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Multiple handlers, several owner changes and short relationship lengths affect horses' responses to novel object tests

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ABSTRACT

Despite numerous studies emerging on the human-horse relationship, significant gaps exist in the identification of the horse and handler factors that influence the quality of their relationship. Here, we explore key factors affecting human-animal relationships: the number of regular handlers an animal has, the length of the relationship with the handler, the number of owner changes, and the familiarity of the handler. A total of 76 horses participated in two novel object tasks (walking on novel surfaces and being touch with a novel object) to determine whether horses react differently to novel situations depending on whether they are handled by a familiar or an unfamiliar person. We observed that having multiple regular handlers negatively affected the horse reluctance towards novel surfaces and novel object. In horses used to be handled by multiple persons, 68% were showed reluctant behaviours towards the novel surfaces while 75% of the horses handled by only one person did not show reluctant behaviours. Similarly, 26% of the horses with multiple regular handlers refused to be touched with a novel object while only 13% of the horses with only one regular handler refused to be touched with the object. The relationship length between the horse and the familiar handler decreased the horse reluctance towards the novel surfaces and the novel object. The longer the relationship the less reluctant were the horses. Horses sold more than once were also more reluctant to the novel object. These horses had higher chances to refuse to be touched with the novel object than the horses still owned by their breeder or their first buyer. Finally, older horses (> 18 yo) had higher success at walking on the surface when led by someone familiar (87%) compared to led by someone unfamiliar (15%). Our findings suggest that the horse-human relationship may take time to develop as it is shaped by multiple factors involving the horse's previous and current interactions with humans that affect their everyday life.

1. Introduction

Domestication can alter the socio-cognitive skills of animals and how they act towards humans. Modern horse domestication and the expansion of the equestrian culture were recently re-dated to approximately 2000 B.C. (Librado et al., 2021). Following the long co-evolution with humans, horses today demonstrate numerous socio-cognitive skills during their interactions with humans: they are receptive to human emotions, can follow human attentional state and use referential communication with them (Jardat and Lansade, 2021). Therefore, it is not surprising that the human use of horses has evolved from working animals to also include athletes and much-appreciated companions in leisure riding. The change in the status and use of horses has also significantly influenced human perception and interaction with horses (Hausberger et al., 2008; Freeman, 2019). Domestic horses may spend several hours daily in imposed close contact with humans which can affect horse welfare, physiology, and behaviour (Kelly et al., 2021). However, despite numerous studies emerging on the human-horse relationship, a recent literature review highlighted significant gaps in our understanding of horses' emotional states during human interactions (Kelly et al., 2021) and both horse and owner factors that influence their relationship (Freeman, 2019).

Here, we explore key factors relevant for understanding behavioural mechanisms driving human-horse cooperation. In 2011, Krueger et al.

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(2011). demonstrated that horses were better at following the focus of attention of familiar humans compared to unfamiliar persons, supporting the handler's familiarity to be of importance. Horses may generalise their perception of humans based on previous and repeated interactions that impact their subsequent interactions with familiar and unfamiliar humans (Fureix et al., 2009; Sankey et al., 2010a, 2010b). In working animals such as horses, at least three factors could influence the quality of the human-animal relationship. First, horses often interact closely and regularly with multiple trainers or riders (from here on referred to as handlers) differing in their experience, their attitude or in the nature of the interaction they have with the horse. Consequently, the quality of the relationship with the horse may also vary. Given horses are highly sensitive to human emotions (Trösch et al., 2019; Merkies and Franzin, 2021), having multiple relationships with humans that can vary in quality and intensity may affect how a horse behaves towards humans in general. Exploring the effect of having multiple regular handlers on the human-horse relationship is therefore necessary. Second, the relationship length between handlers and animals may play a key role in working relationships and cooperation. Working Asian elephants agreed more often to step on a novel surface when they were called by familiar handlers known for over a year (Liehrmann et al., 2021). They also responded faster to handlers known for longer (Crawley et al., 2021). Third, the number of times the animals have changed owners could also influence relationship quality. In dogs, changing owners may affect their behaviour towards humans (Thielke and Udell, 2019). Interestingly, even though horses often face owner changes, very little is known about how it affects them. Going through multiple owner changes in life could negatively alter a horse's behaviour towards humans in a new relationship. Consequently, it seems important to consider the relationship length between horses and the owner, as well as the number of past owner changes when investigating the human-horse relationship.

