Reactions to objects and humans in Przewalski's horses; a first step to assess domestication effects on behaviour in horses

Reaktioner på föremål och människor hos Przewalskis vildhäst; ett första steg för att undersöka domesticeringseffekter på beteende hos häst

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Etologi och djurskyddsprogrammet

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Table of contents

Abstract
Despite the increasing interest in equine science little research has focused on possible differences in behaviour between domestic horses and their wild ancestors. Understanding if or how domestication has changed horse behaviour, along with better comprehension of behavioural differences in equine species, can improve housing, handling and training of equids as well as increase the safety of their human handlers. The wild progenitors of today’s domestic horses are now extinct, but the Przewalksi's horse could serve as an alternative when we want to compare the behaviour of the domestic horse with a wild equid. The aim of this study was to develop and evaluate tests that can be used on both domestic and wild equids, in order to examine fear reactions, social transmission of behaviour and reactions to novel objects and humans. For this purpose, we need tests that can be conducted on groups of individuals held in zoos or similar domestic settings. It is important to be able to test equids in groups as separating them from their social group for testing can lead to misleading results.

A group of 3 Przewalski's horses housed in a zoo environment were used in this study. Three tests were applied: 1) a novel object test, where the horses were exposed to a familiar and an unfamiliar object, 2) a voluntary approach test, where the horses were exposed to a familiar and an unfamiliar human, and 3) a target training test, where one individual, while remaining in the group, was trained with positive reinforcement to touch a target. Individual variation was evident, despite the horses being tested together. This suggests that group testing does not introduce a significant bias to individual differences in behaviour, and provides an alternative to isolate individuals in future tests. The horses showed different behaviours depending on if the object or human was familiar to them or not, and signs of habituation to the novel stimuli were noted. During the target training the horse was active, attentive and learned the behaviour instantly.

Tests that have been used to show domestication effects in dogs have also been used on horses, but I question their relevance since the dog has had a different role as a human companion. Instead I suggest similar tests as the ones in this study to be used on different equine species in order to extend the knowledge of variation and similarity in equine behaviour.

1. Introduction
The relationship between domestic horses and humans has been investigated repeatedly from different angles (e.g. Fureix *et al.*, 2009; Hausberger *et al.*, 2011; Lampe & Andre, 2012; Maros *et al.*, 2008 and Maros *et al.*, 2010). However, few conclusions have been drawn on the effects of domestication on the behaviour of horses, perhaps because it is unclear what to focus on when looking for these effects.

What makes a domestic horse, *Equus caballus,* more suited for keeping and training by humans? Is the domestic horse better at coping in a human environment compared to a wild horse? Those are intriguing questions that could be further assessed by learning more about possible domestication effects on the behaviour of horses.

Since the beginning of domestication horses have been selectively bred for a variety of physical traits, such as size, build, speed and strength (Ramey, 2011). What behavioural traits we have selected for are seemingly more difficult to determine, even though it is likely that traits such as low fearfulness have been desired. Change in behaviour might also have occurred without being intentionally selected for.

Wolff & Hausberger (1996) found that learning ability to some extent seem to have a genetic origin and Visser *et al.* (2003) mentioned learning ability as an important trait to consider when choosing horses for different horse - human activities. This opens up for a possible change in learning ability through domestication. Are the learning capacities of the domestic horse different from that of wild equids and how can we test this? Is the human - horse relationship a factor, and do domestic horses have an advantage when learning in a context together with humans?

There is also a lack of detailed behavioural research in equids other than the domestic horse, for example the Przewalski's horse (*Equus przewalskii*). Instead the domestic counterpart often serves as a model for this and other equine species. Since Przewalski's horses and other nondomestic equids such as zebras, are commonly held in zoos, more studies of their behaviour and their interactions with humans should be undertaken. These equids may differ in their behavioural needs compared to the domestic horse and therefore perhaps different aspects should be considered in terms of handling, group composition and housing. Moreover, to compare behaviour and cognitive skills of domestic horses with those of Przewalski's horse offers a possibility to assess effects of domestication.

1.1. Przewalski's horse

There is an ongoing debate whether the Przewalski's horse is a separate species or a subspecies of the wild horse, *Equus ferus* (Kavar & Dovč, 2008; Lau *et al.*, 2009). Therefore the literature is inconsistent in the use of scientific names for these equid groups. In this paper I will refer to the (now extinct) wild horse as *Equus ferus*, the domestic horse as *Equus caballus* and the Przewalski's horse as *Equus przewalskii.*

Wild horses *(Equus ferus and Equus przewalskii)* were once common on the Eurasian steppes but got extinct in the wild in the 1960s (van Dierendonck & de Vries, 1996). Today the Przewalski's horse (*Equus przewalskii)* is the only remaining population of wild horse (IUCN, 2015; Orlando *et al.*, 2013) and has never been domesticated (Wakefield *et al.*, 2002). The population has survived in captivity and individuals have recently also been reintroduced to the wild (IUCN, 2015). The Przewalski's horse is considered endangered on the IUCN Red List of Threatened Species (IUCN, 2015).

1.1.1. Genetics and relationship to domestic horses

The genetic relationship between the Przewalski's horse and the domestic horse is still under debate (Lau *et al.*, 2009). They are however undoubtedly closely related and can produce fertile offspring (Short *et al.*, 1974).

The Przewalski's horse has passed through a population bottleneck, and todays population of about 1900 individuals (by 2008 according to IUCN Red List 2015), originates from only 13 individuals, and among these founders one female was a domestic horse and one was a domestic/Przewalski hybrid (Oakenfull & Ryder, 1998). There are also suggestions of intermixing due to mating between Przewalski and domestic stock from Mongol horse herds (Olsen, 2006). In contrast, Orlando *et al.* (2013) found no signs of recent mixing of the two when comparing the genomes, they instead claim that the Przewalski and domestic lineages diverged around 38 – 78 000 years ago and have remained separated since then. Przewalski's horse is likely a sister taxon to the now extinct wild ancestor of the domestic horse (Kavar & Dovč 2008), and hence the domestic horse did not originate directly from the Przewalski's horse (Vilà 2001).

1.1.2. Social behaviour

Behavioural studies of the Przewalski's horses have been very limited and there are no records of their behaviour in the wild prior to their extinction (Houpt & Boyd, 1994). In the literature, the behaviour of *Equus caballus* has often been used to describe and understand Przewalski’s horse behaviour and cognition (Houpt & Boyd, 1994), but this may be partly premature, as differences in behaviour could have arisen due to domestication effects. These effects have been seen in other domestic species such as chicken (*Gallus gallus*) (Schütz *et al.*, 2001), dogs (*Canis familiaris*) (Hare *et al.*, 2002; Miklósi *et al.*, 2003) and pigs (*Sus domesticus*) (Gustafsson, *et al.*, 1999).

The social organisation of Przewalski’s horses are thought to be similar to that of feral populations of domestic horses and in the wild the Przewalski's horses form harems and bachelor groups much like feral horses do (Houpt & Boyd, 1994; Linklater, 2000). Christensen *et al.* (2002) studied group behaviour and social interactions in Przewalski's stallions reared in natural conditions and domestic stallions reared in typical domestic setting respectively. Similarities in play behaviour were evident while differences in social grooming and social investigative behaviour was noted (Christensen *et al.* (2002). Although these differences were likely due to environmental factors and the amount of time the stallions had spent together before the study. Przewalski's stallions engaged in more social behaviour in general and maintained a shorter distance to others compared to the domestic stallions, according to the same study. Another study that compared Przewalski's horses and domestic horses found species-specific characteristics of separation calls emitted by mothers and foals of the two different species (Alberghina *et al.*, 2014), while the duration of stallion calls seems to be similar in the domestic horse and the Przewalski's horses (Policht *et al.* 2008).

1.2. Cognition and learning in horses

In comparison to other species cognition in horses have only been investigated on a fairly basic level (Murphy & Arkins, 2007). Nothing however suggests that horses learn differently than other animals (Mills, 1998).

1.2.1. Cognition, perception and attention

Investigations of equine visual systems, perceptual ability and particularly attention studies may still have much more to yield (Murphy & Arkins, 2007).

