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Generalization of fear in farm mink, *Mustela vison*, genetically selected for behaviour towards humans

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Mink offspring from two genetic lines, selected over 10 generations for confident (C) or fearful (F) reaction towards humans, were exposed to six different tests. The aim was to investigate whether this behavioural selection in mink has affected their reaction in other potentially fear-eliciting situations. A total of 192 naïve mink, males and females, were tested over 6 weeks. C-mink had a shorter latency to get near and establish exploratory contact with a human than F-mink. F-mink maintained 6–10 times the distance to a human than C-mink. Similarly, C-mink had a markedly shorter latency than F-mink to approach and make contact with a novel object. C-mink also manipulated the object sooner and more often. In encounters with unfamiliar mink, C-mink were quicker to approach and establish nonaggressive contact than F-mink. C-mink had a shorter latency than F-mink to enter tubes within an X maze, and were more likely to visit these tubes. In contrast, F-mink made the most visits to other parts of the maze; number of visits may not, however, reflect just exploration. When presented with novel food, F-mink changed their behaviour more often than C-mink, indicating a higher degree of behavioural conflict. C-mink were also less hesitant than F-mink to approach and eat the novel food. In conclusion, offspring from a confident breeding line reacted with more exploratory behaviour than offspring from a fearful breeding line. Mink lines selected for behaviour towards humans thus generalized their fear responses across several social and nonsocial situations.

In large-scale animal production, such as farming of mink, individuals may be handled only sporadically. This situation weakens the basis for reducing their reactivity by habituation. Nevertheless, during the production year, it is sometimes necessary to handle farm animals, for example during mating, weaning and medical treatment. Besides handling, farm animals are exposed to other social and nonsocial changes, resulting from relocations, mixing with new animals and fluctuations in feed composition. Therefore, an animal's inherent characteristics of fearfulness (i.e. its threshold level for experiencing fear) and adaptability seem important, affecting its final welfare in captivity.

Approach and avoidance are thought to reflect the fear level of an animal (Gray 1987; Oliverio & Castellano 1990; Hughes 1997). The extent to which exploration towards a given stimulus occurs depends in part on how much it is inhibited by fear (Hughes 1997), and novelty may invoke competing states of fear and curiosity (Russell 1983). Surveys on Danish farms show a range in variation in the fear-related reactions of mink to humans. An early study reported that on average 47.9% of 1128 mink from 22 farms reacted fearfully (Hansen & Møller 1988), whereas this proportion was lower, 23.0%, in a recent study with 1768 mink on six farms (Hansen & Møller 2001). In one randomly selected line of mink, the progeny of which were tested for 8 years (275–325 mink yearly in 1992–1999, in total ca. 2400 mink), a mean of 51.3% reacted fearfully (Malmkvist & Hansen 2001). These evaluations are all based on a Stick test, where the mink's immediate reaction (approach/avoidance) to a stick presented by a human is scored in its home cage.

A long-term experiment has shown that it is possible to reduce the mink's fear level by controlled selection (Hansen 1996). This selection experiment was initiated in 1988 at the Danish Institute of Agricultural Sciences. Today, two genetic lines of mink exist, showing a high degree of either approach (Confident line) or avoidance (Fearful line) towards humans compared with an unselected control line (Malmkvist & Hansen 2001). The breeding lines originated from the same population of mink prior to the behavioural selection experiment and the observed behavioural changes in reaction towards humans are due to genetic selection rather than results of

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Table 1. Summary of the six tests used to evaluate fearful reactions in mink

Test	Stimuli	Duration	Comment
Stick test	Human	30 s	Voluntary approach
Trapezov's hand test	Human	Variable	Attempts to handle mink (see Table 2)
Novel Object test	Cube	6 min	Dimensions 10×10×10 cm
Social test	Mink	6 min	Trained male mink
Novel Food test	Cat food	10 min	Canned wet food
X-maze test	Environmental	10 min	Transfer to cage system with tubes

processes such as random drift or inbreeding (Hansen et al. 2000). Whether this behavioural selection has caused changes in the situation-specific response rather than in general fearfulness is important, because an overall reduced level of fearfulness is believed to improve the welfare of farmed mink.

Previous studies on mink from the selection lines used in the present experiment showed that confident and fearful mink reacted differently towards a novel object (Hansen 1997; Malmkvist & Hansen 1999), and towards an unknown intruder mink placed in the home cage (Hansen 1997). However, data presented in Hansen (1997) were obtained only after six generations of breeding, and a human observer was present during testing; thus the mink's reaction towards the novel stimuli could not with certainty be separated from reactions caused by human presence in this study. The study by Malmkvist & Hansen (1999) included only a few animals, and in both studies mink females were exposed only to a limited number of fear-releasing stimuli. In the present experiment we aimed to extend these earlier investigations on fear generalization in mink.

Other studies on generalization of reactions in farm animals towards novel stimuli have been criticized because a human observer was present during testing, which may affect the reaction of the animals, thereby impeding interpretation of the test results (Lyons et al. 1988; Hemsworth & Coleman 1998). Handling stress prior to, for example, an open-field test may similarly affect the results (e.g. silver foxes, *Vulpes vulpes*: Pedersen & Jeppesen 1990; cattle, *Bos taurus*: Munksgaard & Jensen 1996). In the present study, we minimized human contact in the tests, the majority of which did not involve a human as a test stimulus. We investigated whether 10 years (equal to 10 generations) of genetic selection in mink for reaction towards humans have affected their behaviour when exposed to a range of potentially fear-eliciting situations, including a novel object, a human, an unfamiliar mink, unknown food and a novel environment.

METHODS

Animals

We used 192 offspring from two genetic lines of the colour type 'Scanblack', selected over 10 generations for confident (C) or fearful (F) reaction towards humans. Behavioural tests used to create these distinct breeding lines since 1988, the Stick test and the Trapezov's hand test, are described in Malmkvist (1996). The adult offspring are referred to as C- and F-mink, according to their origin in these two breeding lines. The experimental animals were born around 1 May 1998, weaned at 49 days of age, and housed individually. In all other aspects they were kept under typical Danish farm conditions in a standard cage (90 × 30 cm and 45 cm high) connected to a covered nestbox (23 × 28 cm and 20 cm high), access to drinking water, and one daily man-driven machine feeding at 1100 hours ± 15 min with commercial wet mink food in amounts close to ad libitum.

After weaning, each mink was assigned a random number to replace its usual identification card. Therefore, the genetic line (C, F) of animals was unknown to the farm staff and experimenters until data collection was complete. The animals were housed in a mixed way, so that cages had alternating C- and F-mink. Experimental mink were naïve, that is they had never been exposed to excessive handling or testing, prior to the behavioural tests from 19 September to 1 November 1998.

Experimental Design

Based on an estimated minimum group size (ca. 46, if behavioural correlations of minimum 0.4 should be detected with $\alpha=0.05$ and a power of 0.80; Cohen 1988), we used a group size of 48 in a balanced 2 × 2 factorial experiment, the factors being sex (male, female) and genetic line of parents (Confident, Fearful), giving a total of 192 mink. Each mink was exposed once to each of six tests (summarized in Table 1), with at least 4 days between tests, during a 6-week period. We assigned the mink randomly to treatments, based on a Latin square design (Box et al. 1979; Miliken & Johnson 1992), so that each test was performed an equal number of times as first, second, third, fourth, fifth or sixth in the sequence of tests, with a balanced distribution of sex and genetic line. Test order therefore should not contribute to systematic or biased effects and is not considered in the statistical analysis.