Only a few studies have investigated the effect of the familiarity with the handler in novel objects and novel surface tests (Ijichi et al., 2018; Hartmann et al., 2021). Although they did not find effects of the familiarity on the horses' reactions to novelty, they did not explore the potential interactions with other variables that could affect the relationship of the horse with the handler. Through similar experiments, this study aims to examine how the number of regular handlers, the relationship length between the familiar handler and the horse and the number of past owner changes may affect the human-horse relationship and how they interact with the familiarity of the handler. 76 horses participated in two novel object tasks (Dai et al., 2015; Lansade et al., 2016). For the first task, horses were led by familiar and unfamiliar handlers in turn to encounter a new type of surface material and asked to walk over it. For the second task, a novel object was presented to the horses either by familiar or unfamiliar handlers. We hypothesised that 1: As found in Ijichi et al. (2018) and Hartmann et al. (2021) studies, the familiarity with the handler alone should not affect the horse response during the tests. 2: Horses with only one regular handler may show less stress behaviours when handled by someone familiar during the tests compared to someone unfamiliar. On the contrary, horses trained by multiple people may not show behavioural differences depending on the handler's familiarity. 3: Horses with longer relationships with the familiar handler may be less hesitant towards novelty when handled by them compared to when handled by an unfamiliar person, whereas horses with shorter relationships with the familiar handler may not show behavioural differences depending on the handler's familiarity. 4: Horses that had changed owner several times may be more habituated to interact with different people and are not expected to show different behaviours depending on the handler's familiarity. Finally, we also controlled for intrinsic factors such as the horse's age and sex.

2. Materials and Methods

2.1. Study population and collected information

A total of 76 horses participated in the study. To be eligible to participate horses were required to have received enough training to walk safely on a leash in a familiar environment. Thirty-eight females and 41 gelded males ranged in age from two to 26 years (mean ± SE = 12.73 ± 5.6). Given the ages were not equally distributed, we divided the age variable into four categories: '2 – 6′ (n = 12), '7 – 12′ (n = 24), '13–17′ (n = 25) and '18 – 26′ years (n = 15). Housing style varied from stalls and paddocks to pastures and all horses were provided with ad libitum hay and water. Their diet could be complemented with pellets depending on their nutritional requirements. The relationship length between the owners and horses ranged from 6 months to 15 years (mean ± SE = 5.24 ± 3.96). The number of owner changes the horses had faced were categorised into two groups: horses still owned by their breeder or bought directly from the breeder (n = 27) and horses sold more than once (n = 49). The number of regular handlers was categorised into two groups: horses handled exclusively by the familiar person (n = 35) and horses that are also trained or ridden by other people at least once a week (e.g. horse rental, or shared with family members) (n = 41, mean ± SE = 3 ± 2). The potential biases that may limit the generalisability of the reported findings were assessed using the guidelines from the STRANGE framework (Webster and Rutz, 2020): The methods and the amount of training varied among the participants as well as the type of activity the horses were used for. The horses' previous habituation training toward novelty was not always possible to assess due to the lack of information regarding the horse experience with previous owners. Moreover, familiar handlers varied in their level of experience with horses. We were not able to control for such variation in the analyses. Additionally, the subjects represented a range of breeds composed of Finnish cold bloods, Estonian breeds, Fjords, Haflingers, Tinkers, Finnish warm bloods and other sports horses, Spanish purebreds, Icelandic horses, Shetlands, Welsh and Gotland ponies (see online resources 1 for details). Potential biases induced by this variation among the subjects will be discussed. However, those variations are likely to be inevitable and reflect on the variety of environments in which horses can evolve, therefore, the sample used in this study can be assumed to be a good representation of the privately owned leisure horses in Finland and Western countries.

2.2. Handlers during the experimentation

The handler we classed as 'familiar' was the main care giver of the horse, 55 volunteer participants (54 women and one man) were recruited for the study using advertisements on social media. Four women (OL, AV, VR and EA) with experience in horse groundwork training acted as the 'unfamiliar' handler to prevent the potential effect one specific person could have on the horses. None of the four experimenters had ever had previous contact with any of the subjects. The identity of the unfamiliar handler was pseudo-randomised depending on the availability of the experimenters (number of horses tested by each experimenter: OL = 35, AV = 10, VR = 11, EA = 23).

2.3. Experimental design

Experiments were performed in March and April 2021 in the stables where the horses lived, 26 locations (private homes or private stables) from Southern Finland. The two tests were performed in a place familiar to the horses and where they are used to be taken for training (empty outdoor paddock/riding arena or indoor riding arena). In total 76 horses participated in the novel surface test and 71 in the novel object test due to practical reasons. The materials were set up in the test area prior to the horses' arrival. Both tests were recorded using cameras fixed on tripods for later analysis of the behaviours using the Behavioural

Observation Research Interactive Software (BORIS) (Friard and Gamba, 2016).

Novel surface tests: The horses were led once by the familiar handler and once by an unfamiliar handler to walk on a novel surface. Two different surfaces were used to avoid habituation between the two tests: a fluffy blue blanket and a white tarp (both 1.40 m x 2.00 m). The order of the surface and the handlers' identity (Familiar/Unfamiliar) were randomised. Each horse was led towards the surface with low pressure on the lead rope whilst the handler first walked onto the surface and continued walking. If the horse stopped, the handler stopped as well, released the pressure from the rope and allowed the horse to choose whether to approach the surface. The horse was free to interact with the surface as much as it wanted and was free to choose to walk on it or to go around. Once the horse had finished interacting with the surface or had stood still for more than 10 s, the handler applied a small pressure on the lead rope to indicate to the horse to step forward and walk on or around the surface. There was no other trial if the horse decided not to walk on the surface. Once the horse passed the first surface, the second handler took the horse, walked a loop of the test arena and led the horse onto the second surface. For consistency, all handlers were asked not to talk to the horse and to avoid eye contact during the test. We recorded the reluctance of the horse towards each surface coded as three ordinal levels (see Table 1). The success of stepping on the surface with at least one front leg was recorded as a binomial response (1: success/0: fail).

