfer, Gary Player, imported five stallions and a group of mares, four of them in foal. His goal was to develop in South Africa a true sporting horse that would excel in polo, show-jumping, eventing, gymkhana and general riding. Of the original registered Quarter horses, only a tiny population of purebred and half-bred animals survived in South Africa. In March 1989, a small group of Quarter horse enthusiasts formed the American Quarter Horse Society of South Africa (AQHSSA) (Anonymous 1998a).

The AQHSSA, which is still struggling for recognition by the American Quarter Horse Association, keeps records of about 380 purebred Quarter horses registered with the South African Stud Book and Livestock Improvement Associations. One hundred and sixty six of those horses are also registered with the AQHA (Anonymous 1998a). With only 45 members, the AQHSSA had 860 Quarter horses registered in 1995. In 1996, AQHSSA became affiliated to the AQHA under the name South African Quarter Horse Association (SAQHA). Recently the AQHSSA has grown to 78 members (Anonymous 1998a).

The pinnacle event for the American Quarter Horse exhibitors is the AQHA World Championship Show held yearly in Oklahoma City. This invitational event draws more than 3 000 open and amateur exhibitors from around the world, competing for an award purse valued at more than $1.2 million, making it the largest and richest world championship horse show in existence (Anonymous 1996a)[1].

1.3.3 American Saddler horses

The American Saddler (Figure 1.4), like many of the American breeds, began as an essentially practical animal. It evolved during the 19th century in the Southern states, particularly around Kentucky, and was initially known as the Kentucky Saddler. Standing at 1.63 m or more, the American Saddler was the result of selective breeding based on the Thoroughbred and the Canadian and Narragansett Pacer, the Morgan horse which, by then, was well established in Vermont. The result was an elegant utility horse. In the early days it was used to plough, carry a man in comfort over rough terrain and doubled up as a smart carriage horse to go to church on Sundays (Edwards 1994).

Figure 1.4: A photograph of an American Saddler horse (Anonymous 1998a).

In 1891 the American Breeders' Society was established in the U.S.A. At first a list of ten of the most imported ancestors was published in 1901 but, in 1908, the list was reduced to only one stallion, Denmark. In the first edition of the breeders' society, 55% of 3000 registered horses could be traced back to purebred Denmark (Van der Merwe 1981). Gaines' Denmark, sired by Denmark and whose dam was a natural

gaited mare, established the Denmark family of the American Saddler. Over 60% of the horses in the first three registry volumes of the American Saddlebred Horse Association trace back to Gaines' Denmark. In 1991, Harrison Chief was designated as a foundation sire, along with Denmark. The Chief family has a similar background, with a dominance of blood coming from the Thoroughbred Messenger and his son, Mambrino Chief (Anonymous 1999a)[2].

Both Denmark's and Chief's families go back eight generations to the ancient Thoroughbred, Blaze. One theory holds that, when these families were crossed back, beginning in the late 1800's, the American Saddler was permanently fixed as a dominant breed. By the time of the Mexican War in 1846, the American Saddler was a well-established breed. Entire companies of American volunteers from Kentucky and Missouri were mounted on these horses. In 1856, St. Louis, the largest city west of the Mississippi, held its first great fair, which also featured the nation's first major horse show (Anonymous 1999a)[2].

Because of the increased popularity and commercial value of the American Saddler, breeders began to call for a breeding association and registry in the 1880's. As a result Charles F. Mills of Springfield, Illinois, began compiling pedigrees and formulating rules for a registry. *The Farmers Home Journal*, a newspaper published in Louisville, Kentucky, called for a meeting on April 7, 1891 to organize the association. The registry was established that day, the first horse breeding association in the U.S.A. (Anonymous 1999a)[2].

The first American Saddler to be imported to South Africa, was the stallion Myer's Kentucky Star, registration number 7675. The horse came from Boston in the U.S.A. and was imported by Mr. Claude Orpen from Barkley-East (Van der Merwe 1981). In South Africa, the first American Saddler Horse Breeders' Society was founded in 1949. At first parentage was determined by blood typing but, since 1997, DNA typing is used. The Breeders' Society has 450 members, with more than 22 000 horses on record. The first South African-bred Saddler horse to be exported to

[2] Anonymous 1999a. The history of the American Saddlebred. American-Saddlerbrem.com. http://www.american-saddlebred.com/asbhist.htm

America was the stallion, Commander in Chief, that won the World championships in the U.S.A. In 1995 (Anonymous 1998a).

The American Saddler has a height of approximately 1.52 - 1.62 m and a body weight of about 550 - 700 kg. The main colours of the breed (Figure 1.2) are brown and bay, but there are a few black, gray and even palomino horses registered (Anonymous 1998a).

The modern American Saddler is shown in the show ring both under saddle or in harness. In the saddle division, it is shown in either three- or five-gait classes. The three-gait horses are shown with a hogged mane and a trimmed tail. The five-gait horse, the supreme Saddler, is shown with a full mane and tail. As well as the first three gaits, it performs the slow gait, a prancing, four-beat motion with a moment of deliberate suspension preceding each footfall and the rack, the full-speed "flashy, four-beat gait free from any lateral movement or pacing" (Edwards 1994).

The American Saddler Horse Breeders' Society in South Africa organizes and presents the National Saddler Horse Championships, the second largest championship for Saddler horses in the world. Over 800 horses compete over a period of three days (Anonymous 1998a).

Today, American Saddlers are dazzling show horses, reliable police mounts, steady companions and celebrated movies stars. Most of the horses used in the film *Gone with the Wind*, were American Saddlers. *Flika, Fury, Black Beauty* and *War Winds* in the Elizabeth Taylor movie "Giant", were all American Saddlers. The American Saddlers show business success can be attributed to a combination of their striking good looks, willing trainability and a simple love of attention. Whether a Saddler is working in the show ring, on the movie set or in the streets of a major metropolis in partnership with a police officer, the horse is a picture of elegance (Gary 2001) .

[3] Gary, T. 2001. Recognizing the American Saddlebred. http://www.azgardfarm.com/recogASB.htm

Despite its relatively short history, the Saddler has evolved into a distinct breeding type with easily recognizable characteristics (Meade *et al.* 1984).

1.3.4 The Cape horse

The development of the Cape Horse is parallel and inseparably connected to the history of the early settlers of South Africa. It started shortly after the arrival of the Dutch at the Cape in 1652 (Anonymous 1998a).

The Dutch East India Company had realised that horses would be needed at the Cape settlement. They, however, did not send any horses from Holland with Van Riebeeck and his party, because of the length of the journey. They had arranged for several animals to be sent to the Cape from their headquarters in Java, a shorter and safer voyage. The first horses to land in South Africa were of eastern type and not European. They were small, hardy beasts only about thirteen and a half hands high. The species known as South-East Asia ponies, were of mixed Arab and Mongolian origin. Their foundation stock had been taken to the East Indies centuries before by traders from Arabia, and as the Java islands were free from horse-sickness they had soon increased in number (Child 1967).

Persian Arabian horses were also imported at a later stage to prevent inbreeding. From this combination, the basic Cape horse was bred (Anonymous 1998a). The Cape horse gained world renown as a cavalry mount for the British army in India, and proved its mettle on many a battle field. It became the nucleus around which the large horse-breeding industry in Australia was built up (Child 1967).

It was also renowned for its endurance and intelligence. Lord Charles Somerset further improved and stimulated the breeding process by importing Thorougbred stallions. At this stage, there were about 200 000 horses in the Cape. The Cape horse was established in the next 150 years as a specific type (Anonymous 1999b)[4].

[4] Anonymous 1999b. Boer. Oklahoma State Univeristy. http://www.ansi.okstate.edu/breeds/horses

The Cape horse has ceased to exist, but of its descendants the Kaapse ryperd and the Boerperd there are still vestiges. The Anglo-Boer War practically destroyed the breed, and a good deal of crossbreeding took place among the few survivors (Burman 1993).

Recently Prof. Piet Roussouw, Mr. Dawie du Plessis and Mr. Hennie Allers started to look for foundation stock to reastablish the Cape Horse breed. They toured throughout South Africa inspecting many horses and tracing back their records. They found 78 horses that could be classified as true Cape horses. These horses will now be used in a breeding programme.

1.3.5 South African boerperd

During the period of the Great Trek (1836) until the beginning of the Anglo-Boer-War (1899), various horse breeds such as Hackneys, Norfolk Trotters and Cleveland Bay horses had an influence on the development of the Boerperd. The basic type of the Boerperd was fixed during this period. During the Anglo-Boer War, the stamina, hardiness and mobility of the Boerperd was tested and refined. Efforts to conserve the Boerperd started soon after the war and an auxiliary register for the Boerperd was included in the Stud Book Register of the Breeders' Association of the Transvaal in 1905. Due to poor support, this register became defunct between 1918 and 1921 (Anonymous 1998a).

In 1973, the Boerperd Society of South Africa was established at Memel in the Free State. The aim of the Society was the restoring of the original Boerperd by means of strict selection. A constitution and breeding standards were compiled. Horses that were phenotypically suitable for breeding were identified (Figure 1.5). The name of the breed and the Society were changed to the Historical Boerperd Breeders' Society in 1977. Official recognition of the breed by the Department of Agriculture and affiliation to the South African Stud Book and Livestock Improvement Association, were achieved in 1980. The Boerperd is one of the truly South African-bred horse breeds and was accorded the status of a fully recognized and developed breed by the Department of Agriculture in 1996 (Anonymous 1998a).

Figure 1.5: A photograph of a S.A. boerperd (Anonymous 1998a).

The membership of the Society has increased from the original 11 in 1973 to 125 and approximately 200 horses are currently registered in the Herd Book. All horses are subjected to inspection at the age of 2½ years. Those which comply with the strict selection criteria are accepted, branded with the Boerperd official brand and recorded in the Herd Book (Anonymous 1998a).

1.3.6 Welsh Ponies

The original home of the Welsh Mountain pony was in the hills and valleys of Wales. They lived there even before the Romans arrived. Their lot was not an easy one. These ponies had to survive the severe winters and the sparse vegetation. Shelter, most often, was an isolated valley or a clump of bare trees. Yet the Welsh pony managed not only to survive but also to flourish (Anonymous 1996b)[5].

The Welsh ponies roamed in a semi-wild state, climbing mountains, leaping ravines and running over rough terrain. Hence, the development of a pony with a remarkable soundness of body, a tremendous endurance, and a high degree of native intelligence (Figure 1.6). Even an edict of Henry VII, that all horses under 1.38m must be destroyed, did not eliminate the Welsh pony. Hiding in desolate areas where their persecutors were reluctant or unable to go, they continued to live and reproduce, preserving for mankind a distinctive strain of pony that today has generated enthusiasm among breeders and pony lovers all over the world (Anonymous 1996b)[5].

The Welsh Pony and Cob Society in Britain was founded in 1901 (Van der Merwe 1981). The founders decided in 1910 to register and record the Welsh Mountain ponies and the larger Welsh ponies together in the Welsh Stud Book, dividing them into four sections according to height and type. Essentially the description for each section is similar (Anonymous 1996b)[5]

There are four types of Welsh ponies: Section A, Section B, Section C and Section D. Section A, the mountain pony, lived for more than two centuries in the mountains of Wales and is a descendant of the prehistoric Celtic. These ponies have a maximum shoulder height of 122 cm. All colours are acceptable except piebald. Only two section A ponies can breed a Section A pony (Van der Merwe 1981).

[5] Anonymous 1996b. Welsh pony and Cob. Oklahoma State University. http://www.ansi.okstate.edu/breeds/horses

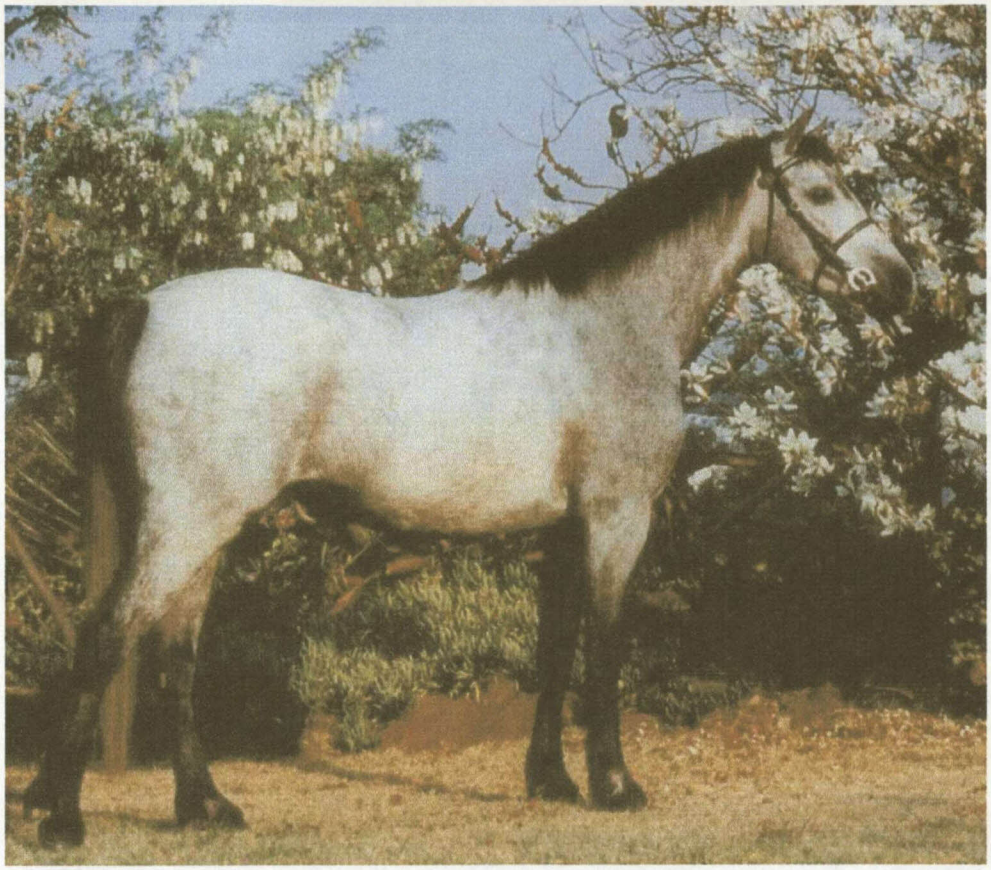


Figure 1.6: A photograph of a Welsh Pony (Anonymous 1998a)

Section B ponies have a maximum height of 1.37 m. The adjudicators pay much attention to the riding abilities of this pony. A cross between two Section B ponies or between a Section A pony and a Section B pony can produce a Section B pony (Van der Merwe 1981).

Section C ponies have a shoulder height of 1.37 m. A cross between Section A and C, B and C, A and D, B and D and C and D, can produce an Section C pony, provided that the shoulder height of the pony is within the boundary (Van der Merwe 1981).

Section D ponies have a shoulder height of 1.47 m to 1.53 m. The adjudicators pay a great deal of attention to the pony characteristics. Any cross between Section D and D, D and B and D and C, can produce a Section D pony, provided that the shoulder height of the pony is higher than 1.47 m (Van der Merwe 1981).

The present day breeding classifications were accepted by the United Kingdom Society in 1949 and are adhered to throughout the world (Anonymous 1998a).

In 1948, Mrs Rosalie Lasbry from Cape Town visited Britain to look for foundation stock from which to breed children's ponies. She returned home with five Welsh Mountain pony mares and the famous stallion *Coed Coch Serwyddr* (Anonymous 1998a). The mares were of five different colours: grey, reddish-grey, bay, black and a dun mare (Van der Merwe 1981).

Thus South Africa started its Welsh pony breeding with the best possible bloodlines, reinforced by later imports of the highest quality. In 1957, the Welsh Pony and Cob Society of South Africa, was founded in Middelburg, in the Eastern Cape. The original high standards are still adhered to and only registered ponies are recognized as purebred ponies (Anonymous 1998a).

At shows, Welsh Cob classes always draw the crowds. In harness, the Welsh Cob is spectacular and has recently proven that, in combined training events under F.E.I. (Federal Equestre Internationale) rules, they can compete against all and win. The Welsh's suitability for dressage in the "Lippizaner" manner is now being realized and demonstrated in Austria (Anonymous 1996b)[5].

The Welsh pony crosses especially well with the Thoroughbred to produce hunters, jumpers and event horses or with the Arabian horses for a riding pony with more bone and substance. At one time cob mares were in great demand as the foundation for polo ponies to obtain the agility and nimbleness necessary (Anonymous 1996b)[5].

1.3.7 Friesian horses

The Roman historian Tacitus (AD 55-120) recorded the existence of the Friesian horse. The Friesian had carried the Friesian knights and their German neighbours to the Crusades. The breed was up-graded further by the introduction of eastern blood, a result of contact made with desert horses during these campaigns and,

later, by breeding with the reknown Andalucian horses when Spain occupied the Netherlands during the 80 Years' War (1568-1648) (Edwards 1994).

The relatively small Friesian, although blessed with an impressive top line, was not in the same class as the Andalucian or the purpose-bred war-horses of Lombardy, but for centuries it was the most practical, up-to-weight war-horse of Europe. It was also the cheapest to keep. For the last few hundred years its versatility has been demonstrated in harness, under saddle, and in every kind of farm work (Edwards 1994).

Not surprisingly, this horse was much in demand not only to improve neighbouring breeds but also as foundation stock. Marbach, the German state stud at which the Württemberger originated, used Friesian horses in the 17th century. At the same time, the Oldenburger was founded largely on Friesian stock from the area between the Netherlands and the River Weser. The Døle Gudbrandsdal of Norway is derived directly from the Friesian. The Friesian's influence was also manifested in the Dales and Fell ponies and in the Old English Black from the Midlands. More importantly, it was, without dispute, the ancestor of the Shire horse (Edwards 1994).

However, despite its eminence, the Friesian nearly became extinct during the early part of the 20th century. A stud book had been opened in 1879 but the popularity of trotting races, in which the Friesian excelled, resulted in outcrosses to increase speed at the expense of the essential type. By 1913 only three Friesian stallions were left in Friesland. The breed was saved when vehicle and fuel shortages in the Second World War caused the Dutch farmers to return to horse-power. A new society was formed, and in 1954 this was granted the title of "Royal" (Edwards 1994).

The Friesian Horse Breeders' Society of South Africa was founded in 1980. Inspectors were appointed and horses throughout the country were screened according to the standards of excellence of the breed and registered in terms of legislation with the South African Stud Book and Livestock improvement Association (Anonymous 1998a).

The first two Friesian horses were imported from Friesland in 1906 by a Mr Hoogendoorn, who wanted to use them to pull the hearse of his funeral undertaking business. The Friesian horse was bred from the northern or cold-blooded type of horse, giving it the easy-going temperament which is so characteristic of the breed (Anonymous 1998a).

Today, Friesians are always black, and stand at around 1.52 m. (Figure 1.7) They are able to cope with a heavy workload on moderate rations without losing condition or the cheerful willingness inherent in the breed's lovable character. Friesians are used for working on the land, are driven in harness and are prized as dressage horses (Edwards 1994).

Figure 1.7: A photograph of a Friesian horse (Anonymous 2000a)[6].

Friesian foals are normally weaned at six months of age, after which they spend much of their time on pastures. At about two years of age they start training. As the Friesian has such a docile nature, there is no need for them to be "broken in", as is the case with warm-blooded horses (Anonymous 1998a).

[6] Anonymous 2000a. Friesian. Manfred Link. Germany. http://www.ansi.okstate.edu/breeds/horses/FRIESIAN/

1.3.8 Przewalski horse

It was originally believed that the Przewalski horse was discovered by the Russian explorer Colonel Przewalski, for whom it is named, in 1881. More recent information from the Przewalski Horse Foundation indicates two Europeans saw these animals much earlier. A Scottish doctor who was sent as an ambassador to China by Peter the Great wrote of his experiences in "*Journey from St. Petersburg to Pekin, 1719-1723*" and included an accurate description of this Asiatic wild horse. Even earlier, Hans Schiltberger, a Bavarian nobleman, was taken prisoner by the Turks and sold to the famous Tamerlane of the Golden Horde, who in turn gave Schiltberger to a Mongol prince named Egedi. Schiltberger spent several years in the Tien Shan mountains. He wrote of the wild horses he observed in his memoirs "*Journey into Heathen Parts*". This unpublished manuscript was written in 1427 and is housed in the Munich Stadtbibliotek (municipal library) (Sweetman 1998b)[7].

Some authorities feel strongly that the Przewalski horse is the ancestor of all modern breeds. Others point out that it is a different species from the domesticated horse, having 66 chromosomes as compared to the 64 of the domestic horse. They further point out that while crosses between the Przewalski and domestic horses result in a fertile hybrid, the offspring has 65 chromosomes. Subsequent crosses result in 64 chromosomes and bear little resemblance toe the Przewalski. The Foundation for the preservation and protection of the Przewalski horse, in Rotterdam, The Netherlands, report that only a few Przewalski horses are tamable, in proportions similar to a Zebra. Blood group testing has found several marks which are unique to the Przewalski in addition to markers which it has in common with other equids (Sweetman 1998b)[7].

The Przewalski's horse is similar to the domestic horse though it has a smaller, more robust build, and upright main, and a low-set tail. The most common colours are sandy tan, dun, and reddish bay. The horses have a dorsal stripe, a shoulder stripe, barring in their legs, and lighter colouring on their muzzles and bellies. (Figure 1.8). Concern about the future of the Przewalski horse led its breeders to form an

[7] Sweetman, T. 1998. Przewalski. Oklahoma State University. www.ansi.okstate.edu/breeds/horses/PRZEW/

international studbook in 1979 to facilitate the goals of increasing the population and reducing inbreeding. The species has also been included in the International Species Inventory System, a computer-based information system for wild animal species in captivity. There have been exchanges of breeding stock between North America and the U.S.S.R., which now has the largest herd of Przewalski horses at Askania Nova (Sweetman 1998b)[7].

Figure 1.8 Photograph of a Przewalski horse (Sweetman 1998b)[7].

1.4 Genetic characterization

During the last ten years, the development of new genetic tools has brought about great advances in individual recognition, and DNA markers, such as microsatellites have proved to be useful in clarifying population structure. They also detect population differentiation better than for example allozymes. The FOOD AND AGRICULTURAL ORGANIZATION OF THE UNITED NATIONS has proposed a global programme for the management of genetic resources using microsatellite methodology for breed characterization (Bjornstad *et al.* 2000).

The genetic diversity of domestic species such as cattle, sheep and goats has been examined by microsatellite analysis. More recently, horse breeds have also been analyzed using a panel of microsatellites recommended for routine parentage testing. Genetic characterization is the first step in breed conservation and may have implication for future breeding strategies (Bjornstad *et al.* 2000).

To keep a breed pure, it is very important to ensure that the parentage of the horses used in this and any other studies are accurate. Since the American Saddlers have been DNA typed, I have found that about 10% of the given parentages tested, is incorrect. Because all breeding programmes assume that the parentage is accurately given, it is of prime importance to ensure that this is actually the case.

1.5 Parentage determination

Initially the farmers indicated the parents of a foal on a certificate. They used visual markings on the horse and book keeping. Blood typing was the first scientific method by which the parents could be established. It is still used but lately most horse breeding societies use DNA tests. These are more accurate and easier since any type of biological material can be used, blood samples or hair samples. The farmers can put a few hair samples in the post and the test can be done without the additional cost of a veterinarian. Since computer programmes and analyzing programmes are used, all results are universal and objective.

1.5.1 Blood typing

For nearly three decades, horse registries have verified pedigree records and resolved queries of parentage using blood group and protein polymorphism tests (blood typing). These tests are rapid, reliable and legally compelling, but the requirement of a fresh blood sample is occasionally a drawback (Bowling *et al.* 1997).

The horse blood typing test uses serological procedures and electrophoresis to detect autosomal genetic variation in seven blood groups (A, C, D, K, P, Q and U) and in eight protein systems (ALB, A1B, ES, GC, HBA, PGD, PI and TF) (Bowling *et al.* 1997).

In 1974, during a meeting of the horse research section held on the 14th Conference of the ISABR (International Society of Animal Blood Group Research) at Davis, California, a committee was appointed for the elaboration of a new international nomenclature for horse erythrocyte antigens. In the seven recognized horse blood group systems, the factors were designated by Aa, Ab, Ac, etc. The absence of a factor is designated by a dash. Table 1.2 shows the nomenclature to be applied (Scott 1994).

Table 1.2: The international nomenclature for horse erythrocyte antigens (Scott 1994).

Table 1.2.1: Protein polymorphism nomenclature for horses.

Locus	Alleles
Albumin	Al^F, Al^S
Transferrin	Tf^D,Tf^F, Tf^{F2}, Tf^G, Tf^H, Tf^M, Tf^O, Tf^R
Esterase	Es^F, Es^G, Es^H, Es^I, Es^S
Prealbumin	Pr^F,Pr^I,Pr^L,Pr^N,Pr^S,Pr^U,Pr^W
Haemoglobin	Hb^A,Hb^B
Carbonic Anhydrase	CA^F,CA^I
6-PGD	PGD^F, PGD^S
PGM	PGM^F, PGM^S, PGM^V
PHI	PHI^F, PHI^I, PHI^S
Catalase	Cat^M,Cat^S

Table 1.2.2: Red-cell grouping nomenclature for horses.

System	Designation
A	Aa
	Ab
	Ac
C	Ca
D	Da
	Db
	Dc
	Dd
	De
	Df
K	Ka
P	Pa
	Pb
Q	Qa
U	Ua

Blood typing has an exclusion probability of approximately 99.2%. The actual effectiveness of blood typing is 97.3%. These values are based on allelic frequencies calculated from 1195 Quarter horses in America (Bowling *et al.* 1997).

The new DNA-based methodologies for genetic marker-testing using polymerase chain reaction (PCR) technology provide an obvious alternative to blood typing, particularly the analysis of short tandem repeat loci (STR's or microsatellites). The STR tests require only small amounts of biological samples and are not restricted to one tissue source (Bowling *et al.*1997).

