Can hair whorls predict temperament in Standardbred trotters? A comparison between questionnaire responses and behavioral tests

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Abstract

Introduction: Compared to the 55–65 million years of evolutionary history of the horse, some 4000 years that horses have been domesticated is quite a short period. During the time they have been in contact with humans, the natural behaviors and needs of horses have not significantly changed, and therefore the new environment and everyday cooperation with humans can conflict with these needs. In the breeding of racehorses, athletic and physical characteristics have long been the main focus, but it has been increasingly noticed how temperament and mental abilities affect not only the horses' athletic performance, but also their general functioning and everyday co-operation with humans.

In order to enable harmonious coexistence, it is critical to understand horses' behavior. During the last decades, the research of behavior and temperament in animals has been a growing field. Both objective behavioral tests and subjective questionnaires have been developed to study tempera-ment traits in animals. For centuries, there has been a belief among equestrians that hair whorls on horse's forehead can be indicative of its behavior and temperament, and recent scientific studies support this assumption.

The aim of this study was to determine whether the results from behavioral tests and the questionnaire responses correlate, and to further investigate the connections between different types of facial hair whorls and horse temperament.

Materials and methods: This study included a total of 56 Standardbred trotters, stabled in seven different professional racing stables in Sweden.

Three objective behavioral tests were used in this study; Reactivity to human, Novel object and Novel surface tests. Reactivity to human, as the name says, assessed the horse's reactivity towards humans, Novel object test measured behavior associated with nervousness, while the fearfulness trait was assessed in Novel surface test.

The subjective method to assess temperament traits in this study was an online questionnaire on Netigate. Temperament traits studied in the questionnaire were nervousness, fearfulness and reactivity to humans. The questionnaire also covered topics related to cognition, cooperation, concentration, learning and memory. Additionally, questions about the horses' background, living conditions, appetite and health status were included.

The facial hair whorls were inspected live and/or from photographs, a protocol was filled out and photos were taken on the horses' forehead.

In order to investigate the associations between questionnaire answers, results from behavioral tests and hair whorls, comprehensive statistical analyses were conducted. Analyses involved various statistical methods including correlation tests and association analysis ANOVA.

Results: This thesis could not find significant correlations between questionnaire responses and behavioral test scores. However, several associations between different hair whorl types and tem-perament traits were revealed. The results indicate the following: feather-shaped facial whorl was linked to higher compliance, courage, and a good appetite. Also, horses with a whorl with longi-tudinal position medium (between the eyes) appeared to have quicker learning abilities compared to horses with a high whorl.

Conclusion: In this thesis, significant correlations between questionnaire responses and behavioral test results were not found. However, some significant correlations between facial hair whorls and certain temperament traits were found. This study represents a small step toward validating the use of questionnaires in horse temperament research, but to get more reliable results more studies with the larger number of horses are needed.

Keywords: behavior, hair whorls, questionnaire, Standardbred, temperament, trotter

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Abbreviations

ANOVA	Analysis of Variance
BCE	Before Common Era
CC	Counterclockwise
CW	Clockwise
FDR	False Discovery Rate
NO	Novel Object test
NS	Novel Surface test
RH	Reactivity to Human test
SNP	Single Nucleotide Polymorphism

1. Introduction

The evolution of the horse began approximately 55-65 million years ago (Goodwin 2007; MacFadden 2005), while the genus *Equus*, which includes all modern horses, emerged around 4 to 4.5 million years ago (Piras *et al*. 2022). In comparison, the roughly 4000 years since horse domestication represent a relatively brief period in the species' evolutionary timeline (Librado *et al*. 2024). General behavior of horses appears to have changed only a little during the time they have been in contact with humans (Goodwin 2007) and even though horses have benefited from domestication by gaining food, as well as shelter, medical care and protection from predators, it also poses some challenges.

By design, domestication always restricts the horses' natural behavior to some extent, such as the freedom to roam and to choose food, shelter, and social companions (Goodwin 2007; Torres Borda *et al*. 2023). Horses are expected to accept humans, and to understand our instructions, even though human behavior has been shaped by a very different evolutionary history than horses'. This conflict can cause stress for the horse and thus affect its welfare.

Research into animal behavior and promoting their well-being has made progress in recent decades, but much is still unknown. Horse sports, dressage in particular, has recently been the target of criticism in the media, and harness racing has not been spared from negative publicity either (Tuomola *et al*. 2019; Hummel & Hummel 2024). Animal welfare issues being brought into focus in public debate, is absolutely a good thing, but it also poses a challenge for the horse industry – animal welfare must be taken into account more than ever, and should be prioritized over, for example, the financial interests of the industry and people involved. Above all, this must be done for the sake of the horses, but also because public opinion about equestrian sports is of paramount importance for its existence.

For centuries, there has been a belief among equestrians that hair whorls on horse's forehead can give indications of its behavior and temperament thus also of its performance. Different whorl patterns in horses have been linked to different characteristics, such as nervousness or general learning ability (Le Scolan *et al*. 1997; Saxe 2022). Recent scientific findings show that there may be truth to this belief; the patterns are believed to be linked to the development of the nervous system not only in horses but also in other species (Furdon & Clark 2003; Jameson *et al.* 2023).

In humans, certain hair whorl patterns have been associated with various developmental disorders of the nervous system (Aksu *et al.* 2013; Korlimarla *et al*. 2021). It is known that the skin and the brain share the same embryonic origin, and genetic mapping in mice has revealed genes with dual roles in brain and skin development (Cinquanta *et al.* 2000; Furdon & Clark 2003; Jameson *et al.* 2023). These facts could explain the association between hair whorls and behavior traits.

Some masters' theses have been done on this topic in recent years (Berglund 2021; Delbos 2024; Saxe 2022). My task with this project was to build on the findings of those theses. Behavioral tests and the classifications of the facial hair whorls had been performed on 44 Standardbred trotting horses in Sweden by Delbos (2024). Also, responses for a questionnaire regarding horses' behavior and temperament had started to be gathered from the trainers of the horses. We continued to collect data from these individuals, but added 12 new horses to the study, from which we collected hair whorl data, hair samples, and trainer-assessed responses to questionnaires.

All horses are not suitable for the same task. For example, riding schools have begun to wake up to the problem that as maximal physical abilities and racing performance have become the main objectives of selective breeding, the lack of calm, good-natured riding school horses has emerged as a contemporary problem (SVT 2022). Therefore, a few breeders have already started breeding horses with the goal of producing a horse that is suitable for riding schools.

The ideal outcome for this thesis is to be useful as one tool to help steer breeding in the direction where optimal performance and animal welfare go hand in hand. The outcome of this thesis could also help to partly explain why some horses compete better than others. A horse is a sensitive animal, the mentally taxing aspects of competing (e.g. stress tolerance) are often overlooked as a major factor in the overall performance of horses.

The main purpose in this thesis was to find out how well behavioral tests and questionnaire responses correlate, as the secondary research aim was to further explore the connection between hair whorls and temperament in horses. Previous studies indicate that questionnaire surveys can be an effective tool when assessing equine temperament traits (Momozawa *et al*. 2003, 2005a), so there is a reason to believe that it is a reliable method in this project as well.

2. Literature review

2.1 The domestication of the horse and the evolution of horse behavior

Horses were domesticated approximately 4000-4500 years ago on the Eurasian steppe, in a region encompassing parts of modern-day southwestern Russia, Ukraine, and western Kazakhstan (Librado *et al*. 2024). Recent ancient DNA studies indicate that modern domestic horses originate from a single genetic lineage that rapidly expanded from the lower Volga-Don region around 2200 BCE. While earlier horse use by the Botai culture in northern Kazakhstan (~3500 BCE) has been documented, these horses do not appear to be the direct ancestors of modern domestic horses (Librado *et al*. 2021). The domestication of horses played a transformative role in human history, enabling new forms of mobility, trade, and warfare across Eurasia.

Since domestication, humans have selectively bred horses for different purposes, leading to the emergence of genetically distinct breeds suited for tasks ranging from agriculture and warfare to racing and leisure riding (Petersen *et al*. 2013).

Over the course of 65 million years of horse evolution, the relatively short period of approximately 4000 - 4500 years during which horses have been domesticated and in contact with humans has not significantly altered their natural behavior (Goodwin 2007). The reproductive success of feral horse populations is a testament to the stability of their innate behaviors. While modern horses may exhibit a higher degree of sociability toward humans and are less inclined to escape than their distant ancestors, their fundamental behaviors and species-typical needs for social interaction, forage and freedom of movement remain largely unchanged from those of 4000 years ago (Hall & Kay 2024).

Despite the protection and sustenance provided by humans, domestication has constrained horses' natural behaviors, such as their freedom to roam, select food, shelter, and companions (Goodwin 2007; Torres Borda *et al.* 2023). These limitations, along with challenges in communication between humans and horses, can induce stress and impact the horse's overall well-being. The environment and the ability to meet their natural needs play a crucial role in shaping a horse's behavior (Wolff *et al.* 1997), which is why this study includes questions about the horse's living conditions.

2.2 The Standardbred trotter

The origins of the Standardbred can be traced back to a stallion named Messenger, born in England in 1780 (Sochocky 2024). Later Messenger was brought to the United States to pass on its genes, especially a strong trotting gait. However, its great grandson Hambletonian 10 (b.1849) is considered to be the real progenitor of Standardbreds. The standardbred gene pool was further refined by selected pacers and trotters as well as thoroughbreds.

The breed eventually diverged into two primary lines based on different gaits: pacers and trotters (Sochocky 2024). Pacers perform a two-beat lateral gait, where the front and hind legs on the same side move forward simultaneously whereas trotters move their legs in diagonal pairs, a more common gait in European harness racing. The National Association of Trotting Horse Breeders established an official registry for Standardbreds in 1879. The requirements to be included was to finish a mile in a time less than 2.5 minutes, either by pacing or by trotting. In 1973, the studbook was closed by United States Trotting Association (USTA) (Esdaile *et al*. 2021).

The Standardbred is mainly developed for harness racing, where horses race on an oval-shaped racetrack with the driver sitting in a cart behind the horse (Svensk Travsport, n.d. b). Along with France and the USA, Sweden is one of the leading trotting countries in the world and trotting is a popular sport to watch as well as a source of employment for a significant amount of people. Currently, there are around 16 000 racehorses in training in Sweden, consisting of around 90% warm-blooded Standardbreds and 10% of cold-blooded breeds. Even though Standardbreds` main purpose is racing, after their racing career it`s also becoming more and more popular to use them in leisure riding and social pedagogical use due to their good temperament and cooperative nature.

According to Swedish trotting association the breeding goal for Swedish Standardbred is to produce trotters that are physically well suited for trotting races, but also have good temperament, are easy to handle and have strong will to win (Svensk Travsport n.d. a).

2.3 Definitions of behavior, temperament and personality in humans and animals

The term behavior is defined as the observable and measurable actions or responses of an individual or group to internal or external stimuli (Heimlich & Ardoin 2008). Those responses can include conscious and unconscious movements as well as physiological responses. The study of human and animal behavior differs in both methodology and underlying assumptions, as behavior in humans is observed only in non-verbal children or in cases of illness or injury – otherwise is behavioral research in humans based on verbal communication (Martin & Bateson 2007; Schlinger 2004).