The visual system of horses seems to be sensitive to dim light and movement (Saslow, 2002), and depth perception studies have shown that they do possess stereopsis (Hanggi, 2005). The visual field is large but has a limited area of focus as a horizontal band, which is aimed by movements of the head and neck (Saslow, 2002) and horses exceed both dogs and cats in visual acuity (Hanggi, 2005). Colour vision in horses is still not fully understood. Studies have shown horses to be able to discriminate red and blue and in some cases also yellow and green, but likely factors such as brightness have played a part in these tests of colour discrimination (Hanggi, 2005). Horses have well adapted anatomical features for olfaction, such as an extensive olfactory epithelium (and thus more receptors), the ability to move and trap large volumes of air in the nasal cavity and a prominent vomeronasal organ (Saslow, 2002). Equines are extremely sensitive to tactile stimuli and can react to pressures that are too light for a human to sense (Saslow, 2002). Horses are able to hear frequencies of up to 33,000 Hz, which exceeds the capacity of the human ear, and in addition the horse directs its attention and hearing by pointing its ears (Saslow, 2002).

1.2.2. The study of learning, fear and investigative behaviour in horses

For a wild animal a rapid flight reaction to something potentially dangerous can be life-saving, but it is also important to be able to habituate and learn not to react to things that do not pose a threat, as fear reactions cost energy. Habituation is a form of learning, which is commonly used when training horses. It is often termed stimulus-specific, which has a significant function for prey animals since small changes in the behaviour of a predator need to trigger a flight response (Griffin, 2001). Despite this some generalization has been seen for habituation in horses. Christensen *et al.* (2008b) found that horses do generalize if the unfamiliar objects are highly similar, for example identical in colour. Furthermore, it is possible to increase object generalization by simultaneously habituating horses to a range of different objects (Christensen *et al.*, 2011). According to Górecka *et al.* (2007) gentle and repeated handling by a human during the test situation has a positive effect on habituation.

By exposing animals to novel stimuli fear and investigative behaviour can be evaluated. Christensen *et al.* (2005) did this for visual, auditory and olfactory stimuli and all of these resulted in increased investigative behaviour in comparison to the control test. Lansade *et al.* (2008) used a novel object test together with three other tests to evaluate fearfulness in horses and concluded that fearfulness was a stable trait across both time and different settings. Antunes & Biala (2012) describe the novel object test as usable for study of memory, learning and the preference for novelty, among other things. As such it is also valuable when intending to measure cognition in animals (Antunes & Biala, 2012). Presenting something unfamiliar to an animal can provoke different responses, such as increased stress level and/or more approach behaviour (Antunes & Biala, 2012). If the stimulus is perceived as fearful immediate flight behaviour is the natural response and increased movement, such as walking, can also be an indication of anxiety when a novel stimuli is presented (McCall *et al.*, 2006).

Discrimination learning (learning that one stimulus and not another yields reinforcement) has been well documented in horses (Hanggi, 2005) and they seem to be especially sensitive to spatial cues in these tests (Nicol, 2002).

Most likely, learning ability differs between individuals and is also context-dependant for the same individual. The latter has been supported by studies by Wolff & Hausberger (1996) and Visser *et al.* (2003).

1.3. The horse – human relationship

There is substantial research concerning the relationship between domestic horses and humans, but none (that I could find) on that of Przewalski's horses and humans.

A study by Fureix *et al.* (2009) supports the theory that horses perceive humans and their relation with them based on their experience (repeated interactions). Horses also seem to generalize between familiar and unfamiliar humans and they may react differently depending on whether the person is passive or active (towards the horse) (Fureix *et al.*, 2009). Stone (2010) suggests that horses are capable of facial recognition of humans and Lampe & Andre (2012) propose that horses are able to distinguish familiar and unfamiliar humans, both only by the voice as well as only by sight and smell.

Voluntary approach tests (where the horse, in either a familiar or novel setting, is free to choose whether to approach a human test person or not) have often been used to test the reaction of horses to humans and could serve as some type of measure of the human-horse relationship. For instance, Søndergaard & Halekoh (2003) used this test on young horses and found that handled horses approached the human sooner than unhandled ones.

Also using a voluntary approach test Maros *et al.* (2010) found that horses with only one handler took less time to approach this person than horses with multiple handlers.

1.4. Operant conditioning and reinforcement training in horses

Target training in this study was done by positive reinforcement training, which is based on operant conditioning. In operant conditioning a certain stimuli is presented to the animal and when a specific behavioural response is offered voluntarily the animal is rewarded (or punished), which means that the reward (or punishment) is associated with the performed response rather than the stimuli (McGreevy, 2004; Pearce, 2008). Operant conditioning has evolved in wild animals through natural selection and is one of the means by which learning commonly occurs (Skinner, 1981).

Training by using operant conditioning gives the animal the ability of choice and hence it gains increased control of the event, which in turn can improve welfare (McGreevy, 2004).

Traditional horse training is largely based on the use of negative reinforcement; a wanted response by the horse is reinforced by the removal of pressure, often that from the reins or the riders legs (McGreevy, 2004; McLean, 2005). Riding and equestrian sport involves complex requirements in terms of the horse's behaviour and movements, which makes this signalling system between rider and horse prone to error and inconsistence (McLean, 2005).

When training captive animals in other contexts, such as wild animals in zoos, positive reinforcement (adding something to the situation to reinforce a behaviour) has in recent years been used in many species, and today the technique is recognized as a valuable tool in handling and management (Laule *et al.*, 2003). Training of zoo animals has many possible benefits, such as increasing movement throughout the enclosure and enabling the animals to actively participate in husbandry and medical procedures (Melfi, 2013).

2. Aim and questions

The aim of this study was to help develop a series of flexible tests that can be used to investigate domestication effects in equine behaviour and the equine - human relationship in a broader perspective, by comparison of several equine species/subspecies/groups.

The behaviour of the Przewalski's horse, being a sister taxon to the ancestor of domestic horses, can be assumed to resemble that of the horse prior to domestication. The best available option to study domestication effects on horses should therefore be to compare the behaviour of Przewalski's horses with that of domestic horses.
The tests in this study were constructed to also give information about learning processes, such as habituation, in horses.
The purpose of the first 2 tests was to study the horses' reactions to something familiar and something unfamiliar respectively, both in the form of an object and as a human. The last test was supposed to evaluate if target training with positive reinforcement could be used on Przewalski's horses.

These tests can be summed up in 3 questions:

- How do Przewalski's horses react to a familiar vs. an unfamiliar object presented by a human, and do the reactions to the unfamiliar object change over repeated tests?

- How do Przewalski's horses react to a familiar looking vs. an unfamiliar looking person, and do the reactions to the unfamiliar person change over repeated tests?

- How do a Przewalski horse respond to target training with positive reinforcement and can it be further used as enrichment and/or as a tool for zookeepers?

3. Materials and method

3.1. Horses and housing

The study was conducted at Nordens Ark Zoo Park, between 14th to the 28th of April. A group of three mares was used for the study (Table 1). The horses were individually recognised by appearance features and brands on the thigh.

Table 1. Przewalski's horses at Nordens Ark used in this study.

Name	Sex	Year of birth	Birthplace	Distinction
Nadine	mare	1993	Nordens Ark	Birthmark shoulder
Xusan	mare	2005	Helsingfors (Helsinki)	Smallest, brand 4550
Sahara	mare	2012	Nordens Ark	More contrast in coat colour, brand 5998

The horses were housed in an outdoor environment with access to dry shelters. The enclosure area was about 3600 m^2. They had free access to water and to hay in a feed rack, but no possibility to graze. Part of their enclosure had trees and there were also logs, branches and fixed brushes placed in the enclosure for rubbing and gnawing on. Around 10 a.m. the horses were fed alfa alfa pellets in stationary feed troughs and around 2 p.m. apples and carrots were spread out in the enclosure as enrichment.

Keepers did not handle the horses. When someone was working inside the enclosure they were supposed to have a quad bike in between them and the horses for protection and the horses were encouraged to keep distance to staff.
All handling that needed physical contact with the horses, such as trimming of hooves or care/examination of injuries, had to be done under anaesthesia.

3.2. Study design

The same person performed all tests, at approximately the same time in the morning between 08.00 and 10.00 before the park opened.

Tests 1 and 2 were each conducted over 4 days, test 1 the first week and test 2 the second. Between these tests the horses had three days without trials. Test 3 was performed on the day following the last day of test 2. All tests were filmed and behaviours were later analysed from the recordings. For test 1 and 2 behaviours were registered for all three individuals, while in test 3 only one horse was chosen for training.

