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Associationsanalyser av hårvirvlar i pannan, beteende och kromosomregioner hos varmblodstravare

Victoria Saxe

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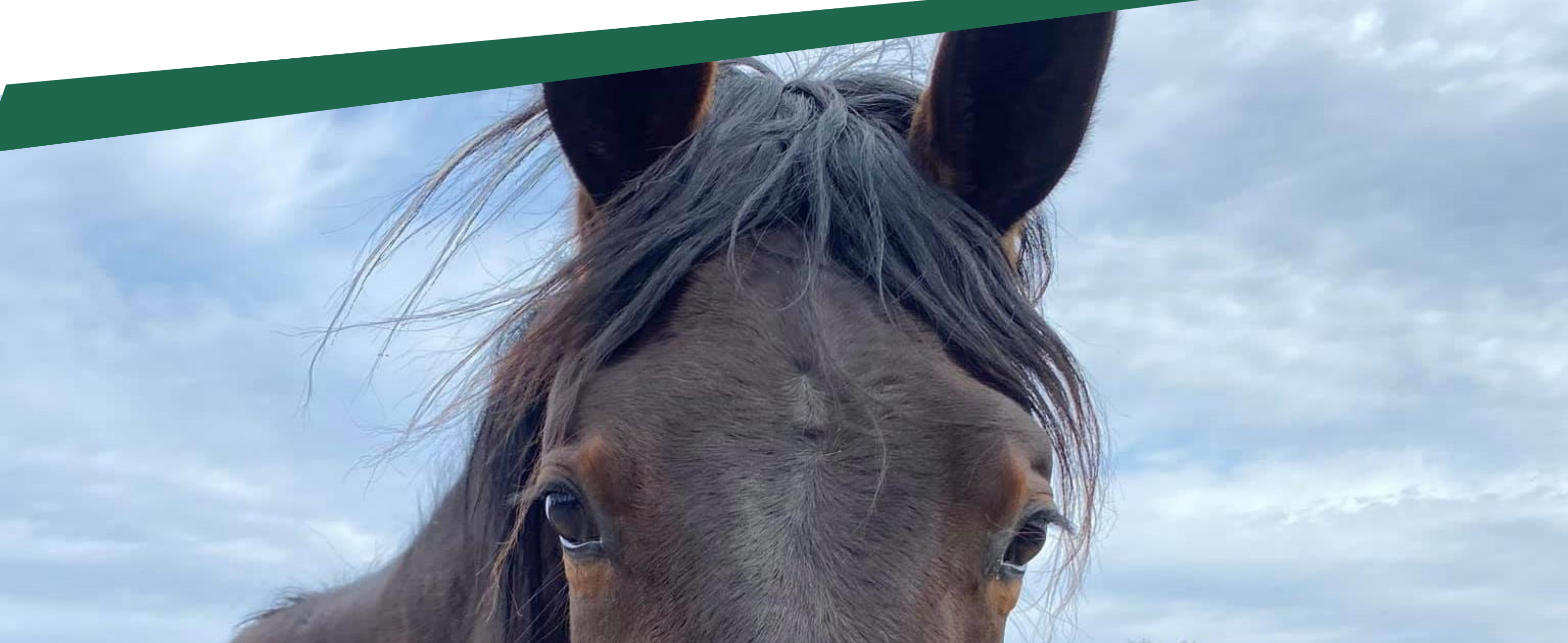

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Victoria Saxe

Supervisor:	**Maria Wilbe, Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics**
Assistant supervisor:	Gabriella Lindgren, Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics
Examiner:	Tomas Bergström, Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics

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Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Animal Breeding and Genetics

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Abstract

Introduction: In horse breeding, athletic skills have been in focus and certain behaviour types have been found to be important for competition. Behaviour is heritable but is also affected by the environment. Just like humans, animals have their own personality and behaviour-traits.

The hair whorls in the equine forehead and their potential meaning have been discussed by people practising horsemanship. They are believed to tell us things about the horse's behaviour and personality. The hair whorls are thought to take form when the skin on the foetus expands during growth in the uterus. At a certain timepoint during development, the hair follicles get set in their angle that gives the hair its direction to grow. But exactly how the hair whorls get their specific appearance and what position they get is unclear. Since both brain and skin cells originate from the ectoderm, there are theories that the brain can be mirrored by the overlying skin.

The aim of this study was to investigate if there is an association between facial hair whorls and behaviour as well as finding regions of interest for the hair whorl types in the horses' DNA.

Material and method: The behaviour data to be analysed in this study had already been collected through a survey in another project about genetics and behaviour. The behaviour traits analysed were nervosity, excitability, fearfulness, concentration, learning, memory, cooperation, will to win, stubbornness, self-control, recovery, appetite and stereotypic behaviour. In that survey the trainer gave the horses scores between 1-7 depending on how often they saw the behaviour during competition. Hair samples from the horses' tails had also been collected.

This study analysed the association between the thirteen different behaviour traits from the survey and nineteen different hair whorl types in 175 Standardbred trotters. Their DNA were extracted, and their genome low pass sequenced and analysed. Their genomic information was run in a computer program that compared the individuals in the different hair whorl groups.

The hair whorls were inspected live or from a photo, a protocol was filled out and a photo was taken on the horses' forehead. The horses were then divided into groups as low (1 and 1-2) and high (7 and 6-7) scores from the survey, and hair whorl groups that were classified as contraries. Statistical association analyses were then performed between individuals that had extreme scores (1 against 7 and 1-2 against 6-7) in the behaviour traits according to the survey, and the differing hair whorl groups. The association were analysed using two by two contingency tables.

The results of this study can indicate the following; if a facial hair whorl is located to the right (to the left from a frontal view), the chance is bigger that it becomes nervous during competition in comparison to a horse with a facial hair whorl to the left (p-value: 0,025). If a horse has more than one facial hair whorl, it more often took longer for it to learn the task of competing (p-value: 0,03). Comparing the horses that had a facial hair whorl looking like a feather, there was a greater chance of the trotter to be focused and concentrated during competition if the feather went in a vertical direction, compared to if it had a horizontal or diagonal direction (p-value: 0,05). If the feather was vertical, they also had a higher chance of having good appetite after competition (p-value: 0,03).

On five chromosome regions, but one especially, there was found a highly significant difference between horses with one hair whorl and horses with two hair whorls in the forehead. For future investigations in this subject, these results can serve as an interesting basis about associations between facial hair whorls, behaviour, and genetics of Standardbred trotters.

Keywords: Behaviour, temperament trait, tricoglyph, hair whorl, swirl, genetics, Standardbred trotter, genotype, phenotype

Sammanfattning

Introduktion: De atletiska egenskaperna har varit i fokus när det kommer till hästavel, men även betéende är viktigt för tävlingshästar. Beteende är genetiskt överförbart, men påverkas även av miljön. Precis som människor har djur olika personligheter och därav olika beteende-typer.

Hårvirvlarna i hästens panna och deras eventuella betydelse har sedan länge diskuterats bland människor som lever och jobbar med hästar. Dessa hårvirvlar tros kunna antyda vilken sorts personlighet och beteenden en häst har.

Hårvirvlarna tros bildas när huden på fostret stretchas ut under tillväxten i livmodern. Hårfolliklarna får då troligen en vinkel som ger upphov till åt vilket håll hårstrået växer. Men exakt varför virvlarna får det utseende de får, och vilken position de får, är oklart. Eftersom samma embryonala celler ger upphov till både hjärnan och skinnet, finns teorier om att hjärnan kan speglas av den överliggande huden.

Studiens syfte var att undersöka om det finns någon association mellan hårvirveltypen i pannan och beteende hos häst, men även att hitta DNA-regioner av intresse för hårvirveltyp hos hästen.

Material och metod: Beteendedata som analyserades i denna studie hade redan samlats in genom en enkät i ett tidigare projekt om genetik och beteende. Beteende-typerna som analyserades var nervositet, upphetsning, rädsla, koncentration, inlärning, minne, samarbete, vilja att vinna, envishet, självbehärskning, återhämtning, aptit och stereotypier. I denna enkät gav travtränaren hästen poäng mellan 1-7 beroende på hur ofta de såg betéendet under tävling. Hårprover från hästarnas svansrötter hade också samlats in i den tidigare studien.

Denna studie analyserade eventuella samband mellan de tretton olika beteende-parametrarna och nitton olika hårvirvel-typer på 175 stycken varmblodstravare i Sverige och Norge. Hästarnas DNA extraherades på labb och sedan analyserades och gensekvenserades DNA:t. Deras genetiska information kördes därefter i ett dator-program som jämförde individerna i de olika hårvirvel-grupperna för att hitta eventuella genetiska faktorer av värde för dessa hårvirvel-typer. Hårvirvlarna inspekterades, ett protokoll fylldes i och ett foto togs på vardera hästpanna. Hästarna blev sedan indelade i grupper som låg (1 och 1-2) och hög (7 och 6-7) poäng-tagare från enkäten, och hårvirvelgrupper som klassificierades som motsatta varandra. Statistisk association beräknades med hjälp av frekvenstabeller, mellan individer som hade extrema beteende-poäng (1 mot 7 och 1-2 mot 6-7) och de motsatta hårvirvelgrupperna.

Resultaten kan indikera följande: Om hårvirveln är till höger i pannan (vänster framifrån sett) är sannolikheten större att hästen blir nervös under tävling, till skillnad från en häst med en virvel till vänster i pannan (p-värde: 0,025). Om en häst har fler än en virvlel i pannan, så tar det längre tid för den att lära sig uppgiften att tävla (p-värde: 0,03). Vid jämförelse av de hästar som hade en hårvirvel som ser ut som en fjäder, var det mer sannolikt att hästen skulle vara fokuserad under tävling om fjädern gick vertikalt i pannan jämfört med om den gick horisontellt eller diagonalt (p-värde: 0,05). Om fjädern gick i vertikal riktning var det också större chans att de skulle ha bra aptit efter tävling (p-värde: 0,03).

På fem men speciellt en kromosom hittades en region av hög signifikans för skillnad mellan hästar som hade en virvel i pannan jämfört med hästar som hade två virvlar i pannan. För framtida undersökningar i detta område kan dessa resultat ge intressant grund och indikationer på associationer mellan hårvirvel, beteende och genetik hos varmblodstravaren.

Nyckelord: Beteende, temperamentsegenskap, trikoglyph, hårvirvel, genetik, varmblodstravare, genotyp, fenotyp

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Abbreviations

CW	Clockwise
CC	Contraclockwise
OR	Odds ratio
∞	Infinity
SNP	Single nucleotide Polymorphism
AF	Allele frequency

1. Introduction

For a time, there has been a conviction that the hair whorls on horses have different meanings depending on the whorl's shape and location (King & Wylie, 2013). The question of whether the hair whorls on an animal can tell us something about that individual's behaviour or not isn't yet completely answered, but studies have been made on the subject (Grandin *et al.* 1995; Lanier *et al.* 2001; Randle *et al.* 2003; Broucek *et al.* 2007; Górecka *et al.* 2007; Shivley *et al.* 2016). Some connections between behaviour and facial hair whorls have been found. One is that horses with a high positioned facial hair whorl were less manageable compared to them with a medium or low positioned whorl (Górecka *et al.* 2007), and a clockwise hair whorl has been associated with turning to the right (Shivley *et al.* 2016). In humans, abnormal hair whorls have been associated with abnormal brain development (David & Osborne 1976; Puri *et al.* 1995; Ruiz-Maldonado 2002; Yousefi-Nooraie & Mortaz-Hedjri 2008).

The curiosity about the meaning of hair whorls have created a society among people practising horsemanship. These people are using both personal experience and scientific literature in their conclusions. They think that a horse with a high whorl has an extrovert personality, which makes them energy-filled and alert, and that a low whorl would indicate a horse that is introvert, typically quiet and secure. If a whorl is laterally placed to the left (horse´s left) it is believed to show a horse with mainly right-brained behaviour characteristics. This means a horse who is fearful and can be seen as nervous. A whorl on the horse's right side of the forehead is said to be usual for a left brained horse. They are the ones to think a lot and can unlock gates and untie themselves. A feathered whorl is thought to be common for a left brained horse, but also give a friendly, calm, and confident horse. However, as in most of biology, according to the experts in this area, there will always be some exceptions (Miller 2021).

The hair whorls are formed before birth and never change. This is the reason why they are also used to identify horses on register papers. The hair whorl on the forehead is considered to be the most indicative of temperament, because the skin and the nervous system originate from the same embryonic cell layer (King & Wylie, 2013).