Behavioural Tests

We carried out the tests between 0800 and 1500 hours, with a pause of 0.5 h before and 1.5 h after the regular feeding time at 1100 hours, except for the Novel Food test. The human tests were done by the same person with direct observation registered on a handheld computer. During the other four tests, no humans were present inside or in the vicinity of the mink shed, and recordings were made on real time video for later behavioural analysis. We chose 1 s as the smallest duration of an event to be registered from the tapes. To minimize the potential effect of, for example, social facilitation caused by mink in the two neighbouring cages, they were shut into their nestboxes during the Stick test, Trapezov's hand test, Novel Object test and Social test. This procedure also ensured that mink did not gain close up experience with the test stimuli (e.g. object, social mink) prior to their testing.

In the Novel Object and Social tests, we recorded the mink for 6 min with an automatic portable video camera on a tripod. To evaluate potential effects of handling, we divided the time before the start of the test into preparatory and waiting time. Preparatory time was the time it took to set the camera ca. 1 m from the cage and to shut the mink into its nestbox. Human presence was hereafter invisible to the mink, as the nestbox was covered. Waiting time was defined as the time from when the mink was confined in the nestbox until the metal shutter was removed, thereby allowing the mink access to the cage, with a novel object, or in the social test, another mink placed nearby.

Stick test

The mink was excluded from the nestbox, and tested in the wire cage. The test person put a tongue spatula through the net in the upper part of the front lid section, and registered the animal's reaction as: (1) explorative if the mink sniffed the stick persistently; (2) fearful if the mink escaped and did not touch the stick; (3) aggressive if the mink attacked and bit the stick; (4) uncertain if the mink showed a mixture of responses, and could not be placed in one of the first three categories. These scores are also used together with results from a human test without a stick (in September–November) each year in choosing the animals to be next year's breeders. In the present experiment, we expanded the standard procedure to include measurement of latency to make contact, number of visits and time spent in different parts of the cage (near, middle, back in relation to stick entry) and nearest distance to human. There was a fixed time limit of 30 s.

Trapezov's hand test

If it was not already in the cage, the mink was guided out. The test person put one hand (with glove) on the open front of the cage gate and moved it slowly into the cage. The reaction of the mink to handling attempts was recorded according to Table 2, modified from Trapezov (1987). Besides the scoring, we recorded the nearest distance of the mink to the human, and all occurrences of defecation, screaming and stereotypy. Test duration was variable, depending on how much the mink interacted with the person.

Novel object test

After the preparatory time, a wooden cube (10 × 10 × 10 cm) was introduced as a novel object into the home cage. A fresh cube was used on each trial, and placed ca. 60 cm from the nestbox entrance, equidistant from the two side walls. The test period of 6 min began when the metal shutter was removed and the mink again had access to its cage, now with a novel object. Table 3 gives the ethogram of behaviours recorded from the tapes.

Table 2. Trapezov's hand test scores, used in categorizing responses of mink towards human intrusion into their cage

Score	Description
+6	The mink could be handled (lifted, moved) without avoiding/biting
+5	The mink could be held around the back, but not lifted from the cage floor (i.e. no free handling), without defensive reactions
+4	After being led into the nestbox, the mink explored the hand moved in through the nestbox entrance
+3	A hand could be moved and the front legs and chest of the mink touched (hand in movement) without the mink reacting with avoidance or aggression
+2	The mink touched the hand; physical contact with its head or chest with the hand held still
+1	The mink explored at a distance (no physical contact)
0	(Start position, a hand was slowly moved into the open cage)
−1	The mink took flight and did not explore
−2	The mink took flight and screamed
−3	The mink took flight to the back part of cage (maintained maximum distance to the hand)
−4	The mink took flight to the back part of cage and screamed
−5	The mink panicked, showing for example intense flight reactions, attacks, persistent screams

Mink were given the highest score they reached.

Social test

After the preparatory time, a trained male mink (the standard opponent) in a small wire-mesh cage (35 × 28 cm and 28 cm high) was attached to the outside back wall of the home cage of the test mink. The test period of 6 min began when the metal shutter was removed from the nestbox entrance, allowing the test mink access to its cage again. Thus, the test mink could see the opponent from its start position in the nestbox and make contact with it through the wire mesh at the opposite end of its home cage. The opponent could withdraw only a few centimetres from the test mink's cage.

We chose eight individuals from a group of 10 male mink as standard opponents, based on their calmness and ease to handle in a pilot study. They were 1.3 years of age, and originated from an unselected control line of 'Scanblack' mink. One week prior to the first confrontation with experimental animals, we trained the standard opponents in similar situations with nonexperimental mink of both sexes for at least 44 min each, distributed on 12 visits over 2 days. This was done to

Table 3. Behaviour of mink recorded during the Novel Object test and Social test

Behavioural variable	Definition
Position	
In nest (O, S)	The mink was inside the nestbox, and invisible but could be audible (start position)
Half out (O, S)	At least the head and front legs of the mink were out of the nestbox, but the mink was not completely out in the wire cage
Out (O, S)	The mink had all four legs in the wire cage (O), and was not in the back of the cage (S)
Back (S)	The mink was within the rear third part of the cage, and thus near the other mink
Event	
Contact (O, S)	The mink touched the object (O) or the back wall/other mink (S) typically with snout/paws
Manipulation (O)	Object was moved (pulled/pushed/tilted) for minimum of ca. 1 cm with snout, mouth, paws or trunk
ΣContact (O)	All contacts and manipulations with object combined, as manipulation was regarded as a subclass of contact
Raid (O, S)	Accelerated running often combined with jumps directed towards object (O) or other mink (S); resembling play behaviour/sham attacks
Marking (O, S)	Rubbing ventral parts of trunk on object (O) and/or on cage wire mesh (O, S), often associated with a vibrating tail movement
Elimination (O, S)	Defecation and urination
Stereotypy (O, S)	A uniform pattern of movement apparently without purpose, e.g. somersaults or fixed pacing with head oriented towards cage wall, repeated minimum three times without interruption
Freezing (O, S)	Mink immobile in a fixed posture for a minimum of 4 s
Conflict (O, S)*	Approach followed by withdrawal (minimum 1 cm each way) with head oriented towards object (O) or other mink (S)
Screaming (O, S)*	Number of audible screams, irrespective of volume and duration
Other (O, S)	Animal not visible (in nestbox), other behaviour than those included in the scheme above, or no activity

O: Novel Object test; S: Social test.
*Only counts, no duration, recorded for this variable.

reduce the difference in stimuli presented in the social test. Each opponent was used in four trials per week, in a balanced way with a male and a female from each of the two lines (C, F), during the 6 weeks of testing. Table 3 gives the ethogram of behaviour recorded from the tapes.

Novel food test

Testing took place at the usual feeding time at 1100 hours. Before each experimental feeding, all mink were food deprived for at least 3 h, as any refusals were removed at 0800 hours. This deprivation also took place the day before. Novel food was delivered as usual food on to the top of the cage by a man-driven feeding machine, to which the animals have been accustomed their entire life. Feeding a shed section of six cages took 6–8 s. Canned cat food was given as the unknown food in approximately the same amount as normal wet mink food. During the initial 10 min with the unknown food, mink were video recorded in real time. Table 4 gives the ethogram of behaviours recorded from the tapes.