3.2.1. Test 1 - familiar vs. unfamiliar object

The familiar object was a green plastic bucket (Figure 1), which was sometimes used to bring food to the horses and the unfamiliar object was a multi-coloured plastic toy tractor (Figure 2). The bucket was presented to the horses on the first day, and the tractor the following three days. The experimenter stood on a platform on the edge of the enclosure. Attached to a rope, the object was hoisted calmly into the enclosure and placed on the ground (Figure 3). After placing the object, the experimenter stood silent and still on the platform (visible to the horses), approximately 1 meter from the rail. The object rested on the ground for a minimum of 6 minutes. Behaviours were analysed for 6 minutes, starting when the experimenter was first visible in both the camera and to the horses and before the object was hoisted into the enclosure. Ethograms (appendix I and II) were constructed (partly based on descriptions from McDonnell (2003)) and the video recordings were analysed for these behaviours. Frequency was registered for some of the behaviours with continuous registration, while for others duration was measured. A chi-square test was used to evaluate if the difference in total number of registered behaviours between horses were significant. General notes on behaviour as perceived by the experimenter were also taken immediately after each trial.

Figure 1. The bucket (with rope) used in test 1.

Figure 2. The tractor (with rope) used in test 1.

Figure 3. Recorded view for test 1. The objects were hoisted from the wooden platform on the right and placed on the ground at the orange mark.

3.2.2. Test 2 - familiar vs. unfamiliar human

This test was carried out as a voluntary approach test. The test person was the same in every trial, but was dressed differently. "The familiar human" was dressed in a zookeeper jacket (Figure 4) and "the unfamiliar human" was dressed in a white mesh overall with hood (Figure 5). The tester was dressed according to this schedule: day 1: jacket, day 2-4: overall. In all trials the test person approached the enclosure in a place where she was clearly visible to the horses and stopped at a certain spot by the fence (Figure 6). The horses were previously used to humans standing in this place. The horses could come all the way up to the fence and thereby be in immediate contact with the tester (but still separated by a metal wire net fence). The person acted neutral, quiet and calm, without giving notice to the horses, and remained at the fence for a minimum of 6 minutes. Behaviours were analysed for 6 minutes onwards from when the person was first visible to the horses. The same ethograms (appendix I and II) as in test 1 were used for analysis. Individual variation in total number of behaviours was evaluated with a chi-square test. General notes on behaviour were also taken after each trial.

Figure 4. Test person as familiar human dressed in zookeeper jacket.

Figure 5. Test person as unfamiliar human dressed in white overall.

Figure 6. Recorded view for test 2. The test person walked through the green gate at the top left corner and took position behind the grey gate at the orange mark.

3.2.3. Test 3 - target training

This test was conducted with the trainer standing at the same spot as in test 2, with the fence separating her from the horses. All the horses were present and free to move around in the enclosure even though the training was only directed at one individual (Sahara). The training consisted of simple target training were the horse was supposed to touch a target with its muzzle to receive a food reward. The target was unfamiliar to the horse and consisted of a tennis ball at the end of a stick, which was held and moved to different locations along the fence by the trainer. The position of the target could be up, down, left or right (in relation to the horse) but within a vertical area of approximately 2x2 meter (Figure 7). The target was presented to the horse 11 times, without any delay in between presentations. The whole session was 2 minutes long. Sliced carrots were used as reward and these were thrown/dropped onto the ground of the enclosure. Before the test the horses were accustomed to the trainer being present in the location. First a clicker was used as a secondary reinforcer, but since the horses showed fearful reactions to the clicker sound, the word "good" (spoken by the trainer) was used instead. Before the start of the test a few successful repetitions (horse touching the target and receiving reward) had been done to see if the horse had any interest in interacting with the trainer.

A separate ethogram (appendix III) was constructed for the training session. The training was analysed from video recordings, and total number of target presentations as well as successful presentations (when the horse touched the target within 5 seconds of it being presented and received reward) was counted.

Figure 7. Setting for target training. The target was placed within the marked area.

4. Results

4. 1. Test 1 - familiar vs. unfamiliar object

There was a notable variation in the horses' overall behaviour across days for test 1 (Table 2).

Table 2. General notes on behaviour for test 1.

Day 1 (bucket)	Slight fear reaction at first but approach quickly. All 3 move together, Nadine initiates approach but looses interest after investigating (Xusan follows her), while Sahara continues to investigate.
Day 2 (tractor)	More distinct flight reaction compared to day 1 that includes cantering along the fence. Approach rather rapidly afterwards, with Sahara first but supported by Nadine right behind her. Nadine and Xusan quickly check the tractor and leave, while Sahara repeatedly investigates from different positions.
Day 3 (tractor)	Weaker flight reactions and show less interest than day 2. Sahara shows the most curiosity, but again needs support from the others (mainly Nadine) to investigate close.
Day 4 (tractor)	More tense in general. Showed some interest but never came close to the tractor.

4.1.1. Behavioural distribution

Both individual variations in behaviours as well as tendencies of coordinated behaviour and movement between individuals were seen (Figure 8a-c). For example Nadine and Xusan had the same number of registrations for flight (these also happened on the same occasion), while Sahara showed flight reactions in addition to these. Wood chewing was only registered for Nadine and Xusan. Most investigative behaviours were seen one day 1, with the bucket. Pawing was only registered once, for Sahara on day 1.

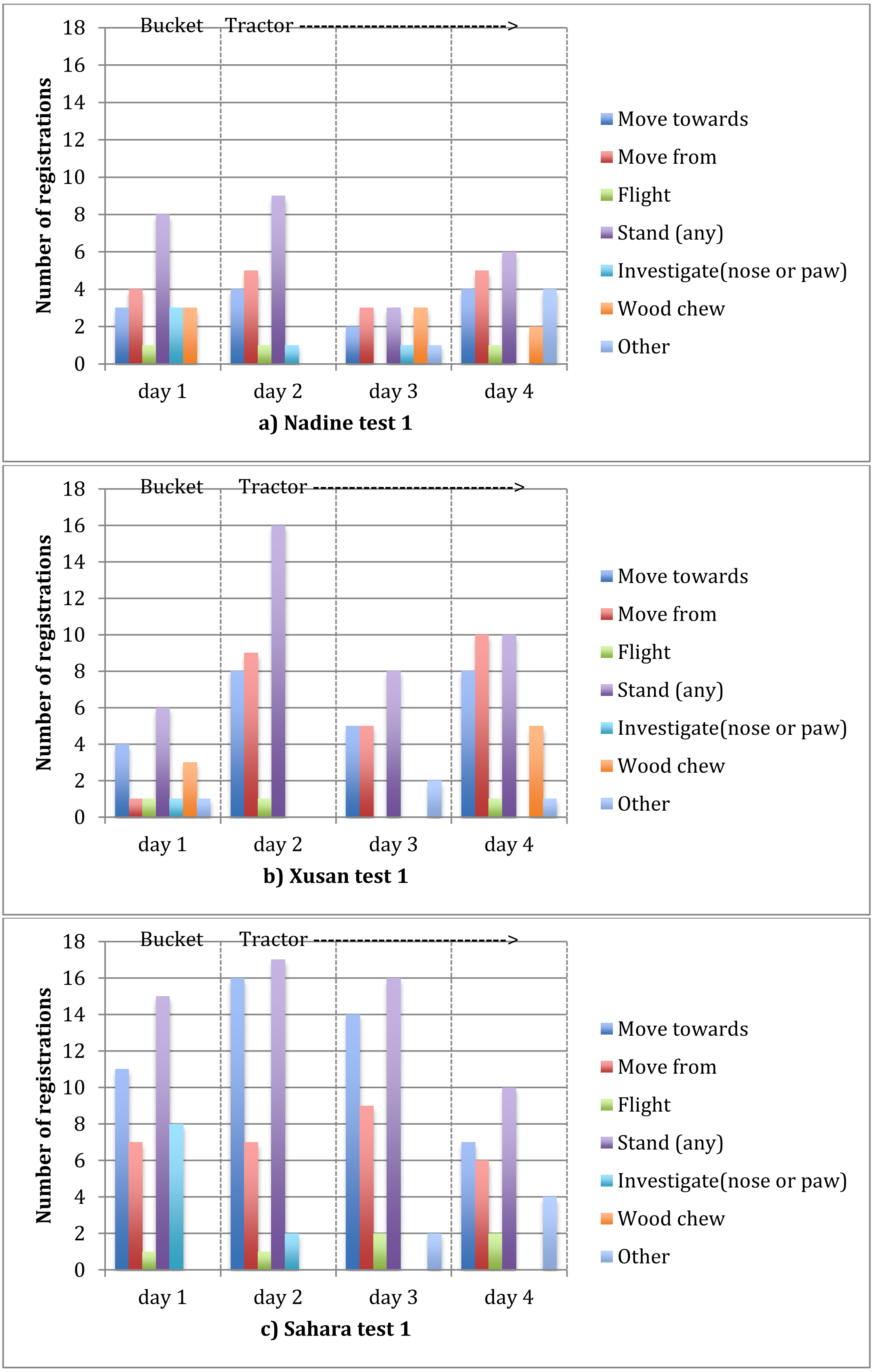

Figure 8a-c. Number of registrations for each behaviour per horse, for test 1. "Stand" includes both alert and relaxed, "investigate" includes both with nose and pawing, "other" also includes defecation.

