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Connection between degree of confident behaviour and social motivation in domestic mink (*Neovison vison*)

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Animal Science - Ethology

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Sammendrag

Dyrevelferdsmessige utfordringer knyttet til gruppehold av husdyr er et viktig tema i den offentlige debatten, særlig av arter som i naturen lever som solitære rovdyr og med svært territoriale instinkter. Oppdrettsmink er en slik art som møter velferdsproblemer i gruppehold, til tross for at gruppehold ellers regnes som godt for dyrevelferden. Ulike studier har antydet at domestiserte arter har økt sosial toleranse, og dermed tolererer de bedre å leve i grupper. I tillegg viser ulike seleksjonsstudier på russiske sølvrev og mink at seleksjon for tamhet kan ha korrelerte positive effekter på intraspesifikk sosial toleranse. Målet med min studie var derfor å undersøke om mink med mest tillitsfull respons mot en menneskelig observatør også er mer motivert for å etablere positiv sosial kontakt med en annen mink.

Denne studien ble utført på en minkfarm i Norge. Ut fra 1969 juvenile mink av begge kjønn ble 14 hanner først valgt som såkalte stimulusdyr, som senere skulle fungere som en sosial stimulus når den sosiale motivasjonen og adferdsrespons til 80 andre mink ble testet. Stimulus-mink var alle hvite albinoer, med en aktiv kontaktrespons mot et menneske i "hånd-testen" (HT-verdi 3,0 ± 00) som måler graden av tamhet, dvs. hvor godt de samhandler med mennesker. De 80 testminkene, som ble valgt ut fra samme hånd-test og en pinnetest, var blitt målt på tillitsfullhet (Confident) og eller aggressiv respons. Dyrene med den mest ekstreme responsen ble dermed valgt, enten i Aggressiv eller Confident-gruppen; 40 i hver gruppe og 20 av hvert kjønn i hver gruppe. Testdyrets atferd i den sosiale testen (5 min) ble gradert ved å måle 7 adferdsresponser, nemlig 1) latens for å kontakte en stimulusmink, 2) agonistisk utforskning, 3) sosial utforskning, 4) nese-til-nese-kontakt med stimulusmink, 5) undersøkelse av miljøet, 5) halvveis ut av redekasse, og 7) tid brukt i redekassen.

Resultatene fra hver test ble analysert og de statistiske analysene viste at de to gruppene, det vil si Aggressive og Confident, hadde signifikant forskjellig atferd ($P \leq 0{,}05$) for alle de målte egenskapene, selv om nese-til-nese-kontakt gav de mest klare forskjellene mellom de to gruppene av dyr ($P < 0{,}0001$), og siden effekten av gruppen forklarte en stor del av variasjonen (ca 50%). Generelt var mink kategorisert som Confident raskere til å nærme seg stimulusminken ($P < 0{,}0001$) og brukte mer tid med den sammenlignet med den Aggressive gruppen ($P < 0{,}001$). Resultatene støtter hypotesen om at mink med høyere grad av tamhet mot mennesker har mer sosial motivasjon og mer positiv atferd mot artsfrender. Derfor har Confident mink økt grad av sosial motivasjon i nye sosiale samspill sammenlignet med mindre tillitsfull mink. Dette kan ha implikasjoner for hvordan mink takler gruppehold og sannsynligvis vil mestre sosial kontakt bedre, noe som igjen kan forbedre dyrevelferden til oppdrettsmink.

Abstract

A public concern is the welfare challenges associated with housing of farm animals in groups, particularly farm animals that in nature live as solitary carnivores, with territorial instincts. Farmed mink is one such species that faces challenges when housed in groups, which would otherwise been considered good for animal welfare. Different studies have indicated that domesticated species have increased social tolerance, hence they better tolerate being housed in groups. In addition, selection experiments on Russian silver foxes and mink, and Swedish poultry, indicate that increased selection for tameness may have correlated positive effects on intraspecific behaviour. The objective of this study was thus to investigate whether mink with more confident response towards a human observer is also more motivated to establish positive social contact with another mink; assessed in a 5 min social test.

This study was carried out at a regular commercial mink farm in Norway. Out of 1969 juvenile mink of both sexes, 14 males were first selected as so-called *stimulus* animals, which would function as a social stimulus when the social motivation and behaviour of 80 other mink were to be tested later. The stimulus mink were all white albinos, with an active contact response toward a human in the specific "hand-catch test" (HT score 3.0 ±00), which measures the degree of tameness i.e. how well they interact with humans. The 80 test-mink, selected among the remaining pups, were chosen on the basis of the same hand-catch test together with a stick test, which measures how confident or aggressive they behave. The animals with the most extreme response were hence chosen, either in the Aggressive or the Confident group; 40 in each group and 20 of each sex in each group. The test animals' responses in the social test were graded by measuring 7 behavioural responses, namely 1) latency to contact a stimulus mink, 2) agonistic exploration, 3) social exploration, 4) nose to nose with stimulus mink, 5) exploration of environment, 5) half out of next box, and 7) time in nest box.

The scores from each test were analysed and the statistical analyses showed that the two groups, i.e. Aggressive and Confident, had significant different behaviours ($P \leq 0.05$) for all the measured traits, although the Nose-to-nose test gave the most distinct responses between the two groups of animals ($P<0.0001$), since the effect of group explained a large part of the variation (ca 50%). In general, mink categorised as Confident, were faster to approach ($P<0.0001$) and spent more time with the stimulus mink compared to the Aggressive group ($P <0.001$). The results support the hypothesis that mink with higher degree of confidence towards humans have more social motivation and more positive behaviour towards conspecifics. Thus, confident mink tend to have increased social motivation in novel social interactions compared

to less confident mink. This might have implication for how mink cope when housed socially, likely more predisposed to cope well with social contact, which can improve the welfare of farmed mink.

1. Introduction

Background

Farming of fur animals have been subject to many concerns in public media, and one of these debates is whether farmed mink (*Neovison vison*) is suitable to be kept together, since they are solitary carnivores and very territorial towards each other. Despite this concern, farmed mink are often housed in groups or in pairs (Clubb & Mason, 2003; Dunstone, 1993; Henriksen, 2015). However, a study has shown that selection for reduced fear of humans also affects minks' social responses across several situations (Malmkvist & Hansen, 2002). This link may have important implications concerning animal welfare issues in animal husbandry and help us better understand the processes of domestication. The animals' behaviour, specifically its *approach* and *avoidance* towards humans, have likely been vital when man have selected which animals to breed, and this increased selection for tameness towards man may have correlated effects on intraspecific social behaviour.

Key terms used in this paper and their definitions

This study addresses various topics of domestication: human and animal relationship, tameness, motivation behaviour, i.e. animal emotions behind the observable behaviour, mink and group housing and behavioural tests used to assess motivational behaviour. Some key terms used frequently throughout this paper are defined in the list below:

- Confident: Classification of animal with high degree of approach towards humans (Malmkvist & Hansen, 2002)
- Domestication: adaptation to man and the environment humans provide, physically and socially, achieved through the combination of genetic change over generations, the environmental influences and the experiences during an animals' lifetime (Price, 1984)
- Motivation: The desire or willingness to engage in a behaviour, can be positive or negative and vary in strength. A motivation in generally influenced by many factors that can be genetic or physiological as well as environmental (Amdam & Hovland, 2011).
- Social reinstatement: the tendency of animals to flock and stay close to conspecifics (François et al., 1998).
- Social tolerance: The probability that individuals can stay in close contact during a competitive situation with little or no aggression (Cronin & Sánchez, 2012)

- Tameness: a behavioural trait that includes a motivation to approach a human as well as calm interaction without fear or aggression. Tameness have two important components, *Active tameness*: motivation to approach humans, and *Passive tameness*: a reluctance to avoid humans (Nagayama et al., 2018; Price, 2002).

Human and animal relationship

The interaction between animals and humans is first of all determined by the degree of tameness of the individual animals. Tameness is composed by the inherited capacity to be tamed and the experience each animal has with humans, as well as with other animals. In captivity, the animal's ability to adapt to the presence of people and handling is an important fitness-determining factor and a vital trait linked to domestication and to the welfare of animals.

Today, selecting tame and confident animals in breeding programs is required by law in Norway (Dyrevelferdsloven, 2009 § 24). A good animal- human relationship is a prerequisite to achieve good welfare in any production system. Any animal living with humans need to cope with being handled, even if this occur only sporadically. Since domestication requires a relationship between humans and animals, both interspecific (between human and animal) and intraspecific (between animal and conspecifics), the link between these two kinds of relationships are likely to give us vital clues about the processes of domestication.

The domestication of animals also represents a unique opportunity to study evolutionary changes caused by man's selection pressures and how animals are being changed to suit our production systems.

Animal husbandry and breeding

The production of domestic livestock and poultry has nearly tripled in the last 100 years, enabled by improved feeding methods and genetic selection. Breeding programs have altered the emotional mechanisms in domestication (Price, 1984; Price, 2002; Sandøe et al., 1999). As a consequence of selectively breeding on farm animals being recently mainly applied to increase economic important traits, it is conceivable that the importance of the underlying mechanisms and heritability of social motivation and confidence in production animals have not been considered sufficiently. Although social behaviour has not been the main attention of the commercial breeding programs, there are studies suggesting that farm animals can change strategies and adapt more easily to different environments due to their behavioural plasticity

(Estevez et al., 2007). E.g. a study on aggression and group size in domestic pig (*Sus scrofa*) done by Andersen et al. (2004) found that the larger groups had decreased number of fights per pig as well as fewer pigs involved in fights. A possible explanation for this trend is that in order for a larger group to maintain social stability, individuals have to refrain themselves, from defending their resources and showing aggression, due the large number of competitors and high cost of injury (Andersen et al., 2004; Estevez et al., 2007; Grandin & Johnson, 2009; Grandin & Deesing, 2014; Jensen & Wright, 2014; Trut, 1999).

Domestication

The first steps towards domestication requires animals that tolerate handling by humans and that are willing or able to breed in captivity. As a consequence, exclusion of animals with non-social behaviour. like fear or aggression towards man, has through direct and/or indirect selection processes implied that domesticates cope better than wild animals in high density populations. A study of Guinea pig (*Cavia porcellus*) concluded that domestication has led to reduced aggressiveness and increased tolerance of conspecifics, and that the domesticated guinea pig showed more positive social behaviour and less aggressive behaviour, than their wild ancestors. It was thus concluded that the capacity to adapt to the presence of humans, had also enabled these guinea pigs to have more positive interaction with each other (Kaiser et al., 2015).

Pre-adaptation for domestication

Most of our farm animals descend from social herbivores or omnivores, which make them more pre-adapted to domestication. Levels of social activity, frequency of aggressive behaviour, social intolerance, and behavioural intensity determines the success of captive breeding. Mink is one of relative few exceptions in this regard, along with other carnivores such as the domestic dog (*Canis lupus familiaris*), domestic cat (*Felis catus*) and ferret (*Mustela putorius furo*), where cats and ferrets are semi-social. (Breed & Moore, 2016a; Breed & Moore, 2016b; Hovland et al., 2017; Price, 2002; Schradin et al., 2010). This entails special challenges when housing mink in groups, even though they have been kept in captivity for many generations.