X-maze test

In the X-maze test, mink were transported in a closed wooden nestbox. This portable box replaced the ordinary nestbox 5 days prior to the X-maze test, allowing mink to get accustomed to it. We could often restrict the mink in this box with little or no handling. During transport

Table 4. Behaviour of mink recorded during the Novel Food test

Behavioural variable	Definition
State	
In cage	The mink had at least the forepart of the body and the front legs out in the wire cage
Position	
Away	The mink was in the two-third of the cage furthest from the nestbox and the food
Underneath	The mink was in the one-third of the cage closest to the nestbox and the food. All four legs were kept on the floor of the cage
Close	The mink was in the one-third of the cage closest to the nestbox and food and was either standing on its hind legs or climbing the walls
Events	
Sniffing	The muzzle of the mink touched the food, but no chewing was observed
Eating	The mink chewed the food
Grooming	The mink licked or scratched its own body
Drinking	The mink touched the drinker with its snout
Stereotypy	A uniform pattern of movement apparently without purpose, e.g. somersaults or fixed pacing with head oriented towards cage wall, repeated minimum three times without interruption

inside this box, mink could not see the handler. In some cases we had to force the mink out into the new environment. We noted duration of catching (handling time), transport from home cage to test arena (waiting time), and use of force (pushing the animal out from the portable nestbox and into the test apparatus with a 20-cm-long plastic stick) before the X-maze test.

The X-maze apparatus consisted of four arms of equal size (148 × 23 cm and 45 cm high) perpendicular to a common central platform (23 × 23 cm), all made of wire mesh for mink cages. The top part of the central platform had a wire lid through which the mink entered. The X-maze was raised 1 m above ground level, in a closed, otherwise empty room with artificial light as the only light source. Two opposite arms contained a clear tube, whereas the other two opposite arms contained a non-transparent dark tube, secured in a fixed position in the middle of the arms. The four tubes were made of PVC with a diameter of 10 cm and length of 90 cm. The mink was fully exposed from all sides in the new environment, except when entering the two dark PVC tubes. Design and test time were based on results from an earlier test of mink's reaction in a new environment (Malmkvist 1998).

We recorded the following variables from video recordings of each individual during 10 min in the X-maze: latencies and number of visits to the two kinds of arms (with dark or clear tube), the clear tube, the dark tube and the central platform. Number of visits and latencies did not include the start position of the mink, because the first arm entry may partly depend on where the mink was placed on the central platform. Each arm in the analysis was divided into three parts of equal size, so the mink could visit a maximum of 13 areas (the central platform included). The mink was recorded as in a certain area when its front end and front legs were there.

Statistical Analysis

We analysed latencies to occurrence of events with methods for survival analysis, considering censored data, estimated using the procedure 'Phreg' in the computer software SAS (SAS Institute 1996) with genetic line and sex as the main independent variables (Allison 1995). Number of events, Trapezov's hand test scores and proportion of visits in the X-maze test were analysed by generalized linear models (GLIM) including effect of sex, genetic line, test week and interactions as independent variables. In relevant cases (Novel Object, Social and X-maze test), estimates of handling time were included in the model as well. Identity of the trained male (number 1–8) was included in the model of behaviour in the Social test. For all measures, the final model was chosen on the basis of maximum likelihood estimation, calculated by means of the procedure Genmod or in the case of normally distributed data the procedure Glm in SAS. Demand for dispersion and variance homogeneity was tested and residuals checked graphically. We occasionally used contrasts to compare groups of observations (McCullagh & Nelder 1989).

For time spent, and in cases where data failed to meet the demands of using the procedures of general/generalized linear models (GLM/GLIM), non-parametric methods such as the Mann–Whitney test (two-group comparisons) and Kruskal–Wallis analysis of variance (multiple-group comparisons) were applied (Siegel & Castellan 1988), calculated in the computer software SigmaStat (SPSS 1997). For significant results with Kruskal–Wallis ANOVA post testing included pair-wise comparisons by Dunn's method (PDIFF option in SAS). Frequencies of animals in different score classes were compared with the χ^2 test between groups (Zar 1984), and correlations between variables tested by Spearman rank order correlation or Pearson correlations (Siegel & Castellan 1988). All statistical tests used are two tailed. Mean are given ± SE, medians with 25 and 75% quartiles.

Not all results include observations from the 192 animals originally assigned to the experiment, one reason being the death of two experimental animals (one male and one female C-mink) and technical failures during the test period. At a post mortem examination hypertrophic fatty liver was found in the dead male and diarrhoea in the dead female. Only behaviour observed in at least five individuals was subjected to further statistical analysis for the Novel Object test (i.e. elimination, N=2, stereotypy, N=4, freezing, N=0 excluded) and the Social test (i.e. raid, N=3, marking, N=1, elimination, N=0, stereotypy, N=2, freezing, N=0 excluded). More F- than C-mink screamed during the Novel Object and Social tests. However, since we could not always decide from the tapes whether screaming originated from the mink under test, or from its neighbours, we did not analyse it in detail.

Note that we tested mink offspring only from the unique breeding lines present at the Danish Institute of Agricultural Sciences; that is, the data are treated statistically within this population and there was no replication of the behavioural selection experiment.

RESULTS

Stick Test

C- and F-mink differed in all scores (Explorative: χ^2_1=54.568, P<0.001; Aggressive: χ^2_1=5.052, P=0.023; Uncertain: χ^2_1=22.809, P<0.001; Fearful: χ^2_1=71.614, P<0.001, N=191; Fig. 1). Within the C-mink, significantly fewer males than females were fearful (χ^2_1=4.375, N=95, P=0.029). Mink from group C had a median latency of 6 s (25–75% quartiles 2–28) to establish exploratory contact with a human (survival analysis: χ^2_1=24.987, N=191, P<0.001; Fig. 2a), and 77.9% of C-mink approached the stick. C-males made contact with the stick significantly sooner (median 3 s) than C-females (median 9 s; survival analysis: χ^2_1=5.654, N=95, P=0.017). The sexes did not differ in latency to approach or make contact in F-mink (survival analysis: χ^2_1<0.001, N=96, P=0.994), reflecting the fact that only 1.0% of mink in the F-group came near the stick within the test time of 30 s. F-mink maintained a larger mean minimum distance to the stick (62 ± 2 cm) than C-mink (6 ± 2 cm; Mann–Whitney: Z= − 11.03, N_1=95, N_2=96, P<0.001), with no sex difference (C-mink:

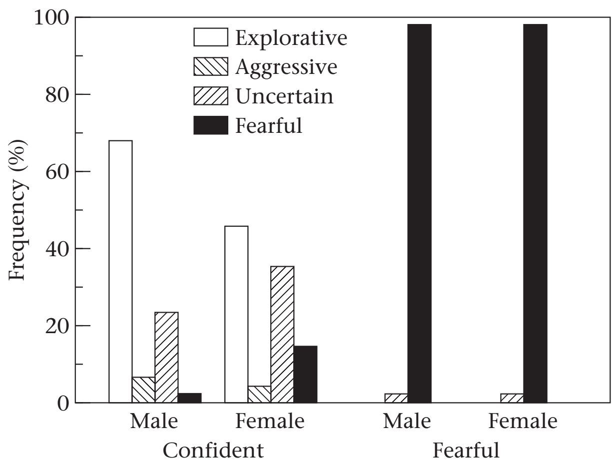

Figure 1. Percentage of mink showing explorative, aggressive, uncertain and fearful behaviour in the Stick test. N=191 mink.

