A comparison of learning behavior between wild type horses (Equus Przewalski) and domesticated horses (Equus Caballus)

Bachelorthesis

Lisa McKenna

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Abstract

In this pilot study, it was investigated whether there is a difference in the speed of learning a discrimination task when comparing domesticated horses with a wild type horse species. Further it was explored whether inbreeding has an influence on the cognitive abilities of the tested horses as suggested by the literature on other species. The possibility that the horses were also 'learning to learn' was investigated by observing a change in the time needed to solve the discrimination task across a sequence of 15 trials.

To investigate the role of domestication on learning ability, a zoo population of Przewalski horses who were rarely handled by humans was compared to a group of Thoroughbred horses. Both groups are known to have high inbreeding coefficients. To investigate the potential impact of inbreeding these two inbred breeds were compared to a group of Paso Finos, a breed with a low inbreeding coefficient and a comparatively high level of heterozygosity. All horses performed a two choice discrimination task, they were asked to discriminate between a designated 'correct' object and a second 'incorrect'. Both objects were three-dimensional and identical in size, texture and colour but differed in shape.

Study outcomes demonstrated that all three breeds showed a significantly different learning profile from one another ($p = 0.008$). The Paso Fino group showed a statistically significant difference in the speed of learning ($p= 0.009$), completing the discrimination tasks successfully in a much shorter time than the other two breeds. The Paso Finos lower inbreeding coefficient was identified as a significant factor in speed of learning ($p=0.0001$). This finding supports outcomes from the existing literature.

The level of domestication of the respective horse breeds had no significant influence in the speed of learning of the discrimination task. It could be hypothesized that the fact that the Przewalski group was not used to the man-made test environment and were therefore wary of the task may have combined with their high levels of inbreeding to limit their performance. Some support for this is provided by looking at the performance of the equally inbred Thoroughbreds. Whilst the Thoroughbreds showed a rapid decrease in the time required to solve the task across the fifteen trials, the Przewalskis speed of learning remained at the same low level throughout the experiment and field notes indicate that the lead mare in this group remained wary of the task throughout. The time required by the Paso Finos to solve the task also failed to decrease significantly over time, but in the context of this breed, the lack of any decrease in time resulted from the very high speed of learning demonstrated at the outset of the task.

According to these findings, inbreeding appears to have a limiting effect on the learning abilities of horses, whilst their degree of domestication does not. These outcomes must, however, be regarded as speculative, as the sample size for this study was comparatively small and the observed differences could stem from individual horse differences. It is possible that the horses recruited for the study were not entirely representative of the characteristics of their respective breed. The interesting outcomes of the study merit further exploration in a larger sample size.

Chapter 1

Introduction

1.1 Background and rationale

There is substantive evidence to suggest that natural selection plays a significant role in the development of conceptual learning ability in both animals and man (Zentall et al 2008). This leads to the possibility that equine breeds subject largely to natural selection may display significantly different learning skills and associated behaviours in comparison to modern highly inbred breeds which are subject to artificial selection (Mader & Price 1980). It has also been suggested by Price (1999) that genetic changes including artificial selection, natural selection in captivity and evironmental changes including relaxed selection under domestic conditions may have an influence on the learning ability of the domestic horse. Research so far has shown that domestication has influenced the animal in body weight, brain size, reproductive traits and temperament traits (Harker and Wishaw 2000); however the effect of domestication on learning and memory in horses has not been researched yet.

According to a systematic review of the literature on learning theory in horses carried out by the Equine Research and Information Centre in 2010, there is little or no scientific data relating to cognitive abilities and, in particular, learning in Przewalski horses. Information in this field would give insight into the domestication event of horses in respect of the impact of artificial breeding selection and its influence on cognitive abilities. The Przewalski horse was chosen for this study because it is one of the only wild type horses left; therefore all information gathered on the breed is vital.

1.2 Aims and objectives

The main aim of the present study was to find out whether there is a difference in the speed with which a discrimination task can be learned when comparing a domestic type (Equus Caballus) with a wild type horse species (Equus Przewalski).

For the purposes of achieving this aim, three test groups were compared. The group of Przewalski horses represented the wild type horse species, which, however, due to captive breeding and a genetic bottleneck are highly inbred. A group of Thoroughbred horses represented the domesticated horses, which are highly inbred through artificial selection for their racing ability. Additionally, the study attempted to look at the relative influence of the degree of inbreeding (and/or 'wild type genome') versus domestication *per se* on cognitive abilities. For this purpose, a third test group was introduced. A group of Paso Finos was chosen for their unusually low inbreeding coefficient. Studies on other species have shown that increasing inbreeding is limiting the learning abilities of mice (Deckard et al, 1988). However, this type of research has not, to the authors knowledge, ever been carried out in horses.

This study is carried out as a pilot study. It has only a limited sample size of eight horses in total. The results of this study can therefore only be assumptions and a bigger sample size would be needed to present stronger evidence.

The aims and objectives are summarized in the following paragraph:

A. The main aim of this study is to find out whether domesticated horses show a disadvantage in the speed of performing a discrimination task.

B. In order to achieve this aim, further objectives were to find out if there is a difference in the speed of learning a discrimination task, when comparing a domestic type with wild type horse species and

C. to find out if inbreeding had an effect on the learning behavior of the horse.

D. To see if the speed of learning changes over the duration of the discrimination task and the horses learn to learn, the time the horses required to reach criterion was observed.

E. Also it was investigated if the learning rate of all three groups improves over the duration of the discrimination task.

1.3 Structure of dissertation

The introduction in chapter one states the central issue of concern and summarizes the research aims of the study. Background information on the domestication of the horse, the history and genetic makeup of the three investigated breeds and an overview of the literature concerning learning behavior of the horse is given in chapter two. In chapter three, the research strategy, the study design and the data collection is stated. All results are presented in chapter four and discussed in chapter five. Finally, a conclusion is drawn in chapter six. All references used in this study can be found in the adjoining reference list.

Chapter 2

Literature Review

2.1 Domestication

This study is investigating the possible impact of domestication on the cognitive abilities of the horse. In order to fully understand this topic, an overview of the domestication event itself and the according literature to date is given in the following section.

Two alternative hypotheses have been used to describe the origin of the modern horse (EquusCaballus). According to the now rejected 'restricted origin' hypothesis, the horse was selectively bred from wild stock within a single limited area of domestication (Vilà et al, 2001). The ´multiple origin´ hypothesis, in contrast, implies that a large number of founder animals were captured from diverse wild populations over a longer period of time, throughout the Eurasian range of the horse (Vilà et al, 2001).

Exploring these competing hypotheses, Jansen et al (2002) established that the degree of genetic diversity observed in the domestic horse could not have originated from a single wild population. Jansen collected mitochondrial DNA from 25 breeds of horse and showed that the DNA clustered into 17 distinct groups. Several of these corresponded both to breed and to geographic areas. Outcomes from this research study indicate that at least 77 breeding mares were recruited from the wild.

It has been suggested that, with increasing knowledge of horse breeding, humans began taking additional horses from the wild to breed with their domestic herds (Levine,2005). This led to the decline and eventual disappearance of true wild populations (Vilà, 2001) through a decrease in wild numbers and through cross-breeding with domesticated populations.

The horses in the Eurasian Steppes of the Ukraine are believed to have been the first domesticated for meat, riding and traction around the end of the 3rd Millennium BC with captive foals kept as pets and later worked or used as meat (Levine, 2005). Archaeological evidence taken from Dereivka, a location north of the Black Sea which is especially rich in respect of domestication data, suggests that horses were ridden at least 500 years before the wheel was invented (Levine, 1998). The taming of wild horses probably started with the hunting of horses for their flesh (Levine, 1998), but the importance of riding horses in human culture was well established by 1000BC (Goodwin, 2005). During the Neolithic and Iron Ages, the evidence suggests that horse domestication even began to influence human social life (Levine, 2005).The importance of riding horses in human culture was established by 1000BC (Goodwin, 2005).

Given the difficulty of successfully breeding wild horses in captivity now, it is likely that in the past controlled breeding over generations must have been established before domestication became possible. The social needs of the animals, their nutrition and appropriate environmental conditions are complex and need to be maintained and managed. If these needs are not met, problems such as pacing, impotence or a larger degree of aggression are observed (Boyd &Houpt, 1994). The relative simplicity of horse breeding today is likely to derive from: 1. Genetic changes predisposing the modern horse to breed in captivity or 2. Advances in the human understanding of horse behavior promoting the conditions which allow horses to reproduce successfully in captivity (Levine, 2005).

2.1.1 Influence of domestication on genetics

In order to understand the effect domestication has had on the horse, it is important to look into the genetic structure of the horse and how it was changed over the course of domestication.
Jansen et al's (2002) key study identified not only the multiple origins of the modern horse, but also the likely genetic effects contributing to current gene pools, namely natural selection *per se*, founder effects and genetic drift. The breakthrough in our understanding of the impact of domestication on modern equine genetics stems from studies such as Jansen's which take advantage of the characteristics of mitochondrial DNA (mtDNA).

MtDNA exists outside the nucleus of the cell in the Mitochondria, which are the 'energy producing' bodies of the cell. Since they exist outside the nucleus, they do not undergo the same 'cross over' as nuclear DNA when two animals mate. Their genetic profile is therefore conserved across generations. In addition MtDNA mutates at such a constant rate that it enables geneticists to identify how closely (in time) two groups are related, more specifically, when their genetic lineages began to diverge.

Geneticists trace the commonality or divergence of lineage back through the maternal line. Initially it was thought that only maternal mtDNA could be passed on to the next generation (both males and females). Now it is recognized that, occasionally, male mtDNA may also pass through, but this remains far less likely. This is because the location of the mtDNA in the tail of the sperm is a vulnerable one, the tails often break off or male mtDNA is destroyed by female cells on entry to the egg. Maternal MtDNA therefore remains the tool of choice in establishing the association between lineages.

Since MtDNA is conserved across generations, any difference in DNA sequences between the mtDNA of groups of animals is due solely to the accumulation of mutations. The number of different sequences therefore indicates how long it is since the groups have separated from their common ancestor (Hall, 2005). If no mutations occur, progeny show the same mtDNA sequence as their mother. If mutations occur, they become fixed in the maternal line. These mutations are 'echoes' of distinct maternal lines right back into the population's furthest history (Kavar&Dovc, 2008)

If there had been a single domestication of the horse, with only one population contributing the founder animals, then today's mtDNA lineages should show that the common ancestor of the horse lived around 5000 years ago. According to the findings of Vilà et al (2001), however, the common ancestor of the modern horse must have lived around 630,000 to 320,000 years ago, which means, that today's domestic horse must have evolved from many different wild populations that have been separated long enough to have developed distinctive mtDNA (Hall, 2005).

Jansen et al (2002) found that most of today's mtDNA lineages group into one of 17 clusters. These clusters suggest the approximate number and type of wild populations contributing to the genetic profile of the domesticated horse. The Northern European ponies form a distinct cluster as well as the Iberian and North African Horses. These three groups for example could each be descended from an independent domestication event.

Similarities and differences between the equine breeds seen today can be accounted for by the patterns of restricted mating imposed on the horse via domestication, superimposed on the genetic profile resulting from previous and ongoing natural selection. The nature and impact of these various processes is described below:

Natural selection is best seen as an active process. Nature favours a particular phenotype, so the genotypes which produce such phenotypes are more likely to survive to the next generation. This process encourages key similarities between breeds which otherwise have a distinct genetic profile. For example, feeding, social behaviour and reproductive strategies are very similar across all equids.

In contrast, **genetic drift** is a chance process with a random fixation or loss of genes in the population gene pool (Price, 2002). If there is only a small number of breeding animals in a population, alleles which are found in pairs on the chromosomes, may be randomly fixed or lost to the gene pool. This drift is common when populations are founded with only a few animals. This phenomenon is called the "**founder effect**". The genetic drift may also occur if the population size is suddenly severely reduced in numbers; a population goes through a genetic "bottleneck" (Price, 2002).

In addition to the largely random impact of the founder effect and genetic drift, deliberate artificial selection of desired traits by humans in recent times has acted as an accelerated 'mimic' of the process of natural selection. A good case in point here is the emergence of breeds such as the American Miniature, which are primarily used for show purposes and can often carry deleterious genes which would in a wild population have been selected against and removed from the population.

Regardless of whether selection is natural or artificial, 'desirable' and 'undesirable' characteristics are coded by particular genes or gene combinations. Restricted mating and/or death prior to reproduction causes a random drift of the genetic structure, so that some genes may be lost in breed A but found in breed B because of its distinct function and identity (Hall, 2005). What is 'designated' desirable or undesirable by humans, or by the evolutionary process, is relative to circumstance, but any impact on the structure of the equine genome is absolute. One key result of selection, if there is pressure in a particular direction, however achieved, is a lessening of genetic variability.

Genetic variability can be measured through the number of different alleles at various gene loci on the chromosomes. Another way of evaluating genetic variability is the percentage of gene loci that are heterozygous (carry different alleles) for an individual or population (Price, 2002). The processes outlined above can all result in a reduction of genetic variability. A 'special case' of this type of process of genetic restriction, which is of particular relevance to the modern horse, is inbreeding. That is, mating between animals which share a close genetic ancestry.

Continuous inbreeding results in a decrease in genetic variability. One possible outcome of inbreeding is that harmful recessive genes which have previously been masked by 'good' dominant genes are expressed through inbreeding ("Inbreeding depression") and cause a reduction of fitness and vitality of the individual animal (Price, 2002). This outcome is not inevitable, however, as it may be that harmful recessives are not present in the lineage, in which case inbreeding may fix other traits in a genetic line without exposing that line to harmful outcomes.

Inbred individuals may carry two genes that are replicates of the same gene in a previous generation. These two genes which have originated from the replication of one single gene of the previous generation are called identical by descent (Falconer, 1981). The level of homozygosity and therewith the degree of relationship between mating pair, is expressed through its inbreeding coefficient (Falconer, 1981). The inbreeding coefficient is the percentage of chances for two alleles to be identical by descent. Inbreeding is sometimes practiced together with artificial selection in order to reach a certain phenotypic goal. Through constant inbreeding, a degree of homogeneity and constancy of characteristics can be attained that is normally not found in wild populations (Price, 2002). Heterozygosity on the other hand describes the amount of alleles available in a gene pool, and therefore symbolizes the genetic variability.

In the context of this study, inbreeding is an important factor because it can have effects on cognitive abilities in mice (Deckard et al., 1988). Deckard et al (1988) mated brother – sister mouse pairs for six generations. The increasing inbreeding coefficient with every generation resulted in an increase in the number of trials required to learn an active avoidance task.

Since two of the three tested groups in this study are affected with inbreeding in the course of their history, it was investigated if the above is also the case in horses. Sufficient pedigree information of all test groups in this study was not given in order to calculate each individuals inbreeding coefficient. All stated inbreeding coefficients of the test breeds are derived from most recent calculations available in the literature. Nevertheless, a more detailed insight in each test groups history and genetic overview is given below.

2.2 Genetics and Behaviour

To what extent genetics plays a role in equine behavior is not properly known yet (Houpt & Kusunose, 2001). Observed behaviour is a composite of physiological changes, such as the pattern of muscle contraction and the cognitive impulses driving these changes, which provide them with their direction and focus (e.g. allowing the horse to run from a threatening situation) and level of intensity with which they are engaged (e.g. stronger contractions will occur if the horse needs to run at high speed). It is shaped both by environmental and genetic characteristics of the horse. In one sense, behaviour is inherently a product of 'genetic determination', since it relies on the development of sensory organs, the neuronal system and the muscular system (Jensen, 2006). Behavioural traits are polygenic, meaning that a large number of genes are generally involved in even the basic elements of a behavioural sequence (Roubertoux et al, 1998). Behaviour is primarily a product of the Central Nervous System but hormones affect behavior as well.

Modern genomics, for example the sequencing of the equine genome, has enabled us to understand genetic codes and ethologists have developed tools for measuring and quantifying behavior. However, the link from DNA to observable behavior remains obscured by its complexity. The understanding of this link is vital in understanding how behavior is shaped by evolution (Jensen, 2006).

What is clear is that artificial selection (such as that to which the horse has been deliberately and accidentally exposed) can have dramatic effects. The experiment of Belyaev et al (1979) draws attention to this. Silver Foxes (*vulpesvulpes*) were selected for tameness towards humans. After only a few generations, they produced progeny which showed friendly behaviour towards humans (comparable to behaviour of domesticated dogs). Also in contrast to their 'wild type' relatives, these offspring had piebald fur, a rolled tail and several behavioural problems among which were abnormal maternal behavior. The selection for one phenotypic trait affected the expression of other traits. This interconnectedness of genes is called pleiotropy. It describes the combinations of genes and the physiological or behavioural traits they support. Belyaevs´ study shows the, sometimes undesired, changes that occur through the selection of traits. It could be assumed that artificial selection during the course of domestication and nowadays used in horse sports could have an effect on the cognitive abilities of horses which leads to the objective of this thesis.

Especially the genetics of learning behaviour are still to be investigated. A systematic review of the literature concerning learning behaviour of horses by the Equine Research and Information Centre in Macclesfield, England, in 2010 has shown that there is no evidence to date that provides information on the link between genetics and learning behaviour in horses.

Only few studies investigated learning behaviour with regards to genetics in horses and if so, mostly through breed comparisons. Mader and Price (1980) compared the learning behaviour of Thoroughbred horses with Quarter Horses with the result that the Quarter Horse learned to perform a three choice discrimination task significantly faster than the Thoroughbreds. The authors of this study assume that the learning differences reflect the different artificial selection pressures on the two breeds for their roles in the horse industry.

The much larger non genetic literature has focused on the psychological dimension of learning in horses (Murphy & Arkins, 2007). Ultimately, behaviour is always produced via the interplay of genetics and environment but there is still a lack of studies which evaluate the role of each of these factors.

2.3 History and genetic overview of the breeds investigated

2.3.1 The Przewalski Horse *(Equusferusprzewalskii)*

Przewalski Horses were first brought to the attention of the western world by Colonel Nikolai Michailovich Przewalski, who came across the breed in 1870 on an expedition to Central Asia. He received the skull of a Przewalski horse as a gift when crossing the Russian-Chinese border. The skull was later taken to the Zoological Museum of the Academy of Science in St. Petersburg, where scientists confirmed it as the skull of a previously unidentified wild horse. In 1879/1880, Colonel Przewalski had the chance to observe two groups of Przewalski Horses and describe their build, their appearance and their social life (Boyd & Houpt, 1994)

Przewalski Horses are native to the plains of Mongolia. Up until the 1940s, they led a relatively safe existence, since they had few natural predators and the Mongolian people were pastoralists who relied on cattle for meat and possessed only primitive one shot firearms which presented little danger to Przewalski numbers. This position changed in 1945-1947 with the settlement of Southwest Mongolia by the Chinese Kazakhs. Equipped with sophisticated firearms and with a culture more prone to hunting than pastoralism, the Kazakh people presented a significant threat to the Przewalskis. This threat was not ameliorated by largely unenforced legislation outlawing the hunting of wild horses (Boyd & Houpt 1994) and the Przewalski population continued to show a substantive decrease in numbers. Prior to recent introduction attempts, the last sighting of a Przewalski horse in the wild was in 1968 by the Mongolian scientist N. Dovchin.