2.3.1. Novel object tests

The second test consisted of presenting a novel object (stuffed narwhal with rainbow colours - 50 cm × 30 cm) to the horses. The object was presented either by the familiar or an unfamiliar handler. The handler identity was randomised. The horse was led by the familiar handler to a 10 m x 10 m arena and let free to roam and explore for three minutes while the familiar person was answering our questions and signed the forms. The horse was then led to the centre of the arena by one of the unfamiliar persons, and then the handler (familiar or unfamiliar) entered the arena with the object hidden behind their back and stood stationary 1.5 m away, in front of the horse. The handler revealed the object and held it in front of them at the level of the hips. The horse was released and for one minute was free to interact with the object. The time from when the horse was released until they freely came to interact with the object was recorded. After one minute, the horse was still free in the arena and the handler slowly approached the horse with the object held in one hand with a slightly stretched arm in the direction of the horse. The handler had one minute to try to touch the horse on the neck with the object. The horse was free to go away at any time. If the horse was clearly stressed about being approached with the object and started running away from the handler, the test was stopped before one minute. We recorded the reluctance of the horse towards the novel object when the handler tried to touch the horse with it on the neck. This was coded with three ordinal levels (see Table 1).

Table 1
Ethogram used for the coding of the reluctance towards the surfaces and the novel object.

Variables	Reluctance Levels		Descriptions
Reluctance towards the novel surfaces	1	None	– Walks on it or next to it without hesitation, the gait is regular (does not slow down or accelerate) – The body is relaxed, the neck is not straightened – No signs of stress such as startles, blows, steps back
	2	Mild	– Stops or slows down before approaching the surface or accelerates when walking on it or next to it – Neck is straightened and the horse keeps the gaze on the surface – No steps back – Possible presence of startles or blows before approaching the surface or on the surface
	3	Strong	– Refuses to approach the surface or walks as far as possible from the surface – The body is tense, the neck is straightened, and the white of the eye can be visible – Presence of signs of stress such as startles, blows, steps back – Jumps out of the surface after stepping on it
Reluctance when approached with the novel object	1	None	Accepts the object on the body without signs of stress: – No signs of stress such as startles, blows, steps back – The body is relaxed, the neck is not straightened
	2	Mild	Accepts the object on the body with signs of stress: – The neck is straightened with ears orientated towards the back – The body is tense when the object touches the body – Steps back before accepting to be touched with the object – Startles or blows when approached with the object
	3	Refused	Refuses to be touched with the object: – Steps back/moves away and refuses to be approached by the handler – Steps back/moves away and refuses to be touched with the novel object – Startles or blows when approached with the object

2.4. Sanitary protocol

To prevent the transmission of horse pathogens, subjects were handled with their own halter, the tarps and the experimenters' shoes were washed and sprayed with ©Virkon S disinfectant and the blankets, novel objects and the experimenters' clothing were washed in a washing machine with neutral scent detergent in between each stable. Prevention measures regarding the circulation of the Covid 19 virus were applied according to the recommendations of the Finnish government at the time of the experiments.

2.5. Ethics

Each participant provided informed consent for them and their horse (s) via the completion of a participant information form provided in English and Finnish. All data provided was stored according to the EU General Data Protection Regulation Act 12–14 (2016/679). Researchers and horse owners had the right to withdraw their consent at any time. This study was conducted following the ethical guidelines of the University of Turku and did not contain any procedure that would require a project license according to the Finnish National legislation Act 497/2013 and Decree 564/2013 on the protection of animals used for scientific or educational purposes or the EU Directive 2010/EU/63 on the protection of animals used for scientific purposes.

2.6. Statistical Analysis

Data are provided in Online Resources (Liehrmann, 2022). All analyses were carried out using the statistical software R, version 3.6.3 (R Core Team, 2021) and figures were created using the '*ggplot2*' package (Wickham, 2016). We checked for potential collinearity with bivariate analyses between our variables of interests (number of regular handlers, number of owner changes, relationship length, age and sex) using Wilcoxon, Spearman and χ^2 independence tests. Correlations appeared

between the relationship length and the age of the horse (as a quantitative variable, $r_s = 0.54$, $P < 0.0001$, $N = 76$). For this reason, if the relationship length appeared to significantly affect the response variable, the models were run again on a smaller portion of the dataset (11–22 years old) with no correlation between the age and the relationship length ($r_s = 0.24$, $P = 0.108$, $N = 48$) to determine if the effect is still present when there is no collinearity with the age. There was also an association between the age and the number of owner changes ($\chi^2 = 14.18$, df = 3, $P = 0.003$) as among the older horses (18 – 26 years old), 14 of the 15 individuals had changed owners at least twice in their life. This is considered in the discussion.