The mink

Farmed mink originates from wild American mink and is today the most common animal used for fur production. The mink belongs to the carnivore family of the Mustelidae, where most species are solitary with the exception of Eurasian badger (*Meles meles*), sea otters (*Enhydra* *lutris*) and some northern river otters (*Lontra candensis*). In solitary species, even interaction between males and females during mating season is limited (Dunstone, 1993; Lariviere, 2015)

Adult farmed mink is housed singly and is only put together for short periods of time to mate(Henriksen, 2015; Hovland et al., 2017). Being solely bred for maximum quantity and quality of fur, the most important traits in mink breeding have been pelt quality, body size, high fertility, maternal quality and high survival rate of offspring (Thirstrup et al., 2019).

Since mink is mostly housed in large-scale production units, with on average more than 2000 breeding females, individuals are only handled sporadically (Thirstrup et al., 2019). Farmed mink breeds well in captivity and as they are housed in cages, it has not been crucial to select animals that go well together, i.e. score high for social behaviour. Still, social contact is unavoidable during the life cycle; e.g. when moved to new cages or during medical treatment, and young animals are commonly housed socially, like in pairs or groups of 3-4 individuals (Bowman et al., 2017; Hansen, 2014; Malmkvist & Hansen, 2002).

Group housing and mink

Group housing is a cost-effective use of space and promotes social interaction and is generally considered to be good for animal welfare, as it is an environmental enrichment, at least for most of our domesticates that are naturally social, i.e. herd animals. However, housing more than two mink together has shown to increase aggression, bitemarks and cause higher mortality (Pedersen et al., 2004). There are thus severe welfare challenges when applying group housing, and increased knowledge about the social behaviours of domestic species is a precondition to maintain good animal welfare and prevent harmful situations within a cultured animal population (Kaiser et al., 2015; Quintavalle Pastorino, 2017; Rault, 2019).

All animals have some degree of social interaction, although in some species it may only be limited to the mating season, like with wild mink, that spend the majority of their lives without contact with conspecifics. Mink’s mating season starts in early March and lasts for about four weeks. The female gives birth in May/June and will feed the kits alone, until the juveniles are totally independent, normally in August. Farmed mink is normally weaned in July (Dunstone, 1993; Henriksen, 2015; Ireland, 1990).

Farmed mink is often housed in pairs after weaning, mostly siblings with one male and one female, and they live paired until pelting in December. There has been concerns whether this is an acceptable practice, since it increases aggression, mortality and tend to disturb their sleeping and foraging patterns (Pedersen et al., 2004). Yet, Schwarzer et al. (2017) found that juvenile mink and their social behaviour in semi-natural habitat used the nest box only 25% of the resting time alone, the rest of the time were with company, mostly resting and sleeping in pairs. The mink thus preferred company on average 75% of the time observed. In another study, it was found that farmed mink housed in pairs and mink housed singly did not show difference in welfare (Damgaard & Hansen, 1996; Schwarzer et al., 2017).

The modern rearing systems and the environment that can be provided for farmed mink is not easily changed. Selection of mink that are tolerant and adaptable to existing conditions and human interaction is thus an important way of improving the animal welfare. If there is a link between confident animals and their motivation for positive interaction, it is possible to breed animals that cope better in farms and consequently increase their welfare.

In the following sections, different subjects and factors that may influence social motivation in farmed mink are introduced and exemplified by results from previous studies.

Tameness

Tameness can be measured by the degree an animal is willing to avoid or approach humans. Tameness have two behavioural components, *passive tameness*: a reluctance to avoid humans, and *active tameness*: motivation to approach humans (Nagayama et al., 2018; Price, 2002). Here, the term "tameness" or "confident" as a behavioural trait includes a motivation to approach another individual, as well as calm interaction without fear and aggression. The more confident an animal is, the more motivated it will be to seek positive social interaction.

Belyaev's' selection experiment with foxes

One of the most recognised selection experiments on domestication is a Russian study of silver foxes (*Vulpes vulpes*), started by geneticist Dmitry K. Belyaev, as it is considered as one of the best demonstrations of the genetic contribution to tameness. Belyaev hypothesised that selection for tame behaviour have been unconsciously or indirectly carried out by humans since the very beginning of animal domestication. To investigate this theory, Belyaev started his classic selection experiments on farmed silver fox in the 1950's at the Institute of Cytology and Genetics, Novosibirsk, Russia (Belyaev, 1979). The foxes were selected from different fur farms and were originally bred for fur traits only, and not specific behavioural traits. His approach was to select individuals that behaved similarly to domestic dogs e.g. approaching in a non-aggressive, calm and explorative manner. Initially, Belyaev and his team found that in their fox population, 30% of the animals were extremely aggressive towards humans, 60% were either fearful or fearfully aggressive, whereas the last 10 % displayed a quiet exploratory reaction towards the humans without fear nor aggression. These 10 %, was selected as a founder population where the main goal was breeding for increased domestication. After forty years, 80 % of the domesticated pups were genetically tame and showed no aggression but affiliative and submissive behaviour towards humans, without any prior training. It is also found that pups from these domesticated foxes is as skilful as domestic dog pups in understanding human gestures (Belyaev, 1979; Hare, Brian et al., 2005; Huang et al., 2015; Price, 2002; Trut, 1999). However, the phenotypes of these Russian domesticated foxes have been altered, in that they resemble more and more a pet dog, and less and less a wild fox. They also found that the tame foxes that were playful with humans, also would play more with other foxes (Hare, B. et al., 2005; Nelson et al., 2017). The selection for low fear towards humans thus seems to have altered their intraspecific social tolerance.

Similar to the silver fox study, long-term selection of farm-bred mink for tame and aggressive defensive reaction towards human has been carried out since the 1980's by Oleg Trapezov, once a student of Belyaev. In each generation, mink have been exposed to human handling, using "Trapezov hand catch test" (explained later in this paper). The selection line of confident mink has shown morphological alterations and traits typical of domestication (Belyaev & Trapezov, 1986; Kizhina et al., 2017; Trapezov et al., 2008; Trapezov et al., 2012; Wilkins et al., 2014). Another similar selection study has been conducted at the Danish Institute of Agricultural Science since 1988 (Malmkvist & Hansen, 2002) This research has created a population of confident mink which exhibit more tame behaviour towards humans and generally show increased curiosity. Furthermore, Malmkvist and Hansen (2002) reported that mink lines selected for confident behaviour towards humans generalised their fear responses across several social and non-social situations.

Tame animals versus domesticated animals

Taming of animals is learned behaviour and is thus different from domestication, where actual tameness is seen from birth, e.g. Belyaev's' foxes where the pups did not show fear towards humans during first introduction. Domesticates can also be less tame than habituated wild species if they do not have much experience with being handled. However, domesticates have decreased fear and stress mechanisms, such as downregulated hypothalamic-pituitary-adrenal axis and serotonin levels (Geiger et al., 2018; Wilkins et al., 2014). Since domesticated animals have been selected for low fear of humans, their stress responses will not be triggered as easily as with wild animals (Bélteky et al., 2018). Belyaev et al. (1985) studied the physiological boundaries of the sensitive period for primary socialization within fox populations selected for tameness and the control group not selected for tameness. They found that unselected fox pups ended their sensitive period for socialization at 40-45 days of age and would show fear in response to novel stimuli, while in pups from the domesticated foxes, the sensitive period was prolonged to 60-65 days; thus the time when the pups are open to form social bonds was longer and explains why taming is easier in domesticated animals (Belyaev et al., 1985).

Motivation behaviour

Animal behaviour is composed of several factors: learned behaviours, biologically based emotion and hardwired instinctual behaviour (Mery & Burns, 2010). Motivational states include e.g. thirst, hunger, fear and the need to migrate, nest-build and dust bathe (Mason & Bateson, 2002). Sociality-motivated behaviour is seen when animals seek companions. Both genetic factors and early rearing environment will affect the intensity of social motivation (Amdam & Hovland, 2011). Variation in motivational strength among individuals can be explained by the influence of genetics or physiology, as well as the animals' environment and the animals' willingness to engage in a behaviour, and it will thus vary between individuals (Hovland et al., 2006). If motivation for social contact is low or the motives for seeking contact are competitive and aggressive, social contact will most likely reduce the animal welfare. Positive and negative motivations can be reasoned or deducted from evidence of behaviour, i.e. approach-behaviour versus avoidance.

Knowledge about the mechanisms underlying motivation enables us to understand the behavioural needs of animals and eventually to provide a better environment for them. A study of play among juvenile mink from Ahloy Dallaire & Mason (2016), showed that object and social play have different motivational bases, hence providing one or the other is not sufficient. It is also important to acknowledge that different species may have different underlying mechanisms for motivation (Ahloy Dallaire & Mason, 2016; Mason et al., 2001; Vinke & Schoemaker, 2012).

Animals emotional reactivity.

Emotional reactivity presents an advantage in avoiding dangers and can be viewed as kind of behavioural advisors. The emotional systems help an animal to predict the outcome of any social interaction and what behaviour that is required to obtain the best outcome (Price, 2002). How an animal experiences a situation, is operated by the emotions and is located in the subcortical parts of the brain (Damasio et al., 2000). Dr. Jaak Panksepp, defined seven core emotion systems in animals; *Seeking, Rage, Fear, Panic, Lust, Care* and *Play* (Grandin & Johnson, 2005; Panksepp, 2004; Walker, 2017). How these core emotional systems interact may vary from species to species, but in general, animals feel *fear* when their survival is threatened; physically, mentally or socially. Panksepp defines *seeking* as: *the basic impulse to search, investigate, and make sense of the environment. Seeking* reflects the anticipation of something positive, e.g. in a study on temperament, stereotypes and anticipatory behaviour in farmed mink, it was showed that expectation of a positive reward increased activity in the cage, while expectation of negative reward resulted in more time spent in the nest box (Hansen & Jeppesen, 2006). The seeking system is activated by neurotransmitter dopamine, (Grandin & Johnson, 2009; Panksepp, J., 2005).

Low level of fearfulness and increased exploratory behaviour towards man, is considered good for the animal welfare, and Price (2002) proposed that these characteristics are the most important behavioural effects in domestication. Also, animals that are less emotional sensitive to changes in their environment generally have higher degree of tameness towards people. It is argued that reduced responsiveness to fear-eliciting stimuli is an adaptation to living in an environment with frequent invasion of personal space with little opportunity to get away from conspecifics or humans (Malmkvist & Hansen, 2002; Price, 2002).

Behavioural tests

There are several validated methods to measure and test connections between an observed behaviour and a hypothesis. In controlled experimental designs researchers can study the value and motivational strength of a resource or interaction.

Scientists started early in the 1970's to use the *preference and choice test*, a test that require animals to choose between two or more options, e.g. different environments or social enrichment. With the preference and choice test, scientist can find the optimal option by looking at the choice the animal actually make, i.e. find out what an animal prefers or needs (Amdam & Hovland, 2011; Fraser & Matthews, 1997).

Another way to assess motivation is to measure how much an animal is willing to work for unlimited access of a resource. Both wild and domestic animals may often show great effort and concentration to accomplish a given task. (Amdam & Hovland, 2011; Cooper & Mason, 2001; Cooper, 2004; Kirkden & Pajor, 2006; Reijgwart et al., 2016).