1.5.2 Microsatellites

Non-coding regions of DNA may be located within genes (where they are called "introns") or between genes. Because they are non-functional, they are not subjected to strong selection and the presence or absence of a particular marker would not affect an individual's survival. This has allowed tremendous genetic diversity to develop in these regions. Much of this non-coding DNA consists of highly repetitive segments of DNA. Repeated sequences can occur as a tandem array. Such sequences, called variable number tandem repeats (VNTRs) are unique to each individual and are the basis for the precise DNA fingerprinting used in forensics (Frank 2001)[8].

VNTR's or minisatellites, were introduced in 1985. These are short DNA sequences which are present in multiple copies and arrayed in tandem. Because of their complexity, questions concerning laboratory accuracy and statistical methods used to analyze VNTRs have been debated since they were discovered. The main problem is that VNTR alleles cannot be exactly distinguished on gels. This has led to a 'binning" approach which relies on grouping together allelic frequencies. Whereas this is accurate if individuals are analyzed at the same time, it is a problem when parent and offspring data are obtained at different times. Since pedigree determinations often rely on comparing data collected over many years, this method has some serious limitations (Anonymous 1999c)[9].

Microsatellites are short tandem repeats (STRs) of genomic sequences. The repeated unit can be a mono-, di-, tri-, or tetra-nucleotide with di-repeats being the most common. Microsatellites generally occur in non-coding regions of the genome and appear to be uniformly distributed (Anonymous 1999c)[9]. Because they are non-functional, they are not subjected to strong selection and the presence or absence of a particular marker would not affect an individual's survival (Straughan 2001)[10].

The length of the repeat is highly variable. This is very significant for detecting differences in a population and between individuals. Microsatellite typing reveals a high degree of polymorphism and is relatively easy to interpret. Parentage verification in later generations can

[8] Frank, T.S. 2001. Topics in Molecular Oncology: Microsatellite Instability. University of Michigan. http://zapruder.pds.med.umich.edu/users/Frank/MIN.html

[9] Anonymous 1999c. DNA typing techniques – a comparison and contrast. ImmGen, Incorporated. http://www.immgen.com/html/techniques.html

[10] Straughan, D. 2001. Microsatellite DNA. http://biology.uoregon.edu/biology/Spring94/Dyan1/Dyan.html

be done since the genotype of parents can be compared with their progeny at a future date (Anonymous 1999c)[9].

The horse microsatellites in this thesis can be represented as simple two-letter repeats. These repeats are embedded in the more typical mixed-letter sequence, in a pattern such as: -GAC TTA GCT AGC TAC TTC ACA CAC ACA CAC ACA CAC ACA CCT TAT CTC G-. In the example here, the microsatellite pattern is 11 consecutive "CA" motifs flanked by unique nucleotide sequences. The length of the repeat is a reliably inherited trait, but can be highly variable within a breed. For example, the systems selected for the horse parentage test each have from 8 to 16 length variants, depending on the breed (Bowling 1999)[11].

Microsatellites can be amplified using the polymerase chain reaction (PCR) and resulting amplicons are sized and scored as alleles. Traditionally, this is done radioactively with labelled amplicons DNA fragments, producing black fragments on a film. Usually only one locus at a time is typed in this way. Alleles that produce amplicons in different size ranges can be multiplexed in the same PCR, or be run together in the same lane on the gel. The sizing of fragments and scoring of alleles are all done manually (Anonymous 2001a)[12].

The use of automated fluorescent methods for analysing microsatellites streamlines the whole process. One of the PCR primers is synthesized with a 5' fluorescent label. The resulting PCR products are thus fluorescently labelled. Only a small amount of each PCR reaction is loaded on to the gel. Detection of the labelled bands is very sensitive. Overloading causes smears, blobs and colour bleeding. The sample is loaded with an internal lane standard, which is usually Pst I cut lambda, labelled with ROX, a red fluorescent dye. The sizes of the Pst I fragments are known and thus the sizes of the PCR products are calculated by the Genescan software. The internal lane standard in each lane eliminates lane-to-lane variation. Thus a gel that runs "smiling" or curved poses no problems (Anonymous 2001a)[12].

As fragments pass the laser window they are detected and their signal is sent to the Macintosh computer to be processed. There is no further handling of the gel. With four different

[11] Bowling, A.T. 1999. DNA test for parentage verificaton in horses. University of California, Davis. http://www.vgl.ucdavis.edu/horse/partest.htm
[12] Anonymous 2001. Microsatellites, Joslin diabetes center. http://134.174.243.149/core/microsats.html

coloured dyes available from ABI, more than one microsatellite locus at a time can be analysed (Anonymous 2001a)[12].

The Genescan software calculates the size of each allele. It draws a picture of the gel showing the alleles and lane standards. It also draws a profile of each lane and alleles are represented as peaks. The peak height and peak area are also calculated and this information can be used for quantitative studies. After processing the data, it can be imported into the ABI Genotyper software which selects alleles as defined and leaves behind unwanted fragments such as non-specific (Anonymous 2001a)[12].

The DNA microsatellite primer set for horses consist of 12 autosomal dinucleotide repeat microsatellites: AHT4, HMS7, HTG4, VHL20 (all blue), AHT5, ASB2, HMS6, HTG6 (all green), HMS2, HMS3, HTG10 and HTG7 (all yellow). The nomenclature for microsatellite alleles is in accord with that recommended for horses by the Horse Standing Committee of the International Society for Animal Genetics at Tours, France, 1996. Alleles are designated with alphabetical symbols, in the order of smallest to largest, based on a middlesexed allele having been designated as *M* by the laboratory producing the microsatellite and comparison testing of reference samples (Bowling *et al.* 1997).

DNA typing can be done on the root of a horsehair sample. Owners can collect the hair sample from the mane or tail, eliminating the expense of a veterinarian to draw the blood. Hair is also easier to transport and unlike blood does not require refrigeration for long-term storage. DNA testing also offers other essential advantages. It provides higher efficiency (e.g., the ability to exclude parentage); and the same basic protocol can be used to test for genetic diseases. Blood typing consists of about 15 different tests, is labour intensive, and difficult to automate. DNA typing is far simpler and much easier to automate and standardize (Anonymous 1998c)[13].

The Veterinary Genetics Laboratory at the University of California in Davis (U.C. Davis) changed from blood typing to DNA typing for Quarter horses in January 1995. DNA typing on other breeds followed shortly thereafter. They analyse 30 000 samples per year to verify parentage for about 17 different registries (Anonymous 1998c)[13].

[13] Anonymous 1998c. Animal studies turn increasingly to DNA for parentage, biodiversity and gentic disease testing. Perkin Elmer. http://www2.perkin-elmer.com/ab/pebio/ie3/jump/ab_frame.html

The Animal Improvement Institute (ARC) in South Africa, started the DNA typing of American Saddler horses in November 1996. The other horse breeding societies changed from blood typing to DNA typing shortly thereafter.

1.6 Computer software for genetic characterization.

There are computer software programmes available on the market to do all kinds of calculations to determine the genetic structure of a population. A few well-known programmes were used in this study to determine the genetic relationships between seven horse breeds in South Africa.

BIOSYS 1 is a computer program for the analysis of electrophoretically detectable allelic variation. The program performs most types of electrophoretic data analysis commonly employed in biochemical population genetics and systematics (Swofford & Selander 1981).

POPGENE is a user-friendly Microsoft Window-based computer package for the analysis of genetic variation among and within natural populations using co-dominant traits. The molecules for co-dominant markers are limited to a maximum of 1 400 populations, 150 groups and 1 000 loci. The number of alleles per locus is limited to 52 if you use the alphabetic letters (Yeh *et al.* 1999).

DISPAN (Genetic Distance and Phylogenetic Analysis) is designed to compute the average heterozygosity, gene diversity and standard genetic distances. It also constructs phylogenetic trees (dendrograms) by using the neighbour-joining method and the unweighted pair group-method with arithmetic mean from matrices or distances (Tatsuya 1993).

1.7 The genetic relationships of seven horse breeds in South Africa

Genetic diversity provides the flexibility for individuals to adapt to diverse enviroments, and the evolutionary potential for population to survive in the face of environmental changes. Comparison of breeds within species reveals distinctive arrays and gene frequencies of genetic

variants of microsatellites. Selective breeding practices, founder effects and random drift are processes which cause breeds to have distinctive profiles of genetic markers. Genetic characterization is the first step in breed conservation and may have implication for future breeding strategies.

The aim of all the breeding societies is to select and breed horses with very specific qualities. A breeding programme cannot succeed if the parentage is not accurate. Therefore, it is very important to have the correct parentage, so that it can be certain that the foal will inherit certain characteristics of the parents.

The aims of this study were to determine whether horse breeds in South Africa are pure and that there is still enough genetic variation in the different horse breeds. It was also my aim to determine whether the Cape horse has evolved enough from the S.A. Boerperd to be classified as a horse breed that was bred in South Africa.

Chapter 2

CHAPTER 2

MATERIAL AND METHODS

2.1 Sampling of DNA

Hair samples were received from various farmers throughout South Africa. To reduce the effect of inbreeding and thus influencing the genotipic frequencies, horses were chosen that were not directly related to each other. The following horses have been send in by horse breeders in South Africa: 80 Quarter horses, 80 American Saddlers, 79 S.A. Boerperde, 81 Welsh ponies and 39 Friesian horses. All the horses that have been used are registered at studbook, or the different breeder's societies.

Hair samples of 80 Arabian horses were sent from breeders in Namibia. Hair samples of 78 Cape horses were received from Prof. Piet Rossouw (Pretoria Technichon, South Africa) and Mr. Dawie du Plessis (a breeder of Cape horses in South Africa). They selected all the horses themselves and classified them as Cape horses.

The Przewalski horses are very rare and wild and a small group was found in South Africa at the Pretoria Zoo. To obtain a hair sample, these horses have to be darted, since they are wild animals. Only one hair sample could be obtained for this study. The Przewalski horse is used as the outgroup in this study.

The following chemicals were used for this study.

- Perkin-Elmer StockMarks for Horses Equine Paternity PCR typing Kit (Stock item no. 402825). The paternity kit consists of:
  - primers (VHL20, HTG4, AHT4, HMS7, HTG6, HMS6, HTG7, HMS 3, AHT5, ASB2, HTG10 and HMS2).
  - StockMarks PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl2, pH 8.3), dNTP mix (1.25 mM each nucleotide),
  - AmpliTaq Gold (5 U/µl),
  - Equine control DNA (20 ng/µl),
  - GeneScan-350 ROX Size Standard
  - Loading buffer
- Long ranger gel (Sterilab services. Stock item no. 50611. Long ranger gel sol.50% BMA product 250ml)
- All other chemicals used were of analytical grade.

2.2 DNA extraction

The method supplied by the Analytical Manual of the ARC AII Animal Genetics Division was used to extract DNA from hair samples. Six hair roots were cut into a reaction tube. The hair samples were incubated for 1½ hour at 56°C in a mixture of 43 µl sterile H_2O, 5 µl Tween™ 100 (undiluted), 1.5 µl Proteinase K (1 mg/ml) and 5 µl 10X buffer (100 mM Tris-HCl pH 8.3 and 500 mM KCl in 1 liter). After incubation the hair samples were boiled for 10 minutes at 100°C to denature the Proteinase-K in the extract. The DNA was stored at -20°C (Anonymous 1999c).

2.3 PCR method

The PCR method described by the protocol of the Paternity kit was used. The following primers were used: VHL20, HTG4, AHT4, HMS7, HTG6, HMS6, HTG7, HMS 3, AHT5, ASB2, HTG10 and HMS2 (See table 2.1) (Anonymous 1996c).

Table 2.1 Primers used for PCR reaction (Anonymous 1996c)

Locus	Size range (bp)	pmol/rxn	Dye label	Sequence
AHT4	146-170	0.72	FAM	Forward:5'[FAM]-AAC CGC CTG AGC AAG GAA GT-3' Reverse:5'-GCT CCC AGA GAG TTT ACC CT-3'
HTG4	120-140	0.9	FAM	Forward:5'[FAM]-CTA TCT CAG TCT TGA TTG CAG GAC-3' Reverse:5'-CTC CCT CCC TCC CTC TGT TCT C-3'
HMS7	170-188	1	FAM	Forward:5'[FAM]-CAG GAA ACT CAT GTT GAT ACC ATC-3' Reverse:5'-TGT TGT TGA AAC ATA CCT TGA CTG T-3'
VHL20	86-105	1.8	FAM	Forward:5'[FAM]-CAA GTC CTC TTA CTT GAA GAC TAG-3' Reverse:5'-AAC TCA GGG AGA ATC TTC CTC AG-3'
AHT5	129-149	1.5	JOE	Forward:5'[JOE]-ACG GAC ACA TCC CTG CCT GC-3' Reverse:5'-GCA GGC TAA GGG GGC TCA GC-3'
ASB2	240-270	2	JOE	Forward:5'[JOE]-CCA CTA AGT GTC GTT TCA GAA GG-3' Reverse:5'-CAC AAC TGA GTT CTC TGA TAG G-3'
HMS6	157-171	2.5	JOE	Forward:5'[JOE]-GAA GCT GCC AGT ATT CAA CCA TTG-3' Reverse:5'-CTC CAT CTT GTG AAG TGT AAC TCA-3'
HTG6	80-107	1	JOE	Forward:5'[JOE]-CCT GCT TGG AGG CTG TGA TAA GAT-3' Reverse:5'-GTT CAC TGA ATG TCA AAT TCT GCT-3'
HMS2	218-238	3	TAMRA	Forward:5'[TAMRA]-CTT GCA GTC GAA TGT GTA TTA AAT-3' Reverse:5'-ACG GTG GCA ACT GCC AAG GAA G-3'
HMS3	149-172	3.4	TAMRA	Forward:5'[TAMRA]-CCA ACT CTT TGT CAC ATA ACA AGA-3' Reverse:5'-CCA TCC TCA CTT TTT CAC TTT GTT-3'
HTG10	92-112	6	TAMRA	Forward:5'[TAMRA]-CAA TTC CCG CCC CAC CCC CGG CA-3' Reverse:5'-TTT TTA TTC TGA TCT GTC ACA TTT-3'
HTG7	118-130	4.5	TAMRA	Forward:5'[TAMRA]-CCT GAA GCA GAA CAT CCC TCC TTG-3' Reverse:5'-ATA AAG TGT CTG GGC AGA GCT GCT-3'

The PCR was prepared in two multiplexes, an eight multiplex and a four multiplex. This minimised the chances of non-specific amplification. The eight-multiplex consisted of the following primers: VHL20, HTG4, AHT4, HMS7 (blue, labelled with FAM), HTG6, HMS6 (green, labelled with JOE), HTG7 and HMS3 (yellow, labelled with TAMRA). The master mix consisted of 2.5 µl StockMarks PCR buffer, 4 µl dNTP mix, 0.5 µl AmpliTaq Gold, 2 µl deionised water and 0.5 µl of each primer. Two micro litre of DNA was added to the master mix before amplification. The four multiplex consists of AHT5, ASB2 (green, labelled with JOE), HTG10 and HMS2 (yellow, labelled with TAMRA). The master mix consisted of 2.5 µl StockMarks PCR buffer, 4 µl dNTP mix, 0.5 µl AmpliTaq Gold, 4 µl deionised water and 0.5 µl of each primer. Two microlitre of DNA was added to the master mix before amplification (Anonymous 1996c).

The Perkin Elmer GeneAmp PCR System 9700 was used to amplify the DNA. The program consisted of 10 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 60°C and 60 sec at 72°C. After the 30 cycles the samples were left for 60 minutes at 72°C. The PCR product was stored at 4°C (Anonymous 1996c).

2.4 Preparing PCR products for electrophoresis

For each DNA sample, 2 µl eight-plex and 2 µl four-plex were mixed with 8 µl deionized water. Of this mixture 1.4 µl was mixed with 2.25 µl formamide, 1.25 µl loading buffer (50 mm EDTA, 50 mg/ml blue dextran) and 0.3 µl GeneScan-350 ROX Size Standard. Before loading, the samples were preheated for 2 minutes at 95°C and immediately cooled on ice (Anonymous 1996c).

2.5 Gel pouring

A 5% Long Ranger /6 M Urea gel was used for the DNA typing of horses. For a 36 cm gel a 50 ml gel solution was used. Five millilitre of 50% Long Ranger gel solution concentrate was added and 5 ml 10X TBE buffer was added to 18 g urea. Thirty-five millilitre of deionised water was slowly added and mixed until all the crystals were dissolved. A 0.2 µm cellulose nitrate filter was used to filter the solution. The filtrate was degassed by applying a vacuum for at least five minutes. Ammonium persulphate (250 µl of a 10% solution) and 25 µl TEMED was added to the mix and the gel poured. The gel was left for at least 2 hours to polymerize (Anonymous 1998b).

2.6 Electrophoresis

The ABI PRISM 377 DNA sequencer and the ABI PRISM® data collection software were used for electrophoresis. The conditions of the gel were as follows: the electrophoresis voltage 1 KV, electrophoresis current 11.8 mA, electrophoresis power 11 W, gel temperature 51°C, laser power 40 mW and the running time 2 hours. One and a half microliters of the DNA mix was loaded onto the gel and analyzed by the GeneScan software (Anonymous 1998b).

2.7 ABI PRISM® GeneScan® analysis software

The GeneScan® analysis software analyses the data collected by the ABI PRISM 377 DNA sequencer to size and quantifies DNA fragments. GeneScan analysis of sample files includes establishing a baseline, adjusting for spectral overlap of the dyes, peak detection, and size calling (Anonymous 1997a).

The GeneScan analysis software sizes and quantifies DNA fragments automatically, allowing for faster and more accurate analysis than traditional methods such as radio labelling. Depending on the running conditions, sufficient resolution can be achieved to differentiate between fragments that have apparent sizes up to 5000 base pairs (Anonymous 1997a).

When the GeneScan system is used, different DNA fragments can be labelled with up to three different colour fluorescent dyes. A fourth colour is reserved for the GeneScan Internal Lane Size Standard. The size standard is used for precise size calling without the problems often encountered using other techniques, such as band-shift artifacts and run-to-run variation (Anonymous 1997a).

The results of an experiment can be displayed as electropherograms, as tabular data or as both. Electropherograms show fluorescence intensity as a function of fragment size or migration time. Each electropherogram represents a single gel lane. The tabular data provides precise sizing and quantitative information. The data can be exported to downstream applications, such as Genotyper® software. The results from the ABI 377 DNA sequencer can also be displayed as a reconstructed gel image. The gel image provides a qualitative picture of the run (Anonymous 1997a).

The intensity of emitted fluorescence is different for each dye. For example, to generate signals of equal intensity eight times as much ROX must be loaded as 6-FAM. Using too little or too much sample can cause problems. The ABI PRISM® instrument can convert a limited range of fluorescent signals into digital values. For optimal results, the fluorescent signal should be kept between approximately 150 and 4000 relative fluorescent units. Too little signal below this range results in the signal-to-noise ratio being too low to discriminate between sample peaks and

background fluctuations. If there is too much signal above this range, the instrument cannot measure the true value of the signal and consequently cannot compensate for the spectral overlap among the dyes. As a result, artifact peaks, called pull-up peaks, can appear in other colours. Artifact peaks can corrupt both automated size-calling (extra peaks in the size standard colour) and the analysis of co-loaded samples (Anonymous 1997a).

Optimising electrophoresis conditions (running time, running voltage, and running temperature) can greatly improve data quality, run-to-run precision, and throughput. When selecting values for these parameters, the following factors must be considered: range of fragment lengths and required degree of resolution. The resolution, Rs, of two peaks in an electropherogram is defined as follows:

$$Ra = |P_1 - P_2| / 0.5 \times (W_1 + W_2)$$

where the P_i are the peak positions measured below the peak apex and the W_i are the peak widths measured at half peak maximum (Anonymous 1997a).

The purpose of control DNA is to determine whether there was a problem with the PCR, or with the sample DNA. It also allows for the monitoring of the sizing precision, since the control DNA's sizing and labelling is known (Anonymous 1997a).

When analyzing a gel, the gel tracking must first be checked. If there is a problem, the misaligned lanes must be manually retraced. The analysis parameter must be set. The analysis range is usually between 800 and 6000 datapoints. The data processing should be marked as a baseline and is multicomponent. There should be zero smooth options. The peak detection thresholds is set for 100 for blue, green and yellow and 50 for red. The minimum peak half width is set as 3 points (Anonymous 1997a).

The size call range is set as all sizes. The size calling method is chosen as a local southern method. The split peak correction is set as zero and the correction limit is set as 30 data points (Anonymous 1997a).

After the parameters are set the size standard is defined. GeneScan ROX 350 is used. The peaks are the following : 35 50 75 100 139 150 160 200 250 300 340 350. The data are then

analyzed. After the analysis is completed, the peak assignments for the size standard are verified in all sample files by using the results control method. A new size standard is defined for those samples with incorrect peak assignments (Anonymous 1997a).

The purpose of a matrix is the following: while the most intence fluorescence emitted by an ABI PRISM™ will fall within a small wavelength detection range, some fluorescence emission in the detection ranges of the other dyes will always occur. The multicomponent matrix compensates for this overlap by subtracting out, in each dye's detection range, the portion of the signal due to fluorescence from other dyes. The A matrix and filter set are used for the analysis of the DNA profiles. (Anonymous 1997a).

Size standards and sample fragments loaded on the same gel undergo the same eletrophoretic forces. Therefore, the relative electrophoretic mobility of any sample fragment is a good indicator of its molecular weight because lane-to-lane variation in electrophoretic forces no longer contribute to measurement errror (Anonymous 1997a).

During the first step of analyzing the GeneScan® analysis software attempts to match the peaks of the internal lane standard with the peaks of the size standard definition, so that the overall fit. To be considered a match, an internal lane standard peak must lie within ± 400 scans of its expected position (as defined by the corresponding size standard definition peak) (Anonymous 1997a).

From the fragment migration times of the internal lane standard, the GeneScan analysis software generates a sizing curve giving size in base pairs or nucleotides as a function of scan number (i.e., migration time) using the chosen sizing method. The local methods, which generate the best-fit curve from nearby internal lane standard data points, are less affected by changes in the electrophoresis conditions or in the analysis range. For the local southern method to work, there must be at least two size standard fragments larger than the largest unknown fragment (Anonymous 1997a).

Accuracy in size calling is a measure of the instrument's ability to generate fragment sizes that are close to the actual size of the fragment as determined by sequencing. Precision, or

reproducibility, in size calling is a measure of the instrument's ability to generate the same size consistently for a given fragment independent of whether the called size is close to the actual size for a given set of running conditions. Because the called size for a fragment can differ from its actual size, fragment sizes must be converted to alleles before comparing microsatellite data generated on different instruments (Anonymous 1997a).

Pony X is a horse that is heterozygotic for all the alleles. All the DNA profiles of horses generated in the USA by U.C. Davis is sized by the pony X profile. To make our data compatible, the DNA of pony X was analyzed on our machine and the alleles were assigned names. The DNA of horses thereafter were standardised to Pony X.

2.8 Genotyper™ software

Genotyper software analyzes the data generated by the GeneScan analysis software. It enables the analysis and interpretation of nucleic acid fragment size and quantitative data by converting it to user-defined results specific to your genotypic studies. For example, Genotyper can convert GeneScan fragment data to called allele symbols (Anonymous 1996d).

The Genotyper software labels sample fragments with identifying labels and displays fragments as labelled peaks in plot displays. The programme also generates statistics and histograms that characterise peak data and creates tables that correlate peak data and marker information for specific genotypic applications. Genotyper can produce a table of alleles that can be exported to a mapping application or a database or a table of alleles that have been checked for Mendelian inheritance for paternity testing (Anonymous 1996d).

The binning system of ISAG was used on the ABI 377.

2.9 BIOSYS 1

BIOSYS 1 is a computer program for the analysis of electrophoretically detectable allelic variation. The program performs most types of electrophoretic data analysis commonly employed in biochemical population genetics and systematics (Swofford & Selander 1981).

The program was used to estimate allelic frequencies and values of diversity, number of alleles per locus, percentage of alleles and proportion of polymorphic loci for each population. The χ^2 test was done to test whether the sample frequencies conform to Hardy-Weinberg equilibrium frequencies.

2.10 POPGENE

POPGENE is a user-friendly Microsoft Window-based computer package for the analysis of genetic variation among and within natural populations using co-dominant traits. Co-dominant markers are limited to a maximum of 1 400 populations, 150 groups and 1 000 loci. The number of alleles per locus is limited to 52 using the alphabetic letters. Popgene was used to do the following diploid data analyses : genotypic frequency, Hardy-Weinberg test, fixation index (Wright 1978), allele frequency, allele number, effective allele number, polymorphic loci, observed homozygosity, expected homozygosity, observed heterozygosity, expected homozygosity, Shannon index, F-statistics, gene flow and genetic distance (Yeh *et al.* 1999).

2.11 DISPAN

DISPAN (Genetic Distance and Phylogenetic Analysis) is designed to compute the average heterozygosity, gene diversity and standard genetic distances. It also constructs phylogenetic trees (dendrograms) by using the neighbour-joining method and the unweighted pair group-method with arithmetic mean from matrices or distances (Tatsuya 1993).

DISPAN was used to estimate the genetic distances among the seven horse populations. The allelic frequencies, calculated from the 12 microsatellites, were used. A dendrogram was constructed and over 1 000 bootstrapping replications was carried out in all cases.

The formule used for the calculations are available in the user manuals of the programmes and the presentation of results will be discussed in the chapters which follow on.

Chapter 3

CHAPTER 3

RESULTS

3.1 Gel electrophoresis results

DNA was extracted from hair samples of 80 Quarter horses, 80 American Saddlers, 79 S.A. Boerperde, 81 Welsh ponies, 39 Friesian horses, 80 Arabian horses, 78 Cape horses and one Przewalski horse. The DNA of these 518 horses were amplified in PCR reactions with the paternity kit. The concentration of the DNA was only tested in cases were a problem was experienced with the PCR reactions.

DNA extraction and amplification proved to be very successful, (Figure 3.1) and results were obtained from all the horses used. The success with the amplification process is cleary visible in the electropherogram (Figure 3.2). The method provided unique DNA profiles for each horse as seen in figure 3.3.