In animals, the research on behavior, temperament and personality began later than in humans but has been growing in the last couple of decades. This has been incentivized by both practical interest of animal owners and scientific interest in the topic as a whole (König v. Borstel 2013). Animal temperament and personality can influence a wide array of factors, such as cognitive abilities, the behavior in a herd and even the expression of pain (Ijichi *et al*. 2014; Finkemeier *et al*. 2018).

The definition of temperament has been a topic of discussion since ancient Greek philosophies and causes still differing opinions (Rettew & McKee 2005). In human psychology, according to general consensus, the term temperament refers to inherited psychological traits and variation in emotional reactivity that can be observed in an individual from early childhood and that are quite stable throughout the individual's life. Temperament forms the basis for an individual's personality.

The term personality in turn refers to the set of mental traits that form the basis for an individual's behavior (Digman 1990; Finkemeier *et al.* 2018). In addition to temperament, personality is also considered to be influenced by environmental factors and the individual's experiences over the course of a lifetime. One of the ways to describe personality in humans is the "Five-factor model of personality", the factors being extraversion, openness to experience, agreeableness, conscientiousness and neuroticism (Digman 1990). Furthermore, these factors consist of several more detailed traits that describe an individual's personality. For example, neuroticism refers to emotional stability, and includes traits such as nervousness, temperamentality and moodiness.

Whereas the Five-factor model used to describe personality in human psychology does not directly translate into animals, some of the traits have been explored in animals as well (Finkemeier *et al*. 2018). Some of these traits are boldness, exploration, activity, aggressiveness and agreeableness. The fifth trait of the human model, conscientiousness, has not been observed in animals other than primates, such as chimpanzees and gorillas.

The two most common ways to describe animal personality are division between bold and shy (Sih *et al*. 2004) or proactive and reactive (Koolhaas *et al.* 1999). The division into bold/shy is based only on behavioral aspects, as research performed on feral animal populations shows repeatedly some individuals to be generally more aggressive, more active or bold, while others are generally less aggressive, active or bold (Sih *et al*. 2004). The definition of personality types, or more specifically stress-coping styles, along a proactive-reactive axis includes besides behavior, also physiological measures (Koolhaas *et al*. 1999). Proactive individuals are typically bold, aggressive, routine-oriented, showing lower stress responses and a higher tendency to take risks and become dominant. In contrast, reactive individuals are more cautious and flexible, become subordinates and are more successful upon environmental changes. These individuals usually exhibit higher physiological stress responses.

The fact that the methods and terminology for the field of animal psychology were borrowed from human psychology may pose some ambiguities. For example, in animal temperament research, the terms “behavior”, "temperament" and “personality” are often used interchangeably (Réale *et al*. 2007). When studying farm animals, the word "temperament" is often used instead of "personality", even in situations where the latter might be more accurate. One potential reason for this is to avoid the humanizing effect of the word "personality", possibly leading into further ethical questions about animal-based products.

In equine research, however, the most used term is "temperament", which is the term we have used in this study as well, meaning a set of inherited mental tendencies that are relatively stable, and observed across different contexts and over time (Lansade & Simon 2010).

2.4 Methods to assess behavior and temperament in horses

2.4.1 Objective behavioral tests for horses

Several experimental tests have been developed to objectively study behavior and temperament in animals (Murphy *et al*. 2014; Finkemeier *et al*. 2018). Criteria for tests include that they must be specific enough to make it clear which temperament trait the test is reflecting. It is also important that the test and variables can be standardized so that valid comparisons can be made between studies and that the tests can be repeated (König v. Borstel 2013).

According to a review article by König v. Borstel (2013) some valid tests are available, even though there is still no consensus on which are the best tests to assess equine behavior and temperament. Examples of tests designed for horses include novel object test, bridge test, physiological measurements that reflect the psychological state of the individual (for example heart rate), as well as tests that assess the horses interaction with humans (Wolff *et al*. 1997; Górecka *et al*. 2007; König v. Borstel 2013).

The three tests selected by Delbos (2024) for this study are named Novel object (NO), Novel surface (NS) and Reactivity to human (RH). These tests were selected based on their reliability according to the literature (Hausberger *et al*. 2004; Lansade & Bouissou 2008; Górecka-Bruzda *et al*. 2011).

The first of these three tests was RH. According to Lansade and Bouissou (2008), one of the best approaches to assess horse’s reactivity to humans is the time taken for an actively acting familiar or unfamiliar human to touch the horse's muzzle and withers. The protocol used in this project was the adaptation of the RH protocol done by Gorecka-Bruzda *et al*. (2011).

The NO test is inspired by the initial experiment of Le Scolan *et al.* (1997) that showed a link between behaviors in the NO and the nervousness trait in riding conditions. This test has been used in several studies to interpret fearfulness and nervousness in horses (da Luz *et al*. 2024; Le Scolan *et al.* 1997; Liehrmann *et al*. 2022). The reliability is increased by heart rate measurements made by Visser *et al.* (2001, 2002). In this test, interpretations of the horse's nervousness are made based on how it approaches a novel object.

The NS test performed in this project was inspired by the Bridge test and NS test protocols in literature (Wolff *et al.* 1997; Hausberger *et al*. 2004; Ijichi *et al*. 2013). The bridge test is used in many studies as a handling test and the protocol includes a human that leads the horse to a bridge (Ijichi *et al*. 2018; Rosselot *et al*. 2019; Liehrmann *et al*. 2022). However, in this project the protocol was designed to interpret fearfulness, and the horse was intended to walk freely on a novel surface without the human's influence on the test result (Delbos 2024).

2.4.2 Questionnaires

Objective behavioral tests usually require a lot of time and resources. When data on a larger number of individuals may increase the reliability of the research, various human-assessed subjective approaches have been developed to study equine temperament (Ijichi *et al*. 2013, Momozawa *et al*. 2003, 2005a). One type of subjective assessment is when trained judges assess horses´ mental abilities when making breeding assessments (König v. Borstel 2013). This concept has been criticized, because the judges can only evaluate the horse's behavior at the time of the test, and the scores often lack consistency and standardized definetions. Therefore, it may be more reliable if the questionnaires are answered by people who have followed the horse's behavior over a longer period of time (König v. Borstel 2013; Momozawa *et al*. 2005a). Due to all of the above, the goal of many researchers has been to develop a standardized questionnaire, in which riders, trainers or grooms would subjectively assess the horse's temperament traits.

One of the leading studies on this topic is by Momozawa *et al*. (2003), who developed the first version of the questionnaire used in this study. In the study by Momozawa *et al.* (2003), the results of the questionnaire were compared with objective tests that were found to be reliable. The questionnaire asked to score on a scale of 1–5 traits of the horse's temperament, including anxiety. Then a behavioral test for interpretation of the horses' anxiety was performed to validate the questionnaire. The results of the study were promising and showed that at least anxiety could be reliably assessed using the questionnaire. Momozawa *et al.* (2005a) continued their work and developed the scoring system to include more questions, now on a 9-point scale, which allowed for a more precise assessment. This time, validation was attempted by comparing two different questionnaires, which were made for two groups of Thoroughbreds with similar backgrounds.

The test results indicated the validity of the questionnaire in question for the traits anxiety, affability and trainability.

Ijichi *et al.* (2013) investigated whether subjective assessments of temperament traits could predict the results of objective tests assessing the same trait. The study included 146 horses, and the results were positive for many traits. For example, neuroticism predicted how reactive horses were to a sudden visual stimulus, and extraversion predicted a shorter time to complete a handling test. In contrast, agreeableness could not be associated with any of the results of the objective tests. The results suggest that combining both objective and subjective assessments provide the most reliable data on temperament. Although several studies have provided indications of the validity of questionnaires (Ijichi *et al*. 2013; Le Scolan *et al*. 1997; Momozawa *et al*. 2003, 2005a) it has not yet been possible to develop a standardized questionnaire that would cover several temperament traits (Delbos 2024).

2.5 Emotional systems and genes

According to the most recent studies in the field, the behavior of human beings is mainly driven by the emotional system in the cerebral subcortex of the brain (Panksepp 2011). Whereas humans have more developed cerebral cortexes than other animals, the aforementioned emotional systems are surprisingly similar across the animal kingdom. The primary goal of an individual is to seek positive emotions while minimizing negative emotions. The main emotional systems are novelty-seeking, fear, rage, and panic (Panksepp 2005; Morris *et al*. 2011) with play, lust and care being secondary systems (Panksepp 2011).

Genes exert a significant influence on one's behavior and temperament through the genetically determined structure of the brain (Grandin 2022). Since these systems of genes affecting behavior are extremely intricate, tracking of individual genes can be challenging. However, certain genes influencing the psychological characteristics of horses have been found. For example, Lima *et al*. (2021) found genes with pleiotropic effects on mental abilities in their study of genomic regions associated with hair whorls in Quarter Horses. In addition, certain single nucleotide polymorphisms (SNPs) have been linked to temperament traits in horses, such as the SNPs in the dopamine receptor D4 being associated with vigilance and curiosity in Thoroughbred horses (Momozawa *et al.* 2005b).

2.6 Hair whorls

2.6.1 Facial hair whorls in horses

Hair whorls, also known as swirls or trichoglyphs, are permanent features of a horse's coat pattern that are formed before birth and remain unchanged throughout the animal's life (Yokomori *et al.* 2019). Most horses have at least one forehead whorl, although it is not uncommon for some individuals to have two or more. Definition of a hair whorl is that the direction of the hair growth differs from that of the surrounding coat.

Hair whorls are classified based on their location, and direction of the hair (Shivley *et al.* 2016). Direction can be radial, where the hair goes out from the central point of the whorl symmetrically, clockwise (CW) or counterclockwise (CC), depending on the direction in which the hair spirals from the root outward (Saxe 2022). A feather or elongated whorl defines as hair growing radially along a line instead of central point. The base direction of such whorls may vary.

Facial hair whorls are classified based on their vertical position as high (located above the eyes), medium (between the eyes) and low (below eye level). In terms of horizontal placement, they may lie directly on the midline or be offset to the left or right. Some examples of the different hair whorls are presented in Fig. 1.

Figure 1. Left: A horse with two whorls; one high-positioned clockwise whorl on midline, and high-medium feather on midline. Middle: A horse with two whorls; one radial and one feather. Right: A clearly visible feather.

2.6.2 Hair whorl development theories

The formation of hair whorls in humans and various animal species has been studied, and there are several alternative theories for their origin (Klar 2003; Samlaska *et al*. 1989; Willems *et al.* 2024). Some studies suggest that the formation of hair whorls is linked to the mechanical tension targeted to the skin and hair follicles during development of the fetus in utero (Paul 2016). Another theory is that hair whorl patterns are determined by genes (Samlaska *et al*. 1989). It is known that characteristics of the hair coat, such as texture and color, are inherited. In humans, the most common hair whorl has been found to be clockwise, and it has been hypothesized that it is caused by a dominant gene (Klar 2005). However, studies suggest that the inheritance of whorls is more complex than that, and it is likely that prenatal environment, epithelial cell migration, and various gene expressions during skull and scalp formation play a role in their origin (Curtiss *et al*. 2002; Ribeiro *et al.* 2003; Willems *et al*. 2024).