Ethologists who study animals' behaviour, have recently started to incorporate genetics and neuroscience in their studies since they are most likely important factors in personality traits (Grandin & Deesing, 2014). Studies have suggested that the position of a hair whorl in the forehead is likely to be passed on from parents to offspring, with a heritability of 0,6-0,8 (Górecka *et al.* 2006; Yokomori *et al.* 2019).

The overall aim of this study was to investigate if there is an association of forehead hair whorls, behaviour, and genetics in Swedish standardbred trotters. More specifically:

- Investigate the association of forehead hair whorls and behaviour based on a behaviour questionnaire and hair whorl-phenotyping.
- Perform a genetic association study to identify possible genes associated with a certain behaviour and/or hair whorl type.

2. Literature review

2.1. Types of hair whorls

There are many varieties of hair whorls (also called swirl or trichoglyph) (Top Horse, n.d.). What defines a hair whorl is that the hair grows in another direction from the hair surrounding it. It can be radial (Figure 1C), where the hair goes out from the centre point (X) of the whorl symmetrically, like the petals in a flower. It can be clockwise (CW) (Figure 1A) or contraclockwise (CC) (Figure 1B), measured from the hair root and out.

A feathered, or elongated whorl is where the hair goes radially in a line, the base of the feather can have different directions though. When more than one whorl is present, a tuft can be seen between them, this is where the hair converges and piles up into a tuft, this can be seen in figure 2 between the two midline placed whorls. The tuft itself is not classified as a hair whorl.

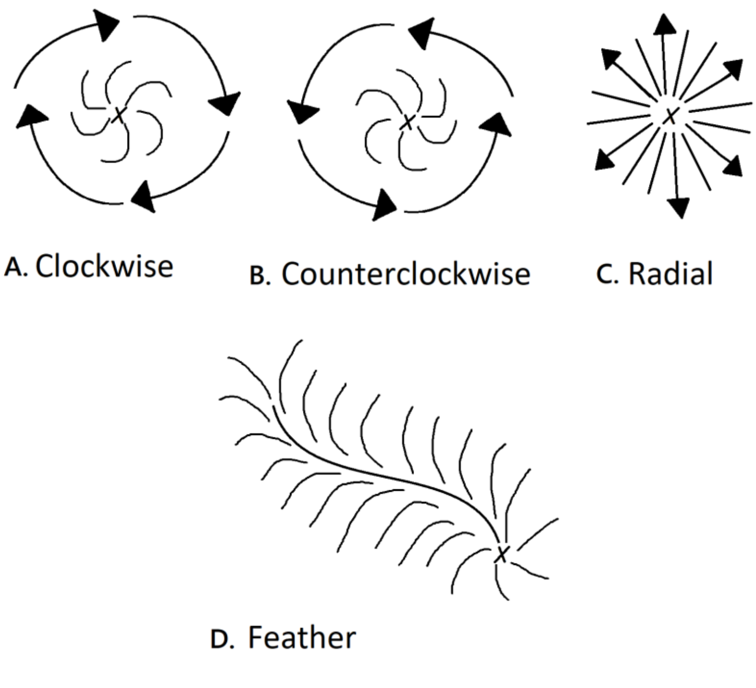

Figure 1. Schematic description of hair whorls. A) Clockwise B) Counterclockwise C) Radial D) feather. Illustrated by the author.

A hair whorl can be located in different places on the forehead (Shivley *et al.* 2016). A high one is set above the eyes, a medium whorl, between the eyes and a low whorl, beneath eye level. A hair whorl can also be placed on the midline or lateral (left or right) to the midline. Most horses have a hair whorl on their forehead and some individuals have more than one.

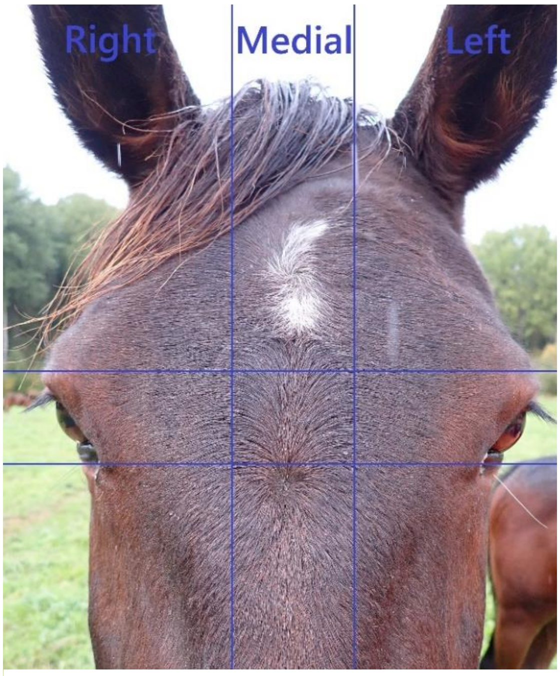

Figure 3. A high whorl with a clockwise direction and a low radial whorl on the midline. Photo by the author.

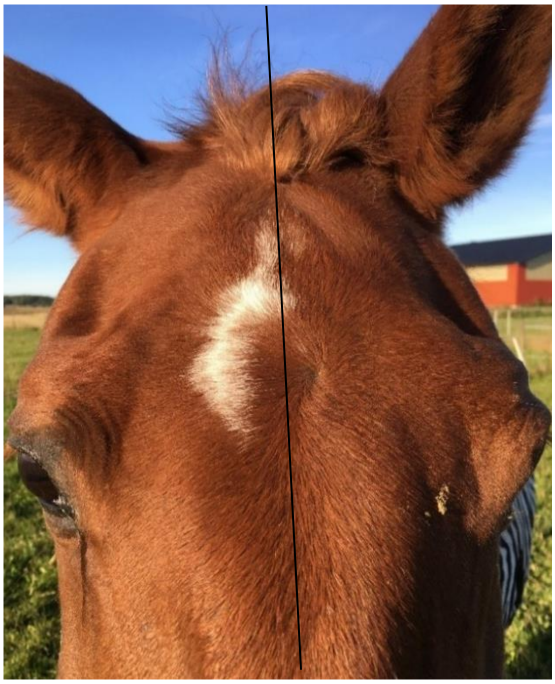

Figure 2. A high positioned, medial left, counter-clockwise whorl. Black line is the midline. Photo by the author.

2.2. Embryology and neurulation

During the preborn life in the uterus, when the foetus is formed, there are three layers of cells that together forms the end product of the mammal, the ectoderm, mesoderm and endoderm (Arora, 2008). These three layers originates from the blastocysts and appears during the gastrulation. Neurulation is the process that forms the neural tube, starting with a neural groove formation. When the three layers ectoderm, mesoderm and endoderm have been formed around week four of gestation in humans, neurulation starts, from a centre point called the notochord (See figure 4). In horses, neurulation starts around day 16 after ovulation (Walter *et al.* 2010; Gibson *et al.* 2017). The ectoderm folds and form a groove that then becomes a tube that dissociates from the rest of the ectoderm that seals itself together again, on the outside and later becomes the epidermis (Arora, 2008). The tube that invades into the mesoderm underneath later becomes the spinal cord, and from the mesoderm, muscle and skeletal cells are formed to make the vertebrae and muscles around it.

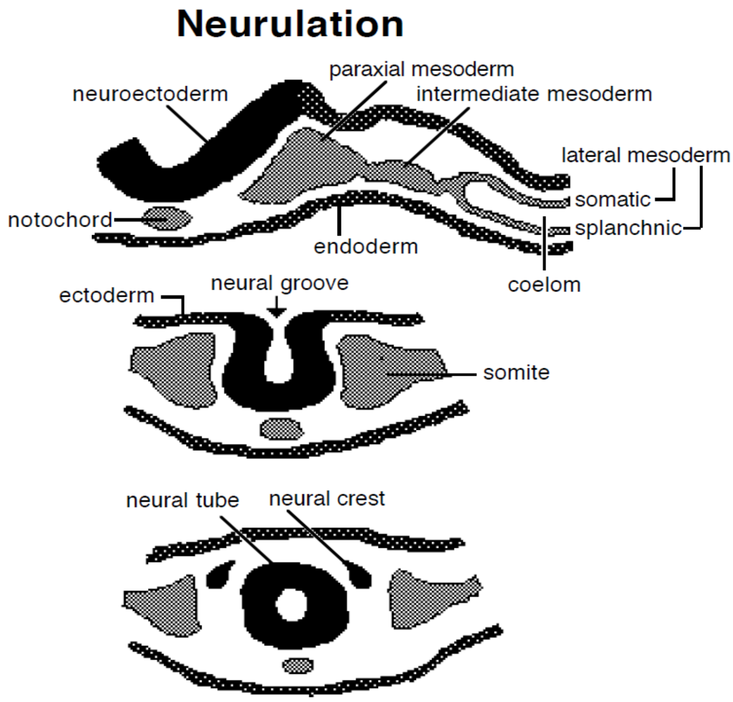

Figure 4. "Neurulation" by Fletcher & Weber, 2013, permitted to use. The ectoderm folds and form a groove that then becomes a tube that dissociates from the rest of the ectoderm that seals itself together again on the outside and later becomes the epidermis. The tube that invades into the mesoderm underneath later becomes the spinal cord, and from the mesoderm, muscle and skeletal cells are formed to make the vertebrae and muscles around it.

The neural structures, as well as the skin, arises from the ectoderm. Between the ectoderm and endoderm sheet the mesoderm lies. In the mesodermal sheet, loosely packed cells (mesenchyme) follow the ectoderm as the embryo "folds" to create a tubular shape. The endoderm becomes the GI-tract and organs such as lungs. The ectodermal cell sheet on the outside is made up of epithelial cells, which are arranged in sheets, often with tight links together. Organs derived from the epithelia are induced by the local mesenchyme beneath it, and the spatial arrangement of the epithelial organs depends on a precise pattern of inductive stimuli from the underlying mesenchyme (Arora, 2008). The epidermis as well as the hair follicles originates from the ectoderm and the dermis originate from the mesenchymal mesoderm (Hardy, 1992). As the foetus grows, the epidermis is stretched out to follow it around, and as the cranium develops quite rapidly, it is believed to be a tension on the skin and hair follicles that grows in the loose underlying mesenchyme. The most commonly accepted theory to explain the scalp hair slope and patterning in humans is this "mechanical tension" (Ruiz-Maldonado 2002).

2.2.1. The hair coat formation and growth in size

According to De Meijere and Carter in Hardy (1992), the coat of fur is starting to form in the uterus in a specific location, such as the scalp of the head, and then extend in a wave-like manner all throughout the body surface. In a study of Franciolli *et al.* (2011), the embryos' skin was translucent, until day 35, when vascularisation of the dermis started. The equine foetus is hairless and the size of a hamster at 60 days of gestation (Acres 2002). The neck straightens between 60-80 days. At around day 100, the foetus has hair on the lips, and is the size of a kitten. On day 150, hair has formed on the chin, muzzle and eyelids and is the size of a rabbit. At day 180, the mane and tail are visible, and it has grown to the size of a beagle. Around day 270 thin hair covers the body and the size is like a German Shepherd's (Acres 2002). When it's 300 days old, the mane and tail have started to develop and when it's 330 days old, the hair coat is fully developed (Burwash Equine Services Ltd. n.d.).

2.2.2. Hair pattern development theories

Some different theories of how the scalp hair pattern develops in humans are present (Samlaska *et al.* 1989 in Randle *et al.* 2003). One is that the mechanical tension on epidermis by expansion and growth of the foetus gives the direction of the hair follicles *in utero*. Another theory is the one of inheritance. Distribution of hair, hair texture and colour are known to be heritable. In studies on humans, the most usual whorl is a clockwise one (Klar 2003, 2009; McDonald 2011). Studies have also found that it is not common to have two hair whorls. Genetical aspects, such as if a clockwise hair whorl is determined by a dominant gene, have been discussed (McDonald 2011). Nevertheless, a study on twins with double occipital hair whorls showed that the intrapair concordance rate was only 1 / 6 in the monozygotic twins. They mean that this indicates that the trait is presumably subject to prenatal environmental influences (Sharma, 1987).

Klar (2009) found that the hair whorl direction in Japanese individuals are random, although their handedness variation is similar to the one in the US (most people being right handed). His hypothesis is that separate decisions are made during embryogenesis for developing handedness and hair-whorl features in at least Japanese individuals. It is probable that the development of hair whorls are influenced by different factors, like epithelial cell migration (Ribeiro *et al.* 2003) before and during the events of neurulation, and gene expression and cell signalling occurring at the site of the developing scalp (Curtiss *et al.* 2002).

On animals with fur, more hair whorls can be located on many more places on the body than humans (Guo *et al.* 2004). Hair orientation has been seen to occur before follicles emerged through the surface of the skin in the foetus of dogs (Wang *et al.* 2006).