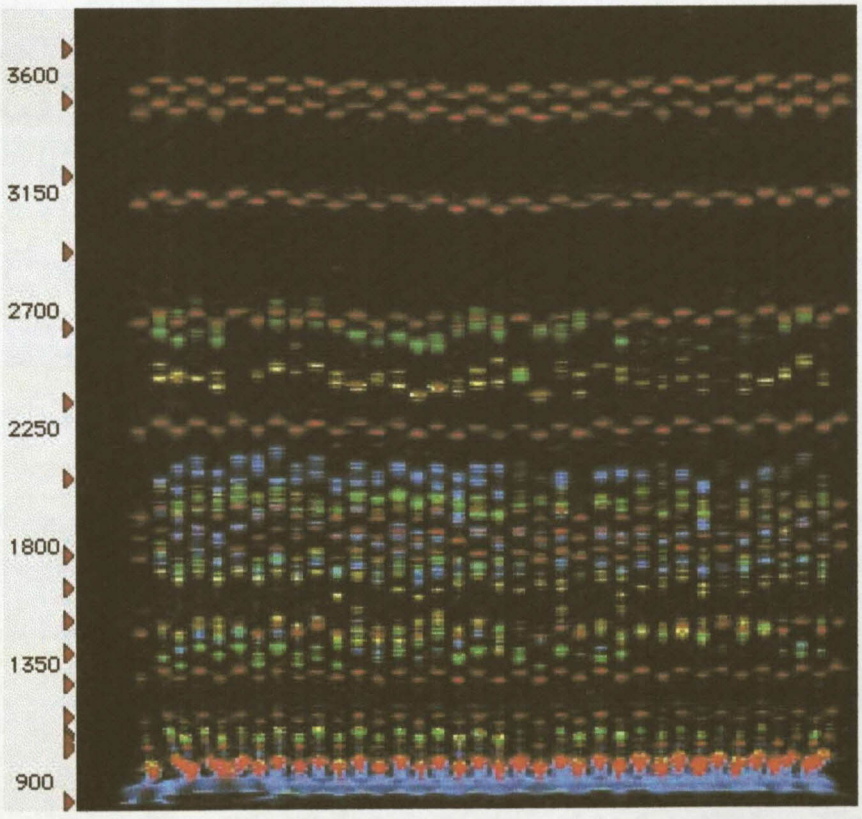


Figure 3.1: GeneScan gel view of 36 horse samples.

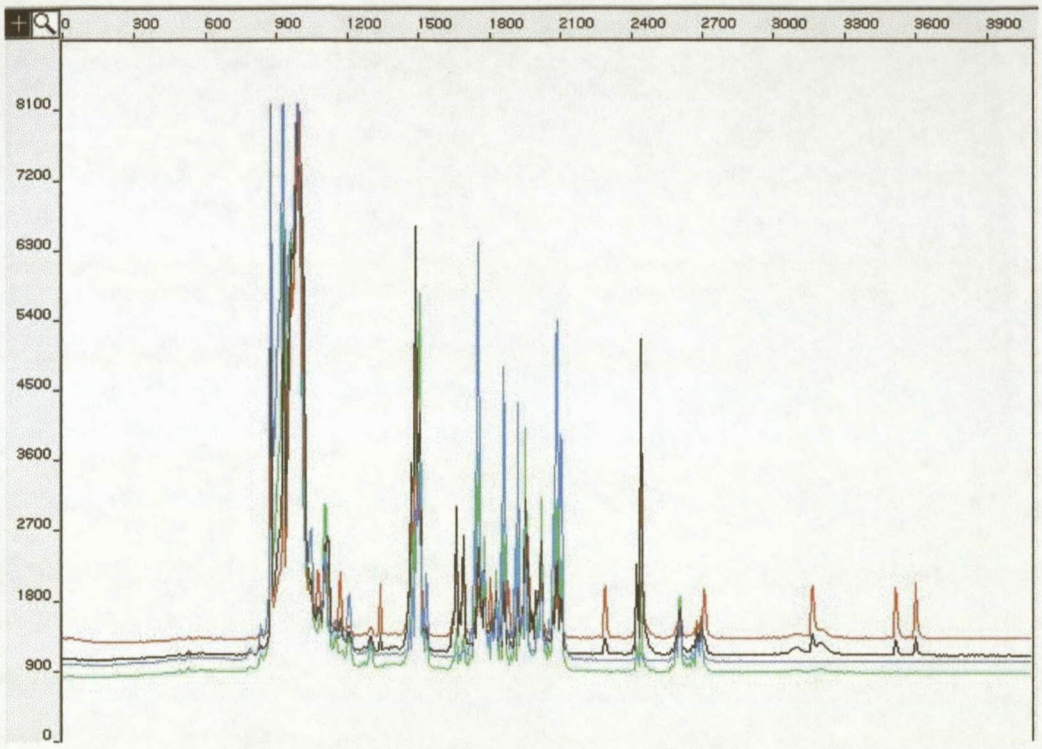


Figure 3.2: Electropherogram from one lane (one sample) of gel.

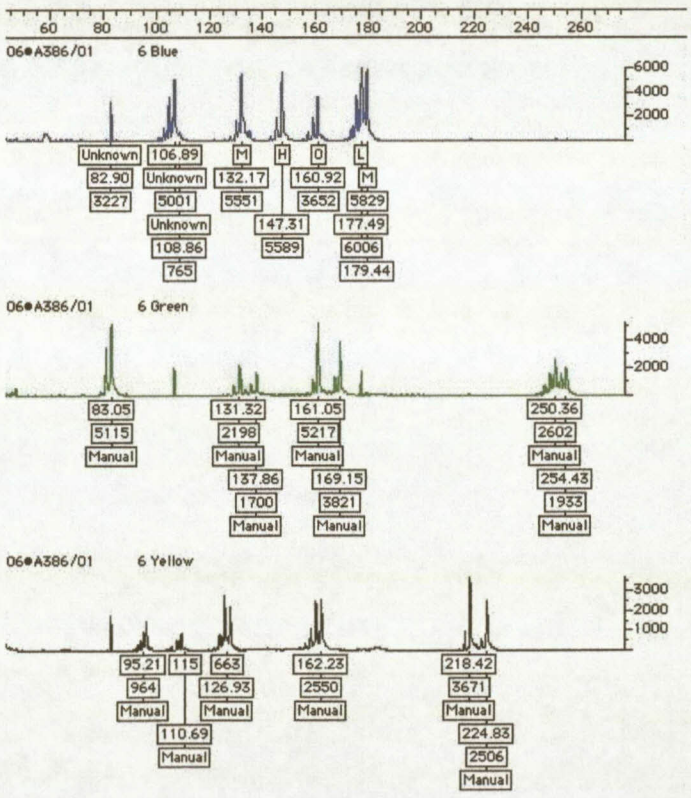


Figure 3.3: Example of DNA profiles constructed by the Genotyper program.

The Animal Research Counsel Irene uses the same method as the one used in this thesis to confirm parentage disputes for horses. Most of the parentage disputes could be resolved by using this DNA test. The method has been proven to be very effective. Laboratories all over the world make use of this test. To be able to compare results, all profiles have to be analysed in the same manner.

The DNA profiles of all horses are given in the ISAG standardized format. The allelic nomenclature is in alphabetical order by size, from smallest to largest in two-base pair increments, according to agreement among participating laboratories at the meeting of the International Society of Animal Genetics (ISAG), July 1996. Size calling is based on ABI 350 ROX, using the local southern algorithm (Anonymous 1996e)[14].

3.2 DNA profiles

DNA of 518 horses was typed and 15 gels were loaded. The peak heights were approximately 4000 relative fluorescent units. The loci HTG7 and HTG6 did not show much variation as others. All the populations had in most of the cases at locus HTG6, alleles E, G or H present. Most horses had at locus HTG7, allele O present, except for the Friesian horses. Allele M was present in most of Friesian horses. An allele at any other loci can thus be considered relatively rare if it is not one of the above for HTG6 and HTG7 in the populations studied.

The Friesian horses had the least variation of alleles. They had a high frequency of the following alleles, whereas the others populations had not: AHT4 allele J, HTG4 allele L, VHL20 allele P and HMS6 allele L. The Przewalski horse was homomorphic for seven of the 12 alleles. The Przewalski horse was polymorphic for allele AHT4 at locus L, which is a relatively scarce allele for the other horse populations.

The ISAG formatted DNA profiles were converted for use in the BIOSYS-1 and the POPGENE programs (Table 3.1) and are listed in this format for all horses profiled in Appendix

[14] Anonymous 1996e. M values for STRs reported by VGL. U.C. Davis. California. http://www.vgl.ucdavis.edu/horse/mvalues.html

A. The results obtained from the different statistical analyses, will be discussed in the chapters which follow.

Table 3.1: The transformation of ISAG standardized profiles (white blocks) to BIOSYS-1 and POPGENE input files (coloured blocks).

VHL 20	I	J	K	L	M	N	O	P	Q	R
	A	B	C	D	E	F	G	H	I	J

HTG4	K	L	M	N	O	P	Q
	A	B	C	D	E	F	G

AHT4	H	I	J	K	L	M	N	O	P
	A	B	C	D	E	F	G	H	I

HMS7	J	K	L	M	N	O	P	Q
	A	B	C	D	E	F	G	H

HTG6	E	F	G	H	I	J	K	L	M	N	O	P
	A	B	C	D	E	F	G	H	I	J	K	L

AHT5	I	J	K	L	M	N	O
	A	B	C	D	E	F	G

HMS6	J	K	L	M	N	O	P
	A	B	C	D	E	F	G

ASB2	I	J	K	L	M	N	O	P	Q	R
	A	B	C	D	E	F	G	H	I	J

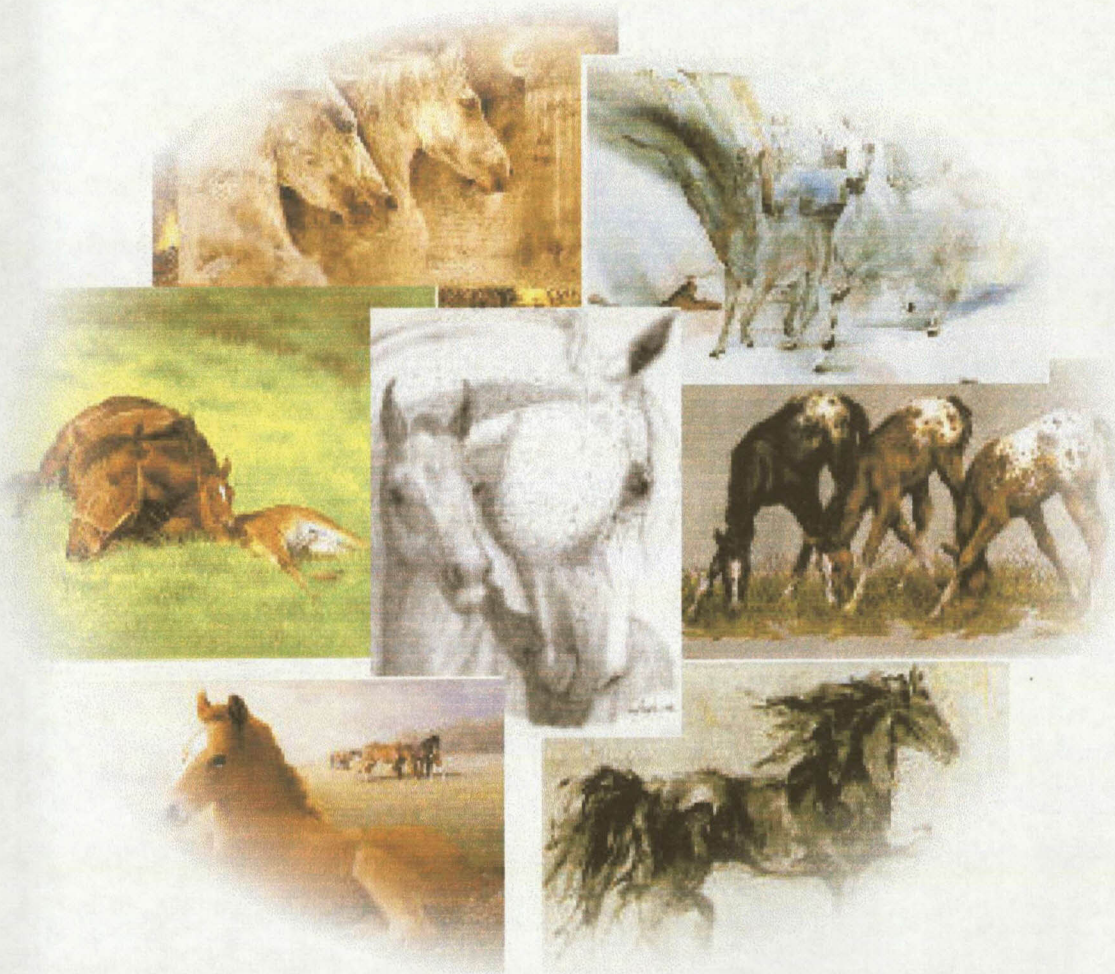
HTG10	I	J	K	L	M	N	O	P	Q	R	S	T
	A	B	C	D	E	F	G	H	I	J	K	L

HTG7	J	K	L	M	N	O	P
	A	B	C	D	E	F	G

HMS3	I	J	K	L	M	N	O	P	Q	R
	A	B	C	D	E	F	G	H	I	J

HMS2	I	J	K	L	M	N	O	P	Q	R	S
	A	B	C	D	E	F	G	H	I	J	K

Chapter 4



CHAPTER 4

HARDY-WEINBERG AND HETEROZYGOSITY

4.1 Introduction

Population genetics, can be simply defined as the study of the properties of genes in populations. Scientist began to formulate a comprehensive theory of how alleles behave in populations and the ways in which changes in allele frequencies lead to evolutionary change. The most fundamental model in the field op population genetics, the Hardy-Weinberg principle, was developed in the early years of the previous century; other aspects of this field can be viewed in relation to it (Raven & Johnson 1991).

The Hardy-Weinberg principle serves as the founding theorem of population genetics. Perhaps its main contribution to evolutionary thought lies in demonstrating that genetic diversity in a randomly breeding population will remain constant unless acted upon by external forces; a point that is contrary to the premendelian concept that heredity involves a blending of traits which become diluted with each generation of interbreeding (Strickberger 1990).

Since the Hardy-Weinberg principle is a basic guideline for the study of the evolutionary state of a population, it is importend to calculate whether the populations are in Hardy-Weinberg equilibrium. These statistics is also helpful in the summerization of data. Statistics takes large masses of data and reduces them to one or two meaningful values that can give you a lot of information about a population. The aim of this chapter is to determine whether the different horse breeds are in Hardy Weinberg equilibrium for the various loci typed.

4.2 The Hardy-Weinberg Equilibrium

When an allele in the homozygous (or in some cases, heterozygous) state leads to a disorder that due to its severity and the time of onset prevents the individual from reproducing, it represents a selective disadvantage for that individual. Although it could be assumed that such a mutant allele would eventually disappear from a population, this is not the case. Rather, a frequency equilibrium develops according to principles and conditions elucidated in 1910 by the

English mathematician Hardy and the German physician Weinberg (Hardy-Weinberg equilibrium principle) (Passarge 1995).

In a large population in which there is random mating and in the absence of forces that change the proportions of the alleles at a given locus, the original proportions of the genotypes will remain constant form generation to generation. Dominant alleles do not in fact replace recessive ones. Because their proportions do not change, the genotypic frequencies are said to be in Hardy-Weinberg equilibrium (Raven & Johnson 1991).

The relationship between gene frequencies and genotypic frequencies is this if the gene frequencies of two alleles among the parents are p and q, then the genotypic frequencies among the progeny are p^2, 2pq, and q^2. The relationship refers to autosomal genes (Falconer & Mackay 1996).

This simple relationship has proved extraordinarily useful in assessing actual situations. For many genes, they prove to be very accurate. Most human popualation , for example, are large and effectively random-mationg, and so are similar to the "ideal" population envisioned by Hardy and Weinberg. For some genes the calculated predictions do not match the actual values. The reasons they do not, do so tell us a great deal about evolution (Raven & Johnson 1991).

The Hardy-Weinberg principle is valid only under certain conditions. Above all, it applies only when the population is sufficiently large and mating is random (panmixi). Selection for a particular allele in mating would lead to an increase in the corresponding allele frequency. In such a population, a particular allele would become more frequent than would be predicted by the equilibrium. Furthermore, if preferential mating of certain genotypes occurs (assortative mating), a shift in the frequency of the corresponding allele will also result (Passarge 1995).

There is a strong selection factor involved with horse breeding. Breeders select only a few stallions to sire their foals. The stallions that are selected, possesse certain characteristic. There are also other factors involved that could influence the Hardy Weinberg equilibrium. Migrating is one of the factors. Horses are also sold and used on different studs. Stallions are also rented to sire the foals of certain mares. A bloodline is created by breeders and can be traced back in

history.

A sample may not be an accurate representation of a population, especially if the sample is small. The larger the sample, the greater the probability that the allelic frequencies of the offspring will accurately represent allelic frequencies in the parent population. When populations are small or when alleles are rare, changes in allelic frequencies take place due to chance alone. These changes are referred to as random genetic drift (Tamarin 1993).

There are several ways to determine whether a given population conforms to the Hardy-Weinberg equilibrium at a particular locus. However, the question usually arises when there is just a single sample from a population, representing only one generation. Can existence of the Hardy-Weinberg equilibrium be determined with just one sample? The answer is that we can determine whether the three genotypes (AA, Aa, and aa) in the case of two alleles, occur with the frequencies p^2, 2pq, and q^2. If they do, then the population is considered to be in Hardy-Weinberg proportions; if they do not, then the population is considered **not** to be in Hardy-Weinberg proportions (Tamarin 1993).

4.3 Hardy-Weinberg tests

Two statistical tests are frequently used to test for deviations from Hardy-Weinberg proportions. The most common is the Chi-square test (χ^2), which is computed by summing the quotients of the squared differences between the observed and the expected numbers and the expected number for each phenotype. $\chi^2 = \sum(\text{obs} - \text{exp})^2 / \text{exp}$. The degrees of freedom are

$$df = (x^2 - x) / 2$$

where x is the number of allelic classes for a locus. It is difficult to test for Hardy-Weinberg proportions when rare alleles are present because low expected values severely inflate the statistic. To avoid this problem, the frequencies of rare alleles can be pooled into one synthetic allelic class so that the expected values are larger than one (Ayala 1982).

The G-test can also be used to detect significant departures from Hardy-Weinberg proportions. G can be calculated using $G=2$ ($\sum$obs ln obs - $\sum$obs ln exp) where ln obs and ln exp are the natural logarhithms of the observed and expected numbers of individuals with a particular

phenotype (Sokal & Rolf 1969).

In the χ^2-test small expected frequencies is a problem. Sokal & Rolf (1969) recommended expected values no smaller than 5 when the χ^2 test is used. The G-statistics is less affected by this problem and occasional expected values as low as one can be used. Smouse and Kojma (1972) present a theoretical framework for using the maximum likelihood analysis G-test on allelic frequency data.

This statistic are distributed as Chi-square and critical values can be found in a Chi-square table with the same degrees of freedom as previously. This test statistic are not inflated as severely as the Chi-square statistic with small expected values.

The BIOSYS-1 program was used to calculate the Chi-square statistic (Table 4.1) for given degrees of freedom and the POPGENE program was used to calculate the G-square statistic (Table 4.2). BIOSYS-1 uses the usual Chi-square goodness-of-fit test and is performed by using observed genotypic frequencies and those expected under Hardy-Weinberg equilibrium. Expected frequencies were calculated by using Levene's (1949) formula for small samples (Swofford 1981).

The POPGENE program was also used to calculate the Chi-square values. The results were exactly the same as that of the BIOSYS-1 program. POPGENE also gave the estimation of G-square values.

All loci were found to be polymorphic in all breeds of horses studied. All loci, except HTG10 were found to be in Hardy-Weinberg equilibrium when tested among different horse breeds ($P>0.05$). When individual breed-locus combinations were tested, most of the breeds were found to deviate from Hardy-Weinberg equilibrium at least at one locus.

Table 4.1 Chi-square test for deviation from Hardy-Weinberg equilibrium.

	Arabian horses			Quareter horses			American Saddlers			Cape horses			S.A. Boerperde			Welsh ponies			Friesian horses		
Allele	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
AHT4	9.86	15	0.83	14.74	10	0.142	44.44	28	0.25	2.841	6	0.828	20.075	21	0.517	83.6	36	0*	5.924	3	0.115
HTG4	6.88	15	0.96	62.16	28	0*	23.67	21	0.309	6.181	15	0.977	223.388	28	0*	7.873	15	0.929	6.762	6	0.343
HMS7	18.7	6	0*	15.02	10	0.131	9.181	15	0.868	12.68	10	0.242	35.628	21	0.24	46.55	15	0*	5.501	10	0.855
VHL20	40	36	0.3	15.44	28	0.973	19.2	45	0*	32.49	36	0.636	67.757	45	0.015*	38.43	45	0.745	36.89	15	0.001*
AHT5	17.5	10	0.07	5.791	15	0.983	10.87	21	0.965	13	15	0.603	48.271	28	0.01*	11.83	15	0.692	23.64	21	0.311
ASB2	14.1	15	0.52	119.1	45	0*	19.35	28	0.887	28.58	28	0.434	60.119	45	0.065	56.44	36	0.016*	16.81	21	0.723
HMS6	9.42	10	0.49	76.96	15	0*	9.381	15	0.857	4.747	10	0.907	14.807	15	0.465	11.09	15	0.746	16.49	10	0.087
HTG6	17.7	10	0.06	31.12	21	0.072	9.86	15	0.828	1.213	6	0.976	8.961	15	0.879	172.8	28	0*	75.13	10	0*
HMS2	16.1	15	0.38	20.2	28	0.857	35.92	28	0.145	13.24	15	0.584	57.620	28	0.001*	64.49	15	0*	98.76	15	0*
HMS3	47.8	15	0*	29.55	28	0.385	12.39	15	0.649	87.86	21	0*	38.613	28	0.087	20.29	21	0.503	21.33	15	0.127
HTG10	62	15	0*	207.3	55	0*	49.93	28	0.007*	27.67	15	0.024*	155.867	45	0*	60.02	45	0.066	25.4	15	0.045
HTG7	2	3	0.57	19.23	10	0.037*	1.443	6	0.617	5.385	6	0.495	16.362	6	0.012*	34	10	0*	4.996	6	0.544

* $P<0.05$

Tabel 4.2 The coefficient of gene differentiation values.

	Arabian horses			Quareter horses			American Saddlers			Cape horses			S.A. Boerperde			Welsh ponies			Friesian horses		
Allele	G	df	P	G	df	P	G	df	P	G	df	P	G	df	P	G	df	P	G	df	P
AHT4	12.7	15	0.63	15.45	10	0.117	23.43	28	0.711	4.418	6	0.62	20.487	21	0.49	34.45	36	0.542	3.909	3	0.271
HTG4	9.87	15	0.83	19.88	28	0.869	19.03	21	0.583	7.509	15	0.941	51.794	28	0.004*	8.473	15	0.903	6.276	6	0.393
HMS7	19.9	6	0*	16.53	10	0.085	10.64	15	0.778	12.26	10	0.268	30.014	21	0.091	15.03	15	0.449	7.778	10	0.65
VHL20	39	36	0.34	17.64	28	0.935	20.59	45	0.999	26.44	36	0.878	55.951	45	0.126	38.89	45	0.727	15.41	15	0.423
AHT5	15.3	10	0.13	7.881	15	0.928	13.56	21	0.888	16.88	15	0.326	35.486	28	0.156	15.21	15	0.436	17.48	15	0.291
ASB2	13.9	15	0.54	60.34	45	0.063	20.26	28	0.855	29.21	28	0.402	46.916	45	0.394	47.35	36	0.097	22.02	21	0.396
HMS6	10.9	10	0.36	30.7	15	0.009*	8.994	15	0.877	7.366	10	0.691	17.034	15	0.317	11.34	15	0.727	8.38	10	0.592
HTG6	9.33	10	0.5	20.39	21	0.496	10.92	15	0.758	1.985	6	0.921	10.007	15	0.819	24.29	28	0.666	12.88	10	0.231
HMS2	14.3	15	0.5	22.07	28	0.778	30.02	28	0.362	13.76	15	0.544	27.194	28	0.507	51.76	45	0.227	26.18	15	0.036*
HMS3	40.5	15	0*	27.88	28	0.47	12.71	15	0.625	58.2	21	0.001*	38.473	28	0.089	22.68	21	0.361	15.16	15	0.44
HTG10	20.8	15	0.14	94.81	55	0.001*	37.58	28	0.107	27.58	15	0.024*	88.265	45	0.001*	49.36	45	0.303	15.75	15	0.399
HTG7	1.93	3	0.59	9.092	10	0.523	3.818	6	0.701	7.402	6	0.285	16.145	6	0.012*	16.79	10	0.079	6.12	6	0.409

*P<0.05

These statistics are distributed as Chi-square and critical values and can be found in a Chi-square table with the same degrees of freedom as previously. These test statistics are not inflated as severely as the chi-square statistic with small expected values.

The S.A. Boerperd was on the brink of not being in Hardy-Weinberg equilibrium with the Chi-Square test. The G-test confirmed however that the S.A. Boerperd were in Hardy-Weinberg equilibrium. These results confirms studies by Dr. E. van Dyk (Faculty of Veterinary Science at the University of Pretoria, South Africa) who indicated that there has been more outbreeding of the S.A. Boerperde than the other horse breeds and that they are the least closely related to one another (Hofmeyr 1999).

4.5 Heterozygosity

The average heterozygosity is calculated in three ways: the proportion of individuals sampled that are actually heterozygous (direct count), the usual estimate based on Hardy-Weinberg expectations and the unbiased estimate based on conditional expectations (Levene, 1949 ; Nei, 1978).

The percentage of loci polymorphic was calculated. A locus is considered polymorphic if any variation was observed in the population and the frequency of the most common allele is 0.99 (Black 1997).

As expected all the microsatellites in all the populations were 100% polimorphic except for the Przewalski horse. Since there was only one individual in the population, it is understandable that the Przewalski population was not 100% polimorphic (Table 4.3).