In humans, hair features and brain development occur primarily between 10 and 16 weeks of gestation (Rees & Harding 2004; Yokomori *et al*. 2019). It is known that these two are linked; the tissues develop from the same cell layer in the embryo, and even genes with dual roles in brain and skin development have been found via genetic mapping in mice (Cinquanta *et al*. 2000; Furdon & Clark 2003; Jameson *et al*. 2023). Because of this connection, it is reasonable to assume that behavior and temperament correlate with facial hair whorls.

In addition to temperament and mental traits, hair whorls have also been found to be associated with laterality, which refers to lateral differences in locomotor activity (sidedness, handedness) in horses as well as humans (Kis *et al*. 2024; Murphy & Arkins 2008; Randle & Elworthy 2006; Shivley *et al.* 2016).

2.6.3 Temperament traits linked with facial hair whorls

In humans, certain types of hair whorls are linked with neurological disorders. For example, humans with trisomy 21 are more likely to have their posterior hair whorl midline (Korlimarla *et al*. 2021). Autistic people in turn have been found to have more hair whorls in number than people without autism (Aksu *et al.* 2013).

Although animals have hair whorls in many different places on their body, most research in horses and cattle focuses on facial hair whorls (Broucek *et al.* 2007; Górecka *et al*. 2007; Murphy & Arkins 2008; Onouchi *et al*. 2024; Randle & Elworthy 2006; Saxe 2022; Shivley *et al*. 2016).

In Murphy and Arkins (2008), the most common facial whorl type in horses was counterclockwise. Randle *et al.* (2003) studied Lundy ponies and found that horses with facial hair whorls on the left side of the head were more friendly, calm and enthusiastic, while those with whorls on the right side were more unfriendly and wary, but also more playful.

In a study by Saxe (2022) of 175 Standardbred trotters, the results indicated that horses with feather shaped whorls were more focused and ate better during competitions, if the feather was vertical compared to diagonal or horizontal. A whorl on the right side of the head was associated with a stronger nervousness trait during competitions. The number of whorls correlated with the horse's learning ability, with horses with more than one whorl appearing to learn the task of competing slower than horses with only one facial whorl.

Randle and Elworthy (2006) studied the correlation between the hair whorls and laterality in 219 ridden horses. The results revealed that horses with a CW whorl were more often right-lateralized and horses with a CC whorl were more often left-lateralized. Similar results were also obtained by Onouchi *et al.* (2024), who studied a small group of Japanese Kiso horses. They found a link between left-lateralized horses and CC whorls.

In addition to the scientific field, the connection between hair whorls and temperament has long been of interest to ordinary equestrians. Oral tradition has been talked among horsemen for a long time and, for example, Miller (2021) has compiled a comprehensive package of information not only about facial hair whorls, but also about those on the whole body. This information is based on both science and empirical experience. According to Miller (2021), horses with a low facial hair whorl indicate a more introverted and quiet nature, while a high whorl would indicate an extroverted nature.

3. Materials and methods

3.1 Materials

3.1.1 Horses

This study included a total of 56 Standardbred trotters. Hair whorl classification and hair samples from tail for possible later DNA-testing were collected for all the 56 horses. The questionnaire was answered for 54 of them, and behavioral tests in field were performed on 44 horses (RH n=44, NO n=37, NS n=26). The horses were located in Sweden, more specifically in Uppsala, Stockholm, Södermanland and Gävleborg counties. The study population was composed of 21 mares, 30 geldings and five stallions, aged between two and ten years and housed in seven professional harness racing stables. The study included mostly horses that were actively competing, but also four retired trotters. Horses participating in the behavioral tests were in training and uninjured at the time of testing.

Some of the data used in this study was collected during a previous study by Delbos (2024) that included 44 Standardbreds. We continued to collect data from these individuals, but added 12 new horses to the study, from which we collected hair whorl data, hair samples, and trainer-assessed responses to questionnaires. Hair whorl data and tail hair samples were double-checked for some of the horses (n= 26). For clarity, the division of tasks is presented in Table 1.

Table 1. The division of responsibilities between Delbos (2024) and Korpi (2025) regarding data collection.

	Tail samples	Behavioral tests	Hair whorl data	Questionnaires
Korpi (2025)	38	0	38	42
Delbos (2024)	44	44 (RH:44, NO:37 NS:26)	44	12
Total n	56	44	56	54

3.2 Methods

3.2.1 Questionnaire

The online questionnaire (©Netigate) used in this study was the same as that used in a previous study by Delbos (2024). The questionnaire used in this study is based on earlier versions developed by Momozawa *et al*. (2005a) and Staiger *et al.* (2016), with adaptations made for the current context. Similar adaptations have been used in several previous studies concerning related topics (Berglund 2021; Saxe 2022). The questionnaire was then further developed and modified by Delbos (2024).

The questionnaire answers were collected during 2024 (March–November). The same questionnaire was offered during the previous study (Delbos 2024) to all then participating six trainers (n=44 horses) but were completed by trainers for only 12 horses. However, there may have been a problem with the website, as some of the survey participants (n=3) reported that they had already completed the survey during the previous study (Delbos 2024), but for some reason their answers had not been saved.

During this study, we were able to collect responses for 42 horses, in addition to those 12 responses collected during the previous study (Delbos 2024). The questionnaire remained unanswered for only two horses of the total of 56 and the final number of responses was 54. That was because the horses in question had changed trainer and stable during the year.

Structure of the questionnaire

The questionnaire took an estimated time of about ten minutes to complete and was divided into three different sections.

The first part included a project description and the person answering the survey signed a form where consent was given to use the collected data in research.

The second part consisted of information about the horse in question. It included general information about the horse (name, ID number, start age for training), questions about horses' living conditions (housing and time spent outside, feeding, social contacts). These were asked because the possible influence of the environment on the horse's behavior (Hausberger *et al.* 2004). In this part trainers were also asked about possible stereotypical behaviors, the horses' health status and if the horse was frequently injured.

The third part included the main information, statements to which the trainers responded by assessing the behavior and temperament of each horse. The statements were answered using a 7-point Likert scale, where 1 described "Strongly Disagree" and 7 "Strongly Agree". Number 4 was neutral.

The statements were designed for interpretation of horses´ behavior and temperament. The temperament traits studied in the questionnaire were: nervousness, fearfulness and reactivity to human. The questionnaire also included statements related to cognitive abilities, concentration, cooperation, learning and memory. In addition, there were statements about tactile, auditory and visual sensitivity, and appetite.

There was a total of 25 statements in the behavioral part of the questionnaire (Table 2). The most difficult temperament traits to assess were estimated to be nervousness, fearfulness, reactivity to human, cooperation, concentration, learning and memory. To help get a reliable answer, at least two opposing questions were asked concerning these traits.

Table 2. Statements in the questionnaire and interpretations based on them.

Questions	Interpretation
On foot, in daily handling, this horse reacts with fear/is not ready to face 'danger' in novel situations? On foot, in daily handling, this horse reacts with courage/is ready to face and endure 'danger' in novel situations? On race days, this horse tends to be easily afraid (e.g. novel environments?)	Fearfulness
On foot, in daily handling, this horse thinks before reacting to external intervention or stimulation? On race days, this horse tends to be nervous?	Nervousness
On foot, in daily handling, with an unfamiliar human, this horse is aggressive/threatening? On foot, in daily handling, this horse welcoming/seeking/very friendly with an unfamiliar human?	Reactivity to unfamiliar human
On foot, in daily handling, this horse is sensitive to [tactile/auditory/visual] stimuli?	Sensory sensitivity
On foot, in daily handling, this horse complies with human request (e.g. hand over the hooves, halter maniability)? On foot, in daily handling, this horse resists human requests and remain obstinate after?	Cooperation
On foot, in daily handling, this horse stays focused and unaffected by environment? On foot, in daily handling, this horse gets distracted easily and tends to look at the surroundings?	Concentration
(In general and race day context) This horse rushes to its hay/eats its hay quickly? This horse rushes to its grains/eats its grains quickly? This horse tends to have a poor appetite between competitions?	Appetite
In general context, this horse is able to learn quickly an information or a behavior? In general context, this horse learns slowly new information or behavior? On race days, this horse tends to learn the task of competing quickly?	Learning
In general context, this horse is able to memorize information or a behavior for a long time? In general context, this horse has difficulty to memorize an information or behavior for a long time? In race day, this horse tends to remember unpleasant events?	Memory

3.2.2 Behavioral tests

Behavioral tests were performed on 44 Standardbred trotters in the previous study by Delbos (2024). Of those 44, RH was performed on all the horses, but for the NO and NS tests, 37 (NO) and 26 (NS) horses, respectively, were tested because of weather conditions. The tests were performed at field, in the locations that were already familiar to the horses. Five to nine horses per stable were tested, and it took one to two afternoons per stable to perform the tests. All tests were filmed.

Reactivity to human

The first test performed was RH. Horses were selected by the trainer, and the test was performed indoors. This protocol included the test parts with both passive and active humans.

Passive human test: The experimenter, who was unfamiliar to the horse, entered an empty box and stood in the corner of the box. The horse was released into the box and the experimenter stood still for 300 seconds. The time taken for the horse to approach the experimenter was measured, and the latency, frequency and duration of horses' all behaviors during the test were recorded. The test concluded when the horse approached and touched the experimenter or when the 300-second time limit was reached. Interpretations about the horse's reactivity to humans along a scale ranging from very friendly to threatening were then made based on measured times and behaviors. The next test then commenced.

Active human test: When the horse had touched the experimenter or 300 seconds had passed, the experimenter started approaching the horse, regardless of the horse's location at that moment. When the experimenter was less than one meter from the horse, they slowly raised their hand with the palm towards the horse and approached the horse at a slow pace (1 m/s). The experimenter tried to first touch the horse's forehead and then its withers. The time to touch the forehead and the time to touch the withers were recorded. The latency, frequency and duration of all horses' behaviors during the test were recorded (Table 3).

Table 3. Behaviors recorded during the RH test and their category classification. Reworked from Delbos (2024).

Category	Behavior	Description of the behavior
Approach movement	Approaches	Walks in the direction of the experimenter and gets closer.
Touch experimenter	Touches experimenter	The horse's muzzle touches the experimenter.
Explore experimenter	Smells experimenter	Brings its muzzle close to the experimenter, and nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Visual attention - unfamiliar human	Looks at the experimenter	Pricks its ears forward towards the experimenter and shows a tense neck. The head may be low or high. Visual attention is high.

Visual attention-environment	Looks at its neighbor	Pricks its ears forward towards the wall open to the neighboring box and shows a tense neck. The head may be low or high.
Visual attention-environment	Looks outside	Pricks its ears forward towards the environment outside and shows a tense neck. The head may be low or high.
Mouth movement	Bits its tongue	The horse is seizing or clenching the tongue with the teeth.
Mouth movement	Chews	Repetitive movement of the jaws as when food or another object is crushed or ground between the teeth.
Mouth movement	Sticks out its tongue	Action of extending the tongue outside of the mouth.
Mouth movement	Yawns	Involuntary act of opening one's mouth wide and inhaling deeply.
Threatening behavior	Ears back	The horse is orientating or pinning its ears flat against its head.
Exploration of the box	Smells its neighbor	Brings its muzzle close to the wall open to the neighboring box, and its nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Exploration of the box	Smells its hay	Brings its muzzle close to the hay in his box, and nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Exploration of the box	Smells the bucket	Brings its muzzle close to the bucket in his box, and nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Snoring	Snoring	Vocalization characterized by a low, rumbling sound emitted during breathing, may be characterized by repeated breath sequences.
Avoidance behavior	Raises its head	When the experimenter approaches the horse's face with his hand, the horse avoids contact by moving his head up.
Avoidance behavior	Turns its head to avoid the experimenter	When the experimenter approaches the horse's face with his hand, the horse avoids contact by moving his head on the side.
Avoidance behavior	Move away	After approaching the experimenter, but before touching him, the horse leaves and go to other activities (looking outside, eating).
Avoidance behavior	Step back	The horse does one or more steps back when the experimenter approaches with his hand.
Vocalization	Sigh	Vocalization characterized by a loud breath in by the nose and a long breathe out by the open mouth.