Both the decimation of the original native population and subsequent attempts to preserve the breed in captivity have resulted in high inbreeding coefficients amongst the remaining Przewalskis and it is highly likely that the breed is subject to the consequences of genetic drift outlined above. To summarise the history of the captive population, which remains the most populous to date: The first attempt to capture Przewalskis alive was carried out in 1897. The first *successful* attempt was carried out in 1901. Since all attempts focused on foals, generally unweaned foals, which were easier to capture, the mortality rate was high. Following the 'successful' attempt in 1901 only 28 foals survived the trip to 10 zoos located in 8 countries (Boyd &Houpt 1994). Of these, only 11 have contributed their genes to the current captive population (Bouman 1977).Despite additional attempts to import 'fresh blood', the adverse impact of the Second World War[1], plus the lack of any consistent exchange and breeding programme resulted in a peak inbreeding coefficient in 1965 of 0.597 of the European zoo population at the time (about 56 horses) (Boyd & Houpt, 1994). The situation subsequently improved slightly, with the introduction of a 12th wild Przewalski (a mare, with low genetic relations to the animals in the zoo population and therefore potentially adding a broader range of alleles to the gene-pool of the zoo population) and, following a 1976 symposium on the Preservation of the Przewalski horse, more concerted efforts have since been

[1]Only 31 horses survived the war, of which only 3 males and 6 females succeeded in reproducing, a situation which took ten years to recover from (Bouman 1977)

made to lower the inbreeding coefficient and improve reproductive outcomes. In 1978 the Foundation for the preservation and the Protection of the Przewalski Horse (FPPPH) was founded. An international group of scientists is now investigating the genetics and hereditary diseases of the Przewalski horse and options for the exchange of stallions is discussed biannually at a meeting at which the current genetic status of the Przewalski horse is also considered (Boyd &Houpt, 1994). The first steps towards 're-wilding' of captive horses have now been taken, with the reintroduction 1992 of 16 Przewalski horses to HustainNuuru, a steppe region, 120 km away from the Mongolian capital Ulaanbaatar (FPPPH, 2004). Today, more than 300 horses roam their natural habitat again.

Notwithstanding recent advances in the genetic conservation of the Przewalski horse, it is clear that the few captured animals initially available went through at least one if not more genetic bottlenecks. Currently available gene pools are substantially smaller than those which would have existed in the original wild herds and gene frequencies will have changed (Bowling & Ryder, 1987). The most recent estimated inbreeding coefficient for the Przewalski Horse are 0.273 for the Munich line 0.273 and 0.142 for the Prague line idem is 0.142 (Bowling & Ryder 1987).

The captive bred Przewalskis present an interesting proposition for testing theories relating to the origins of equine behaviour and function. Whilst they are highly inbred, for the above reasons, they retain the original wild-type genes of their founder population ancestors. There has to date been no consistent attempt at artificial selection for any specific purpose (other than breed conservation) and also no successful attempts at domestication (strictly defined). With regard to equine (as opposed to equid) breed types therefore, they provide us with one of the closest models we have to a horse in the 'natural state'.

Technically, the Przewalski Horse can be regarded as a separate species rather than a breed, although it is able to interbreed with other horse types. It is not only distinct from the domestic horse in its outer appearance and likely genetic profile, but also in its genetic structure or karyotype. It is excluded from common ancestry with the domestic horse due to this unusual karyotype. The Przewalski horse has a set of 66 chromosomes whereas the domesticated EquusCaballus only has a set of 64 chromosomes (Bowling &Ruvinsky, 2000). As noted above, today's population of Przewalski horses is derived from 13 founder animals. Only descendants of the captured wild animals were karyotyped. If however, a translocation occurred recently (over the last 1000 years), other Asian populations of Holocene wild horses might still have 64 chromosomes (Kavar & Dovc, 2008). Identifying such horses would provide even greater opportunity for exploring the origins of modern equine behavioural and physical characteristics.

2.3.2 The Thoroughbred *(Equuscaballus)*

During the reign of King James I (1603-1625) Arabian horses were imported to England and crossed with native light horses. By around 1660-1685, 50 foundation mares of the breed ("Royal Mares") were imported. Between 1689 and 1728 three stallions called Byerly Turk, Darley Arabian and Godolphin Barb were brought to England. They are the foundation sires of the Thoroughbred horses (Thiruvenkadan et al, 2009). All English and American Thoroughbreds are descendants of one of these stallions. Every name in pedigree can be traced to one of the 50 "Royal Mares" on the female side, and to one of the three sires on the male side (Thiruvenkadan et al, 2009).

The first official recording of Thoroughbreds in England was made in 1791 by James Weatherby in his first volume of the General Stud Book. The breed has had a closed studbook for 200 years (Bowling & Clark, 1985).

The Thoroughbred horse has primarily been bred for racing purposes. They have been developed for speed at intermediate distances. Other than that, the Thoroughbred is well suited for activities such as hunting, dressage, polo and jumping and has also influenced the development of many other breeds, as it is used as a foundation for the development of lighter breeds (Thiruvenkadan et al, 2009). For the most part though, it is used for galloping at speed. Bowling and Clark (1985) found out that the Thoroughbred has comparatively little variation in blood type markers (A, B, AB and 0 plus the rhesus factor) which stems from the fact that they have been selected for a single trait of moderate to high heritability combined with 200 years of a closed studbook. Compared to six other breeds, they showed the lowest heterozygosity value of 0.378. This heterozygosity value describes the diversity of alleles within the genome in contrary to the inbreeding coefficient which is based on estimates of the identity by descent of the alleles in relation to ancestral alleles (Falconer, 1981).

2.3.3 The Paso Fino

The Ice Age about 10,000 years ago caused the deterioration of grasslands and therewith the extinction of Equidae on the American continent. The first horses to repopulate this continent were brought by Christopher Columbus on his second journey to the New World. 25 horses of Barb-type landed in what is today the Dominican Republic at the end of 1493 (Bravo, 2007).

The horses were previously interbred in North Africa and the Iberian Peninsula which resulted in multiple gaits (trot, pace, gallop). The pacing groups of the Barb breeds which are characterized by a two beat, lateral gait, were crossed with horses of Iberian breeds such as the Andalusian, the Lusitano and the Spanish Jennet. The Paso Finos natural isochronic gait was characteristic to most riding horses before the 17^{th} century. It is highly distinct to the square trotting gait of the modern horse (Bowling & Clark, 1985) which was developed to enable the horse to pull a carriage or cart smoothly. Breeding selection in respect of the Paso Finos was based on the ability to work and travel many hours daily over mountainous terrains whilst maintaining a smooth gait which would allow the rider to show equal endurance.

These horses and horses from future trips to the New World produced offspring which, from 1500-1520, spread throughout the territory of the Spaniards (Bravo, 2007). The possession of horses was an enormous advantage for the invading Spaniards, allowing them to dominate the native tribes both physically and psychologically. Over the next five centuries these horses have developed into the Paso Fino Horse we know today (Bravo, 2007).

The Paso Fino Horses were first introduced to the United States in 1950. They were imported from Puerto Rico, Colombia, Venezuela and the Dominican Republic and are now a steadily growing breed in numerous countries worldwide (Bravo, 2007).

The fact that the Paso Fino is a relatively young breed and has descended from the interbreeding of a variety of distinct breed types, has resulted in fairly high levels of heterozygosity (0.551) (Bowling, 2000). As a research model, the Paso Fino therefore presents as a horse breed which has been fully domesticated, but which, as yet, has not experienced the genetic ‘streamlining’ associated either with genetic bottlenecks or with artificial selection. It was not possible to locate an inbreeding coefficient for the Paso Fino group. An estimated value was calculated using the same procedure as Goodloe et al (1991), who used a formula based on heterozygosity to calculate inbreeding coefficient, which is the net difference between observed and expected numbers of heterozygotes at a locus divided by the number of heterozygotes observed. Luis et al (2006), provide heterozygosity values for the paso fino which are 0.450 for observed and 0.446 for expected. 0.450-0.446/0.450 = 0.008 inbreeding coefficient, which is low. The Thoroughbred group had a high inbreeding coefficient (Mahon and Cunningham, 1982).

2.4 Principles of learning

In the context of this study literature on learning behaviour in horses has been thoroughly searched and summarized below.

All learning processes can be allocated to two major categories: 1) Non-associative learning, where there is a single stimulus to which the horse can become habituated. 2) Associative learning where a relationship between at least two stimuli is established (McGreevy, 2004).

2.4.1 Habituation

Non associative learning (Habituation) can be seen as the simplest form of learning. It involves the repeated presentation of a stimulus, which eventually causes a diminution in response. This form of learning is commonly used in the training of horses. A good example is the education of police horses, where horses are 'acclimatised' to a variety of sights and sounds that they may encounter whilst out on patrol.

Jeziersky et al (1999) showed that habituation to handling can reduce reactivity. In their study, two groups of Konic horses were compared. One group were handled daily from two weeks of age and the other group was reared, up to weaning age, in a forest environment free from human contact. It was found that horses which were handled daily had a reduced heart rate and increased manageability in comparison to the horses receiving no human contact.

2.4.2 Classical and operant conditioning

Associative learning is subdivided into classical and operant conditioning. In classical conditioning a response to a new stimulus is established by association with an already established stimulus. Essentially, classical conditioning couples a stimulus with a physiological response (McGreevy, 2004). Classical conditioning has its origins in the experiments of Ivan Pavlov in 1901.Pavlov found that experimental dogs started salivating when seeing the technicians who normally fed them in their white lab coats, even before any food was presented to them. The dogs were associating the technicians with the food. In subsequent investigation, Pavlov conditioned the dogs to a buzzer before presenting the food. He did this by ringing a buzzer each time the dogs were about to be fed (Mills & Nankervis, 1999). The dogs eventually began to salivate on hearing the sound of the buzzer. This is known as a conditioned response. The buzzer, which had previously been irrelevant, became a conditioned stimulus. The food itself is known as an unconditioned stimulus, as the animal responds to it with no conditioning.

With regard to horses, conditioned responses can be illustrated in a number of common training situations. Breeding stallions, for example, can become conditioned to their breeding bridles. If the same bridle is used each time the stallion is taken to cover a mare, the sight of the bridle alone will soon produce a sexual response in the stallion (Hart, 2008).

In the case of operant conditioning, the operant response is a voluntary activity on the part of the animal that results in a reward. In 1911, Thorndike placed cats in puzzle boxes which could only be opened by pulling a specific mechanism. The cats initially stumbled across the correct way to escape by chance, but with ongoing trials they learnt to pull the correct mechanism. In a similar way, Skinner (1938) trained rats to pull levers or negotiate for food rewards through trial and error.

Another example would be a horse which has to open the lid of a box in order to get the food placed in the box. The horse sees the cue (the box), performs a response (opens the lid) and gets the reward (food).

The effect of the reward is to strengthen the response. The reward is a **reinforcer** which follows a particular behavior so that the frequency of that behavior increases (McGreevy, 2004).

The difference between classical and operant conditioning is that in operant conditioning the animal is able to link a set of events over which it has some control. This gives the animal the possibility to control its environment. In classical conditioning, innate behavioural responses are directly linked with a stimulus in such a way that reflex responses result (McGreevy, 2004).

2.4.3 Reinforcement

To increase the likelihood of a desired response, animal trainers use a reward immediately preceding the behavior to indicate to the animal that it has made the correct response. This is known as reinforcement. Reinforcement can be divided into positive reinforcement (giving the animal something it likes) e.g. giving a food reward or stroking a horse for displaying a desired behavior and negative reinforcement (removing something the animal doesn't like) e.g. releasing the pressure of the rider's leg when the horse moves in the desired direction (McCall, 1990).

Innes and McBride (2007) trained two groups of welsh ponies to be led and to stand whilst being groomed, to negotiate an obstacle course and to be loaded onto a trailer. One group was taught using positive reinforcement, whilst the other group learned through negative reinforcement. Significant differences were found in regard to the training technique used, with the positive reinforcement group being more motivated to participate, exhibiting more exploratory behavior using a 'trial and error' approach in new situations and thus were more likely to obtain the correct response.

Reinforcement can be further divided into primary and secondary reinforcement. Primary reinforcers (natural reinforcers) are resources the horse has evolved to seek. For example food, water, sex, play, liberty or companionship (McGreevy, 2004).The horse does not have to learn that primary reinforcers are valuable, so they can function in a context involving these reinforcers without previous experience (Hart, 2008). Secondary reinforcers are neutral stimuli and are not in themselves rewarding, but by pairing them with primary reinforcers the horse can be conditioned to respond to them (Warren Smith, 2006). A popular secondary reinforcer is the Clicker which is a small plastic box contains a metal strip which emits a distinct double clicking sound when pressed. If the horse performs the desired behavior this is marked with the click and the horse is subsequently rewarded. Clicker training is a method of increasing the frequency of the desired behavior, the clicking sound serves as a bridging stimulus which fills the gap between the desired behavior and the delivery of the primary reinforcement. When the clicker is first used, the association is established just before the food is delivered. The clicker is a signal to inform the horse that it has performed the correct response which is always followed by the reward (Hart, 2008).

2.4.4 Shaping

Behaviour patterns are often shaped with a clicker. By rewarding successive approximations, a desired behavior can be formed (McGreevy, 2004). The shaping of a behavior pattern can be illustrated using the example of teaching a horse to target an object (for example a lid of a feed bucket). The horse is firstly rewarded for approaching the target and then for interacting with the target e.g. sniffing and eventually touching and following the object. It is important to reward desired responses as soon as they happen. A delay will lessen the effect of the reward (McGreevy, 2004). Target training has been found to be effective in teaching horses to enter confined areas such as trailers. For example Ferguson and Ruiz (2001) used targeting to train horses to load into a trailer and found that undesirable behaviours associated with reluctance to load reduced to zero with the target training.These effects continue to be observed when the situation has been generalized to different trailers and different trainers.

2.4.5 Learning ability

Learning abilities in horses have been tested in various ways such as Maze learning (McCall, 1981, Marinier, 1994), observational learning (Baker & Crawford, 1985), concept formation (Hanggi, 2003), categorization learning (Sappington & Goldman, 1994, Hanggi, 1999), avoidance learning (Visser, 2003), reversal learning (Fiske & Potter, 2010) and matching to sample tasks (Samuelsson, 2008).

Discrimination tasks are a commonly used method to evaluate learning in horses. The horses have to learn which feature of the task is relevant and remember this over a number of trials (Proops, 2008). Horses learn to concentrate and to react to rewarding stimuli and also learn that irrelevant stimuli can be ignored. This permits the behavioural developments that make discrimination possible (McGreevy, 2004).

Gardner began a series of experiments on the discrimination ability of horses in 1933. The horses were required to choose one of three covered feed boxes. A black cloth signaled in which box a food reinforcer could be found. This study showed that the horses were capable of learning this simple choice discrimination task. Sappington and Goldman (1994) tested four horses using a task in which the horses had to discriminate between black and white cards. All four horses were able to reach criterion although a wide variation in the number of sessions needed was shown. In a second task, the horses had to discriminate between a white card with a black cross and a white card with a black circle. Only three out of four test horses met criterion in this task but the mean number of sessions reduced from the first to the subsequent problem.

Hanggi (2003) investigated the discrimination ability of horses using three-dimensional objects. The horses were required to choose the larger of two objects over a series of tasks (i.e. objects of different materials and colours). One horse showed a correct response significantly above chance no matter which material, colour or combination. Studies like this show that horses are capable of higher learning ability.

Other discrimination abilities like human facial discrimination (Stone, 2009), colour discrimination (Hanggi, 2007), tactile discrimination (Dougherty & Lewis, 1993) and peak shift in visual discrimination (Dougherty & Lewis, 1991) are examples of further stimulus discrimination experiments that have been carried out. These interesting studies notwithstanding, research on the cognition and the learning ability of horses remains limited.

Thomas (1986) produced a hierarchy of learning abilities which consists of eight levels. The hierarchy ranges from habituation, classical conditioning, simple operant conditioning, chaining operant conditioning, concurrent discrimination, concept learning and conditional concept formation to biconditional concept formation. Horses have been shown to be able to successfully form concepts (Hanggi, 2003) and therefore reach level seven on the Thomas' hierarchy of learning.

Although it often takes time for horses to understand the concept behind discrimination tasks, once they have understood the learning set, their accuracy and speed increases. This phenomenon is described as "learning to learn" (McGreevy, 2004).

Chapter 3

Materials and Methods

3.1. Animals, location, housing and management

A total of nine horses were initially used in this study consisting of three Przewalski horses, three Thoroughbreds and three Paso Fino's. During the study it was found that one of the Przewalskis had previously had a stroke, which may have affected her learning ability so was withdrawn from the study, leaving a total of eight horses. Due to the variety of breeds it was necessary to source horses from different locations; details of these three groups of horses are outlined below.

3.1.1 Group One: Przewalski Horses

This group consisted of two mares located at the Welsh Mountain Zoo, ColwynBay, North Wales in a herd consisting of five horses and a large herd of red deer. Anna aged 20 is a captive born Przewalski and is the lowest animal in rank. Katrina the alpha mare of the herd was also born in captivity and is 19 years old. The horses are kept in two paddocks on rotation. The smaller paddock is of approximately 2 acres and the larger paddock is of approximately 6 acres. They are kept outdoors all year round and provided with ad lib hay, grass and water. Additionally, they are fed with concentrates twice a day. The horses are not handled whatsoever, they are darted for veterinary and farriery procedures. This experimental group only consists of two horses whereas the other groups consist of three horses. It is important to note that this study is a pilot study. In order to conduct a future full study, it would be advisable to identify a situation in which Przewalski horses could be worked on an individual basis. A useful location would have access to a confined area in view of the herd.

3.1.2 Group Two: Thoroughbreds

This group consisted of two geldings and one mare located at Bondwood Farm in Repton, Derbyshire in the UK. Eddie, a retired racehorse, is 15 years old. Donnie, 13 years old, is also a retired racehorse and still gets ridden as a leisure ride. Sky is a retired broodmare and is age 18. All three of these horses are kept out in group paddocks day and night. They are fed concentrates twice a day plus ad hoc hay at night. The horses are handled by staff on a daily basis. Prior to their retirement as racehorses, all have been involved in active racing training. They were started with walking and trotting before going on to gallops of increasing distances and speeds. Donnie and Eddie were also schooled over hurdles and jumps as they were National Hunt horses. Sky was a sprinter but similarly trained on a daily basis.

3.1.3 Group Three: Paso Finos

This group consisted of a stallion, gelding and a mare located at the Equine Research and Information Centre, Wildboarclough. Raphael a 7 year old stallion is kept in a field with a retired Arab gelding. The mare (Quito) aged 11 and the gelding (Pincel) aged 11 are housed in a field together with two native breed ponies (a Highland gelding and a Welsh section A mare). The horses are housed in the pasture day and night all round the year. They are provided with free access to grass and water, supplemented with hay and concentrates during the winter. The horses are not ridden and currently are not involved in any physical training. However, they are handled on a daily basis.

3.2 Test areas

Due to the nature of the different breeds of horses and their locations, it was not possible to use the same test area for all the horses.

Group one: Przewalski's

As the Przewalski's were kept unhandled in an open field setting, it was necessary to carry out the data collection on location in their home paddocks. It was also not possible to isolate an individual horse to work with, due to their strong herd instinct, so research was carried out within the herd.

Group Two: Thoroughbreds

It was decided not to use the home paddock in which the thoroughbreds were kept to conduct the data collection for two reasons. Firstly the horses were kept in large mixed groups including youngsters and secondly the horses are used to being handled and fed, so gather round humans when they enter the paddocks. This situation could have been dangerous and would not have been conducive to data collection, so it was decided that data collection would be carried out in a large foaling box approximately $10m^2$. Each horse was worked with on an individual basis.

Group Three: Paso Fino's

Data collection was conducted in a small paddock, approximately $15m^2$, adjoining the horses' home fields. This allowed each horse to be isolated from but in sight of his/her companions, providing a safe working environment.

Within all three groups, all horses have been tested individually. However, it was more difficult to collect data of the Przewalski horses as they could not be individually confined. It was particularly difficult to gain and keep the Przewalskis attention as they were free to roam across a substantially sized paddock. As the herd bond was extremely close, horses that were participating in a trial were always watching the herd in order to follow the other horses should they move on. The Paso Fino horses as well as the Thoroughbreds were able to be confined to smaller Paddocks or boxes and so there attention was more focused on the task.