4.1.2. Total number of behaviours - "activity"

General activity was outlined by comparing total number of registrations of all behaviours for each horse and day. The results suggested that the horses behaved to some extent independent from each other (Figure 9), and a chi-square test supported this conclusion (the chi-square statistic was 13.8 and the p value 0.03 and the result is statistically significant at $p < 0.05$).

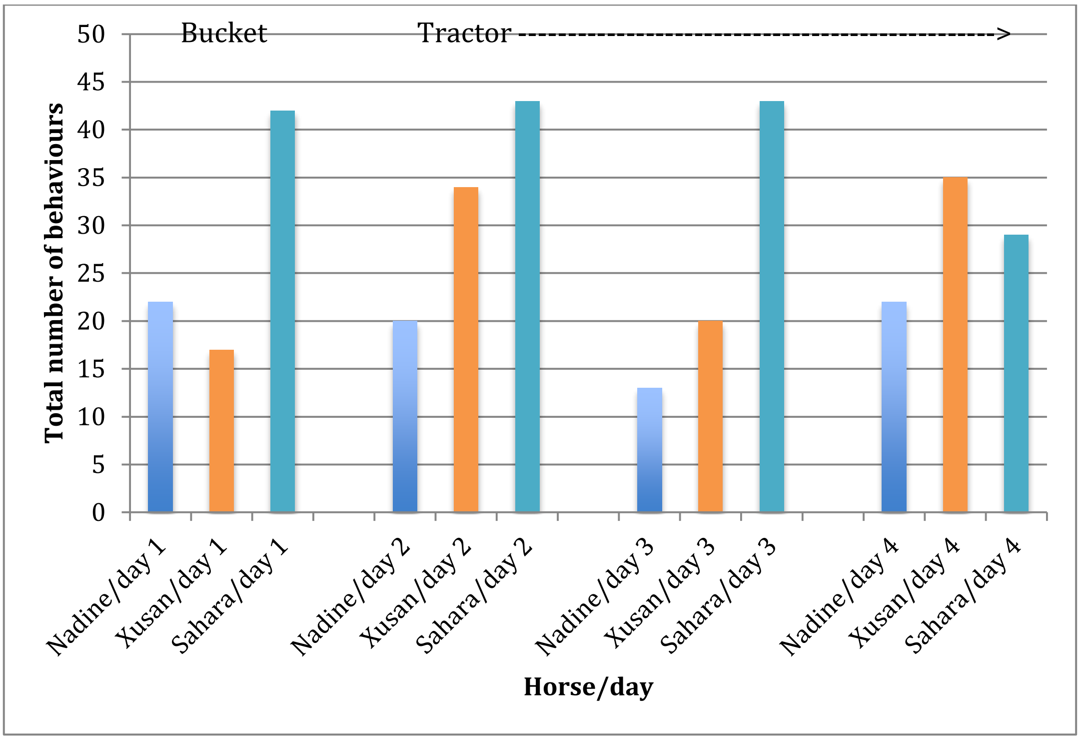

Figure 9. Total number of behaviours per horse and day for test 1.

4.1.3. Distance to object

The horses spent more time far away from the object, but there were also a tendency for individual variation along with variation in distance between days (Figure 10).

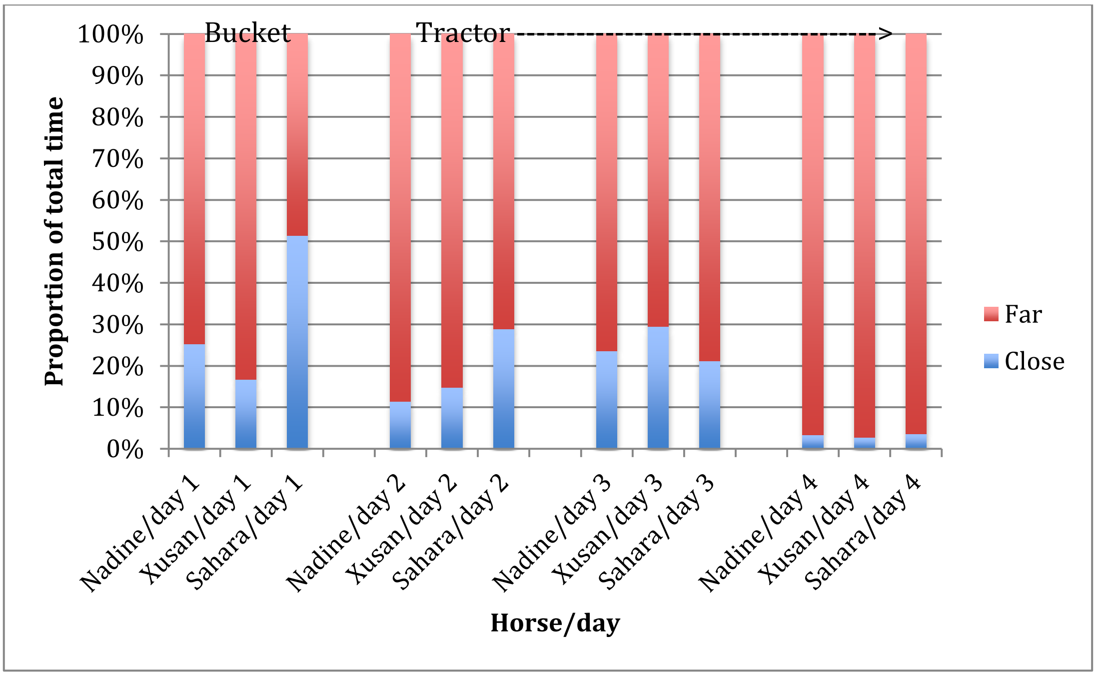

Figure 10. Proportion of time spent far and close respectively for each horse.

<u>4.1.4. Recover from flight</u>

Recover from flight (appendix II for definition) was only measured for all horses on day 2 of test 1 (the day when the tractor was first presented), because after the other flight reactions the horses remained out of sight. Recover from flight on day 2 was 0 seconds (Nadine), 17 seconds (Xusan) and 7 seconds (Sahara).

4.2. Test 2 - familiar vs. unfamiliar human

The horses' overall behaviour for test 2 varied across days (Table 3).

Table 3. General notes on behaviour for test 2.

Day 1 (jacket)	Notice the experimenter but shows no particular interest. Move towards, but not all the way to the person.
Day 2 (overall)	Shows much more fearful behaviour (including flight) compared to day 1. Still somewhat curious and move around the experimenter from different angles but never get close.
Day 3 (overall)	No fearful behaviours are noted. Comes up to the experimenter rather quickly with Xusan first and Sahara right behind. Sahara shows the most investigative behaviour towards the experimenter, for example pawing at the fence and sniffing at the person, but all 3 stay close to the experimenter for much of the recording.
Day 4 (overall)	No fearful reactions and shows very little interest in the experimenter. Are passive and moves only little.