Horse reluctance towards the surface (a 3-level ordinal variable, see Table 1) was investigated using Cumulative Linked Mixed Models (CLMM) from the package '*ordinal*' (Christensen, 2019). The individual horse identity was included as a random factor to account for individual variation in paired data as each horse was tested twice. First, a simple model with only the surface order as an independent variable was run to test for potential habituation between the first and second surface. There was no significant difference in the reluctance levels between the two surfaces, confirming that there was no habituation (Estimate ± SE = −0.65 ± 0.42, $Z = -1.57$, $P = 0.116$). The success of stepping on the surface (1: success/0: fail) was investigated using Generalized Linear Mixed Models (GLMM) from the package '*glmmTMB*' (Brooks et al., 2017). The best suited distribution was selected using the '*DHARMa*' package (Hartig, 2021) to be a complementary log-log binomial family. Again, we controlled for potential habituation between the first and second surface and there was no significant difference in a horse's success at stepping on surface 1 or surface 2 (Estimate ± SE = 0.44 ± 0.29, $Z = 1.51$, $P = 0.129$).

The latency to touch the novel object was investigated using survival analyses from the package 'coxme' (Therneau, 2020). This process was selected because 19 horses did not approach the object at all and therefore had no timing recorded. The survival analysis allows these individuals to be considered in the analyses. The reluctance when approached with the novel object (3-level ordinal variables, see Table 1) were investigated using Cumulative Linked Models (CLM) from the package '*ordinal*' (Christensen, 2019).

To avoid over-parameterisation of the models due to the sample size of the datasets, for each of the four response variables – the reluctance to the novel surface (ordinal: None <Mild <Strong), the success to step on the surface (binomial 0.1), the reluctance to be touched with the novel object (ordinal: None <Mild <Refused) and the latency to touch the novel object (sec) – six small models were always run assessing the effect of the handlers' familiarity in interaction with one of the six other independent variables (age, sex, relationship length, number of owner changes, number of regular handlers, breed and previous habituation to novelty) plus one model with the effect of the handlers' familiarity alone. The most appropriate version of each of the 28 models was selected using a model comparison with two tailed ANOVA (Rouder et al., 2016) comparing the null model against the original model (variable of interest in interactions with the handler familiarity) and simpler models (without the interaction and then without the variable of interest). For each of this selection process, the selected models are presented in Tables 2 and 3. When necessary, a post hoc test based on Tukey's methods was performed with the function *emmeans ()* from the package '*emmeans*' (Lenth, 2021). Significance was evaluated at $P < 0.05$, with tendencies recognised at $P < 0.10$.

3. Results

3.1. Number of regular handlers

The number of regular handlers affected the horses' reluctance towards the novel surfaces regardless of the handler's familiarity to the horse (estimate ± SE = 1.90 ± 0.64, Z = 2.98, $\boldsymbol{P = 0.003}$). Tukey's post hoc test revealed that horses with only one regular handler were significantly more likely to approach the surface without reluctance (reluctance level = None). In total 75% of them were non-reluctant, while 23% were mildly reluctant and 2% were strongly reluctant (None/Mild: estimate ± SE = 0.64 ± 0.25, Z = 2.53, $\boldsymbol{P = 0.0001}$; None/Strong: estimate ± SE = 0.81 ± 0.41, Z = 5.71, $\boldsymbol{P < 0.0001}$) (Fig. 1a-left). Most horses with more than one handler showed significantly more mild reluctance towards the novel surfaces. In total 56% of the horses were mildly reluctant, while 32% were non-reluctant and 12% were strongly reluctant (Mild/Strong: estimate ± SE = 0.46 ± 0.08, Z = 5.98, $\boldsymbol{P < 0.0001}$; Mild/None: estimate ± SE = −0.23 ± 0.23, Z = −1.04, $P = 0.549$) and the proportion of non-reluctant horses was not significantly different from the proportion of strongly reluctant (None/Strong: estimate ± SE = 0.22 ± 0.19, Z = 1.17, $P = 0.471$) (Fig. 1a-right). Similarly, the number of handlers significantly affected the horses' reluctance towards the novel object regardless of the familiarity of the handler presenting it (estimate ± SE = 0.94 ± 0.47, Z = 2.01, $\boldsymbol{P = 0.044}$). Tukey's post hoc test revealed that significantly fewer of the horses with only one regular handler refused to be touched with the novel object (13%) compared to non-reluctant (45%) and Mildly reluctant (42%) (Refused/None: estimate ± SE = 0.33 ± 0.12, Z = 2.76, $\boldsymbol{P = 0.016}$; Refused/Mild: estimate ± SE = 0.28 ± 0.08, Z = 3.61, $\boldsymbol{P = 0.0009}$) (Fig. 1b-left). This was not the case for horses with more than one rider as there were no significant differences between the three different levels of reluctance (None = 25%, Mild = 49%, Refused = 26%) (Fig. 1b-right). The number of handlers did not affect the horses' success at stepping on the novel surfaces nor their latency to approach the novel object. The number of handlers was not part of the selected model indicating that it was not suited to explain the variation in these

Table 2
Results of the model selections from the statistical analyses of the two response variables (Reluctance and Success) analysed from the novel surfaces test. Bold text highlights the models that are selected in the ANOVA because they were significantly different from the null model. When none of the models were significantly different from the null model, the results from the comparison with the simplest model are presented.