The direct count and the expected values (Levene 1978) were very simmilar. The populations show a high value of heterozygosity, but one must not forget that the markers used were specifily selected for it's polymorphism, and that these values cannot be used to give an accurate account of what is going on in the population. There were no monomorphisms found.

The values of genetic distances obtained from microsatellite data can be more precise

when compared with genetic distance values obtained with protein markers. Arranz (1996) concluded that microssatellite loci provide more accurate values than protein markers of the time when species or populations are separated from a common ancestor. These authors have also suggested that microsatellites might be a better indicator than protein polymorphisms of evolutionary relationships within a particular species. The high level of polymorphism displayed by microsatellites makes it possible to obtain a better resolution in genetic analysis using a lower number of markers. Such results are in accordance with previous suggestions regarding the usefulness of microsatellites when examining genetic relationships (Arranz et.al 1996).

Table 4.3 Genetic variablility at 12 loci in all populations

Population	**Mean sample size per locus**	**Mean number of per locus**	**Percentage of loci (%)**	**Mean** Direct count (H_0)	**Heterozygosity** H/W expected (H_e)
Arabian horses	80	5.6	100	0.575	0.64
American Quarter	80	7.3	100	0.657	0.699
American Saddler	80	7	100	0.661	0.695
Kaapse Ryperd	77	5.8	100	0.61	0.634
SA Boerperd	128	7.7	100	0.686	0.744
Welsh ponies	81	7.7	100	0.707	0.766
Friesian Horses	39	5.3	100	0.534	0.576
Przewalski	3	1.4	41.7	0.417	0.25

The microsatellites had a mean of 6.6 alleles per locus (excluding the Przewalski horse, since there was only one sample available). The largest number of alleles was found in the S.A. Boerperd and the Welsh ponies and the lowest in the Friesian horses. The average number of alleles and heterozygosity levels for the various breeds are shown in Table 4.3. There were no significant differences in the number of alleles among the breeds surveyed.

The S.A Boerperd and the Welsh ponies display the greatest degree of heterozygosity (genetic variation between individuals). This indicates that there has been more outbreeding of Boerperde and that the breed has not been a closed herd, as closed herds tend to become more homozygous over such a long period of time. These results confirm results given by Dr. E van Dyk (Hofmeyr 1999).

4.6 Allelic Frequencies

The allele frequencey were calculated for the seven horse breeds with the BIOSYS-1 program. (Appendix B) The frequency of allele HTG 7 at locus O, (converted to F for BIOSYS-1 input file) had the highest frequency for all the populations. The average frequency for all the alleles for each population is given in Table 4.4.

Table 4.4 Average frequency for each allele for all seven horse populations except the Przewalski horse.

Alleel\Frequency	A	B	C	D	E	F	G	H	I	J	K	L
AHT4	0.287	0.017	0.323	0.086	0.018	0.0008	0.0009	0.24	0.011			
HMS7	0.123	0.082	0.383	0.057	0.158	0.18	0.002	0.015				
HTG4	0.196	0.189	0.469	0.033	0.036	0.076	0.0009	0	0.0006			
VHL20	0.175	0.051	0.005	0.119	0.209	0.109	0.036	0.158	0.095			
AHT5	0.0006	0.231	0.114	0.115	0.14	0.099	0.001					
ASB2	0.076	0.008	0.158	0.017	0.126	0.223	0.133	0.019	0.189	0.051		
HMS6	0	0.081	0.188	0.282	0.044	0.132	0.273					
HTG6	0.305	0	0.033	0.157	0	0	0.004	0.0008	0.468	0.012	0	0.016
HMS2	0.183	0.084	0.008	0.228	0.29	0.062	0.005	0.013	0.049	0.004	0.07	
HMS3	0.091	0	0	0	0.105	0.208	0.133	0.253	0.023	0.178	0.01	
HTG10	0.07	0.119	0.685	0.112	0.143	0.067	0.298	0.065	0.014	0.223	0.02	
HTG7	0.003	0.169	0	0.144	0.078	0.605	0.0008					

The highest average frequency for an allele is for HTG7. The rest of the alleles had an average of 0.114. It is of great importance, because these microsatellites are used for determining parentage. If the frequency of an allele becomes to high, it cannot be used to distinquised between two individuals. There were differences found in the frequencies between the different populations (Appendix B).

The chromosomal or genomic location of a gene is called a locus, and alternative forms of the gene at a given locus is called alleles. In a population, more than one allele may be present at a locus, and their relative proportions are referred to as the allele frequencies or gene frequencies. Allelic frequencies are used in studies of populations instead of phenotypic

frequencies because phenotypic frequencies arise anew each generation and may be subject to short-term changes from selection or random population events. The most frequently used method of estimating allelic frequencies from genotypic data is "gene" counting, where the numbers of allelic doses are counted and a relative frequency for each allele is calcultated against the total number of gene doses or twice the number of individuals in the sample (Kirby 1973).

In many studies of natural populations it is of interest to know if allele-frequency differences are due to chance errors in estimation the frequencies from a finite sample of the populations, or whether the samples were drawn from populations with different allele frequencies. The inferrence in the last instance is that the populations are reproductively isolated from one another to some degree and that allelic frequencies have diverged because of genetic drift, mutation or selection. The null hypothesis (H_0) is that the allelic frequencies are not different. It is frequently assumed that if no differences ar found, that the samples were drawn from one panmictic population (one randomly-mating population). This may not be true, because isolation may have occurred recently but without time for genetic divergence (Kirby 1973).

Sokal and Rohlf (1969) present a method for finding the sample size required to detect a "true" difference between two frequencies. For calculating allelelic frequencies the sample size is twice the number of individuals assayed (2N) because, with the assumption of random mating, alleles are merely carried around in packets of two in diploid organisms. In most genetic studies of natural populations sample sizes range from 50 - 100 mainly for economic reasons, but are adequate to detect only very large allele-frequency differences. Levene (1949) noted that when a finite sample of diploids is drawn from a population in Hardy-Weinberg equilibrium, then on average, the frequency of hetreozygotes in the sample will exceed the Hardy-Weinberg expectation for the sample (Kirby 1973).

However Roberson (1965) showed that is was not valid to expect Hardy-Weinberg frequencies in a population if there were a small number of parents for the individuals in the population. Robertson (1965) demonstrated that if the variance effective number of parents was N, then because of the gene frequency difference between the sexes that arises by chance the frequency of heterozygotes in the progeny population will exceed Hardy-Weinberg expectations by a proportion ½N, on average. For this reason genotypic frequencies in small populations may

show an excess of heterozygotes when tested by the methods of Levene (1949), Kirby (1973).

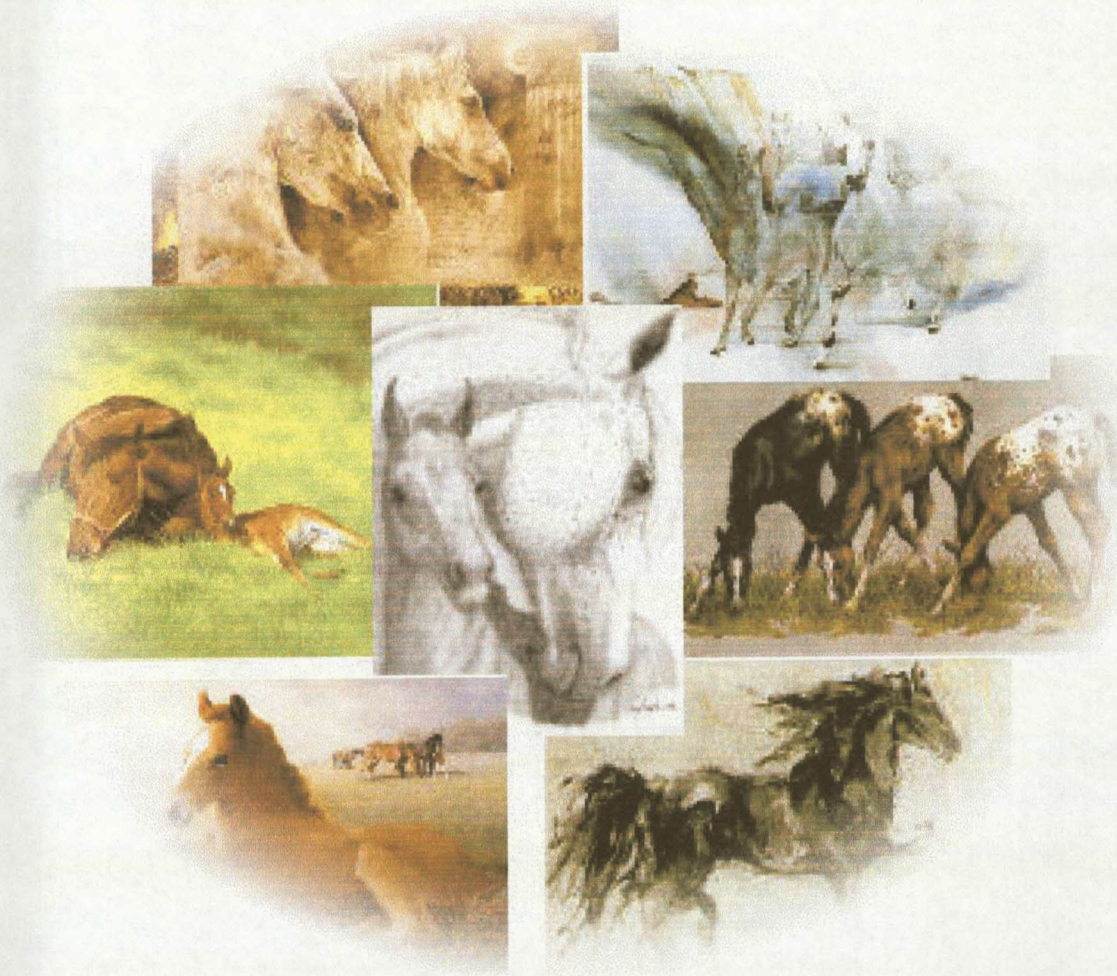
Inbreeding is a factor that can influence the alleel frequency and the Hardy-Weinberg equilibrium. There are a lot of cases in stud breeding where a father is used on his daughter to produce a foal that have very spesific characteristics.

The allelic frequencies at an autosomal locus in a population will not change from one generation to the next. Inbreeding is the mating of related individuals and outbreeding is the mating of genetically unrelated individuals. Inbreeding is a consequence of either pedigree relatedness or small population size. One of the first counterintuitive observations of population genetics is that deviations from random mating alter genotypic frequencies but not allelic frequencies. Assortative mating and inbreeding will change the zygotic (genotypic) combinations from one generation to the next but will not change which alleles are passed into the next generation. Thus genotipic, but not allelic frequencies change under non-random mating (Tamarin 1993).

Inbreeding increases the proportion of homozygotes in the population. With complete inbreeding (F=1), only homozygotes will occur in the population. Inbreeding does not change allelic frequencies.The result of inbreeding can be seen by the appearance of recessive traits that are often deleterious. Inbreeding increases the rate of fetal deaths and congenital malformations (Tamarin 1993).

The seven horse breeds studied in this thesis are all in Hardy-Weinberg equilibrium. It is of great importance since the Hardy-Weinberg equilibrium is a guideline of the evolutionary state of the populations.

Chapter 5



CHAPTER 5

F-STATISTICS

5.1 Introduction

Most breeders are very specific in selecting stallions and mares in their breeding programmes. Since the value of a horse depends upon his pedigree, inbreeding is a definite concern for breeders. The aim of this chapter is to determine whether inbreeding has taken effect in the different horse breeds and what the genetic differentiation is between the different horse breeds.

Mating among relatives is termed inbreeding and is represented by F, the inbreeding coefficient, which measures the probability of autozygosity (homozygosity by descent). It can be calculated from pedigrees by using the formula: $F=\sum[(1/2)^n(1+F_J)]$ where n is the number of ancestor of that path. Inbreeding exposes recessive deleterious traits already present in the population and causes homozygosity throughout the genome. It does not by itself change allelic frequencies. F can also be calculated from the reduction in heterozygosity in a population (Tamarin 1993)

The hierarchical F-statistics defined, are all types of fixation indexes, but they differ in the reference populations. F_{SR} is concerned with subpopulations (S) relative to the regional aggregates, F_{RT} is concerned with the regional groupings relative to the total population (T), and F_{ST} is the most inclusive measure of population substructure (Hartl 1987).

5.2 F_{ST} values

The F_{ST} values were calculated pair wise with the BIOSYS-1 program and these values were used to calculate the N_{em} values (Table 5.1).

Table 5.1 Population differentiation of horse breeds based on F_{ST} values (below diagonal) and N_{em} values (above diagonal). The F_{ST} values were calculated pair wise.

	Arabian	Quarter	Saddlers	Cape horse	Boerperd	Welsh	Friesians	Przewalski
Arabian horses	****	2.294	2.753	1.597	2.753	2.504	1.196	0.357
Quarter horses	0.077	****	3.715	2.395	4.846	5.277	1.206	0.382
American Saddlers	0.065	0.049	****	3.167	6.404	3.881	1.316	0.392
Cape horse	0.107	0.074	0.057	****	6.898	3.489	0.969	0.325
S.A. Boerperde	0.065	0.038	0.029	0.027	****	7.784	1.431	0.434
Welsh ponies	0.071	0.035	0.047	0.052	0.024	****	1.867	0.507
Friesians	0.138	0.137	0.127	0.165	0.118	0.093	****	0.455
Przewalski	0.349	0.334	0.328	0.371	0.306	0.274	0.296	****

Population differentiation estimates are shown in Table 5.1. Most pair-wise breed combinations were significantly different from zero. Least differentiation was detected between the S.A.Boerperd and the Welsh ponies with a value of 0.024. Relatively small differentiation values were observed for the American Saddler and the S.A. Boerperd and between the Boerperd and the Cape horse. The most distinct difference was observed between the Friesian horse and the Cape horse. The Friesian horses showed a F_{ST} value that indicates a great genetic differentiation from the rest of the breeds, but the Przewalski horse's F_{ST} value indicate a very great genetic differentiation from all the other horse breeds. The Przewalski horse can thus be used as an outgroup for the dendrogram, and the Friesian horse will be the next in line.

The effective number of exchang between populations in each generation (N_{em}) is given in Table 5.1, using the relationship between the F_{ST} value and the N_{em}. These high values suggest that there is substantial gene flow between the populations, since a N_{em} value of one is interpreted as evidence of sufficient gene flow between populations to counter the effects of genetic drift.

Population subdivision entails an inbreeding-like effect in terms of excess homozygosity. A subdivided population, however, has three distinct levels of complexity: individual organisms (I), subpopulations (S), and the total population (T).

H_I = the heterozygosity of an individual in a subpopulation

H_S = the expected heterozygosity of an individual in an equivalent random mating subpopulation

H_T = the expected heterozygosity of an individual in an equivalent random mating total population.

H_I can be interpreted as the average heterozygosity of all the genes in an individual or as the probability of heterozygosity of any one gene. H_I is the observed heterozygosity averaged across subpopulations. H_S represents the level of heterozygosity that would be found in a subpopulation if the subpopulation were undergoing random mating; therefore, H_S always equals $2p_iq_i$ for a subpopulation with allelic frequency p_i. The quantity H_T represents what the heterozygosity would be if all subpopulations were pooled together and mated randomly; if the average allelic frequency among subpopulation is denoted p_0, then $H_T = 2p_0q_0$ With multiple alleles the definitions of H_I, H_S, and H_T remain the same (Hartl 1987).

The effects of population subdivision are measured by a quantity called the fixation index (symbolized F_{ST}), which is the reduction in heterozygosity of a subpopulation due to random genetic drift. F_{ST} is always greater than (or equal to) zero, because the Wahlund effect assures that $H_T > H_S$, if all subpopulations are in Hardy-Weinberg equilibrium with the same allelic frequencies, $F_{ST} = 0$ (Hartl 1987).

For examining the overall level of genetic divergence amongst subpopulations, F_{ST} is the most informative statistic. Although F_{ST} has a theoretical minimum of 0 (indicating no genetic divergence) and a theoretical maximum of 1 (indicating fixation for alternative alleles in different subpopulations), the observed maximum is usually much less than 1. Wright (1978) has suggested the following qualitative guidelines for the interpretation of F_{ST}. The range 0 to 0.05 may be considered as indicating little genetic differentiation. The range 0.05 to 0.15 indicates moderate genetic differentiation and the range 0.15 to 0.25 indicates great genetic differentiation. Values of F_{ST} above 0.25 indicate very great genetic differentiation (Table 5.1). On the other hand, Wright (1978) also noted that, amongst subpopulations, differentiation is by no means negligible if F_{ST} is as small as 0.05 or even less (Hartl 1987).

5.3 F_{IS} and F_{IT} values

The hierarchical F-statistics defined, are all types of inbreeding coefficients, but they differ according to the reference populations. F_{IS} is concerned with inbreeding in individuals (I), relative to the subpopulation (S) to which they belong; F_{ST} is concerned with inbreeding in

subpopulations (S), relative to the total population (T) of which they are a part; and F_{IT} is concerned with inbreeding in individuals (I), relative to the total population (T) (Hartl 1987).

Except for plants that have a high frequency of self-fertilization or for certain insects that regularly undergo parent-offspring or brother-sister mating, values of F_{IS} in most natural populations are typically close to zero, which indicates random mating within subpopulations. For many natural populations, therefore, particularly in animals and out crossing plants, it is reasonable to assume $F_{IS} = 0$. This assumption is equivalent to the assumption of random mating within subpopulations. Furthermore, when $F_{IS} = 0$, then $F_{ST} = F_{IT}$, and there is no longer need for the subscripts. In the context of multi allelic loci, F_{ST} is denoted G_{ST} (Nei 1973; Hartl 1987).

All the F_{IS} values are relatively close to zero, which indicates random mating within subpopulations. The F_{IT} values are relatively similar to the F_{ST} values, with a mean difference of about 0.072 (Table 5.2).

Table 5.2 F_{IS} (below diagonal) and F_{IT} (above diagonal) totals F-statistics and gene flow for all loci. The F-statistics were calculated pair wise.

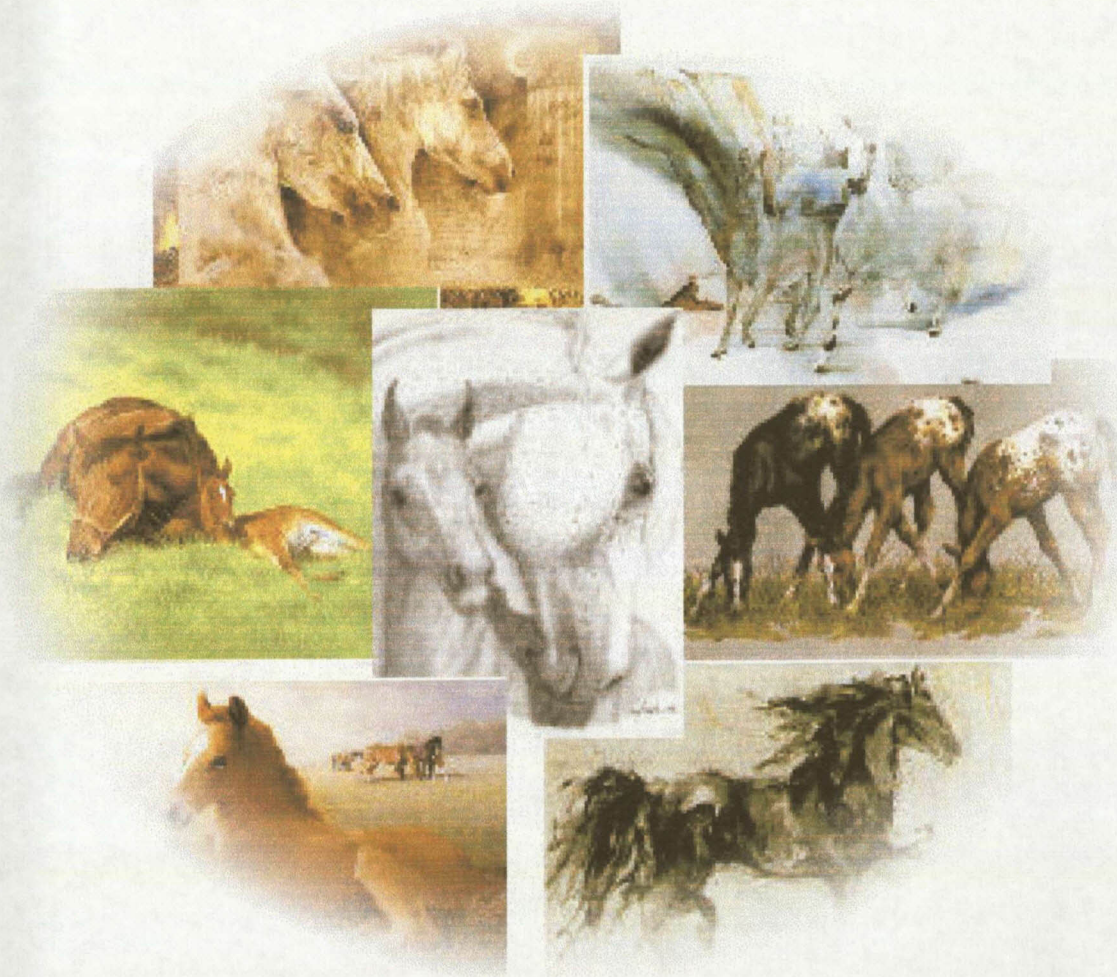
	Arabian	Quarter	Saddlers	Cape horse	Boerperd	Welsh	Friesians	Przewalski
Arabian horses	****	0.145	0.129	0.164	0.145	0.148	0.207	0.236
Quarter horses	0.074	****	0.095	0.113	0.101	0.096	0.186	0.208
American Saddlers	0.068	0.048	****	0.091	0.086	0.101	0.171	0.194
Cape horse	0.064	0.043	0.036	****	0.08	0.102	0.203	0.229
S.A. Boerperde	0.085	0.065	0.059	0.054	****	0.095	0.179	0.195
Welsh ponies	0.083	0.063	0.057	0.053	0.073	****	0.154	0.158
Friesians	0.08	0.057	0.05	0.045	0.069	0.067	****	
Przewalski	-0.174	-0.189	-0.2	-0.226	-0.161	-0.159		****

The allelic frequencies, F-statistics and the genetic distance and identity of Nei (1972) results calculated by the POPGENE program, were the same as indicated in the BIOSYS-1 program. Both programs were used to confirm that there were no mistakes made in the inputfile of both the programmes.

The F_{IS} values are relatively close to zero, thus the populations can be considered as a random mating population. It must not be forgotten that the horses came from different stud

breeders and inbreeding can take its toll in a stud if the breeder is not careful in his selection of breeding stock. The Cape horse and the S.A. Boerperd had very little genetic differentiation.

Chapter 6



CHAPTER 6

GENETIC DIVERSITY IN THE DIFFERENT HORSE BREEDS

6.1 Genetic diversity

The distinction between breeds is usually not simply discerned by the presence or absence of particular genes but is in many instances a matter of gene frequencies. The horse populations have not reached the point where one population is fixed for one allele at a particular locus and another population is fixed for a different allele. When a population shows fixation for one allele, other populations are always polymorphic for it (Strickberger 1990).

The criterion for evaluating the differences between populations of a single specie is based essentially upon gene-frequency differences. When these differences are numerous and it is advantageous to consider populations as separate entities we may categorise them broadly as different breeds. It is essential for horse breeders to keep the breed as pure as possible, especially for breeds like the Cape horse. The Cape horse almost lost its identity due to the fact that breeders used American Saddlers and Thoroughbreds to upgrade their horses. The aim of this chapter is to determine whether the Cape horse and the S.A. Boerperd can be considered as different horse breeds as well as to determine the genetic diversity amongst the seven horse breeds studied.

Several measures of genetic distance using electrophoretic data have been devised, but Nei's (1972) coefficients of identity (I) and distance (D) are used most frequently. Values of I range form 0 (total dissimilarity) to 1 (identical), and values of D range from 0 (no genetic distance) to infinity, but the useful range of D is only from 0 to about 1.5. Beyond this range D is compressed because of convergence in allelic mobilities with new mutations and because of the inability of electrophoretic methods to detect multiple mutation events (Ayala 1982).

Table 6.1 Genetic distance, Nei (1972) (above diagonal) and unbiased genetic distance (1978) (below diagonal).

	Arabian	Quarter	Saddlers	Cape horse	Boerperd	Welsh	Friesians	Przewalski
1 Arabian horses	****	0.397	0.319	0.536	0.355	0.417	0.663	1.463
2 Quarter horses	0.384	****	0.266	0.368	0.224	0.207	0.773	1.632
3 American Saddlers	0.306	0.252	****	0.264	0.158	0.291	0.669	1.491
4 Cape horse	0.524	0.355	0.251	****	0.113	0.265	0.893	1.824
5 SA Boerperde	0.344	0.211	0.145	0.102	****	0.157	0.68	1.454
6 Welsh Ponies	0.401	0.19	0.274	0.249	0.142	****	0.481	1.065
7 Friesians	0.649	0.757	0.653	0.879	0.666	0.462	****	0.719
8 Przewalski	1.431	1.598	1.457	1.791	1.421	1.028	0.684	****

The S.A. Boerperd and the Cape horse are the closest related breeds with a value of 0.102. The Friesian is the least related to the Cape horse with a value of 0.879. This can be expected since the Friesian originated in Europe and was imported to South Africa in 1906. The S.A. Boerperd and Cape horse are two indigenous breeds.

DISPAN was used to calculate the standard genetic distances (D) between populations using Nei (1972) (Table 6.1) and the standard errors of standard genetic distances by using Nei (1978) (Table 6.2).

Table 6.2 Standard genetic distances (above diagonal) standard error of standard genetic distances (Below diagonal).