2.3. Ethology

Personality is defined as the combined factors behaviour, cognition and emotional patterns that comes from biological and environmental influences. Behaviour is how someone react in response to different situations (Cherry, 2020). The mystery of behaviours has been discussed by many scientists throughout history. Today scientists have agreed that animals have feelings and that they are individuals with their own personality and express themselves mostly by body language (Desire *et al.* 2002 & Siniscalchi *et al.*, 2021 in Grandin & Deesing 2014). Several different behaviour-observations have been developed to be able to interpret emotional actions. Temperament traits that have been studied in a number of studies on horses are fearfulness, nervousness, reactivity and curiosity (Desire *et al.* 2002 & Hausberger *et al.*, 2004 in Grandin & Deesing, 2014).

It was quite recently behaviourists and ethologists also got involved with neuroscience (Panksepp 2011). According to neuroscience literature, emotional systems in the brain drives behaviour. All mammals have the same basic brain design: brainstem, limbic system, cerebellum, and cerebral cortex. The cerebral cortex is the part used for thinking and problem solving. The main difference between the human brain and most of the animals' brain is the size and complexity of the cortex. The emotional systems serving as drivers for behaviour are located in the subcortex, and are similar in all mammals (Panksepp 2011). The main difference between emotions and feelings is that feelings are experienced consciously, while emotions are subconscious but can become conscious (Wake Forest University n.d.). Emotion is a feedback system that affects behaviour indirectly. The behaviour is applied to pursue or prevent the anticipated emotional outcomes. Behaviour also provides feedback and stimulate retrospective evaluation of actions, so that learning come to be possible.

2.3.1. Environmental influence on behaviour

Behaviour and the personality of a horse is dependent on genetics but also the environment. How a horse behaves in different situations cannot be predicted by only looking at the DNA. The behaviour can also develop into different actions depending on which environment the horse is exposed to and what it is taught (Wolff *et al.* 1997, Desire *et al.* 2002). Environmental changes can influence the emotion of fear. A stressful treatment of a pregnant mother or her offspring can upregulate the fearfulness. When handling and stressing pregnant rat mothers, the gestational environment changed so that the offspring resulted in being nervous. This shows that emotional reactivity develops in the nervous system during early gestation. Handling rats when they were newly born reduced their emotional reactivity and they were calmer as adult rats. (Denenberg & Whimbey, 1968 in Grandin & Deesing 2014).

2.3.2. Neuroscience and behaviour

When scientists started studying brain systems that control emotions, they started to understand how genetic factors affect behaviour. Panksepp (2005) defined the major emotional systems located in the subcortical areas of the brain. Fear, rage, panic (separation distress) and novelty seeking are the four main emotions (Panksepp 2005; Morris *et al.* 2011). Three additional emotional systems of lust, care (mother-young nurturing behaviour) and play, were added by Panksepp himself in 2011. Each of these systems are thought to be associated with a genetically based subcortical brain network. The subcortical circuit in the brain bypasses the sensory cortex and goes straight to the amygdala from thalamus (LeDoux *et al.* 1998). This means that it is a strong and fast connection (hardwired) which gives the animal a chance to respond quickly and therefore a great chance to survive (Rogan & LeDoux 1996).

Amygdala in the limbic system is a small structure in the brain that has triggers for the flight-or-fight-response (autonomous, sympatic nervous system). In rats and cats, stress hormones increases when the amygdala is electrically stimulated (Matheson *et al*., 1971; Setchkliev *et al*., 1961in Grandin & Deesing 2014) and when the amygdala is destroyed in a wild rat it becomes tame (Kemble *et al*., 1984 in Grandin & Deesing 2014). Furthermore, a destroyed amygdala makes it impossible to provoke a fear response. This applies to both learned fear of an electrical shock and innate fear of a predator (LeDoux *et al.* 1988, 1990; Davis 1992, Blanchard and Blanchard, 1972, in Grandin & Deesing 2014).

The human brain has a bigger frontal lobe, compared to the equine brain. In humans, the frontal lobe is used for abstract thinking, strategy, planning and predict future events. In horses, this area is used for voluntary movement and not abstract thinking. When training a horse, many people know to release pressure to give a reward when the horse is doing what it is asked to. This relief is believed to trigger a release of dopamine and allows a growth of dendrite cells. On the contrary, constant stress and pressure could decrease brain size and short-circuit learning. (Stewart, 2020)

2.4. Genetical aspects

2.4.1. Behaviour and genetics

Researchers have discovered that only a few genomic regions control many dog appearance traits (Boyko *et al*. 2010) but when it comes to behaviour and genetics that controls brain development, they are much more complex (Grandin & Deesing 2014). When breeding for a calm tempered fox, this selection also ended up giving black and white foxes. Non-coding genes that aren't encoding for a protein, have been found not to always be near the regions they regulate. There are long-range interactions. Some traits are linked in unexpected ways and to isolate one gene effect may be impossible (Grandin & Deesing 2014).

Wolff & Hausberger (1994) found that foals related to certain studs showed similar behaviour which would indicate genetical factors contributing to the behaviour. Cattle with excitable temperament had lower weight gains and more problems with meat quality (Voisinet *et al.* 1997; Cafe *et al.* 2011). According to Belyaev (1979) breeding for tameness in foxes reduced their mothering skills and neurological problems appeared.

A single nucleotide polymorphism (SNP) is a variation in the DNA. In the horse, there are a few SNPs studied concerning temperament traits. Hori et al (2016) found for example a SNP in the serotonin receptor 1A (*HTR1A*) in thoroughbred horses which correlated to manageability. A SNP in the Dopamine D4 receptor gene (*DRD4*) have been found to correlate to vigilance and curiosity (Momozawa *et al.* 2005b). Ninomiya *et al*. (2013) found an association to frustration level during feeding time at the SNP in this *DRD4* gene. In another study, a SNP in monoamine oxidase A (*MAOA*) was found to cause more aggressive horses and a SNP in the androgen receptor (*AR*) seemed to cause more docile horses (Song *et al*. 2018). The Hydroxysteroid 17-Beta Dehydrogenase 3 (*HSD17B3)* gene is engaged in the metabolism of testosterone (Yazawa *et al.* 2020).

2.4.2. Health and heritability

There is a link between personality and health in animals where fearful animals are more easily stressed and then may have weaker immune system (Koolhas, 2008; Mehta and Gosling, 2008 in Grandin & Deesing 2014). In Fel *et al.* 2003 in Grandin & Deesing 2014 a negative association between immune system function and fear related actions was found in cattle. However, in young pigs with a strong emotional reaction in stressful situations, there were shown that they had a stronger immune response several weeks later compared to the pigs with less stressful activity.

Wolff *et al.* 1997 found that horses that were related showed similar reactions during tests of fear and panic in an open field. Other studies have suggested that genetic factors have a strong effect on both fearfulness and novelty seeking (Stead *et al.* 2006; Clinton *et al.* 2007; Campler *et al.* 2009). Rats that express novelty seeking a lot have more dopaminergic activity in the nucleus accumbens (Dellu *et al.*, 1996 in Grandin & Deesing 2014). Novelty seeking is also shown to have a high heritability rate (0,4 ± 0,07). Stress seems to be able to break the appetitive system in the nucleus accumbens, so stress could be a reason an animal won't explore because the stress takes over the novelty seeking function (Stead *et al*., 2006).

On a study made on Tennessee Walking horses in 2016, Staiger *et al*. found two genes of value for the horses' temperament traits. These were 5-hydroxytryptamine receptor 1A (*ALDH18A*) and *HSD17B3*. Both these genes were associated with anxious horses. The *ALDH18A1* gene have been correlated to a mutation causing degradation of neurons and spasticity in humans (Koh *et al.* 2018) and Alzheimer's disease as well as Down's syndrome (Patel *et al.* 2011).

Examples of genetic diseases concerning neurology and skin

The "Letal white foal syndrome" (LWFS) is a genetically caused disease in Paint, Quarter horses, miniature horses and rarely Thoroughbreds (Edwards & Finno 2020). It is displayed as a colic, abdominal swelling, ileus, and failure to pass faeces. The mall intestines are constricted and there is a complete absence of the intrinsic myenteric plexus in the intestines, mainly ileum affected. The foal also has a white coat, this is because of non-existing melanocytes in the skin. The LWFS is caused by a missense mutation in the Endothelin receptor B (*EDNRB*) gene on chromosome 17. In the embryo, at the neural crest, this leads to abnormal development of enteric ganglia and melanocytes. A genetic test for this disease is available. (Edwards & Finno 2020)

The Lavender Foal Syndrome (LFS) affects Egyptian Arabian neonates. The coat on the foals is silver, lavender, or pinkish and the new-born foals have episodes with opisthotonos, paddling and extensor stiffness, they cannot stand or be in sternal position. The paddling episodes are either due to seizure or attempts to stand. Reflexes can be present but are exaggerated. The central nervous system does not have lesions, but an abnormal choroid plexus can be found, as well as vacuolisation of the neurons in the CNS. In skin biopsies normal tissue or abnormal clumping of melanin can be found. The LFS is caused by a mutation on one or more genes in chromosome 1, the hypothesis is that the vesicle traffic (melanosomes and dendritic cargo) is lost which gives the failing function of melanocytes and neurons (Edwards & Finno, 2020).

In American Paint Horses there is a disease called Sensory Deafness which is associated with extensive facial markings and at least one blue eye. The hearing is reduced or absent. A small population of melanocytes in the inner ear is essential for the development of a zone with blood vessels in the cochlea, which is necessary for hearing. It is thought that the migration of these melanocytes from the neural crest, and poor survival in the inner ears helps to cause this. A mutation in the *EDNRB* gene has also been found to correlate to the deafness (Edwards & Finno, 2020).

2.4.3. Hair whorls and genetics

In a recent study by Lima *et al.* (2021) they looked at the hair whorls (number and position) of Quarter Horses, on their forehead and neck, and the association to genomic regions. They found that genes associated with hair whorl position in the forehead are some that affects the formation and growth of hair follicles, which could be linked to hair whorl formation. The genes identified here, have a pleiotropic effect (gives more than one phenotype) on psychiatric characteristics. This could give us a plausible explanation of hair whorl position and behaviour indication.

One gene they found to be associated with the number of whorls is called *SIRT1* and is expressed in the hair follicle. This gene has also been correlated to schizophrenia and depression in humans. Another interesting gene, the *CD47* gene, is known to be active in the development of neurons in the central nervous system (Numakawa *et al.* 2004), and participate in follicular development and hair follicle formation (Gnedeva *et al.* 2015).

A gene called Fz6 has been found to be important in the determination of a hair whorl's location in mice. Without the Fz6 gene the hair whorls got located in many random places. A natural variation of hair whorls could therefore be due to different sequence variations in genes such as Fz6 (Guo *et al.* 2004). The gene called Fz3 is believed to act similarly as Fz6 (Wang *et al.*, 2016 in Whishaw & Kolb 2017).

2.5. Studied Hair whorl phenotypes

2.5.1. Human hair whorls and associated disease phenotypes

In humans there are studies made on the hair whorls on the scalp (David & Osborne 1976; Puri *et al.* 1995; Ruiz-Maldonado 2002; Smith 2005; Yousefi-Nooraie & Mortaz-Hedjri 2008; Malathi *et al.* 2013). These studies have suggested that the hair whorl can have unusual appearance in cases of neuro-disease like bipolarity, Down's Syndrome and schizophrenia (David & Osborne 1976; Smith & Greely 1978; Puri *et al.* 1995; Yousefi-Nooraie; Mortaz-Hedjri 2008). Alexander *et al.* (1992) in Tomkins *et al.* (2012) discovered a higher prevalence of anti-clockwise whorls in schizophrenics. Sechi *et al.* (2020) found an association between the localisation of a frontal hair whorl and Neurofibromatosis type 1 (NF1).

2.5.2. Hair whorls and laterality

There have also been suggestions that the hair whorl indicates whether a person is left-handed or not. Klar (2003) found that right-handed people in the US had a consistency in that the majority of them had a clockwise scalp whorl, while in the group of left-handed people, there was a big variation, they could have either a clockwise or contraclockwise whorl. However Çetkin *et al.* (2020) did not find a significant association between hair whorl direction and handedness, footedness or eyedness in their study. They imply that hair whorl direction is not an evident marker of functional laterality. It is suggested that human hair whorl patterns and handedness is a result from a genetical mechanism, and not something that forms "undecided" *in utero* (Klar 2003). However, Klar was later (2009) surprised to find that the whorl orientation in Japanese individuals were random, even though their handedness variation is similar to that of earlier studies made in the US. Therefore, according to Klar (2009), the whorl orientation trait is not necessarily (only) genetically determined in the Japanese population.