3.3. Ethics

As all procedures were conducted with the animals at liberty in their home environment, the horses were not exposed to any novel adverse factors. The methods involved the use of positive reinforcement and the horses were never forced to interact with the humans or objects. All establishments were informed of the nature of the study and their permission was granted. Researchers had substantive experience of handling horses and particular care was taken to avoid exposure to potentially hazardous situations when working in the field setting with the Przewalskis.

3.4. Training Procedure

Prior training was necessary to ensure the three groups of horses had a common baseline. The three groups were kept at different locations and used for different purposes and had therefore received differing amounts and types of training and had varying experiences with humans and novel situations.

The Przewalskis are rarely handled and are often darted, making them wary of people and unusual objects, it was therefore necessary to gain their trust. This was achieved using a clicker training programme, this programme also served to bring all the research horses to the same learning baseline.

3.4.1 Clicker training

The Clicker training programme was based on the methods used by Bruce (2009) who has developed a manual on how to clicker train horses. The tasks were designed to be achievable and not fear -provoking for the horses. Since the Przewalski horses used for this study are not handled by humans whatsoever, safety measures needed to be taken. That is why the clicker training methods described in the manual by Bruce could only be carried out to an extent that ensured safety for handler and animal. The three tasks used were: look away from handler, touch a cone and touch a target stick. All three tasks discourage the horse from 'barging' the human for food, whilst still allowing the horse to be sufficiently close to the human to develop confidence in interaction.

Horses were initially introduced to the clicking noise and given a reward immediately after the click in order to establish an association between the noise and the food reward. After a number of repetitions, the handler positioned herself next to the horse and started clicking as soon as the horse turned its head away from the handler. The horses were required to deliberately turn their head away from the handler (Picture 1). After this movement had been established, the handler stood on the other side of the horse which was now required to turn its head in the direction that had previously been incorrect. This formed the first task within the clicker training scheme.

Picture 1: Paso Fino mare Quito looking away

The horse was thereafter introduced to a red cone approximately 20cm in height and 11cm in diameter. The handler held the cone very close and waited for the horse to sniff it. As soon as the horse touched the cone with its nose, the handler clicked and rewarded the horse. Through successive approximations, the behavior of the horse was shaped towards deliberately touching the cone which was moved closer to the ground (Picture 2). Thereafter, the cone was placed on the ground, the horse touched it, rewarded and then the cone was moved to a different position, the horse went to touch it, was rewarded and so forth. This formed the second of the clicker training tasks.

Picture 2: Paso Fino Pincel touching the cone

The third task required the horses to touch a target stick (Picture 3). The tool used in this experiment originated from dog training. It is a 60cm long, blue stick with a handle on one end and a yellow ball on the other end. The horse is required to touch the yellow ball at the end of the target stick. The handler is holding the stick in a way that the yellow ball is very close. As soon as the horse touches the ball it gets clicked and rewarded. The yellow ball is now positioned further and further away from the handler. The horse is required to go up to the ball and touch it no matter in which position it is placed (i.e. close to the ground or higher up).

Picture 3: Przewalski Horse Anna touching the Target Stick

The horses were required to perform 10 consecutive correct behaviours for each task prior to commencing with the discrimination task. Each individual was granted the different number of attempts to reach this criterion.

3.5 Discrimination test design

The discrimination was designed as a simple two choice task. Two objects identical in size, colour and texture but differing in shape were used as the stimuli. One object was a plastic plantpot which was presented upside down and the other object was a plastic box. From the horses view the plantpot had a round shape, whereas the box had a rectangular shape. Both objects were covered with silver gaffer tape to ensure their similarity. Prior to commencing the discrimination task, a preference test was conducted to ensure the horse did not have a bias towards the correct object. Whichever object the horse initially selected, the alternative object was designated as designated as the 'correct' choice for the trial.

3.5.1 Test Procedure

The experimenter simultaneously placed the two objects approximately 50cm apart, one meter in front of the horse. The horse was then required to touch the object it had not selected during the preference test. Each correct touch was marked with a click and followed by a reward, the clicker was chosen to mark the correct response as it had been used in the training phase and it also allowed the experimenter to feed the horse between the two objects. If a food reward had been used in the absence of the clicker, the horse would have needed to be rewarded near the correct object, thus creating a side preference. To avoid such bias, the experimenter always gave the food reward with the horses head between the two objects. A hand-delivered food reward was used rather than an automated system due to the nature of the Przewalskis. An automated system could have startled them and also caused a risk of mobbing.

As soon as the horse had reached ten consecutive touches, the objects were picked up by the handler and their sides were exchanged. This was done in order to rule out the bias of sidedness. The objects were placed back down on the ground and the horse had to continue touching the correct object which was now on the other side. Once ten consecutive touches were achieved a trial was designated as complete. Each horse had to perform 15 trials in total.

All trials were video-taped. The taped material gave the experimenter the opportunity to accurately measure the response phases of each horse and it ensured that no response a horse made was accidentally missed. The video-taping was used to ensure objectivity and accuracy.

During the video analysis of the discrimination task, the total number of hits, divided into the number of correct hits and the number of errors, was counted. After that, the percentage of correct hits and the according percentage of errors were calculated. Additionally, the time the horses needed from the moment the objects were placed on the ground until the horse reached ten consecutive correct hits and the time taken to touch a correct object within each trial was measured. This was done individually for each horse and for every trial. All horses performed fifteen trials in total. These variables were chosen according to the research objective.

3.6 Testing Materials:

- Canon XL1s 3CCD Digital Video Camcorder
- Clicker
- Treats (Badminton Horsetreats)
- Objects
- Videotapes

3.7 Data Analysis

Data were analysed using SPSS V.18.

Identified Variables

The meaning of the identified variables is described in the following paragraph:

'Trial': This variable described the trial number (1-15).

'Subtrial': This variable described the subtrial number within each of the 15 trials. The measure of a subtrial is described below.

'**Subtrial time'**: Measured time from placing the objects on the ground until the horse performs the first touch and thereafter the time from receiving food reward to the next performed touch. This variable varied for each horse depending on how quick the horse was to touch the correct object ten times in a row without touching the wrong object in between.

'**Average time to touch correct object within trials'**: This variable describes the time measurements of all subtrials added together and then divided by the amount, giving an average time for each trial.

'**Time to reach criterion'**: This variable is the core variable of the study. It presents the time the horses required to touch the correct object ten times in a row. Time from placing the objects on the ground until the horse touched the correct object ten consecutive times. Every trial contained a varying number of subtrials.

'Number of total hits': This variable counts the total number of touches (both incorrect and correct object) the horse performed within one trial.

'**Number of correct hits'** The number of times the horse touched the correct object within one trial is described.

'Number of errors': The number of times the horse touched the incorrect object and therefore made an error within one trial was counted in this variable.

'Percentage of correct hits': The percentage of correct touches within one trial was calculated here.

'**Percentage of errors'**: The percentage of errors made by touching the incorrect object was counted.

'Age': Age of horses

'Gender': Gender of horses

'Breed': Respective breed of the horse

'Correct Object': Plantpot or box, if the horse initially chose the box, the plantpot was designated correct.

'Side of correct object': Respective side (left or right) of the designated correct object

'**Wild Type'**: This variable stated if the horse still has a wild type genome.

'**Inbreeding**': This variable describes the level of inbreeding the horse shows.

'Heterozygosity': This variable describes the level of heterozygosity of each horse.

'Domestication': Here the domestication background of the horse is described.

The table below shows an overview of the nature of essential variables used in the study:

Variable	Continuous/Categorical	Dependent/Independent
Time To Criterion	Continuous	Dependent
Number of Total Hits	Continuous	Dependent
Number of Correct Hits	Continuous	Dependent
Number of Errors	Continuous	Dependent
Percentage of Correct Hits	Continuous	Dependent
Percentage of Errors	Continuous	Dependent
Age	Categorical	Controlled
Gender	Categorical	Controlled
Correct Object	Categorical	Independent
Side of Correct Object	Categorical	Independent
Breed	Categorical	Controlled
Trial	Continuous	Dependent
Subtrial	Continuous	Dependent

Table 4: Nature of the variables used in the study

A Chi square analysis of association was done to find possible correlations of different variables (a detailed description can be found further along in this chapter) and then, a multivariate analysis was carried out where four factors for each breed were investigated.

Firstly, the breeding **selection** is considered. The Paso Fino group still has a wild type genome because of the breeding practices of their South American origin that did not until recently, artificially select which mare to breed to which stallion. The Przewalski group remains its wild type genome as well because it is not artificially selected. The Thoroughbred on the contrary does undergo artificial selection which is focused on a limited number of desirable traits for a substantial period of time already.

The second factor is **inbreeding**. The Przewalski group as a high inbreeding coefficient, which for the Munich line is 0.273 and for the Prague line is 0.142. Due to its natural selection prior to capture, 'key traits' like learning ability are likely to have been conserved as they are important to survival. It was not possible to locate an inbreeding coefficient for the Paso Fino group. An estimated value was calculated using the same procedure as Goodloe et al (1991), with an inbreeding coefficient value of 0.008 inbreeding coefficient, which is low. The Thoroughbred group had a high inbreeding coefficient (Mahon and Cunningham, 1982).

The **heterozygosity** level of the breeds represents the third factor in this analysis. The Przewalski horses and the Thoroughbreds have a relatively low heterozygosity level of 0.474 and 0.38 respectively, whilst the Paso Finos have a comparatively high heterozygosity level of 0.551 (Bowling, 2000).

Each horse was used as a 'breed representative' and not as an individual in terms of their cognitive ability. For this reason inbreeding coefficients and heterozygosity levels of the breeds as a whole were used instead of calculating each horses individual inbreeding coefficient and heterozygosity level. Further, pedigree information was not sufficiently available for all horses in order to calculate each horses individual values.

The last factor is **domestication**. The Thoroughbred and the Paso Fino horse both are domesticated horse breeds whereas the Przewalski is not.

These factors undergo the following comparisons:

Wild type can be compared with non wild type: This would be a comparison of the Thoroughbreds with the combined Paso Finos and Przewalskis.

High inbreeding can be compared with low inbreeding: Compares the Przewalski and the Thoroughbred with the Paso Fino.

High heterozygosity compared to low heterozygosity: Again, compares the Przewalski and the Thorougbred with the Paso Fino.

Domesticated breeds with non domesticated breeds: Compares Thorougbreds and Paso Finos with Przewalskis.

It is important to note, in setting out the results of this study, that the sample size reflects the fact that this study is a pilot study only. In total, 8 horses were available for the study; therefore all results are speculative at some level. It is assumed, that the horses recruited are, firstly, representative of the equine species as a whole (where data are analysed as a whole) and, secondly, that each horse is representative of its breed characteristics (where data are analysed on a within or between-breed basis). Where possible, these assumptions have been tested, for example by analysing data for individual horses within a breed to assess whether values differ significantly between horses or whether the horses in a breed group can reasonably be assumed to represent the same population.

Categories of variables

The first category contains the variables 'average time to touch correct object' and 'time to reach criterion' and represents the **speed of learning**. This category is the core category of the study as the main question was to find out whether there is a difference in the speed of learning between the three investigated breeds.

Category two describes the **responsiveness** of the horses to the task. It evaluates how open the horse is to exploring the situation and its motivation to perform the task. The variable 'total number of responses' is measuring the general responsiveness of each horse in terms of touches no matter if their correct or incorrect.

Category three is looking at the **accuracy** of the horses when performing the task. Variables in this category are 'number of correct responses', 'number of errors', 'percent correct' and 'percent errors'.

All categories correlate with each other. For example if a horse is very vary of the object and takes more time to motivate itself to touch an object its responsiveness is decreased and the time variables are increased. However, if it thinks through the task more in depth, its accuracy level may be better than that of another horse that is highly motivated but therefore makes numerous errors as well.

Category four is **learning to learn**. It looks at the change of speed the horses show when performing the task over the duration of the trials. This variable looks at the improvement rate of the three breeds.

Testing the assumption of normality

The sample size for this study was too small to apply the Kolmogorov-Smirnov test for normality as this test becomes unstable with a sample size less N=50. Instead, absolute values for skewness and kurtosis and their standard errors were used to establish an overview over the distribution of the data. To apply parametric tests, the main focus lies on the degree of skewness. The rule of thumb is that the ratio of skewness to its respective standard error should be less than two. In this case the assumption of normality can be accepted. A similar rule applies to the evaluation of degree of kurtosis, but this ratio has less impact on the decision to use parametric tests, as a distribution can be kurtotic but nevertheless symmetrical.

All dependent variables were tested for normality for 1) all the horses combined together, 2) individual breeds and 3) individual trials. The logic here was that distributional data might help to clarify the nature of the learning variables within horses *as such* but also within breeds, should the breeds differ from one another in respect of one or more of the learning variables. For example, a normal distribution for horses as a whole might imply that breed differences are less important than individual differences, whilst a modal distribution, with modes express individual breeds would imply that learning characteristics are very strongly breed specific. The distribution of the data within a given trial was of potential importance since learning may occur both independently within a given trial, but also across the course of a number of trials, thus affecting the distribution of subsequent trials. For example, time to criterion data may become increasingly skewed towards quick response times as the number of trials increases.

Independent variables (age, breed, gender, correct object and side/position of object) were not evaluated for normality. Age is known to be normally distributed in horses as in other mammalian populations and, as sample values are used only as a proxy for actual population values, the assumption of normality in respect of age is better tested against actual population data. The remaining independent variables were all categorical and therefore not open to any evaluation of distributional characteristics.

In addition to providing initial exploratory data against which to evaluate the hypothesis, the distribution of the data also determined how the analysis should progress in statistical terms. If it was found that the distribution of a particular variable was positively skewed, it was transformed using square root, log10 or 1/x transformations of the raw data, depending on the level of skewness. If the variable was negatively skewed, transformations x^2, x^3 or antilog x were used again depending on the level of skewness. If the variable was still skewed after transformation with 1/x or antilog x in the negative case (the most substantive transformations), the decision was taken to run non-parametric tests on this variable. If however, the variable could be transformed to normality, parametric tests were performed.

Descriptive statistics

Descriptive statistics were applied in order to get an overview over the outcomes of the data. Here it was looked at the mean, the median, the standard deviation, the minimum and the maximum value if the variable was parametric. If the variable was non-parametric, the median, the minimum and the maximum value were looked at.

Analysis of improvement rate

In the next stage of analysis it was investigated whether the speed of learning shown by the horses improved over the duration of the fifteen trials. The variable 'Subtrial' is investigated in this context. This variable describes the time to touch the correct object from the point at which the objects are placed on the ground for the first time in each trial to the point at which the horse touches the correct object and, subsequently, the time from when the horse receives the food reward until the horse touches the correct object again. Where the speed of correct response increases from trial to trial, it can be said that the horse is 'learning to learn', that is, it begins to recognize in each new trial the same learning situation presented to it previously and so is able to respond correctly within a shorter period of time. For this variable a ROC (Receiver Operating Curve) analysis was used. The value of using a ROC analysis in this context is that it allows direct comparison between trials which have different number of subtrials within them. The area under the ROC curve (for a plot of time to criterion by subtrial) is used as a value which can be directly compared for each horse against itself, from trial to trial and also between horses from trial to trial. The null hypothesis tested is that the area under the curve is 0.5, which indicates that there is no difference between trial one and the rest of the trials, trial two and the rest of the trials and so on, for the time taken to touch the object.

Analysis of association between variables

The Chi square test was chosen as the measure of association for all variables except age (age was the only continuously distributed independent variable) firstly, because (used with the appropriate statistic for significance testing) Chi square is neutral to the nature of the distribution (parametric or non parametric). Secondly, chi square tests are relatively robust to situations in which there are only a limited number of data points.

In the following stage of data analysis, the associations between individual variables was investigated, with a view to identifying variables which would carry sufficient strength of association to justify further evaluation using multivariate statistical analysis. In line with the main focus of the research (speed of learning), 'time to criterion' and 'subtrial' were the two core dependent variables for which we explored associations with the independent variables 'Breed', 'Age', 'Correct Object', 'Side of correct Object', 'Gender', 'Wild Type', 'Heterozygosity' 'Domestication' and 'Inbreeding'.

For these analyses only a small number of data points were available, because of the limited number of tested horses and average values between trials had to be used. The format for the dependent variables (the way in which these were measured) were chosen following the analysis of the ROC curves. It was decided to use values of the dependent variables 'time to criterion' and 'time touching correct object' for trial one only. This is the *de novo* context, where horses are presented with the task for the first time, hence placing all horses on a 'level playing field' for the purposes of comparative analysis. For the same reasons, the alternative measures 'time to criterion' averaged over all 15 trials and 'time to touch the correct object' averaged over all 15 trials were chosen, rather than evaluating these variables on the absolute basis of trial by trial differences. The measure of association used in the context of age was the Pearson Product-Moment correlation coefficient in the case of the parametric independent 'time to criterion' variables and Kendalls tau b in the case of the non-parametric 'subtrial' variables.

For all Chi square measures of association, a continuous dependent variable was being compared with a nominal independent variable. The appropriate statistic to use as the measure of association was therefore eta. Eta does not return a significance level as such. However, according to Cohen (1988), when using eta, values for eta^2 can be used to estimate the strength of the association between variables. Small associations range from 0.02 to 0.13, medium associations from 0.13 to 0.26 and large associations from 0.26 to anything above. The correlation coefficient used to evaluate the association between age and the dependent variables returns a standard level of significance figure.

Regression Analysis

Having established the variables which showed a significant degree of association with the 'core' dependent variables, the next stage in the analysis was to explore further the nature of these relationships using multivariate analyses. The first focus here was to explore which of the independent variables, identified above as showing a statistically significant association with 'time to crtiterion' or 'subtrials', accounted for the greatest degree of variance in these variables. Since both of the 'core' variables were continuous and there was no *a priori* reason to assume a non-linear association between dependent and independent variables, linear regression was chosen for this purpose. Whilst the 'subtrial' variable was non-parametric and did not respond to transformation, linear regression is sufficiently robust to the violation of assumptions regarding normality to justify use in this context (Stoddard, 2010) and all other key assumptions made by this procedure were met by the data. Linear regression is also a procedure which is open to the mix of categorical and continuous variables which needed analysing. In line with the requirements of the linear regression procedure in SPSS, continuous variables were left in their original format, but categorical variables were re-coded into 'dummy' (0 or 1) variables. So, for example, 'breed' was coded using three sets of dummy variables (is the horse a Paso Fino: yes/no; is the horse a Przewalski: yes/no and is the horse a Thoroughbred: yes/no). For each of the dependent variables ('Time to criterion' and 'Subtrial'), the individual horses, of course, contributed more than one value each, since there were 15 trials in total with a varying number of subtrials for each horse. In statistical terms then, the values from a single horse are therefore not independent. To maintain sufficient datapoints in order to explore variance in the dependent variables, each datapoint was treated *as if* it were independent. However, this lack of independence was controlled for by including horse and trial number as independent variables, thus ensuring that the assumptions behind the regression equation were not in fact violated.

Discriminant Function Analysis

Up to this point, it has been looked at the learning variables as the key focus and tried to establish which variables could account for the variance observed in various aspects of learning. With the discriminate function analysis instead breed category was identified as the key focus. It was tried to establish whether using all of the various learning variables could arrive at a 'learning profile' model which would allow classifying individual horses into their correct breed category. If such a model can be successfully developed, then it provides good support for the hypothesis that the breeds, for whatever reason, do show qualitative differences in their learning styles.

Chapter 4

Results

The paragraph below reminds of the original aims of the study and the following chapter is built upon the objectives which were set out to reach.