The "price to pay" can be a naturalistic task that the animal must execute. E.g. in a study with mink, a weighted door was used to measure the motivation to access food, swimming pool, toys and other enrichments, demonstrating that the mink would work more to get access to food or swimming pool (Cooper & Mason, 2001). In another study, on silver fox vixens (*Vulpes vulpes*) housed in an operant apparatus where they could pull a loop to access physical contact with another vixen of same age, social motivation was higher in younger vixens. And in yet another study on social reinstatement on Japanese quail chicks (*Coturnix japonica*), they used a five minutes treadmill test, where the time spent trying to reinstate contact with the flock and how many meters a chick would walk was recorded to measure the "price" they were willing to pay, or the motivational strength (Cooper & Mason, 2001; François et al., 1998; Hovland et al., 2011).

Mink and temperament testing

Various studies have been completed to test the temperament of mink, including social motivation and behaviour. The most frequently used behavioural test to assess fearfulness, and this emotion's impact on or ability to develop stereotypes, is the "Stick test" (Hansen, 1996; Ring et al., 2018).

Stick test

The stick test involves the mink's immediate response to a spatula (stick) being introduced though the cage wire. This test was developed and validated to make it possible to select confident mink for breeding (Hansen, 1996). With this test it is possible to evaluate the temperament of the mink and to detect the mink's reaction to novelties as well as the animal's stress level during handling (Malmkvist & Hansen, 2002).

Glove test

Researchers in Canada developed and validated a modified version of the stick test, called the "Glove test" since they found that the original stick test was unsuited on mink from Ontario farms (Meagher et al., 2011). The Glove test was used in a study where the research team investigated differences in fearfulness among mink with different coat colours; concluding that black coated minks were more fearful than pastel coloured individuals. The new glove test was validated, as mink classified as "fearful", also used more time expressing general fear-related behaviour.

Hand catch test

The idea to put a glove on the stick was inspired by Trapezov's hand-catch-test. In Trapezov's hand-catch-test, the experimenter uses a protecting glove, opens the animals cage and slowly reaches for the animal. How the mink responds to human intrusion shows the confidence level and tameness towards humans. If the animal is tame, it will investigate the experimenter and allow the human to touch, pet and even lift up the animal (Trapezov, 2000).

The social test

To determine intraspecific behaviour in mink, the "Social test" is considered a good method. The mink is here introduced to an unknown mink through wire mesh, and the behaviour of the test mink is characterised together with the duration of social interaction. The test is developed by Malmkvist and Hansen (2002).

Domestication alters physiological traits

It is reported that domestication of farm mink has altered several physiological traits such as fur colour, skull dimensions, brain size, body size and temperament (Kizhina et al., 2017). E.g. scientist Melinda Zeder found that mink, as well as other well-known domesticated species like dog and cat, had a brain reduction of about 20% (Zeder, 2012). In another study on genetic variability, samples from farmed black mink and from wild captured mink in Eastern Canada, and found that black herds were more closely related to the wild mink than to the coloured mink (Belliveau et al., 1999). Further, Dieter Kruska (1996) measured the total brain size as well as the five fundamental brain parts and structures in the telencephalon of wild minks (*Mustela vison energumenos*) and compared them to dark standard ranch mink. Also, the volumes of the brain parts were compared in both groups, and it is concluded that brain and all structures measured were smaller in ranch mink, corrected for body size, age and sex, although there were differences in how much the various brain parts had decreased (Kruska, 1996). Similarly, skull sizes of adult mink from populations of American mink in Eurasia that originated from feral ranch mink captured in, were compared with wild Canadian mink's skull size and total body weight, and the results showed that the feral mink skulls were significantly different from the Canadian. Independent of body size, the feral mink skulls were clearly shorter and smaller in brain cavity size. These differences are also seen as intraspecific changes due to the process of domestication i.e. leading from wild to the ranched mink (Kruska & Sidorovich, 2003).

Alterations in behavioural traits

Malmkvist and Hansen (2002) investigated the reactions of mink offspring in two breeding lines, one selected for low fear of humans, confident, and the other fearful. In six different tests that could intimidate the mink, they recorded fear and confidence behaviour. Their conclusion was that mink from these breeding lines generalise their fear response across several social situations and non-social situations (Malmkvist & Hansen, 2002).

Objective, hypothesis and predictions

Since studies on domestication has demonstrated several alterations in farm animals, one of them being the social behaviour, it suggests a link between increased confidence and social motivation. Since wild mink is solitary, only social during the mating season and when the females have cubs, evidence of increased social confidence is a strong confirmation that domestication alters social behaviours and that the plasticity in the mechanisms behind these behaviours are not rigid.

The objective of this study was thus to investigate the link between levels of confidence towards humans and intraspecific social motivation behaviour in farmed mink. The hypothesis is that mink with a confident response towards a human, will also be more motivated to establish positive social contact with another mink in a social test. The main hypothesis and associated predictions are listed below.

Hypothesis: Mink with more confident response towards a human observer is also more motivated to establish positive social contact with another mink in a social test

- Confident mink will have shorter latency to contact the stimulus mink
- Confident mink will show more positive and/or neutral social behaviours
- Confident mink will show lower levels of aggression and agonistic behaviours
- Confident mink will spend more time in contact with the stimulus mink

2 Material and methods

Farm

This study was performed at a fur farm in Klepp municipality, in the county of Rogaland, Norway. The farm had three conventional buildings for housing of fur animals. The animals were fed around 7:30 – 08: 00 and again between 16:00- 17:00. The animals were housed in pairs, mostly brother and sister, in standard mink cages (45 cm 30.5 cm x 90 cm) distributed among the three animal houses. Besides the feedings, the animals were checked twice each day by the farmhand or the farmer himself.

Experimental setup

Three tests were used:

Test 1: The stick test

Test 2: Trapezov's hand-test

Test 3: Social test; the test mink (either Confident or Aggressive) is introduced to an unknown mink (stimulus mink) through wire mesh at the opposite side of its nest box. See setup in Figure 1

Test 1 and 2 were part of the screening of all animals and were used to select animals for the two distinct groups that were to be usedin test 3.

Figure 1 : The social motivation test for mink setup. The male stimulus mink was kept in the stimulus cage (to the right in the cage) attached to the test mink's cage.

Animals and test environment

The mink population (N = 1969 pups), or the sampling population, for the animals used in this project were all born and kept at the farm, i.e. born during the spring 2018 and weaned the same summer. The coat colours were; white, palomino, wild-cross, mahogany and wildtype. To create two distinct groups with confident or aggressive reaction towards humans, the stick test (Table 4) and the Trapezov hand-test (Table 5) was used. 80 mink (40 Confident + 40 Aggressive) were used for the social test, and comprised 20 males and 20 females in each group. The colour types were: mahogany (N = 20), wildtype (N = 28), white coated (N = 31) and wild cross (N = 1). The average hand test scores of each Group and sex combination, and the stimulus animals, are given in Table 1.

Stimulus animals

The stimulus animals were selected based on the "hand-catch-test" (see Table 5). Only confident animals were chosen as stimulus animals as they accept handling and transport better and are hence less likely to experience stress prior to the experimental test. The 14 stimulus animals were distributed into two evenly sized groups. All animals selected as stimulus animals had a "hand-catch-test" score of +3 and all were white males (Table 1) Three of the stimulus animals had the same father. When setting up which animals that were to be paired in the social test, it was made sure that the stimulus animal and the animal tested was not siblings nor had been housed together. Each stimulus group was used as stimulus animals only every other day (Table 2). The stimulus animals were housed in the same building as the test animals, but on the opposite side of the house, separated by 4 cage rows, i.e. about 24 meters apart. The stimulus animals were given a treat (dried chicken, dog bone and a knot from cattle skin, (Appendix 1) each time they had been used in the social test after being put back in their home cage.

Table 1. Hand catch test scores (mean ± SD) of the experimental animals, females and males, from the two groups and the stimulus animals.

Confident females (N = 20)	Confident males (N = 20)	Aggressive females (N = 20)	Aggressive males (N = 20)	Stimulus mink (N = 14)
3.40 ± 0.60	3.55 ± 0.51	-2.00 ± 0.00	-1.60 ± 0.60	3 ± 0.00

Table 2. The stimulus animals from stimulus group (G) 1 and 2 and which days they were used.

G	17.sep	18. sep	19. sep	20. sep	21. sep	22. sep	23. sep	24. sep	25. sep	26. sep	27. sep	28. sep	29. sep	30. sep	01. okt	02. okt
1	7/7	Off	7/7	Off	7/7	Off	Off	Off	7/7	Off	Off	7/7	Off	5/7	Off	5/7
2	Off	7/7	Off	7/7	Off	7/7	Off	Off	Off	Off	7/7	Off	6/7	Off	6/7	Off

Stimulus cage

The stimulus cage was made from wire mesh (Figure 2 and Figure 3). To ensure that the stimulus animal and test animal could not have any physical contact, e.g. due to possible aggressive response by the tested mink, the stimulus cage had an extra mesh wire on the side that was attached to the test animal's home cage. The stimulus animals spend on an average 12 minutes in this cage, depending on how long it took to prepare the test animals for the social test.

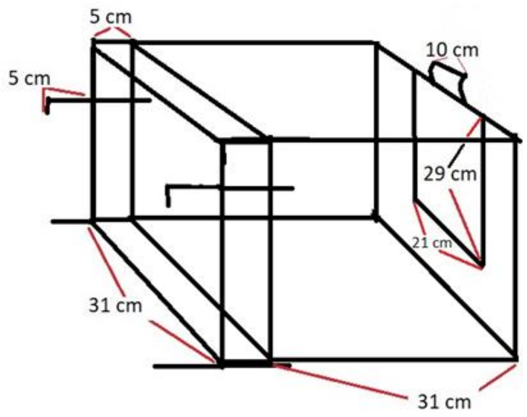

Figure 2 Measurements (cm) of stimulus cage.

Figure 3 The stimulus cage for testing social motivation in mink.

Experimental cages

The social test animals were relocated to the experimental test cages in the third animal building at the farm, two days before the social testing period (Figure 5 and Figure 4). Each mink had access to two identical cage units (h:48 x w:24 x l:62 cm) connected with a hatch (12.5 cm x 15 cm), with a total floor area of 0,29 m^2 (0,48 x 0,62 m) and two nest boxes (h: 20 x w:20.5 x l: 28 cm) mounted 26 cm up at the back end of the cage. Each experimental cage had a golf ball and a small plastic tube (diameter = 30 mm) as environmental enrichment. The animals were exposed to natural lighting and the nest boxes were filled with straw on both sides and on top

Figure 5. Photo of test mink in experimental cage

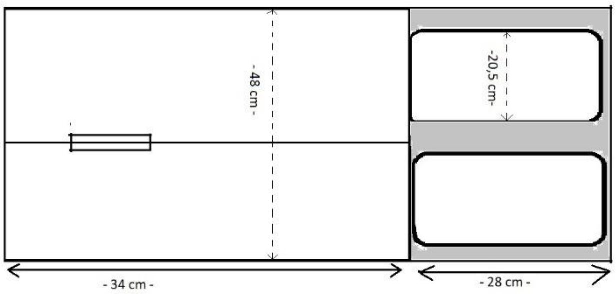

Figure 4. Sketch and measures (cm) of experimental cage used to house the animals used in the social test.

Genetic relationship among test animals

Some of the chosen animals were parental half-sibs and two of them full-sibs (see Table 3). *Table 3* also present how these animals are distributed among the five test groups described above; Confident females (CF), Confident males (CM), Aggressive females (AF), Aggressive males (AM) and the stimulus animals (Stim.) but does not include the animals that did not share parents with others.