As well as humans, horses have appeared to have a preferred side of motor laterality (Murphy & Arkins 2008). It is hard to establish an exact idea of how much humans has influenced the horse's laterality with training and handling (Dalin *et al.* 1985). A CW hair whorl has been associated with turning to the right and CC whorls with turning to the left (Shivley *et al.* 2016). It is reported that more emotional horses are more likely to view novel objects with their left eye than the right eye (Larose *et al.* 2006). But McGreevy & Rogers (2005) did not find a correlation between motor and sensory lateralisation in thoroughbreds, which they mean indicates an at least two levelled neural organisation in the equine brain - sensory and motor. They also found that the motor laterality grade increased with age. This finding is supported by McGreevy & Thomson (2006).

2.5.3. Hair whorls and behaviour in animals

According to literature, there are some associations between an animals' hair whorls and their behaviour. Studies have been made looking at hair whorls in the forehead and on other parts of the body. When it comes to horses and cattle, most studies are made on looking at the hair whorls in the forehead (Abe *et al*., 2004 in

Broucek *et al.* 2007; Górecka *et al.* 2007; Murphy & Arkins 2008; Shivley *et al.* 2016) while in studies on dogs the whorls on the whole body is often included (Tomkins & McGreevy 2010; Tomkins *et al.* 2012, Lillebø S., 2013). See table 1 for facial hair whorls' association to temperament traits in different animal studies.

Dogs

In dogs, cephalic and ischiatic whorls were CW on the right side, and CC on the left side (Tomkins & McGreevy 2010). However, the brachial axillary whorls were CC on the right side and CW on the left. The whorl on the chest were mainly CC (91%). The direction of the elbow whorls was found to be influenced by coat length and thickness. They also found a trend where dogs with fine to medium coat thickness were to be more consistent in the direction of their elbow whorls than dogs with dense coats. Fewer follicles are present in a fine coat compared to a dense coat.

In another study (Tomkins *et al.* 2012), dogs with a CW whorl at the right elbow were more successful in a training program for guide dogs. The direction of a chest whorl was also associated with success in this study, where dogs with a CC chest whorl were rated higher (61%) than dogs with a CW chest whorl (29%). The ventral mandibular whorls on the dogs of Tomkins & McGreevy's study (2010) were correlated to the sex of the dog. CW whorls were more common in male dogs while in female dogs, CC whorls were more frequent.

Cattle

The most common facial hair whorl on cattle, in published studies, have been a middle whorl (Grandin *et al.* 1995; Lanier *et al.* 2001; Aierqing *et al.* 2020). In the study by Lanier *et al.* 2001, a laterally placed whorl was more frequently located below the eyes rather than between or above eye level. A double facial hair whorl does not seem to be common in cattle (6,6%) (Aierqing *et al.* 2020).

Cattle with a high facial hair whorl became more agitated during restraint compared to cattle with a low hair whorl according to a study of Grandin (1995). If the cattle had a low hair whorl, they were less disturbed by the presence of, and interaction with unfamiliar humans (Randle, 1998). Other studies on Red Angus beef cows found that cows with a high hair whorl could be more vigilant (Flörcke *et al*., 2012). No association was found between maternal protective behaviour and the facial hair whorl in Zebu cattle (Pérez-Torres *et al.* 2014).

Cattle with whorls on the midline had more intermediate and stable temperament than those with whorls placed to the right or left of the facial midline, who then were found to be more extreme in their temperamental characteristics (Lanier *et al.* 2001)

Horses

A CC whorl in the forehead of horses was most common in Murphy and Arkins study (2008) (114 versus 82 horses). Feral Lundy ponies with two facial hair whorls have been found to be more ‘enthusiastic’ and less ‘wary’ than those with 1 or 3 facial hair whorls (Webb & Gill, 2003). Ponies with hair whorls placed to the left of the midline were more calm, enthusiastic, and friendly, than those with the whorl placed to the right, who were more flighty, wary, and unfriendly. Ponies with the whorls to the left in the forehead also scored higher in placidity and the ponies with the whorls to the right scored higher in playfulness. If the pony had the whorl in the midline, it tended to be intermediate in these ratings.

Table 1. Facial hair whorl type and associated temperaments in horses and cattle. The frequency is listed as percent of the studied population in the also listed reference.

Hair whorl type	**Species**	**Associated temperament**	**Frequency**	**Reference**
Clockwise	Horse	Turning to the right	63%	Shivley *et al.* 2016
Counterclockwise	Horse	Turning to the left	37%	
Clockwise	Horse	Right lateralised	37%	Murphy & Arkins 2008
Counterclockwise	Horse	Left lateralised	52%	
High position	Horse	Less manageable	16%	Górecka *et al*. 2006
Elongated or double whorl	Horse	took longer to approach novel object (compared to medium and low whorl)	11%	Górecka *et al.* 2007
Two whorls	Horse	More enthusiastic and less wary	30%	Randle *et al.* 2003
1 or 3 whorls	Horse	less enthusiastic and more wary	70%	
laterally left placed (frontal view)	Horse	High scores in calmness, placidness, enthusiasm and friendliness	33%	
laterally right placed (frontal view)	Horse	High scores in wariness, associated with flightiness and unfriendliness	16%	
High position	Cattle	more agitated during restraint	25%	Grandin *et al.* 1995
High position	Cattle	Higher body weight and average daily gain	10%	Broucek *et al.* 2007
High position	Cattle	higher self-grooming behaviour, resting behaviour serum dopamine and lower serum cortisol compared to middle positioned		Abe *et al*. 2004
High position	Cattle	High in temperament scores	12%	Lanier *et al.* 2001
Round epicenters	Cattle	higher percentage of normal spermatozoa compared to nonround epicenters and long lines.	71%	
Round spirals		higher percentage of normal spermatozoa compared to nonround epicenters and long lines.	62%	Meola *et al.* 2004

Before making conclusions about the connection between hair whorls and temperament it is important to rule out possible confounding factors that could influence temperament like age, gender and coat colour (Visser *et al.* 2001). Few studies have been made on relationship between coat colour and behaviour, Mills *et al.* (2002) did not find that stereotypic behaviour were connected to coat colour in horses.

2.6. Heritability of the hair whorl

The heritability of a horse´s hair whorl has been investigated by Górecka *et al*. (2006), where the hair whorl position on the sire, dam and foals were studied on Konik horses. They found a high heritability of 0,8 for the position (low to high) of the facial hair whorl. In another study made on thoroughbreds, it was found that the heritability of the number of hair whorls were low (h^2=0,16) and the heritability of the position (high, medium or low) of the hair whorl was high (h^2=0,643) (Yokomori *et al.* 2019).

3. Material & methods

3.1. Material

Horses

This study included 175 Standardbred trotters who had started at least one race and been in the previous study "Genetic background of temperament traits in Standardbred trotters" by Berglund (2021). The horses were born between 1995-2018 and the data included 70 mares, 82 geldings and 23 stallions. The horses were a mix of active and retired trotters. 78% (136) of these horses were trained by a professional trainer and 13% (23) by an amateur trainer, and 9% (16) of the horses had been trained by both professional and amateur trainers.

Survey

The behaviour survey that was used in the study of Berglund (2021) is a survey adapted from Momozawa *et al.* (2005a) and Staiger *et al.* (2016) and is the survey from where data was taken for this study. It includes 13 different traits that were "Nervousness", "Excitability", "Fearfulness", "Concentration", "Learning", "Memory", "Cooperation", "Will to win", "Stubbornness", "Self-control", "Recovery" and "Appetite". The trainer had rated how often they had observed the temperament trait in competition where 1 corresponded to never, 2 rarely, 3 occasionally, 4 sometimes, 5 often, 6 usually and 7 always, see table 8 in the Appendix. Data collection for the survey and hair samples for DNA extraction from the horses were collected in the period of April 2019 to March 2021 (Berglund, 2021).

Hair whorls

The collection of the horses' hair whorls was made either from photos sent in by the horses´ owner or by live visual investigation. A hair whorl protocol was filled out for each individual, see Appendix 2, as well as a photo taken on each of the visited horses' forehead. This data was obtained during September to October 2021. Thirty-five horses in Norway had already been phenotyped during the spring of 2021. The whorls were classified as high (above eye level), medium (in between the eyeline) and low (beneath eye level). The whorls were also classified into laterally placed or placed on midline. The hair whorls were also classified, as radial, clockwise or contraclockwise, and feathered (elongated) or not. All whorls that formed a line in a radial matter, with or without a turn in the line were classified as a feathered whorl. These whorls could have a starting whorl going either clockwise, contraclockwise, or radially and therefore the horses with a feather always had two or more whorls because the feather itself counted as two whorls. The feather was then also classified as going vertical, horizontal, or diagonal as well as the vertical position of it in the forehead. All the data were handled in Excel and a pivot-table were made in the program; this gave a good capability to find the different groups for the tests.

3.2. Statistical analyses of hair whorls and behaviour

Study material

The 13 behaviour groups were divided into high scores (6-7) and as control group for these, the ones that scored 1-2. For the hair whorls, 19 different groups were made in order to compare behaviour and genetics, see table 2.

Table 2. List of the different hair whorl groups to be compared with behaviour.

Hair whorl groups
One whorl
Two whorls (no feather)
Two whorls or more (incl. feather)
High positioned (of the ones with one whorl)
Medium positioned (of the ones with one whorl)
Low positioned (of the ones with one whorl)
CW (of the ones with one whorl)
CC (one whorl)
Radial (one whorl)
Midline (one whorl)
Medial left (one whorl)
Medial right (one whorl)
Feather
No feather
High feather
Medium feather
Feather on midline
Laterally placed feather
Vertical feather
Horizontal / diagonal feather

The hair whorl groups were partially designed after how many individuals of each that were present and partially after study-interest after literature analysis. The threshold was set to a minimum of five horses in a group, and therefore the “medium whorl” and “low whorl” groups were fused, as well as the “left feather” and “right feather” groups. There was an interest in seeing if there was an association between high versus medium/low hair whorls for the temperament traits because of earlier findings about high positioned hair whorls being harder to handle. The hair whorl of a feather has not been studied yet but is described as showing a typical behaviour in non-scientific literature by an author passionate about hair whorls (Miller, 2021).

Statistical test

The website VassarStats (Lowry, n.d.) was used to calculate associations between the 19 different hair whorl groups and the 13 different Behaviour groups (Nervosity, Excitability, Fearfulness, Concentration, Learning, Memory, Cooperation, Will to win, Stubbornness, Self-control, Recovery, Appetite and Stereotype). Odds ratio and p-value were calculated in a two by two cross table, with Fischer´s Exact Probability test. These statistical analyses were performed to find associations between a hair whorl group and a behaviour trait (high 6-7 or low 1-2 scored).

The risk of always getting at least some (5%) significant test results by chance becomes higher when performing multiple testing. To counteract this problem of multiple comparisons, a Bonferroni correction method was performed.

3.3. Genetic association analysis of hair whorls

3.3.1. DNA extraction

Twenty of each of the horse's hair roots were cut and placed into wells on a 96-plate. Then the plate was centrifuged. Thereafter 186 µl 5% Chelex (styrene-divinylbenzene copolymer containing paired iminodiacetate ions) and 14 µl Proteinase K (20 mg/ml) were added to each well. The plates were incubated at 56 degrees Celsius (˚C) on a shaker for two hours at 600 revolutions per minute (rpm) and then incubated in 95˚C for ten minutes to inactivate Proteinase K. To cool down, the plates were then left in room temperature for half an hour. After that the plates were centrifuged again and the supernatant in each well was transferred to a new plate while the Chelex in the bottom was discharged.

Qubit protocol to measure DNA concentration

A working solution was mixed, containing Qubit Buffer (199 × n µl) and Qubit reagent (1 × n µl), where n is the number of samples, plus two standards for calibration of the Qubit instrument. Into each Qbit-tube, 199 µl working solution was dispensed with 1 µl of sample solution. For the standards 10 µl "Standard Double stranded DNA High Sensitivity" were added into 190 µl working solution. Before the reading of concentration, all tubes were vortexed for 2-3 seconds. The concentration of each tube was read in the Qubit fluorometer and noted. Samples with low concentration were left to evaporate overnight and concentrations were measured again with Qubit.

Dilution of DNA

All samples were diluted into a new plate to the correct input concentration of 50 ng with a maximum volume of 4 µl. The needed volume of sample DNA was calculated by $V_{sample} = 50/C_{sample}$. Nuclease free water was added to reach 4 µl with 50 ng DNA in each well, $V_{Nuclease\ free\ water\ to\ add} = 4 - V_{sample}$.