Novel object and Novel surface tests

The NO and NS tests were performed outdoors in a location that was familiar to the horse and were filmed with a camera. For both tests, a starting line was marked on the ground, and a bucket containing treats was placed 15 meters from the starting line. The horse was led by an experimenter using a halter and lead rope to the bucket. The time to walk these 15 meters was recorded, and the horse was then allowed to eat from the bucket for 10 to 30 seconds. The horse was then led away from the location and the set up for NO or NS was prepared. Half of the horses started with NO and other half with NS.

The NO test has its origin in the study of LeScolan *et al*. (1997) and is used to estimate horses' nervousness during riding. However, in this study the test was performed by leading the horse. The novel object used in this study was a multicolored buoy (~1x1x0.3 m). The buoy was placed next to the food bucket and the time taken by the horse to reach the bucket was recorded. This time was compared to time measured before the object was placed. Also, the latency, frequency and duration of all the horses' behavior during the test was recorded (Table 4).

Table 4. Behaviors recorded during the NO test and their category classification. Reworked from Delbos (2024).

Category	**Behavior**	**Description of the behavior**
Direct to the bucket	Direct to the bucket	The horse does not show behavior linked to the buoy, and does not even look at the buoy. Walks straight and relaxed to the buoy.
Vocalization	Neigh	Vocalization characterized by the expulsion of air through the nostrils along with vocal cord vibrations.
Vocalization	Snoring	Vocalization characterized by a low, rumbling sound emitted during breathing, may be characterized by repeated breath sequences.
Visual attention	Looks at the buoy	Pricks its ears forward towards the buoy and shows a tense neck. The head may be low or high. Visual attention is high.
Exploration close to the buoy	Touches the buoy	The horse's muzzle in contact with the buoy.
Exploration close to the buoy	Smells the bucket	Brings its muzzle close to the bucket, and its nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Exploration close to the buoy	Smells the buoy	Brings its muzzle close to the buoy, nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Exploration close to the buoy	Smells the ground	Brings its muzzle close to the ground, and nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Startled when touching the bucket	Startled when touching the bucket	Suddenly moves or jumps because of surprise or fright contact with the bucket.

Stop	Stop near the buoy	The horse ceases or halts the act of walking when he reaches the buoy (1–2 meters before the buoy).
Stop	Stop	The horse ceases or halts the act of walking before reaching the buoy for at least 2 seconds (at least 2 meters before the buoy).
Avoidance behavior	Step back	Steps back when facing the buoy.
Avoidance behavior	Deviate	Starts to pull on the leash to go back or to bypass the buoy, walking or trotting, or jumps on the side.
Vigilant posture	Vigilance	This posture involves raised head and neck, forward-focused eyes, pricked ears, tense body, and heightened physiological responses.

The NS test was performed in order to interpret fearfulness in the horse on the scale from very brave to very fearful. The protocol was inspired by the bridge test and new surface test performed in earlier studies (Hausberger *et al*. 2004; Ijichi *et al.* 2013). As novel surface in this test was a blue tarpaulin (2x3 meters). The tarpaulin was placed between the starting line and the bucket, and the horse was meant to cross the tarpaulin to reach the bucket. The time to reach the bucket was recorded and the results were compared to the baseline time required for the horse to reach the bucket. If the horse tried to approach the bucket in any other direction, it was led back in front of the tarpaulin. The test was finished when the horse crossed the tarpaulin with at least three feet or after 10 minutes. The latency, frequency and duration of all the horses' behavior was recorded during the test (Table 5).

Table 5. Behaviors recorded during the NS test and their category classification. Reworked from Delbos (2024).

Category	**Behavior**	**Description of the behavior**
Hoof on the tarp	One hoof on the tarp	The horse makes a forward movement with the foreleg and places the first hoof on the tarp. The entire sole of the horse's hoof touches the tarp.
Hoof on the tarp	Two hooves on the tarp	The horse makes a forward movement with the foreleg and places a second hoof on the tarp. The entire sole of the horse's hoof touches the tarp.
Crossing the tarp	Three hooves on the tarp	The horse makes a forward movement with the hind leg and places a third hoof on the tarp. The entire sole of the horse's hoof touches the tarp
Defecation	Defecates	Act of expelling feces from the body, through the anus.
Exploration of the tarp	Touches the tarp	The horse's muzzle is in contact with the tarp.

Exploration of the tarp	Paws the tarp	Striking or scraping its hoof against the tarp.
Exploration of the tarp	Explores the tarp with muzzle	The horse touches the tarp with its muzzle and executes right-left movements pressing on the tarp with the upper lip.
Exploration of the tarp	Smells the tarp	The horse brings its nose close to the tarp, and its nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Exploration in the test zone	Smells the ground	The horse brings its nose close to the ground, and its nostrils dilate and contract in synchrony with the horse's respiratory rhythm.
Vigilant posture	Vigilance	A posture involving raised head and neck, forward-focused eyes, pricked ears, tense body, and heightened physiological responses.
Locomotor activity	Jambette	The horse raises a foreleg forward, between 45 and 90 degrees, and holds it in the air for a few milliseconds.
Locomotor activity	Moves	The horse moves in place without going forward or backward, paws or sways its hind legs from right to left.
Locomotor activity	Pawing ground	Repeatedly scraping its hoof against the ground.
Head movement	Head up and down	Up-and-down movement of the head and neck.
Head movement	Shaking head	Quick movement where the horse moves its head back and forth or from side to side without a defined axis of rotation.
Shaking	Shaking	An involuntary and repetitive movement of the horse's body, characterized by rapid vibrations.
Repositioning	Repositioning	The experimenter has to replace the horse in front of the tarp.
Visual attention	Looks at the tarp	The horse pricks its ears forward towards the tarp and shows a tense neck. The head may be low or high. Visual attention is high.
Stop	Stops	The horse ceases or halts the act of walking before reaching the tarp for at least 2 seconds (at least 2 meters before the tarp).
Startle	Startles	The horse suddenly moves or jumps because of surprise or fright.
Avoidance behavior	Steps back	The horse takes one or more steps back quickly, when facing the tarp.
Avoidance behavior	Deviates	The horse starts to pull on the leash to go back or to bypass the tarp, walking or trotting, or jumping on the side.
Vocalization	Snorts	Loud exhalation causing the horse's nostrils to vibrate.

Vocalization	Neighs	Vocalization characterized by the expulsion of air through the nostrils along with vocal cord vibrations.
Vocalization	Breathes out loud	Exhalation with noticeable sound.
Vocalization	Blows	Short and very strong exhalation of the horse, often heard in a vigilant posture.
Vocalization	Snores	Vocalization characterized by a low, rumbling sound emitted during breathing, may be characterized by repeated breath sequences.

Scoring

The three behavioral tests were scored according to the scoring scales (Tables 6, 7 and 8) and numerical scores were obtained for three behavioral traits (reactivity to human, nervousness and fearfulness). The scoring scales were created by Delbos (2024), based on previous research and literature on the subject (Hausberger *et al.* 2004; Lansade & Bouissou 2008; Le Scolan *et al*. 1997). Scores ranged from one to seven, corresponding to the questionnaire results. The scores were based on both behavioral data recorded during the tests and time-based data.

Table 6. The scoring scale for the RH test (Delbos 2024).

Horse – Human Relationship in a box standard size		
Welcoming/Seeking/ Very friendly	1	Approached and touched the experimenter in less than 5s with ears forward. Smells/touch experimenter and seek for scratches or food. And the experimenter touched the face and withers of the horse (in less than 3s for face) without observing ears back or avoidance behavior (steps back, raises his head, turning his head to avoid).
Friendly and Approaches	2	Approached and touched the experimenter in less than 10s with ears forward. Smells/touch experimenter and seek for scratches or food. And the experimenter touched the face and withers of the horse (in less than 6s for face) without observing ears back or avoidance behavior.
Quite Friendly	3	Approached and touched the experimenter in less than 1min with ears forward or axial. And the experimenter touched the face and withers of the horse (in less than 20s for face) without observing ears back and more than 1 avoidance behavior.
Moderatly Friendly	4	Approached and touched the experimenter in more than 1min with ears forward or axial. And the experimenter touched the face and withers of the horse (in less than 20s for face) without observing more than 3 of the following behaviors: ears back or avoidance behavior.
Passive/Distant	5	Didn't approach the experimenter. And/or the experimenter touched the face and withers of the horse (in less than 20s for the face) without observing more than 3 of the following behaviors: ears back or avoidance behavior.
Avoid	6	Didn't approach the experimenter. And/or the experimenter touched the face and withers of the horse (in less than 20s for the face) with observing more than 3 of the following behaviors: ears back or avoidance behavior.
Aggressive/ Threatening	7	Tries to bite, ears back, pushes or turns back to hit. The experimenter couldn't touch the face and/or withers of the horse.

Table 7. The scoring scale for the NO test (Delbos 2024).

Nervousness (Novel object test)		
Very Calm – don't react to external stimulation + relax	1	Reached the bucket with + or – 2.50s compared to the trial without the buoy. Didn't look at the buoy. No behavioral indicators as deviate, stop, step back or vigilant posture.
Calm	2	Reached the bucket with + or – 2.50s compared to the trial without the buoy. Pays attention to the buoy (looks at/smells/touches the buoy) quickly (less than 2s). No behavioral indicators as deviate, stop, step back or vigilant posture.
Quite Calm	3	Reached the bucket in less than 20s, pays attention to the buoy (looks at/smells/touches the buoy) quite quickly (less than 10s). No behavioral indicators as deviate, stop, step back or vigilant posture.
Moderately nervous	4	Reached the bucket in less than 1min, pays attention to the buoy but shows maximum 1 behavior indicators among step back, deviation, stop.
Quite nervous	5	Reached the bucket in less than 1min, pays attention to the buoy and shows more than 1 behavior indicators among step back, deviation, stop. Can feel not confident/ shy to eat in the bucket (looks at the buoy while eating).
Nervous	6	Reached the bucket in more than 1min and shows more than 1 behavior indicators among step back, deviation, stop. Can feel not confident/ shy to eat in the bucket (looks at the buoy while eating).
Very Nervous – React quickly and strongly + muscle tension	7	Didn't reached the buoy and didn't approach it. Tried to escape. Very Stressed.

Table 8. The scoring scale for the NS test (Delbos 2024).