4.2.1. Behavioural distribution

As in test 1, both individual and collective behaviours were observed when the horses were exposed to a familiar or an unfamiliar human (Figure 11a-c). More social interactions (in figure 11a-c included in category "other") were observed in test 2 than in test 1. Sahara was the only one for whom pawing was registered (5 times during day 3). Investigative behaviour towards the human was only seen on day 3. Wood chewing was registered much less than for test 1.

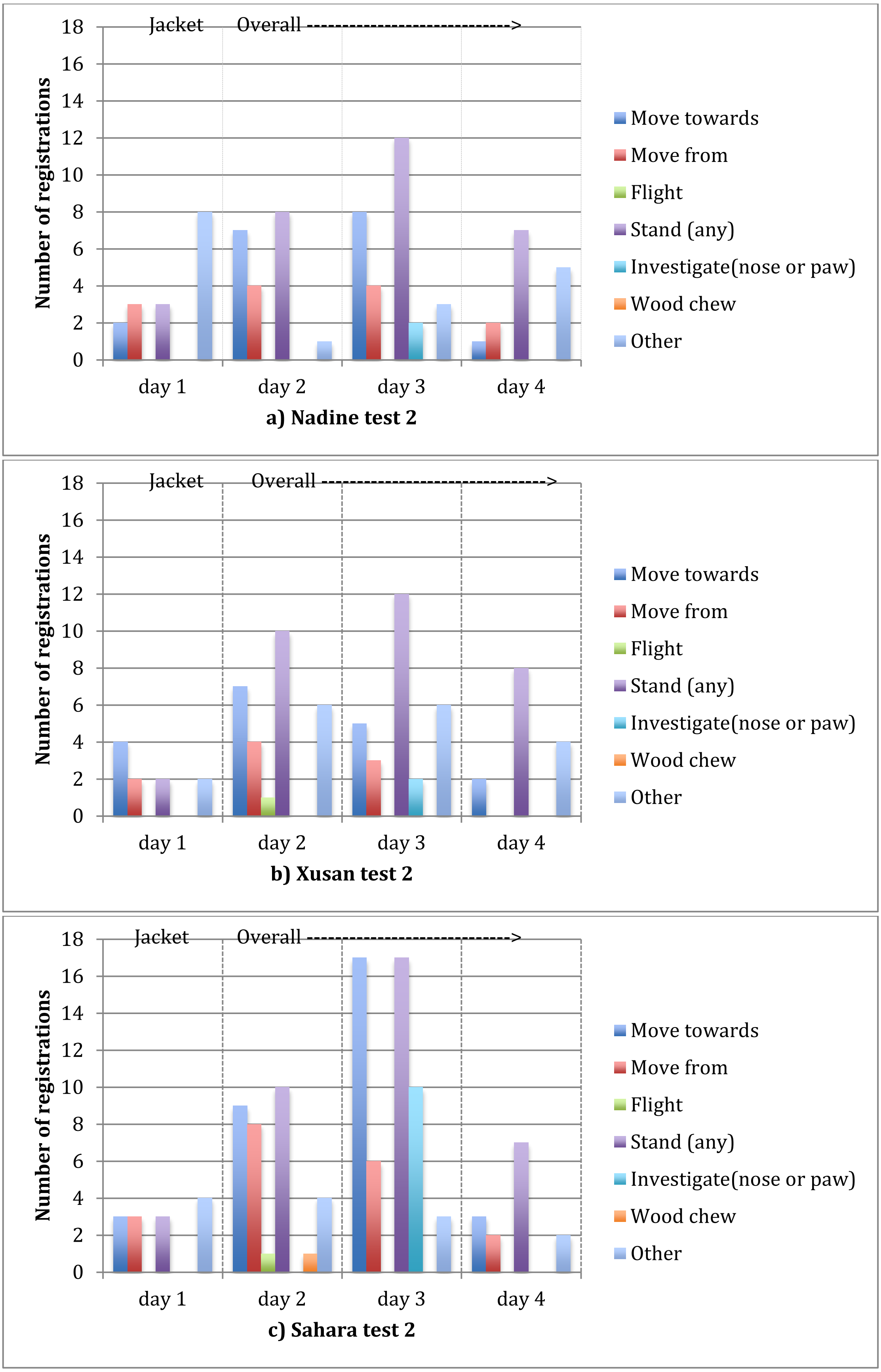

Figure 11a-c. Number of registrations for each behaviour per horse, for test 2. "Stand" includes both alert and relaxed, "investigate" includes both with nose and pawing, "other" also includes defecation.

4.2.2. Total number of behaviours - "activity"

There seemed to be an individual variation in the total number of behaviours, especially for day 3 (Figure 12), however this trend was not significant according to a chi-square test (chi-square statistic was 7.51 and p value 0.28, and the result is statistically significant at $p < 0.05$).

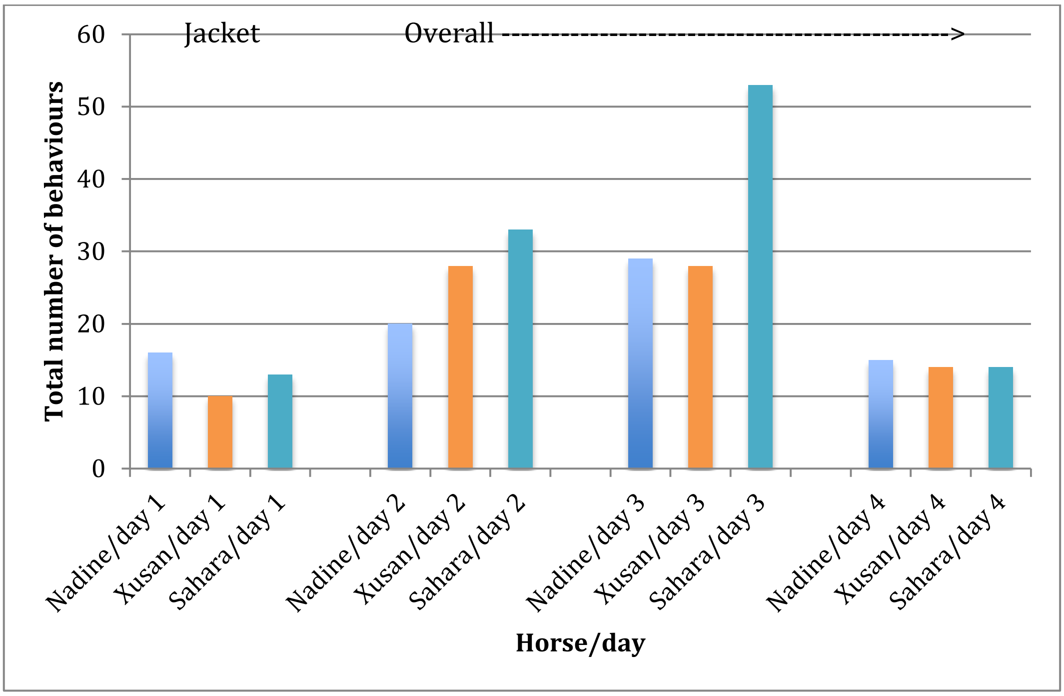

Figure 12. Total number of behaviours per horse and day for test 2.

4.2.3. Distance to human

The horses approached the test person only on day 3 (Figure 13) and this was also the only occasion when investigative behaviour was seen in test 2. This day all horses showed investigative behaviour, during 7 seconds (Nadine), 15 seconds (Xusan) and 41 seconds (Sahara).