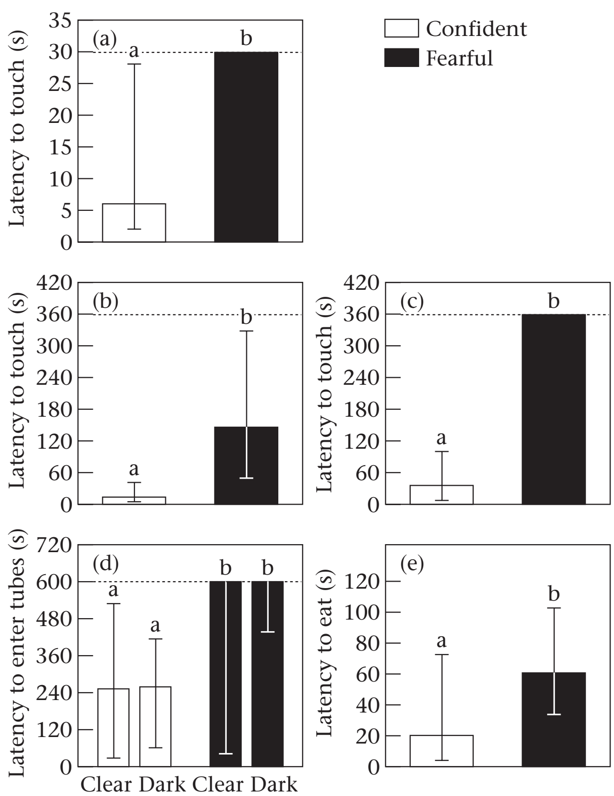

Figure 2. Latencies to mink's reaction in (a) Stick test, (b) Novel Object test, (c) Social test, (d) X-maze test and (e) Novel food test. Medians and 25–75% quartiles are given. Bars with different letters differ significantly (P<0.05), in tests of offspring from confident (N=90–95 mink) and fearful (N=92–96 mink) breeding lines. The dotted line indicates the test time.

$Z=-1.28$, N_1=47, N_2=48, P=0.197; F-mink: Z=0.71, N_1=48, N_2=48, P=0.293).

The genetic lines differed in the number of visits and time spent in different parts of the cage (number of visits: near: GLIM: $\chi^2_{1,189}$=234.22, P<0.001; middle: $\chi^2_{1,187}$=71.38, P<0.001; back: $\chi^2_{1,187}$=150.14, P<0.001; time spent: near: Mann–Whitney: Z=10.68, N_1=95, N_2=96, P<0.001; middle: $Z=-6.02$, N_1=95, N_2=96, P<0.001; back: $Z=-11.08$, N_1=95, N_2=96, P<0.001). C-mink visited areas near the human more frequently (2.8 ± 0.16 visits) and for longer (15.1 ± 0.83 s) than F-mink (0.3 ± 0.06 visits, 1.1 ± 0.34 s; visits: $\chi^2_{1,189}$= 234.22, P<0.001; time spent: Mann–Whitney: Z=10.68, N_1=95, N_2=96, P<0.001). In this test, females visited the middle and back of the cage more frequently than males, in both the C- and F-group (C-mink: middle: $\chi^2_{1,83}$=39, P<0.001; back: $\chi^2_{1,93}$=17.97, P<0.001; F-mink: middle: $\chi^2_{1,94}$=5.23, P=0.022, back: $\chi^2_{1,94}$=5.19, P=0.023). This may indicate that females more actively sought to avoid the human, while the males were more stationary. In C-mink, males spent more time in contact with the stick than females (13.2 ± 1.31 versus 8.4 ± 1.21 s; Mann–Whitney: Z=2.60, N_1=47, N_2=48, P=0.009), whereas the sexes did not differ in time spent in different cage areas for F-mink ($Z=-0.52$, N_1=48, N_2=48, P=0.865).

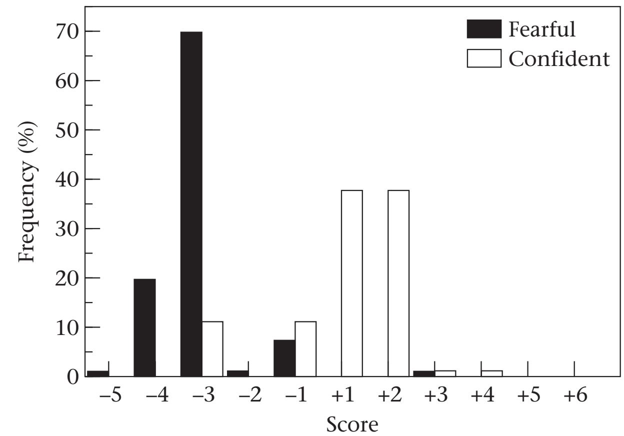

Figure 3. Trapezov's hand test. Percentage of offspring from confident (N=90 mink) and fearful (N=96 mink) breeding lines reaching different scores, where negative scores indicate avoidance of, and positive scores interaction with, a human (see Table 2 for definitions). One aggressive fearful mink was scored −5, and five confident mink were not scored because of aggression towards the handler.

Trapezov's Hand Test

The median score for C-mink was +1 (25–75% quartiles +1–+2), and higher (GLIM: $\chi^2_{1,184}$=95.24, P<0.001) than the score of F-mink: −3 (−3–−3; Fig. 3). The two sexes did not score differently ($\chi^2_{1,162}$=0.22, P=0.638). A few mink reacted aggressively in this test: 5.3% (5/95) of C-mink and 1.0% (1/96) of F-mink, but aggression did not differ significantly between the genetic lines (chi-square test: χ^2_1=2.709, N=191, P=0.100).

C-mink kept a shorter mean minimum distance of 12 ± 3 cm to humans than F-mink (69 ± 2 cm; GLM: $F_{1,184}$=321.40, P<0.001), whereas the distance did not depend on sex ($F_{1,167}$=0.91, P=0.341). Some F-mink (25.0%), but no C-mink, screamed during the Trapezov's hand test, with no difference between males and females (GLIM: $\chi^2_{1,167}$=0.89, P=0.344). In 83 out of 191 cases mink defecated during the Trapezov's hand test, and this

Table 5. Number of and time spent in positions and events by confident and fearful mink in a 6-min Novel Object test (N=187)

	Confident		Fearful	
	Male	Female	Male	Female
In nest (s)	26.7±4.74[a]	37.2±4.59[a]	141.6±15.14[b]	167.7±17.78[b]
Half out (s)	8.9±1.18[a]	11.7±1.82[a]	38.6±4.98[b]	25.3±3.50[b]
Out (s)	323.7±5.37[a]	310.7±5.71[a]	174.4±17.52[b]	165.4±18.06[b]
No. of contacts	23.3±1.11[a]	19.0±0.90[a]	9.6±1.18[b]	8.9±1.21[b]
Time spent in contact (s)	195.3±6.93[a]	205.9±6.53[a]	97.1±11.82[b]	98.1±11.77[b]
No. of manipulations	11.8±1.04[a]	7.8±0.83[b]	2.3±0.69[c]	0.9±0.34[d]
Time spent in manipulation (s)	54.6±5.19[a]	30.8±3.87[b]	9.0±2.79[c]	3.0±1.35[c]
No. of Σcontact	13.5±0.70[a]	12.6±0.57[a]	7.8±0.83[b]	8.2±1.04[b]
Time spent in Σcontact (s)	249.9±8.21[a]	236.7±7.44[a]	106.1±13.48[b]	101.1±12.29[b]
No. of raids	3.2±0.62[a]	3.9±0.88[a]	0.2±0.12[b]	0.0±0.04[b]
Time spent in raid (s)	8.8±1.95[a]	8.5±1.95[a]	0.4±0.30[b]	0.1±0.09[b]
No. of markings	0.2±0.07[a]	0.4±0.14[a]	0.0±0.02[b]	0.0±0.04[b]
Time spent in marking (s)	0.4±0.19[a]	0.6±0.25[a]	0.0±0.02[a]	0.2±0.13[a]
No. of conflicts	4.2±0.95[a]	6.3±1.21[b]	19.6±2.81[c]	17.7±2.34[d]

Means are given±SE. Values within a row lacking a common letter differ (P<0.05). Behaviours observed in fewer than five animals are not included. See Table 3 for definitions of behaviours.

was observed more frequently in F-mink than in C-mink ($\chi^2_{1,187}$=36.34, P<0.001), with an interaction between genetic line and sex ($\chi^2_{1,187}$=6.15, P=0.013). One animal (an F-female) showed stereotypical behaviour during the test session.