Fearfulness (Novel surface test)		
Very Courageous/Brave – Ready to face danger in novel situations	1	Crossed the tarp in less than 1min with or without exploration behaviors (smells tarp). No behavioral indicators among step back, deviation, stop.
Courageous	2	Crossed the tarp in less than 5min with mainly exploration behaviors (look at the tarp, explore/smell/paw/touch the tarp), may have shown 3 behavioral indicators maximum among step back, deviation, stop.
Quite Courageous	3	Crossed the tarp in less than 5 min showing exploration behaviors and behavioral indicators among step back, deviation, stop.
Moderately fearful	4	Crossed the tarp after 5 min with stress and/or crossed quickly.
Quite Fearful	5	Didn't cross the tarp but approached it and put at least 1 hoof on the tarp.
Fearful	6	Didn't cross the tarp. Approached it and smells it but never put 1 hoof on the tarp. Shows stress.
Very Fearful – Cannot face danger in a novel situation	7	Didn't cross the tarp and didn't approach it. Shaking. Tried to escape.

3.2.3 Facial hair whorls

Information on facial hair whorls was collected either from photographs sent by owners or by live observation followed by taking photographs in the stables. The hair whorls had been photographed and categorized on 44 horses during the previous study (Delbos 2024), but we photographed and re-evaluated all whorls from the horses that were present at the time we visited the stables (n=26), as well as new (n=12) horses that were not photographed during the previous study (total n=38). This was done because some of the images were of poor quality, and because we wanted to double-check the categorization of the whorls. Hair data was collected by us during September–October 2024, while the previous hair data had been collected during the spring of 2024, at the same time point as the behavioral tests were performed. Each horse's facial hair whorls were photographed (whole face and close-ups of the whorls; Fig. 2). A hair whorl classification protocol was filled out for each horse (Appendix 1) and a hair sample was also taken from the tail for possible later DNA testing.

Figure 2. Examples of how the photographs on each horse were taken during this study; whole face image (left) and stepwise close-ups (middle and right). Photos by Fangyuan Qian.

The hair whorls were classified based on their number, location and direction of the hair. The vertical location of the whorls was classified as high (above the eye level), medium (eye level) or low (below the eyes). The horizontal position was determined using the midline of the face as a reference. Whorls were classified as either midline, or (horses) right or left. In addition, the whorls laterally to the nasal bone were classified as lateral and those on the area of the nasal bone as medial (Fig. 3).

The whorls with a center point were classified based on the direction of the hair growth as either clockwise, counterclockwise or radial, in which case the direction of the hairs was directly outwards from the center. If the whorl pattern had a central axis instead of a center point, it was classified as a feather. These feather whorls can have an origin that can be classified as either clockwise, counterclockwise or radial, and a feather was always counted as two whorls.

In a small number of the whorls (n=4), the direction was difficult to determine, as they had features of both CC, CW and radial. We decided to classify these whorls as radial/bidirectional in this study.

Figure 3. Guidelines for identifying the location of the whorl. Photo and edit by Fangyuan Qian.

3.2.4 Statistical analyses

Statistical analyses of the data were performed by assistant supervisor of this master's thesis, Mahmoud Amiri Roudbar. Data from the questionnaires, behavioral tests, and hair whorl observations were compiled using Microsoft Excel®, and statistical analyses were conducted in R. The primary aim of the statistical analysis was to explore potential correlations between questionnaire responses and behavioral test outcomes. Secondary aim was to investigate whether different temperament traits could be associated with certain facial hair patterns. A range of statistical methods was applied.

Initially, correlation tests were conducted to identify statistically significant relationships within and between the different datasets. Spearman's rank correlation coefficient was chosen for this analysis due to its suitability for ordinal data. P-values were calculated to assess the statistical significance of the correlations. To control the false discovery rate due to multiple comparisons, the p.adjust function was used with the "fdr" method.

Finally, associations between variables were examined using linear regression models and ANOVA. Regression coefficients were calculated to better understand the strength and direction of these associations, particularly between temperament traits and different types of hair whorls. Only statistically significant correlations and associations with an adjusted p-value below 0.05 were considered for further interpretation.

4. Results

4.1 Questionnaire

The questionnaire was completed for a total of 54 horses; 21 mares, 28 geldings and 5 stallions, aged between 2 and 10 years. Most of them had started to be trained at 1.5 years of age (87%). For the remaining 13% was starting age for training one year.

Regarding the frequency of hay meals, most of the horses had unlimited access to hay (67%). Twenty percent of the horses got their hay meals twice a day and 13% three to four times a day. The majority of the horses (72%) were stabled at night and outside by day, 17% lived in a free range/outside and 11% was reported living mostly in the box. The majority of the horses (91%) spent their time outside in groups, and remaining 9% were in the paddock alone. The most common time spent outdoors was 5–9 hours per day (69%). Options ”10–14h” and ”over 15 hours” were next most popular answers (11% and 11% respectively), 6% of the horses lived in free-range and 3% spent 1–4 hours per day outdoors.

When asked about health, 17% answered that their horse was frequently injured with the majority of causes (56%) being orthopedic issues.

The third part of the questionnaire was designed to interpret temperament traits in participating horses. The averages and variability of the responses are presented in Figure 4 and 5. Generally, the highest scores in the questionnaire were given to compliance and a welcoming nature (averages of 5.4 and 5.7, respectively). In contrast, the lowest average scores were assigned to aggressivity (1.6), uncooperative behavior (resists requests, 2.5) and often expressed stereotypical behaviors (2.3).

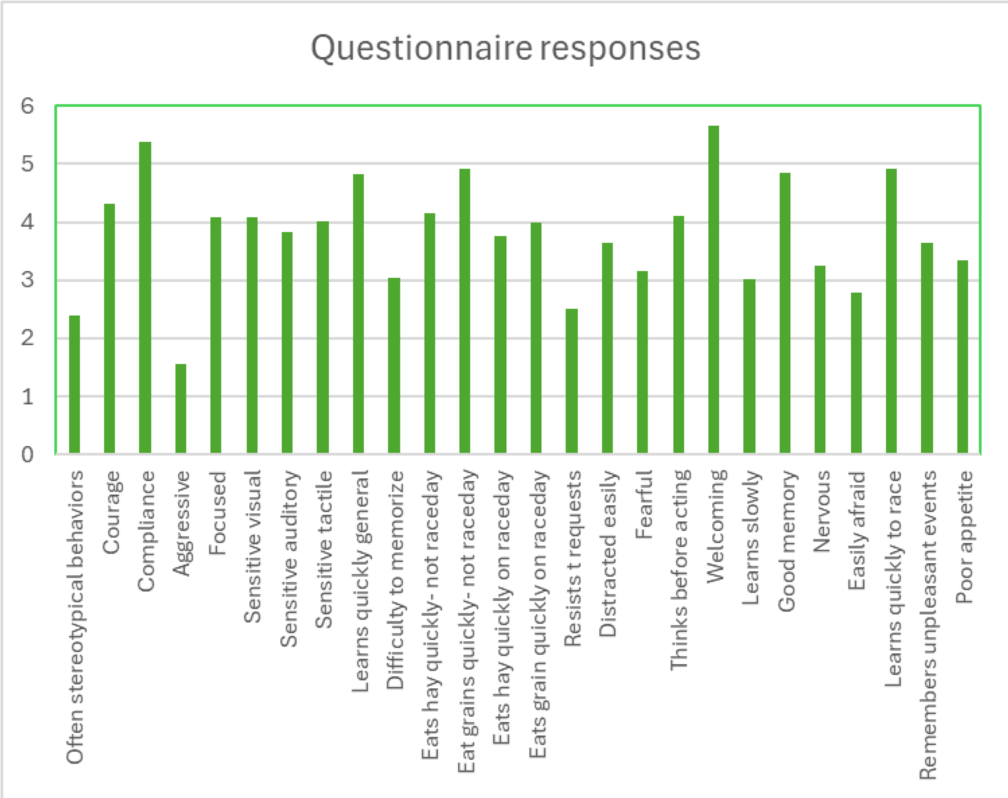

Figure 4. The average scores of the responses in questionnaires behavioral part. Number 1 on the scoring scale corresponds to "strongly disagree" and number 7 corresponds to "strongly agree".

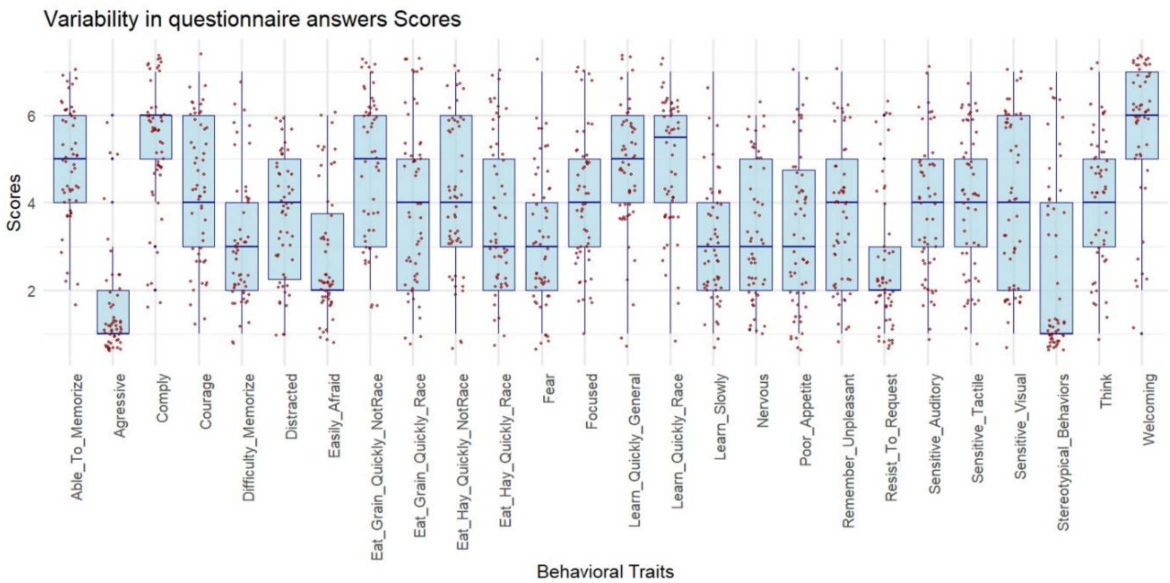

Figure 5. A boxplot illustrates the variability of the questionnaire scores. Figure by Mahmoud Amiri Roudbar.

4.2 Behavioral tests

The behavioral tests RH, NO and NS were performed on 44, 37 and 26 horses respectively. Figure 6 shows average scores for nervousness (NO), fearfulness (NS) and reactivity to human (RH). Figure 7 shows variability of the scores from behavioral tests. From these three tests, and temperament traits interpreted from those, highest average scores were given for fearfulness (4.2 corresponding moderately fearful on the scoring scale). Fearfulness scores had also the highest variability between the horses. Average score for nervousness was 3.2 (quite calm on the scoring scale) and for reactivity to human was average score 2.9, which corresponds "quite friendly" on the scoring scale.