3.3.2. Library preparation

So that the 96-well plates that later would be sent for whole genome sequencing, were to be filled, additional horses' DNA from the previous study of Berglund (2020) were included. Two plates consisting of DNA samples of 192 horses were prepared for sequencing. Low density whole genome sequencing was performed with riptide DNA library preparation protocol (Riptide[TM] High Throughput Rapid DNA Library prep) (iGenomX n.d.). The protocol consists of four steps:

1: Extension and termination of primer A
2: Bead capture and extension of primer B
3: PCR and amplification
4: Size selection

In the first step, primers were added to new 96-plates. According to Kalbfleisch *et al.* (2018), the protocol recommended to use was a combination of low GC primer and high GC primer. Enzyme buffer, dNTP mix and enzyme were combined into a master mix which was added to the plates. After incubation at 98 ˚C for one minute, the DNA samples were added into each well. Then the plates were put in a thermal cycle and this program was run:

1. 92˚C for 3 minutes
2. 16˚C for 5 minutes
3. Ramp: 0,1˚C per second up to 68˚C
4. Hold at 68˚C for 15 minutes
5. Hold at 4˚C

Now each horse had an individual barcode with 8 nucleotides followed by a random sequence with 12 nucleotides. The products from the 96 wells were transferred to one single tube (Eppendorf) containing EDTA. After this step, three tubes each containing products from one 96 well-plate were obtained. SPRI beads was added to the tubes to collect the amplicons, the tubes were placed on a magnetic stand and the supernatant containing all that was not attached to the DNA was discarded.

After washing the beads with ethanol and thereafter dissolve the DNA from the SPRI beads with Tris-HCl, the eluted DNA was transferred to a new tube.

In step two Capture beads was added to new tubes. The tubes were placed on the magnetic stand and the supernatant was discarded. A buffer was used to resuspend the capture beads and the tubes were centrifuged again and the supernatant was discarded. The DNA was heated up to 95˚C for 3 minutes. Thereafter the beads with DNA attached were washed with sodium hydroxide. Then Enzyme buffer 2, dNTP 2, primer B was added, and the tubes were incubated at 24˚C for 20 minutes. After this step, the beads were washed again.

Step three was a PCR reaction. Universal primers, barcodes, and PCR amplification mix was added to each tube. The three tubes were placed in the PCR machine with the following settings:

1 cycle 98˚C for 2 minutes
8 cycles 98˚C for 20 seconds
60˚C for 30 seconds
72˚C for 30 seconds
1 cycle 72˚C for 5 minutes
4˚C hold

After this step, each tube had a unique barcode and the DNA strands had been amplified.

The fourth step was a size selection, depending on the Illumina sequencing read length, the amount of SPRI beads 2 volumes differed. Since the read length for the current protocol was 2x150, 70 µl followed by 30 µl of SPRI beads 2 was added to remove small fragments ending up in the supernatant. The beads were washed with ethanol and Tris HCl was added to resuspend the DNA from the beads. The tubes were placed on a magnetic stand and the supernatant containing the library could be transferred to new tubes and sent for sequencing (iGenomX n.d.).

3.3.3. Lowpass whole genome sequencing

The DNA was sequenced with Illumina Novaseq6000 with S4 Flow cells. The aim was to have 2x coverage. Haploid genome length of the horse is 2.41 Gb (Kalbfleisch *et al.* 2018). The Illumina S4 flow cell can generate from 80 Gb and 800 M reads to 3 Tb and 10 B reads of data in single flow cell mode.

The Quality Control (QC) was done with FastQC software package (De Sena Brandine & Smith 2021). The alignment of the data was done following Li & Durbin (2009)´s work (Andrews 2010).

The hair whorl groups were divided into comparison groups. Bam files were generated by aligning the fastq files to the EquCab3.0 using BWA and GATK 3.7 tools. The allele frequency (AF) was calculated separately for each group using PCAngsd. The AF calculations were based on genotype likelihood. Thereafter, the delta AF (dAF) was calculated for the case-control pairs as [AF(cases) minus AF(controls)]. All SNPs with dAF<0.05 were excluded. The dAF was Bonferroni-corrected and z-standardized so that the standard score (z-score) value which corresponded to the p-value of 0,05 could be calculated. Then the significance threshold on the dAF scale could be re-calculated (this is seen as a horizontal line on the Manhattan plot) (Auwera *et al.* 2013).

4. Results

4.1. Distribution of individuals

4.1.1. Hair whorl groups

To be able to compare the different hair whorl groups with the behaviour types, and to have groups opposing each other, the horses were divided into groups according to what kind of facial hair whorl(s) they had. The most common facial hair whorl of the 175 Standardbred trotters in this study was a single, high positioned whorl on the midline and they were 53 (30%). Out of the horses with a single whorl, there was only one horse (0,8%) that had a low hair whorl, 31% had a medium whorl and 68% had a high whorl. There were no whorls in this study that were outside the medial left or medial right lateral line. The distribution of single, laterally placed hair whorls was 24 (20%) for left and 17 (14%) for right, and a midline whorl was seen in 81 (66%) horses. It was slightly more common with a radial whorl (37%) than a CW (32%) or CC (30%). Two whorls and no feather were seen in 11 horses (6%). Two whorls or more, with or without feather(s) was seen in 53 horses (30%), while the most common was horses with one whorl and no feather in the forehead (70%). See table 3. Out of all the 175 horses, 122 (70%) had at least one high hair whorl, 52 (30%) had at least one medium positioned, and 2 (0,1%) horses had low whorls.

Table 3. Hair whorl groups and their quantity of individuals.

Hair whorl group	Quantity (N)
One whorl	122
Two whorls (no feather)	11
Two whorls or more (incl. feather)	53
High positioned (of the ones with one whorl)	83
Medium positioned (of the ones with one whorl)	38
Low positioned (of the ones with one whorl)	1
CW (of the ones with one whorl)	39
CC (of the ones with one whorl)	37
Radial (of the ones with one whorl)	45
Midline (of the ones with one whorl)	81
Medial left (of the ones with one whorl)	24
Medial right (of the ones with one whorl)	17
Feather	42
No feather	124
High feather	20
Medium feather	21
Feather on midline	35
Laterally placed feather	7
Vertical feather	33
Horizontal / diagonal feather	9

On one horse, the direction of the whorl was not able to be identified, why the sum of direction in the group of one whorl is 121 instead of 122. The sum of the groups “Feather” and “No feather” is 166 because in ten individuals it was not decided if they had a feather or not. (This is because some hair whorl data were collected before it was decided to also look at the feathers, and/or a bad quality picture was held from these individuals, so that it could not be seen properly). One horse had two feathers, one high positioned and one medium positioned and were thus not included in either of the groups “High feather” and “Medium feather”, hence the sum of individuals in these groups became 41.

4.1.2. Behaviour groups

Groups were made with individuals only scoring high (6-7) and low (1-2) in the behaviour survey. In the behaviour groups, as seen in table 4, the distribution of individuals was not very even. The groups that had the most distribution between high and low scores were; “Nervousness” (with 26 horses scoring 1-2 who tended not to be nervous during competition and 23 horses scoring 6-7 who tended to become nervous during competition), and “Excitability” (where 64 horses scored 1-2 who did not tend to become excited during competition and 26 horses scoring 6-7 who tended to become excited). The distribution of “Fearfulness” was 103 horses that did not usually get afraid during competition (low scores) and 10 horses that usually got scared during competition (high scores). For “Concentration” the distribution was 18 horses who tended to be unfocused during competition (low scores) and 70 horses that usually had good focus during competition (high scores). There were a few horses that had low scores in “Learning” and “Will to win”. No horse had a score of 1 in these categories. On the other hand, there were a few horses with high scores in “Stubbornness”, “Self-control” and “Stereotype”. In the category of “Memory” 97 horses showed a bad memory (low scores) and 22 horses showed an extremely good memory (high scores). For “Cooperation” there was only 6 horses scoring 1-2 with bad cooperation skills, and 115 horses scoring 6-7 having good cooperation skills during competition. Twelve horses showed a bad recovery after competition (low scores) and 114 horses had good recovery (high scores). In the category of “Appetite” there were 113 horses scoring low, showing a good appetite between competitions and 12 horses who had bad appetite (high scores).

Table 4. Number of horses having behaviour scores 1-2 and 6-7.

Behaviour group	**Low scores (1-2)**	**High scores (6-7)**
Nervousness	79	22
Excitability	64	26
Fearfulness	103	10
Concentration	18	70
Learning	9	119
Memory	97	22
Cooperation	6	115
Will to win	24	88
Stubbornness	153	2
Self-control (bad)	130	9
Recovery	12	114
Appetite (bad)	113	12
Stereotype	147	8

4.2. Association analysis of hair whorls and behaviour

Statistical analyses were performed with a 2x2 contingency table for each of the whorl groups and temperament traits listed (whorl groups' opposites against each other and the behaviour group's low and high scores against each other). Because of the small number held in some of the groups, the Fischer Exact Probability Test were performed for all comparisons, see table 6 and 7 for each test's p-value (two tailed), odds ratio, and number of individuals in each group. Highlighted is p-values close to 0,05 and the p-values of 0,05 or less is in bold. Below is seen one two-by-two table that were able to hold a p-value of significance ($p < 0,05$).

Table 5. Number of whorls and Learning Contingency table, with the p-value of 0,03 and Odds ratio of 4,6.

P-value: **0,03,** OR: 4,6		**Learning**		
		Low scores (1-2)	High scores (6-7)	**Total**
Number of whorls	One whorl	3	83	86
	2 whorls or more (incl feather)	6	36	42
	Total	9	119	128

This result shown in the table above indicates that it is more probable that the trotter is more prone to learn or learn the task of competing quicker if it has one whorl than if it has two or more whorls in the forehead.

Table 6. Compared groups and p-values as well as odds ratio for the 2x2 Cross table tests where low score is 1 and high score is 7. When a value could not be held: -.

High: 7	**P-value (Fischer's, two-tailed), Odds ratio**												
Low: 1	**(Group 1 low scores (n), group 1 high scores (n), Group 2 low score (n), group 2 high scores (n)).**												
	Nervosity	**Excitability**	**Fearfulness**	**Concentration**	**Learning**	**Memory**	**Cooperation**	**Will to win**	**Stubbornness**	**Self-control**	**Recovery**	**Appetite**	**Stereotype**
1 whorl VS 2 whorls (excl. Feather)	1, ∞, (16, 8, 1, 0)	1, ∞, (17, 7, 2, 0)	1, ∞, (27, 1, 3, 0)	1, 1,1, (6, 20, 1, 3)	1, 0, (1, 39, 0, 4)	1, ∞, (31, 8, 1, 0)	1, 0, (1, 44, 0, 5)	1, -, (0, 39, 0, 4)	I, ∞, (84, 1, 6, 0)	1, ∞, (68, 1, 5, 0)	1, 0, (5, 35, 0, 3)	1, 0,7, (54, 8, 5, 1)	1, ∞, (99, 2, 11, 0)
1 whorl VS 2 whorls (incl. feather)	1, 1,3 (16, 8, 9, 4)	0,4, 2,4, (17, 7, 12, 2)	1, 0,6, (27, 1, 15, 1)	0,7, 0,6, (6, 20, 2, 11)	1, 0, (1, 39, 0, 16)	1, 1,3, (31, 8, 15, 3)	1, 0, (1, 44, 0, 21)	1, -, (0, 39, 0, 21)	1, ∞, (84, 1, 36, 0)	1, 0,4, (68, 1, 30, 1)	1, 0,5, (5, 35, 1, 13)	0,5, 2, (54, 8, 28, 2)	1, 0,9, (99, 2, 45, 1)
A high whorl VS medium / low whorl	0,3, 0,3, (14, 5, 2, 3)	1, 0,7, (11, 4, 6, 3)	1, ∞, (21, 1, 6, 0)	0,3, 0, (6, 15, 0, 5)	0,35, ∞, (0, 26, 1, 13)	1, 0,9, (20, 5, 11, 3)	1, 0, (1, 35, 0, 9)	1, -, (0, 27, 0, 12)	0,3, 0, (61, 0, 23, 1)	1, ∞, (49, 1, 19, 0)	0,6, 2,7, (3, 28, 2, 7)	1, 1,4, (37, 6, 17, 2)	1, 0,5, (67, 1, 32, 1)
Feather VS No feathe	1, 0,6, (8, 4, 17, 8)	0,7, 0,5, (10, 2, 18, 7)	1, 2,4, (12, 1, 29, 1)	0,7, 2,7, (1, 8, 7, 21)	1, ∞, (0, 12, 1, 41)	1, 0,9, (14, 3, 32, 7)	0,3, 0, (1, 16, 0, 45)	1, -, (0, 17, 0, 42)	1, 0, (30, 0, 86, 1)	1, 2,8, (25, 1, 69, 1)	1, 1,1, (1, 10, 4, 35)	1, 0,8, (18, 2, 52, 7)	1, 0,9, (34, 1, 32, 1)
High VS Medium feather	1, 1, (2 ,1, 6, 3)	1, 1,17, (3, 1, 7, 2)	0,5, 0, (7, 0, 5, 1)	1, 0, (1, 4, 0, 4)	1, -, (0, 6, 0, 6)	0,5, 0, (5, 0, 9, 3)	0,4, ∞, (0, 10, 1 6)	1, ∞, (0, 8, 0, 8)	1, -, (16, 0, 14, 0)	1, 0, (11, 0, 14, 1)	1, ∞, (0, 5, 1, 5)	1, 1,6, (7, 1, 11, 1)	0,5, ∞, (15, 1, 19, 0)