- **A.** The main aim of this study is to find out whether domesticated horses show a disadvantage in the speed of performing a discrimination task.
- **B.** In order to achieve this aim, further objectives were to find out if there is a difference in the speed of learning a discrimination task, when comparing a domestic type with wild type horse species and
- **C.** To find out if inbreeding had an effect on the learning behavior of the horse.
- **D.** To see if the speed of learning changes over the duration of the discrimination task and the horses learn to learn, the time the horses required to reach criterion was observed.
- **E.** Also it was investigated if the learning rate of all three groups improves over the duration of the discrimination task.

Prior to the initial data analysis, an overview is given overleaf which describes whether it was appropriate to use parametric or non parametric statistics on the different variables used in the study. This decision was based on the results from the skewness values and examination of the histograms which can be found in the annex.

Table 5: illustrates whether Parametric or Non Parametric statistics were appropriate for each of the variables used in the study; this is illustrated for all horses, group comparisons and individual breeds.

Variable	All Horses	*Group Comparisons*	Individual breeds		
			Paso Fino	Przewalski	Thoroughbred
Time To Criterion	Parametric – successfully transformed	*Non Parametric – not able to transform for the Paso Fino or Przewalski group*	Non Parametric – non transformable	Non Parametric – non transformable	Parametric – successfully transformed
Total Hits	Parametric – successfully transformed	*Parametric – successfully transformed for all groups*	Parametric – successfully transformed	Parametric – successfully transformed	Parametric – successfully transformed
Number Correct Hits	Non Parametric – unable to transform, skewness ratio was too high (-3.87)	*Parametric – successfully transformed for all groups*	Parametric successfully transformed	Parametric – successfully transformed	Parametric Raw data was parametric
Number Errors	Non Parametric – Transformation failed	*Non Parametric* *Unsuccessfully transformed*	Non Parametric unsuccessfully transformed	Non Parametric – non transformable	Non Parametric Transformation failed
% Correct	Parametric Transformation successful	*Parametric* *Successfully transformed*	Parametric raw data was parametric already	Parametric – Raw data was parametric already	Parametric – Raw data was parametric already
% Errors	Non Parametric transformation failed	*Parametric* *successfully transformed*	Parametric raw data was parametric already	Parametric – Raw data was parametric already	Parametric – Raw data was parametric already
Time to touch correct object within trials	Transformation successful only for trial 4. As group variable retain all as Non Parametric	*Non Parametric* *Transformation failed*	Non Parametric Trial 14 did not need transformation, raw data was parametric. Trials 1-4 were successfully transformed, trials 8-9 were successfully transformed and trials 12 and 15 were successfully transformed. 5-7, 10-11 and 13 were not successfully transformed	Non Parametric Trial 1, 3-4, 7,9-10 and trials 13/15 successfully transformed however trials 2, 5-6, 8, 10-11 all failed transformation	Non Parametric Trials 12 and 14 were successfully transformed, all others failed transformation
Average Time to touch correct object within trials	Non Parametric transformation failed	*Non Parametric* *Transformation failed*	Parametric successfully transformed	Non Parametric – transformation failed	Non Parametric Transformation failed

4.1 Difference in the speed of learning a discrimination task, when comparing domestic type with a wild type horse species

<u>*Speed of learning*[2]</u>

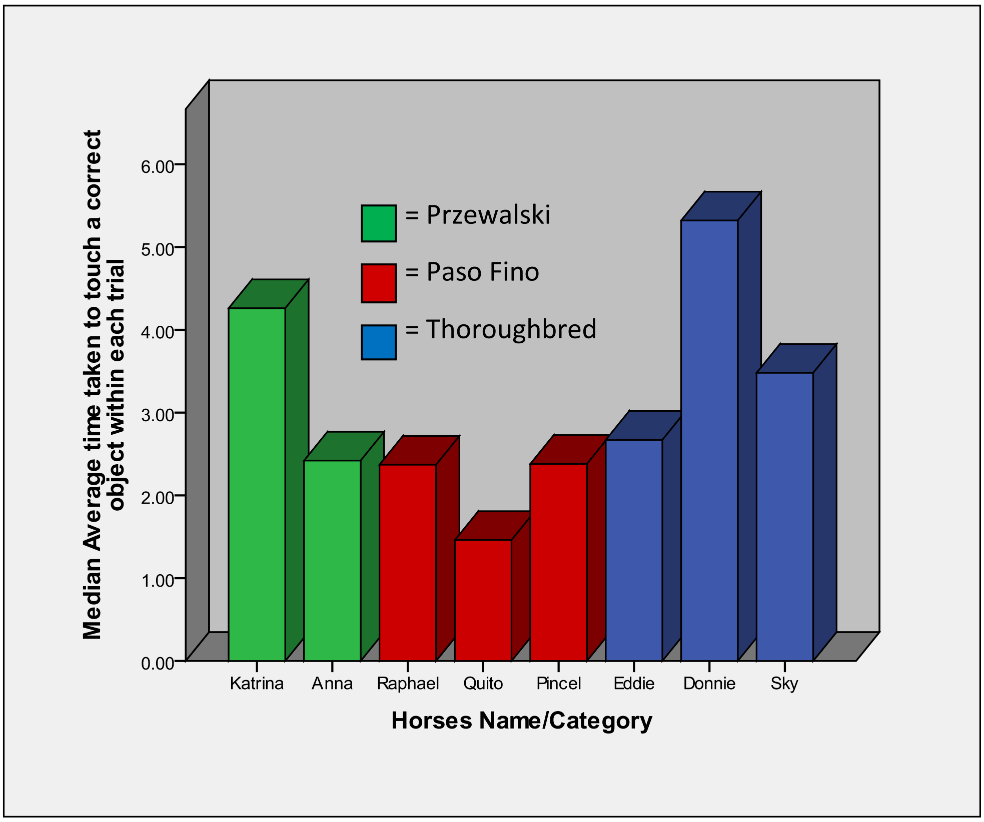
- *Average time to touch correct object within each trial*

The following table (Table 3) and graph (graph 1) shows the average time taken, for all horses combined together and for every individual horse separately, to touch the correct object, for the first time, within each trial so n= 15. The time measured was the time from placing the objects on the ground until the horse touched the correct object and subsequently the time from receiving the food reward until the horse touched the correct object again and so forth. This variable is non-parametric. The horses name in the table is followed by its breed in brackets: PF = Paso Fino, TB = Thoroughbred, PZ = Przewalski. It can be seen that Quito, a Paso Fino, has the lowest median and was quickest to touch the correct object again after being fed. Katrina, a Przewalski horse, showed the highest minimum and maximum value meaning it took her the longest time overall to touch the correct object again after being fed. However, Donnie, a Thoroughbred showed the highest median length of time.

	All horses	Pincel (PF)	Quito (PF)	Raphael (PF)	Donnie (TB)	Eddie (TB)	Sky (TB)	Anna (PZ)	Katrina (PZ)
Median	3.065	2.38	1.46	2.37	5.32	2.67	3.48	2.42	4.26
Minimum	1.13	1.46	1.13	1.16	2.21	1.25	2.25	1.30	3.16
Maximum	28.40	4.53	6.88	4.94	28.17	12.44	28.24	4.58	28.81

Table 3: Average time to touch correct object within each trial, for each individual horse

[2] Time in seconds



Graph 5: Median average time to touch a correct object within each trail for each individual horse.

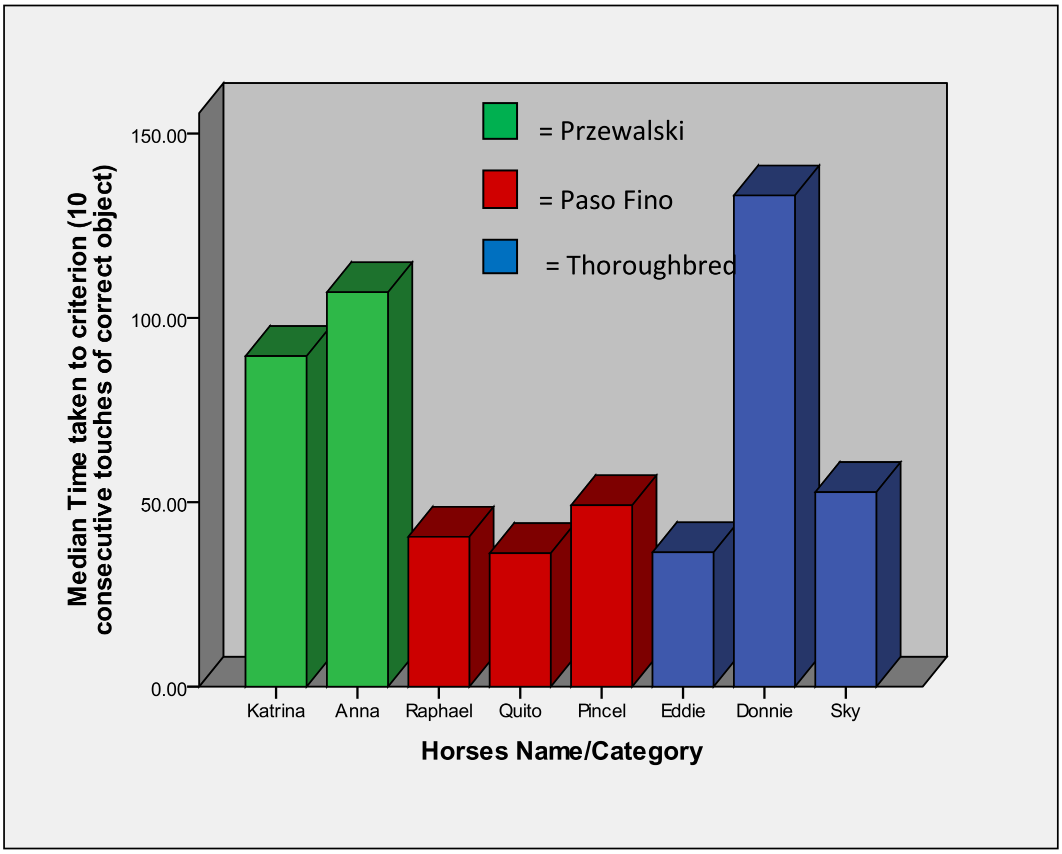
4.2 Speed of learning over the duration of the discrimination task

- *Average Time to criterion (time to touch correct object ten times consecutively per trial over 15 trials)*

Table 4 and graph 2 gives an overview of the time taken by the horses to touch the correct object ten times in a row. This was set as the criterion a horse had to achieve in order to move on to the next side exchange and therefore the next trial. This variable is parametric. Parametric in this context refers to the fact that the values for the variable follow a normal distribution. Parametric statistics make assumptions regarding the distribution of a variable; generally a key assumption is that the data are symmetrically distributed around a normal curve. It can be seen that Quito, a Paso Fino, was quickest to reach criterion with the lowest median, mean and standard deviation. Anna, a Przewalski, scored the lowest minimum value compared to the other horses, whereas her herd mate Katrina showed the highest minimum value. Donnie, a Thoroughbred, took longest to reach criterion with the highest scores for mean, median, standard deviation and maximum. Raphael, a Paso Fino, showed the lowest maximum score.

	All Horses	Pincel (PF)	Quito (PF)	Raphael (PF)	Donnie (TB)	Eddie (TB)	Sky (TB)	Anna (PZ)	Katrina (PZ)
Mean	90.57	61.06	44.29	46.17	180.69	64.39	80.51	124.65	122.23
Median	55.2	49.17	36.10	40.66	133.24	36.42	52.75	106.95	89.63
Std Dev	86.61	50.88	18.01	15.65	149.38	36.56	67.61	79.04	94.62
Minimum	28.27	31.51	30.76	28.17	36.50	30.86	36.29	28.71	44.97
Maximum	637.71	229.15	88.68	72.97	637.71	128.09	88.38	285.47	383.16

Table 4: Average time to criterion (10 consecutive correct hits per trial) over the 15 trials for each individual horse.



Graph 2: Average time to reach criterion (10 consecutive correct hits per trial) over the 15 trials for each horse.

4.3 Differences between the breeds with regards to responsiveness, accuracy, number of errors and number of correct hits

Responsiveness to task

- *Total number of responses(number of times any object touched in a trial)*

Table 5 describes the general responsiveness of the horses to the task presented to them. It shows the number of times each horse (and all horses combined) touched an object, whether the correct or incorrect object. This variable is also a parametric variable. Whilst, again, it can be seen that Quito, a Paso Fino, gave the lowest number of responses, this is in fact an artefact of the criterion we introduced, rather than an indication that she was not responsive to the task. Each horse's responsiveness needs to be judged in the context of their overall accuracy and the time taken to make a response. The criterion was ten consecutive correct touches; hence the minimum value is ten for all horses. Quito's median is ten but, again, this reflects the fact that, in most of the trials, she did not make an error. In contrast, Anna, a Przewalski, showed an overall very high number of responses, with a median of 30 and a maximum value of 65, but (as per Tables 1 and 2) she was slow to reach criterion. Putting the responsiveness of these horses in context then, Quito is a responsive horse showing a high level of accuracy (see Table 6), whilst Anna is a responsive horse who nevertheless had difficulties grasping the concept of the 'correct' object. Our later multivariate analyses partition out the variance attributable to 'responsiveness' and 'accuracy', so that the impact of 'responsiveness' as such can be considered independently of the constraints imposed by our criterion of 10 consecutive touches. Field research notes are also informative in this context and relevant comments taken from these will be considered in the discussion section.

	All Horses	Pincel	Quito	Raphael	Donnie	Eddie	Sky	Anna	Katrina
Mean	21.00	15.6	11.60	13.53	25.00	14.93	14.33	33.33	21.00
Median	18.00	12.00	10.00	12.00	25.00	13.00	13.00	30.00	18.00
Std Dev	10.68	9.99	2.89	4.89	9.44	4.55	5.09	18.80	10.68
Minimum	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Maximum	46.00	48.00	18.00	25.00	41.00	22.00	26.00	65.00	46.00
Total Nr of responses	2240	234	174	203	375	224	215	500	315
% of all responses		10.4%	7.77%	9.10%	16.7%	10%	9.6%	22.3%	14.1%

Table 5: Total responses (number of touches of any object) made over the course of the 15 trials by each horse.

Accuracy

- *Number of correct responses*

Table 6 below shows the correct responses the horses made in the task. The minimum is ten, again because a minimum of ten touches was required to move to the next trial. This variable is non-parametric. It can be seen that all Paso Fino horses had a median of ten, meaning that they made very few errors. The Przewalskis and Donnie, a Thoroughbred, on the other hand made more errors and therefore also increased their correct response rate, because ten consecutive correct touches were required for criterion but these horses had the opportunity to intersperse individual correct responses within a series of incorrect responses. This highlights the importance of using 'percentage correct' rather than 'absolute number correct' in our later multivariate analyses.

	All horses	Pincel (PF)	Quito (PF)	Raphael (PF)	Donnie (TB)	Eddie (TB)	Sky (TB)	Anna (PZ)	Katrina (PZ)
Median	11.00	10.00	10.00	10.00	16.00	10.00	11.00	25.00	16.00
Minimum	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Maximum	55.00	38.00	12.00	20.00	32.00	18.00	20.00	55.00	38.00
Total Number of correct responses	1809 (80.76% of all responses)	202.00 11.2%	154.00 8.5%	172.00 9.5%	269.00 14.9%	184.00 10.2%	175.00 9.7%	391.00 21.6%	262.00 14.5%

Table 6: Number of correct responses made by each individual horse

- *Percent correct*

The percent correct variable was parametric. The table below (table 7) shows the percentage of correct responses for each horse and for all horses combined. Quito, a Paso Fino again performed outstandingly, with a median of 100% correct responses and a mean of 91.66%. Donnie, a Thoroughbred, showed the lowest percentage of correct responses overall. The Przewalskis (Anna and Katrina) ranged around the average values. Note here, that the maximum of 100% is the same for all horses because all horses had at least one trial in which they made no errors.

	All Horses	Pincel (PF)	Quito (PF)	Raphael (PF)	Donnie (TB)	Eddie (TB)	Sky (TB)	Anna (PZ)	Katrina (PZ)
Mean	84.64	88.58	91.66	87.19	73.33	84.41	84.93	82.46	84.56
Median	83.66	83.33	100.00	83.33	71.43	85.71	84.62	83.33	83.33
Std Dev	12.77	8.78	13.70	10.74	13.45	11.69	13.84	12.68	10.81
Minimum	50.00	76.92	61.11	70.59	50.00	63.16	54.16	52.54	62.50
Maximum	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 7: Percentage of correct hits over all trials made by each individual horse

- *Number of errors*

Table 8 below shows the number of errors the horses made throughout all trials. This variable is non-parametric. Anna (PZ) made the most errors (109) whilst Quito (PF) only made 20 errors overall. Note that the number of errors is not simply the inverse of the number of correct responses because the criterion of ten consecutive correct responses was set, which meant that after each error, the horse was required to touch the correct object ten times consecutively again.

	All horses	Pincel (PF)	Quito (PF)	Raphael (PF)	Donnie (TB)	Eddie (TB)	Sky (TB)	Anna (PZ)	Katrina (PZ)
Median	2.00	2.00	0.00	2.00	5.00	2.00	2.00	7.00	3.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	28.00	10.00	7.00	6.00	16.00	7.00	11.00	28.00	8.00
Total number of incorrect responses	432 19.3%	32.00 7.4%	20.00 4.6%	31.00 7.2%	106.00 24.5%	40.00 9.26%	40.00 9.26%	109.00 25.2%	54.001 2.5%

Table 8: Number of errors in total over all 15 trials made by each individual horse

- *Percent errors*

In this table (table 9), it can be seen that Quito (PF) showed a median of 0% indicating a very low error rate. Donnie (TB) scored the highest error rate of all horses. The variable 'percent errors' is non-parametric.