	Arabian	Quarter	Saddlers	Cape horse	Boerperd	Welsh	Friesians	Przewalski
Arabian horses	****	0.3842	0.3064	0.5247	0.3443	0.4008	0.6488	1.4309
Quarter horses	0.1294	****	0.2517	0.3555	0.2108	0.1897	0.7569	1.5986
Saddlers	0.0805	0.0656	****	0.2512	0.1449	0.2738	0.653	1.4569
Cape horse	0.1485	0.1132	0.072	****	0.102	0.2496	0.8794	1.7928
S.A. Boerperde	0.0871	0.0634	0.0392	0.0374	****	0.1417	0.6655	1.4214
Welsh ponies	0.0885	0.0487	0.0474	0.0711	0.0348	****	0.4619	1.0276
Friesians	0.1822	0.1414	0.1484	0.1297	0.0966	0.1197	****	0.6833
Przewalski	0.3826	0.3417	0.3959	0.4359	0.3166	0.2023	0.2508	****

The standard error of genetic distance is large when average heterozygosity is high (Nei 1978). The standard genetic distances calculated by DISPAN confirm that the Cape horse and the S.A. Boerperd are closely related. The Welsh ponies and the S.A. Boerperd also showed a close relationship.

A recently published study (Dr. E. van Dyk, Faculty of Veterinary Science at the University of Pretoria, South Africa) of the relationship between three Southern African horse breeds – S.A. Boerperd, the Nooitgedachter and the Basuto pony – has dispelled some of the speculation about their origins. Amongst other things, this research has confirmed that:

- They are indeed separate breeds.
- They are more closely related to one another than to any other horse breed in the world.
- The Nooitgedachter and the Basuto pony are more closely related to the breeds with a strong English Thoroughbred influence. The Boerperd is clearly related to the gaited North American breeds.
- None of these breeds are closely related to the Arab horse.

This study confirms that the Arab horse is not closely related to any other of the horse breeds studied in this thesis.

To give an accurate justification whether the Cape horse, as compiled by Prof. Roussouw and Mr du Plesssis, is the real Cape horse, the following breeds will have to be entered into the study, the Nooitgedacht pony, Basuto pony and the Kaapse Boerperd. Dr. E. van Dyk already confirmed that these horse breeds are separate breeds (Hofmeyr 1999).

Dr. F. van der Merwe (Hofmeyr 1999) points out that breeders should bear in mind that the present Nooitgedacht and SA Boerperd studbooks only came into being about 25 years ago. He believes that they should not shy away from the reality that these two breeds are of mixed origin, and that closed studbook breeding of the Nooitgedachter and the Boerperd began relatively recently (Hofmeyr 1999).

Nei *et al.* (1983) suggest from theoretical considerations, that when the object of using a genetic distance is to infer phylogenetic relationship, at least 30 loci should be included in the sample. Nei (1978) and Gorman and Renzi (1979) show that it is more important to examine a

large number of loci than to examine a large number of individuals when estimating genetic distances between taxa. D should also be based on a "random" sample of loci without prior knowledge of their degrees of polymorphism or amount of differentiation between taxa. In practice, however, both of these requirements are seldom met. Genetic distances appear in the literatures that are based on as few as 10 loci and enzymes are often chosen for genetic studies because they are polymorphic in related organisms (Ayala 1982).

In this study 12 loci were used. These loci can be used to compare findings found in different laboratories. The loci used here, are also used for parentage verification in laboratories around the world.

Since these horse breeds are so closely related, it is better to use the microsatellite markers than the bloodtyping markers. The values of genetic distances obtained from microsatellite data can be more precise when compared with genetic distance values obtained with protein markers. Microsatellite loci provide more accurate values than protein markers of the time when species or populations are separated from a common ancestor. Microsatellites might also be a better indicator than protein polymorphisms of evolutionary relationships within a particular species. The high level of polymorphism displayed by microsatellites makes it possible to obtain a better resolution in genetic analysis using a lower number of markers (Arranz *et.al.* 1996).

6.2 Relationships between the different breeds

Dendrograms using Nei (1972) and Nei (1978) were calculated with the BIOSYS-1 and the POPGENE programs. Both dendrograms (Figure 6.1) grouped the Cape horse and the S.A. Boerperd together with the American Saddler closest to them. The Friesian and the Przewalski horse are the furthest related from the other horse breeds.

The dendrograms corresponded with the history of South African horse breeds. The dendogram published by Dr. I. van Dyk (Onderstepoort, South Africa) was done with markers used for bloodtyping and their results correspond with the results found in this study.

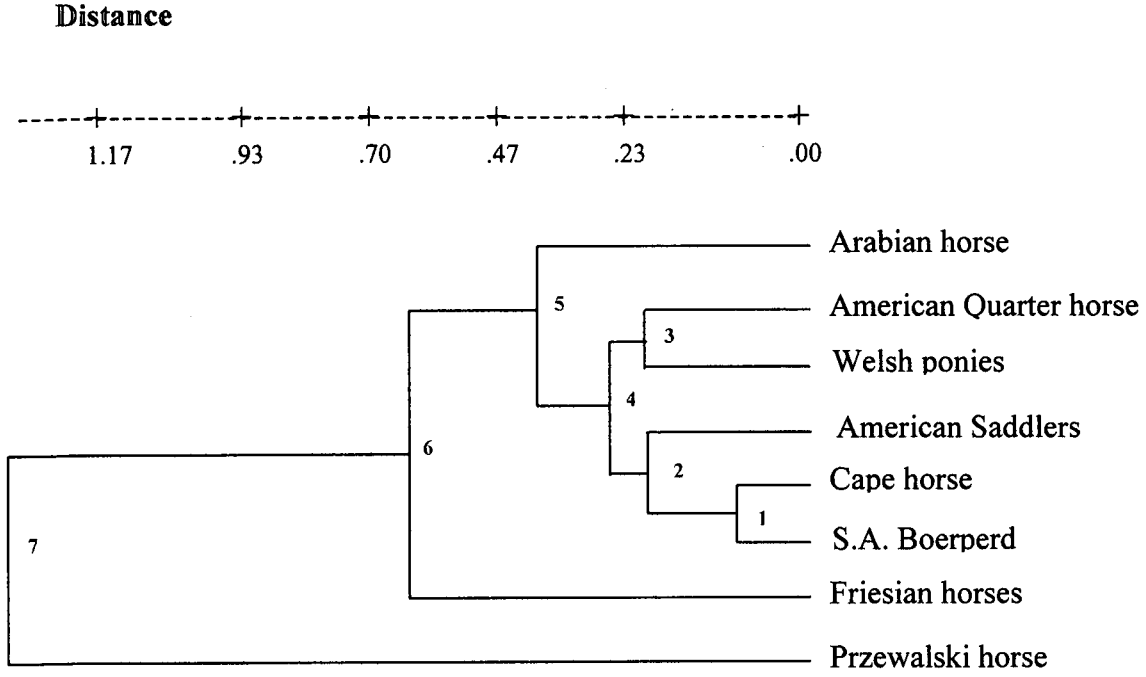


Fig 6.1 Dendrogram of Nei's (1972) genetic distance: method = unweighted pair group (UPGMA), modified from NEIGHBOR procedure of PHYLIP version 3.5.

The American Saddler has a close relationship with the South African horse breeds since most of these breeds evolved from the American Saddler and the Thoroughbred. The Quarter horse arrived in South Africa in1988 and thus has not contributed to the development of the South African horse breeds. Even though the Welsh pony and the Quarter horse are grouped together, the genetic distance between the two breeds is still commendable.

When a dendrogram for a group of species is constructed from genetic distance estimates, the reliability of the topology of the dendogram depends on the differences in genetic distance amongst different pairs of species. If these differences are small, the genetic distances must be estimated accurately, thus a considerable number of individuals should be examined for each locus. On the other hand, if the differences are large, even a single individual may be sufficient for obtaining the correct topology of a dendogram (Nei 1978).

A phylogenetic tree (dendogram) using the neighbour-joining (NJ), unweighted pair group-method with arithmetic mean (UPGMA) from matrices of D or DA distances was calculated by the DISPAN program (Figure 6.3). Bootstrap tests for these trees were performed.

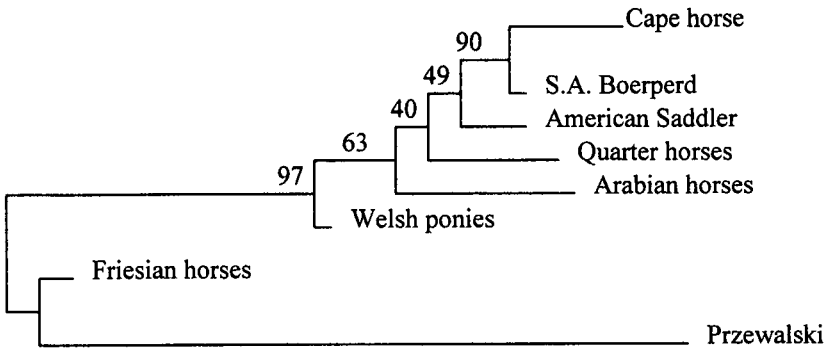
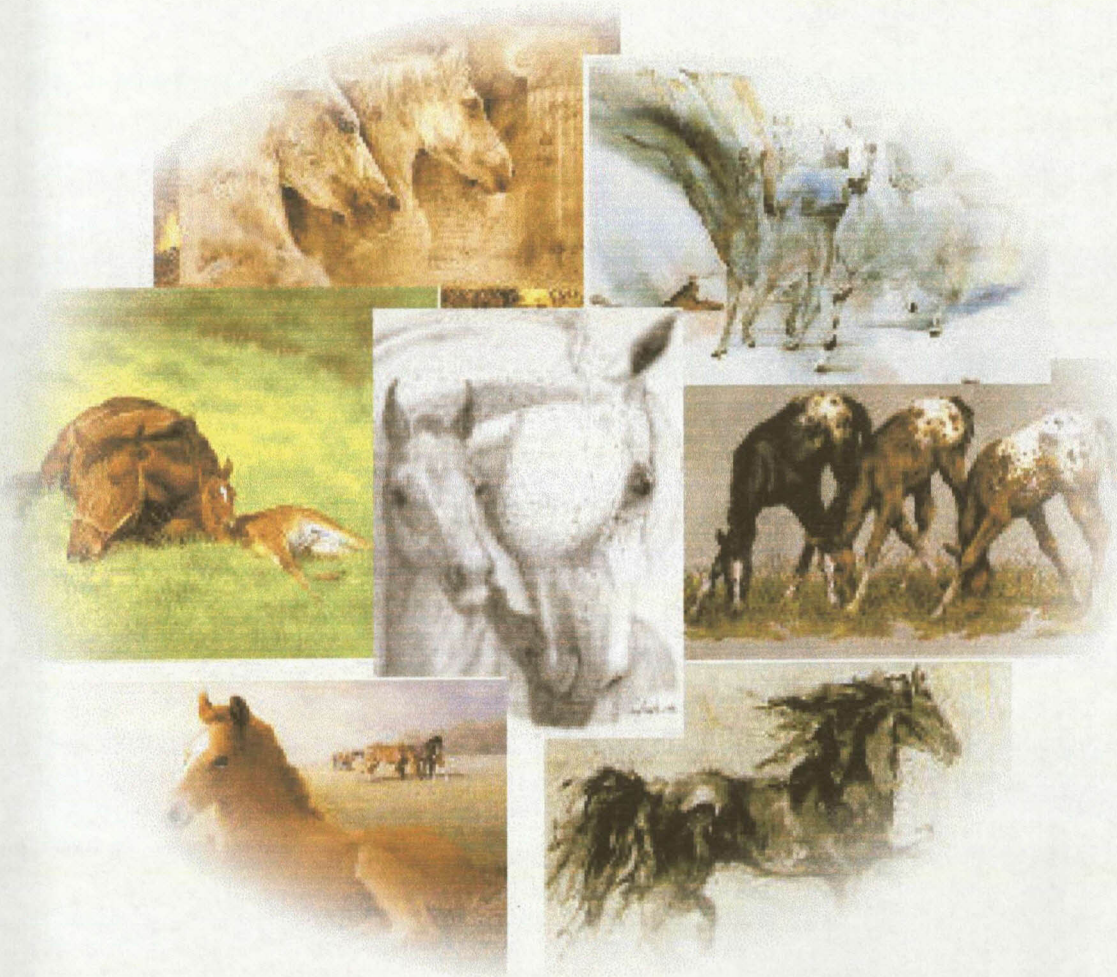


Fig. 6.3 Dendrogram of relationships among different horse breeds using 12 microsatellites. (Standard tree)

The dendrograms showed that the S.A. Boerperd and the Cape horse are very closely related. The Friesian is the least related of all the horse breeds, except for the Przewalski horse. These results correspond with those find by Dr. E. van Dyk and with the history of the different breeds. The Arabian, Quarter, Saddler, S.A. Boerperd, Cape horse and Welsh ponies are all riding horses, while the Friesian horse is a workhorse and the Przewalski horse is a wild horse. Both these horse breeds can be considered as an outgroup in all of the dendrograms.

There is still enough genetic diversity between the different horse breeds to distinguish between the breeds. Even though the genetic differentiation between the Cape horse and the S.A. Boerperd is small, it can still be considered as a different breed.

Chapter 7



CHAPTER 7

CONCLUSION

Genetic characterization and DNA typing for horses is a very important service to farmers. DNA profiles are used to accurately identify an animal and to confirm parentage. The parentage of a horse is very important since the value of the animal depends upon its pedigree.

DNA tests enable farmers to run a stud more accurately than before. The parentage can be verified and inbreeding can thus be avoided. Genetic characterization studies help a farmer to determine what the impact of a stallion is on his stud. He can thus make more informative decisions based on these results.

This thesis supports the program of FACT (Farm animal conservation trust) and contributes to the breed characterization of indigenous farm animals like the South African Boerperd and the Cape horse.

During the last ten years, the development of new genetic tools has brought about great advances in individual identification, and DNA markers such as microsatellites have proved to be useful in clarifying the structure of populations. They also detect population differentiation better than for example allozymes. The FOOD AND AGRICULTURAL ORGANIZATION OF THE UNITED NATIONS (1993) has proposed a global programme for the management of genetic resources using microsatellite methodology for breed characterization (Bjornstad *et al.* 2000).

The genetic diversity of domestic species such as cattle, sheep and goats has been examined by microsatellite analysis. More recently, horse breeds have also been analyzed using a panel of microsatellites recommended for routine parentage testing. Genetic characterization is the first step in breed conservation and may have implications for future breeding strategies (Bjornstad *et al.* 2000).

The population sample sizes range from 78 – 128, mainly because of economic constraints. Only one Przewalski horse could be obtained because these horses have to be darted, as they are wild. The Przewalski horse was only used as an outgroup.

The DNA profiles were all ISAG standardized. The aim of ISAG (International Society for Animal Genetics) is to encourage the study of genetically influenced characters of animal tissues and fluids and to facilitate the exchange of ideas and materials between research workers. The Society supports efficient exchange of research ideas, results and applications by organising conferences and workshops.

All except the S.A. Boerperd were in Hardy-Weinberg equilibrium. Genotypic frequencies deviated from Hardy-Weinberg proportions at the loci HTG10. The Hardy-Weinberg equilibrium is only valid in random mating populations. The microsatellites are not linked to a specific characteristic of a horse and are thus not influenced by selection. The populations can be considered as random mating populations, even though strong selection is being administered.

All the microsatellites were 100% polymorphic. The microsatellites were however selected for their polymorphisms. Arranz (1996) concluded that the high level of polymorphism in terms of the number of alleles per locus displayed by microsatellites makes it possible to obtain a better resolution in genetic analysis using a lower number of markers. The heterozygosity values calculated by BIOSYS-1 and POPGENE will, however, be higher than in the populations, since no monomorphic microsatellites were used. It is important to note that the values of the genetic distances obtained will be precise and will therefore give an accurate account of the relationships between the populations studied.

The highest average frequency value for an allele was found to be 60.5% for O at the locus HTG7 (ISAG standardized). It is of great importance for the alleles not to have a high frequency value, since it is used for parentage determination. If the frequency of an allele becomes too high, it cannot be used to distinguish between individuals.

The F_{ST} and the N_{em} values were calculated pairwise. The least differentiation was calculated between the S.A. Boerperd and the Welsh ponies with a value of 0.024. The most

distinct difference found was between the Friesian horse and the Cape horse. The high N_{em} values suggest that there is substantial gene flow between the populations.

The genetic distances were calculated by Nei (1972) and Nei's unbiased genetic distance (1978). The S.A. Boerperd and the Cape horse were found to be the most related breeds of horses with a value of 0.102. The history of these two horse breeds support these values. To get a more accurate answer whether the horses selected as Cape horses are indeed the Cape horse, one should include Basuto ponies, Nooitgedacht horses and the Kaapse Boerperd. History has shown that these horse breeds evolved from the Cape horse.

Genetic diversity provides the flexibility for horse populations to adapt to diverse environments, and the evolutionary potential of a population to survive in the face of environmental changes. Comparison of breeds within species reveals distinctive arrays and allele frequencies of microsatellite loci. Selective breeding practices, founder effects and random drift are processes which cause breeds to have distinctive profiles of genetic markers. Additional studies are needed to determine whether small herd management units demonstrate as much genetic variability as seen in these relatively large units. Data are also needed from feral horse groups in other countries to assess whether the conclusions of this theses are generally applicable to other populations of domesticated horses.

To give an accurate justification whether the Cape horse, as compiled by Prof. Roussouw and Mr du Plesssis is the real Cape horse, the following breeds will have to be entered into the study: the Nooitgedacht ponies, Basuto ponies and the Kaapse Boerperd. Dr. E. van Dyk already confirmed that these horse breeds are separate breeds.

Since these horse breeds are closely related, it is better to use the microsatellite markers than the bloodtyping markers. The values of genetic distances obtained from microsatellite data can be more precise when compared with genetic distance values obtained with protein markers. Microsatellite loci provide more accurate values than protein markers of the time when species or populations from a common ancestor are separated. Microsatellites might also be a better indicator than protein polymorphisms of evolutionary relationships within a particular species. The high level of polymorphism displayed by microsatellites makes it possible to obtain a better resolution in genetic analysis using a lower number of markers.

This thesis is a contribution to the FAO and their global programme as well as to all horse breeders in South Africa.

Chapter 8

CHAPTER 8

SUMMARY

The story of the horse begins nearly 60 million years ago. Herds of horses were initially a source of food for humans. Between 5 000 - 6 000 years ago on the Eurasian steppes, nomadic Aryan people began the process of domesticating the horse. Domestication resulted in different breeds, which served a variety of purposes. Horses are now mainly used for pleasure and sport of which there are many different varieties.

Genetic characterization is the first step in breed conservation and may have implication for future breeding strategies. The genetic diversity of domestic species such as cattle, sheep and goats has been examined by microsatellite analysis. More recently, horse breeds have also been analyzed using a panel of microsatellites recommended for routine parentage testing.

The aim of this study was to determine whether the horse breeds in South Africa is being kept pure and whether there is still enough genetic variation in the different horse breeds. It was also my aim to determine whether the Cape horse has genetically evolved far enough from the S.A. Boerperd to be classified as a horse breed that was bred in South Africa.

All the horse populations were in Hardy-Weinberg equilibrium except for the S.A. Boerperd. The Chi-square test showed that the S.A. Boerperd was not in Hardy-Weinberg equilibrium, but the G-test showed otherwise. Studies by Dr. E. van Dyk (Faculty of Veterinary Science at the University of Pretoria, South Africa) indicated that there has been more outbreeding of Boerperde than the other horse breeds and that they are the least closely related to one another than the other horse breeds.

The S.A Boerperd and the Welsh ponies display the greatest degree of heterozygosity (genetic variation between individuals). Which suggests that there has been more outbreeding of Boerperde and that the breed has not been a closed herd, as closed herds tend to become more homozygous over such a long period of time. These results support results given by Dr. E van Dyk done with bloodtyping.

The highest average frequency for an allele is for HTG7. Other alleles had an average of 0.114. It is of great importance, because these microsatellites are used for determining parentage. If the frequency of an allele becomes to high, it cannot be use to distinguished between two individuals.

Relatively small differentiation values were observed for the American Saddler and the S.A. Boerperd and between the Boerperd and the Cape horse. The most distinct difference was observed between the Friesian horse and the Cape horse.

The dendrograms showed that the S.A. Boerperd and the Cape horse are very closely related. The Friesian is the least related of all the horse breeds, except for the Przewalski horse. These results correspond with those find by E van Dyk and with the history of the different breeds.

This thesis is a contribution to the FAO and their global programme as well as to all horse breeders in South Africa.

***Keywords**: Equus, genetic characterization, microsatellites, electrophoresis, ISAG, BIOSYS-1, POPGENE, DISPAN*

Chapter 9

CHAPTER 9

OPSOMMING

Die verhaal van die perd begin ongeveer 60 miljoen jaar gelede. Perde was aanvanklik 'n bron van voedsel vir die mens. Tussen 5 000 – 6 000 jaar gelede in die Eurasiese Steppe het die nomadiese Ariese ras begin om perde te tem. Die tem van perde het gelei tot die ontwikkeling van verskillende perde rasse, wat 'n verskeidenheid van doele gedien het. Vandag word perde hoofsaaklik vir verskillende sportsoorte gebruik.

Genetiese karakterisering is die eerste stap vir die behoud van 'n spesie en kan toekomstige teling strategieë beinvloed. Die genetiese diversiteit van plaasdiere soos beeste, skape en bokke is alreeds geondersoek met mikrosatelliet analyses. Eers onlangs is perde ondersoek met mikrosatelliete wat ook gebruik word vir ouerskap bepaling.

Die doel van hierdie studie is om vas te stel of die perde rasse in Suid Afrika suiwer gehou word en of daar steeds genoeg genetiese variasie teenwoordig is. Dit is ook my doel om vas te stel of die Kaapse perde genetiese ver genoeg van die S.A. Boerperd is om as 'n ras geklassifiseer te word wat onwikkel is in Suid Afrika.

All die perde populasies was in Hardy-Weinberg ewewig, behalwe die S.A. Boerperd. Die Chi kwadraat toets het aangedui dat die S.A. Boerperd nie in Hardy-Weinberg ewewig is nie, maar die G-toets het die omgekeerde aangedui. Studies deur dr. E. van Dyk (Fakulteit van veearts snykunde, Universiteit van Pretoria) het aangedui dat die S.A. Boerperde die meeste uitgeteel is en dat hierdie perde die minste aanmekaar verwant is vergeleke met ander perde rasse.

Die S.A. Boerperd en die Walliese ponies het die meeste heterosigositeit getoon. Hierdie is 'n indikasie dat daar in die S.A. Boerperde baie uitkruising teenwoordig was en dat die ras nog nie vir lank geslote was van ander rasse nie. Geslote rasse word meer homosigoties na 'n tydperk. Hierdie resultate bevestig resultate wat gepubliseer is deur dr. E van Dyk wat merkers gebruik het wat vir bloedtipering gebruik word.

Die hoogste gemiddelde frekwensie vir 'n alleel was gevind vir HTG7. Die res van die allele het 'n gemiddelde frekwensie van 0.114 gehad. Dit is baie belangrik dat die frekwensies nie te hoog is nie, aangesien die mikrosatelliete gebruik word vir ouerskap bepaling. As die frekwensie van 'n alleel te hoog is, kan dit nie gebruik word om tussen individue te onderskei nie.

Relatiewe klein differensiasie waardes is waargeneem vir die Amerikaanse Saalperde en die S.A. Boerperde en tussen die S.A. Boerperde en die Kaapse perde. The grootste verskil is waargeneem tussen die Friesperde en die Kaapse perde.

Die dendrogramme toon aan dat die S.A. Boerperde en die Kaapse perde baie naby verwant is aanmekaar. Die Friesperde is die minste aan die ander rasse wat ondersoek is verwant, die Przewalski perd uitgesluit. Hierdie resultate stem ooreen met die bevindings van Dr. E. van Dyk en dit wat geskiedkundig aangeteken is.

Hierdie tesis lewer 'n bydrae tot die FAO en hulle wêreld wye program, sowel as alle Suid Afrikaanse perdetelers.

Sleutelwoorde: *Equus, genetiese karakterisering, mikrosatelliete, elektroforese, ISAG, BIOSYS-1, POPGENE, DISPAN*

Chapter 10

CHAPTER 10

LITERATURE CITED

ANONYMOUS, 1998a. *South African Livestock Breeding.* South African Stud Book And Livestock Improvement Association.

ANONYMOUS, 1999c. Analytical Methods Manual. No: DNA20 ARC AII. Animal Genetics Division. (See Higuchi, R et al. (1988) DNA typing from single hairs. Nature 332: 543-546.

ANONYMOUS, 1996c. StockMarks® for Horses Equine Paternity PCR Typing Kit. Part number 402828 Revision A. Perkin Elmer applied biosystems.

ANONYMOUS, 1998b ABI Prism®377 DNA Sequencer User's Manual. The Perkin-Elmer Corporation Perkin Elmer applied biosystems.

ANONYMOUS, 1997a. GeneScan®Reference Guide. The Perkin-Elmer Corporation. The Perkin-Elmer Corporation Perkin Elmer applied biosystems.

ANONYMOUS, 1996d. ABI Prism™ Genotyper® 2.0 User's Manual. The Perkin-Elmer Corporation Perkin Elmer applied biosystems.

ARRANZ J.J., BAYóN Y., and SAN PRIMITIVO F. 1996. Comparison of protein markers and microsatellites in differentiation of cattle populations. *Animal Genetics* 27: 415-419.

AYALA, F.J. 1982. Population and evolutionary genetics: A Primer. Benjamin/Cummings Publ.co.

BJORNSTAD, G. GUNBY, E. and ROED, K.H. 2000. Genetic structure of Norwegian horse breeds. *Journal of animal breeding and genetics* 117 (5): 307-315

BLACK, W.C. 1997. Users manual for Biosys-2. Department of Microbiology Colorado State University.

BOWLING, A.T & CLARK, R.S. 1985. Blood group and protein polymorphism gene frequencies for seven breeds of horses in the United States. *Animal Blood Groups Biochemical Genetics* 16: 93-108.

BOWLING, A.T., EGGLESTON-SCOTT, M.L., BYRNS, G., CLARK, R.S., DILEANIS, S. & WICTUM, E. 1997. Validation of microsatellite markers for routine horse parentage testing. *Animal Genetics* 28: 247-252.

BURMAN, J. 1993. *To horse and away.* Human & Rousseau, Cape Town.

BöKöNYI, S. 1974. *The Przevalsky horse.* Souvenir press, Ontario, Canada.