Plots did not indicate any changes in scores during the 6 test weeks, which were confirmed in the statistical analysis (GLIM: $\chi^2_{5,162}$=3.63, P=0.605). The distance to humans was significantly higher (GLM: $F_{5,184}$=3.54, P=0.004; post testing: T=2.409, N=64, P=0.017) on the first day, 21 September (C-mink: 31 ± 10 cm; F-mink: 74 ± 6 cm), than on the last test day, 26 October (C-mink: 2 ± 2 cm; F-mink: 59 ± 7 cm). However, the observed distance to humans did not differ between any of the other test weeks and there was no interaction between genetic line and test weeks. Significantly fewer mink screamed in the last 2 weeks than during the first 3 weeks of testing (GLIM: $\chi^2_{5,184}$=15.92, P=0.007; post testing: week 6 versus week 1: χ^2_1=3.962, P=0.046; week 6 versus week 2: χ^2_1=5.062, P=0.025; week 6 versus week 3: χ^2_1=5.062, P=0.025; week 5 versus week 1: χ^2_1=5.062, P=0.025; week 5 versus week 2: χ^2_1=3.962, P=0.047; week 5 versus week 3: χ^2_1=5.062, P=0.025).

In most cases (139/191=72.8%), the test mink was guided from the nestbox and out into the cage prior to the Trapezov's hand test, and this happened more frequently in F-mink (85.4%) than in C-mink (60%; GLIM $\chi^2_{1,189}$=16.02, P<0.001), with no difference between sexes or test weeks.

Novel Object Test

C-mink had a shorter latency to approach the object than F-mink (half out: C-mink 3 s, 2–11; F-mink: 45 s, 15–131; survival analysis: χ^2_1=88.757, N=191, P<0.001; out: C-mink: 6 s, 2–25; F-mink: 102 s, 32–269; χ^2_1=94.480, N=191, P<0.001). All C-mink and 75.3% of the F-mink touched the novel object placed in their home cage. The median latency to touch the object was shorter for C-mink (χ^2_1=104.354, N=191, P<0.001; Fig. 2b). C-mink also manipulated the object sooner than F-mink (χ^2_1=99.317, N=191, P<0.001). The latency to C-male manipulation of the object (67 s, 28–133) was shorter than for C-females (124 s, 69–242; χ^2_1=6.456, N=95, P=0.011). Otherwise, the sexes did not differ in the latencies during the test.

C- and F-mink differed in time spent in the nestbox (C<F; Kruskal–Wallis: H_3=85.086, P<0.001), half out (C<F; H_3=33.050, P<0.001) and completely out (C>F; H_3=89.910, P<0.001) in the cage with the novel object, whereas males and females did not differ in the time spent in these three positions (Table 5).

In behavioural events (Table 5), C-mink touched (typically with the snout) the object more than F-mink (GLIM: $\chi^2_{1,163}$=442.31, P<0.001). Similarly, C-mink manipulated the object more than F-mink ($\chi^2_{1,163}$=615.86, P<0.001), and males manipulated more than females ($\chi^2_{1,163}$=62.80, P<0.001). The combined ordinary contacts and manipulations (Σ contact) occurred more frequently in C-than in F-mink ($\chi^2_{1,168}$=129.08, P<0.001). C-mink performed more raids towards the object ($\chi^2_{1,168}$=384.54, P<0.001) than F-mink. C-mink rarely marked, but significantly more so than F-mink ($\chi^2_{1,179}$=22.34, P<0.001), when the novel object was present in the cage. Number of conflicts differed between the genetic lines ($\chi^2_{1,183}$=750.81, P<0.001) and sexes ($\chi^2_{1,183}$=8.59, P=0.003) with an interaction between these two main effects ($\chi^2_{1,183}$=24.98, P<0.001; Table 5).

C-mink spent much more time than F-mink (P<0.013) in contact (Kruskal–Wallis: H_3=72.484, P<0.001), manipulation (H_3=91.961, P<0.001) and Σ contact (H_3=95.031, P<0.001) with the novel object, and also in raids towards the object (H_3=54.629, P<0.001; Table 5). C-mink marked more during the test than F-mink (H_3=10.507, P=0.015). In manipulation with the novel object, C-males spent significantly more time than

Table 6. Number of and time spent in positions and events by confident and fearful mink in a 6-min Social test (N=186)

	Confident		Fearful	
	Male	Female	Male	Female
In nest (s)	48.8±12.91[a]	66.0±13.30[a]	181.4±17.58[b]	228.4±17.39[b]
Half out (s)	11.5±3.90[a]	16.7±4.01[a]	46.1±7.58[b]	34.6±4.92[b]
Out (s)	41.6±4.70[a]	48.98±5.61[a]	65.9±9.60[a]	71.36±12.91[a]
Back (s)	257.6±14.40[a]	227.7±16.15[a]	66.3±14.72[b]	24.7±9.46[b]
No. of contacts	8.8±0.72[a]	7.5±0.70[a]	2.6±0.61[b]	0.6±0.28[c]
Time spent in contact (s)	227.8±13.97[a]	196.3±15.70[a]	43.0±11.45[b]	14.4±8.59[b]
No. of conflicts	7.6±2.10[a]	14.7±2.63[b]	22.9±2.54[c]	22.6±2.68[c]

Means are given±SE. Values within a row lacking a common letter differ (P<0.05). Behaviours observed in fewer than five animals are not included. See Table 3 for definitions of behaviours.

C-females (t test: t_{91}=3.689, P<0.001), otherwise the sexes did not differ in time spent in the different behavioural events.

Effects of test week existed for latencies to positions half out (survival analysis: χ^2_1=8.676, N=191, P=0.003), out (χ^2_1=10.030, N=191, P<0.001), and the events contact (χ^2_1=8.278, N=191, P=0.004) and manipulation (χ^2_1=17.062, N=191, P<0.001); these latencies tended to decrease with increasing number of tests performed (half out: r_S= − 0.13, N=181, P=0.091; out: r_S= − 0.16, N=178, P=0.034; contact: r_S= − 0.16, N=173, P=0.035; manipulation: r_S= − 0.15, N=128, P=0.096). Furthermore, for F-mink, the number of total contacts (Σ contact) tended to increase over test week. In the last 2 weeks, F-mink had a significantly higher number of Σ contact (week 5: 8.9 ± 1.69; week 6: 12.6 ± 1.76) than in the first (4.9 ± 1.58) or second week (5.6 ± 1.15) of testing (contrast week 5 versus 1: $\chi^2_{1,82}$=18.95, N=94, P<0.001; contrast week 5 versus 2: $\chi^2_{1,82}$=12.98, N=94, P<0.001; contrast week 6 versus 1: $\chi^2_{1,82}$=53.31, N=94, P<0.001, contrast week 6 versus 2: $\chi^2_{1,82}$=43.11, N=94, P<0.001). However, the observed differences between the genetic lines in latencies and number of total contacts remained significant over the 6 weeks of testing.