	All horses	Pincel (PF)	Quito (PF)	Raphael (PF)	Donnie (TB)	Eddie (TB)	Sky (TB)	Anna (PZ)	Katrina (PZ)
Median	16.33	16.67	0.00	16.00	28.57	14.28	15.37	16.66	16.66
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	50.00	22.07	38.88	29.41	50.00	36.84	45.80	48.46	37.5

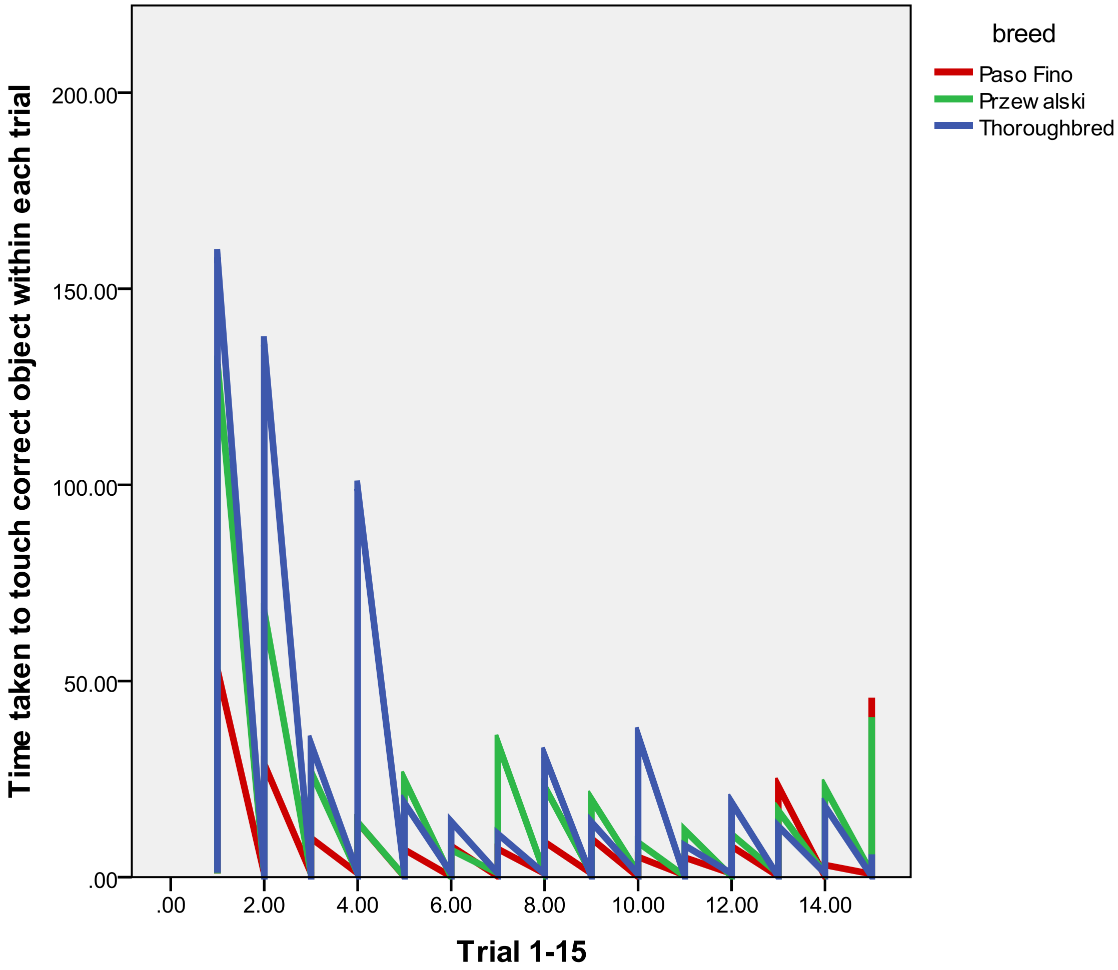
Table 9: Percentage of errors throughout the trials made by each individual horse

4.4 Changes in the learning rate of all three groups over the duration of the discrimination task

<u>Learning to learn</u>

- *Comparison across trials 1 to 15*

The ROC curve for all horses shows a significant downwards tendency from trial one to four which is then interrupted. The decrease equates to a decrease in the time required by the horses to touch the correct object again after being rewarded for the previous correct touch. The ROC curves for the Paso Fino horses show an inconsistent pattern, but this may in part be due to the fact that their early trials already demonstrated a high base rate for the speed of response. The Thoroughbreds' ROC curves, on the whole, show a consistent decrease over the duration of the 15 trials. The ROC curves for the Przewalski horses are inconsistent and it can be seen that the response pattern does not appear to improve across trials. Graph 3 shows the differences between the three groups over the 15 trials.



Graph 3: Changes in time to touch a correct object over the 15 trials for the three different breed groups: Przewalski, Paso Fino and thoroughbred.

4.5 Analysis of association between variables

	Time to criterion Trial 1	Time to criterion average across Trial 1-15	'Subtrial' Trial 1	'Subtrial' average across Trial 1-15
Breed	0.52(eta) 0.27 (eta^2)	0.70(eta)0.49(eta^2)	0.44(eta)0.19(eta^2)	0.71(eta)0.50(eta^2)
Age	0.19(ns, pearson)	0.49(ns, pearson)	0.40(ns, kendalls tau b)	0.40(ns, kendalls tau b)
Correct Object	0.08(eta)0.01(eta^2)	0.12(eta)0.02(eta^2)	0.06(eta)0.00(eta^2)	0.20(eta)0.04(eta^2)
Side of correct object	0.49(eta)0.24(eta^2)	0.48(eta)0.23(eta^2)	0.53(eta)0.28(eta^2)	0.22(eta)0.05(eta^2)
Gender	0.12(eta)0.01(eta^2)	0.05(eta)0.00(eta^2)	0.63(eta)0.40(eta^2)	0.02(eta)0.00(eta^2)
Wild Type	0.39(eta)0.15(eta^2)	0.31(eta)0.09(eta^2)	0.42(eta)0.18(eta^2)	0.63(eta)0.39(eta^2)
Heterozygosity	0.51(eta)0.26(eta^2)	0.69(eta)0.47(eta^2)	0.15(eta)0.02(eta^2)	0.68(eta)0.46(eta^2)
Domestication	0.13(eta)0.02(eta^2)	0.42(eta)0.17(eta^2)	0.31(eta)0.09(eta^2)	0.03(eta)0.00(eta^2)
Inbreeding	0.51(eta)0.26(eta^2)	0.69(eta)0.47(eta^2)	0.14(eta)0.02(eta^2)	0.67(eta)0.45(eta^2)

Table 11: Chi square analysis of associations

From table 11 it can be seen that Breed showed a significant association with all dependent variables. Age and Correct Object however showed no statistically significant association with either the 'time to criterion' or the 'subtrial' variables. 'Side of correct object', 'Gender' and 'Wildtype' showed a significant association with the 'Subtrial' variable. Both 'Heterozygosity' and 'Inbreeding' showed significant associations with at least one of each variable measuring 'time to criterion' and 'subtrial', whilst 'Domestication' showed no significant degree of association with any of the dependent variables evaluated.

4.6 Effect of inbreeding on the learning behavior of the horse

Regression Analysis

Table 12 below shows which variables were included in the regression analysis, the decision to include was based on the outcome of the chi square analysis above, independent variables showing no association with the core dependent variables were excluded from further analysis.

	Time To Criterion	Average Subtrial per trial
Horse	+	+
Trial Number	+	+
Breed	+	+
Correct Object	-	-
Side of correct object	-	+
Gender	-	+
Wild Type	-	+
Heterozygosity	+	+
Domestication	-	-
Inbreeding	+	+

Table 12: Overview of included and not included variables in the regression analysis

Regression Values for ‘time to criterion’

		R squared	Cumulative R sqaured	Adjusted R squared	Cumulative Adjusted R Squared	Standardized Beta	t	Significance of t
First variable entered into the model	Horse is Donnie	0.16	0.16	0.15	0.15	0.39	4.67	0.0001
Second variable entered into the model	Number of Trial	0.14	0.30	0.14	0.29	-0.38	-4.96	0.0001
Third variable entered into the model	Horse breed is Przewalski	0.10	0.40	0.10	0.39	0.32	4.37	0.0001
Total variance in ‘time to criterion’ accounted for by the above model: **38.6% (F= 25.89, p=0.0001)**								

Table 13: The regression values for the variable ‘time to criterion’

Table 13 sets out the outcome of the regression analysis in respect of the variable ‘time to criterion’. The value ‘R squared’ describes the proportion of variance explained (e.g. a value of 0.16 indicates that 16% of the variance in the dependent variable is accounted for by variance in a particular independent variable). However, the value ‘adjusted r square’ is a more reliable value to use, because it takes into account the statistical profile of the data, for example providing an outcome which takes into account the need to standardize a particular variable. ‘Standardized beta’ is a value which indicates the relative strength of effect of the variables included in the model. The ‘t’ value and ‘significance of t’ confirm that the variable plays a statistically significant and independent (that is, taking all other variables in the model into account, this variable still, independently, accounts for variance in the dependent variable). Regression analysis then provides two valuable pieces of information. Firstly, it indicates the strength of association between a dependent and an independent variable, taking into account any covariance which may be observed between independent variables. Secondly, it provides a model, comprising one or more independent variables, to account for the variance observed in the dependent variable. In doing so, it provides both an individual estimate of the variance accounted for by each independent variable and a composite estimate which gives the amount of variance in the dependent variable accounted for *overall* by a combination of variables which each have an individual impact on the dependent variable. So, in relation to the hypothesis, it was investigated how much of the observed variance in the core ‘speed of learning’ variables was accounted for by variance in the set of independent variables that were identified as being associated with speed of learning. It was also investigated which of these variables were the best predictors for likely speed of learning.

The method used to enter independent variables into the equation was ‘stepwise’.

The Beta value cited in the tables also indicates the direction of effect of any association. So, for example, as the beta value for Donnie (TB) is positive, that means that adding Donnie into the equation increased the overall ‘time to criterion’ observed in the data. In contrast, as the ‘trial number' increased, the 'time to criterion' decreased, which is to be expected since the horses were learning to learn and so as the trials continued they became quicker at the task. In addition to Donnie, it can be seen that the Przewalski group also increased the overall 'time to criterion'.

		R squared	Cumulative R sqaured	Adjusted R squared	Cumulative Adjusted R Squared	Standardized Beta	t	Significance of t
First variable entered into the model	Breed is Przewalski	0.20	0.20	0.19	0.19	0.45	5.06	0.0001
Second variable entered into the model	Trial Number	0.14	0.34	0.13	0.32	-0.37	-4.63	0.0001
Total variance in 'time to criterion' accounted for by the above model: **32.0%** (F = 26.07, p= 0.0001)								

Table 14: Regression values for the variable 'time to criterion' without outlying variable

As table 14 shows, removing the 'outlier' horse (Donnie, TB) did not have any substantial impact on the structure of the model. No new variables were drawn in as independent predictors of the dependent variables. The variables 'breed category' and 'trial number' remained the sole important factors. The amount of variance explained in both of the above models is quite substantial given the small number of variables included in the final model. The beta values indicate that horses improved the speed of 'time to criterion' as the number of trials increased, but that membership of the Przewalski breed group equated to a slower speed of learning (in contrast to the original hypothesis).

		R squared	Cumulative R sqaured	Adjusted R squared	Cumulative Adjusted R Squared	Standardized Beta	t	Significance of t
First variable entered into the model	Horse is Donnie	0.12	0.12	0.11	0.11	0.34	3.94	0.003
Second variable entered into the model	Horse breed is Paso Fino	0.05	0.17	0.04	0.15	-0.23	-2.65	0.009
Third variable entered into the model	Is the horse Anna	0.03	0.20	0.02	0.18	-0.19	-2.09	0.040
Total variance in 'time to criterion' accounted for by the above model: **18% (F= 9.45, p=0.0001)**								

Table 15: Regression values for the variable 'subtrial'

Table 15 describes the outcome of the regression analysis in respect of the variable 'subtrial'. The three factors identified as predictors of the 'subtrial' variable were Donnie (TB), the Paso Fino Breed as a whole and Anna (PZ). It can be seen that the Paso Fino breed and the Przewalski horse Anna significantly contributed to a decrease in the time to the first correct touch in each subtrial. In contrast, the Thoroughbred Donnie significantly increased the time required within each subtrial. The fact that individual horses influenced outcomes to the extent suggested above strongly implies the need for a larger sample size in any subsequent research in this area. With a larger sample size individual differences are more likely to be cancelled out, leaving a clearer pattern of association between the main dependent and independent variables.

		R squared	Cumulative R sqaured	Adjusted R squared	Cumulative Adjusted R Squared	Standardized Beta	t	Significance of t
First variable entered into the model	Inbreeding high or low	0.13	0.13	0.121	0.12	0.36	3.56	0.0001
Second variable entered into the model	Is the horse Eddie	0.03	0.17	0.03	0.15	-0.22	-2.04	0.045
Total variance in 'time to criterion' accounted for by the above model: **15% (F= 8.75, p=0.0001)**								

Table 16: Regression values for the variable 'subtrial' without the outlying variable

Table 16 sets out the regression values for the variable 'subtrial' once the outlier data points represented by Donnie and Anna are removed. It can be seen that once these outliers are removed, the factor 'inbreeding', but also a further factor representing individual differences (Eddie (TB) provided a model accounting for more modest, but nevertheless still quite substantial proportions of variance in the 'subtrial' variable. 'Eddie' had a negative beta value, indicating that including his datapoints 'improved' the subtrial times significantly. To see if an improved fit of the model to the data could be achieved by, again, removing a variable representing individual differences, the regression was run again also removing 'Eddie' as a factor. The outcome can be seen in table 17 below. The total variance in subtrial times explained by the variable 'inbreeding' alone is 17% with a significance value of p= 0.0001. For a single variable, this is a very substantive amount of variance explained. Of course, it must be noted here that, particularly in this last model, the number of datapoints included is relatively small. Again, replication with a much larger sample size is needed.

		R squared	Cumulative R sqaured	Adjusted R squared	Cumulative Adjusted R Squared	Standardized Beta	t	Significance of t
First variable entered into the model	Inbreeding high or low	0.18	0.18	0.17	0.17	0.43	4.05	0.0001
Total variance in 'time to criterion' accounted for by the above model: **17% (F= 16.41, p=0.0001)**								

Table 17: Regression values for the variable 'subtrial' when removing all outlyers

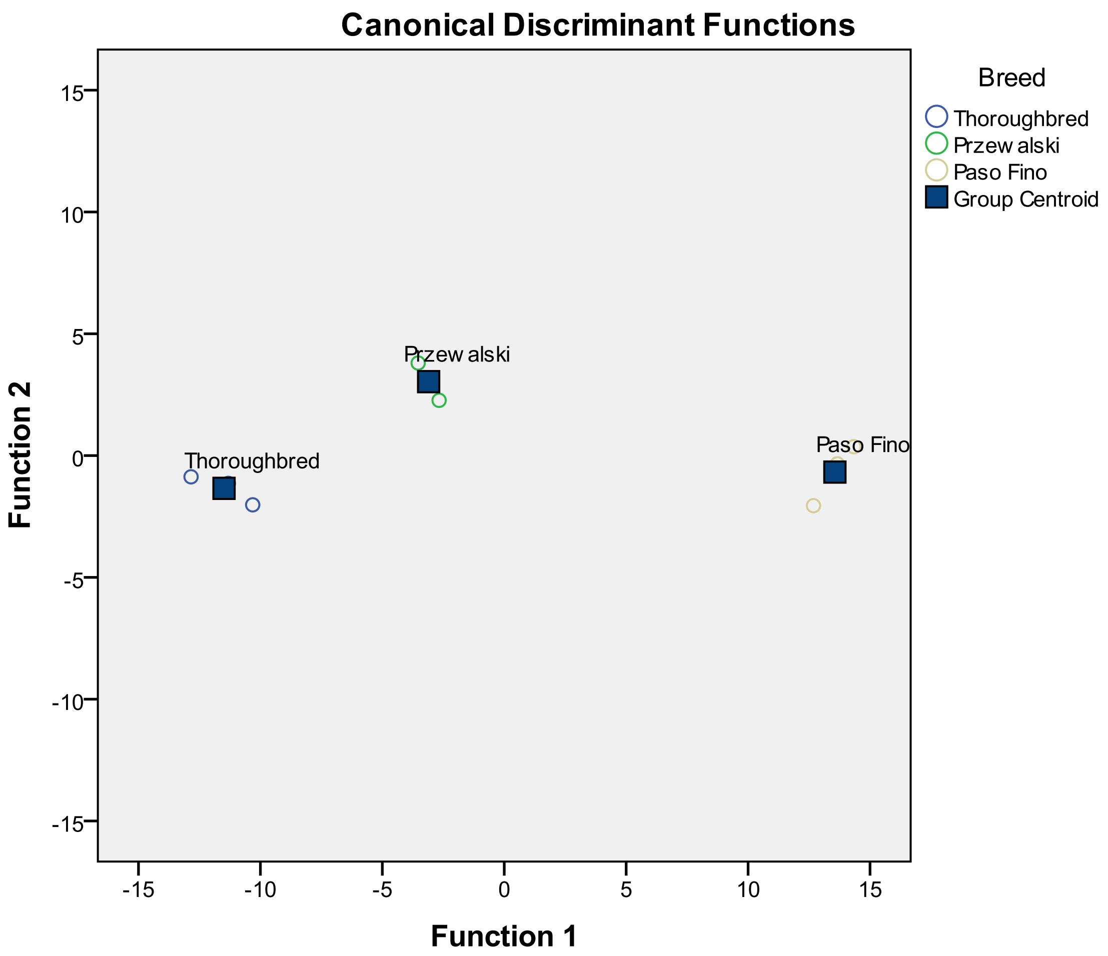
4.7 Discriminant Function Analysis

Table 18 is a reminder of the individual learning variables identified on page 25 and how these have been classified as components of the learning process. Age and gender were included in the model as a control since these values differed between the breed categories.

Variable Category	Variable
1. Responsiveness	Total number of hits overall Average number of hits per trial
2. Accuracy	Percentage of total hits correct Average percent correct across 15 trials Percentage of total hits incorrect Average percent incorrect across 15 trials
3. Speed of learning	Time to criterion for trial 1 Average time to touch correct object for the first time in trial 1
4. Learning to learn	Average time to criterion across 15 trials Average time to touch object for the first time across 15 trials
5. Potential confounding variables	Age Gender

Table 18: Established categories and the respective variables used for measurement

The discriminant function analysis returned a two function solution for how the independent variables differentiated between breed groupings. 'Functions' can be seen as clusters of associated variables which, taken as a whole, show little or no overlap between one another. Each function is defined by the variables which contribute to the cluster. So, for example, two functions used to describe eye colours might be, respectively 'blue clear eyes' and 'brown cloudy eyes'.



Graph 4: Discriminant function solution

With regard to the two identified learning profile clusters identified by this discriminant function analysis, as the graph above indicates (graph 4) and as demonstrated by the previous analyses, the Paso Fino Breed was most clearly distinct from the other two groups. All three groups clustered around the mid range for function two although Przewalskis were slightly away from the other two breeds in this function having slightly higher values. The Paso Finos were very well described by function one, having mid range values on function two but very high values on function one. The following paragraphs will describe function one and two and what the final discriminant function model tells us about breed characteristics.

The structure matrix diagram below (table 19) shows how all of the independent variables initially correlated with function one and function two before the stepwise selection process was used to evaluate the independence of association. A number of the variables which failed to show independent explanatory value were discarded following this initial descriptive stage in the analysis. The values presented in table 19 are correlation coefficients.

Structure Matrix

	Function	
	1	2
Average % Wrong[a]	-.776*	-.037
Gender[a]	-.485*	.314
Time To Criterion in Trial 1[a]	.257*	-.091
Average Time to Touch in Trial 1[a]	.177*	-.098
% Correct	.087*	-.072
Average Number of Touches in Subtrials	-.076*	-.051
Age	-.121	.668*
Average % Correct[a]	-.052	.528*
Average Number Hits	-.044	.456*
Number of Total Hits[a]	-.046	.455*
Average Time Taken To Touch the Correct Object 10 Consecutive Times Over The 15 trials[a]	.049	.237*
% Wrong Touches[a]	-.138	.152*

Table 19: Correlation of independent variables with each function

Standardized Canonical Discriminant Function Coefficients

	Function	
	1	2
Average Number Hits	9.950	2.472
% Correct	14.265	2.462
Average Number of Touches in Subtrials	5.139	1.123
Age	-4.875	.157

Table 20: Indication of the variables used in the final model

Table 20 above shows the standardized discriminant function coefficients. It indicates the variables in the final model which were shown to have independent explanatory values in respect of the two functions. Looking at the nature of the variables included in terms of the established categories, it can be seen that function one is best described as relating to horses which are "younger, responsive, accurate and good at learning to learn". In contrast, function two describes the opposite profile in which horses are "older, less responsive, less accurate and struggle with learning to learn". It is important to note, in respect of age in particular, that each of the included variables shows an independent association with the function. So it is not the case that the younger age accounts for the responsiveness. These are two separate components of function one. This final model was highly statistically significant in differentiating between the three breeds (Wilks Lambda = 16.64, p= 0.008).

Classification Function Coefficients

	Breed		
	Thoroughbred	Przewalski	Paso Fino
Average Number of hits	576.899	593.788	621.830
% Correct	1195.850	1230.019	1289.869
Average Number of Touches in Subtrials	964.101	991.444	1037.807
Age	-681.399	-699.821	-737.294
(Constant)	-49363.890	-52251.758	-57384.357

Table 21: Relative numerical distances between key variables

The above table (table 21) sets out the relative distances numerically between each of the horse breeds on the key factors. The absolute value of the numbers is not relevant. The relative value shows where the breeds are located in space and therefore where they stand in relation to one another in respect of the two factors, as per graph 19.