Table 3. Test and stimulus animals with the same father or mother.

Father ID	Test Animal	Mother ID
147-7	CF18, CM10, CM11, CM17, AF10	
208-7	CM5, AF1	
218-7	CF15, AM11	
251-7	CF3, AM3, Stim.9	
290-7	CM1, Stim.7	
339-7	CM6, AM1	
395-7	CF8, AF4	**3362-7**
4046-7	CF5, AF6, Stim.8	
4068-7	CM18, AF19	
422-7	AM2, AM8	
545-7	CM7, Stim.10, Stim.11, Stim.13	
556-7	AM9, AM10	
562-7	CM13, CM14	
564-7	CF13, AF7	
636-7	CM20, AF8	
93-7	CF11, AF8	

Behavioural tests

The animals were screened using two behavioral tests; the *Stick test*, where all animals (N=1960) were introduced to a spatula through the cage wire and the *Trapezov's hand-catch-test*. The scores from these tests were used to select experimental animals, both the test as well as the stimulus animals. The initial behaviour tests were performed from 09:20 to 16:20 with a break between 12:00 and 12:45.

During the experimental test period, animals were tested each day the first week, but after one of the stimulus animals had to be discarded, every other day only six animals could be tested. On the 30th of September, only five animals were tested since the random selection of animals to be tested had caused the remaining animals to be located too close to each other, and they were not electable as test animals at the same day (they should not be able to observe a social test being performed on a neighbouring mink).

Stick test

The first test performed was the stick test. All animals were then introduced to a stick (spatula) held by a person, and the immediate response was noted and given a score as described in Table 4, in accordance with Malmkvist and Hansen (2002). The mink was tested in its home cage and since they were housed in pairs, both of them were evaluated. The stick (spatula) is inserted through the mesh of the cage, over the nest box for 30 seconds. (Hansen & Jeppesen, 2006; Zieliński et al., 2018). The purpose of this test is to evaluate the temperament of the mink and to detect the minks reaction to a new experience, *novelty* (Malmkvist & Hansen, 2002).

Table 4. The Stick test scores used in categorizing responses to the stick

Score	Behaviour
1	Investigative; sniffing the stick and examine it, physical contact
2	Fearful; timidly backs away from the stick and/or hides in the nest box
3	Aggressive; attacks the stick and bites it
4	None of the behaviours above or several

Trapezov's hand-catch-test

After the first screening, the second test performed was the "Hand-catch-test" (Table 5). The purpose of this test is to observe the animal's reaction towards humans. The test is a sequence of simple and progressive steps where the experimenter slowly reaches for the animal and try to catch it while using a well-padded mitten, suited to protect the hand from mink bites. This implies that the human slowly opens the cage, and calmly introduces the protected hand to the mink. This introduction will normally lead to three typical defensive behaviours by the animal: Aggressive, Fearful or Tame. The behaviours were assigned different scores, according to the criteria given in Table 5.

Table 5. The hand catch test: Behaviors registered, and the respective scores used in categorizing responses of mink when humans are trying to touch them (Kizhina et al., 2017; Trapezov, 2000)

Score	***Behaviour***
-4	*Onset of attack in response to approach of a human*. Even before the test began, the animal loudly shrieked, ran around in the cage and attacked the bars.
-3	*Active attack outside the shelter*. When attempts were made to handle the animal, it attacked the hand instead of hiding
-2	*Attack from nest box*. The animal would jump to the entrance of the nest box, and from there hide and attack the hand
-1	*Defensive response towards humans*. The animal rapidly retreated, hid in the nest box, gaped and bared its teeth, cried shrilly or hissed and shriek or tighten the muscles around the mouth, preparing for a shriek. The animal would have a tense posture and show severe emotional stress.
0	*Avoid contact with a man*. The animal is fearful and is running about in panic, shrieking
+1	*Exploratory responses.* The animal would calmly respond to the hand by showing exploratory response, sniffing the hand and quivering the vibrissae
+2	*A calm response to contact with the human hand.* Animal allowed the handler to touch its face, chest and paws
+3	*Active contact on the part of the animal*. When the cage was opened, the animal leaned against the opened door and reached out with its snout to the human hand. When the hand was inside the cage, the animal actively investigated and sniffed the hand and could even lean against the hand with its paws
+4	*The animal allowed any part of the body to be touched.* Apart from being lifted or held, the animal allowed any interaction with the hand, even playful interaction and cuddling/petting
+5	The animal allowed humans to handle it. The animal would show outstanding tame behaviour, allowing handling without showing any signs of fear or aggression

Social test

The social motivation test is based on a previous test used in mink by Malmkvist and Hansen (2002). In the beginning of each social test, the stimulus mink was taken out of its home cage and transported in the stimulus cage (see Figure 2 and Figure 3**Feil! Fant ikke referansekilden.**), next to the home cage of the test animals. The test animal was then enclosed inside its nest box, as were the two neighbouring animals on each side, to block their view and to avoid disturbance. The stimulus cage was then attached to the outside of the home cage of the test mink. After attaching the stimulus cage to the opposite side of the nest box, the blockage was removed from the nest box entrance, allowing the test mink access to its home cage again, and the subsequent observation lasted for five minutes. The test mink and stimulus animal were never capable of making physical contact due to the double cage wall of the stimulus cage. The stimulus mink could retreat only a few centimetres from the test mink's cage wall. After five minutes, the stimulus animal was detached and put carefully down, and the neighbours of the test animal could be released from their nest boxes. Then, the stimulus animals were immediately brought back to its own cage and given a treat. The cameras were mounted prior to the social tests, to ensure that the test animal could investigate and habituate to the cameras before the monitored test. All test animals got at least five minutes to investigate the cameras before the test started. This was to hinder any novelty that could distract the animal, taking the focus away from the stimulus animal. While the test animals were put in start position, the stimulus animal was placed out of sight, in a shielded place, about 5 meters away from the test animals' row.

Behaviour recording and the variables

The social test sessions were recorded by two cameras (Gopro Hero3 and Sony FDR-X3000 4K-Action), one above the cage of the test animal and one on the side to record the interaction and the behaviour of the stimulus animal, 110 cm away. The recordings were then analysed through Solomon coder (https://solomoncoder.com/). The behaviours were evaluated according to the criteria given in Table 6. Behaviours such as grooming, marking, attack, screaming, hind-leg-thumping and drinking were excluded due to low occurrences or lack of connection with fear in mink (Malmkvist & Hansen, 2002).

Table 6. The ethogram of the different behaviours assessed in in the social test.

Behaviours	Description
Half out 	At least the head and shoulders of the mink were out of the nest box, but the mink was not completely out
Exploration of environment	The animal cease to inspect the stimulus animal or delay interactive exploration and walks around the cage; sniffing and investigating the surroundings, often accompanied with grooming
Nose to nose contact 	Mutual social exploration, where both animals have a calm and inquisitive focus towards the other mink, leading to synchronized and parallel patterns of movement
Agonistic exploration	Aroused and intense focus towards the stimulus mink with an arched body posture; staring and rapid, staccato movements
Social exploration 	Inquisitive focus towards the stimulus mink with a neutral body posture; sniffing and calm, balanced movements
Nest box	The animal is inside the nest box with the whole body

Ethical note

The experiment was approved by Forsøksdyrutvalget (Norwegian Food Safety Authority). To maintain good animal welfare throughout the project, it was decided before the project started that any animal would be taken out of the experiment if showing any signs of not coping well with being used in the social tests This evaluation should be based on their fear response to humans. It was a priority to maintain the animal's normal activity budget, and preserve the animal's motivation to interact with humans, normal species-specific grooming behaviour and curiosity and anticipating behaviour. If the animal did not go back to relaxed curious behaviour after the social test, but stayed stressed and fearful, the animals would no longer be used in the experiment. Stimulus animals that were taken out of the study, remained housed in their given enclosures until the experiment was done, but not handled, and still given treats during the test-day they were meant to be used. Out of the 14 selected stimulus animals, two were taken out during the period of testing, one from each of the two stimulus groups.

Potential sources of error

Due to a misunderstanding regarding when the animals were to be moved back after use, the farmer removed all the animals that already had gone through the social test at the 22th of September. Therefore, the row was a lot quieter during the rest of the test period. Further, at the following Sunday, on the 30th of September, even more animals were removed.

Data transformation, statistical tests and models

To examine the degree of social interaction, the number and duration of each behaviours were recorded as variables (Martin & Bateson, 2007). The behavioural traits thus observed were *Social latency (1), Agonistic exploration (2), Social exploration (3), Nose to nose (4), Exploration of environment (5), Half out (6),* and *Nest box (7).* Box cox transformation was used to ensure normal distribution for behaviours *1, 6* and *7,* whereas a square root transformation was used for behaviour *5*.

For *Agonistic exploration*, the significant p-values were calculated using Fishers exact test, because there were few observed individuals that showed the behaviours (Confident group N= 3, Aggressive group N=18) and in *Nose to nose,* although this behaviour had a lot more observations (Confident group N= 40, Aggressive group N=31, i.e. 9 did not show the behaviour).

The effect of Group and Sex were analysed by comparing their least square means (LSM) and their standard errors (SE). JMP®14 and RStudio Version 1.1.463 were used for the statistical analyses.

To study the probability that the behavioural responses could be explained by group (Confident vs. Aggressive) and/or sex, a linear mixed model was used, where stimulus animal was set as a random effect. Also the interaction between these two factors, Group and sex, was included in the model.

The mixed model used can be presented as:

$\boldsymbol{y}_i = \boldsymbol{X}\boldsymbol{b}_i + \boldsymbol{Z}\boldsymbol{a}_i + \boldsymbol{e}_i$,

where $\boldsymbol{y}_i$ is a vector of direct or transformed observations, as described above, for trait i (i = 1, 7), $\boldsymbol{b}_i$ is a vector of the fixed effect as described above, whereas $\boldsymbol{a}_i$, and $\boldsymbol{e}_i$ are vectors of stimulus animal and residual effects, respectively. The matrices $\boldsymbol{X}$ and $\boldsymbol{Z}$ are the incidence matrixes relating $\boldsymbol{y}_i$ to $\boldsymbol{b}_i$ and $\boldsymbol{a}_i$. Random effects were assumed to be independent and normally distributed with expected mean equal to zero. The results are presented as mean ± SE and minimum statistical significance was set to P = 0.05. The F-statistics are presented in the results with each behaviour measured, in text and in Appendix 5.

The stimulus animal as a random effect gave negative variance component in behaviour *1*, *3* and *6* (-59.59, -6.563, -21.91) and very high variance component in behaviour *4* and *7* (9.996, 52.45). Thus, stimulus animal does not fit the assumptions and criteria to be used as a random effect, but I have chosen to set them as random since I am interested in the test animals' reaction towards the stimulus mink and not the interaction between test animal and stimulus animal. One explanation to why the variance component is negative, can be the small sample size (N=80) and the effect of outliers. Also, it is important to consider that each stimulus animals were only few times within each group. For example, *Stim12* was used in total only five times; being introduced to one confident female, two confident males, one aggressive female and one aggressive male. The Group and Sex effects (fixed factors) are in this case unbalanced (unequal number of observations per subclass), and is not enough to accomplish normal distribution, especially if there are any outliers (Brown, 2016).