The total time before testing (preparatory+waiting time) was 67 ± 2.3 s per mink, and did not differ significantly between the genetic lines (GLM: $F_{1,163}$=3.88, P=0.051), sexes ($F_{1,163}$=0.26, P=0.612) or test weeks ($F_{1,163}$=0.56, P=0.729). The mean preparatory time with human visible to the mink was 15 ± 1.8 s, and did not differ between C- and F-mink ($F_{1,163}$=1.06, P=0.305). However, the average waiting time differed between the two genetic lines ($F_{1,185}$=17.42, P<0.001), in that C-mink waited a shorter time (46 ± 1.6 s) than F-mink (59 ± 2.7 s). Based on this, waiting time within the genetic lines and preparatory time were both included in the statistical models of behavioural events. No effect of preparatory and waiting time was found for the events contact, manipulation, conflict, or marking. Σ Contact correlated positively with waiting time (r_S=0.26, N=187, P<0.001) but not with the preparatory time. Effects of preparatory time on number of raids were significant (GLIM: $\chi^2_{1,166}$=16.04, P<0.001) for both genetic lines. However, no correlation could be found for the groups of C-mink (r_S= − 0.15, N=93, P=0.138) and F-mink (r_S=0.10, N=94, P=0.361). Similarly, plots did not confirm a general trend in number of raids with the preparatory time.

Social Test

C-mink had a shorter latency than F-mink to approach the social mink (half out: C-mink: 3 s, 1–16; F-mink: 72 s, 23–213; survival analysis: χ^2_1=56.614, N=186, P<0.001; out: C-mink 6 s, 2–32; F-mink: 111 s, 29–335; survival analysis: χ^2_1=52.918, N=186, P<0.001; back: C-mink: 17 s, 4–59; F-mink: 360 s, 360–360; χ^2_1=81.023, N=186, P<0.001). The majority of the C-mink (88.3%) and 23.9% of the F-mink made contact with the opponent and the median latency to contact was 35 s (7–99) for C-mink and 360 s (360–360) for F-mink (P<0.001 between lines; χ^2_1=90.133, N=186; Fig. 2c). No differences existed between the sexes in latencies to approach (half out: χ^2_1=0.442, N=186, P=0.506; out: χ^2_1=0.023, N=186, P=0.880; back: χ^2_1=1.791, N=186, P=0.786), but males made contact sooner than females (χ^2_1=10.128, N=186, P=0.002) in the Social test.

C-mink and F-mink differed in the time spent in the nestbox (C<F; Kruskal–Wallis: H_3=75.761, P<0.001), half out (C<F; H_3=21.660, P<0.001) and in the back third of the cage closest to the opponent (C>F; H_3=87.355, P<0.001; Table 6). C-mink made contact with the standard opponent more frequently than F-mink (GLIM: $\chi^2_{1,149}$=435.55, P<0.001), with an effect of sex ($\chi^2_{1,149}$=23.99, P<0.001), and an interaction between sex and genetic line ($\chi^2_{1,149}$=38.61, P<0.001). C-mink spent more time than F-mink in contact with the opponent (H_3=96.009, P<0.001), with no difference between the sexes. There was a significant effect of genetic line ($\chi^2_{1,149}$=360.23, P<0.001) and sex ($\chi^2_{1,149}$=29.92, P<0.001) for the counts of conflicts (Table 6).

Effects of test week existed for latencies (half out: survival analysis: χ^2_1=7.731, N=186, P=0.005; out: χ^2_1=12.039, N=186, P<0.001; back: χ^2_1=19.993, N=185, P<0.001; contact: χ^2_1=10.991, N=183, P<0.001). Latencies to approach and make contact decreased over the 6 test weeks for line C (half out: r_S= − 0.35, N=90, P<0.001; out: r_S= − 0.39, N=90, P<0.001; back: r_S= − 0.35, N=85, P=0.001; contact: r_S= − 0.26, N=83, P=0.016) and line F

Table 7. Number and proportion of visits by confident and fearful mink to different areas in an X-maze test (N=182)

Number of visits	Confident		Fearful	
	Male	Female	Male	Female
Arms with clear tube	8.9±0.79[a]	8.9±0.88[a]	12.5±1.80[b]	15.6±1.99[c]
Arms with dark tube	10.2±0.93[a]	11.0±1.34[a]	13.4±1.87[b]	17.5±2.40[c]
Central platform	19.0±1.57[a]	19.5±2.14[a]	26.3±3.63[b]	33.3±4.19[c]
Clear tubes	3.4±0.45[a]	4.5±0.61[b]	1.8±0.49[c]	1.6±0.41[d]
Dark tubes	3.9±0.43[a]	5.4±0.81[a]	0.6±0.19[b]	1.3±0.30[b]
In total*	45.4±3.37[a]	49.31±5.17[a]	54.5±7.46[b]	69.4±8.52[c]
P(clear tubes)†	0.49±0.087[a]	0.59±0.083[a]	0.17±0.049[b]	0.24±0.104[b]
P(dark tubes)‡	0.46±0.062[a]	0.60±0.082[a]	0.05±0.017[b]	0.16±0.066[b]
P(tubes)§	0.19±0.025[a]	0.21±0.018[a]	0.05±0.010[b]	0.06±0.019[b]

Means are given±SE. Values within rows lacking a common superscript letter differ (P<0.05).
*In total=(visits in arms with clear tube+in arms with dark tube+in central platform+in clear tubes+in dark tubes).
†*P* (clear tubes)=visits in clear tubes/in arms with clear tube.
‡*P* (dark tubes)=visits in dark tubes/in arms with dark tube.
§*P*(tubes)=(visits in clear tubes+in dark tubes)/in total.

(half out: $r_S=-0.37$, N=73, P=0.001; out: $r_S=-0.35$, N=71, P=0.002; back: $r_S=-0.10$, N=40, P=0.523; contact: r_S=0.16, N=22, P=0.487). In week 5, the latency to half out and out was not significantly different between C- and F-mink. However, for the other latencies and test weeks, the effect of genetic line remained significant (week 1: χ^2_1=14.73, N=32, P<0.001; week 2: χ^2_1=10.36, N=31, P=0.001; week 3: χ^2_1=13.42, N=28, P<0.001; week 4: χ^2_1=18.58, N=32, P<0.001; week 6: χ^2_1=8.81, N=31, P=0.003) with C-mink showing the lowest values at each test. In number of conflicts, an effect of test week existed in C-mink (conflicts falling from 27.6 ± 6.60 in the first week to 5.9 ± 2.19 in the last test week; $r_S=-0.31$, N=94, P=0.002). In contrast, the number of conflicts in F-mink remained at a relatively high level over the test weeks (e.g. 23.4 ± 5.18 in week 1 and 24.9 ± 4.16 in week 6; r_S=0.14, N=92, P=0.179). With the exception of the first test week, C-mink showed significantly fewer conflicts than F-mink (week 2: GLIM: $\chi^2_{1,25}$=30.73, P<0.001; week 3: $\chi^2_{1,23}$=75.00, P<0.001; week 4: $\chi^2_{1,25}$=80.05, P<0.001; week 5: $\chi^2_{1,27}$=247.55, P<0.001; week 6: $\chi^2_{1,28}$=195.98, P<0.001). Number of contacts was positively correlated with test weeks only for C-females (Pearson: r_{45}=0.31, P=0.035).

On average, the preparatory time was 16 ± 1.5 s and the waiting time 96 ± 4.7 s. Preparatory and waiting time did not differ between the genetic lines, the sexes or the test weeks. In C-mink, but not in F-mink, a weak positive correlation (r_S=+0.20, N=94, P=0.049) existed between number of conflicts and waiting time, whereas no correlations were found with preparatory time. In number of contacts, no significant or unidirectional correlations with preparatory and waiting time existed (C-mink: $r_S=-0.16$–+0.16, N=94, P=0.113; F-mink: $r_S=-0.03$–+0.18, N=92, P=0.099).