Functions at Group Centroids

Breed	Function	
	1	2
Thoroughbred	-11.492	-1.345
Przewalski	-3.102	3.034
Paso Fino	13.560	-.678

Table 22: Mean values of the three breeds in relation to functions

Table 22 indicates how the three breeds are differentiated in terms of their mean values (the combined values of each horse contributing to a particular breed) in relation to the functions. The model used to classify the individual horses into breed groups, returned a perfect (100%) allocation of all horses to their correct group. This can be seen in table 23 below. Whilst this is clearly a highly successful model and indicates that the breeds are indeed differentiated on the base of their learning profile, within the confines of this pilot study it must be borne in mind that the sample size in this study was very small. The model should be tested with a larger sample size to establish fully its level of accuracy.

Classification Results[a]

		Breed	Predicted Group Membership			Total
			Thoroughbred	Przewalski	Paso Fino	
Original	Count	Thoroughbred	3	0	0	3
		Przewalski	0	2	0	2
		Paso Fino	0	0	3	3
	%	Thoroughbred	100.0	.0	.0	100.0
		Przewalski	.0	100.0	.0	100.0
		Paso Fino	.0	.0	100.0	100.0

a. 100.0% of original grouped cases correctly classified.

Table 26: Classification of horses into breed groups

Chapter 5

Discussion

The study described in this thesis is a pilot study, designed to evaluate and inform the objectives of a proposed larger scale study which address potential cognitive differences between primitive or 'wild type' horse breeds and highly domesticated breeds.

The primary research objective of the study reported here was to investigate whether domesticated horses show a disadvantage in the speed with which they learn a discrimination task in comparison with wild type breeds. This hypothesis is based on findings in the literature such as the study by Rohrs and Ebinger (1993) who found that the brain case volume of the domesticated horses is 14% lower than that of the Przewalski wild horse. It could be suggested that domestication might therefore have reduced cognitive abilities. There is substantive evidence to suggest that natural selection plays a significant role in the development of conceptual learning ability in both animals and man (Zentall et al 2008). This leads to the possibility that artificially selected equine breeds may display significantly different learning skills and associated behaviours in comparison to modern highly inbred breeds (Mader & Price 1980).

The second aim of the study was to establish whether inbreeding has an effect on the learning abilities of horses. This supplementary hypothesis was introduced because both chosen domesticated horse groups, namely the Thoroughbred and the wild type horse group, the Przewalski horse, have high inbreeding coefficients. Deckard et al (1988) found that mice who were inbred for a number of generations took longer to learn a task than outbred mice. To investigate this subsidiary hypothesis, a comparator group of Paso Fino horses was included in the study. This breed of horse is known to have a low coefficient of inbreeding and also one of the highest heterozygosity coefficients amongst extant horse breeds.

Since the sample size of this study was comparatively small (N=8), data analysis was not only focussed on the comparison of breed clusters, but also on the performance of each horse individually. This focus on the individual also allowed the comparison between horses within each breed group. This approach had the advantage of allowing potential outliers to be identified, since, notably with such a small sample, it may have been the case that individual horses were not good representatives of general breed characteristics.

The first learning category to be investigated was the speed of learning. The variables chosen to reflect speed of learning were 'Subtrial' and 'Time to criterion' (a description of all key variables is given in the methods section). When looking at the average time it took each horse to touch the correct object within one trial, it can be seen that the Paso Fino group and Quito (PF) in particular, showed the quickest times to touch the correct object again after being fed the reward for the previous correct touch. In contrast, Katrina (PZ) took the longest time to respond correctly in this context. It is worth noting that Katrina is the alpha mare of the Przewalski herd in the Welsh Mountain Zoo. In line with this, she is very conscious of her surroundings and is wary of any novelties. When collecting the data, it could be seen that she needed a larger amount of time to approach the discrimination objects and confidently touch them, since she was at the same time also keeping an eye on the herd, animal and visitor movements and all resulting background noises. Discrimination trials were sometimes also intruded on by herd mates in the case of the Przewalski horses. This could have had an effect on the learning curve of these animals. Quito (PF), as an example for the Paso Fino breed group, on the other hand, focused intently on the task and was not afraid of the objects from the outset. There is research in the literature which suggests that even slight differences in background noise and lighting variations can result in changes in perception and therefore interfere with the formation of associations (Hothersall, 2007). In the current study it has to be noted that three different locations were used in the evaluation of the three breeds of horses. When data was collected, weather conditions also varied and, especially while collecting the Przewalski horse data, situations changed with the movement of the herd. These pragmatic constraints on data collection need to be taken into account when evaluating the outcomes of this study.

When looking at the time the horses required to reach the criterion of ten consecutive touches in order to move on to the next trial, it was found that Quito (PF) required the least amount of time. As mentioned she, as well as the other Paso Finos, was focused on the task and showed a quick understanding of what she was required to do. The Thoroughbreds and, in particular, Donnie (TB) took a substantially longer time to touch the correct object ten times in a row. Donnie was easily distracted from the task and took a long time to understand what was required. He lost interest very easily and walked off to look out of the window or nibble on some hay. The other Thoroughbreds Eddie and Sky initially showed a similar pattern but, as the task went on, they began to understand what was required and were keen to go on.

The learning category "Responsiveness" was represented by the variable 'total number of hits'. In this context, all responses the horses made were counted, whether or not they touched a correct or incorrect object. Taken as a 'stand alone' variable, there are some issues with this variable as a true measure of responsiveness. For example, Quito's median was ten; however, she was a very responsive horse. The issue here was that Quito made so few errors that she had little opportunity to explore objects and exhibit responsiveness, since the criterion to move on to the next trial was 10 responses. Anna (PZ) in contrast made 65 responses within one trial alone, which reflects her learning profile. She was overly keen on interacting with the experimenter but did not understand the task properly. The varying responsiveness of the horses may therefore have multiple origins. In Katrinas (PZ) case it was due to nervousness as mentioned above. Anna (PZ) was very keen on the interaction with humans whereas Eddie (TB) and Raphael (PF) were overly keen on the food reward. Sky (TB) and Donnie (TB) were not majorly interested in the food reward and were very hesitant regarding the task. The two Paso Finos Pincel and Quito were keen on the food as well, but managed to focus on the task so that they received the reward quickly and at a constant rate. In the multivariate analyses these differences in profile are in part controlled for, allowing responsiveness as such to be evaluated.

The above findings, which reflect the important role of individual differences between the horses, are supported by Visser et al (2001) who mentioned that it is arguable whether comprehension of a learning task in horses is due to greater sensitivity to human cues, lower reactivity in an experimental situation or an underlying willingness to perform. The Paso Fino test group was comprised of horses used to receiving a food reward prior to the study. It could be argued that this resulted in a different approach to start with. General breed differences do however have to be kept in mind and different behavioural characteristics within the breed groups were also seen. It could on this basis be argued equally that the groups that had not received any food rewards prior to this study might have been more resourceful and highly motivated to look for food.

A further point of consideration is the impact of previous training on the motivation of the horses. Literature suggests that horses that are used to being trained through negative reinforcement are often less motivated to think and therefore also less likely to take the initiative by offering responses because of the risk of making a mistake (Hart, 2008). Sankey et al (2009) tested the effect of negative and positive reinforcement on horses while learning a task. The results show that the horses used in the positive reinforcement schedule required less time to complete training than the control horses. On top of which, the horses in the positive reinforcement group displayed more “positive” behaviour, such as licking and sniffing the experimenter whereas the horses trained by negative reinforcement expressed six times more “negative behaviours” such as biting and kicking. In this study, horses come from different backgrounds and their degree of interaction with humans ranged from rarely handled at all (the Przewalskis) to moderately and highly handled. It has to be taken into account that all participating Thoroughbreds were riding and racing horses and therefore are likely to have been taught, to some extent, through negative reinforcement, if only in the context of their early training. When setting these facts in context with the results of this study, it is worth noting that apart from Donnie, the mean responses in terms of responsiveness of the other tested Thoroughbreds Eddie and Sky did not differ from the means of the other horses to any great extent.

In terms of the learning category “Accuracy”, the variables ‘number of correct responses’, ‘percentage of correct responses’, ‘number of errors’ and ‘percentage of errors’ were investigated. When looking at the correct responses the horses made over the course of all trials and the associated percentage, it was found that the most correct responses were made by the Przewalski group. This implies that they have equally made most of the errors because of the criterion of ten consecutive correct touches. For example, if a horse touched the correct object for seven times and is then making an error, it has o touch the correct object for ten times again, which makes a total of 18 touches. The Paso Finos all had a median of correct responses of ten, meaning they hardly made any mistakes. This was supported by the investigation of the variable ‘number of errors’ and ‘percentage of errors’. It could be argued that the Paso Finos showed a higher accuracy because they were able to observe each other’s performance. However, the Przewalskis were equally able to observe each other, but had a much lower accuracy. This notwithstanding, the literature does not fully support the idea of observational learning in horses. Researchers evaluating this concept have shown that observational learning does not occur when testing learning tasks in horses (Baker et al. 1985, Baer et al. 1982, Clarke et al. 1992).

In the fourth learning category evaluated, the concept of ‘learning to learn’ was explored. The focus of interest was whether the number of attempts taken to reach criterion decreased across the course of 15 trials. This was done by investigating the variable ‘subtrial’. McGreevy (2004) mentioned that “Horses often take time to grasp the concept of discrimination tasks. Once they have established the learning set, their responses become more rapid and accurate. Such horses are described as having learned to learn.” Only the Thoroughbred group showed a decrease in the time required to touch the correct object again after receiving the food reward for the previous correct touch. The Paso Finos curve is rather inconsistent, although it has to be borne in mind that the Paso Fino group started off on a higher speed of response than the Thoroughbreds. The Przewalski’s curve shows inconsistency across trials also and failed and failed to improve substantially over the course of the fifteen trials (see Graph 18) According to these findings, it could be assumed that the Paso Finos posses higher cognitive abilities, although it should be noted that the conditions under which the data for the Przewalskis was collected were far more distracting for the horses than was the case in the other breed groups. The Thoroughbred group showed a lower motivation to start with, so if they were not successful right away they lost interest and went on to exhibit other behaviours such as attempting to acquire the food rewards by mugging the experimenter or going back to their previously learned clicker tasks such as ‘look away’. The literature suggests that the Thoroughbred is generally a fussy feeder and may be less motivated to solve problems for food rewards (McGreevy, 2004). Within this category the ‘time to criterion’ was explored for the first trial only in order to assess the horses response to novelty. The data analysis showed a statistically significant difference between the breeds when looking at the time to criterion on their first trial. This outcome supported observations when the data was collected.

The above outcomes all relate to the evaluation of individual variables. In the final stage of the data analysis, a multivariate analyses (specifically regression and discriminant function analysis) was employed firstly, to identify the extent to which individual variables could account, independently, for the variance observed in the core ‘speed of learning’ dependent variables and secondly, to identify whether a ‘learning profile’ could be used to reliably classify each horse into its correct breed grouping.

Through the above multivariate analyses, it was found that ‘breed’ had a highly significant influence on the speed of learning and that breeds were clearly differentiated on the base of their learning profile. It must be kept in mind that this study only had a small sample size and larger samples are required to confirm these findings. Age was not found to influence the learning curve of the horses. This finding is in line with the literature, which suggests that age does not influence the learning of a task in horses (Wolff and Hausberger, 1995). Further findings showed a decrease in time required to touch the correct object. This confirms again, that the horses were learning to learn. However, the fact that the Przewalskis accounted for an increase of the time to criterion is counter to the suggested hypothesis.

‘Inbreeding’ showed a statistically significant and independent association with speed of learning, whereas ‘Domestication’ did not. The fact that the domestication background of the horses did not affect the speed of their learning may be due to previous interactions of the Przewalski horses with humans that were not known to their current owner. This would undermine the assumption that these horses are entirely non-domesticated. In a study of Lewejohann et al. in 2010, domestic guinea pigs outperformed their wild type conspecifics significantly. The authors concluded that the domestic guinea pigs had an advantage in contrary to the wild guinea pigs because they were adapted to man-made environment which allowed them to solve the task more efficiently. Maybe this assumption could be related to this study as well.

The total variance in subtrial times explained by inbreeding is 17% with a significance value of p= 0.0001.On this basis there is some limited support for the hypothesis that speed of learning is faster in breeds of horse with a lower inbreeding coefficients. Again, it has to be noted that with a sample size this small, all conclusions must be regarded as speculative.

The discriminant function demonstrated, as *per* the previous analyses, that the Paso Finos were significantly distinct from the other breeds. Literature suggests that horses with the greatest ability to understand and conceptualise are these, which are better equipped to deal with the demands of modern and future training schemes (McCall, 1990).

The results of this study indicate that inbreeding may have an effect on the learning ability of the horse. However, it is necessary to carry out a full study which includes a larger sample size in order to strengthen the outcomes.

In this study it was observed that the level of food motivation influences learning behaviour immensely, especially when positive reinforcement is used. Unfortunately, the literature to date does not provide any information on this particular topic. As this information would be transferable to the learning of other species, such as dogs, it could be even more worthwhile looking into.

Chapter 6

Conclusion

The outcomes of this study showed that there is a significant difference in the learning profile of the three horse breeds investigated. A difference in speed of learning was evident when comparing a domesticated with a wild type horse species. Domestication as such, however, did not account for these differences. Either the domestication event as such did not have an impact on the cognitive abilities of the horse or the particular Przewalski horses used in this study had at some point in the past been handled to a greater extent than was known to the current owner.

In contrast, the potential impact of inbreeding on learning ability was identified as a significant factor in this study. The only participating breed group with a low inbreeding coefficient included in the study was the Paso Fino group. This group showed a learning speed which was significantly faster than the other two groups. Even at the beginning of the trials the horses in the Paso group exhibited a clear focus on the task and quickly understood what was required from them in order to receive the food reward. Since multivariate analyses indicated that inbreeding was a 'stand alone' factor in accounting for the variance in speed of learning, this finding supports, although cannot entirely confirm, that inbreeding may have had an effect on the learning ability of these horses. It is valuable knowledge for breeders that non-inbred horses, which show an heterogeneous genetic structure may show better learning abilities which could reduce time spent on training the horse.

With regard to 'learning to learn', which is of as much importance as speed of learning, it was found that the time the Thoroughbreds needed to reach the discrimination task criterion for each trial decreased significantly over the duration of the 15 trials. This indicates that even horses with a limited absolute capacity in respect of speed of learning can be trained, given sufficient persistence. It also suggests that 'learning to learn' may be an ability which is conserved even in the most highly inbred breeds. The learning curve of the Paso Fino group remained the same across the 15 trials, but this is a function of their significantly higher baseline speed of learning. The Przewalski horses also failed to show an improvement over the ongoing trials. In this context, the lack of any observed increase in speed over time appears to result from a failure to 'learn to learn'. Possible explanations of this pattern may be their higher inbreeding coefficient, but equally, given comments in the field notes, could be the horses' natural wariness of the novel objects, exacerbated by their lack of handling.

The main limitation of this pilot study was its small sample size. Given which, all outcomes need to be tested further in the context of a larger trial. The individual horses tested may not be good representatives of their respective breeds. However, despite only being a small pilot study, the data analysis identified statistically significant outcomes which are amongst the first in the literature to provide information on the cognitive abilities of wild horses and to explore the potential influence of genetic factors on learning in the horse.

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Annex

1. Testing the assumption of normality

What the Shape of the distribution can tell us about a trait

A normal distribution curve ('Gaussian' distribution) implies a multi factorial origin of the trait, meaning that the trait can be influenced by many things. Therefore it is a quantitative trait which is characterized by a large number of contributing genes. A normal distribution suggests that selection for a particular trait value is not particularly strong (Schilling et al, 2002, Spiegel, 1992, Monod, 1974).

If the distribution curve is shaped in distinct 'modes' (more than one peak in the distribution curve), the trait has several sub-populations. If these modes in itself are normally distributed the above applies. If however, the distribution curve of an individual mode shows for example a very steep peak, this would indicate that this trait may be under strong selective pressures because the values do not vary very much from individual to individual. The steepness of the distribution is expressed through the Kurtosis level, describing how flat a distribution is (Schilling et al, 2002, Spiegel, 1992, Monod, 1974).

Distribution curves may as well be 'skewed', indicating a 'tail' to the left or the right of the distribution. A tail to the right of the distribution would be a positive skew whilst a tail on the left of the distribution would indicate a negative skew. The direction of the skewness indicates what the likely pressure on a trait may be. A distribution with a positive skew would indicate that natural selection favours low values of the trait and vice versa (Schilling et al, 2002, Spiegel, 1992, Monod, 1974).

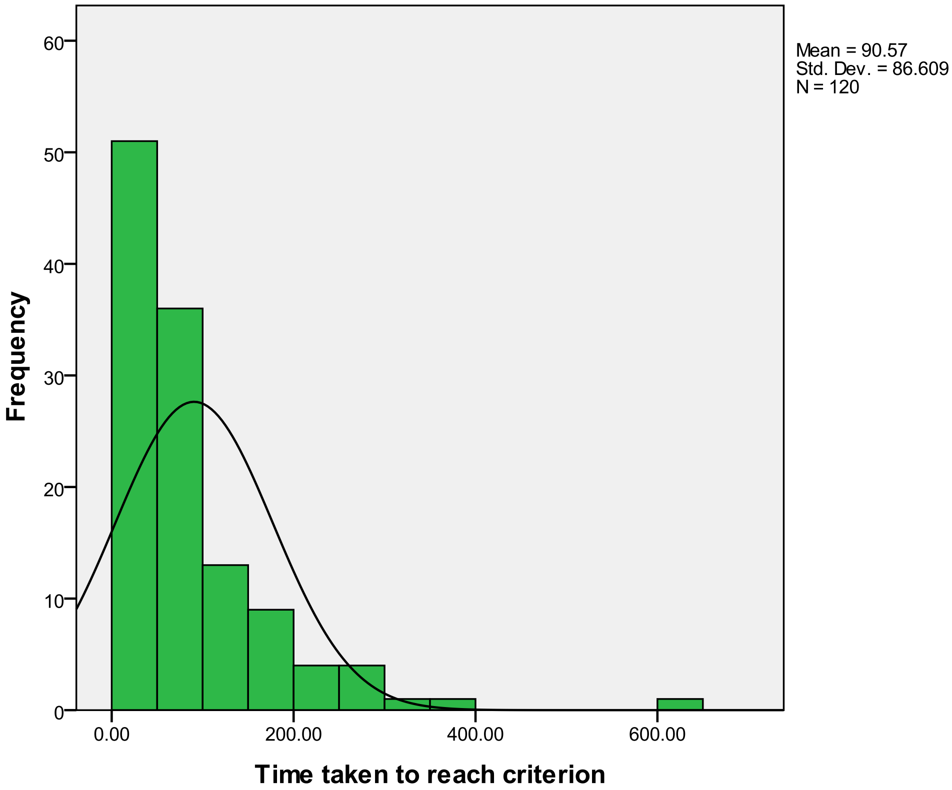
Determining the appropriate format and statistical tests for each variable

The structure of the distribution for each variable is also used to determine the appropriate statistical tests to use in subsequent analyses. In order to know whether to apply parametric or non-parametric statistical tests, it is important to evaluate whether the variable in questions conforms to a normal distribution. Parametric tests make assumptions about the form of the data, in particular, these test assume that the data are symmetrical and follow the profile of a normal (or 'Gaussian') curve. If the data match these assumptions, parametric tests are generally regarded as having greater statistical power and therefore are regarded as preferable to non-parametric tests.