The correlation between hand-test-score and latency to contact was tested using Spearman's rank correlation coefficient.

3 Results

The two groups of mink, Confident and Aggressive, differed in all the recorded behavioural traits. The effects of group were estimated using mixed models and Table 7 shows that the total variation that were explained by the models varied. The only behaviour where sex had significant effect was *time spent in nest box*, where females spent more time in the nest box ($F_{1.67}$= 6.05, P=0.017). All figures (Figure 7-15) are presented with non-transformed data (sec, mean ± SE); non-transformed means for each trait are given in Appendix 2.

The latency to approach the stimulus mink were shorter in the Confident mink (N = 40) compared to the Aggressive mink (N=39), and there was significant effect of group ($F_{1.70}$=43.3, P<0.0001) but no significant effect between sexes ($F_{1.74}$=2.85, P=0.096) although the females made contact sooner than males within both groups (Figure 6).

Table 7. The duration (sec, LSM ± SE) of social and agonistic behaviours performed during the 5 min social test and the significance levels for Group and sex, and the total variation explained by the effects in the mixed model.

	LSM±SE		*Statistics*		
Trait	Confident	Aggressive	Group	Sex	R^2_{adj} (%)
Social latency	41.5± 3.7	77.5±3.92	P<0.0001	P=0.096	0.153 (15%)
Agonistic exploration	0.37± 0.21	2.26±0.4	P<0.0001	P=0.170	0.195 (20%)
Social exploration	168 ± 7.5	123 ± 9.95	P<0.001	P=0.055	0.109 (11%)
Nose to nose	22.2 ± 1.1	11.5 ± 1.34	P<0.0001	P=0.379	0.512 (51%)
Exploration of environment	7.84± 0.42	5.86 ± 0.40	P<0.01	P=0.106	0.185(19%)
Half out	26 ± 2.59	42 ± 3.12	P<0.001	P=0.899	-0.011 (0%)
Nest box	28.6 ± 3.66	55.6 ± 3.6	P<0.0001	P=0.017	0.412 (41%)

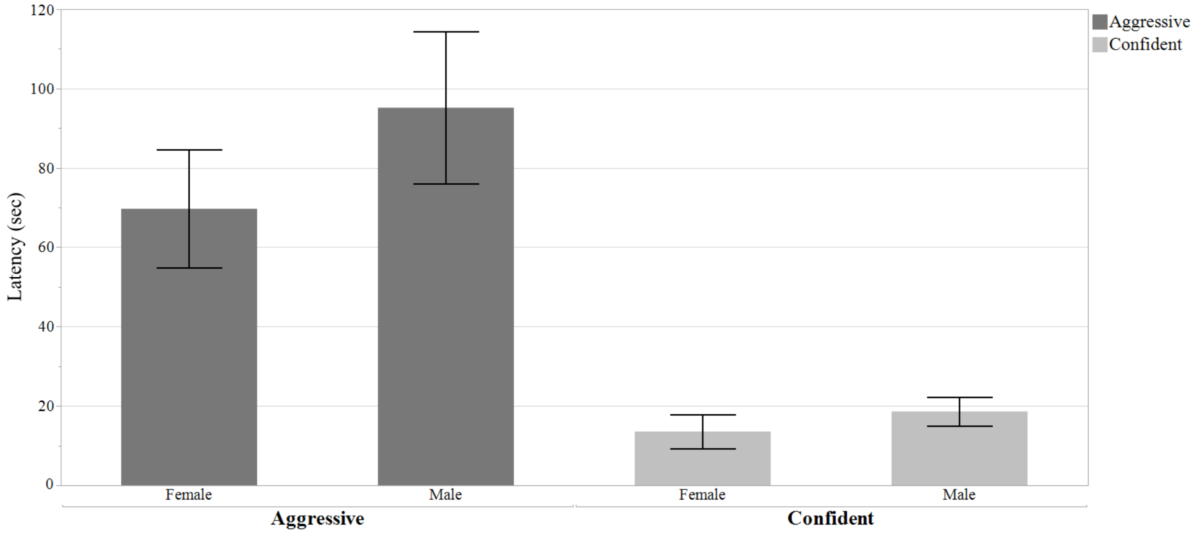

Figure 6. The latency (sec, mean ± SE) to the first close contact with the stimulus mink during the 5 min social test for mink from the different groups. Confident mink used significant shorter time to approach the stimulus mink.

Social exploration

Confident mink showed more social exploration than the aggressive group (Figure 7), and there was significant effect of Group ($F_{1.68}$ = 12.61, P <0.001) but no significant effect of Sex ($F_{1.68}$ = 0.4, P = 0.55). In both groups, males showed more social exploration.

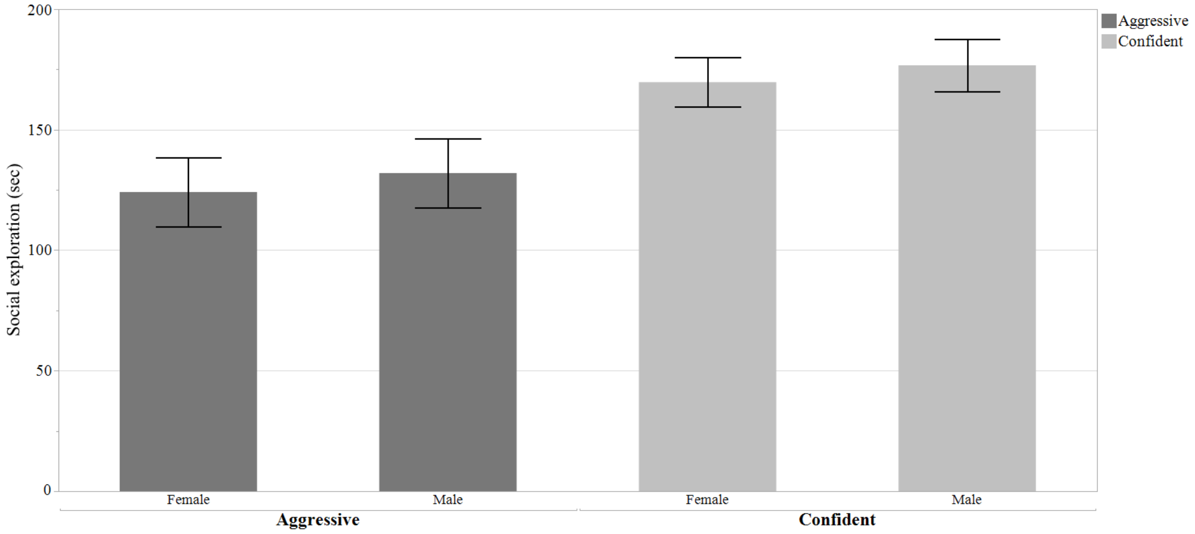

Figure 7. The duration of social exploration (sec, mean ± SE) during the 5-minute test from mink from aggressive and confident group. Confident mink used significant more time to explore the stimulus mink.

Agonistic exploration

The number of minks showing agonistic exploration was higher in the aggressive group than in the confident group with significant difference between the groups (Aggressive: N= 18; Confident: N= 3; $F_{1.67}$ = 17.5, Fisher's exact test P <.0.001). In line with the hypothesis and predictions, the confident group showed less agonistic behaviour (Figure 8).

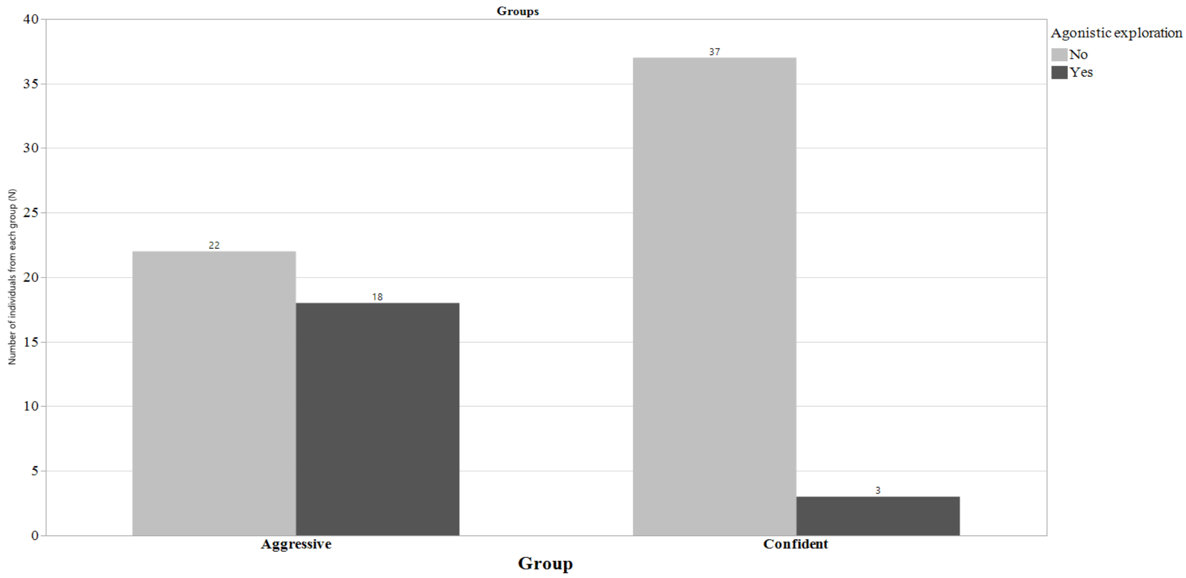

Figure 8. The number of minks from aggressive and confident group that were showing agonistic exploration (Yes- dark bars) or did not show agonistic exploration (No – light grey bars) towards the stimulus animal during the five-minute social test. Significantly fewer of the Confident mink showed agonistic exploration towards the stimulus mink.

Nose to nose

Nose to nose behaviour was observed in both groups (Aggressive N = 29, Confident N= 40) ( ). but was much more frequent in the Confident group, where all of them showed this behaviour ($F_{1.\ 68} = 7.9$, Fishers exact test $P < 0.001$).

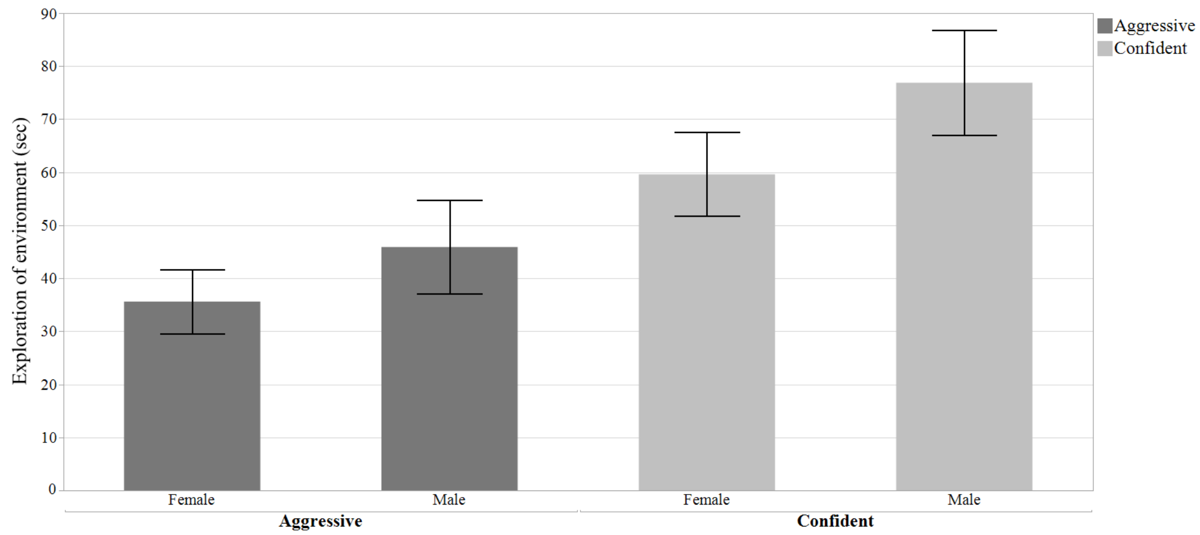

Figure 9 . The number of minks from aggressive and confident group that performed nose to nose contact during the 5 min social test. Significantly more (all) of the Confident mink showed nose to nose contact with the stimulus mink.