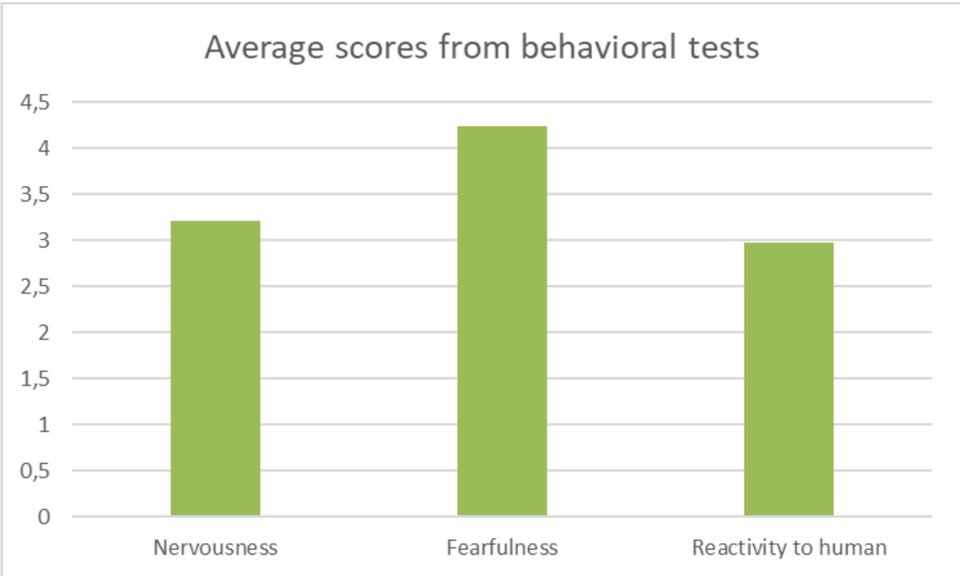

Figure 6. The averages of the scores from behavioral tests. The average score for nervousness was 3.2 which corresponds "quite calm" on the scoring scale. For fearfulness was average score 4.2 corresponding moderately fearful. Average score for reactivity to human was 2.9 corresponding "quite friendly".

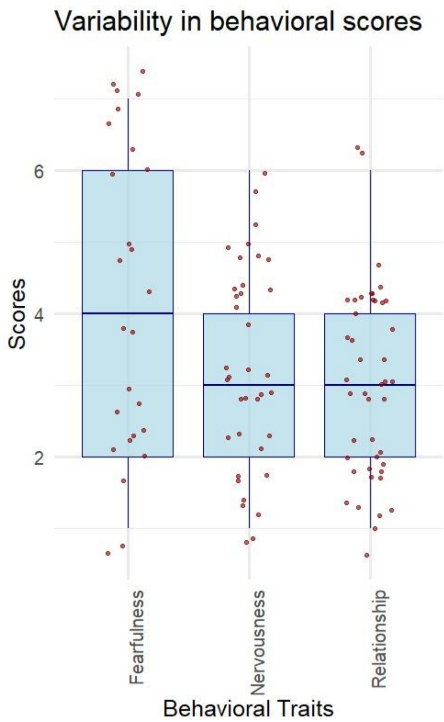

Figure 7. A boxplot shows variability of the scores from behavioral tests. Figure by Mahmoud Amiri Roudbar.

4.3 Hair whorls

Hair whorl data was collected from 56 horses during this project. The distribution of hair whorl types in the study population is presented in Table 9. The most common whorl in the study population was one radial whorl positioned high and medial left (12 horses, 22%). The bare majority of the horses, 30 (54%), had only one facial whorl (including feathers), 24 horses (43%) had two whorls and only one horse had three and one horse four whorls.

Of the horses with one whorl (incl. feather), in 28 horses (93%) was longitudinal position of the whorl high. In the remaining 7% was longitudinal position medium, as low positioned whorls were not found in this group. Of the horses with one whorl, 16 horses (53%) had lateral position medial left, seven (23%) medial right and seven (23%) midline position.

The most common direction of the whorl was radial (19 horses, 63%). Eight horses (28%) had a CW whorl and two horses a CC whorl. A total of 24 horses (43%) had at least one feather shaped whorl, and of the feathers, 18 horses (75%) had vertical and six (25%) diagonal direction.

The whorls (n=4) that had features of both CW, CC and radial whorl types were classified as radial/bidirectional in this study.

Table 9. The distribution of hair whorl types in the study population.

Hair whorl type	Number of horses
One whorl (excl feather)	51 (92%)
Two whorls (excl. feather)	4 (7%)
One whorl (incl. feather)	30 (54%)
Two whorls (incl. feather)	24 (43%)
Three whorls (incl. feather)	1 (2%)
Four whorls (incl. feather)	1 (2%)
Longitudinal position high (of the horses with one whorl, incl. feather)	28 (93%)
Longitudinal position medium (of the horses with one whorl, incl. feather)	2 (7%)
Lateral position midline (of the horses with one whorl, incl. feather)	7 (23%)
Lateral position medial left (of the horses with one whorl, incl. feather)	16 (53%)
Lateral position medial right (of the horses with one whorl, incl. feather)	7 (23%)
Radial (of the horses with one whorl, incl. feather)	19 (63%)
Clockwise (of the horses with one whorl, incl. feather)	8 (27%)
Counterclockwise (of the horses with one whorl, incl. feather)	2 (7%)
Feather	24 (43%)
No feather	32 (57%)
Vertical feather (of the horses with feather)	18 (75%)
Diagonal feather (of the horses with feather)	6 (25%)
Midline feather (of the horses with feather)	12 (50%)
Medial left feather (of the horses with feather)	9 (38%)
Medial right feather	3 (13%)

4.4 Associations between the questionnaire, behavioral tests and hair whorls

4.4.1 Correlations within and between the data sets

Correlation tests performed with Spearman's rank correlation coefficient revealed several statistically significant correlations within both the questionnaire responses (Fig. 9) and the behavioral test results (Fig. 10). Heatmaps (Fig. 9 and Fig. 10) were generated to visually represent the correlation matrix. Statistically significant correlations within the questionnaire data (Fig. 9) and within the data from the behavioral tests (Fig. 10) are highlighted by displaying their correlation values. However, when looked for relationships between the questionnaire data and the behavioral data, no significant correlations remained after adjusting for multiple testing using the false discovery rate (FDR) method (Fig. 11). Figure 11 is a heatmap illustrating the correlation between behavioral data and questionnaire score raw values. This suggests that, based on the current sample size, we cannot confidently detect links between questionnaire results and behavioral test outcomes.

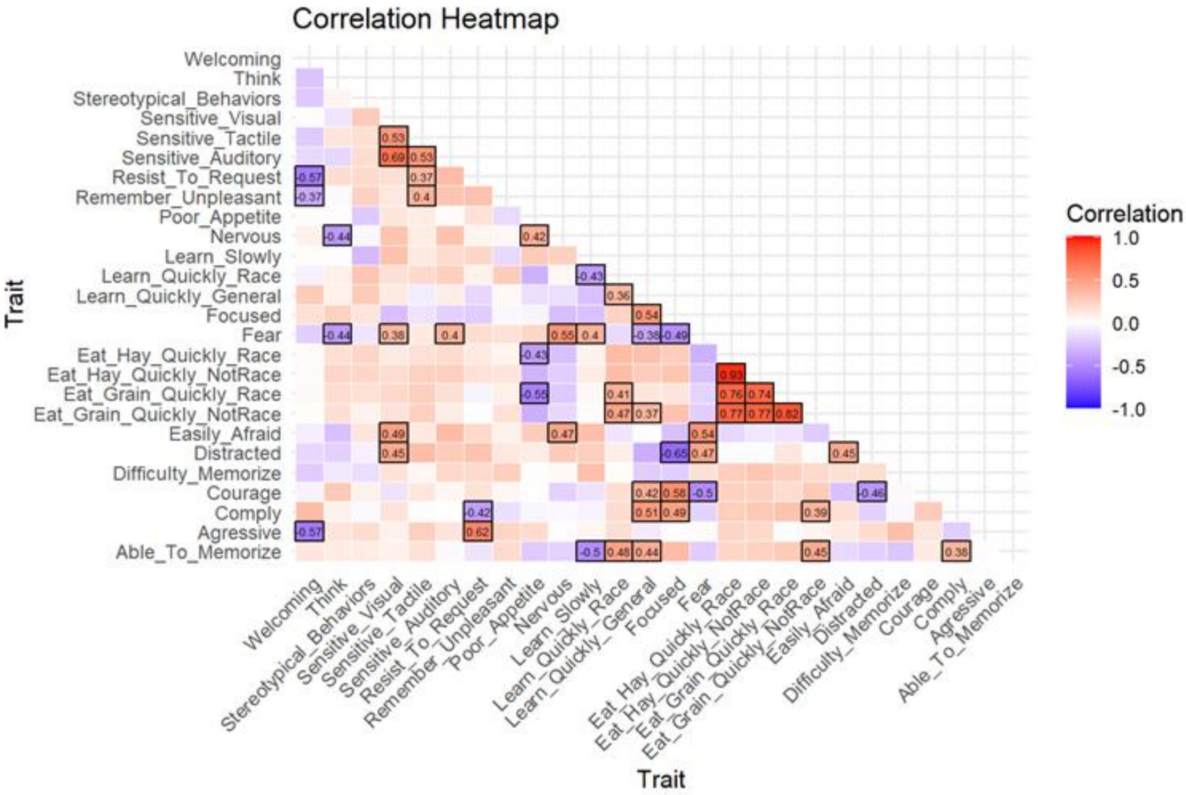

Figure 8. This heatmap represents statistically significant correlations within the questionnaire data. Significant correlation values are highlighted in the map. Figure by Mahmoud Amiri Roudbar.

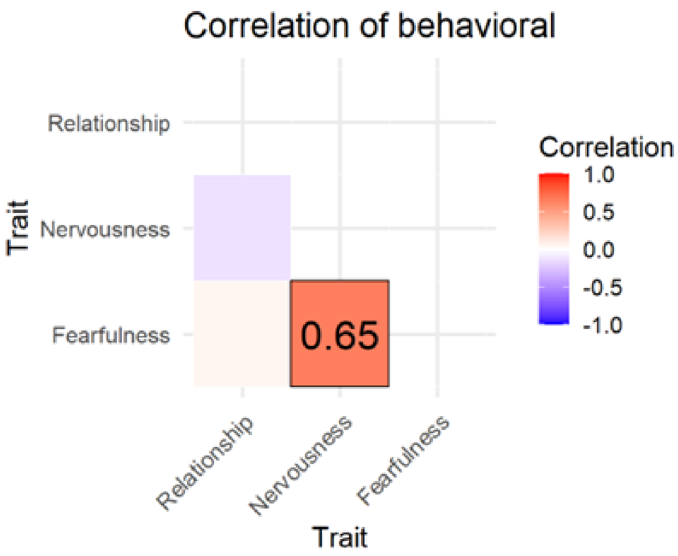

Figure 9. A heatmap illustrating the correlations within behavioral test values. Figure by Mahmoud Amiri Roudbar.