2. Overview of the distribution of key variables

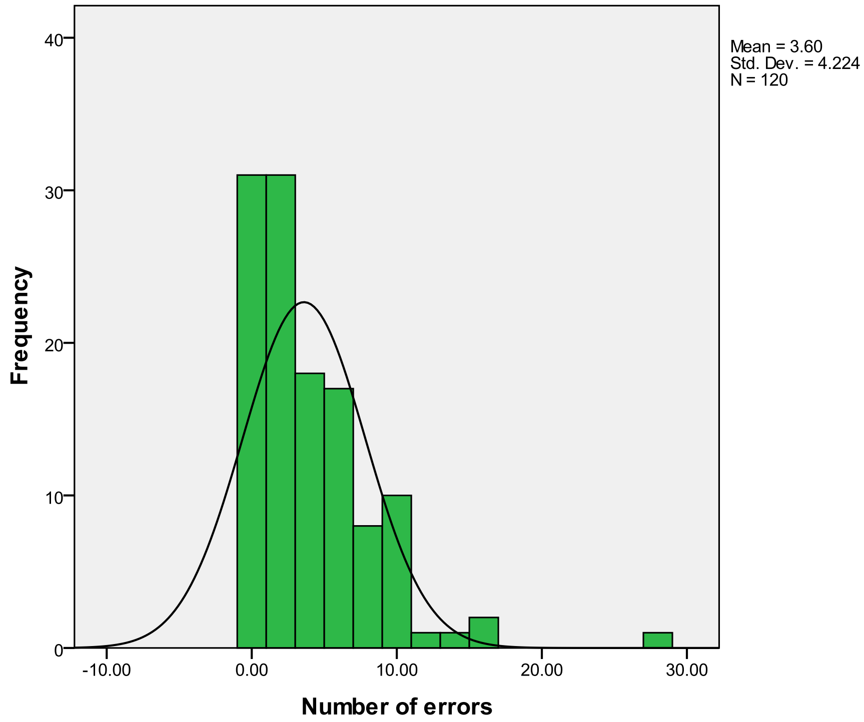
The displayed graphs were chosen because they prove a particular point. The remaining graphs that are not seen in the text can be found in the annex.

a) All horses combined:

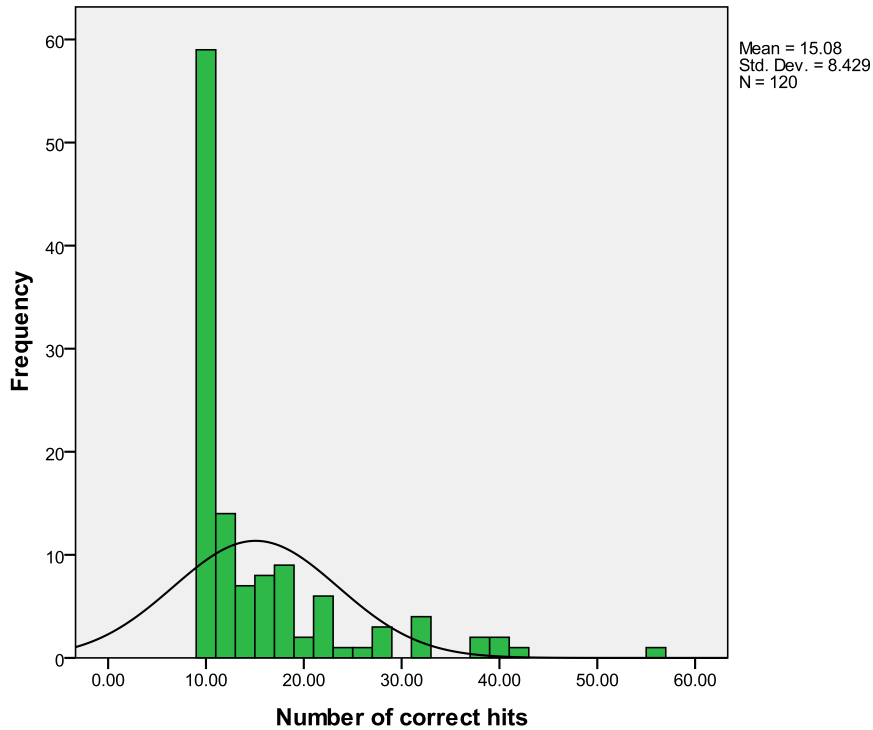


Graph 6: distribution of the time the horses needed to reach criterion

Data showed a positive skew for the time taken to reach criterion (Graph 1) with a skewness ratio of 13.90, total number of hits with a skewness ratio of 8.63, total number of correct hits (Graph 2) with a skewness ratio of 10.17, total number of errors (Graph 3) with a skewness ratio of 10.42 and the percent of errors with a skewness ratio of 24.96.



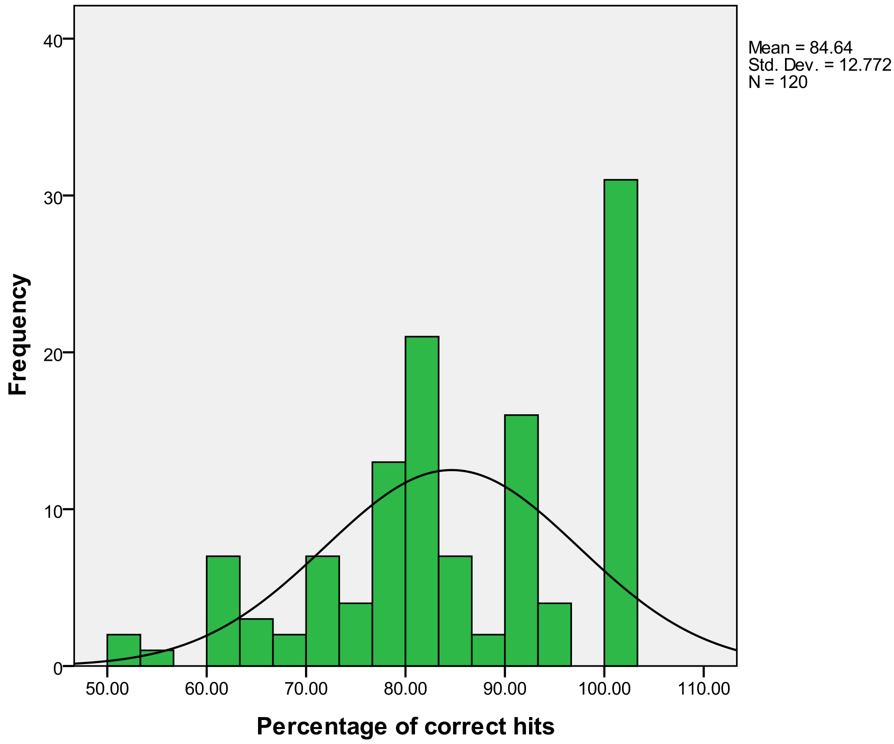
Graph 7: distribution of the number of errors the horses made



Graph 8: Number of correct hits of all horses

Graph three indicates that the horses learned the task very quickly as the single bar at the ten mark is very distinct (ten consecutive touches were required to move on to the next trial). The positive skew of these data suggests that natural selection favours speed of learning in horses.

Transformations were used for all variables but were only successful for 'time to reach criterion' and 'total number of hits'. The variable 'percent correct' showed a negative skew with a skewness ratio of -2.45 (Graph 4). With data clustering at 100% again good cognitive abilities were indicated. Transformation was successful for this variable and 'average time o touch the correct object' within each trial.

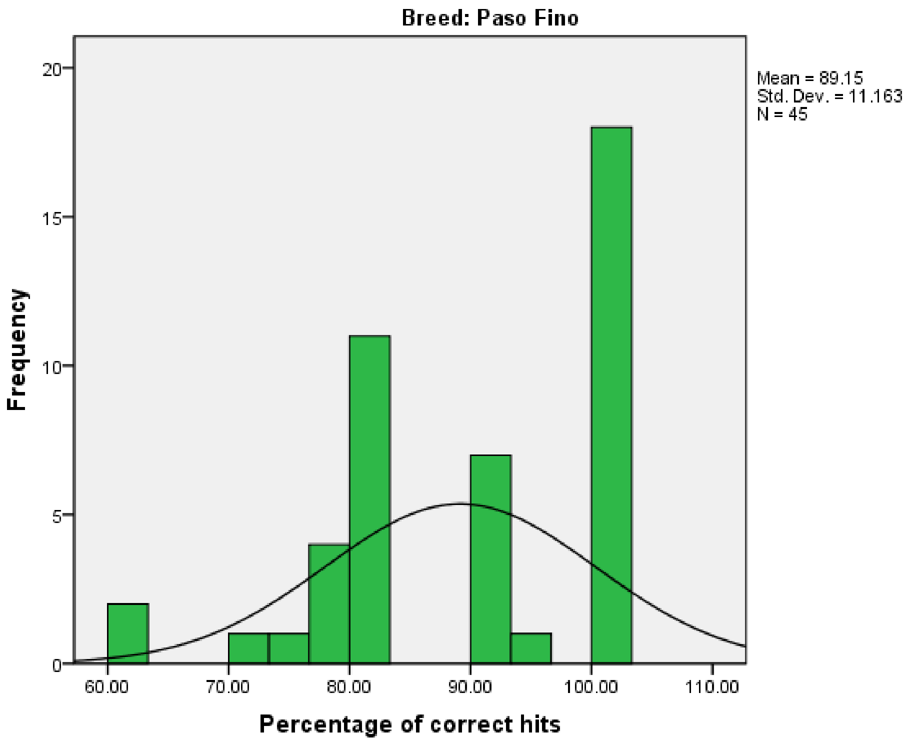


Graph 9: Percentage of the correct responses of all horses

b) Paso Fino Group

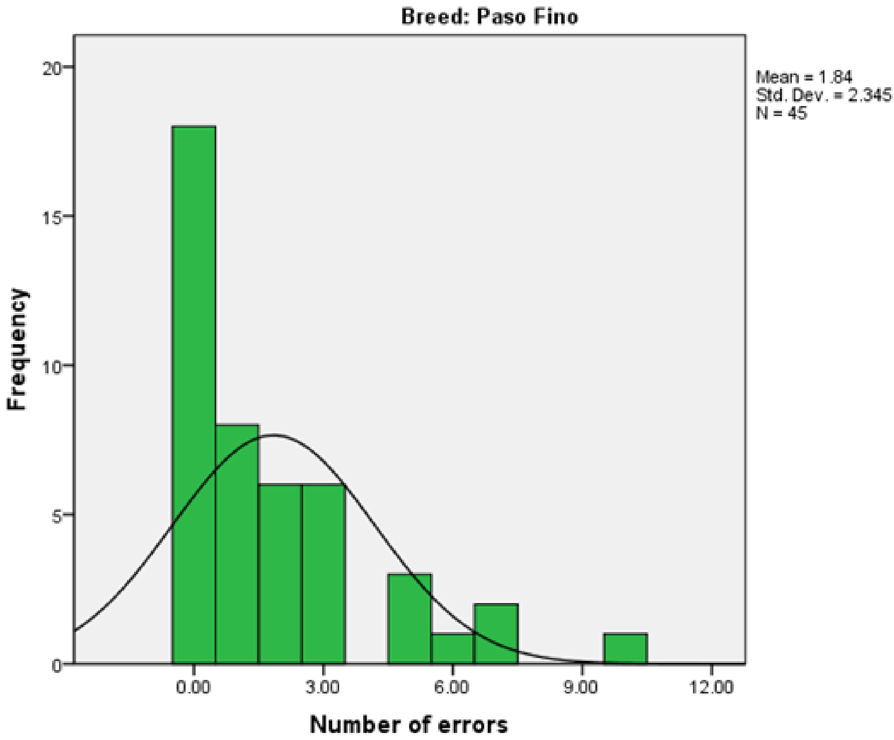
In the Paso Fino group the variables 'Time taken to reach criterion' with a skewness ratio of 3.03, 'Number of correct hits' with a skewness ratio of 2.43, 'Total number of hits' with a skewness ratio of 2.03, 'Number of errors'(Graph 6) with a skewness ratio of 5.20 and 'Average time to touch correct object within trials' with a skewness ratio of 4.94 showed positive skews, with total number of hits and correct hits having a mode of 10 and number of errors zero. 'Total number of hits' and 'Number of correct hits' were successfully transformed with the 1/x transformation.

The variables 'percent correct' (Graph 5) and 'percent errors' for the Paso Fino group were not skewed and did not need any transformation.



Graph 10: Percentage of correct hits for the Paso Fino group

Graph five shows that the Paso Finos as a group mostly had 100% correct hits.



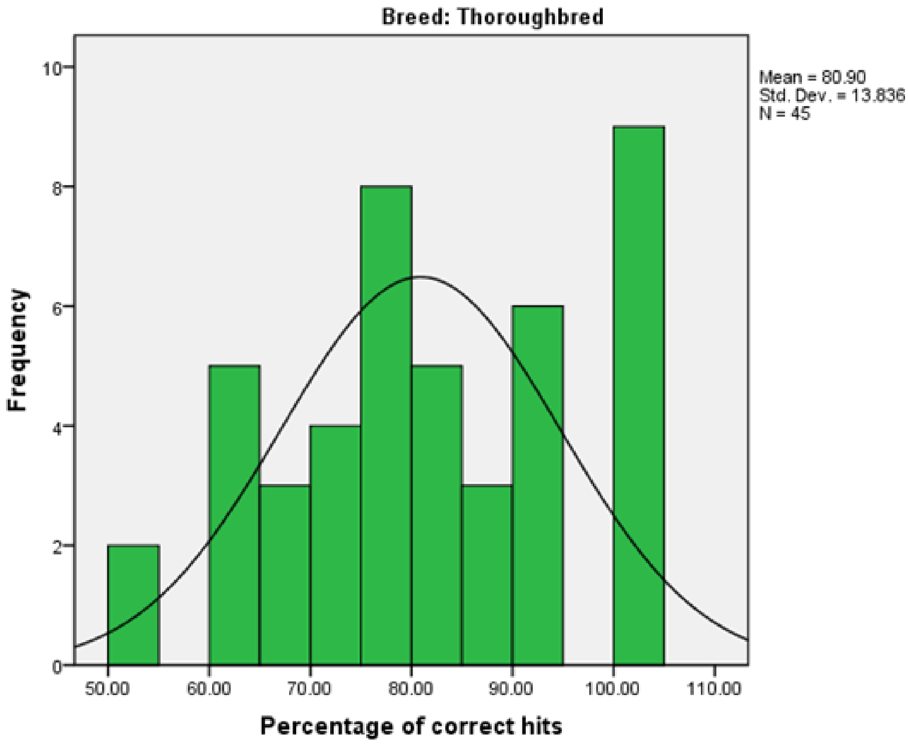
Graph 11: Number of errors made by the Paso Fino group

Graph six shows that their error rate was very low.

c) Thoroughbreds

In case of the Thoroughbred group the data for the variables ' time to criterion' with a skewness ratio of 8.72, 'total number of hits' with a skewness ratio of 2.93, 'number of errors' with a skewness ratio of 3.24 and 'the average time to touch a correct object within trials' with a skewness ratio of 7.92 showed positive skews. The mode for total number of hits was 10 and errors zero. The variables 'time to criterion' and 'total number of hits' were successfully transformed through the 1/x transformation to an acceptable level of skewness.

The variables 'Number of correct hits', 'Percent correct' (Graph 7) and 'Percent Errors' showed normal distributions. The graphs showed slight skews for these variables, with 'number of correct hits' and 'percent of errors' showing a slight positive skew and the percent of correct hits a minor negative skew.



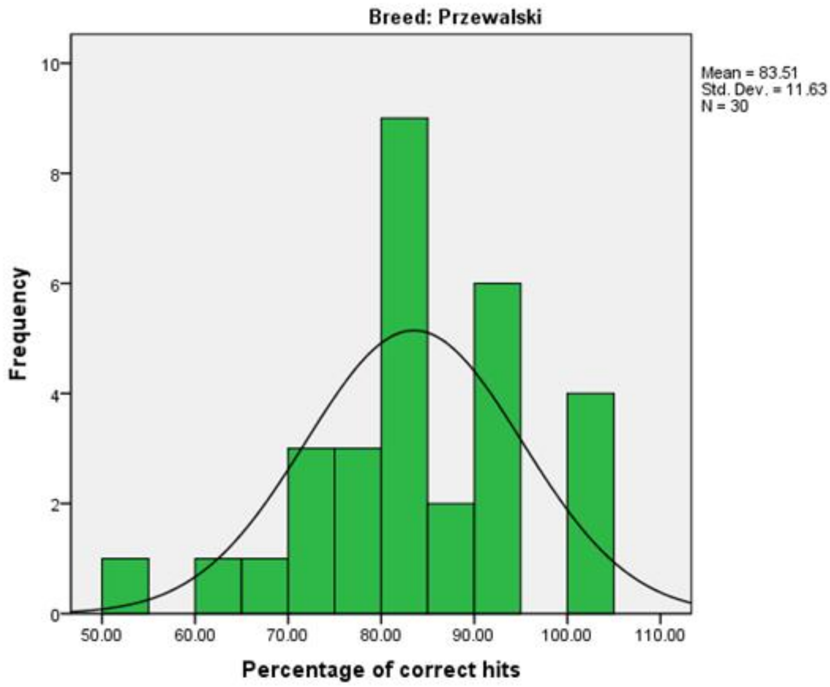
Graph 12: Percentage of correct hits of the Thorougbred horse Group

Graph seven shows that the Thoroughbred group had its peak at 100% as well, however there were many trials with just 80% correct as well.

d) Przewalskis

In the Przewalski group the variables 'Number of correct hits' with a skewness ratio of 2.43, 'Total number of hits' with a skewness ratio of 2.03, 'Time taken to reach criterion' with a skewness ratio of 3,03, 'Total number of errors' with a skewness ratio of 5.20 and 'Average time to touch the correct object within trials' with a skewness ratio of 8.11 all showed positive skews. The variables 'Number of correct hits' and 'Total number of hits' could be transformed.

The variables 'percent errors' and 'percent correct' (Graph 8) did not need any transforming as the skewness ratios indicated normal distributions.



Graph 13: Percentage of correct hits of the Przewalski horse group

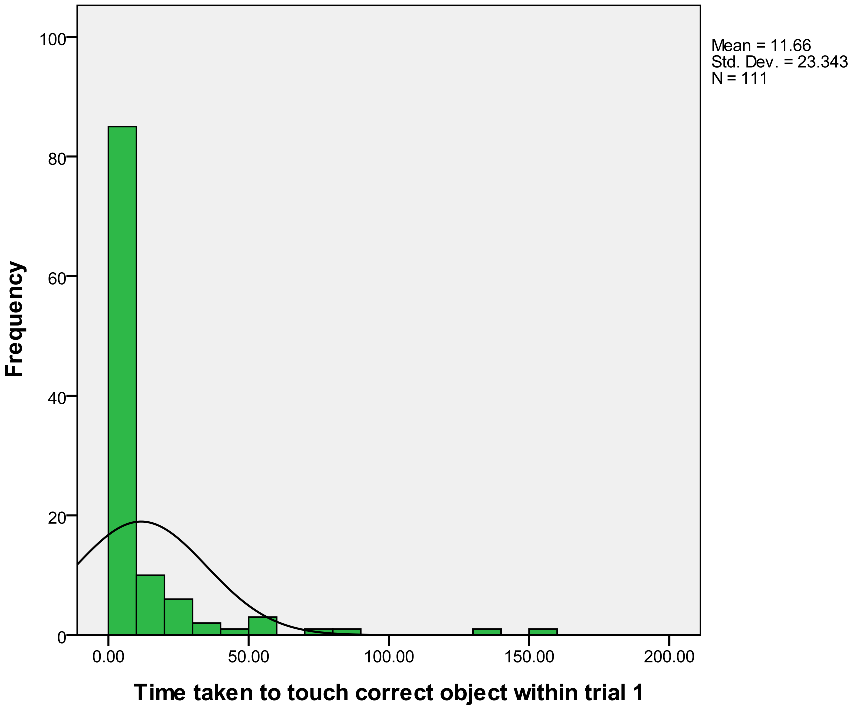
Graph eight illustrated that the Przewalski group had 80% correct hits over the duration of the trials.