Exploration of the environment

The Confident group also did significant more exploration of the environment than the Aggressive group ($F_{1.66} = 10.7$, $P = 0.002$) (Figure 10). Confident males tend to explore the cage and orientate themselves within the environment more than confident females (CM = 8.36 ±0.61, CF = 7.33 ± 0.56), and this was also observed in the aggressive group (AM = 6.19 ± 0.63, AF= 5.53 ± 0.52).

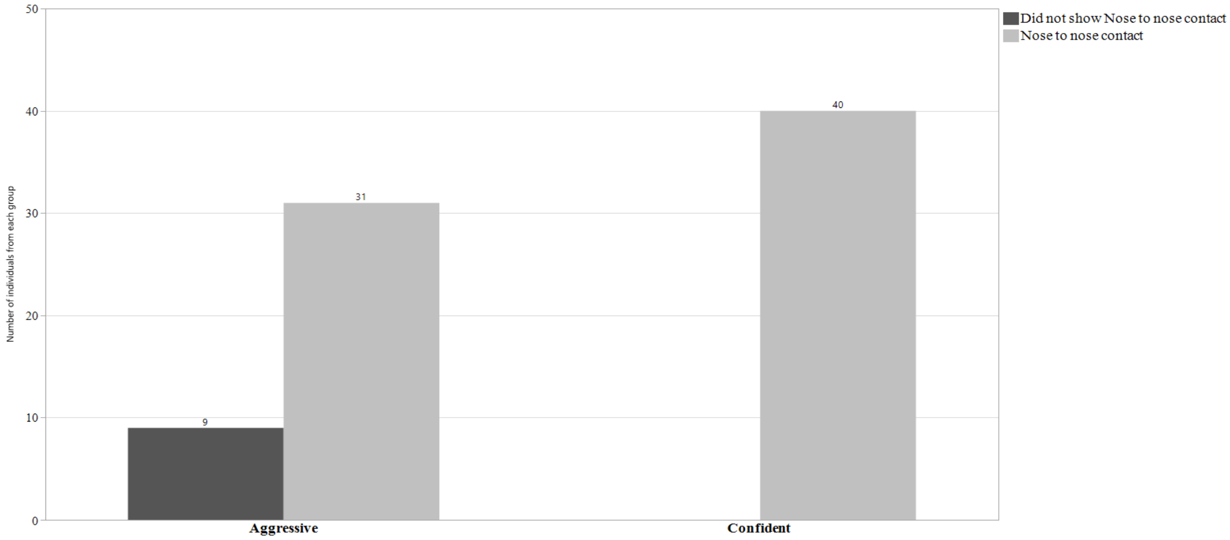

Figure 10. The duration of exploration of environment (sec, mean ± SE) where the mink would not focus on the stimulus animal during the 5 min social test for mink from the different groups. Confident mink used significant more time to explore the environment.

Half out and time used in nest box

Significant differences between groups were found in *half out* and *nest box* (*half out*: $F_{1.69} = 14.3$, $P<0.001$, nest box: $F_{1.67}=30.7$, $P<0.0001$). No difference existed between sexes in *half out* behaviour ($F_{1.70} = 0.02$, $P= 0.899$) (Figure 11), but sex had effect on time used in *nest box* ($F_{.1.67}= 6.05$, $P= 0.017$; CF= 36.8 ± 5.36, CM= 20.4 ± 4.39, AF= 59.7 ± 4.52, AM=51.4 ± 5.56) (Figure 12).

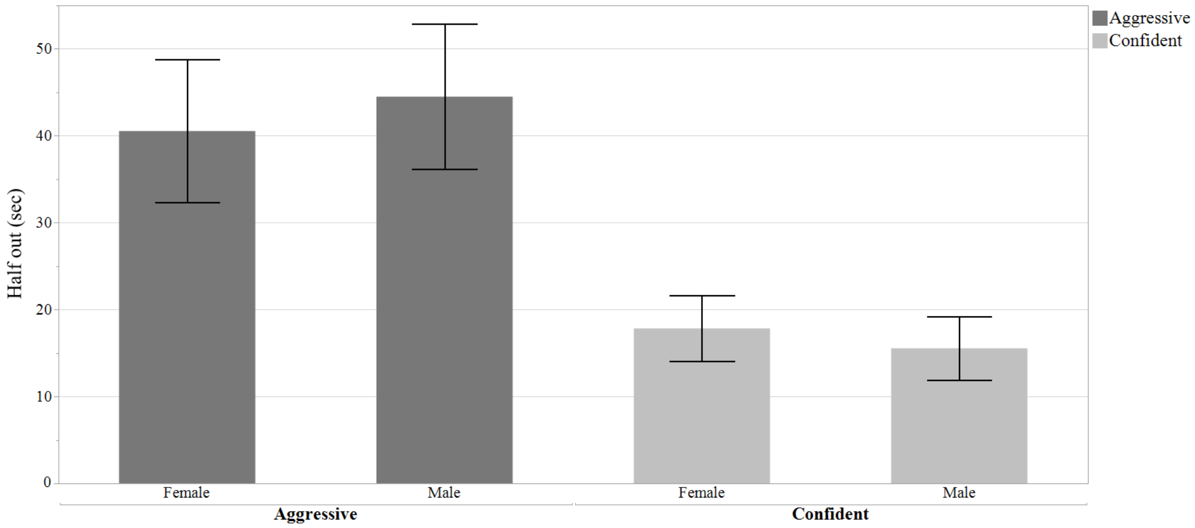

Figure 11. The duration (sec, mean ± SE) of half out behaviour observed in mink from the two groups during the 5 min social test. Confident mink used significant less time on half out behaviour.

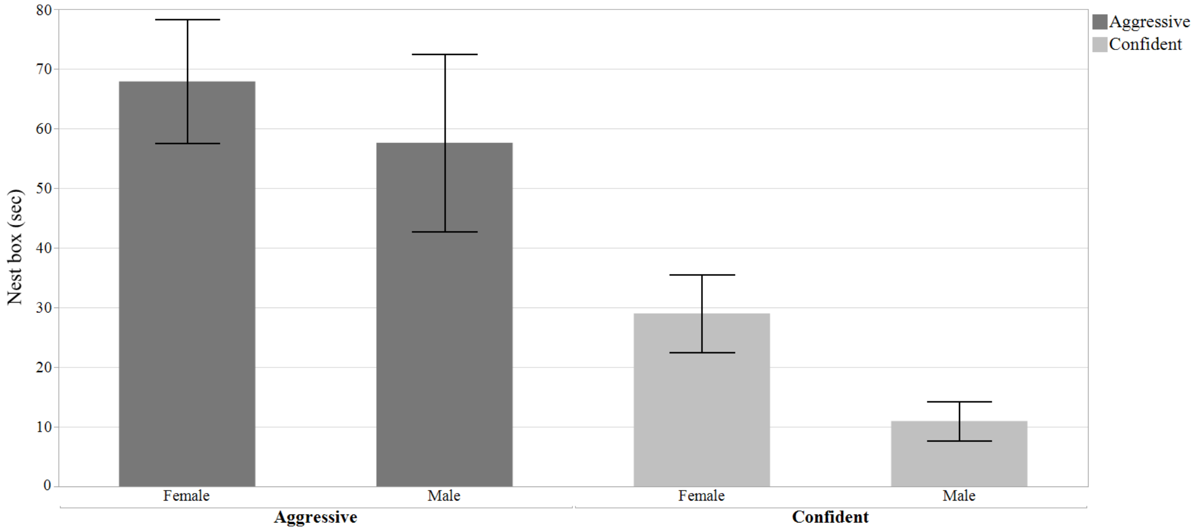

Figure 12. The time used in nest box (sec, mean ± SE) observed in mink from the two groups during the 5 min social test. Confident mink used significant less time in the nest box.

There was a significant negative correlation between latency to contact the stimulus mink and the outcome of the hand test score (Spearman rho = -0.58, P<0.0001) (Figure 13). The mink that scored low on the hand test, would use significant longer time to approach the unknown stimulus mink (Figure 14).

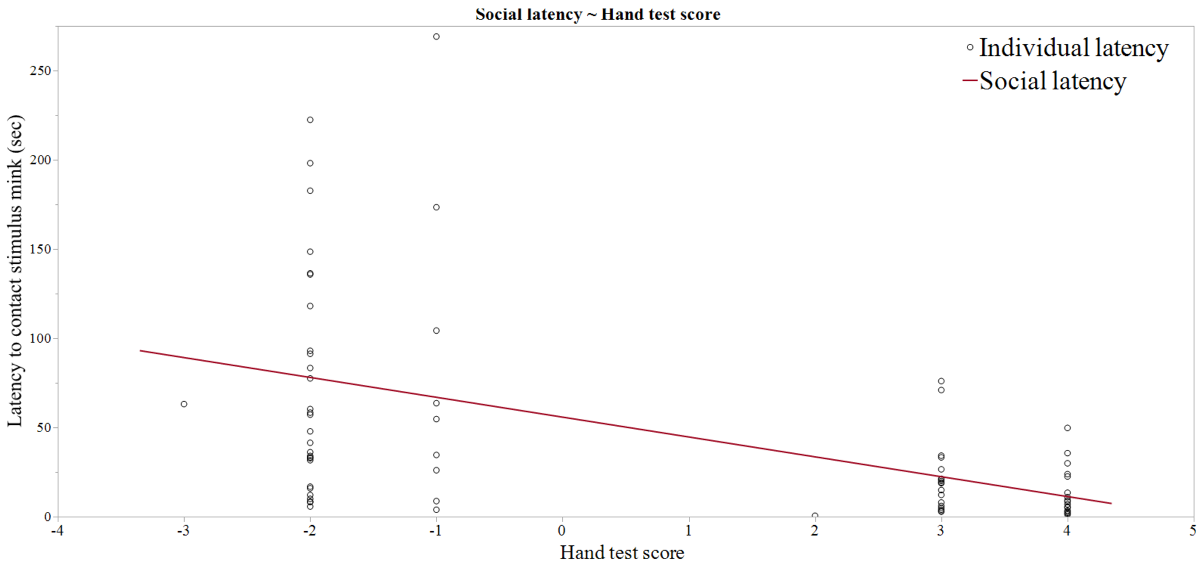

Figure 13. Correlation between latency to contact (Y-axis) hand test behaviour score (X-axis). The graph shows scatterplot of individual latency to contact stimulus mink and the regression line which shows how the hand test score affects latency to contact the stimulus animal in the 5 min social test.