	Nervosity	Excitability	Fearfulness	Concentration	Learning	Memory	Cooperation	Will to win	Stubbornness	Self-control	Recovery	Appetite	Stereotype
Vertical VS horizontal / diagonal feather	0,5, 0,14 (7, 2, 1, 2)	0,1, 0,06, (9, 1, 1, 2)	1, ∞, (9, 1, 3, 0)	0,2, ∞, (0, 7, 1, 1)	1, -, (0, 9, 0, 3)	1, 0, (12, 3, 2, 0)	1, 0, (1, 11, 0, 5)	1, -, (0, 13, 0, 4)	1, -, (23, 0, 7, 0)	1, ∞, (19, 1, 6, 0)	0,3, 0, (1, 2, 0, 8)	**0,05**, 0, (15, 0, 3, 2)	1, ∞, (28, 1, 6, 0)
CW VS CC	0,2, 5,3 (3, 4, 8, 2)	0,99, 1,9, (4, 3, 5, 2)	1, ∞, (8, 1, 8, 0)	0,6, 0,3. (3, 6, 1, 7)	0,4, 0, (1, 10, 0, 15)	1, 0,8, (11, 3, 9, 3)	1, -, (0, 16, 0, 13)	1, -, (0, 13, 0, 14)	0,5, 0, (27, 0, 23, 1)	1, ∞, (20, 1, 18, 0)	1, 2,2, (1, 12, 2, 11)	0,7, 1,9, (17, 4, 16, 2)	1, 0,97, (31, 1, 30, 1)
CW VS Radial	0,6, 3,3 (3, 4, 5, 2)	0,6, 3,33, (4, 3, 8, 2)	0,45, ∞, (8, 1, 11, 0)	1, 0,6, (3, 6, 2, 7)	0,4, 0, (1, 10, 0, 14)	1, 1,5, (11, 3, 11, 2)	1, ∞, (0, 16, 1, 15)	1, -, (0, 13, 0, 11)	1, -, (27, 0, 34, 0)	0,4, ∞, (20, 1, 29, 0)	1, 2, (1, 12, 2, 12)	0,4, 3, (14, 4, 21, 2)	0,5, ∞,, (31, 1, 38, 0)
CC VS Radial	1, 0,6, (8, 2, 5, 2)	1, 1,6, (5, 2, 8, 2)	1, -, (8, 0, 11, 0)	1, 2, (1, 7, 2, 7)	1, -, (0, 15, 0, 14)	0,6, 1,8, (9, 3, 11, 2)	1, ∞, (0, 13, 1, 15)	1, -, (0, 14. 0, 11)	0,4, ∞, (23, 1, 34, 0)	0,4, ∞, (18, 0, 29, 0)	1, 0,9, (2, 11, 2, 12)	1, 1,3, (16, 2, 21, 2,)	0,4, ∞, (30, 1, 38, 0)
Midline VS lateral	1, 1,4, (11, 6, 5, 2)	0,2, 5,3, (9, 6, 8, 1)	0,3, 0, (19, 0, 8, 1)	0,65, 0,5, (5, 14, 1, 6)	1, 0, (1, 27, 0, 12)	0,7, 0,7, (22, 5, 9, 3)	0,35, ∞, (0, 29, 1, 15)	1, -, (0, 28, 0, 11)	0,3, 0, (58, 0, 26, 1)	0,3, 0, (45, 0, 23, 1)	0,3, 2,9, (2, 23, 3, 12)	0,7, 0,7 (38, 5, 16, 3)	1, 0,5, (68, 1, 31, 1)
Medial left VS medial right	1, 0,25, (4, 1, 1, 1)	0,1, 0, (8, 0, 0, 1)	0,3, 0, (6, 0, 2, 1)	0,14, ∞, (0, 6, 1, 0)	1, -, (0, 9, 0, 3)	0,5, 0,1, (7, 1, 2, 2)	0,3, ∞, (0, 11, 1, 4)	1, -, (0, 7, 0, 4)	1, ∞, (16, 1, 10, 0)	0,4, 0, (15, 0, 8, 1)	0,5, 6, (1, 9, 2, 3)	0,2, 0,2, (12, 1, 4, 2)	0,4, 0, (19, 0, 12, 1)
Feather on midline VS lateral feather	0,5, ∞, (5, 4, 3, 0)	0,5, ∞, (7, 3, 3, 0)	1, ∞, (8, 1, 4, 0)	1, 0, (1, 5, 0, 3)	1, -, (0, 9, 0, 3)	0,5, ∞, (9, 3, 5, 0)	1, 0, (1, 12, 0, 4)	1, -, (0, 15, 0, 2)	1, -, (23, 0, 7, 0)	1, ∞, (19, 1, 6, 0)	0,3, ∞, (0, 8, 1, 2)	-, 0,3, (14, 1, 4, 1)	1, ∞, (28, 1, 6, 0)

Table 7. Compared groups and p-values as well as odds ratio for the 2x2 Contingency table tests where low score is 1-2 and high score is 6-7. Highlighted is p-values 0,05 or less.

High: 6-7 **Low: 1-2**	**P-value (Fischer's, two-tailed), Odds ratio** **(Group 1 low scores (n), group 1 high scores (n), Group 2 low score (n), group 2 high scores (n)).**												
	Nervosity	**Excitability**	**Fearfulness**	**Concentration**	**Learning**	**Memory**	**Cooperation**	**Will to win**	**Stubbornness**	**Self-control**	**Recovery**	**Appetite**	**Stereotype**
1 whorl VS 2 whorls (without feather)	0,7, 2, (51, 16, 7, 1)	1, 1,25, (42, 21, 5, 2)	1, ∞, (69, 8, 6, 0)	1, 0,9, (14, 50, 1, 4)	0,07, 7,9, (3, 83, 2, 7)	0,4, 0,4, (68, 12, 8, 3)	0,3, 3,4, (4, 82, 1, 6)	0,2, 2,7, (13, 62, 4, 7)	0,1, 0,06 (104, 1, 6, 1)	0,1, 0,5, (89, 6, 8, 1)	0,6, 2,1, (9, 77, 2, 8)	1, 0,9, (76, 9, 8, 1)	1, ∞, (103, 6, 11, 0)
1 whorl VS 2 whorls (incl. feather)	0,6, 1,5 (51, 16, 28, 6)	0,2, 2,2, (42, 21, 22, 5)	0,5, 1,9, (69, 8, 34, 2)	0,8, 0,7, (14, 50, 4, 20)	**0,03**, 4,6, (3, 83, 6, 36)	0,2, 0,5, (68, 12, 29, 10)	1, 1,2, (4, 82, 2, 33)	0,8, 1,3, (13, 62, 7, 26)	1, 0,4, (104, 1, 49, 1)	1, 0,9, (89, 6, 41, 3)	0,5, 0,5, (9, 77, 3, 47)	0,7, 1,5, (76, 9, 37, 3)	1, 1,3, (103, 6, 45, 2)
A high whorl VS medium / low whorl	1, 1, (37, 12, 14, 4)	1, 1, (21, 14, 11, 7)	1, 1, (51, 6, 18, 2)	1, 1,1, (10, 37, 4, 13)	0,2, 4,9, (1, 59, 2, 24)	1, 0,9, (47, 8, 21, 4)	0,6, 2,6, (2, 59, 2, 23)	0,7, 0,7, (10, 43, 3, 19)	0,3, 0, (72, 0, 32, 1)	1, 0,7, (65, 4, 24, 2)	0,2, 2,6, (5, 59, 4, 18)	0,7, 1,5, (53, 7, 23, 2)	1, 0,9, (70, 4, 33, 2)
Feather OR No feather	1, 0,9, (21, 5, 54, 13)	0,2 0,4, (17, 3, 45, 21)	1, 0,8, (28, 2, 70, 6)	1, 1,3, (3, 16, 12, 51)	0,09, 0,3, (4, 29, 3, 84)	0,14, 2, (21, 7, 74, 11)	1, 1, (1, 27, 3, 82)	0,4, 0,6, (7, 19, 14, 66)	1, 0, (38, 0, 110, 1)	1, 1, (33, 2, 90, 5)	0,45, 2,9, (1, 29, 8, 81)	0,7, 0,6, (29, 2, 76, 9)	1, 1,3, (34, 2, 107, 5)
High VS Medium feather	0,6, 0,5 (7 ,1, 13, 4)	1, 0,7, (7, 1, 10, 2)	0,2, 0, (15, 0, 12, 2)	1, 1,3, (1, 6, 2, 9)	0,11, ∞, (0, 14, 4, 14)	1, 1,2, (8, 3, 13, 4)	1, ∞, (4, 12, 1, 15)	0,7, 0,6, (4, 8, 3, 10)	1, -, (18, 0, 19, 0)	0,5, 0, (16, 0, 17, 2)	1, ∞, (0, 14, 1, 14)	1, 1,3, (12, 1, 16, 1)	0,2, ∞, (15, 2, 19, 0)

	Nervosity	Excitability	Fearfulness	Concentration	Learning	Memory	Cooperation	Will to win	Stubbornness	Self-control	Recovery	Appetite	Stereotype
Vertical VS horizontal / diagonal feather	0,6, 0,4 (17, 3, 4, 2)	0,14, 0,1, (14, 1, 3, 2)	1, ∞, (21, 2, 7, 0)	**0,05**, 30, (1, 15, 2, 1)	1, 1,6, (3, 24, 1, 5)	1, 1,4, (17, 6, 4, 1)	1, 0, (1, 22, 0, 5)	1, 1,5, (5, 15, 2, 4)	1, -, (31, 0, 7, 0)	1, ∞, (27, 2, 6, 0)	1, 0, (1, 26, 0, 5)	**0,03,** 0, (25, 0, 4, 2)	0,3, 0,2, (29, 1, 6, 1)
CW VS CC (1 whorl)	0,5, 1,9 (14, 7, 15, 4)	0,5, 1,9, (16, 10, 12, 4)	1, 1,3, (21, 4, 21, 3)	1, 0,75. (5, 16, 4, 17)	1, 0,5, (2, 26, 1, 27)	0,7, 0,6, (24, 4, 18, 5)	1, -, (0, 26, 1, 25)	1, 1,1, (4, 20, 4, 18)	0,5, 0, (35, 0, 29, 1)	1, 1,4, (28, 3, 25, 2)	0,65, 1,8, (2, 27, 3, 22)	0,7, 2, (22, 4, 24, 2)	0,6, 2,8, (32, 3, 30, 1)
CW VS Radial (1 whorl)	0,3, 2, (14, 7, 22, 5)	0,8, 1,5, (16, 10, 14, 6)	0,2, 5, (21, 4, 26, 1)	1, 0,9, (5, 16, 5, 17)	0,2, 0, (2, 26, 0, 29)	1, 1,4, (24, 4, 25, 3)	0,25, ∞, (0, 26, 3, 30)	1, 1, (4, 20, 5, 23)	1, -, (31, 0, 39, 0)	0,3, 3,8, (28, 3, 35, 1)	0,7, 1,9, (2, 27, 4, 28)	0,7, 1,8, (22, 4, 29, 3)	0,65, 1,9, (32, 3, 40, 2)
CC VS Radial (1 whorl)	1, 1, (15, 4, 22, 5)	1, 0,8, (12, 4, 14, 6)	0,3, 3,7, (21, 3, 26, 1)	1, 1,25, (4, 17, 5, 17)	0,5, 0, (1, 27, 0, 29)	0,4, 2, (18, 5, 25, 3)	0,6, 2,5, (1, 25, 3, 30)	1, 0,9, (4, 18. 5, 23)	0,4, ∞, (29, 1, 39, 0)	0,6, 2,8, 25, 2, 35, 1)	1, 1, (3, 22, 4, 28)	1, 0,8, (24, 2, 29, 3)	1, 0,7, (30, 1, 40, 2)
Midline VS lateral (1 whorl)	1, 1, (35, 11, 16, 5)	0,4, 2, (28, 17, 14, 4)	1, 0,9, (44, 5, 25, 3)	1, 0,85, (10, 34, 4, 16)	1, 1, (2, 56, 1, 27)	0,7, 1,5, (45, 9, 23, 3)	1, 0,7, (3, 56, 1, 26)	0,5, 1,7, (7, 41, 6, 21)	0,3, 0, (71, 0, 33, 1)	1, 1, (58, 4, 31, 2)	0,7, 1,4, (5, 49, 4, 28)	0,7, 0,7, (56, 8, 20, 4)	0,4, 0,4 (70, 3, 31, 3)
Medial left VS medial right (1 whorl)	**0,025**, 0,06, (13, 1, 3, 4)	0,08, 0,09, (11, 1, 3, 3)	0,5, 0,28, (16, 1, 9, 2)	0,5, 4, (2, 13, 2, 3)	0,36, ∞, (0, 18, 1, 9)	0,6, 0,3, (14, 1, 9, 2)	0,4, ∞, (0, 16, 1, 10)	0,6, 0,4, (5, 14, 1, 7)	1, ∞, (19, 1, 14, 0)	0,2, 0, (19, 0, 12, 2)	1, 1,5, (2, 17, 2, 11)	0,06, 0,08, (16, 1, 4, 3)	0,6, 0,3, (19, 1, 12, 2)
Feather on midline VS lateral feather	0,5, ∞, (16, 5, 5, 0)	0,6, ∞, (13, 3, 4, 0)	1, ∞, (23, 2, 5, 0)	0,6, 0, (3, 12, 0, 4)	1, -, (4, 25, 0, 4)	0,3, ∞, (16, 7, 5, 0)	1, 0, (1, 22, 0, 5)	0,55, 3,4, (5, 17, 2, 2)	1, -, (31, 0, 7, 0)	1, ∞, (27, 2, 6, 0)	0,13, ∞, (0, 26, 1, 3)	0,4, 0,2, (24, 1, 5, 1)	1, ∞, (29, 2, 6, 0)