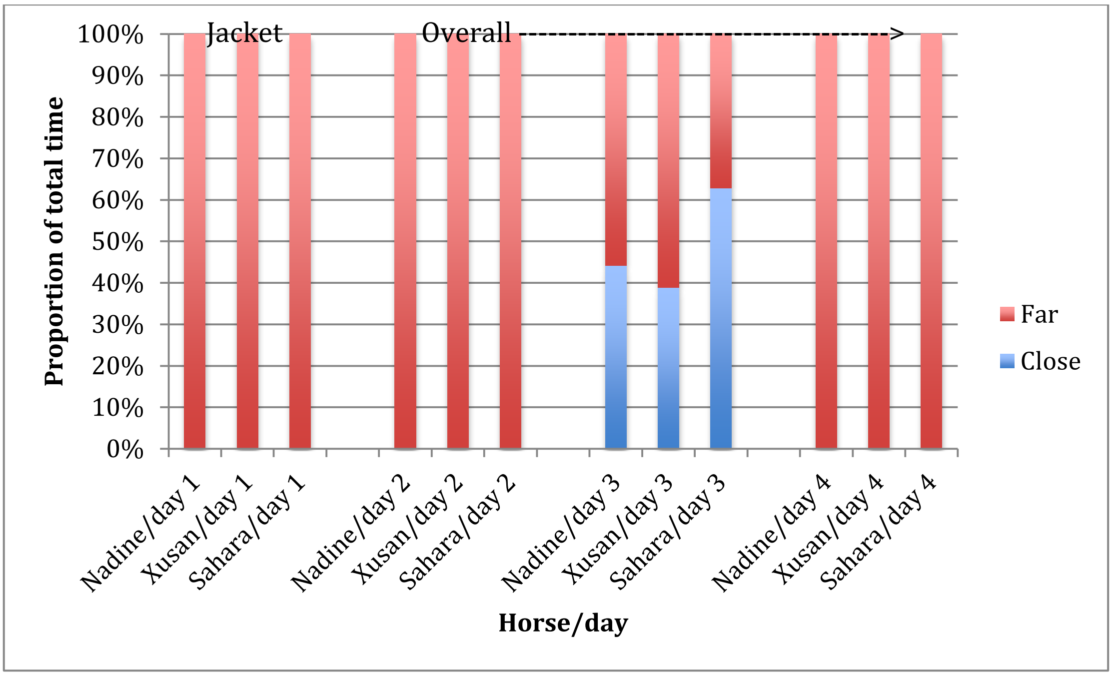

Figure 13. Proportion of time spent far and close respectively for each horse.

4.3. Test 3 - target training

The number of successful trials (defined as when the horse touched the target with its nose within 5 seconds of the target being presented and receiving the reward) was 11 out of 11. The average target time (time from presentation to touch) was 1.95 seconds. The horse had her attention (head/neck oriented towards and following the movements of target/trainer) throughout the whole session.

5. Discussion

5.1. Fear reactions

Christensen *et al.* (2008b) claim that the threshold for experiencing fear is elevated in domestic horses compared to their wild ancestors. However, they provide no reference for this statement and I haven't been able to find any scientific studies that actually compare fear reactions of wild and domestic equids. Price (1984) describes reduced reactions to changes in the environment as a typical change in behaviour due to domestication, and it seems like reduced fearfulness is commonly thought of as a product of domestication in animals in general. In horses it would be of interest to investigate how much previous habituation influences fearfulness, and to see if there is indeed a difference between domestic and wild horses. In the present study fear (flight) reactions were one of the least registered behaviours, especially in test 2. Leiner & Fendt (2011) did a novel object test on domestic horses, which included an umbrella and a tarp presented to the horses at separate occasions. Flight behaviour was only registered for 7% of the tested horses when first exposed to either of the novel stimuli. When the tractor was presented for the first time to the horses in my study (day 2, test 1) all horses showed a flight response, but this reaction did not prevent the horses from approaching the object soon after, and no more flight reactions were registered on that day. The 3 registered flight reactions equals only 3% of all behaviours registered during that day. Even though all 3 Przewalski's horses showed flight when the tractor was presented, I would not draw the conclusion that they are necessarily more fearful than the horses in the study by Leiner & Fendt (2011). A comparison between my study and theirs is difficult since the conditions differ. The flight reactions of the horses in my study were synchronized, which was possible since they were tested in a group, while Leiner & Fendt (2011) tested their horses alone. My study and theirs did however use horses brought up under similar conditions. Leiner & Fendt (2011) used horses that were mostly unhandled, but brought up close to humans in a domestic environment, which is comparable to that of the Przewalski's horses in the present study. This is crucial if one is to evaluate effects of domestication on behaviour. In the otherwise well performed study by Christensen *et al.* (2002) the Przewalski's horses lived under semi-wild conditions while the domestic horses were brought up in a domestic setting, which made the results difficult to interpret.

The Przewalski's horses in the current study mostly showed synchronized flight reactions, and this was possibly due to social transmission of behaviour within the group. In line with their study, Christensen *et al.* (2008a) argue that domestic horses, when in a social context, tend to act as their conspecifics do. This also makes sense in a wild setting since synchronized flight behaviour can decrease antipredation costs (Kendal *et al.*, 2005). However, a couple of times flight reactions were registered for Sahara alone, and not for the other two. It is also possible that the company of other, calm, horses represses flight behaviour of individuals when exposed to novel objects or humans. Christensen *et al.* (2008a) found that the presence of a calm (previously habituated) companion horse decreased fearful behaviour, as well as heart rate, in test horses. In a study on heifers that were exposed to a novel environment the animals in groups had fewer fear reactions compared to those tested alone (Veissier & Le Neindre, 1992). In the present study I noted that Nadine, the oldest, seemed to have a calming effect on the others. She commonly was the one that initiated movement after a synchronized flight reaction, and the younger two, especially Xusan, would often retreat to a position close behind her.

The study of fear in animals is challenging and includes many aspects. In an extensive article Forkman *et al.* (2007) elucidates many important issues, such as the lack of validated fear tests for use on horses, the probability that fear is not a unitary concept as well as the difficulty to distinguish fear from indifference in a novel object test.

5.2. Testing horses in groups

An important concern is that horses, despite being a group living animal, routinely are tested alone. If this is the best way to get a truthful, unbiased behavioural response should be discussed. According to Forkman *et al.* (2007) horses are generally tested alone when taking part in a novel object test. In my study the horses were tested in their normal social group. Even though it was evident that the reactions of the Przewalski's horses were depending on and also often synchronized with the others of the group, my data also showed that individual variation was present and can thus be measured.

Observational learning is something to take into account when conducting tests on several horses acting together. Clarke *et al.* (1996) found no effect on learning if the test horses, previous to the trials, had been allowed to watch a “demonstrator horse” perform the task. However, what they did record was a shorter latency period before the horse approached the area where the task was to be performed, for those horses that had observed a demonstrator. This suggests that watching other horses complete a task (and gain reinforcement) motivates observing horses to explore that certain area. This could influence the results when horses are tested in groups, for instance exploration by one individual might motivate the same behaviour in others. In the present study the horses often initially tended to explore the object/human together, but with one horse (often Nadine) slightly ahead of the others. Then Nadine often seemed to lose interest in the object or human quickly and left the area, while the other two (Sahara especially) continued exploring. This could be interpreted as a preference to explore together, for safety, right after the novelty was presented.

To be able to perform tests on wild equids in a zoo environment, or under wild or semi-wild conditions, it is often a prerequisite that the animals can be tested in a group, which is why I see the need for behavioural tests that are applicable under these conditions. Mechanisms of behavioural facilitation and how group dynamics otherwise can affect the results must be taken into account in these studies.

5.3. Wood chewing

Wood chewing behaviour was shown repeatedly by Nadine and Xusan in test 1, but not in test 2. Wood chewing in horses is often considered as an abnormal behaviour (Redbo *et al.*, 1998; Waters *et al.*, 2002). Development of this behaviour might be due to horses being kept in barns rather than on grass after weaning (Waters *et al.*, 2002), a general lack of forage or fiber in the diet (Willard *et al.*, 1977) or the number of times they receive concentrate feed (Nagy *et al.*, 2008). Why these Przewalski's horses showed wood chewing behaviour is unclear but it likely arose from a combination of factors. In the test situations it may have served as a displacement behaviour for dealing with an unfamiliar situation, since one function of abnormal behaviour could be to give the animal some immediate control over their environment in a stressful situation (Cooper & Albentosa, 2005). The bouts of wood chewing for Nadine and Xusan in test 1 always followed exploration and/or focus towards the presented object, which could imply that the behaviour was triggered by the novel object.

5.4. Approach behaviour and interest

In test 2, a certain pattern of the horses' behaviours was noted. On day 1 (jacket), the horses mostly ignored the test person and no flight reactions were noted. On day 2 (white overall) the general activity (total number of behaviours registered) for all horses was higher and flight reactions were recorded for Xusan and Sahara. This seems to indicate an elevated level of vigilance on day 2, which could be a result of the test person's white overall. On day 3 however, all individuals investigated the person and spent a substantial amount of time close to her. When investigating the person, the horses also repeatedly searched the ground at the person's feet, suggesting that they were anticipating food.