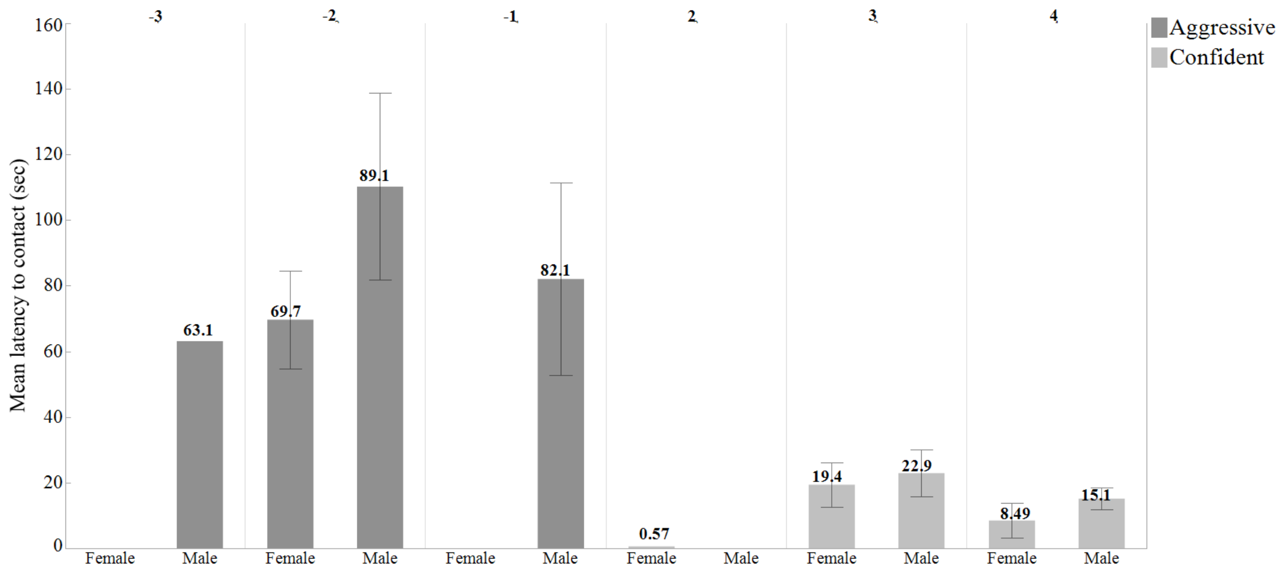

Figure 14. Latencies (mean sec ± SE), to mink's reaction in social test to make contact with a male stimulus mink sorted by Trapezov hand test score (given in the upper row of the figure) and sex.

4 Discussion

Overview

The aim of this study was to examine whether mink classified as confident, would have different intraspecific social motivation and behaviour, compared with mink showing reluctance to interact with a human, the aggressive mink. The study demonstrates a likely link between confident response towards a human and motivation to establish positive contact with conspecifics in the social test. The method comprised a social test measuring the two groups' approach to an unfamiliar male mink, as well as analysis of their agonistic and social behaviour. The results indicate that confident mink will have an overall more positive social interaction with other mink. Noteworthy, the analysis of *Exploration of environment* suggests that Confident mink also explored more in general, exhibiting more *Seeking* behaviour, possible indicating that confident animals have more impulses to investigate and make sense of the environment (Panksepp, J., 2005). Exploration will be discussed in more detail later. Confident and Aggressive mink differed in all the measured behaviours, suggesting that within the same population on a farm; the confidence level is determined by several factors and not only the environment.

In the current study, the model had different descriptive power for each behaviour during the five-minute social test, although Group had significant effect in all observed behaviours. The results suggest that the Group effect explained most of the variation in the *Nose to nose* test, time used in *Nest box* and *Agonistic behaviour* (*Table 7*), where the first these is the behaviour that reflects social motivation the most, the second is associated with fear or indifference towards the stimulus mink and the last is the behaviour that is closely linked to aggression. The fact that these three behaviours are the ones where Group explains the larger part of the variation in the model, further confirms the hypothesis and predictions of this study. *Half out* behaviour was the behaviour which could be most poorly predicted by the model, although Group had a significant effect, suggesting that there are several other factors that affects this behaviour. It is possible that the set-up of the cages has affected the *half out* behaviour; to be discussed later in the "limitations" section.

Approach and positive social exploration

Consistent with the predictions, the results also show that Confident mink were faster to approach and spent more time with the stimulus mink outside of nest box, compared to the Aggressive mink. This is consistent with the conclusion of Malmkvist and Hansen's (2002) study on social motivation in two different breeding lines of mink selected for confidence and fear towards humans for 10 generations.

The analysis implies that being an *Aggressive* mink gives an estimated latency to make contact with the stimulus mink of about 18 seconds, compared to *Confident* mink. When an animal is reluctant to contact the stimulus, it might be because it considers the stimuli as threatening, i.e. due to fearfulness, or it may be indifferent to the stimuli, either way, it indicates that the test animal does not have social motivation. Fearfulness is a psychological trait in individuals that predisposes them to perceive and react in similar manners to a wide range of potentially frightening events (Boissy, 1995), hence, if an animal is fearful towards a human, it will most likely also be more fearful towards other novel stimuli. In line with the hypothesis, the lowest levels of conflict behaviour were observed in the *confident* group. Thus, the analysis indicate that Confident mink and Aggressive mink had divergent motivation to seek social interaction. However, latency to make contact with the stimulus animals does not measure the sociability on its own. To make sure that the mink's latency to make contact with another conspecific is due to social motivation, the latency needs to be examined with the subsequent positive behaviours, such as *social exploration* and *nose to nose contact,* as well as negative behaviours, such as *agonistic exploration* and *half out* and *nest box*. And as expected, Confident mink responded to the stimulus animals with less agonistic exploration and reluctance to investigate and more curiosity and explorative behaviour, as well as more frequent *nose to nose contact*.

Aggressive mink had less frequent contact with stimulus mink in both *social exploration* and *nose to nose contact.* The findings show that Confident mink spent on average more time on *social exploration,* compared to Aggressive mink. However, social exploration might just be an indication that *confidence* in animals is just the absence of fear, therefore the amount of actual positive interaction is important to include. *Nose to nose contact* is considered a strong indicator for positive social interaction, and Confident mink spent on average significantly more time in *nose to nose contact* compared to the *aggressive* mink. *Nose to nose* contact is not accomplished unless the stimulus animals also interact with the test mink, so the test mink would have to show non-threatening behaviour and a calm state. The mean duration shows that the Confident mink spent significantly more time on nose to nose behaviour compared to the Aggressive mink.

Agonistic exploration

Only 21 mink showed agonistic exploration, of which 18 were from the Aggressive group (86%). Both groups had more agonistic exploration among females (Aggressive N= 11, Confident N=3). The few occurrences in general might be due to the fact that all the mink were juveniles and will thus naturally be less aggressive than territorial adults.

The mink used were juveniles and not sexually mature and therefore it may be presumed that reproductive motivation would not interfere significantly with any of the behaviours studied. This could explain the tendency for no significant effect of sex in any of the groups, except for the trait's *social latency* and *nest box.* These two behaviours are likely linked to fear, as the mink's latency to contact the stimulus animals is initiated by the drive to explore, and exploration behaviour is supressed by fear (Hughes, 1997; Montgomery, 1955). In nature, male mink has to occupy large territories in order to access females to mate and one male territory can contain several smaller female territories (Birks & Dunstone, 1985; Dunstone, 1993; Thirstrup et al., 2019). Therefore, evolutionary, males with reduced fear and higher exploratory behaviour is likely to have advantageous in finding mates and reproduce successfully, hence males tend to be less inhibited by fear. It could be argued that since stimulus animals used in the social test were males, females on average used the nest box more due to male dominance and that females thus also tend to be reserved to approach, but the results contradict this theory, since Confident females had the shortest *latency* to contact.

Exploratory behaviour

The data suggest that the Confident group showed generally more positive exploratory behaviour, both social and towards the environment, which is consistent with previous studies which have shown that confident mink exhibit more exploratory behaviour (Malmkvist & Hansen, 2002). When encountered with an unknown mink, *confident* mink is not inhibited by fear and the anticipation of danger, thus it will not only focus on the stimulus mink but investigate the environment as well.

Confident mink had higher frequency in both of the positive social behaviours (*social exploration* and *nose to nose*) measured and lowest frequency in the negative associated behaviours (*agonistic exploration, half out* and *nest box*) and it demonstrates a tendency that the Aggressive animals are inhibited to approach due to fear. Malmkvist and Hansen (2002) have suggested that fearful mink change their behaviour more due to behavioural conflict. The present study does not support that theory, and the observed higher frequency of shifting behaviour may also just reflect exploration and curiosity in general and that overall the Confident group explore more, i.e. *social exploration* and *exploration of environment*.

In line with the hypothesis set in this study, the findings provide evidence that mink categorised as confident towards man, did perform significantly different in the social test. Confident mink in the present study had some social-behavioural characteristics, like shorter latency to contact stimulus mink, fewer mink showed *agonistic exploration*, less time in the *half out* and *nest box* position and more *social exploration*. Curious behaviour and friendly approach such as *Nose to nose*, were observed in all the Confident mink. Close proximity and lower outcome of aggression or fear has been concluded to be a consistent measure of social tolerance (Fichtel et al., 2018). This present study shows that Confident mink had more close encounters with the stimulus mink without aggression or fear compared to individuals in the Aggressive group, therefore this study suggests that social tolerance is higher in confident mink.

In addition, this study demonstrates a correlation between confidence and exploratory behaviour, on the grounds that the Confident group would use more time investigating the cage, and other elements in the environment, than the Aggressive group. It seems that exploration in mink is affected by fear, or rather the absence of it (Hughes, 1997; Montgomery, 1955). The parts of the brain that contains dopamine, is the most responsive to the expectation of reward, and unexpected rewards or positive novel stimuli increases the chance of dopamine levels rising (Panksepp, J., 2005; Panksepp, Jaak, 2005; Savory & Maros, 1993). In this study, the behaviour *Exploration of environment*, seems to capture this, as the confident mink were in general more explorative. One explanation can be that with decrease in fear and increase in confidence, exploration in general is increased, hence the confident mink will spend time, not only focusing on the stimulus animal, but to focus on the environment as well. To be able to focus on other elements in the environment would imply that the actual experienced threat, by having a stranger in sight, is decreased in the confident mink.

Social Plasticity

All species that have flexible developmental plasticity have and adaptive advantage to changing selection pressures. Wild populations adapt through developmental and evolutionary processes, where some individuals become social, others solitary, whereas in domestication processes, where humans are the selective force, social traits tend to be more beneficial and hence subject to stronger selection (Koene & Ipema, 2014). However, species with few pre-adaptations to captive environments, requires great changes in selective pressures, both regarding direction of selection, the selection intensity and number of traits affected (Price, 2002).