Response variable	Variable of interest	Model Type	Formula	χ^2	df	*P-value*
Reluctance towards the surface	null model	CLMM	Surface reluctance ~ 1			
	Handler familiarity	Random effect: Horse ID	Surface reluctance ~ Handler familiarity	0.68	1	0.411
	Age	N = 152	Surface reluctance ~ Age	1.66	3	0.65
	Sex		Surface reluctance ~ sex	0.22	1	0.64
	Relationship length		**Surface reluctance ~ Relationship length**	**5.00**	**1**	**0.025** *
	Owner changes		Surface reluctance ~ Owner changes	0.14	1	0.704
	Regular handlers		**Surface reluctance ~ Regular handlers**	**5.13**	**1**	**0.023** *
Success at stepping on the surface	null model	GLMM	Success~ 1			
	Handler familiarity	Random effect: Horse ID	Success ~ Handler familiarity	0.04	1	0.841
	Age	Family:	**Success ~ Handler familiarity * Age**	**15.25**	**3**	**0.002** * *
	Sex	Binomial (link = cloglog)	Success ~ Sex	1.71	1	0.19
	Relationship length	N = 152	Success ~ Relationship length	1.50	1	0.22
	Owner changes		Success ~ Owner changes	0.04	1	0.731
	Regular handlers		Success ~ Regular handlers	3.38	1	0.066.

Table 3
Results of the model selections from the statistical analyses of the two response variables (latency to touch and reluctance) analysed from the novel object test. Bold text highlights the models that are selected in the ANOVA because they were significantly different from the null model. When none of the models were significantly different from the null model, the results from the comparison with the simplest model are presented, although the selected model is the null model.

Response variable	Variable of interest	Model Type	Formula	χ^2	df	*P-value*
Latency to first touch of the novel object	null model	cox model	(Latency, censor) ~ 1			
	Handler familiarity	N = 69	(Latency, censor) ~ Handler familiarity	0.09	1	0.847
	Age	censor = 19	(Latency, censor) ~ **Age**	**4.47**	**3**	**0.007** * *
	Sex		(Latency, censor) ~ Sex	1.71	1	0.196
	Relationship length		(Latency, censor) ~ Relationship length	0.20	1	0.670
	Owner changes		(Latency, censor) ~ Owner changes	7.63	1	0.063
	Regular handlers		(Latency, censor) ~ Regular handlers	2.79	1	0.088
Reluctance towards the novel object	null model	CLM	Object reluctance ~ 1			
	Handler familiarity	N = 69	Object reluctance ~ Handler familiarity	1.39	1	0.238
	Age		Object reluctance ~ Age	3.06	3	0.382
	Sex		Object reluctance ~ Sex	0.03	1	0.872
	Relationship length		**Object reluctance ~ Relationship length**	**4.79**	**1**	**0.029** *
	Owner changes		**Object reluctance ~ Owner changes**	**5.05**	**1**	**0.025** *
	Regular handlers		**Object reluctance ~ Regular handlers**	**4.17**	**1**	**0.041** *

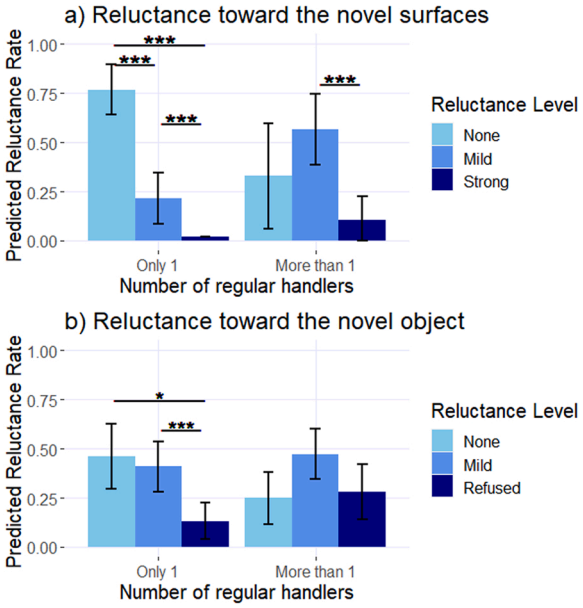

Fig. 1. Effect of the number of regular handlers on the probability to display one of the three levels of reluctance towards the novel surfaces (a), or towards the novel object (b). The error bars represent the standard errors from the models. '* ' indicates significant differences with a p-value < 0.05. The graphs are extracted from the corresponding models presented in Tables 2 and 3.