On day 4 the horses again mostly ignored the person, perhaps because they learned from the previous day that no benefit was gained from being close to the test person. Approach behaviour in animals is a product of several different emotions and motivations, which can sometimes be conflicting (Waiblinger *et al.*, 2003). Novelty is often associated with fear in prey animals, however it can also motivate investigative behaviours.

According to Waiblinger *et al.* (2003) a test person who lacks novelty could make cows unmotivated to approach. Perhaps when the overall was worn for the second time, it was familiar enough to not be too frightening, while still novel enough to encourage approach and investigation. As the horses did neither flee from nor investigated the person when wearing a familiar jacket, it suggests that the novelty of the overall motivated approach behaviour in this case.

The lack of fearful behaviour of the horses in my study on the last 2 days with the overall compared to when it was first worn, suggests a rapid habituation to this stimuli and the test procedure. A similar tendency was also seen with the tractor in test 1.

5.5. Target training

The one session of target training in this study was successful in that Sahara was very interested in the training, and learned the behaviour quickly. Also the delivery of the reward worked well. These results are promising for the trainability of this individual and could point towards good learning capacity in this context (for this horse).

When training 17 species of New world primates the number of training sessions required for each animal to perform a behaviour varied among tested individuals (Savastano *et al.*, 2003). Each session consisted of about 10 minutes of training of animals within a group. For those individuals who had reached the criteria of learning a target behaviour, this had taken 1 to 8 sessions to achieve, while some animals had not yet learned the behaviour at the end of the experiment (Savastano *et al.*, 2003). In comparison Sahara seemed to learn the target behaviour rather fast. Few studies are available regarding how much training is needed to learn target behaviour, especially for horses or animals trained in a group. This could be due to the difficulty to determine when learning has occurred in a target-training situation, as the investigative behaviour may play a role in motivating the animal to examine the target. Thus, during the learning process it is hard to tell when the motivation for the individual switches from being driven by investigation to instead be driven by the insight of how to receive reinforcement.

Williams *et al.* (2004) used the following criteria for learning of target behaviour: A horse had learned the behaviour when it made a correct response within 5 seconds, in 9 out of 10 consecutive trials on two consecutive days. This was similar to what the Przewalski's horse in my study accomplished. Unfortunately, it was not possible to follow up the training the next day to test if the high success rate remained, which would have been preferable. Training additional individuals had also given the results more credibility.

The Przewalski's horse in this study was motivated to participate in the training, as she did not leave the trainer even when disturbed by the other horses. It seemed like the horses in general had been taught to seek for pieces of food thrown on the ground, since they all readily searched for and found pieces of carrot this way. This turned out an appropriate way to deliver the reward through the fence without hand feeding. Since only one individual was trained, during only one session, no general conclusions can be drawn regarding how training works on Przewalski's horses. But I believe training could be used in this particular group, both to increase activity and to stimulate cognitive processes, but also as a tool when handling and/or examining the horses. There are many situations when training with positive reinforcement and target training, in particular, are beneficial, such as when there is a need to move, separate or station animals. Perhaps also handling such as examination of body condition score could be done without the need to anaesthetize or restrain the horses, if target training was applied. This issue relates to animal welfare since equine anaesthesia imposes a higher risk of morbidity and death compared to that of many other species (Senior, 2013).

At the Maryland Zoo in Baltimore a group of plains zebras (*Equus burchelli)* was trained with an operant conditioning program and positive reinforcement (Capiro, 2009). According to the author the zebras quickly learned target behaviour and this was then helpful when they were to be moved or separated. The training was also used to motivate the zebras to move out from a concealed area on the cue of ringing a bell (Capiro, 2009). Aside from this each zebra also got an individual training program based on their abilities, health status and personalities, for example to open mouth on cue and to prepare for muscular injection (Capiro, 2009).

In my study the two individuals not involved in the target study also showed interest in the trainer and target, so to train all individuals in this group seemed indeed possible. There are challenges when training animals in a group, such as some individuals being more motivated and/or express more dominant behaviour, which may make it difficult to train others. This problem was noted by Savastano *et al.* (2003) and was dealt with by asking the most eager animals to station at a distance and/or offer a time-consuming reward to keep them occupied. When target training animals in a group each individual could have their own distinct target to avoid confusion and competition. To have multiple trainers work simultaneously with each individual might also be advantageous.

5.6. Domestication effects on behaviour in horses

It may be argued if today's population of Przewalski's horses are representative of wild horses and thus useful for the study of domestication effects. The population has likely been intermixed with domestic horses (Oakenfull & Ryder, 1998; Olsen, 2006), which suggest they are less appropriate for these studies. Genetics have however also shown prominent differences between Przewalski's horse and the domestic horse (Orlando *et al.*, 2013), and among today's equids Przewalski's horse is likely the best option for comparison when we look for consequences of domestication, but I think that studies on other wild equids, such as zebras and wild asses, are valuable as a complement.

Perhaps domestication effects on behaviour in horses are not that prominent, and beneficial traits for interacting with humans are possessed also by non-domestic equids. Proops & McComb (2010) bring up pre-existing factors that in combination with aspects of domestication and enculturation may make horses exceptionally prone to read human cues of attention. For example, intraspecific communication in horses mainly consists of subtle visual cues such as movements of body, head, ears and/or eyes (Waring, 2003) and wild equids are known to live with and use communicative signals from other species (Goodwin, 2002). Proops & McComb (2010) concluded in their study that domestic horses indeed were sensitive to cues of attention given by humans, including gaze.

Studies on ability to read human cues are common when looking for proof of domestication effects. In horses these studies have, however, given contradicting results. McKinley & Sambrook (2000) showed that two out of four horses were able to find hidden food when a human experimenter touched the correct bucket, and one horse succeeded in using human pointing as a cue. The authors bring up the individual factor as playing a role in the object choice task. The successful horses had been trained using cooperative training, which the authors think might have improved their ability to read human cues.

In a study by Maros *et al.* (2008) horses could successfully locate hidden food by the use of different pointing gestures. In a similar study Proops *et al.* (2010) showed that horses were able to use human (sustained) pointing or the placement of a marker, but not momentary tapping of the correct bucket or alteration in gaze or body posture, to find hidden food.

These studies have all been on domestic horses and it could indeed be interesting to compare the ability to read human cues and/or learning in a human context, of wild and domestic equids. However, I think the methods of these studies should first be reconsidered. In the above studies were horses sometimes were able to locate food by human pointing I find it perhaps more probable that these individuals had learned to associate human hands and arms with the delivery of food. This is supported by the results from Proops *et al.* (2010) were 14 of the 23 horses that correctly used a human pointing cue did so by first examining the arm of the experimenter before following it to the bucket. Proops *et al.* (2010) also concluded that, despite them being able to successfully use some human cues, horses might not actually have an understanding of the communicative features of these.

To investigate the ability to understand human pointing, and/or other bodily cues to locate food seems relevant when looking for domestication effects in dogs. Throughout the common human-dog history dogs have lived close to us, sharing our resources (including food) and they have been valuable as cooperative partners for hunting and scavenging. With this in mind it seems likely that dogs are good at locating resources based on human gestures. Hare & Tomasello (2005) takes this as far as suggesting that convergent evolution has occurred between dog and man and thereby similarities can be seen in our social and communicative skills.

In contrast the horse in human history started out as being hunted as a food source, and also in the early days of horse domestication it is thought that horses were kept for meat as well as perhaps occasional riding (Ramey, 2011). Horses later started to be used for riding and traction, but were still also likely kept for milk and meat in many places (Ramey, 2011). Interbreeding of early domestic horses and wild horses most likely occurred, as well as domesticated horses escaping and becoming feral (Ramey, 2011). The fact that horses are not commonly sharing important resources with humans as dogs do, and that they have been kept in (sometimes semi-feral) herds throughout domestication, speaks of a different historical relationship compared to that of humans and dogs. McKinley & Sambrook (2000) mention the fact that horses do not live as part of the human family (as for example dogs do). Most horses do interact more with conspecifics than with humans, and this could partly explain why domestication effects in the ability to interact with humans are seen more in dogs than in horses. Generation time is also slightly longer in horses compared to dogs (Martin & Palumbi, 1993), while number of offspring per generation is much higher in dogs, which also affects the evolvement of these effects.