CHILD, D. 1967. *Saga of the South African Horse.* Howard Timmons, Cape Town.

EDWARDS, E.H. 1994. *The encyclopedia of the horse.* Dorling Kindersly Limited, London.

FALCONER, D.S. & MACKAY 1996. Introduction to Quantitative Genetics.

HARTL, D.L. & CLARK, A.G. 1987. Principles of population genetics, 2nd edition. Sinaner Associats, Massachusetts.

HOFMEYR, I. 1999. Unravelling the bloodknot – SA's horse breeds. Farmer's Weekly.

KIRBY, G.C. 1973 Heterozygote Frequencies in small subpopulations. *Theoretical population Biology 8* Academic Press Inc.

MEADE, R., FOSTER, C., MCBANE, S., HASTIE, S., RUSSELL, V., HOLDERNESS, J., DAWES, A., KIDD, J., GORDON, M,. BLAZER, D., BEAUMONT, J., RAMSEY, M., MACDONALD, J., COOMBS, T., GEDDES, C. & ROBBINS, J. 1984. *The world of horses and ponies.* Hennerwood Publications Limited, London.

NEI, M. 1972. Genetic distance between populations. *The American Naturealist 106*: 283-292.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics 89*: 583-590

PASSARGE, E. 1995. *Color Atlas of Genetics.* Thieme Medical Publishers, Inc. New York.

RAVEN, P.H. and JOHNSON, G.B. 1991. Biology. Third editon. Mosby year book.

SCOTT, A.M. 1974. Horse blood typing comparison test. *Animal Blood Groups Biochemical Genetics* 5: 195-196.

SOKAL, R.R & ROHLF, F.J. 1969. *Biometry.* Freeman, San Francisco.

STRICKBERGER, M.W. 1990. *Genetics Third Edition.* Macmillan Publishing Company. New York.

SWOFFORD, D.L. SELANDER, R.B. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis for electrophoretic data in population genetics and systematics. J. Jered 72:281-283.

TAMARIN, R.H. 1993. *Principles of Genetics, fourth edition.* Boston University. Wm.C. Brown Publishers.

TATSUYA, O. 1993. DISPAN : Genetics distance and phylogenetic analysis. Pennsylvania State University.

VAN DER MERWE, F.J. 1981. *Ken ons perde rasse* Human & Rousseau, Cape Town.

YEH, F.C., BOYLE, T. RONGCAI, Y., YE, Z. and XIYAN, J.M. 1999 .University of Alberta, Centre for International Forestry Research. *Popgene version 1.31 Microsoft Window-based Freeware for Population Genetic Analysis. Quick User Guide.* Edmonton, ABCanada.

FOOTNOTES.

[1] Anonymous 1996a. Quarter horse. American Quarter horse Association. Oklahoma State university . http://www.ansi.okstate.edu/breeds/horses

[2] Anonymous 1999a. The history of the American Saddlebred. American-Saddlerbrem.com. http://www.american-saddlebred.com/asbhist.htm

[3] Gary, T. 2001. Recognizing the American Saddlebred. http://www.azgardfarm.com/recogASB.htm

[4] Anonymous 1999b. Boer. Oklahoma State Univeristy. http://www.ansi.okstate.edu/breeds/horses

[5] Anonymous 1996b. Welsh pony and Cob. Oklahoma State University. http://www.ansi.okstate.edu/breeds/horses

[6] Anonymous 2000a. Friesian. Manfred Link. Germany. http://www.ansi.okstate.edu/breeds/horses/FRIESIAN/

[7] Sweetman, T. 1998. Przewalski. Oklahoma State University. www.ansi.okstate.edu/breeds/horses/PRZEW/

[8] Frank, T.S. 2001. Topics in Molecular Oncology: Microsatellite Instability. University of Michigan. http://zapruder.pds.med.umich.edu/users/Frank/MIN.html

[9] Anonymous 1999c. DNA typing techniques – a comparison and contrast. ImmGen, Incorporated. http://www.immgen.com/html/techniques.html

[10] Straughan, D. 2001. Microsatellite DNA. http://biology.uoregon.edu/biology/Spring94/Dyan1/Dyan.html

[11] Bowling, A.T. 1999. DNA test for parentage verificaton in horses. University of California, Davis. http://www.vgl.ucdavis.edu/horse/partest.htm

[12] Anonymous 2001. Microsatellites, Joslin diabetes center. http://134.174.243.149/core/microsats.html

[13] Anonymous 1999. Animal studies turn increasingly to DNA for parentage, biodiversity and gentic disease testing. Perkin Elmer. http://www2.perkin-elmer.com/ab/pebio/ie3/jump/ab_frame.html

[14] Anonymous 1996e. M values for STRs reported by VGL. U.C. Davis. California. http://www.vgl.ucdavis.edu/horse/mvalues.html

CHAPTER 11

APPENDICES

Appendix A.1: DNA profiles of 80 Arabian Horses (BIOSYS-1 and POPGENE nomenclature)

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
1	B	D	C	C	C	C	J	J	B	C	I	I	C	D	A	I	E	E	H	H	D	H	B	F
2	B	D	C	C	C	C	J	J	B	C	C	I	C	D	I	I	E	E	H	H	H	H	B	F
3	D	H	C	C	C	C	F	J	B	C	C	I	C	C	A	I	D	E	H	H	D	H	B	F
4	B	H	B	C	C	C	J	J	B	F	G	I	C	C	A	A	E	E	H	H	D	G	B	F
5	C	C	A	C	A	B	D	J	E	F	I	I	C	G	A	I	E	K	F	F	C	C	B	F
6	C	H	C	F	A	C	F	J	E	F	I	I	C	D	A	I	F	I	E	E	I	J	B	F
7	C	H	B	C	A	A	A	J	B	F	I	I	C	G	D	I	F	I	F	F	D	D	F	F
8	C	H	B	C	A	A	F	F	C	E	C	I	D	G	I	I	D	F	F	F	G	J	B	F
9	C	H	A	B	C	C	J	J	F	F	G	I	C	G	A	I	E	E	F	H	D	G	B	F
10	C	F	C	E	A	B	D	J	E	F	C	I	F	G	A	I	E	F	A	E	C	C	B	E
11	C	H	C	C	B	C	F	J	E	F	I	I	C	G	A	D	F	I	F	F	D	D	B	F
12	C	H	C	E	B	C	J	J	F	F	I	I	C	F	A	I	E	E	A	E	G	G	B	F
13	C	H	C	C	A	A	E	J	F	F	G	I	C	G	A	I	I	K	H	I	D	D	B	F
14	B	H	C	F	A	B	J	J	F	F	G	I	C	F	A	I	E	I	E	E	D	G	F	F
15	B	H	C	C	C	C	A	B	E	E	E	I	C	D	I	I	F	K	F	F	D	G	F	F
16	C	H	B	C	C	C	F	J	E	E	E	G	C	G	A	I	F	F	F	H	G	G	F	F
17	C	D	B	B	A	C	J	J	F	G	I	I	C	F	D	G	E	F	E	F	C	D	B	B
18	C	C	B	E	A	C	D	J	E	F	E	I	G	G	A	I	F	K	F	H	D	G	B	F
19	H	H	B	C	C	C	F	J	E	F	G	G	C	C	A	A	F	I	F	H	D	G	F	F
20	C	C	A	C	D	D	F	J	B	F	I	I	C	F	A	I	K	K	F	H	G	G	F	F
21	C	H	C	E	A	B	A	F	F	G	E	I	C	F	A	A	F	K	E	H	D	G	F	F
22	C	C	A	C	A	A	J	J	F	F	I	I	C	G	A	A	E	E	H	H	D	D	F	F
23	A	C	B	F	A	A	B	J	B	F	G	I	D	D	A	I	E	I	E	F	D	G	B	B
24	C	H	A	C	B	C	D	D	F	F	I	I	C	F	A	A	E	I	F	F	C	G	B	F
25	A	C	C	E	C	C	A	F	E	F	I	I	C	F	A	I	F	K	F	F	D	D	B	F
26	C	H	A	C	A	A	J	J	B	F	I	I	F	G	A	I	D	E	F	F	D	D	F	F
27	B	C	C	C	A	C	A	D	E	F	E	I	D	G	I	I	D	I	F	F	G	G	F	F
28	C	H	C	C	A	A	A	D	E	F	E	I	D	G	I	I	D	D	E	F	D	G	F	F
29	F	H	C	E	B	C	F	J	B	E	I	I	C	C	A	I	F	I	E	E	C	G	B	F
30	D	H	B	C	B	C	J	J	E	F	G	I	C	C	D	I	E	K	F	H	D	G	B	F
31	C	H	B	F	B	C	D	D	B	B	E	E	B	G	A	I	F	F	A	A	G	G	F	F
32	B	H	C	F	C	C	D	J	B	F	E	G	B	C	A	A	F	F	A	H	G	G	F	F
33	H	H	A	C	A	D	D	J	F	F	G	I	F	F	I	I	E	I	F	F	C	G	F	F
34	H	H	B	C	C	C	A	A	F	G	I	I	C	C	I	I	F	I	H	H	G	G	F	F
35	H	H	C	C	A	C	A	J	B	G	I	I	C	C	I	I	E	F	F	H	D	G	F	F
36	C	C	B	C	C	C	F	J	F	G	F	F	D	G	A	A	F	K	F	F	G	G	B	B
37	C	C	A	C	B	C	F	J	F	G	F	I	D	D	A	A	E	K	A	F	G	G	B	B

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
38	C	C	B	B	C	D	A	H	E	F	E	I	C	D	A	I	F	F	F	F	D	G	B	F
39	C	C	B	B	B	C	F	H	F	F	E	G	D	F	A	A	E	F	F	F	D	G	B	B
40	C	C	B	E	A	C	J	J	B	F	E	I	G	G	A	I	F	I	F	F	D	D	B	F
41	C	D	A	B	C	C	J	J	E	F	F	I	C	F	I	I	F	K	E	H	C	D	F	F
42	C	C	A	B	B	C	A	A	F	G	F	I	C	C	A	I	E	E	F	H	D	G	F	F
43	A	H	B	C	C	C	A	A	F	G	G	I	C	C	I	I	I	K	H	H	G	G	F	F
44	C	C	A	C	A	B	A	J	F	G	I	I	B	C	I	I	F	K	F	F	G	G	F	F
45	C	H	B	E	B	B	A	D	E	F	E	I	B	G	I	I	F	K	A	A	G	G	F	F
46	C	D	C	E	A	B	F	F	F	F	I	I	B	F	A	A	E	I	F	F	C	G	F	F
47	C	H	B	C	A	A	J	J	F	F	I	I	C	D	A	I	I	I	H	H	G	G	B	F
48	C	D	B	C	A	D	J	J	E	F	I	I	C	C	I	I	E	K	H	I	C	J	F	F
49	C	D	B	B	A	A	J	J	F	F	I	I	D	D	A	I	E	E	E	E	C	G	F	F
50	D	D	B	B	B	C	D	F	E	F	E	E	C	D	D	I	I	I	E	F	C	G	F	F
51	A	C	B	C	A	C	D	D	E	F	I	I	C	D	D	I	E	K	E	F	C	G	B	B
52	C	C	B	B	C	C	A	J	F	F	E	I	C	C	A	I	I	I	F	H	C	G	B	F
53	C	D	B	B	B	C	J	J	E	F	I	I	C	D	A	K	F	K	A	F	C	G	B	F
54	A	C	B	C	A	A	F	F	E	F	G	I	G	G	A	I	E	K	E	F	G	G	B	B
55	C	C	C	E	A	A	A	D	B	F	I	I	C	F	A	I	F	I	A	F	G	G	F	F
56	C	C	C	C	A	C	A	F	B	E	G	I	C	F	I	I	E	F	A	E	G	G	F	F
57	C	F	B	C	A	D	J	J	F	F	I	I	C	C	A	I	E	K	E	I	C	G	F	F
58	B	C	B	C	A	A	J	J	E	F	I	I	D	D	A	A	E	E	E	F	G	G	B	F
59	B	C	B	D	A	A	B	D	F	F	I	I	F	G	A	D	E	K	E	E	G	G	E	F
60	D	H	B	C	A	C	J	J	C	F	G	I	C	D	A	K	F	I	E	H	C	G	F	F
61	C	H	B	E	B	C	D	J	E	F	E	I	C	G	A	I	F	K	F	H	D	G	F	F
62	C	H	C	C	A	C	F	J	F	F	I	I	C	C	A	A	E	F	F	H	D	G	F	F
63	C	C	B	C	A	B	A	F	F	F	E	G	C	C	A	A	E	F	A	A	G	G	F	F
64	C	C	B	B	A	A	A	F	F	F	E	E	B	C	A	A	F	F	A	H	G	G	F	F
65	C	H	B	C	A	A	F	I	E	F	C	I	C	D	A	A	E	G	E	E	D	G	F	F
66	C	H	C	C	C	C	J	J	F	F	C	I	C	C	A	A	E	F	E	H	D	J	B	F
67	C	F	C	C	A	B	J	J	E	F	I	I	C	C	A	I	E	E	E	E	G	G	B	F
68	A	H	A	B	A	A	F	J	F	F	I	I	C	C	A	I	E	F	F	F	C	D	F	F
69	D	H	B	E	A	A	J	J	C	G	I	I	C	C	A	D	E	I	F	H	C	D	B	F
70	C	H	B	C	C	C	J	J	E	F	E	I	F	G	A	I	I	K	F	F	G	G	F	F
71	C	H	A	B	C	D	A	F	F	G	I	I	C	C	I	I	I	K	F	H	G	G	F	F
72	C	F	A	C	A	B	D	J	F	F	I	I	C	F	A	I	F	I	F	F	D	G	B	B
73	F	H	B	C	A	D	J	J	E	F	F	I	B	C	A	I	F	F	F	F	D	G	F	F
74	C	F	C	C	A	C	F	J	E	E	I	I	C	F	I	I	E	K	F	F	D	D	B	B
75	D	H	B	C	A	D	J	J	F	F	I	I	C	C	A	I	E	E	H	H	G	J	F	F
76	D	D	B	C	C	C	J	J	E	G	F	I	C	D	A	I	I	K	F	H	C	G	B	F
77	C	H	B	C	A	A	A	J	C	C	E	I	C	C	I	I	D	I	H	H	C	D	F	F
78	D	H	C	C	C	C	J	J	E	E	I	I	C	D	A	D	E	F	E	H	G	G	B	F
79	D	H	B	B	B	B	F	F	B	F	H	I	B	F	A	I	I	K	F	F	G	G	B	F
80	A	H	C	C	A	C	J	J	E	F	I	I	C	C	D	I	F	I	E	E	G	G	F	F

Appendix A.2: DNA profiles of 80 Quarter Horses

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
81	C	D	A	D	B	C	F	F	E	F	F	H	G	G	I	I	D	E	F	J	B	H	B	F
82	A	A	C	C	A	A	D	H	B	F	E	F	D	G	A	I	A	E	J	J	B	C	B	F
83	A	C	C	C	A	A	D	E	B	F	F	G	D	G	A	I	D	F	G	J	B	B	B	D
84	A	A	A	C	A	B	A	F	B	G	F	H	G	G	I	I	D	K	F	J	B	H	B	F
85	A	A	C	C	A	A	A	F	C	G	C	F	G	G	I	I	D	D	G	J	B	H	F	F
86	A	H	C	C	C	C	E	G	B	B	F	G	F	G	I	L	D	E	F	H	D	J	D	F
87	A	H	A	D	C	C	D	H	F	F	F	G	G	G	I	J	B	D	F	H	A	D	B	F
88	A	H	A	A	C	C	A	I	G	G	E	F	G	G	D	L	A	D	A	H	D	J	B	B
89	C	H	A	E	C	C	A	I	F	G	F	G	G	G	I	L	D	D	A	H	A	D	B	F
90	A	C	A	C	C	C	H	I	B	B	A	G	D	G	I	L	D	D	A	H	D	J	B	F
91	D	H	C	D	A	B	D	J	B	F	E	F	G	G	A	A	D	F	F	F	D	J	F	F
92	D	D	C	D	A	B	A	D	E	F	F	F	G	G	A	I	D	D	F	F	E	J	E	F
93	D	H	D	D	A	C	A	F	B	G	F	G	C	F	C	J	E	H	E	H	E	J	E	F
94	D	D	D	H	A	B	A	A	E	G	F	G	G	G	A	I	D	D	A	F	E	J	E	F
95	A	H	A	C	C	C	A	E	F	G	G	G	D	G	I	I	A	D	H	H	A	J	B	F
96	H	H	E	F	B	C	A	D	D	F	C	G	F	F	D	I	E	E	F	I	J	J	B	E
97	A	H	A	A	C	C	I	I	C	F	C	I	G	G	A	D	D	H	A	J	G	G	F	F
98	A	A	C	D	C	C	D	I	B	G	G	G	D	F	J	L	D	D	H	H	D	J	B	F
99	A	C	A	F	A	A	A	E	B	B	D	F	G	G	A	I	D	E	E	F	A	D	F	F
100	H	H	A	C	A	B	D	D	F	F	D	J	G	G	I	I	D	D	F	H	J	J	F	F
101	C	H	D	F	A	A	A	D	B	C	D	F	G	G	A	I	D	D	F	F	D	J	F	F
102	A	D	C	D	B	C	A	D	F	G	G	G	F	G	D	I	D	H	A	F	J	J	E	F
103	D	D	C	C	B	C	D	D	F	G	E	G	G	G	A	I	D	H	A	F	E	E	E	F
104	A	A	A	C	A	F	D	H	B	F	E	F	D	G	A	A	B	E	I	J	E	E	D	F
105	A	H	C	G	A	B	A	D	F	G	E	F	G	G	A	I	E	K	A	A	E	E	D	F
106	A	H	C	C	B	F	D	D	B	F	E	G	D	F	A	I	B	D	F	J	D	D	D	F
107	C	C	C	D	A	A	A	D	C	C	F	H	D	G	I	I	D	K	F	G	D	H	F	F
108	A	A	A	A	A	C	I	J	F	F	I	I	G	G	A	D	D	D	F	H	D	G	F	F
109	D	H	C	F	A	F	D	D	B	B	B	B	F	G	I	I	A	E	F	F		J	B	F
110	A	H	C	E	A	A	A	D	D	F	C	E	D	G	A	I	D	D	E	I		J	B	F
111	A	H	A	C	A	B	F	I	F	G	B	G	G	G	I	I	D	D	G	J	D	J	B	F
112	A	H	A	C	C	F	D	D	B	F	G	G	G	G	A	I	B	D	E	J	D	J	D	F
113	A	H	A	C	A	F	D	F	F	G	C	G	G	G	A	I	B	K	A	J	D	G	D	F
114	A	H	A	C	A	C	D	D	F	F	G	G	D	G	A	A	B	D	E	E	D	J	D	F
115	A	H	C	H	A	C	E	I	B	D	E	F	F	G	A	I	D	K	F	I	D	J	B	F
116	D	H	C	E	B	B	A	F	F	F	F	G	D	F	D	I	E	E	F	F	J	J	E	F
117	A	D	C	C	A	C	A	I	C	G	C	G	B	F	A	I	D	D	F	J	D	D	B	F
118	A	H	A	C	C	F	G	I	B	C	C	C	B	G	A	A	D	D	H	J	D	D	B	F
119	D	D	C	H	B	C	A	E	C	F	G	G	F	G	A	I	D	E	F	F	E	S	F	F
120	A	D	C	D	A	B	F	H	F	F	E	F	G	G	I	I	D	E	F	J	H	F	F	F
121	C	H	C	C	A	C	D	E	C	F	G	I	F	G	I	J	D	D	H	H	A	J	F	F

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
122	C	C	B	B	C	C	E	F	B	F	F	G	C	C	I	I	D	D	E	F	J	J	F	F
123	H	H	A	C	B	C	D	I	C	F	C	I	G	G	A	I	D	H	F	J	G	J	F	F
124	H	H	C	D	A	B	D	E	C	F	D	F	G	G	A	I	D	D	F	F		J	F	F
125	A	A	A	D	A	F	E	I	C	F	C	C	D	G	A	L	D	F	A	S	D	D	F	F
126	A	G	A	C	C	C	D	D	B	F	C	C	E	F	I	L	E	H	G	H	A	J	F	F
127	H	H	A	C	A	C	D	I	C	G	C	I	B	G	A	I	D	H	F	H	A	A	F	F
128	C	H	C	D	C	C	D	F	C	F	F	G	D	G	I	I	B	D	F	F	A	J	F	F
129	A	H	C	C	A	C	D	I	B	G	G	I	B	D	I	J	D	D	A	H	A	A	F	F
130	A	C	A	C	A	C	A	A	G	G	F	I	D	G	D	D	E	E	F	F	A	A	B	E
131	D	D	C	D	A	B	D	E	F	G	C	G	G	G	I	I	D	F	F	F	J	J	F	F
132	H	H	A	C	A	C	D	I	C	G	C	I	B	G	A	I	D	H	F	H	A	A	F	F
133	C	H	C	D	C	C	D	F	C	F	F	G	D	G	I	I	B	D	F	F	A	J	F	F
134	A	H	A	E	B	F	A	D	D	F	C	G	F	G	D	L	D	E	F	J	A	J	B	B
135	A	A	A	A	C	C	I	I	F	F	C	C	G	G	D	L	D	D	J	J	A	G	F	F
136	A	H	D	F	A	B	A	E	B	C	F	F	G	G	A	A	D	E	E	F	A	J	F	F
137	H	H	C	D	A	B	D	E	C	F	D	F	G	G	A	I	D	D	F	F	J	J	F	F
138	C	H	C	F	A	B	A	E	B	F	D	F	G	G	A	A	D	E	E	F	D	J	F	F
139	A	D	C	D	B	C	D	D	F	F	G	G	F	G	K	I	D	H	A	F	J	J	F	F
140	D	H	C	C	B	C	A	D	F	G	G	G	F	G	I	I	D	H	F	F	J	J	F	F
141	D	H	C	C	A	B	D	D	B	F	C	G	E	F	A	I	A	D	F	F	D	J	F	F
142	A	G	A	C	C	C	D	D	B	F	C	C	E	F	I	L	E	H	G	H	A	J	F	F
143	C	H	A	C	B	F	D	I	F	F	C	C	G	G	I	L	A	H	F	J	D	D	F	F
144	C	H	A	C	B	F	D	D	B	F	C	G	G	G	I	L	A	H	F	J	D	D	F	F
145	A	D	A	A	C	E	A	A	C	G	D	E	D	G	A	A	E	E	F	F	K	K	F	F
146	D	H	A	C	C	C	A	E	B	C	G	G	D	F	A	I	A	D	F	F	K	K	B	F
147	D	D	D	D	A	A	A	D	B	G	E	F	G	G	A	I	D	F	A	F	E	J	F	F
148	D	H	B	C	A	A	D	J	B	C	E	E	G	G	I	I	D	D	E	F	K	J	F	F
149	A	A	C	E	A	E	A	E	B	F	E	F	G	G	A	I	B	D	E	F	E	J	B	F
150	D	H	A	D	B	C	D	F	F	G	D	G	B	G	I	I	D	D	F	G	H	J	F	F
151	D	H	A	H	A	B	F	F	G	G	C	F	G	G	I	I	K	K	A	A	E	G	F	F
152	C	C	A	C	C	C	D	G	C	F	C	G	D	G	A	I	D	D	A	A	J	J	F	F
153	C	H	C	D	A	A	E	J	C	D	C	F	F	G	A	I	D	K	E	G	D	J	B	F
154	C	H	A	D	C	F	E	H	B	F	F	G	D	F	I	N	D	D	H	J	D	D	D	F
155	A	C	C	D	C	C	D	F	F	F	C	F	D	F	D	I	D	D	H	H	J	J	F	F
156	A	C	A	C	C	F	D	I	B	F	G	G	D	G	D	N	D	D	H	J	I	J	B	F
157	A	D	A	C	C	F	D	E	F	F	C	C	F	G	I	L	D	H	I	J	A	A	B	F
158	D	H	C	D	C	C	D	E	B	F	C	G	D	F	I	I	D	E	A	I	J	J	B	F
159	D	H	A	F	C	E	A	E	C	F	E	F	D	G	I	N	D	E	F	F	J	J	B	F
160	A	H	A	A	A	C	D	G	F	G	C	F	G	G	A	L	D	J	A	J	C	G	B	F