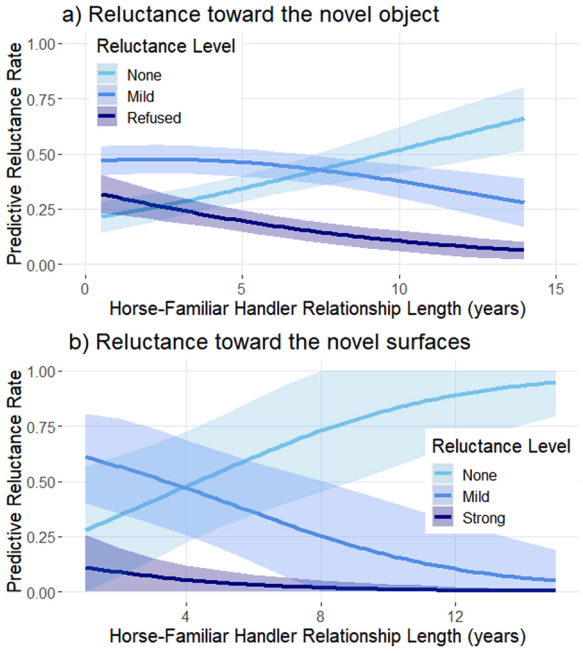

Fig. 2. Effects of the relationship length on the probability to display one of the three levels of reluctance towards the novel object (a) and towards the novel surfaces (b). The graphs are extracted from the corresponding models presented in Tables 2 and 3.

response variables Tables 2 and 3.

3.2. Relationship length

The relationship length significantly affected horse reluctance towards the novel object regardless of the handler's familiarity (estimate ±SE = −0.15 ± 0.07, $Z = -2.14$, $\boldsymbol{P = 0.033}$). The probability for the horses to accept to be approached with the novel object without reluctance (reluctance level = None) increased with the relationship length between the horse and the familiar person while the probability for the horses to display reluctant behaviours (reluctance level = Mild) or to refuse to be touched with the object (reluctance level = Refused) decreased with the relationship length. We can summarise by saying that after eight years of relationship horses had significantly more chances to be touched with the novel object without showing reluctant behaviours (Fig. 2a). The relationship length between the familiar handler and the horse tended to affect the reluctance towards the novel surfaces regardless of the handler's familiarity (estimate ±SE = −0.27 ±0.14. $Z = -1.93$. P=0.054). The probability for the horses to approach the novel surfaces without reluctant behaviour (reluctance level = None) increased with the relationship length between the horse and the familiar handler while the probability for the horses to display mild reluctant behaviours and strong reluctant behaviours decreased with the relationship length. We can summarise by saying that after six years of relationship horses had significantly more chances to approach the novel surfaces without showing reluctant behaviours (Fig. 2b). The results were similar when the models were run with the smaller subsets of the dataset without collinearity with the horses' age: Novel object (estimate ± SE = −0.20 + 0.09 $Z = -2.32$. $\boldsymbol{P = 0.021}$) and Novel surfaces (estimate ± SE = −0.32 + 0.18 $Z = -1.79$. P= 0.074). Finally, the relationship length did not affect the horses' success at stepping on the novel surfaces nor the horses' latency to approach the novel object as the variable was not included in the selected models indicating that it was not suited to explaining the variation in these response variables Tables 2 and 3.

3.3. Number of owner changes

The number of owner changes affected their reluctance to be touched on the neck with the novel object regardless of the handler's familiarity (estimate ± SE = −1.09 ± 0.49, Z=−2.20, $\boldsymbol{P = 0.028}$). Tukey's post hoc test revealed that within the horses with no or one owner change, significantly fewer horses refused to be touched with the novel object (None = 51%, Mild = 38% and Refused = 11%; Refused/None: estimate ± SE = 0.41 ± 0.13, Z= 3.02, $\boldsymbol{P = 0.007}$. Refused/Mild: estimate ± SE = 0.27 ± 0.07, Z= 3.86, $\boldsymbol{P = 0.0003}$) (Fig. 3-left). Within horses which changed owners more than once, there were no significant differences between the three levels of reluctance (None = 26 %, Mild = 48 % and Refused = 26 %) (Fig. 3 – right). The number of owner changes did not affect the horses' reluctance towards the novel surfaces nor their success at stepping on it or the latency to approach the novel object. The number of owner changes was not included in the selected models indicating that it was not suited to explain the variation in these response variables (Table 2).

3.4. Handlers' familiarity during the tests

The handlers' familiarity significantly affected the horses' success at stepping on the surfaces in interaction with the age of the horses. Older horses (18 – 26 yo) succeeded significantly more at stepping on the surface when being led by the familiar handler (87%) than when being led by an unfamiliar handler (15%) (estimate ± SE = 2.55 ± 1.05, t=2.43, $\boldsymbol{P = 0.016}$). The success at stepping on the surfaces did not significantly depend on the handler's familiarity in the three other age categories (2–6: Unfamiliar = 45%, Familiar = 30%, estimate ± SE = −0.52 ± 0.91, t= −0.58 P=0.56; 7–12: Unfamiliar = 65%, Familiar = 35%, estimate ± SE = −0.91 ± 0.80, t= −1.13, P= 0.261; 13–17: Unfamiliar = 69%, Familiar = 32%, estimate ± SE = −1.12 ± 0.713, t= −1.52, P= 0.130). The handlers' familiarity did not affect the horses' reluctance towards the novel surfaces or the novel object, nor the latency to approach the novel object. The handlers' familiarity was not part of the selected models indicating that it was not suited to explain the variation in these response variables (Tables 2 and 3).