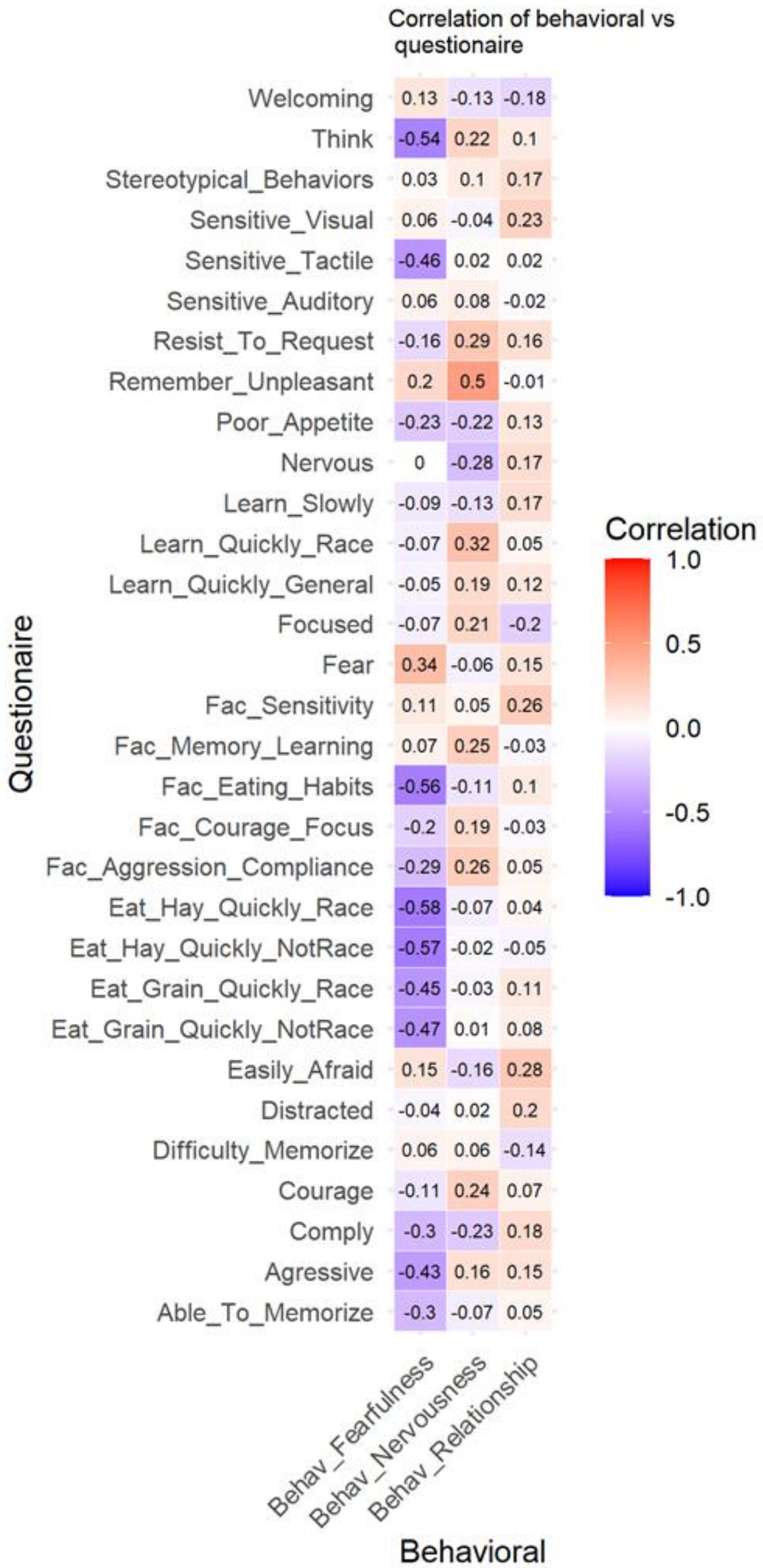

Figure 10. Visual representation of the correlations between the raw values of questionnaire scores and the scores from behavioral tests. Figure by Mahmoud Amiri Roudbar.

4.4.2 Associations between temperament traits and hair whorls

The association analysis (linear regression models and ANOVA) revealed several statistically significant links between behavioral traits assessed both in the questionnaire the behavioral tests, and facial hair patterns ($p < 0.05$). In other words, certain types of hair whorls were connected to specific behaviors in the horses. These findings are summarized in Tables 11 and 12, which show the strength of each association (regression coefficients) and how likely they are to be real (p-values).

Table 10. Significant associations between behavioral trait scores from the objective tests (reactivity to human, fearfulness, nervousness) and hair whorl patterns.

Behavioral trait	Hair pattern	Beta ± SE (P-value)	P-value (ANOVA)
Fearfulness	Feather	yes: -2.38 ± 0.71 (0.003)	0.003

Table 11. Significant associations between behavioral traits measured in the questionnaire and different hair whorl patterns.

Behavioral trait	Hair pattern	Beta ± SE (P-value)	P-value (ANOVA)
Eats grain quickly on race day	Feather	yes: 1.56± 0.46 (0.001)	0.001
Fearfulness	Feather	yes: -2.38± 0.71 (0.003)	0.003
Learns quickly	Whorl 1 longitudinal position	Medium: 1.19 ± 0.40 (0.004)	0.004
Eats grain quickly on race day	Whorl 1 lateral position	Medial right: -0.7 ± 0.56 (0.22); Midline: 1.17± 0.58 (0.048)	0.018
Compliance	Feather	yes: 0.87 ± 0.37 (0.024)	0.024
Eats grain quickly, not race day	Feather	yes: 0.89 ± 0.38 (0.025)	0.025
Eating habits	Feather	yes: 0.56 ± 0.25 (0.03)	0.029
Thinks before reacting	Feather longitudinal position	High/Low: 2.76 ± 1.55 (0.098); High/Medium: 1.76 ± 1.12 (0.14); Medium: 2.41 ± 1.05 (0.038); Medium/Low: -0.47 ± 1.50 (0.76)	0.043
Sensitive to tactile stimuli	Whorl 1 direction	Counterclockwise: -0.19 ± 0.58 (0.74); Feather: -3.05 ± 1.22 (0.02); Radial: 0.42 ± 0.39 (0.29)	0.026

We found several interesting connections between the horses' behavior and their facial hair whorl patterns. Horses with a feather-shaped whorl were less fearful during the behavioral tests compared to those without a feather. This difference was statistically significant, with a p-value of 0.003. The beta value for this association was -2, meaning that horses with a feather-shaped whorl scored, on average, two points lower on the fearfulness scale. Similar results were also observed in the questionnaire data, which showed the same significant correlation (p=0.003). For clarity, feather shaped whorls could be linked to fearfulness trait, as measured by both the behavioral tests and the questionnaire.

In terms of learning ability, we found that among horses with only one facial whorl, their ability to learn quickly was linked to the position of the whorl. Horses with a whorl positioned in the middle scored, on average, one point higher in learning ability compared to those with a high-positioned whorl (p=0.004). Notably, there were no horses in this group with low-positioned whorls.

Regarding compliance, horses with a feather-shaped whorl were significantly (p=0.024) more willing to follow human instructions than those without a feather.

When examining eating habits, we found that this factor, which included questions about how quickly horses rushed to their hay or grains, was also related to the presence of a feather-shaped whorl. Horses with a feather-shaped whorl were more likely to rush to food, and they also tended to eat their grains more quickly. Specifically, horses with a feather-shaped whorl had a beta value of 0.9 for general feeding habits and 1.6 for race days (p=0.025 and p=0.001, respectively). This indicates that horses with feather-shaped whorls were significantly more likely to eat their grains quickly both in general and during races.

Furthermore, among horses with only one whorl, the lateral position of the whorl also had an impact on eating habits. Horses with a whorl in the midline position had the highest scores for the question "eats grains quickly on race day" while those with whorls located on the medial right side had the lowest scores for the same question (p=0.018).

5. Discussion

5.1 Study population

The study population, a total of 56 Standardbred trotters, was relatively small. However, there are many studies regarding this topic with a smaller sample size that have managed to find significant correlations between temperament traits and hair whorls (Randle *et al*. 2003; Onouchi *et al*. 2024). Furthermore, in my opinion, the sample represented quite well the population of Swedish Standardbred trotters, including horses with different sex and age. Horses were aged between two and ten years and the temperament traits studied in behavioral tests (fearfulness, nervousness and reactivity to human) are stable in horses from the age of eight months onwards (Lansade & Bouissou 2008). Horses were also from several different stables, with varying stable sizes.

5.2 Reflection of the results

5.2.1 Temperament of Standardbred horses

According to Svensk Travsport, a part of the breeding goal for Standardbred horses is to produce good-tempered horses that are easy to handle and have strong will to win (Svensk Travsport n.d. a). In this questionnaire, will to win was not studied, but the results regarding good temperament and manageability show that the breeding goal has been achieved quite well. This is indicated by low questionnaire scores in aggressiveness (mean 1.6), and relatively high scores in compliance (mean 5.4) and welcoming/friendly temperament (mean 5.7). The results of the behavioral tests were also similar in terms of aggression, as average score for reactivity to human was 2.9, which corresponds "quite friendly" on the scoring scale. The results are in line with other studies (Saxe 2022) and indicate that these temperament traits are most likely to some extent heritable, as several studies have shown (Samlaska *et al*. 1989; Berglund 2021).

5.2.2 Hair whorl distribution

In this study, the majority of horses had a high-positioned whorl, with 93% of those possessing a single whorl displaying this trait. A similar pattern was observed in Saxe's (2022) study on Standardbreds, where 68% of the horses had a high-positioned whorl. Yokomori *et al.* (2019) estimated the heritability of hair whorl position to be high in Thoroughbred horses ($h^2 = 0.643$), with comparable results reported in Konik horses (Górecka *et al.* 2006). To date, there are relatively few studies on hair whorl distribution in Standardbreds. However, the findings herein together with a previous study (Saxe 2022) support the assumption of high heritability in this breed as well.

5.2.3 Questionnaire vs. behavioral tests

The main objective of this thesis was to determine whether a connection exists between behavioral test results and questionnaire responses on horse behavior. When comparing the scores from the behavioral tests and the questionnaire, could be concluded that the results are quite consistent. For example, fearful temperament received a score of 4.2 in the behavioral tests, while in the questionnaire was average score 3.2 for the same trait. Nervousness was scored on average 3.2 in the behavioral tests and averaged 3.3 in the questionnaire. Reactivity to human got an average score of 2.9 in the behavioral tests, which corresponds to "quite friendly" on the scoring scale. The same trait, as assessed in the questionnaire, resulted in a low average score for aggressiveness (1.6) and a high average for friendly temperament (5.7). The scoring scales for behavioral tests were designed to correspond the scores from the questionnaire, which is not a simple task—but it appears that this has been accomplished quite successfully. However, correlation analyses between these two datasets revealed that, after adjusting for FDR, no significant correlations were found given the sample size in this study.

Previous studies by Momozawa *et al*. (2003, 2005a) have demonstrated that certain temperament traits, such as anxiety, affability, and trainability, can be reliably assessed through questionnaires. Therefore, future research would benefit from collecting a larger dataset, potentially using an improved questionnaire and refined test protocols to enhance the accuracy and reliability of the findings.

5.2.4 Associations between hair whorls and behavioral traits

The secondary aim of the study was to explore potential correlations between different facial hair whorls and behavioral traits or temperament. Notably, several significant associations between the behavioral traits and hair whorls were identified. Due to a relatively small sample size, some of the hair whorl groups only contained a few horses, which could affect the reliability of the results. However, specifically the feather-shaped whorls could be associated with several behavioral traits measured both in behavioral tests and with the questionnaire. Miller (2021) describes that horses with a feather are often friendly, calm and confident and affectionate towards people. The results of this study support these assumptions, as the feather shaped whorls could be associated with higher compliance and lower fearfulness compared to the horses without feather.

In a study by Saxe (2022) the results indicated that horses with feather had a better appetite during competitions if the feather was vertical compared to diagonal or horizontal. In this study, eating habits were also connected with feather shaped whorls, as the horses with feather were more likely to eat their feed quickly compared to the horses without feather.