Selective breeding experiments in foxes (Belyaev, 1979; Trut et al., 2009), quail (François et al., 1998) and rats (*Rattus norvegicus*)(Albert et al., 2009; Blanchard et al., 1994) have shown that levels of defensive reaction and fearfulness to a large part can be accounted for by genetics. An obvious fact is that it is not possible to select a trait that does not exhibit genetic variation in a species, hence, these species have to have had some genes coding for tameness. Wild mink is a solitary species without any obvious predisposition to thrive with humans or other conspecifics company. Yet, selection experiments in mink has shown that behavioural changes and more tameness towards humans are obtainable, thus there is likely a genetic predisposition for these traits within mink. (Hansen, 1996; Hovland et al., 2019; Kizhina et al., 2017; Ring et al., 2018; Thirstrup et al., 2019; Trapezov, 2000).Therefore, despite having solitary ancestors and few pre-adaptions, tameness in mink is indeed inheritable, suggesting that solitary behaviour is not an evolutionary fixed trait or character.

Another Mustelidae that has been domesticated is the ferret (*Mustela putorius furo*), which has been subject to selective breeding. The ferret originates from the European polecat (*Mustela putorius*), and the polecat is solitary and territorial, whereas the ferret is social with humans and conspecifics, although they should be housed in same-sex groups (Bament, 2013). Hernádi et al. (2012) found evidence that the domestic ferret is more comparable to dogs than their wild counterparts. In their study, using 17 domestic ferrets, 16 hand reared wild Mustela hybrids and 18 domestic dogs, both the domestic ferret and domestic dog would establish eye-contact with owner, and not the experimenter, and choose food from the owner's hand rather than the experimenter. The authors thus implied that the ferret, through domestication, has developed mechanisms that adapted them to have close social relationship with humans and *dog-like-social-cognitive skills* (Hernádi et al., 2012). These results should be taken into account when speculating if tameness is only innate in animals that to this day show social motivation and tameness.

All of the mink in the present study were juveniles and from the same population, where several individuals from each "Group" had the same father and two females (Confident and Aggressive) were even fullsibs. Since behaviour is also influenced by non-genetic factors (Alemu et al., 2016), such as early life history and the effect of mother and habituation after weaning, the level of confidence in an individual is not only inherited genetically. Moreover, a previous study on rats found that the quality of maternal care affects offspring fearfulness (Francis et al., 1999), although this is not examined in mink.

Limitations

Great effort should be made to decrease the large non-explained variation in a study. But the layout of the experimental cages in this study differed somewhat from the ones used in previous similar studies (Hovland et al., 2019; Malmkvist & Hansen, 2002). Malmkvist and Hansen (2002) used standard cage (90 x 30 cm and 45 cm high) connected to a nest box at the very end; and Hovland et al. (2019) used cages with similar measurements (90 x 45 x 45) connected with a nest box at the end. The present study on the other hand, had the nest box covering a larger part of the cage’s length, giving the mink only 34 cm from nest box to stimulus cage. This have most likely affected the *half out* behaviour in the test animals, as they could investigate the stimulus mink quite close without leaving the nest box. Our test cage also had walls and sections that made it difficult to examine zones and time used in the different zones, as Malmkvist & Hansen (2002) and Hovland et al. (2019) did.

The effect of stimulus animal in the Mixed model often had negative variance components, which might suggest that the effect of individual stimulus mink is a dependent variable. The stimulus mink were all juvenile, were selected on the basis of the hand test score and got two days to habituate to the temporary housing used during the social test weeks. But to reduce the effect of each stimulus animal, it is preferable to copy Malmkvist & Hansen (2002): Their stimulus mink were 1.3 years of age and were trained in similar situations with opponents from both sexes for 44 min each, distributed on 12 visits over two days (Malmkvist & Hansen, 2002).

The stick test was used as a part of the screening to select the most confident and aggressive individuals. But the mink that were screened were housed together, making it difficult to measure the actual score of their response to the stick. The result from the stick test was therefore not used to select test animals. This uncertainty regarding the stick test result is confirmed in a report by (Henriksen et al. (2017) who emphasise how important it is to perform the stick test in a certain way to actually get representative scores.

Implications

Hovland et al. (2019) found that there is a significant difference between mink selected for aggressiveness towards humans and the line selected for tameness, and the difference in behaviour was thus explained by genetic differences. The conclusion was that it is possible through selective breeding to achieve increased confidence and social motivation within an animal population. These two lines had been selected for 17 generation and Hovland et al. (2019) had a much higher average hand test score in their confident group (CM: 5.61 ± 0.91, CF: 5.20 ± 0.70), compared to the present study (CM: 3.55 ± 0.51, CF: 3.40 ± 0.60). However, the analysis from the present study in a single population without intense selection for confidence, indicates that hand test score with an average around +3 is enough to observe increased social motivation between confident mink and aggression. Thus, traits such as positive social intraspecific interaction and less aggression may be achieved when selecting confident animals. These results should be taken into account when considering how to increase animal welfare in farmed animals. Animal that cope well with human handling and group housing will have reduced levels of fear and distress, which is vital to secure good animal welfare and a requirement in "The Five Freedoms" for all animals (Conklin, 2014). Furthermore, a recent study from Denmark has found that selection for more confident mink will not have any negative effects on important fur production traits, such as pelt quality, reproduction or pelt size (Thirstrup et al., 2019). Hence, it should be possible to include selection for more confident mink in the overall breeding goal.

5 Conclusion

Mink categorised as Confident, were faster to approach and spent more time with the stimulus mink, compared to the Aggressive group. Also, as Confident mink also showed less fear and more *nose to nose* contact towards the stimulus mink, our results support the hypothesis that mink with higher degree of confidence towards humans have more social motivation and more positive behaviour towards conspecifics. Therefore, this study also favours the hypothesis that the animal's urge to approach and calmly interact with humans, likely has its biological basis in motivational systems controlling intraspecific social behaviour as well. Confident mink has increased social motivation in novel social interactions, thus may be more predisposed to cope well with social housing. An overall increased confidence in farmed mink populations may thus result in improved animal welfare.

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Appendix 1

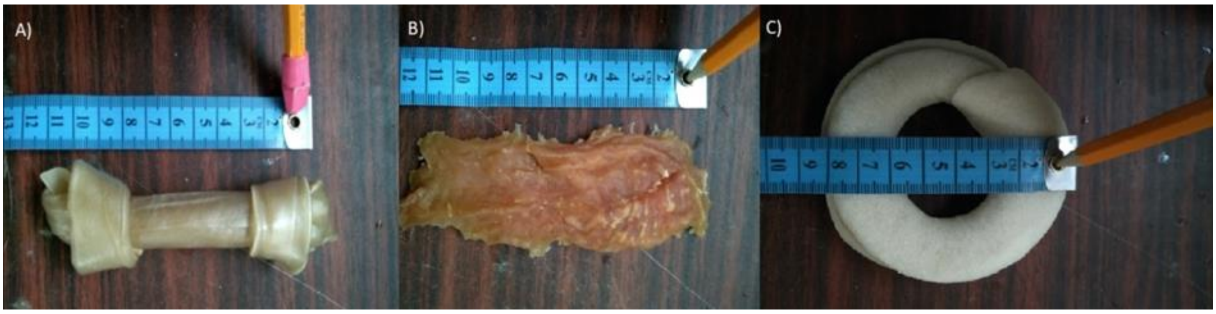

Photo: The treats given to stimulus animals and measures (cm). A) dog bone, B) dried chicken and C) knot from cattle skin.

Appendix 2

Duration (sec, mean ± SE) of each trait observed during the 5 min social test

	Confident		Aggressive	
Behaviour	**Female**	**Male**	**Female**	**Male**
Social latency	13.6± 4.25	18.6 ±3.71	69.7± 14.8	95.2± 19.2
Agonistic exploration	1.26 ± 0.89	0.98 ±0.98	21.6±6.74	10.3±5.42
Social exploration	170 ± 10.2	177 ± 11	124 ± 14.3	132 ± 14.4
Nose to nose	21.71 ± 2.96	17.53 ± 2.23	7.83 ± 1.68	7.81 ± 1.66
Exploration of environment	59.6 ± 7.92	76.9 ± 9.9	35.6 ± 6.05	45.9 ± 8.87
Half out	17.8 ± 3.76	15.6 ± 3.65	40.6 ± 8.24	44.5 ± 8.33
Nest box	29 ± 6.5	11 ± 3.3	67.9 ± 10.4	57.6 ± 14.9

Appendix 3

Selection for tameness towards humans and effects on conspecific social motivation in farm animals: domesticated mink (*Neovison vison*) as a model

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Abstract

There are serious welfare challenges linked with group housing of farm animals due to consequences of aggression. In addition, revealing the factors determining harmful behaviours proves difficult. However, an important feature of animal domestication deserving attention in the discussion on social dynamics is the concept of confidence and tameness. There is a general perception that domestication alters farm animals' social behaviours, but only a few studies indicate that selection for tameness may have correlated effects on animals' social tolerance. If there is a link between these two traits, improved selection for tameness may not only reduce fear towards humans but also positively affect social dynamics in animal groups through reduced aggression and increased tolerance towards conspecifics. To investigate whether selection for tameness affected animals' social motivation 80 farmed mink from two selection lines; one line selected for tameness (N=40, 20/20 male/female) and one aggressive control line (N=40, 20/20 male/female), were chosen as a model animal. Mink from both lines were examined for their motivation to approach a stimulus mink in a 5 min social motivation test. Sixteen highly confident female mink was used as opponent stimulus animals. The test minks' latency to approach, their time spent in proximity to and their social behaviour towards the stimulus mink were recorded. Mink selected for tameness were faster to approach ($P \leq 0.0001$) and spent more time with ($P=0.045$), the stimulus mink compared to control mink from the aggressive selection line. These results, in addition to the finding that confident mink also showed less agonistic exploration ($P=0.014$) and more social sniffing ($P \leq 0.0001$) towards the stimulus mink supports our hypothesis that selection for tameness modify minks' social motivation and behaviour towards conspecifics. To explain this link, we argue that tameness, the animal's drive to approach and calmly interact with humans, likely have its basis in motivational systems governing intraspecific social behaviour. The experiment provides a new insight into the relationship between increased tameness and the correlated effects on intraspecific social behaviour in animals. Our study needs replication that includes physical interaction tests to further examine the effects on social tolerance. However, our findings are intriguing, not only from a basic biological perspective but also as a contribution to our knowledge on factors potentially affecting farm animal social dynamics.

Appendix 4

F-statistics

Behaviour	Nparm			DF			DFDen			F Ratio			Prob > F		
	Group	Sex	Group*Sex	Group	Sex	Group*Sex	Group	Sex	Group*Sex	Group	Sex	Group*Sex	Group	Sex	Group*Sex
Social latency	1	1	1	1	1	1	70.18	74.04	72.851	30.732	6.047	0.667	<.0001	0.096	0.417
Agonistic exploration	1	1	1	1	1	1	67.39	67.28	72.11	17.548	1.921	0.763	<.0001	0.170	0.385
Social exploration	1	1	1	1	1	1	67.89	67.91	72.74	12.615	0.361	0.001	0.0007	0.550	0.973
Nose to nose	1	1	1	1	1	1	66.77	65.97	68.71	46.571	0.785	0.227	<.0001	0.379	0.636
Exploration of environment	1	1	1	1	1	1	66.47	66.06	70.7	10.665	2.686	0.194	0.0017	0.106	0.661
Half out	1	1	1	1	1	1	69.34	70.58	75.42	14.311	0.016	0.418	0.0003	0.899	0.520
Nest box	1	1	1	1	1	1	67.33	66.68	69.95	30.73	6.047	0.667	<.0001	0.017	0.417

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