3.5. Horse age and sex

Age significantly affected the horses' success at stepping on the surfaces in interaction with the handlers' familiarity (see section 'Handlers' familiarity'). Age significantly affected the horses' latency to first touch the novel object. The survival analyses revealed that young horses "2–6″ were faster to touch the object, 87% of them touched it within 12 s. This was significantly faster than the age group "7–12″ (56% touched the object within 57 s; coef ± SE = −1.46 ± 0.43, Z= −3.39, $\boldsymbol{P = 0.0007}$) and the age group "18–26″ (63% touched the object within 44 s; coef ± SE = −1.29 ± 0.48, Z= −2.66, $\boldsymbol{P = 0.008}$). Young horses also tended to be faster than the age group "13–17″ (82% touched the object within 51 s; coef ± SE = −0.76 ± 0.40, Z= −1.92, P= 0.056). Age did not affect the horses' reluctance towards the novel surfaces or the novel object. The age was not included in the selected models indicating that it was not suited to explain the variation in these response variables (Tables 2 and 3).

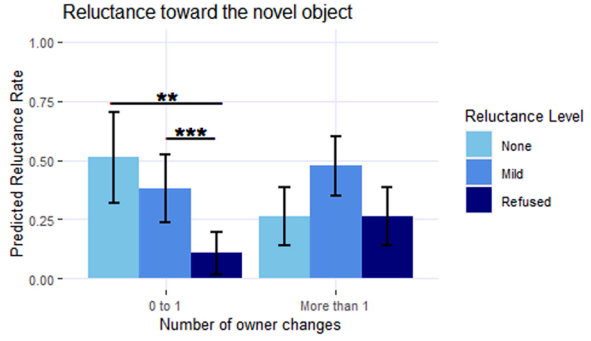

Fig. 3. Effects of number owner changes on the reluctance towards the object. The error bars represent the standard error from the models. '* ' indicates significant differences with a p-value < 0.05. The graph is extracted from the corresponding model presented in Table 3.

The sex variable did not affect the horses' reluctance towards the novel surfaces, the novel object, their success at stepping on the surfaces or the latency to first touch the novel object. The sex was not included in the selected models indicating that it was not suited to explain the variation in these response variables (Tables 2 and 3).

4. Discussion

This study shows that in accordance with our hypotheses, a horse's past experiences with humans had an influence on the quality of their current and future relationships with humans. Horses with multiple regular handlers were more reluctant towards novel objects and surfaces and similarly horses that had changed owner multiple times or had shorter relationships with the familiar handler were less comfortable in novel situations. None of these results were associated with the handler's familiarity suggesting that when exposed to novelty, the horse reaction does not depend on the handler's identity but rather on its general experiences with humans in the past. The handler's familiarity only interacted with the age during the novel surface test: older horses agreed more often to step on the surfaces when led by the familiar handler compared to when they were led by the unfamiliar handler. Among the analyses of age and sex, younger horses were faster to approach the novel object and the sex did not affect horse behaviour in the tests.

We expected horses mostly handled by their someone familiar to be more reluctant to novelty when handled by an unfamiliar person, and horses with multiple handlers to act similarly in both situations. Horses with multiple relationships could be more habituated to experience different situations with various handlers. Surprisingly, our results contrasted with these expectations: horses with multiple handlers showed more reluctant behaviours towards the novel object in both tests regardless of the familiarity of the handler. Horses with only one handler were less reluctant towards the objects also regardless of the familiarity of the handler. It might be that these horses with only one handler had more opportunities to develop a strong bond with one specific person and therefore generalise their behaviour and show trust with other humans as they already have a strong emotional base in their relationship with their handler. On the other hand, it is known that inappropriately applied training and handling methods can lead to negative emotional experiences for horses (McLean and McGreevy, 2010; McGreevy et al., 2011), and riding sessions may involve musculoskeletal injuries such as back or teeth pain due to badly fitted gear (Bondi et al., 2020; Dyson et al., 2020). Therefore, being handled/ridden by different humans who vary in their abilities to respond appropriately to horse behaviour increases the chances of having negative emotional experiences, especially if some handlers are only involved in riding/training sessions and not in everyday caregiving. Horses experiencing multiple relationships with various handlers may be more prone to develop apprehensive behaviours in novel situations, regardless of the handler identity, as the level of reluctance did not vary depending on the familiarity of the handler.

Interestingly, horses which had experienced multiple changes of owners showed more reluctant behaviours when approached with the novel object. On the one hand, horses differ in their temperament, and we cannot exclude this result to be due to individual horse temperaments (Lansade et al., 2017; Rankins and Wickens, 2020) leading to horses considered as fearful and difficult to handle potentially being sold more often than 'easy' horses. On the other hand, changes of owners are often associated with a change of physical and social environment,

which can be considered as a stressful event for the horses. Therefore, it may take time for the horse and the new owner to build their relationship and for the horse to feel comfortable in human company again. In working Asian elephants, mahouts (elephant handlers) suggest that a three-year relationship is necessary to understand an elephant's behaviour, and eight years is needed to develop trust (Srinivasaiah et al., 2014). There is also a common thought that building a strong partnership between the horse and the rider promotes success in stressful and challenging events such as horse eventing (Wipper, 2000; Dashper, 2014). This coincides with our findings that horses with longer relationship lengths with the familiar handler were less reluctant towards novel objects. Considering our results, most horses showed no reluctance towards the object or surfaces when they had a relationship of at least four to six years with the familiar handler. This supports the general assumption that the development of a bond between a horse and its caretaker may take time.