Findings from studies on both horses and cattle (Lanier *et al*. 2001; Górecka *et al.* 2007) suggest that animals with a high-positioned whorl tend to be more vigilant and difficult to handle compared to those with a low or medium-positioned whorl. However, no such connection was found in this study. Also, both this study and in the study by Saxe (2022) a high-positioned whorl was the most common in Standardbreds.

Miller (2021) described horses with a high-positioned whorl as extroverted, curious, energetic, and active—traits that, in principle, align well with the temperament desirable in a racehorse. However, in this study, a high-positioned whorl was only significantly associated with slower learning abilities compared to horses with a medium-positioned whorl.

5.3 Limitations of the study

5.3.1 Questionnaire

We were able to collect questionnaire answers for a total of 54 horses, compared to 12 answers which was the starting position for the study (answers collected in previous study by Delbos 2024). However, there was little confusion among the trainers due to the fact that at least three of them had completed the questionnaire already under spring 2024 alongside the behavioral tests. For some reason the answers were not saved by Netigate. Partly for this reason, we could not obtain questionnaire data for all 56 horses, as two of the participating horses had already changed trainer by the time of this study.

The author was present with some of the trainers filling out questionnaires and noticed that some of the questions were not as clear as they could be. Discussions with respondents about how they interpret the statements could be valuable in ensuring that the statements are capable of accurately capturing the temperamental traits they are intended to assess. For example, the term stereotypical behavior might have required an explanation along with the question. Also, the answer options to the question about housing conditions (“free-range/outside”, “stabled in a box” or “stabled at night, outside by day”) were a bit confusing, even misleading, because hardly any horse in Sweden lives only in stable without access to outdoor activities. Also, it was noticed that some of the trainers found it difficult to answer the questions assessing horses’ memory. These observations are consistent with Delbos (2024), where they noticed poor intra-evaluator consistency for memory traits. There is clearly room for improvement in the formulation of the questions. However, since the aim in this study was to complete the project initiated by Delbos (2024) and to use the same online questionnaire, we chose not to modify the statements. Additionally, the time available for this thesis was limited.

5.3.2 Hair whorl classifications

The majority of the hair whorl classifications were done using a combination of images and live observation at the stables. For horses that we were not able to see during our visits to the stables, the assessment was made solely via photographs. The quality of the images is very important for a reliable classification, and it is worth paying attention to this. However, obtaining accurate images of the whorls is not always that simple. Also, we noticed that weather conditions (rain), grooming and clipping the horse and for example changes from winter coat to summer coat can pose challenges for classification, especially when determining the direction of the whorls.

5.4 Future perspective

5.4.1 Questionnaire respondents

Promising results have been obtained from using questionnaires to assess a horse's temperament (Momozawa *et al*. 2003, 2005a). However, one important aspect to take into account is that the answers are always a respondent's subjective view of the horse, and this view may vary between respondents. The reliability of the questionnaire could be affected by the fact that the trainers tend to have busy schedules, and they might not have time to think about the answers so carefully. In order to get the most reliable answers possible, it is important to consider who is the best person to answer the questions for each horse. Often, especially in large units, it is the grooms that take care of all the daily routines of the horses, and travel with them to the competitions. One trainer has often several dozen horses in training, whereas one groom usually takes care of about eight horses daily. Therefore, the grooms often know the horse best of all and could often answer questions about the horse's character most reliably.

5.4.2 The suitability of the behavioral tests

A total of 44 horses participated in the behavioral tests, although the NO and NS tests could not be performed on all of the horses due to weather conditions. As a result, the data obtained from the behavioral tests was unfortunately incomplete.

The RH test has been found to be a reliable method for assessing the temperament trait “horse's reactivity to humans” (Lansade & Bouissou 2008). However, it must be acknowledged that even when the test is conducted according to a standardized protocol, the experimenter’s demeanor and energy can influence the outcome. For instance, if the experimenter is not experienced with horses and appears uncertain around them, the horse may sense this and respond by being more avoidant.

In this study, significant correlations between the behavioral tests and questionnaire responses could not be found. Delbos (2024) in turn, with the limited dataset (12 questionnaire answers) came to the conclusion that for fearfulness, a moderate positive correlation between the questionnaire answers and behavioral test scores was found. However, nervousness had a negative correlation in this regard, which raises questions about whether the NO test used in this study is suitable for measuring nervousness in racehorses. Alternatively, the statements regarding nervousness in the questionnaire might not be functioning as intended. It is clear that the statements still have considerable room for improvement, and work on developing a validated questionnaire should be continued.

The NO test has been used to study both nervousness and fearfulness (Le Scolan *et al.* 1997; Visser *et al*. 2001, 2002), and these traits are not always easy to distinguish from each other. Therefore, it could be useful to re-evaluate whether this test is suitable for the study. However, the results (Delbos 2024) were likely influenced by the small sample size, as statistical analyses conducted with a larger set of questionnaire responses did not reveal any significant correlations between the questionnaire answers and behavioral test scores.

5.4.3 Conclusions

This thesis did not find significant correlations between questionnaire responses and behavioral test results. However, it did reveal significant associations between different facial hair whorl patterns and certain behavioral traits. In particular feather-shaped whorls were linked to higher compliance, courage, and a good appetite.

The results were likely influenced by the relatively small number of horses, limitations in the formulation of the statements, and possibly the selection of tests. It is also possible that it was overly optimistic to expect such a complex test battery to yield reliable results when studying a subject as complex as equine temperament.

However, this study represents a small step toward validating the use of questionnaires in horse temperament research. In the future, hair whorls may play an increasingly important role in understanding equine temperament, helping equestrians foster more harmonious interactions with their horses.

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Popular science summary

Sweden is one of the leading trotting countries in the world. There are about 16,000 horses in training, and approximately 875 competition events are held each year. The main breed used in trotting is the warm-blooded Standardbred, and currently around 90% of the races are aimed for Standardbreds. The breeding goal for the Standardbreds is to produce horses with great athletic qualities, but also good temperament, easy-going nature and strong will to win are desired. Good mental abilities help horses to compete better, adapt to different environments and are also important for the safety of both the horses themselves and humans taking care of them.

In recent decades, the field of researching temperament and behavior in animals has been growing. The findings of the said research can be used to improve the cooperation between animals and humans.

For measuring behavioral traits in horses, various tests have been developed. These methods include objective tests, in which a horse's behavior is observed in different situations with the aim to assess its different temperament traits on a sliding scale. Another method to find out horses' behavioral traits is through different surveys, where the owners, trainers and caretakers answer questions about the horse's behavior. These responses are, of course, persons' subjective views, but especially in certain traits- such as anxiety and trainability- have been found to give a fairly good picture if horse's temperament.

For a long time, there has been a belief among equestrians that hair whorls on horse's forehead can give indications of its temperament and behavior, and therefore also of its performance. Recent findings from scientific studies show that there might be scientific evidence for this belief. Namely, the development of the brain and skin during the fetal development are connected to each other, showing that whorls in the hair coat can reflect the development of the nervous system and thereby individuals' behavior.

Different whorl patterns in horses' forehead have been linked to different characteristics. The whorls are classified based on their location in the forehead, and the direction of the whorl (clockwise, counterclockwise or radial, which mean that the hairs point straight outwards from the center of the whorl). For example, if the whorl is located high on the forehead, above the eyes, the horse often has energetic and even ”explosive” temperament. In turn, if the whorl is located low, below the eyes, it often reflects calmness and stable temperament. The number of whorls seems to be correlated with the horse's learning ability, as horses with more than one whorl appear to learn the task of competing slower than horses with only one facial whorl.

In summary, it seems that hair whorls could be one of the ways to understand a horse's temperament. However, there will always be individuals to whom these generalizations do not apply.

The aim of this study was to find out whether the results from behavioral tests and the questionnaire responses were correlated, as well as to further investigate the connections between horse behavior and different types of facial hair whorls.

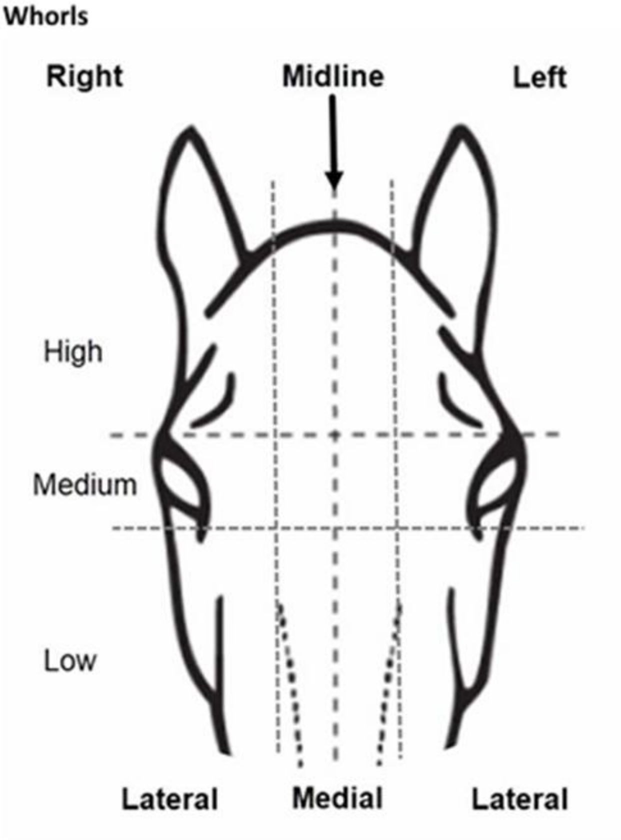
In this study, 56 warm-blooded trotters (Standardbreds) participated. Some of the research materials had already been collected in a previous study. Three different objective behavioral tests were performed on 44 horses in order to evaluate the horse's behavior, more specifically the traits fearfulness, nervousness and reactivity to humans. Then, the trainers were asked to fill out a questionnaire with questions regarding the following traits: nervousness, fearfulness, reactivity to humans, cooperation, concentration, learning, memory and appetite. In these tests, the horse's behavioral traits were rated on a scale of 1-7. In addition, the whorls on the horses' foreheads were photographed either by us or by the owners/trainers. The whorls were evaluated using a specific protocol, which noted the location, number and direction of the swirls. Then, a statistical analysis was performed, with the goal of determining whether there were significant connections between the whorls and the different tests.

In this study, we could not find significant connections between the questionnaire responses and the behavioral test results. However, it did reveal some interesting correlations between the horses' hair whorl types and certain behaviors. Horses with feather-shaped whorls tended to be more compliant, braver, as well as having a better appetite. Additionally, horses with a whorl located between the eyes were found to learn faster compared to horses with a high (above eyes) whorl. However, more studies with a larger number of participants are needed in order to get more reliable results, and research on this topic continues.

Appendix 1

Name: Date:

Whorls



Instruktioner

- Mark the center of the whorl with an "x".
- Plot the whorl around the x, the direction clearly visible
- Utgå från nosbenets laterala kant som skiljelinje mellan lat och med placering
- Numrera virvlarna (1-3)
- Virvelns nummer fylls i tabellen nedan
- Kryssa i riktningen för varje virvel
- Skriv ev kommentarer längst ner
- Fotografera virveln om frågetecken. Hästens namn på en vit tejp synlig på fotot.
- Lyft pannluggen vid fotografering för att göra pannan väl synling

	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:____

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