In table 6, where comparison was made between low score individuals scored 1 and high score individuals scoring 7, there was one test giving the p-value of 0,05. This was; horses with lower points in the “Appetite” behaviour-group -good appetite after competition that is, had more often a vertical feather than a horizontal or diagonal feather.

In table 7, where low scores were represented as 1-2 points and high scores as 6-7, the following results of significance or close to significance was held: Nervosity: Lower scores if the trotter has a (medially) left placed whorl, than if it has a (medially) right placed whorl, (p-value: 0,025). This indicates that if the horse has the whorl placed to the right (horse’s right) in the forehead, it is more prone to become nervous during competition compared to a horse with a facial hair whorl to the left.

Learning: Higher scores, which means the horse learned the task of competing faster if he or she had one facial hair whorl compared to if the horse had two or more forehead hair whorls (p-value of 0,03 (and 0,07)).

Concentration: The horses had better focus during competition if the feather’s direction was vertical compared to the horses with a horizontal or diagonal feather (p-value: 0,05, OR > 1).

Appetite: Horses had lower scores (which means good appetite after competition) if they had a vertical feather, compared to if they had a horizontal or diagonal feather (p-value: 0,05,). The horses also seemed to have good appetite after competition if they had a medially left placed facial whorl compared to a medially right facial whorl (p-value: 0,06, OR < 1).

No associations could be found between high or medium/low hair whorls and the thirteen temperament traits. There were no associations between the hair whorl direction and the temperament traits, apart from the direction of feather. The position of a feather did not show any associations to the behaviour traits either.

4.3. Association between mane and whorl direction

Since the direction of facial hair whorl has been shown to indicate laterality in horses (Shivley *et al.* 2016), to see if there was an association between which side the mane fell on and possible laterality in the horse, the CC and CW hair whorls were analysed in groups against each other in a two-by-two cross table contingency test. Only the horses with one facial hair whorl, and the horses which had the data of mane side, took part in this test. They were 19 horses with a CW hair whorl and mane to the right, and 14 horses with a CC whorl and right mane. 17 horses had a CW whorl and mane to the left, and 13 CC whorl and left mane. However, this did not show any association at all, towards either right or left mane (p-value: 1 and OR: 0,96), see table 8.

Table 8. Number of horses in the comparison of direction of whorl and mane side, showing no association between right or left mane and CW or CC facial hair whorl.

Number of individuals	**Mane to the right**	**Mane to the left**	**Total**
CW whorl	19	17	36
CC whorl	14	13	27
Total	33	30	63

4.4. Correction for multiple testing

A simple method of Bonferroni was used to correct for multiple testing, where the p-value of significance (0,05) was divided by number of tests (12 x 13 = 156) for each table. This gave the p-value of 0,0003 for a test to have significance, which none of the tests in this study showed.

4.5. Association analysis of chromosome regions and hair whorl types

After obtaining the genomic data from the horses and the allele frequency (AF) was calculated for the different hair whorl types, a Manhattan Plot was made. Figure 5 shows a Manhattan plot for the groups “One whorl” and “Two whorls (No feather)” contrast, showing the delta AF (dAF) which should be highly correlated with Fst. Fst is used for calculating different alleles (gene variants) between populations.

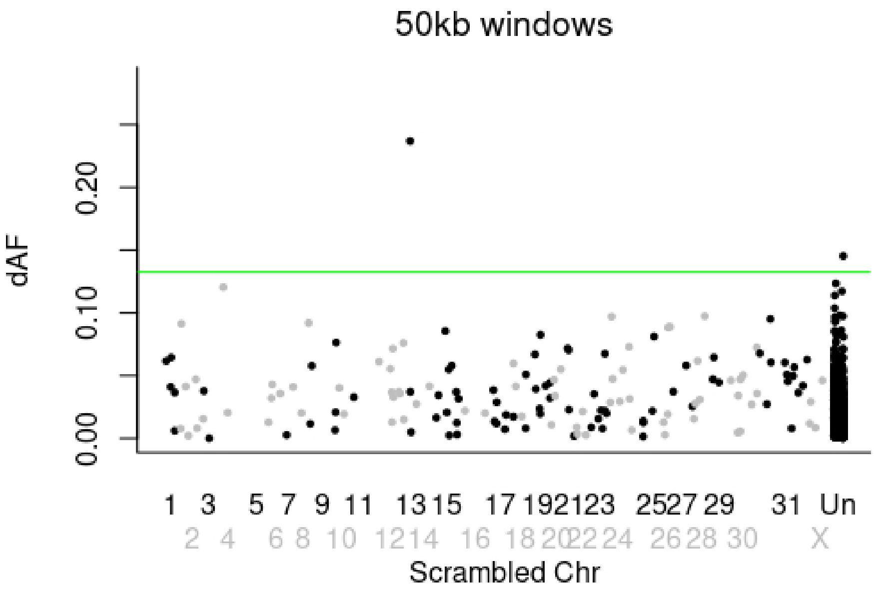

Figure 5. Manhattan plot for the groups "One whorl" and "Two whorls (No feather)" One dot representing the average dAF (Y-axis) of the SNPs in a 50Kb window. The X-axis showing the chromosomes, here scrambled. The horizontal line is the threshold of significance.

There are five significantly different 50Kb windows. This result shows that there is a significant difference between the two populations of horses with "One whorl" and "Two whorls", in a comparison of their DNA. The major genetic difference seems to be located on one of the chromosome regions.

5. Discussion

5.1. Heritability of hair whorl and behaviour

The Swedish standardbred trotter breeding goal is to produce easily handled horses whom are strong in distance-running with a clean trot and also healthy with a good mentality for competition (Avelsförbundet för Svenska Varmblodiga Travhästen (ASVT), 2003). The fact that most of the trotters in this study had a high positioned hair whorl (68%) would support the findings of Górecka *et al.* (2007) and Yokomori *et al.* (2019) that the heritability of facial hair whorl's position is high (h^2 = 0,643 and 0,743). This because the Standardbred horse is continually bred with other standardbreds, and the inbreeding coefficient was as high as 12% in the US in 2003 (ASVT). The closer the number is to 1, the higher the heritability and lower environmentally affected the trait is noticed to be (Wray and Visscher, 2008).

In this study, it could be suspected that the heritability of a lateral position also is high because it was much more common with a midline facial hair whorl than laterally placed hair whorls, and no horse had a hair whorl outside of the medial lines. Since Yokomori *et al.* (2019) did not find the number of hair whorls to be highly heritable, but rather low (h^2 = 0,160) and the majority of the horses in this study only had one hair whorl (70%) it could indicate that the origin of a hair whorl is not only controlled by simple genetics. Instead, it might be polygenic, controlled by more complicated genetics and possibly the environment during development of the foetus.

Many horses tended to be similar in their behaviour in this study, and looking at the horses with extreme temperament traits, it can be noticed that most of the horses have a temper agreeing with the goals of the Breeding Centre for Swedish Standardbred Trotters (ASVT, 2003). This indicates success in their breeding effort, and that these temperament traits most likely are heritable at some level. But to investigate this completely, a more focused study on it is needed.

5.2. Associations between behaviour and hair whorls

Association analyses have been made regarding the facial hair whorl types and the behaviour traits in the 175 standardbred trotters. Because the spreading of individuals among the behaviour-groups was so low, and the fact that some hair whorl groups did not contain many horses, a p-value or odds ratio for all of these tests were not possible to get. However, for most of the tests a p-value and odds ratio could be held.

Even though none of the results from the behaviour analyses did not have a p-value < 0,0003 the results from these association tests could give us some indications of association. Horses with a vertical feather seem to have better appetite after competition compared to horses with a horizontal or diagonal feather. This result was shown in both the test where low score was 1 and high score 7 (p-value: 0,05), and the test where low score was 1-2 and high score was 6-7 (p-value: 0,03). A vertical feather in this study also indicated a horse that was more focused and not affected by the environment (p-value: 0,05). In this study there was no associations between a feathered whorl's presence and a typical behaviour. In another study, horses with an elongated whorl (or double whorls) looked longer at a novel object before they investigated it, after being presented by it, compared to horses with a single whorl (Górecka *et al*. 2007). A double whorl (or more) in this study, indicated a horse who took longer to learn the task of competing (p-value: 0,03). An assumption could be made that perhaps these horses who tend to stop and look at a novel object needs time to loosen up and be comfortable in new environments before they can understand something and learn. Randle *et al*. (2003) found that ponies with two whorls were the most enthusiastic, and ponies with three facial whorls more wary.

5.2.1. High whorl and vigilance

Górecka *et al.* 2007 found, as well as some studies on cattle, that individuals with a high whorl are harder to handle than those with a medium or low facial hair whorl. In this study however, no such associations were found. The high facial hair whorl though was the most common trait in the studied population, and it could be assumed that trotters are performing good if they are confident and "goey" with a lot of energy. This as opposed to horses with a low hair whorl that are thought to be introvert, slow and quiet according to Miller (2021) who has the hypothesis that the closer the whorl is to a certain position the more apparent the behaviour trait. Hence, a medium facial whorl wouldn't indicate much behaviour-wise, according to Miller (2021). In this study there was only one horse in the group "low whorl" (one whorl), why the comparison is in fact mainly made between horses with "medium whorl" and "high whorl". The result may have looked different if there were more horses with low hair whorls to compare the high whorls with.

5.2.2. Left- and right-brained personalities

In human brains, measures have been made on grey versus white matter in the different regions of the brain (Good *et al.* 2001). The right frontal and temporal lobe had more grey matter on the right side compared to the opposite frontal and temporal sides that had more white matter. The two halves of the brain work together, but extrapolated from human studies, the horse's left side of the brain is thought to process logic and facts (Miller, 2020). This part of the brain controls the thinking before acting. "Left brained personality horses" usually have a whorl on their right side (caudal view). In this study, it could be found (with 97,5% confidence interval) that the horses were less nervous during competition if the whorl was placed to the left, compared to if it was placed to the right. This does not support Miller's conclusion (2021) that if a whorl is placed to the left, it shows a horse with tendencies to be emotional, reactive to stimuli and can be seen as nervous. However, Miller has not made scientific studies on her own, but is building her writings on scientific studies and own experience. It is of worth to discuss though because her writings seem to inspire and influence many people.