Appendix A.3: DNA profiles of 80 American Saddler horses

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
161	C	H	A	F	C	C	D	E	B	B	C	I	B	E	A	I	D	E	F	J	E	F	F	F
162	C	C	A	C	C	C	E	E	C	E	C	F	D	G	D	I	C	D	E	F	H	J	F	F
163	C	H	C	F	C	E	D	I	C	F	E	E	D	F	A	D	E	E	E	J	G	J	B	F
164	A	C	A	F	C	D	A	D	B	C	C	J	F	G	A	A	K	K	E	J	E	H	F	F
165	H	H	A	B	B	F	A	A	A	B	C	F	B	B	A	D	E	K	H	H	D	G	B	D
166	C	H	A	E	C	E	D	E	C	E	G	I	D	E	D	I	A	E	F	J	G	G	F	F
167	C	D	C	C	C	F	E	E	C	E	E	F	F	G	D	D	E	E	H	J	G	G	F	F
168	C	H	B	C	C	E	A	J	E	F	A	C	E	F	A	D	C	K	E	H	G	G	F	F
169	H	H	A	C	C	E	A	B	B	D	E	E	G	G	I	I	D	I	H	H	G	H	B	F
170	C	H	A	B	C	C	D	E	D	D	F	I	E	F	A	D	B	E	H	J	D	J	B	F
171	C	I	C	E	E	E	D	D	B	B	F	J	D	E	A	I	E	E	E	H	C	F	F	F
172	C	D	A	C	A	C	E	I	C	E	E	F	E	F	A	D	E	E	F	F	F	H	F	F
173	C	H	C	C	C	E	E	E	B	C	I	I	E	F	D	I	E	E	H	J	E	G	B	F
174	C	H	C	F	B	F	A	G	C	E	C	F	E	G	I	I	A	K	H	J	D	J	F	F
175	A	H	C	C	D	E	E	E	C	E	E	I	E	E	D	D	E	E	H	H	G	H	F	F
176	C	H	A	C	C	E	D	J	D	F	A	G	D	E	A	D	D	K	E	H	G	G	F	F
177	H	I	A	D	C	F	E	G	B	F	E	G	F	G	I	I	E	I	A	H	G	G	E	F
178	H	H	A	C	C	D	E	I	C	F	F	I	D	D	A	D	E	E	F	H	G	G	F	F
179	H	H	A	C	B	D	D	F	D	E	I	J	E	G	D	G	E	E	F	H	J	J	F	F
180	A	I	A	C	A	D	D	E	A	E	F	G	B	G	D	D	D	E	F	H	G	H	F	F
181	C	C	A	C	C	C	B	F	E	G	E	E	D	G	D	I	D	D	F	H	F	F	F	F
182	H	I	A	C	C	E	B	E	D	E	C	E	D	C	A	I	C	D	E	H	G	G	F	F
183	A	G	C	F	E	F	E	I	B	D	C	F	B	F	I	I	E	E	F	H	G	J	F	F
184	H	H	A	C	C	C	E	E	D	G	E	F	D	G	A	D	E	E	H	J	D	F	F	F
185	C	H	C	C	B	E	A	E	D	F	C	G	D	G	D	I	A	D	E	H	G	G	F	F
186	C	H	F	F	C	D	E	F	C	F	C	D	D	E	D	I	B	F	H	H	G	G	B	F
187	A	C	C	F	C	C	D	D	C	E	C	C	B	B	A	D	E	K	J	J	G	H	F	F
188	A	I	A	E	C	D	A	D	B	E	F	I	F	G	A	A	D	D	G	J	F	F	D	F
189	C	I	A	A	A	C	D	F	B	B	I	J	E	G	D	I	E	E	G	H	D	F	F	F
190	A	H	F	F	C	C	A	I	B	C	C	E	B	B	D	I	E	K	H	J	G	I	F	F
191	C	D	A	A	C	C	C	E	B	C	E	G	F	G	I	I	E	I	F	H	G	G	F	F
192	C	H	C	C	C	C	D	I	B	E	E	I	G	G	D	L	A	C	H	J	G	G	F	F
193	C	C	B	C	C	C	A	G	B	B	C	E	G	G	A	A	D	E	H	H	I	I	B	F
194	C	H	C	C	C	C	D	E	F	F	C	G	E	F	A	D	D	E	F	F	D	E	F	F
195	C	D	C	C	C	E	B	I	E	G	E	I	D	F	I	I	D	E	F	F	D	G	F	F
196	B	C	C	C	C	E	A	E	E	E	A	F	B	E	D	D	B	E	H	H	G	G	F	F
197	A	H	A	A	C	D	A	F	C	G	C	J	D	F	A	A	A	K	J	J	D	E	F	F
198	H	H	C	C	E	E	D	E	F	G	C	I	B	G	A	A	C	D	F	F	F	J	F	F
199	A	H	A	C	C	C	A	E	B	E	A	E	D	F	D	D	A	I	E	H	H	H	F	F
200	C	I	A	C	D	E	E	I	C	F	C	F	E	F	A	D	E	E	G	H	E	G	B	F
201	C	C	A	C	C	E	D	D	C	G	C	F	G	G	A	I	D	E	F	F	F	G	B	F

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
202	C	C	A	C	C	C	D	E	B	C	J	J	G	G	A	D	D	E	F	H	D	F	F	F
203	H	H	A	C	B	B	A	D	E	F	C	F	B	F	A	D	D	K	H	H	D	J	F	F
204	C	C	C	E	C	F	D	E	B	C	F	F	D	F	A	D	E	E	F	J	E	G	F	F
205	B	C	A	H	C	D	D	E	B	D	F	I	E	G	D	I	E	E	F	H	E	G	F	F
206	A	D	C	C	C	E	E	E	C	F	E	E	B	D	A	D	D	E	F	H	G	G	F	F
207	H	H	C	C	C	F	A	I	B	E	E	E	E	F	D	I	E	E	F	J	G	H	F	F
208	A	C	A	D	C	C	D	E	C	F	G	G	B	G	D	D	C	D	H	J	G	G	F	F
209	A	H	C	C	B	C	D	F	D	E	F	I	B	G	D	D	E	E	E	H	G	G	B	F
210	H	H	C	F	C	E	D	I	B	F	C	F	D	F	A	D	B	I	H	H	G	G	F	F
211	C	C	C	E	C	F	E	E	B	E	E	I	F	G	D	D	E	E	H	H	G	H	F	F
212	E	H	C	D	C	C	E	E	F	G	E	F	D	G	A	I	E	E	H	H	G	H	F	F
213	A	H	A	A	B	D	A	D	B	E	A	F	D	G	D	D	D	K	E	H	E	H	F	F
214	C	E	C	C	C	C	A	E	F	F	G	I	B	B	D	I	D	E	F	G	G	J	B	F
215	H	H	A	C	E	F	E	E	B	D	F	J	E	F	A	I	D	E	E	J	G	G	F	F
216	C	I	A	E	C	D	A	D	E	F	E	I	D	E	D	I	D	D	F	H	D	G	F	F
217	C	H	C	D	B	C	E	E	F	F	I	I	D	G	D	D	A	K	H	H	G	G	F	F
218	A	C	C	C	B	C	A	E	D	D	F	I	D	D	A	D	D	E	H	H	E	G	F	F
219	H	H	A	C	C	C	A	E	C	E	A	I	B	F	D	K	E	K	H	H	G	J	F	F
220	D	H	C	F	C	C	E	I	B	E	C	F	B	G	A	I	D	K	H	H	G	I	F	F
221	H	H	A	F	B	C	F	I	C	D	C	E	B	F	A	D	E	E	J	J	G	I	F	F
222	C	H	F	F	B	C	E	J	B	F	A	F	B	G	I	I	E	K	A	H	F	F	B	F
223	C	H	C	C	D	E	E	I	C	E	E	G	B	D	A	D	E	E	E	J	G	J	B	F
224	A	H	A	C	C	D	D	F	C	D	F	J	E	E	A	D	D	E	E	F	D	D	F	F
225	A	C	C	C	C	C	E	F	B	E	E	J	B	E	A	A	D	D	F	F	G	G	B	F
226	A	C	A	C	B	C	A	A	C	C	E	I	D	E	D	D	C	E	H	J	D	G	F	F
227	C	C	E	H	C	F	E	H	B	E	F	I	D	F	A	D	D	E	G	J	E	G	F	F
228	C	C	C	F	B	B	E	E	B	D	A	E	B	D	A	D	E	K	H	H	E	J	F	F
229	C	H	A	A	D	F	F	J	B	C	A	C	D	G	I	I	D	E	F	H	E	E	F	F
230	C	H	A	A	D	F	E	F	B	D	A	F	E	G	I	I	A	E	E	F	E	G	F	F
231	C	H	C	C	C	E	E	E	C	F	F	G	D	D	D	I	D	E	E	J	F	G	B	F
232	C	C	C	C	C	C	A	E	B	F	C	G	B	D	A	I	D	K	J	J	F	F	F	F
233	C	C	C	E	C	C	E	G	B	E	F	G	F	G	A	A	E	F	F	H	E	F	F	F
234	C	H	C	E	B	D	E	E	E	F	I	J	D	G	D	D	E	F	H	J	E	G	F	F
235	C	C	C	C	C	F	D	E	C	D	G	J	D	E	D	D	E	E	J	J	E	E	F	F
236	C	D	F	F	A	E	E	F	D	F	C	E	D	E	A	D	D	E	E	E	E	F	B	F
237	C	I	A	C	C	C	E	E	E	F	E	F	F	F	D	D	E	E	E	H	G	G	F	F
238	C	H	A	C	A	C	E	E	B	F	C	E	D	D	D	I	B	E	F	F	G	G	F	F
239	C	C	A	C	C	E	E	E	E	E	E	F	D	F	D	L	A	E	H	J	E	G	F	F
240	H	H	C	F	C	C	E	I	B	E	C	G	D	D	A	I	A	E	H	H	F	F	F	F

Appendix A.4: DNA profiles of 78 Cape horses

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
241	A	C	F	F	A	F	B	E	B	F	E	J	B	D	I	I	E	K	G	G	D	F	E	E
242	A	A	C	C	A	C	E	E	B	F	C	F	D	F	C	I	A	A	E	E	G	J	B	F
243	A	C	F	F	A	C	A	E	B	E	E	J	D	D	I	I	A	K	G	G	F	F	B	D
244	A	D	B	F	C	C	E	E	B	D	C	C	D	F	A	A	E	E	E	G	A	G	B	F
245	A	C	B	C	C	C	B	B	B	B	C	J	D	D	A	I	A	E	G	G	D	J	F	F
246	C	H	C	F	A	A	B	E	B	G	E	F	B	D	I	I	A	B	G	G	A	A	E	F
247	A	C	F	F	B	C	A	E	B	B	C	C	D	F	D	I	A	D	G	H	G	J	B	F
248	A	H	F	F	C	C	A	E	B	B	E	G	E	F	A	I	E	K	G	G	E	F	E	F
249	C	D	B	C	A	C	A	A	B	G	C	C	D	F	A	I	E	E	J	J	D	G	F	F
250	C	H	C	F	C	C	A	A	B	C	F	I	D	F	A	I	A	E	G	H	F	J	E	F
251	A	A	F	F	C	F	E	F	B	D	F	I	D	G	A	I	E	E	G	H	A	J	F	F
252	A	A	F	F	C	F	B	F	B	F	F	J	D	G	A	I	A	E	E	H	F	J	F	F
253	A	A	F	F	A	C	E	E	B	F	I	J	G	G	A	I	E	E	G	G	G	J	F	F
254	C	H	C	F	C	C	B	B	B	F	C	G	D	D	A	D	A	A	F	G	A	A	F	F
255	A	A	F	F	C	C	E	E	F	F	G	H	D	D	D	I	A	E	G	G	E	G	F	F
256	A	A	F	F	A	C	E	E	F	F	F	G	D	D	A	D	E	E	G	J	A	A	F	F
257	A	H	C	F	C	C	E	H	B	F	G	J	D	E	A	I	A	E	H	H	E	E	F	F
258	A	A	D	F	C	C	B	E	B	D	F	G	D	D	A	A	E	E	G	G	G	J	F	F
259	A	A	F	F	B	C	E	E	B	F	F	I	D	G	A	I	E	E	F	J	A	A	B	F
260	A	C	C	F	C	C	B	E	B	F	G	G	D	D	A	I	A	A	G	H	A	J	B	F
261	A	C	E	F	A	A	E	I	C	D	I	I	D	D	A	A	A	E	G	G	G	G	D	F
262	A	C	F	F	C	C	A	E	B	B	C	J	D	D	I	I	A	E	G	H	F	G	D	F
263	A	A	C	F	A	C	B	G	B	F	C	I	D	D	A	I	A	E	G	H	E	F	F	F
264	A	H	C	D	B	C	E	E	B	D	C	J	D	F	A	I	E	E	J	J	F	G	F	F
265	A	A	C	D	B	C	A	B	B	F	F	J	D	G	A	I	A	E	E	H	G	J	B	F
266	A	A	C	H	F	F	A	E	B	C	F	J	D	D	I	I	E	E	H	H	E	E	E	F
267	A	A	C	C	C	C	B	E	B	G	C	F	D	E	I	I	E	E	G	H	F	F	E	F
268	H	H	C	F	C	C	E	I	B	E	C	G	D	D	A	I	A	E	H	H	F	F	F	F
269	A	D	B	C	A	C	A	E	B	E	C	G	D	F	A	A	A	E	G	J	A	E	F	F
270	A	A	C	F	B	C	E	I	B	D	C	F	D	D	A	A	E	E	J	J	G	J	B	F
271	A	A	B	F	C	F	B	E	D	G	C	F	D	D	A	I	B	K	A	J	F	G	F	F
272	A	H	C	F	C	C	E	E	B	E	C	J	D	D	I	I	A	A	H	J	F	F	F	F
273	A	A	C	D	C	C	B	G	B	B	C	C	D	G	I	I	B	E	G	H	F	J	F	F
274	A	H	D	F	C	C	E	I	B	E	G	J	D	D	A	I	A	E	E	H	F	J	D	F
275	A	A	C	F	C	C	E	E	B	B	C	G	F	G	A	I	A	E	E	G	A	A	F	F
276	A	D	C	F	C	C	D	D	F	G	C	C	D	D	D	I	A	E	E	J	E	E	F	F
277	A	H	F	F	A	F	B	B	F	G	F	J	B	D	I	I	A	E	A	A	F	F	E	F
278	D	H	C	F	A	A	A	E	C	E	C	F	B	F	A	I	E	E	G	J	F	G	F	F
279	A	D	B	F	C	C	A	H	B	G	F	G	D	D	A	I	E	E	A	J	E	F	F	F
280	A	A	D	F	C	C	B	E	B	B	C	F	D	D	A	I	A	E	G	H	F	J	F	F
281	H	H	C	F	C	C	E	I	B	E	C	G	D	D	A	I	A	E	H	H	F	F	F	F

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
282	A	H	C	F	C	C	E	E	B	B	C	I	B	D	A	A	E	K	E	G	A	A	B	F
283	C	C	C	F	C	C	E	E	B	B	C	G	B	D	A	I	A	E	G	G	J	J	F	F
284	A	C	C	F	C	D	A	E	B	F	C	G	B	D	A	I	A	A	H	H	A	J	B	F
285	A	C	C	F	A	B	A	C	B	B	C	I	B	F	A	D	A	D	G	G	G	J	B	F
286	A	C	F	F	B	C	A	B	B	B	C	J	D	F	A	I	A	D	G	K	A	J	B	F
287	C	C	C	F	A	C	A	E	D	G	F	G	F	F	C	I	A	A	E	F	E	E	B	F
288	C	H	F	F	A	A	A	A	D	F	G	J	D	F	I	I	A	E	E	K	A	E	F	F
289	A	H	C	C	C	F	A	B	C	G	F	F	D	E	A	I	E	E	G	H	F	J	E	F
290	A	H	C	F	C	C	A	E	C	F	F	G	D	D	A	D	E	E	G	G	G	J	F	F
291	A	H	D	F	C	C	B	G	B	C	C	F	D	G	A	I	B	E	F	F	J	J	F	F
292	A	A	F	F	C	C	E	G	C	D	C	I	D	D	D	I	B	E	F	F	A	J	F	F
293	C	C	E	F	A	F	F	I	C	F	H	I	B	D	A	A	A	D	G	G	D	G	D	F
294	A	C	F	F	A	A	B	F	F	F	G	I	B	D	A	A	A	E	G	G	A	D	B	F
295	A	A	D	F	A	C	A	H	C	E	F	G	D	G	A	A	B	E	E	J	E	F	F	F
296	A	C	F	F	A	B	A	E	B	E	G	G	D	G	A	A	E	E	G	G	A	F	F	F
297	A	C	C	C	A	C	D	E	F	G	C	F	D	F	A	C	A	E	E	G	E	G	B	F
298	C	H	C	F	C	C	B	D	B	F	F	G	D	F	A	C	A	A	G	G	F	F	B	F
299	A	H	F	F	C	C	B	E	B	B	F	J	D	E	I	I	A	E	F	F	F	J	E	F
300	A	H	F	F	C	C	B	E	B	F	G	J	D	G	I	I	E	E	F	K	A	F	F	F
301	A	A	F	F	A	B	A	E	B	F	F	G	D	G	A	A	E	E	F	K	A	G	B	F
302	A	C	F	F	A	B	A	E	B	F	G	I	D	G	A	A	E	E	J	K	A	A	B	F
303	A	D	D	F	C	C	D	E	B	E	G	G	D	D	A	A	E	E	H	J	F	G	F	F
304	A	A	F	F	A	C	E	E	D	E	G	G	D	G	A	D	E	E	G	H	A	G	F	F
305	A	D	B	D	C	C	A	E	D	G	F	G	D	F	A	A	A	E	E	J	E	F	F	F
306	C	D	B	F	C	C	A	E	B	D	F	G	F	G	A	I	E	E	G	J	F	F	F	F
307	A	A	D	F	C	C	E	G	B	C	F	J	D	D	A	I	E	E	E	G	F	F	F	F
308	A	A	D	F	C	C	E	E	C	F	F	I	D	G	A	A	E	E	G	G	G	G	F	F
309	A	H	C	F	C	C	B	H	B	D	H	J	D	E	A	I	A	B	F	F	E	F	F	F
310	A	H	C	F	C	C	E	H	D	F	I	J	D	G	A	A	B	E	G	H	E	G	F	F
311	A	H	C	F	C	C	B	E	D	F	H	J	D	G	A	I	A	B	F	F	E	J	B	F
312	A	A	F	F	C	C	E	E	D	F	H	I	D	D	A	I	B	E	F	F	A	E	F	F
313	A	A	C	F	A	C	E	E	B	F	F	J	D	D	A	I	E	E	H	H	A	J	F	F
314	A	A	F	F	C	C	E	E	B	F	F	I	D	D	A	A	E	E	G	G	A	J	F	F
315	A	C	C	C	C	C	A	D	B	F	C	G	D	D	A	D	A	E	E	G	E	E	F	F
316	A	A	C	F	C	C	A	B	B	F	C	G	D	D	D	I	A	A	G	G	E	F	F	F
317	A	A	D	F	C	C	B	I	B	D	F	J	D	D	A	I	E	E	E	H	J	J	F	F
318	A	A	D	F	A	C	E	I	B	D	F	G	D	D	D	I	E	E	G	H	A	J	F	F

Appendix A.5: DNA profiles of 79 S.A. Boerperde

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
319	C	H	E	E	A	C	A	E	E	F	C	C	D	G	D	I	A	D	H	J	A	E	F	F
320	A	C	F	F	A	B	A	F	D	F	C	G	D	D	A	I	E	E	F	H	C	H	D	F
321	G	H	B	B	C	C	A	B	B	C	I	J	D	D	I	I	A	A	H	S	C	E	D	F
322	A	C	F	H	C	C	A	A	B	G	C	F	D	E	A	I	A	E	A	H	D	E	D	D
323	D	H	A	C	F	F	F	F	B	D	F	G	B	D	A	A	B	E	F	F	H	H	B	F
324	A	H	C	F	C	C	F	F	F	F	C	G	D	G	A	I	A	A	E	H	A	H	F	F
325	A	A	C	E	B	C	A	H	B	F	F	F	D	G	I	I	D	E	A	A	A	E	F	F
326	C	D	E	E	B	C	A	E	F	G	A	G	D	G	A	I	B	D	A	H	B	B	F	F
327	D	H	A	A	F	F	E	F	D	E	G	I	B	B	A	I	B	B	F	G	H	H	B	F
328	A	C	C	D	B	F	H	J	B	F	C	I	E	G	A	D	A	E	E	F	D	D	F	F
329	A	A	E	F	C	C	F	G	C	G	G	J	C	F	D	D	B	E	A	G	D	D	B	E
330	A	H	C	F	C	F	B	F	F	G	C	F	D	E	A	D	A	E	A	A	D	D	F	F
331	A	G	B	C	B	F	A	D	F	F	F	F	D	G	D	D	A	E	A	G	D	H	F	F
332	A	C	C	C	A	C	D	D	C	G	F	F	D	D	A	D	B	K	A	H	E	H	F	F
333	A	C	C	C	D	D	E	H	E	F	C	I	D	G	D	I	D	F	F	G	E	H	B	F
334	A	A	C	C	B	C	D	H	B	E	F	J	D	D	A	I	E	K	A	E	B	H	F	F
335	A	H	F	F	A	F	E	F	B	G	G	I	D	E	A	I	E	I	F	F	D	D	D	F
336	A	A	F	F	B	C	D	E	C	G	E	H	D	F	A	D	A	A	A	G	E	H	D	F
337	A	H	F	F	C	F	B	E	B	G	C	H	C	D	A	I	E	K	F	H	H	H	F	F
338	A	A	C	C	C	C	I	J	D	F	C	C	F	F	A	A	A	E	A	H	E	H	D	F
339	A	A	C	F	C	E	A	H	B	E	F	H	D	G	I	I	A	D	A	F	H	H	D	F
340	D	H	C	F	C	D	A	E	D	F	H	I	E	G	D	I	A	E	H	H	B	B	F	F
341	A	C	C	F	C	E	A	F	E	F	C	I	D	D	A	I	E	K	E	H	E	H	B	E
342	A	D	C	H	A	C	G	J	C	F	C	I	G	G	A	A	D	E	H	S	C	E	B	F
343	A	A	C	C	C	C	E	E	C	F	C	C	D	G	A	A	E	E	A	A	C	C	D	F
344	A	C	F	H	C	E	A	F	E	F	C	E	G	G	D	I	E	F	F	G	C	H	F	F
345	C	C	C	F	C	D	B	H	A	G	H	I	D	D	A	I	B	I	A	G	E	H	E	F
346	A	H	C	F	B	C	A	B	C	F	C	I	B	G	A	I	A	E	A	A	H	C	E	E
347	A	A	E	F	C	E	A	C	B	F	C	I	B	D	G	I	E	E	H	H	D	E	B	E
348	A	A	C	C	B	B	D	D	C	F	C	G	D	D	D	I	A	E	A	G	E	H	D	F
349	A	H	C	F	C	E	E	F	B	E	I	I	D	E	I	I	A	E	A	A	E	G	E	E
350	D	H	A	F	C	D	A	E	B	F	I	I	E	G	D	I	A	A	H	H	C	F	D	F
351	C	H	C	C	C	C	E	E	C	D	H	I	B	D	D	I	E	K	H	H	D	E	F	F
352	C	C	C	D	C	C	F	H	B	D	C	E	B	D	A	A	D	E	A	G	H	H	D	F
353	A	H	F	H	A	C	B	H	C	F	F	J	D	G	D	I	I	K	E	H	D	D	B	F
354	A	H	C	F	C	C	E	F	B	D	I	I	B	G	I	I	B	D	A	A	E	E	E	F
355	A	H	C	D	F	F	A	C	B	C	C	C	D	G	I	I	D	D	A	A	E	E	F	F
356	B	H	C	H	C	C	A	E	B	C	E	E	D	D	D	I	E	K	H	H	D	E	F	F
357	A	H	C	C	C	E	F	F	B	F	I	I	C	D	A	I	A	E	H	J	H	H	F	F
358	A	H	C	H	C	C	E	J	C	D	I	J	D	F	A	A	E	E	J	J	C	E	F	F
359	H	H	A	A	C	F	E	G	D	G	F	G	B	G	A	I	B	E	F	H	H	H	B	F

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
360	C	H	A	F	C	C	E	F	B	D	F	J	B	F	A	I	A	D	F	G	H	H	B	F
361	D	D	A	A	A	F	E	I	B	D	G	H	B	G	A	I	B	E	E	F	E	H	F	F
362	A	G	A	C	C	F	A	H	C	G	C	E	D	D	D	I	E	E	E	S	G	H	B	D
363	A	C	C	C	C	C	C	D	B	C	F	F	D	D	A	I	E	K	A	H	E	E	F	F
364	C	H	B	F	A	C	B	B	C	F	C	E	D	G	A	I	A	D	A	A	E	H	B	E
365	A	H	B	C	A	B	E	G	F	F	G	G	B	G	A	D	D	D	H	J	E	E	F	F
366	A	A	C	C	B	B	D	E	B	G	C	G	D	D	A	D	A	K	F	G	E	H	B	F
367	A	E	C	C	A	A	E	G	B	C	C	C	D	F	A	A	D	E	H	J	E	E	F	F
368	A	D	F	F	C	E	A	D	B	C	C	I	D	D	I	I	E	K	F	G	E	H	B	F
369	A	G	C	F	C	F	A	A	B	G	C	A	B	F	D	I	A	E	G	H	E	G	F	F
370	A	H	F	H	C	C	A	A	D	F	C	J	E	G	D	I	D	K	A	H	D	E	F	F
371	A	C	E	F	C	C	A	E	F	F	E	J	E	G	A	I	E	K	H	K	E	G	F	F
372	A	H	F	F	C	C	D	E	B	D	F	F	E	E	A	D	B	K	F	H	G	H	F	F
373	A	C	F	F	F	F	D	E	G	G	F	G	C	D	A	C	A	K	H	H	E	F	D	F
374	A	H	C	H	C	C	F	H	E	F	G	I	D	G	D	I	E	I	A	G	I	J	B	F
375	A	H	F	D	C	I	B	B	E	F	J	F	G	D	I	I	A	E	A	F	E	J	D	F
376	C	C	A	A	A	F	E	F	D	D	F	I	B	B	A	I	A	E	F	F	A	J	F	F
377	A	H	C	H	A	C	A	E	C	G	F	F	B	D	A	A	B	E	G	H	G	F	F	F
378	A	H	E	E	A	C	A	E	D	G	C	F	B	E	A	I	D	E	A	J	G	G	F	F
379	A	H	A	C	C	F	B	B	B	F	F	F	B	G	D	I	E	K	A	H	G	G	B	F
380	A	H	C	F	A	C	E	F	B	B	H	H	D	G	A	C	B	E	F	I	G	G	D	F
381	A	C	C	E	C	C	B	F	A	A	B	E	D	D	A	D	F	F	A	G	E	H	E	F
382	A	C	C	H	C	C	B	B	H	B	D	H	D	D	I	I	C	F	H	H	F	F	F	F
383	A	H	F	F	C	C	F	G	B	F			C	G	A	D	F	F	A	G	C	J	E	E
384	A	A	C	E	C	C	B	B	D	G	C	F	D	D	A	I	E	E	G	H	D	G	F	F
385	A	C	C	C	A	C	F	H	A	E	F	I	D	G	D	I	A	I	A	A	G	J	E	F
386	C	H	A	F	C	C	E	F	G	G	F	F	E	G	A	D	A	B	A	G	E	G	E	F
387	A	H	A	A	B	E	A	E	B	C	C	I	C	G	A	I	D	D	A	G	G	G	B	F
388	A	H	A	C	C	C	A	F	F	F	C	I	C	D	A	I	D	I	A	G	D	D	B	F
389	A	G	C	F	C	C	G	I	G	G	F	F	G	G	D	I	D	E	G	H	C	J	F	F
390	A	H	F	F	C	C	A	F	E	E	F	I	B	C	I	I	A	E	F	H	A	J	F	F
391	A	A	C	F	B	B	D	E	F	F	E	J	D	D	D	I	A	B	A	F	J	J	F	F
392	A	H	F	F	A	B	A	A	B	G	C	F	D	D	I	I	E	E	H	H	F	G	B	F
393	H	H	C	C	C	C	A	E	B	E	H	J	C	D	A	D	B	E	F	H	A	J	F	F
394	A	C	G	G	A	A	B	E	C	G	C	H	D	F	A	D	B	D	G	H	E	G	B	F
395	A	H	C	C	C	D	E	E	B	F	H	I	C	G	D	D	B	E	F	H	A	G	D	F
396	A	A	C	F	F	F	A	E	G	G	C	C	B	F	A	I	E	E	H	H	F	G	B	F
397	C	C	C	F	C	C	B	E	B	D	F	F	B	D	A	I	B	E	A	H	G	J	B	F
398	A	C	C	C	C	F	B	E	B	B	F	J	D	D	A	I	A	E	A	A	F	F	F	F
399	C	E	C	F	C	C	A	E	C	C	G	J	B	G	A		E	E	G	J	F	J	D	D