X-maze Test

C-mink entered the tubes placed in the maze sooner than F-mink (clear tube: survival analysis: χ^2_1=15.507, N=182, P<0.001; dark tube: χ^2_1=60.395, N=182, P<0.001; Fig. 2d), whereas the genetic lines did not differ in latency to enter arms with a clear tube (C-mink: 25 s, 9–73; F-mink: 30 s, 7–93; χ^2_1=0.621, N=182, P=0.431), arms with a dark tube (C-mink: 24 s, 9–68; F-mink: 34 s, 8–92; χ^2_1=1.099, N=182, P=0.295), or the central platform (C-mink: 8 s, 4–20; F-mink: 7 s, 7–37; χ^2_1=1.199, N=182, P=0.274). There were no sex differences in the latencies.

F-mink had more visits to both arms (with clear tube: GLIM: $\chi^2_{1,177}$=106.24, P<0.001; with dark tube: $\chi^2_{1,172}$= 83.36, P<0.001), the central platform ($\chi^2_{1,172}$=209.96, P<0.001) and in total ($\chi^2_{1,172}$=178.97, P<0.001; Table 7). In F-mink only, the sexes differed in number of visits to these areas (arms with clear tube: $\chi^2_{1,89}$=16.53, P<0.001; arms with dark tube: $\chi^2_{1,88}$=24.96, P<0.001; central platform: $\chi^2_{1,88}$=38.63, P<0.001) with females having more visits than males. Even though F-mink had the most visits to the arms, C-mink had more entries into the tubes within the arms (clear tube: $\chi^2_{1,174}$=83.37, P<0.001; dark tube: $\chi^2_{1,179}$=234.88, P<0.001; Table 7). There was a significant difference between the genetic lines in the proportion of visits that were to the tubes (clear tubes: $\chi^2_{1,173}$=15.50, P<0.001; dark tubes: $\chi^2_{1,171}$=41.03, P<0.001; tubes: $\chi^2_{1,177}$=51.65, P<0.001; Table 7). The number of areas explored (maximum 13) did not differ between genetic lines ($\chi^2_{1,152}$=0.34, P=0.558) or between sexes ($\chi^2_{1,152}$=0.10, P=0.686); the median was 11 (2–13) for all mink.

During test weeks 1–6 there was a weakly decreasing trend in latency to enter arms with the clear tube ($r_S=-0.16$, N=176, P=0.04) and with the dark tube ($r_S=-0.17$, N=173, P=0.03); none of the other latencies in the X-maze changed during the test weeks. Number of visits in the different areas of the X-maze differed between some weeks, but no systematic change (increase or decrease) during the test period existed ($r_S=-0.01$–+0.02, N=182, P=0.82–0.97).

It took longer to handle C- than F-mink (39 ± 9.3 versus 9 ± 0.8 s; GLM: $F_{1,173}$=10.91, P=0.001), and within the

Table 8. Number of and time spent in positions and events by confident and fearful mink in a 6-min Novel Food test (*N*=192)

	Confident		Fearful	
	Male	Female	Male	Female
Away (s)	137.9±16.95[a]	159.1±14.65[a]	196.6±13.68[b]	205.4±14.83[b]
Underneath (s)	229.4±18.13[a]	227.0±14.98[a]	234.6±14.61[b]	215.9±12.37[a]
Close (s)	41.6±4.70[a]	48.98±5.61[a]	65.9±9.60[a]	71.36±12.91[a]
No. of sniffing bouts	11.0±0.94[ac]	9.9±0.93[a]	12.4±0.86[b]	11.8±0.98[bc]
Time spent sniffing (s)	31.7±2.95[a]	26.2±2.65[a]	38.4±4.27a	28.5±2.84[a]
No. of eating bouts	5.7±0.83[a]	7.0±1.03[b]	3.1±0.67[c]	2.1±0.52[d]
Time spent eating (s)	125.9±22.77[a]	114.7±19.29[a]	44.9±13.15[b]	24.9±8.36[b]
No. of grooming bouts	1.8±0.31[a]	2.1±0.31[a]	2.5±0.38[a]	1.8±0.24[a]
Time spent grooming (s)	14.2±4.23[a]	14.6±3.26[a]	15.5±2.67[a]	14.8±3.17[a]
No. of drinking bouts	1.0±0.22[a]	2.1±0.41[b]	2.2±0.41[b]	1.9±0.42[b]
Time spent drinking (s)	3.5±0.89[a]	6.3±1.56[a]	6.9±1.62[a]	4.4±1.04[a]
No. of stereotypical bouts	2.0±0.79[a]	3.3±0.89[b]	2.7±0.80[b]	5.8±1.03[c]
Time spent in stereotypy (s)	16.5±6.31[a]	35.5±9.84[a]	22.6±7.17[a]	45.3±10.42[b]

Means are given±SE. Values within a row lacking a common letter differ (*P*<0.05). Behaviours observed in fewer than five animals are not included. See Table 4 for definitions of behaviours.

C-group the males had a longer mean handling time than females (58 ± 17.3 versus 20 ± 5.9 s; $F_{1,88}$=4.25, *P*=0.042), the main reason for this being two extreme C-males, which took 400 and 600 s to get into the transport box. Transport duration to the test arena did not differ between the genetic lines ($F_{1,174}$=0.42, *P*=0.518) or sexes ($F_{1,174}$=0.20, *P*=0.657), and was 157 ± 5.9 s. Handling and transport duration did not affect or correlate with the latencies of mink to enter different areas in the X-maze. Similarly, no correlation existed between number of visits in any parts of the X-maze arena and handling duration (r_S= − 0.06–+0.10, *N*=182, *P*=0.17–0.61) or waiting time (r_S= − 0.01–+0.08, *N*=182, *P*=0.31–0.87). No effect of handling could be detected on the proportion of visits into tubes.

The use of force (pushing the animal out from the portable nestbox and into the test apparatus with a 20-cm-long plastic stick) did not affect the behaviour in the X-maze. A Mann–Whitney rank sum test comparing number of visits between groupings with 'force used' (*N*=108) and 'no force used' (*N*=74) showed no effect of force use on number of visits (*Z*= − 3.24– − 2.79, N_1=91, N_2=92, *P*=0.107–0.350). In summary, duration of handling, transport and use of force had minor effects on the behaviour of mink during 10 min of X-maze exposure.

Novel Food Test

C-mink approached the novel food sooner (close: C-mink: 4 s, 0–14; F-mink: 21 s, 8–41; survival analysis: χ^2_1=11.266, *N*=190, *P*<0.001). The sexes did not differ in latency to enter the cage and get close to the food (χ^2_1=2.277, *N*=190, *P*=0.131 and χ^2_1=0.894, *N*=190, *P*=0.344, respectively). C-mink also sniffed the novel food sooner than F-mink within each sex (χ^2_1=4.450, *N*=190, *P*=0.035). Within the genetic lines, males (C-mink: 3 s, 1–14; F-mink: 13 s, 4–29) sniffed significantly (χ^2_1=8.943, *N*=190, *P*=0.003) sooner than females (C-mink: 15 s, 2–38; F-mink: 35 s, 25–63). The latency to eat was lower (χ^2_1=21.913, *N*=190, *P*<0.001) for C-mink than F-mink (Fig. 2e) without any differences between the sexes (χ^2_1=0.477, *N*=190, *P*=0.490).