In the study of Randle *et al.* (2003) they found that a hair whorl to the left showed a horse more prone to be wary, flighty, and unfriendly, however they did not see any associations between nervousness and laterally placed hair whorls. They did find however that if a horse had a laterally placed whorl, the temperament trait became more extreme, which is consistent with the finding of Lanier *et al.*'s study on cattle where the whorls in the middle indicated less extreme temperaments. With a p-value of 0,08 this study could show that a horse with a hair whorl to the left was less excited during competition, compared to horses with a hair whorl to the right, where the excitability scores was evenly distributed. In the study of Randle *et al*. (2003) enthusiastic ponies had a hair whorl to the right, which would indicate the opposite. But then again, this finding had a p-value above 0,05 in this study. My conclusion of this is that these results are different and that it is hard to establish an exact consistency, especially when the studies are made following different protocols, and perhaps observing different behaviour traits, so there could be a risk of misinterpretation.

5.3. Chromosome regions associated to hair whorl phenotypes

On five chromosome regions, a significant difference could be found between horses with one whorl compared to horses with two whorls (No feathers included). One other study has been made on Quarter Horses' genetics and hair whorls (Lima *et al.* 2021). They included the whole head and not only the forehead, because their outline diagram of hair whorls appears to include any hair whorls on the muzzle, and cheeks. It is unclear if they included eventual hair whorls ventrally on the chin as well. Chromosome regions of importance for number of hair whorls on the head, found by Lima *et al*. (2021) were on chromosomes 1, 19 and 23. These are different chromosome regions compared to this present study.

5.4. Future perspective

It is an idea that following studies on this topic investigate foals or semi-feral animals, not to get the human influence on their behaviour as a confounding factor. To investigate the height of the whorls more closely it could be an idea to measure exactly the position height of the whorl as well as the exact length of the feather for example and compare these groups' behaviour to see what results it gives. This kind of study would require a higher number of individuals in the study population since it would give more narrowed and focused groups. It is good if the study population is bigger, so that each group gets a decent number of individuals in them. Another problem in this study was that two of the behaviour-traits' survey answers were not complete, this is because some horses were so young, or the trainer had not had them for long, so that the trainer could not answer how much will to win they had. The other group "Stereotype" were added a bit later in and so some horses had blank data on it as well. This gave an even lower number of horses in those two traits.

Collecting behaviour data through a survey investigates the trainers' personal opinions and perceptions about the horses' behaviour during competition. The trainer would know the horse well and therefore give valid information. It could be the case that this (behaviour data) differs from another person's opinion, like for example the owner of the horse. Problematic factors would be the already mentioned behaviour traits like "Will to win" if the horse had not been competing for a time long enough to tell yet, but also possibly the time the horse had been with the trainer in that stable and whether it had gotten to know the environment, stable friends and become comfortable or not. Another interesting adding in future studies in this subject could be to not only investigate the horses' behaviour during one situation but also include situations in the stable, in the yard, feeding time and so on, if there is an interest in investigating if the behaviour of a horse easily switched ("double personality") or not. It could also be of value then, to collect this data from the grooms who meet the horses in most situations.

Another thing worth to discuss is the fact that all horses in this study with a whorl looking like a feather (or elongated whorl) were classified into the group "two or more hair whorls incl. feather". This is because the feather could have a direction (CW, CC or radial) in one end, but the feather itself is radial. So even if the horse only had a feather, it was classified as having two whorls; one feather (radial) and one whorl which would be the "starting point" of the feather, which could be CW, CC or radial. This gave a problem in the comparison of direction of the whorl. Perhaps it these horses with one feather should have been classified into the “one whorl” group and mark them with the direction as the one end of the feather. If so, these horses could also go into comparison of the directions of whorls and therefore give more individuals in these groups, and may have given another result. But that would change the result on the “one” versus “two or more” whorls as well. Better yet, if three new groups were added named “feather starting CW/CC or radial”, this would give more focused and clear groups to compare. As already mentioned, this would probably be more doable with a higher number of horses in the study population. It could also be an idea to classify the groups with two or more facial hair whorls into subgroups depending on if they are positioned stacked above and below each other or beside each other for example. For future studies, a suggestion can be to perform a regression analysis as well. It was not done in this study because of limitations in both experience about such test and time.

Results from studies of the equine hair whorl and an insight of their meaning could be of assistance to all people handling and training horses if it could give indications of how to create the optimal environment and conditions for a specific individual. This since the horse will learn better if it is relaxed, and the relationship between human and horse will be safer and nicer if harmony and understanding can be found. Results of the studies about Standardbred trotters could also affect the breeding, to get manageable and valuable behaviour characteristics for the harness racing sport.

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Populärvetenskaplig sammanfattning

Tillsammans med USA och Frankrike, räknas Sverige som en av världens främsta travsportsnationer. Av de lopp som körs varje år, är 90 % sprungna av varmblodstravare och 10 % av kallblodstravare. Den Svenska varmblodstravaren har avlats fram under en tid på mer än 200 år, det är en blandning av mestadels amerikanskt och franskt blod. Aveln har gett en häst som travar snabbt men också är säker i hantering. För en säker hantering och tävlingsmiljö är det av vikt att hästens beteende är sådant som ej bringar risk för olyckor. En stabil och fokuserad häst med energi och vilja att vinna är av intresse.

Sedan lång tid har hårvirveln i hästens panna misstänkts kunna ha betydelse för hästens beteende och personlighet. Om virveln sitter högt, ovanför ögonen, verkar den vara mer benägen till att vara energifylld med eventuellt temperament. Om virveln i stället sitter lågt, under ögonnivå, tros den istället vara lugn och stabil. I studier har undersökningar gjorts på om virveln är placerad till vänster eller höger i pannan. Där har skillnader setts på beteenden som oro, flyktighet och vänlighet. Om en häst har dubbla eller en lång virvel, kan de vara mer avvaktande, än nyfikna att gå fram till ett föremål de precis blivit skrämda av. Skillnader i beteende har även setts hos hästar som har en eller tre jämtemot två håvirvlar i pannan, där de med två virvlar var mer entusiastiska. Hårvirvlars position i pannan har visat sig ha hög arvbarhet.

Det finns travtränare och hästmänniskor som tittar på hårvirvlar i pannan på hästarna och genom erfarenheter bildat sig en uppfattning om detta fenomen. Vid hantering av hästar kan det vara av värde för både häst och människa att skapa en harmonisk miljö där båda trivs för att kunna utvecklas, och detta kan vara enklare att få till om man snabbt kan lära känna hästen ordentligt. Om hårvirveln kan indikera en viss sorts beteende-typ, kan detta på ett simpelt sätt hjälpa människan i dess hantering av hästen. Dock kommer det alltid finnas undantagsfall. Syftet med denna studie var att undersöka om samband fanns mellan hårvirveltyp och beteende, samt att undersöka hästarnas DNA för att hitta regioner i DNAt som eventuellt har betydelse för uppkomsten av en viss hårvirveltyp. Detta inför framtida forksning om förståelsen för om huden kan spegla hjärnan och angående frågor hur genetiskt styrd vävnad organiserar sig under fosterstadiet.

I denna studie har olika beteenden som bland annat nervositet, inlärning och koncentration studerats genom att travtränare sedan tidigare fyllt i en enkät där hästarna fått olika poäng för de olika beteende-typerna. Sedan har hårvirvlarna i hästarnas panna undersökts och hästarna har klassificierats in i olika hårvirvel-grupper beroende på deras hårvirvel-typ. Hästarnas DNA har även undersökts och individer med olika hårveltyper har jämförts mot varandra för att hitta regioner i DNAt av eventuell betydelse för en viss hårvirveltyp.

Studien involverar 175 stycken travhästar i Sverige och Norge. Hårvirvelinspektion utfördes genom att besöka travstall och fota, eller genom att få inskickat foton på hästarnas panna. Klassificieringen av hårvirvlarna gjordes enligt ett protokoll, med tillägg av hårvirveln som kallas för fjäder, vilken går i en linje, med eller utan sväng i detta fall. Med togs även vilken riktning hårvirveln hade, medsols, motsols eller radiär (symmetrisk).

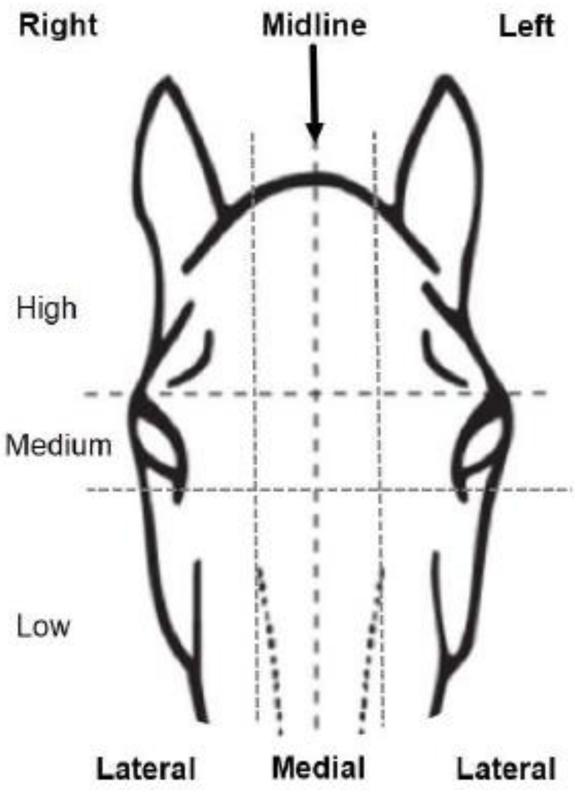
Resultaten indikerade att om hästen hade en hårvirvel som satt lite till höger (hästens höger) i pannan, så var den mer benägen att bli nervös under tävling, än om virveln satt lite till vänster, då den istället fick lägre poäng i nervositet. Om hästen hade en fjäderlik virvel, och denna fjäder gick i vertikal riktning i pannan, hade den större chans att vara koncentrerad under tävling, i jämförelse med en som hade en horisontell eller diagonalt riktad fjäder. Hästarna med vertikal fjäder hade även bättre aptit efter tävling. En enstaka virvel i pannan visade en travare som snabbare lärde sig uppgiften att tävla, jämfört med travarna som hade två eller fler virvlar i pannan som hade lägre poäng på inlärning. Efter statistisk korrektion för flertal tester gav dock dessa resultat inget sannolikt samband, men de kan ändå ge tydliga indikationer för framtida studier. Större och kanske mer inriktade studier med ytterligare hårvirvelgrupperingar kan krävas för att få säkrare resultat.

Vad gäller genetik-delen i arbetet så gjordes ett fynd som verkar vara av hög betydelse när det kommer till hästar som hade en jämfört mot de som hade två hårvirvlar i pannan. På fem kromosomer, speciellt en av dem, sågs en tydlig skillnad i deras DNA. Detta är ett spännande resultat och intressant för vidare forskning.

Appendix 1

Name: Date:

Whorls



Instructions

- Mark the center of the whorl with an "x".
- Plot the whorl around the x, the direction clearly visible
- Use the lateral edge of the nose bone to classify the whorl as either having lateral or medial placement.
- Give the whorl a number (1-3)
- The number of the whorl is filled in the table below
- Mark the direction of every whorl
- Write eventual comments at the bottom
- Take a photo of the hair whorl. The horse's name on a white tape visible on the photo.
- Lift the forelock away during photographing to make the forehead clearly visible.

	Right		Midline		Left
	Lateral	Medial		Medial	Lateral
High					
Medium					
Low					

Direction: = Clockwise = Counterclockwise = radial

1:
- ◻ Counterclockwise
- ◻ Clockwise
- ◻ Radial

2:
- ◻ Counterclockwise
- ◻ Clockwise
- ◻ Radial

3:
- ◻ Counterclockwise
- ◻ Clockwise
- ◻ Radial

Comments:____

____

Appendix 2

Table 9. The survey used in the thesis with trait names, trait descriptions and response options on a scale from 1-7. The trainer was asked to rate how often he or she observe/d the specific behaviour in competition.

	Description	1 Never	2 Rarely	3 Occasionally	4 Sometimes	5 Often	6 Usually	7 Always
Nervousness	Tends/tended to become nervous							
Excitability	Tends/tended to get excited or agitated easily							
Fearfulness	Tends/tended to be afraid easily (e.g. novel environments)							
Concentration	Tends/tended to be focused and unaffected by the environment							
Learning	Tends/tended to learn the task of competing quickly							
Memory	Tends/tended to memorize/remember unpleasant events							
Cooperation	Tends/tended to be cooperative, have good attitude (e.g., willing to work/no resistance)							
Will to win	Tends/tended to desire to win							

	Description	1 Never	2 Rarely	3 Occasionally	4 Sometimes	5 Often	6 Usually	7 Always
Stubbornness	Tends/tended to be obstinate once it resists a command							
Self-control	Tends/tended to panic, escape, and lose control (e.g., impossible to handle or stop / damage itself)							
Recovery	Tends/tended to relax quickly							
Appetite	Tends/tended to have poor appetite between competitions events							