Appendix A.6: DNA profiles of 81 Welsh ponies

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
400	A	B	A	C	B	C	A	H	D	F	F	F	F	F	A	A	D	D	H	H	A	B	F	F
401	A	E	B	E	B	C	F	H	D	E	A	F	C	G	A	I	D	D	G	H	G	H	B	D
402	A	H	A	B	B	C	F	H	E	G	C	I	B	D	C	C	A	D	E	I	C	I	B	F
403	A	E	C	C	C	C	H	H	B	G	A	G	D	G	A	I	D	D	F	G	G	J	E	E
404	A	I	C	D	C	F	H	H	B	G	F	F	F	G	C	D	A	D	F	F	C	G	F	F
405	E	H	C	E	B	C	A	H	B	D	C	G	B	G	I	I	A	D	G	H	D	D	B	E
406	A	H	A	E	C	C	A	D	C	D	F	I	G	G	I	I	D	D	E	I	C	I	F	F
407	A	H	B	C	C	C	A	G	B	C	F	F	F	G	A	I	D	E	E	F	G	J	E	F
408	A	H	E	E	C	C	A	H	B	C	A	F	G	G	A	I	D	D	F	H	C	D	E	F
409	A	A	B	C	B	C	F	I	C	F	A	E	B	G	D	I	A	K	A	H	E	G	F	F
410	A	B	A	B	C	C	B	H	B	F	F	G	C	F	A	C	A	D	G	G	C	D	F	F
411	C	D	B	C	C	D	A	F	C	F	E	I	B	F	G	I	D	K	G	H	G	I	B	D
412	A	A	C	E	B	C	A	F	B	D	C	C	G	G	I	I	D	E	H	I	G	G	E	F
413	H	I	B	D	A	B	F	F	C	F	F	I	F	G	I	I	D	K	F	H	G	G	F	F
414	C	H	C	D	A	C	E	E	C	C	E	J	D	D	A	D	D	E	F	G	E	J	B	F
415	A	C	E	F	C	F	E	F	B	E	I	I	D	D	C	C	B	E	H	H	G	G	D	D
416	A	D	C	E	D	G	H	J	F	G	F	F	D	G	A	A	D	E	H	I	G	G	D	F
417	A	C	E	F	A	A	D	E	B	G	E	I	B	G	I	I	B	K	G	H	F	J	B	B
418	A	D	A	C	C	F	A	A	D	F	C	C	F	G	C	I	A	F	H	I	D	E	E	F
419	A	A	E	E	C	C	H	H	E	F	D	I	C	G	I	I	A	C	H	I	C	C	B	D
420	B	C	A	E	B	C	H	J	C	F	A	C	D	G	A	I	A	H	G	H	D	J	E	E
421	A	C	E	E	A	C	H	J	C	G	E	F	D	D	A	I	A	E	A	H	E	J	B	E
422	A	H	E	F	A	F	A	E	D	E	C	F	D	D	I	I	D	E	E	E	C	D	F	F
423	A	C	B	E	A	C	F	H	C	G	A	E	C	D	A	I	D	E	A	G	G	J	B	E
424	A	E	D	F	B	C	H	I	B	B	A	C	D	F	D	I	B	E	G	H	G	G	B	F
425	A	A	C	C	C	C	H	H	D	G	A	C	C	D	C	I	D	D	G	H	C	D	B	E
426	A	A	E	F	C	C	A	A	B	F	C	C	D	F	I	I	D	D	H	J	G	J	B	F
427	A	D	C	E	A	B	B	H	B	F	C	C	D	F	A	I	A	A	I	J	C	E	B	F
428	E	H	A	C	C	C	A	D	E	G	A	G	G	G	C	I	A	F	E	H	E	E	B	F
429	C	F	B	E	A	C	E	E	C	C	E	F	D	G	A	I	E	E	G	G	G	J	E	F
430	D	D	E	E	C	C	A	C	B	G	D	D	F	G	I	I	D	F	G	H	A	G	B	F
431	A	D	C	E	A	C	A	B	B	C	C	C	B	D	I	I	A	A	I	J	A	A	B	E
432	D	H	E	E	B	C	A	H	B	D	B	F	G	G	A	I	D	D	F	G	A	C	E	E
433	A	A	B	E	B	C	A	A	B	F	E	I	B	F	G	I	D	D	E	H	A	E	E	F
434	A	A	C	C	C	C	A	H	B	F	A	F	G	G	I	I	D	D	G	I	E	G	D	E
435	A	H	A	C	A	C	A	J	E	E	J	J	G	G	A	A	A	I	E	H	C	E	F	F
436	G	G	B	D	B	C	A	F	F	F	A	F	G	G	A	D	A	E	H	H	E	E	A	B
437	A	A	E	F	A	C	D	H	D	G	C	F	C	D	C	I	B	E	F	H	A	A	D	F
438	A	H	C	E	B	B	H	H	B	D	G	G	B	G	I	I	A	A	G	I	E	G	A	B
439	A	E	C	E	C	F	H	H	G	G	A	F	F	G	A	D	A	D	E	G	E	G	E	F
440	H	I	C	F	A	A	A	B	F	G	F	I	C	G	H	I	D	E	G	H	E	E	F	F

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
441	A	A	E	E	C	C	A	H	B	G	C	F	F	F	C	I	D	D	G	H	A	G	E	F
442	A	C	C	D	B	D	F	J	F	F	A	I	G	G	A	I	F	K	F	H	D	E	B	F
443	A	E	C	E	C	C	A	F	B	B	A	A	G	G	A	I	D	D	G	G	E	E	D	E
444	E	H	C	E	C	C	A	A	B	D	C	C	B	G	I	I	A	D	H	H	A	C	B	B
445	A	A	B	E	A	C	H	H	E	F	A	C	F	G	A	I	A	F	G	J	A	C	B	E
446	H	H	E	F	C	F	E	E	C	F	F	J	F	G	D	I	D	D	E	F	A	C	B	D
447	C	H	C	F	F	F	E	J	B	G	C	F	F	G	A	I	D	F	H	J	E	E	B	B
448	A	H	E	E	B	C	A	H	F	G	B	E	D	G	I	I	A	D	E	F	C	E	E	F
449	C	E	A	A	A	D	F	I	D	F	I	I	F	G	A	I	F	G	I	I	E	H	A	A
450	A	A	A	D	C	C	F	H	B	F	F	F	F	G	D	D	A	A	E	H	C	G	B	F
451	D	D	D	E	C	C	A	E	F	F	C	D	D	F	A	D	B	F	E	E	E	G	E	F
452	D	D	C	E	A	C	E	I	E	F	A	D	D	F	C	D	B	F	A	A	G	H	E	F
453	A	D	A	C	A	A	A	E	F	G	A	E	D	F	C	D	B	E	A	H	A	J	E	F
454	D	E	A	F	A	A	A	D	B	C	C	J	C	D	D	I	C	G	F	F	A	E	F	F
455	E	G	A	A	A	A	A	A	C	F	J	J	D	G	A	D	A	G	A	G	A	G	B	F
456	C	H	C	C	B	B	E	F	B	C	F	J	D	D	D	I	D	I	F	H	C	G	D	E
457	A	A	C	C	B	C	H	J	E	F	C	F	F	F	C	D	A	A	E	E	C	G	B	B
458	A	A	C	C	B	C	A	H	B	D	B	F	D	G	D	D	D	F	G	H	C	C	B	F
459	A	C	A	C	A	C	A	A	B	E	C	F	F	F	D	D	D	H	H	I	E	H	B	F
460	A	E	C	E	A	C	A	H	B	C	F	F	G	G	I	I	D	D	F	G	E	G	B	F
461	D	H	C	D	C	C	E	E	C	F	D	E	D	D	A	D	A	F	A	E	E	G	E	F
462	A	H	C	D	A	C	E	F	C	F	D	F	D	G	D	I	D	F	A	E	A	G	E	F
463	D	H	D	E	A	C	A	E	C	G	E	G	D	G	D	D	A	D	E	F	A	A	E	E
464	A	A	E	F	A	C	D	H	D	F	F	I	C	F	C	I	D	G	G	I	C	G	B	F
465	A	D	C	F	C	C	A	F	E	G	C	F	F	G	D	I	B	E	G	I	E	E	B	F
466	A	F	B	F	C	C	E	F	B	D	C	F	G	G	C	D	B	D	E	F	C	G	F	F
467	A	H	F	F	C	F	A	E	C	G	F	I	D	F	C	I	B	K	E	H	G	G	B	D
468	A	A	E	F	B	C	E	H	C	G	G	J	E	F	I	I	D	I	F	H	G	G	D	E
469	B	B	C	E	B	C	F	F	E	F	I	I	F	F	L	L	B	E	F	F	G	J	B	B
470	A	E	C	F	B	F	A	H	D	G	E	F	G	G	A	K	A	D	E	H	C	I	E	E
471	A	H	C	F	B	C	H	H	D	F	F	I	B	G	A	I	A	A	A	I	C	J	D	E
472	A	C	C	E	B	F	H	H	D	G	F	F	C	F	D	I	A	D	G	G	E	I	E	F
473	A	A	C	C	A	C	A	D	B	F	C	I	G	G	A	A	A	K	H	H	C	G	B	F
474	B	H	A	E	B	B	A	J	D	G	C	F	C	C	I	I	A	A	H	H	I	I	E	E
475	A	A	B	C	C	C	A	E	B	B	F	F	G	G	I	I	D	F	G	G	C	G	E	F
476	A	C	C	E	A	C	H	J	B	F	G	G	D	G	C	I	D	E	E	G	G	G	B	E
477	A	A	C	E	C	C	F	H	B	B	F	G	D	G	C	I	D	D	G	G	G	G	E	F
478	A	A	B	C	C	C	E	J	B	B	A	G	C	G	A	C	D	D	G	H	C	C	D	E
479	A	B	C	C	C	C	H	J	B	F	A	G	G	G	C	I	D	D	G	G	C	I	D	E
480	A	E	B	C	C	C	E	H	B	B	A	F	C	D	A	C	D	D	G	G	C	C	D	F

Appendix A.7: DNA profiles of 39 Friesian horses

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
481	C	C	C	E	B	B	H	H	B	D	A	A	C	F	I	O	A	A	H	J	G	G	D	D
482	C	C	E	E	A	B	G	H	A	B	A	E	C	G	A	D	A	B	J	J	G	J	D	F
483	C	C	E	E	A	B	E	H	B	E	A	E	G	G	I	I	A	A	H	J	G	K	D	D
484	C	C	C	E	B	B	H	H	D	E	E	F	C	C	I	O	A	B	J	J	G	G	B	D
485	C	C	E	E	B	B	G	H	B	B	F	F	C	G	A	I	A	B	J	J	G	G	D	D
486	C	C	E	E	B	C	H	H	D	G	A	A	F	F	I	O	A	A	J	J	G	G	D	D
487	C	C	E	F	B	C	H	H	D	D	A	A	C	C	I	I	A	B	J	J	J	K	B	F
488	C	C	E	E	B	F	A	H	E	F	A	A	C	C	I	I	B	B	J	J	G	K	D	F
489	C	H	E	E	B	B	H	H	D	D	E	F	C	G	A	I	A	D	F	H	G	G	B	D
490	C	H	E	F	A	C	H	H	F	F	E	E	B	C	C	I	J	J	H	J	C	F	B	F
491	C	C	C	C	B	F	H	H	D	E	A	F	C	G	I	I	A	B	J	J	G	G	D	F
492	C	C	E	F	C	C	G	H	C	E	A	F	D	G	C	I	A	B	E	J	E	E	D	D
493	C	C	E	E	B	C	H	H	D	E	A	F	B	F	I	I	A	D	J	J	K	K	B	D
494	C	C	E	E	A	B	G	H	D	D	A	F	C	C	I	I	A	B	H	J	G	K	D	F
495	C	H	C	E	B	B	H	H	E	E	A	F	C	C	I	I	A	D	J	J	G	G	B	F
496	C	H	E	E	B	B	H	H	D	D	E	F	C	G	A	I	A	D	F	H	G	G	B	D
497	C	H	C	F	B	B	H	H	B	E	E	F	C	G	D	I	B	D	J	J	G	G	D	D
498	C	C	E	E	C	C	H	H	F	F	C	F	C	D	I	I	A	A	A	J	F	G	D	E
499	C	C	C	C	B	C	E	H	B	E	A	F	C	D	I	I	A	D	J	J	E	E	D	F
500	C	H	C	E	A	C	A	H	D	F	A	F	C	C	D	I	A	D	J	J	G	G	B	D
501	H	H	C	D	B	F	J	J	C	D	A	F	C	D	I	I	D	I	A	J	F	J	B	E
502	C	C	C	F	B	B	H	H	E	G	A	E	C	C	I	I	B	D	J	J	E	G	D	D
503	C	C	E	E	B	F	E	H	F	F	E	F	C	C	D	I	B	D	J	J	C	G	D	D
504	D	H	E	E	B	E	E	F	E	E	A	F	C	C	I	I	D	E	J	J	G	J	D	D
505	C	C	C	E	A	B	H	H	D	E	A	F	C	D	I	I	A	A	J	J	G	G	D	F
506	C	C	E	E	B	B	H	H	C	F	F	F	C	C	I	I	A	A	H	J	E	G	D	D
507	C	C	C	E	A	B	F	H	E	F	A	F	C	C	I	I	A	B	H	J	E	G	D	D
508	C	C	C	C	B	B	A	H	E	F	A	A	C	C	I	I	B	B	G	H	E	G	D	F
509	C	H	C	E	B	B	H	H	D	E	E	G	C	C	I	I	A	D	H	J	E	K	D	D
510	C	H	C	F	B	C	F	H	B	F	C	E	C	G	A	I	A	D	H	J	E	G	D	F
511	C	C	C	C	B	C	H	H	D	E	A	I	B	C	C	I	B	B	H	J	E	J	D	D
512	C	H	C	C	B	F	A	H	F	F	F	F	B	C	C	I	D	I	H	J	G	J	D	E
513	C	H	C	E	B	B	H	H	D	E	E	G	C	C	I	I	A	D	H	J	E	K	D	D
514	C	C	C	E	C	C	H	H	F	F	E	E	C	C	I	I	I	I	A	J	C	F	E	F
515	C	C	C	E	C	F	A	H	F	F	E	E	B	G	I	I	A	D	E	J	C	G	D	D
516	C	C	C	E	B	F	A	H	E	E	E	F	B	G	I	I	B	D	J	J	G	G	E	E
517	C	C	C	E	C	F	H	J	E	G	A	F	C	C	C	I	B	E	A	J	G	G	D	D
518	C	C	C	C	B	F	H	H	D	E	A	F	C	G	I	I	A	B	J	J	G	G	D	F
519	C	C	C	F	B	B	A	H	E	F	E	E	C	C	I	I	A	B	H	J	G	G	D	D
520	C	C	C	F	B	B	H	H	E	G	A	E	C	C	I	I	B	D	J	J	E	G	D	D

Appendix A.8: DNA profiles of Przewalski horse

	AHT4		HMS7		HTG4		VHL20		AHT5		ASB2		HMS6		HTG6		HMS2		HMS3		HTG10		HTG7	
521	C	C	C	E	B	B	H	H	B	D	A	A	C	F	I	O	A	A	H	J	G	G	D	D

Appendix B Allele frequencies of 12 microsatellites in 8 horse breeds.

AHT 4

	A	B	C	D	E	F	G	H	I
Arabian horses	0.044	0.056	0.45	0.199	0	0.044	0	0.287	0
American Quarter horses	0.313	0	0.144	0.194	0	0	0.013	0.338	0
American Saddlers	0.106	0.013	0.406	0.044	0.025	0	0.006	0.344	0.056
Cape horses	0.623	0	0.182	0.058	0	0	0	0.136	0
S.A. Boerperd	0.462	0.006	0.184	0.057	0.012	0	0.031	0.246	0
Welsh ponies	0.469	0.043	0.093	0.105	0.086	0.012	0.019	0.154	0.019
Friesian horses	0	0	0.813	0.013	0	0	0	0.175	0
Przewalski	0	0	0	0	1	0	0	0	0

HMS 7

	A	B	C	D	E	F	G	H
Arabian horses	0.087	0.338	0.463	0.006	0.075	0.031	0	0
American Quarter horses	0.275	0.019	0.425	0.169	0.038	0.044	0.006	0.025
American Saddlers	0.287	0.025	0.481	0.025	0.056	0.112	0	0.013
Cape horses	0	0.052	0.266	0.091	0.013	0.571	0	0.006
S.A. Boerperd	0.105	0.032	0.406	0.027	0.074	0.277	0.012	0.063
Welsh ponies	0.105	0.099	0.327	0.068	0.29	0.111	0	0
Friesian horses	0	0	0.313	0.013	0.563	0.112	0	0
Przewalski	0	0	0	0	1	0	0	0

HTG 4

	A	B	C	D	E	F	G	H	I
Arabian horses	0.387	0.156	0.4	0.056	0	0	0	0	0
American Quarter horses	0.319	0.188	0.394	0	0.019	0.081	0	0	0
American Saddlers	0.031	0.1	0.525	0.1	0.162	0.081	0	0	0
Cape horses	0.195	0.065	0.675	0.006	0	0.058	0	0	0
S.A. Boerperd	0.117	0.117	0.52	0.043	0.059	0.141	0	0	0.004
Welsh ponies	0.185	0.173	0.543	0.025	0	0.068	0.006	0	0
Friesian horses	0.138	0.525	0.225	0	0.013	0.1	0	0	0
Przewalski	0	0	0.5	0	0	0.5	0	0	0

VHL 20

	A	B	C	D	E	F	G	H	I	J
Arabian horses	0.144	0.019	0	0.119	0.006	0.213	0.013	0.006	0.006	0.475
American Quarter horses	0.194	0	0	0.363	0.131	0.094	0.044	0.025	0.125	0.025
American Saddlers	0.162	0.025	0.006	0.188	0.412	0.075	0.025	0.006	0.075	0.025
Cape horses	0.188	0.182	0.006	0.039	0.448	0.026	0.032	0.032	0.045	0
S.A. Boerperd	0.207	0.109	0.023	0.082	0.246	0.160	0.043	0.078	0.02	0.031
Welsh ponies	0.265	0.025	0.006	0.043	0.148	0.130	0.019	0.272	0.025	0.068
Friesian horses	0.063	0	0	0	0.075	0.063	0.075	0.688	0	0.038
Przewalski	0	0	0	0	0	0	0	1	0	0

AHT 5

	A	B	C	D	E	F	G	H
Arabian horses	0	0.106	0.05	0	0.231	0.538	0.075	0
American Quarter horses	0	0.219	0.144	0.038	0.019	0.406	0.175	0
American Saddlers	0.013	0.244	0.181	0.125	0.219	0.175	0.044	0
Cape horses	0	0.435	0.078	0.123	0.065	0.227	0.071	0
S.A. Boerperd	0.02	0.227	0.156	0.121	0.086	0.246	.137	0.008
Welsh ponies	0	0.259	0.148	0.123	0.086	0.228	0.154	0
Friesian horses	0.013	0.125	0.038	0.275	0.275	0.237	0.038	0
Przewalski	0	0	0.50	0	0	0.50	0	0

ASB 2

	A	B	C	D	E	F	G	H	I	J
Arabian horses	0	0	0.038	0	0.138	0.044	0.106	0.006	0.669	0
American Quarter horses	0.006	0.019	0.206	0.05	0.1	0.244	0.394	0.019	0.056	0.006
American Saddlers	0.069	0	0.162	0.013	0.225	0.206	0.094	0	0.156	0.075
Cape horses	0	0	0.221	0.006	0.026	0.234	0.227	0.032	0.110	0.143
S.A. Boerperd	0.012	0.004	0.258	0.004	0.066	0.191	0.117	0.074	0.199	0.074
Welsh ponies	0.130	0.019	0.185	0.043	0.080	0.290	0.080	0	0.117	0.056
Friesian horses	0.316	0.013	0.039	0	0.25	0.355	0.013	0	0.013	0
Przewalski	0	0	0	0	1	0	0	0	0	0

HMS 6

	A	B	C	D	E	F	G
Arabian horses	0	0.05	0.506	0.162	0	0.131	0.15
American Quarter horses	0	0.038	0.0019	0.15	0.019	0.156	0.619
American Saddlers	0	0.156	0.006	0.256	0.169	0.181	0.231
Cape horses	0	0.065	0	0.649	0.039	0.123	0.123
S.A. Boerperd	0	0.133	0.047	0.453	0.074	0.066	0.227
Welsh ponies	0	0.062	0.086	0.228	0.006	0.216	0.401
Friesian horses	0	0.063	0.65	0.075	0	0.05	0.162
Przewalski	0	0	1	0	0	0	0

HTG 6

HTG6	A	B	C	D	E	F	G	H	I	J	K	L
Arabian horses	0.456	0	0	0.063	0	0	0.006	0	0.463	0	0.013	0
American Quarter horses	0.275	0	0.006	0.075	0	0	0	0	0.50	0.05	0.006	0.087
American Saddlers	0.275	0	0	0.444	0	0	0.006	0	0.256	0	0.006	0.013
Cape horses	0.481	0	0.026	0.078	0	0	.0	0	0.416	0	0	0
S.A. Boerperd	0.355	0	0.008	0.207	0	0	0.008	0	0.418	0.004	0	0
Welsh ponies	0.21	0	0.136	0.167	0	0	0.012	0.006	0.451	0	0.006	0.012
Friesian horses	0.081	0	0.054	0.068	0	0	0	0	0.770	0.027	0	0
Przewalski	0	0	0.50	0	0	0	0	0	0	0	0	0.50

HMS2

HMS2	A	B	C	D	E	F	G	H	I	J	K	L
Arabian horses	0	0	0	0.044	0.313	0.275	0.006	0	0.194	0	0.169	0
American Quarter horses	0.05	0.056	0	0.563	0.156	0.031	0	0.087	0	0.006	0.05	0
American Saddlers	0.063	0.031	0.044	0.225	0.469	0.019	0	0	0.044	0	0.106	0
Cape horses	0.299	0.065	0	0.026	0.571	0	0	0	0.006	0	0.032	0
S.A. Boerperd	0.211	0.109	0.004	0.145	0.375	0.027	0	0	0.035	0	0.094	0
Welsh ponies	0.222	0.068	0.012	0.407	0.111	0.08	0.031	0.006	0.019	0	0.043	0
Friesian horses	0.438	0.262	0	0.188	0.038	0	0	0	0.05	0.025	0	0
Przewalski	0	0	0	0	0	0	0	0	0	0.50	0	0.50

HMS 3

HMS3	A	B	C	D	E	F	G	H	I	J	K
Arabian horses	0.087	0	0	0	0.2	0.425	0.013	0.256	0.019	0	0
American Quarter horses	0.125	0	0	0	0.075	0.4	0.044	0.156	0.038	0.156	0.006
American Saddlers	0.013	0	0	0	0.125	0.219	0.025	0.419	0	0.2	0
Cape horses	0.026	0	0	0	0.11	0.11	0.409	0.188	0	0.123	0.032
S.A. Boerperd	0.277	0	0	0	0.055	0.16	0.133	0.289	0.004	0.055	0.027
Welsh ponies	0.062	0	0	0	0.142	0.13	0.259	0.272	0.099	0.037	0
Friesian horses	0.05	0	0	0	0.025	0.013	0.05	0.188	0	0.675	0
Przewalski	0	0	0	0	0	0	0	1	0	0	0

HTG 10

HTG10	A	B	C	D	E	F	G	H	I	J	K
Arabian horses	0	0	0.138	0.269	0	0	0.531	0.025	0.006	0.031	0
American Quarter horses	0.138	0.038	0.013	0.206	0.087	0.006	0.05	0.038	0.006	0.387	0.031
American Saddlers	0	0	0.006	0.087	0.125	0.119	0.45	0.1	0.031	0.081	0
Cape horses	0.201	0	0	0.032	0.156	0.247	0.162	0	0	0.201	0
S.A. Boerperd	0.039	0.039	0.074	0.133	0.262	0.043	0.098	0.266	0.004	0.043	0
Welsh ponies	0.111	0.006	0.198	0.056	0.191	0.006	0.284	0.025	0.049	0.074	0
Friesian horses	0	0	0.05	0	0.175	0.050	0.512	0	0	0.075	0.138
Przewalski	0	0	0	0	0	0.50	0	0	0	0.50	0

HTG 7

HTG7	A	B	C	D	E	F	G
Arabian horses	0	0.294	0	0	0.013	0.694	0
American Quarter horses	0	0.181	0	0.056	0.056	0.7	0.006
American Saddlers	0	.1	0	0.013	0.006	0.881	0
Cape horses	0	0.123	0	0.032	0.065	0.779	0
S.A. Boerperd	0	0.141	0	0.125	0.09	0.645	0
Welsh ponies	0.025	0.241	0	0.105	0.265	0.364	0
Friesian horses	0	.1	0	0.675	0.05	0.175	0
Przewalski	0	0	0	1	0	0	0