The above results were not affected by the familiarity of the handler during the tests. This finding supports the theory that horses build different memories of humans from previous interactions which lead them to attribute a general significance to humans based on the valence of those memories (Fureix et al., 2009; Sankey et al., 2010a, 2010b). However, it is known that horses can discriminate their handler from strangers, even based on pictures (Lansade et al., 2020a, 2020b) and in (Marsbøll and Christensen, 2015), Marsbøll and Christensen observed less stress behaviours in young Icelandic horses when led by a familiar handler compared to an unfamiliar handler during fear tests. However, in the Marsbøll and Christensen experiment, familiar and unfamiliar handlers were leading the horses in two respective different fear tests, and therefore the behavioural variation could also come from the two tests not being equally fearful. Our results mostly corroborate those from Ijichi et al. (2018) and Hartmann et al. (2021) who did not find any effect of the familiarity of the handler in novel object and novel surface tests, but their sample size did not offer them the opportunity to explore the potential interactions of handler familiarity with other variables. We found that the handler's familiarity mattered for older horses. Horses older than 18 years old (n = 15) refused more often to step on the novel surfaces when they were led by an unfamiliar handler compared to when they were led by the familiar handler. One could argue that older horses may also have the longest relationship with the familiar person. In our dataset, horses older than 18 years had relationship lengths with their owners ranging from 1 to 15 years and the relationship length variable did not affect the horses' success at stepping on the surfaces. Geriatric horses often suffer from corneal degeneration and loss of eyesight (Berryhill et al., 2017), and it has been shown that older horses may feel more anxiety towards novelty than younger horses (Lee et al., 2021). Therefore, older horses may perceive someone familiar as a secure base, feeling safer to walk over an unknown material when led by a familiar person. This may also explain our findings from the novel object test: when horses were free to approach the object, younger horses (2–6 years old) were the fastest to approach the object. These results corroborate the findings of Baragli et al. (2014) who observed that younger horses (4–6 years old), confronted by a sudden unfamiliar stimulus, showed more frequent explorative behaviour that started sooner and lasted longer than in older horses. A recent study showed that a horse's exploratory behaviour is likely to reflect the animal's curiosity and intrinsic motivation towards novelty, suggesting that it would be favourable for learning performance in young individuals (Christensen et al., 2021). Moreover, many other species show the same pattern of curiosity and exploratory behaviour being higher in the juvenile period than in later adulthood (Sherratt and Morand-Ferron, 2018).

4.1. Limits of the Study

Investigating factors such as the relationship length, the number of past owners or the number of regular handlers involves recruiting participants with different background traits which are difficult to control for. The human participants could differ in their experience with horses (e.g., recreational or competition riders, horse breeder). The experience and the level of knowledge on horse management practices can affect the behaviour and attitude toward horses in the everyday life (Hemsworth et al., 2015) and therefore could impact the quality of the relationship. The familiar person experience can also affect training the horses have received which could have influenced our results. For example, it is known that the use of positive reinforcement can affect subsequent reactions towards humans (Sankey et al., 2010b; Lundberg et al., 2020; Hartmann et al., 2021). Some horses were kept in private or public stables and some persons had their horses at home meaning that the daily interactions and the time they spend with the horse could differ. Moreover, the tested horses were from various breeds that we could not control for, and it is known that there are differences between breeds in emotionality levels and personality (Hausberger et al., 2004; Lesimple et al., 2011; Vidament et al., 2021). If horses differ in their personalities, it is possible that more fearful horses were much more likely to have experienced habituation training, lowering their initial level of fearfulness. Also, horses from different breeds are not always used for the same activities, although Hausberger et al. (2011) observed that horses from different types of work did not differ much in their overall emotional levels during handling and fear situation. Despite the potential confounded factors that could have interfered with the results, this study used a sample that is representative of the common life of leisure horses to highlight the importance of factors often overlooked when studying the human animal relationship. We encourage future research to investigate such factors within more controlled environment.

5. Conclusion

Our study shows that having multiple handlers, numerous owner changes and a short relationship length increased reluctance to novel objects and surfaces and therefore may negatively impact the horse-human interactions during novel tasks. Our findings suggest that a positive horse-human relationship may take time to develop as it is shaped by multiple factors involving the horse's previous interactions and events with humans as well as repeated interactions that affect the everyday life of the horse. The results of this study contribute much-needed knowledge on human-animal relationships which should be considered when investigating animal welfare.

CRediT authorship contribution statement

OL: Conception and design; Acquisition of data; Analysis and interpretation of data; Project administration; Writing – original draft. AV: Acquisition of data; Analysis and interpretation of data; Writing – review and editing. VR: Acquisition of data; Writing – review and editing. EA: Acquisition of data; Writing – review and editing. SEK: Supervision; Writing – review & editing. VL: Acquisition of data; Supervision; Writing – review and editing. LL: Conception and design; Supervision; Writing – review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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