5.7. Ideas for future research

McGreevy & McLean (2007) propose that selective breeding has made the domestic horse less hyper-reactive and has increased its capacity of habituation. On the contrary my study suggests that Przewalski's horses habituate both to a novel object and to a strangely dressed person rather quickly. It is difficult to say if this is due to the tested Przewalski's horses being brought up in a zoo environment and, perhaps even more than the average domestic horse, are therefore already habituated to people in different clothing, and to some extent also to different objects in their enclosure. The fact that it is important to not be overly anxious and thus waste energy in the wild suggests that wild animals not necessarily should be more difficult to habituate. Another interesting note is that horses trained for dressage have been seen to develop increased anxiousness and reactivity, compared to domestic horses used for other purposes (Hausberger, *et al.* 2011), which indicates that certain training of riding horses can make them increasingly reactive and fearful. Thus the matter of how domestication and training/handling affect fearfulness and the ability to habituate are complex, and an interesting topic for further studies.

The training session in this study showed that this Przewalski mare was able to keep attention and focus for more than 10 repetitions of a new behaviour. In an unpublished pilot study on 3 domestic horses (Johannesson, 2015) all individuals lost interest and focus after only 2-3 repetitions when presented with the same target as used in this study. The same domestic horses all showed signs of stress and anxiety during the training sessions, behaviours I did not see when training the Przewalski mare. The individual variation in traits such as attention, focus, "mental stamina", ability to relax during training and others connected to learning, could be prominent enough to possibly be the target of selective breeding in horses. If we can further develop tests with which we can compare these abilities in equids, we could look for effects of domestication on these traits. A target-training test such as the one used in my study, could be useful in this context.

Future studies in the area could also focus on the social behaviour, and its possible differences between wild and domestic equids. Christensen *et al.* (2002) propose that domestication in horses has had a quantitative effect rather than a qualitative on the behaviour of horses, such as a reduction of aggressive behaviour since the selective advantage of aggressive behaviour decreases in a domestic environment. Christensen *et al.* (2002) also noted more aggressive behaviour in the studied Przewalski group, but this was likely due to two older stallions being present. As the authors also points out, the Przewalski's stallions also engaged in more friendly social interactions, and I would say even if aggression could have a selective advantage for wild equids, so does indeed social bonding. Reasons for group living, and hence the importance of social relationships, in horses include more individuals to watch out for predators and look for resources, better ability to defend these resources against rivalling groups, an increased chance to intimidate or confuse attacking predators, and the ability of cross-fostering of offspring within the group (Mills & Nankervis, 1999). So rather than being more aggressive, a more likely tendency could be that wild horses tend to be more socially active in general, which is also proposed by Christensen *et al.* (2002).

In their study Christensen *et al.* (2002) noticed more mutual grooming in the Przewalski's stallions compared to domestic ones, however this was likely because the Przewalski's horses were still shedding their winter coat and also seemed to be more exposed to insects. Comparative studies of domestic and Przewalski's horses are difficult, because they are usually kept under very different conditions. With the present study the aim was to develop tests suitable for wild equids kept in a zoo environment, which often is similar to outdoor loose housing of domestic horses and thus these tests could be performed both on domestic and wild equids reared and kept under the same conditions.

6. Conclusions

This study showed that Przewalski's horses may be tested in groups without loosing individual variation in behaviour. All 3 tests used in this study are flexible and can easily be used in other equid groups in different housing systems.

Przewalki's horses reacted differently when presented with an unfamiliar object compared to a familiar one. All horses investigated a familiar object more, compared to an unfamiliar one when it was first presented. The unfamiliar object provoked a stronger flight reaction. I found a tendency for habituation to the unfamiliar object shown as a lack of interest and fear when presented a second and third time.

The horses mostly ignored a person dressed in a familiar zookeeper jacket, while they first fled (first day) then investigated (second day) and lastly ignored (third day) the same person dressed in an unfamiliar white overall. This suggests that novelty in this case could provoke interest and the process of habituation to the overall was clear.

The trained horse responded well to the training and the two horses that were not trained also showed interest in the activity. Further use of training in this group of Przewalski's horses could increase the horses' activity and improve the relationship with handlers, but also serve as a tool when horses need to be examined or moved.

Popular science summary/populärvetenskaplig sammanfattning

Trots ett ökande intresse för forskning på häst, har få studier fokuserat på möjliga skillnader i beteende mellan domesticerade hästar och dess vilda föregångare. Förståelse för om och i så fall hur domesticeringen har förändrat hästars beteende, tillsammans med ökad kunskap om skillnader mellan olika hästdjur, kan förbättra djurhållning, hantering och träning av hästdjur i fångenskap, samt öka säkerheten för människorna som hanterar dem.
Den domesticerade hästens vilda förfader är idag utrotad, men Przewalskis vildhäst är dess nära släkting och kan fungera som alternativ när vi vill jämföra den domesticerade hästens beteende med det hos ett vilt hästdjur.
Syftet med denna studie var att ta fram tester som kan undersöka rädsla, social överföring av beteenden, reaktion på främmande föremål och människor och som kan användas både på domesticerade och vilda hästdjur. För detta behövs tester som kan utföras på grupper av hästar som hålls på zoo eller i en liknande miljö. Det är viktigt att kunna testa hästar i grupp eftersom de är utpräglade flockdjur och resultaten kan bli vilseledande om de isoleras i en testsituation.
En grupp på 3 Przewalskihästar användes i studien. Tre test gjordes: 1) hästarna exponerades för ett känt och ett främmande föremål, 2) hästarna exponerades för en känd och en främmande människa, och 3) en av hästarna tränades till att sätta mulen på en "target". Individuell variation var tydlig, trots att hästarna testades tillsammans, vilket antyder att man inte behöver testa hästar en och en för att få individuella resultat. Hästarnas beteende skiljde sig beroende på om objektet eller människan var främmande eller inte, och de vande sig vid det främmande efter upprepade tester. Under träningen var hästen aktiv och lärde sig det önskade beteendet direkt.

Tester som har använts för att visa skillnader i beteende mellan hund och varg har också använts på häst, men jag ifrågasätter om de verkligen är relevanta eftersom hundens roll i människans historia ser annorlunda ut. Istället föreslår jag att liknande tester som de i denna studie görs på flera arter av hästdjur för att öka kunskapen om likheter och skillnader i hästars beteende.

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Appendix I.

Frequency of the following behaviours was recorded for each individual with continuous registration during test 1 and 2.

Movement towards	At least one full step with any front hoof in a direction that decreases the distance to the object/human.
Movement from	At least one full step with any front hoof in a direction that increases the distance to the object/human.
Flight	Quick turn-around away from the object/human and/or suddenly "jumping" into a trot or canter to increase the distance to object/human.
Standing, alert	Standing for at least 2 seconds with head oriented towards object of focus, ears forward, elevated or stretched neck and tense body posture.
Standing, relaxed	Standing for at least 2 seconds with relaxed ears, lowered neck and relaxed body posture. May include one hind leg slightly lifted.
Investigating with nose/mouth	Exploring the object/human closely with nose (flared nostrils, nose 40 cm or less from object/human) or mouth/teeth.
Pawing	Pawing with one front hoof at object or fence in front of human.
Wood chewing	Gnawing with teeth on one of the logs or other wooden surface in enclosure.
Defecation	
Other	This includes social interactions, rubbing, grooming, investigating something else in the enclosure, foraging, eating, etc.

Appendix II.

Duration of the following behaviours was recorded for each individual during test 1 and 2.

Investigative behaviour	Exploring the object/human closely with nose (flared nostrils, nose 40 cm or less from object/human) or mouth/teeth and/or pawing with one front hoof at object or fence in front of human.
Close	The horse is, with at least half its body, within a distance of 2 horse lengths or less of the object/human. This can be combined with any other behaviour.
Far	The horse is further away than 2 horse lengths from object/human. This can be combined with any other behaviour.
Recover from flight	Duration from end of flight reaction (when not running away from the object anymore) to movement towards object/human.
Out of sight	Not visible in camera view.

Appendix III.

Definitions used for test 3 - target training.

Number of presentations	Number of times the target is presented.
Successful trials	Number of times the horse successfully touches the target (within 5 seconds of it being presented) and receives reinforcement.
Average target time	Time from when target is presented to when horse touches it with nose. Mean value of all trials.
Attention	Head/neck oriented towards and following trainer/target.

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