Within trial data: time taken to touch correct object

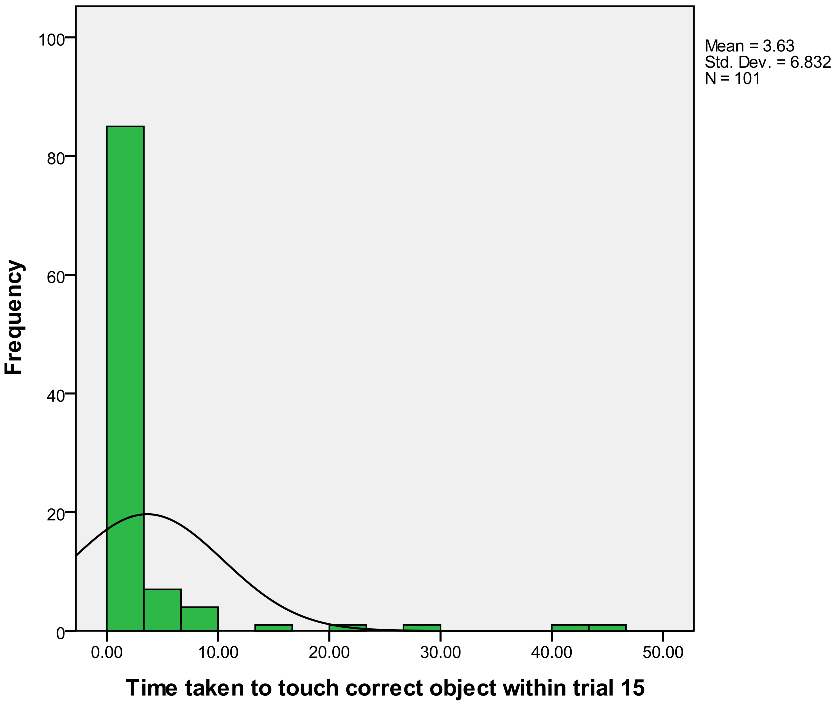
The variable 'time to criterion' was further investigated as it presents the core of the study. The time the horses needed to touch an object again after receiving a reward was measured and documented. For example, the above mentioned time each horse took to touch an object after being fed the reward was collected for all horses, then clustered within each breed and then split into each of the fifteen trials. So each Thoroughbreds first trial was added together and averaged and so forth for all fifteen trials. For these data tests for normality were carried out as well.

a) All horses

All trials were skewed ranging from 10.09 to 30.36 and only trial four could be transformed to reach an acceptable skewness ratio of 1.83. Graph nine and ten show that the mode decreased from 10 to 3.33 over the duration of the 15 trials for all horses.



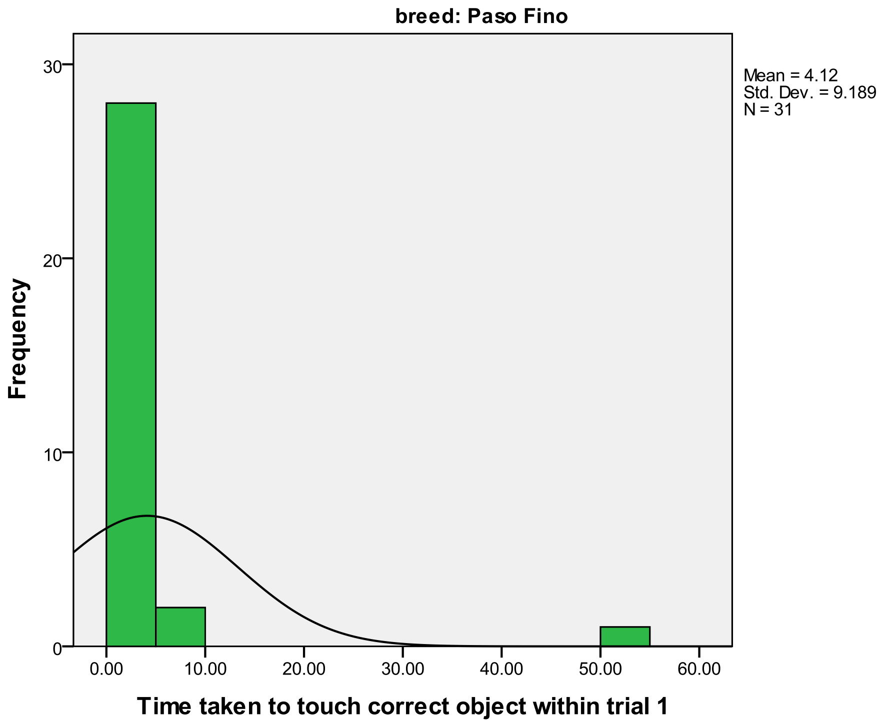
Graph 14: Time needed o touch the correct object of all horses in their first trial



Graph 15: Time needed to touch the correct object of all horses in their 15th trial

b) Paso Fino group

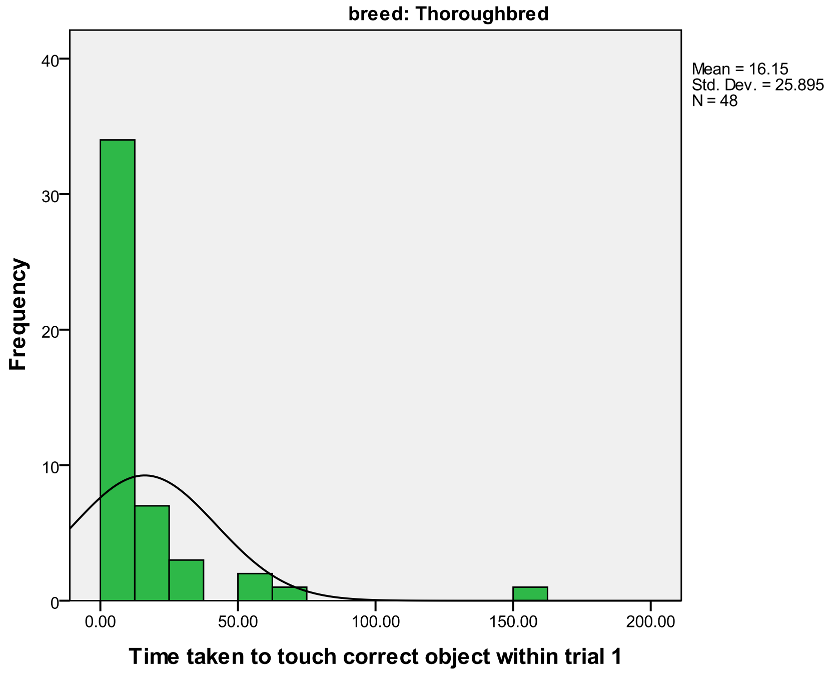
In the Paso Fino group the data of all trials were skewed apart from 14. Trial 1 to 4, 8, 9, 12 and 15 have successfully been transformed to a normal distribution. All remaining trials could not be successfully transformed. Graph 11 shows the mode of 5 for the first trial. Over the duration of the 15 trials for the Paso Fino Group, the mode did not undergo major changes. In trial 15, the mode was 5 again.



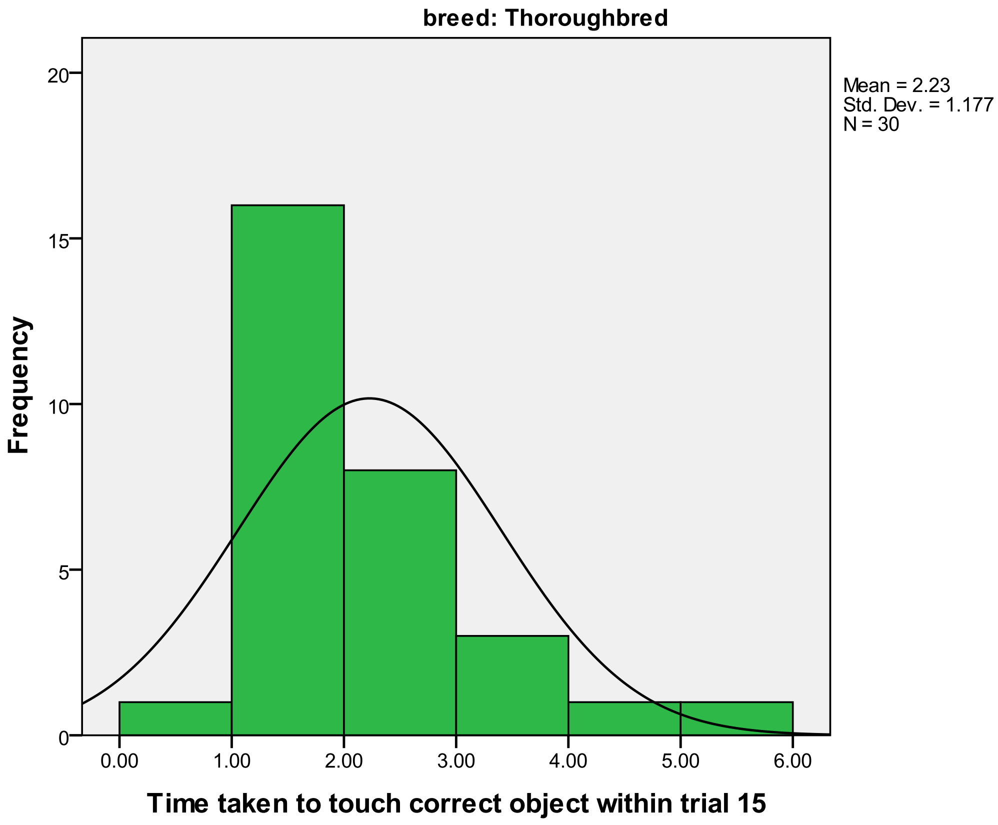
Graph 16: Time needed to touch the correct object of the Paso Fino Horses in their first trial

c) Thoroughbred group

In the Thoroughbred group the data of trial 12 and 14 could be transformed successfully whilst all other trials showed a highly skewed distribution ranging from 2.40 to 11.80 that could not be transformed. Graph 12 and graph 13 show the decrease of the mode over the duration of the 15 trials for the Thoroughbred group from 12.5 to 1.



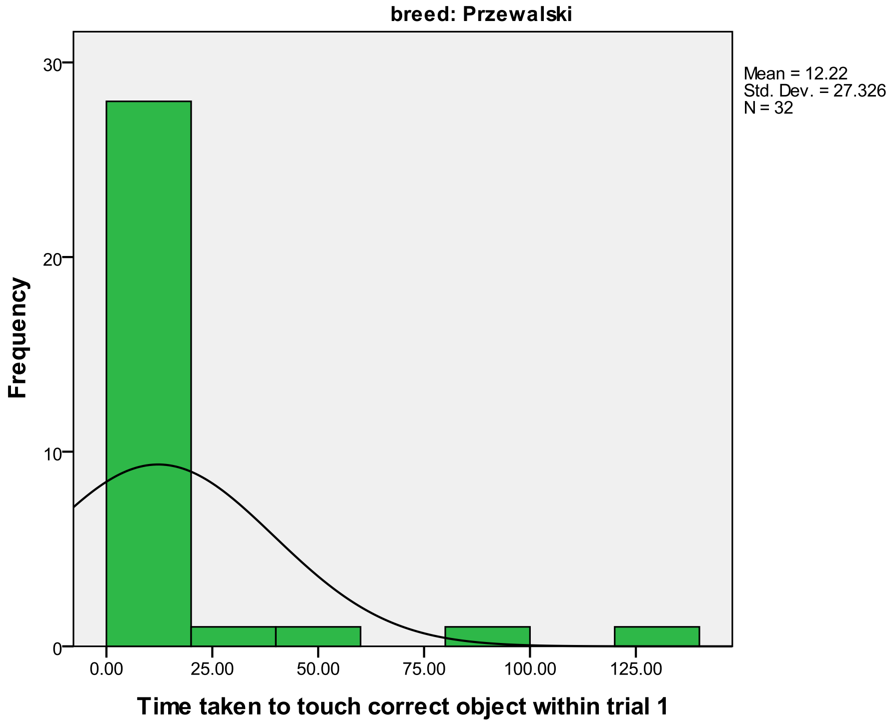
Graph 17: The time the Thoroughbred group needed to touch the correct object within their first trial



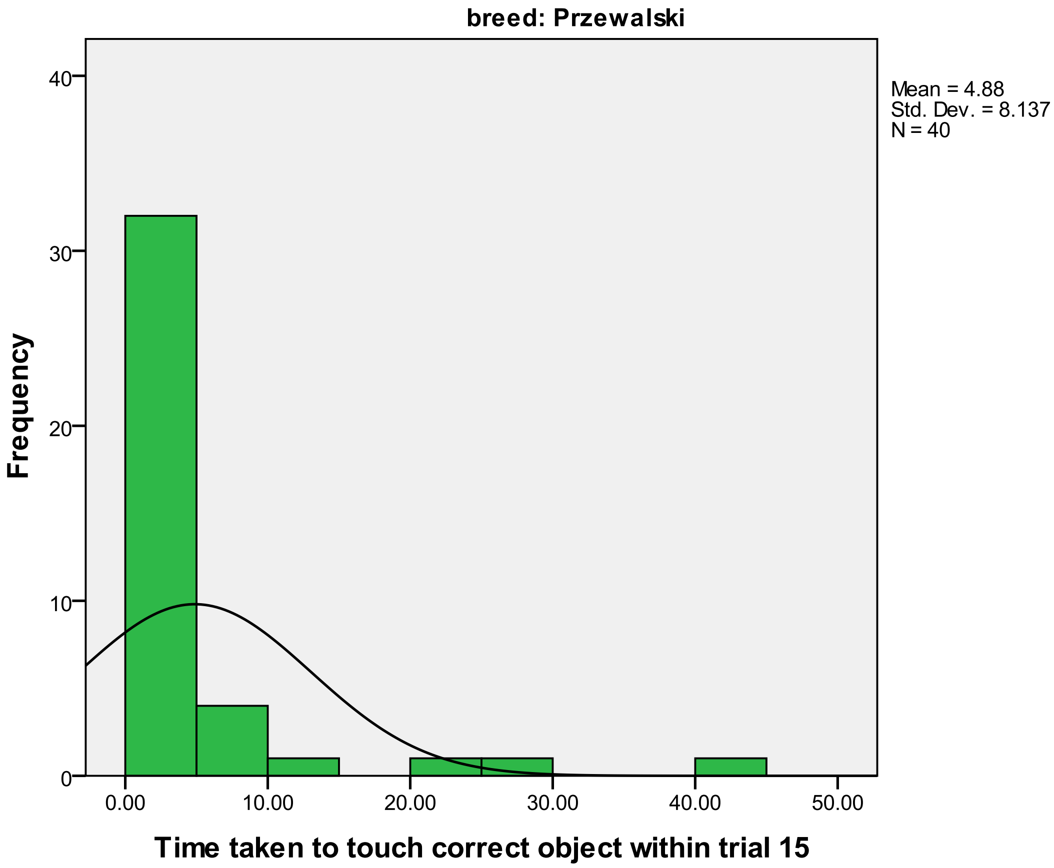
Graph 18: Time the Thoroughbred group needed to touch the correct object in trial 15

d) Przewalski Group

No trial of the Przewalski group showed a normal distribution at the start. The skewness ratios ranged from 2.31 to 20.36. Trials 1, 3, 4, 7, 9, 10 and 13-15 could successfully be transformed whereas all other trials still remained skewed after transformation. Graph 14 and graph 15 show the decrease of the mode over the duration of the 15 trials for the Przewalski group from 23 to 5.



Graph 19: Time the Przewalski group needed to touch the correct object in trial 1



Graph 20: Time the Przewalski group needed to touch the correct object in trial 15

2. Overview over ROC curve results

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10	Trial 11	Trial 12	Trial 13	Trial 14	Trial 15
N	111	170	117	118	107	118	128	138	119	104	128	121	123	98	101
All horses	0.66(0.0001[3])	0.64(0.001)	0.58(0.006)	0.53(NS)	0.48(NS)	0.51(NS)	0.47(NS)	0.53(NS)	0.39(0.0001)	0.48(NS)	0.43(0.009)	0.42(0.003)	0.48(NS)	0.40(0.001)	0.43(0.03)
N	12	38	22	10	10	10	18	10	11	10	10	10	10	10	10
Pincel	0.58(NS)	0.67(0.001)	0.63(0.004)	0.64(NS)	0.39(NS)	0.46(NS)	0.43(NS)	0.62(NS)	0.21(0.001)	0.50(NS)	0.46(NS)	0.26(0.011)	0.52(NS)	0.30(0.033)	0.32(NS)
N	10	12	10	10	10	10	10	10	10	10	10	10	10	10	11
Quito	0.54(NS)	0.81(0.001)	0.50(NS)	0.44(NS)	0.43(NS)	0.45(NS)	0.34(NS)	0.50(NS)	0.42(NS)	0.32(NS)	0.65(NS)	0.56(NS)	0.36(NS)	0.54(NS)	0.52(NS)
N	9	10	10	10	10	12	10	12	18	10	10	20	10	10	10
Raphael	0.67(NS)	0.42(NS)	0.59(NS)	0.46(NS)	0.59(NS)	0.55(NS)	0.56(NS)	0.41(NS)	0.61(NS)	0.23(0.005)	0.47(NS)	0.46(NS)	0.47(NS)	0.30(0.043)	0.60(NS)
N	20	16	10	16	15	32	21	22	10	22	18	14	32	10	10
Donnie	0.88(0.001)	0.84(0.001)	0.62(NS)	0.59(NS)	0.55(NS)	0.46(NS)	0.44(NS)	0.62(NS)	0.51(NS)	0.50(NS)	0.33(0.019)	0.29(0.008)	0.31(0.001)	0.23(0.004)	0.17(0.001)
N	17	13	13	12	13	10	10	18	10	16	10	10	10	10	10
Eddie	0.79(0.001)	0.69(0.018)	0.79(0.001)	0.53(NS)	0.62(NS)	0.35(NS)	0.58(NS)	0.39(NS)	0.28(0.021	0.52(NS)	0.27(0.018)	0.43(NS)	0.32(NS)	0.34(NS)	0.22(0.004)
N	11	13	11	20	10	16	11	10	10	11	10	11	10	10	10
Sky	0.73(0.011)	0.90(0.001)	0.69(0.033)	0.63(0.049)	0.66(NS)	0.51(NS)	0.45(NS)	0.35(NS)	0.37(NS)	0.38(NS)	0.34(NS)	0.21(0.002)	0.41(NS)	0.18(0.001)	0.39(NS)
N	16	55	31	28	23	18	10	40	40	15	42	25	10	10	28
Anna	0.53(NS)	0.62(0.002	0.57(NS)	0.43(NS)	0.63(0.025)	0.34(0.025)	0.22(0.003)	0.58(NS)	0.40(0.041)	0.47(NS)	0.51(NS)	0.49(NS)	0.44(NS)	0.37(NS)	0.63(0.019)
N	16	13	10	12	16	10	38	16	10	10	18	21	31	28	12
Katrina	0.57(NS)	0.74(0.003)	0.59(NS)	0.56(NS)	0.34(0.044)	0.60(NS)	0.40(0.050)	0.68(0.013)	0.40(NS)	0.52(NS)	0.40(NS)	0.50(NS)	0.50(NS)	0.47(NS)	0.38(NS)

Table 10: ROC curves for all horses over the fifteen trials

(ns in the table above indicates 'not significant').

[3] The significance value in brackets is the asymptotic significance, as the ROC is tested on a non parametric basis since the majority of the trial variables were not responsive to transformation.