Mink spent on average 77.9% (467.2 ± 7.55 s) of the test time out in the cage, regardless of genetic line and sex (Kruskal–Wallis: H_3=0.433, *P*=0.933). F-males sniffed more than C-females, with no difference between the sexes within each genetic line (Table 8). C-mink had a higher number of eating bouts than F-mink (GLIM: $\chi^2_{1,166}$=152.33, *P*<0.001). The sex difference was not the same within the two lines (genetic line and sex interaction: $\chi^2_{1,166}$=15.28, *P*<0.001), since in group C (in contrast to group F) the females ate more frequently than the males. Number of grooming bouts did not differ between the genetic lines ($\chi^2_{1,166}$=2.29, *P*=0.130) or the two sexes ($\chi^2_{1,166}$=0.93, *P*=0.334). C-males drank significantly fewer times than other mink (genetic line and sex interaction: $\chi^2_{1,166}$=17.51, *P*<0.001). In stereotypical activity, F-females had the most and C-males the fewest bouts. F-females spent longer than the others performing stereotypical behaviour (Kruskal–Wallis: H_3=12.7, *P*=0.005; Table 8).

F-mink spent more time away from the food (GLM: $F_{1,184}$=12.175, *P*<0.001) and less time eating the novel food than C-mink ($F_{1,186}$=25.773, *P*<0.001). The number of behavioural shifts out in the cage differed between genetic lines (C<F; $F_{1,186}$=12.892, *P*<0.001) and sex (male<females; $F_{1,186}$=7.316, *P*=0.008). C-males showed 80.7 ± 6.17 shifts, C-females 97.3 ± 6.41 shifts, F-males 102.8 ± 6.61 shifts and F-females 120.2 ± 5.89 shifts within the 10 min of the Novel Food test.

Test week did not differ for the latencies, except for one negative correlation of r_S= − 0.41 (*N*=95, *P*<0.001) between latency to eat and test week valid for C-mink only. For sniffing, eating, drinking and stereotypy, but not for grooming, numbers of bouts differed significantly in some test weeks. However, there was no general trend since correlations between bouts of each variable and test

Table 9. Proportion of significant intratest (in parentheses) and intertest correlations in Spearman correlation analysis between variables selected as fear indicators in six test situations

	Stick test	Trapezov's hand test	Novel object	Social	X-maze	Novel food
Stick test*	(0.96)	0.80	0.89	0.82	0.52	0.62
Trapezov's hand test†		(1.00)	1.00	1.00	0.40	0.82
Novel Object test‡			(0.93)	0.95	0.49	0.55
Social test§				(0.94)	0.51	0.40
X-maze test**					(0.80)	0.26
Novel food test††						(0.64)

*Latency to approach (position near), contact, no. of approaches, contacts, avoidance (position away), time spent near, away, in contact, distance and score (excluding aggressive).
†Score, minimum distance.
‡Latency to half out, out, contact, manipulate, no. of approaches (position 'Out'), Σcontacts, manipulations, conflicts, time in nest, Σcontacts, manipulation.
§Latency to out, back, contact, no. of approaches (position back), contacts, conflicts, time in nest, close (position back), contact.
**Latency to enter tubes, no. of areas explored, visits in total, probability to visit clear tubes, dark tubes.
††Latency to be out in cage, close, sniff, eat, no. of visits away, close, sniffing, eating bouts, time out in cage, away, close, sniffing, eating, no. of behavioural shifts.

week were not significant. For F-males only, sniffing increased (r_S=0.28, N=48, P=0.050) during the test weeks.

From test weeks 1–6, time spent underneath (r_S=0.24, N=189, P<0.001) and drinking (r_S=0.16, N=183, P=0.026) tended to increase, whereas time spent close (r_S= − 0.17, N=189, P=0.021) and grooming (r_S= − 0.16, N=186, P=0.033) tended to decrease. No correlation existed between test week and time spent away, sniffing, eating and stereotypy. Test week affected the number of behavioural shifts out in the cage (GLIM: $\chi^2_{5,166}$=153.21, P<0.001), since for females the number of behavioural shifts decreased over test weeks (C-mink: r_S= − 0.32, N=48, P=0.026; F-mink: r_S= − 0.45, N=47, P=0.001).

Intertest Correlations

The majority of the variables within the six tests were chosen based on hypotheses of being either negatively or positively related to fear in mink. Of these, we selected 51 variables as reliable fear indicators, including measures of approach or avoidance towards test stimuli (e.g. latencies to approach and make contact, number and time spent in contact, conflict, manipulation and scores); the rest of the variables were excluded owing to their complementary nature (such as being 'in nest' or 'out in cage'), low occurrence (e.g. freezing, elimination), or lack of obvious directional connection to fear in mink (e.g. grooming, raid, marking, drinking, stereotypical behaviour). Testing correlations between the six tests using the 51 selected variables gives 1037 intertest possibilities. At a significance level (α) of 0.05, ca. 52 of these could be correlated purely by chance in a pairwise comparison. A higher number of significant correlations between two tests may be interpreted as an indicator of generalization across situations (Table 9). Besides the significance, the directions of the correlations are of importance. In the comparisons between Stick test, Trapezov's hand test, Novel Object test and Social test, for all 305 of the significantly correlated variables the r_S values had the expected sign. Between all tests (including X-maze test and Novel food test) 86.6% (562 out of 659) of the significant r_S values had the expected sign. The reason for this value not being 100% is that one variable in the X-maze test (probability of visiting dark tubes) and four variables in the Novel Food test (number of visits and time spent close to food, number of sniffs and time spent sniffing) consistently had the opposite sign to that predicted, questioning the a priori interpretation of the direction of these variables as fear indicators.

DISCUSSION

Generalization across Situations

F-mink were more fearful than C-mink in all test situations. In particular, mink showed similar responses to various stimuli in the home cage (human, object, another mink), and also, although to a lesser extent, to novel food or in a novel environment (Table 9). Within individuals, correlation in response across different threatening situations may serve as evidence for general fearfulness, using the definition of Boissy (1995): 'Fearfulness is a basic psychological characteristic of the individual that predisposes it to perceive and react in similar manner to a wide range of potentially frightening events'. In the present study, C- and F-mink differed significantly in 94.1% (48 of 51) of test variables selected to quantify fear. F-mink were significantly more fearful than C-mink in all except five variables (three in the X-maze test, and two in the Novel Food test). In one out of 12 significant variables in the Novel Food test, the difference between C- and F-mink was valid only for females. Overall, C-mink reacted with less avoidance and more approaching behaviour than F-mink when confronted with voluntary or forced human contact, a novel object, an unknown mink, novel food or in a novel environment (Figs 1–3).

Since the experimental animals from the two breeding lines were housed and treated the same way, this difference in fearfulness must be genetically based; congenital (including innate and prenatal causes) rather than postnatal causes of the responses in a Stick test have previously been demonstrated in a cross-over experiment with kits from the fearful and the confident breeding line (Malmkvist & Hansen 2001). Similarly, studies on other species have shown that levels of defensive reactions and fearfulness are partly under genetic control, for example determined by selective breeding experiments in foxes, *Vulpes vulpes* (Belyaev & Trut 1987) and rats, *Rattus norvegicus* (Gray 1987; Oliverio & Castellano 1990; Wada & Makino 1997; Henninger et al. 2000; Pollier et al. 2000).

In accordance with our finding of a generalized response as an outcome of selective breeding, rats selectively bred for avoidance behaviour differed in response to novelty and different types of conditioning (Flaherty & Rowan 1989). Furthermore, rats genetically selected for reactions (high or low anxiety-related behaviour) in the elevated plus-maze for several years reacted differently in several tests. Differences in a social interaction test, however, were primarily due to locomotor activity (Henninger et al. 2000). In one study on laboratory-bred rats, no evidence for a generalized state of fear was found, since responders and nonresponders to cat odour did not differ in other tests of novelty (Hogg & File 1994).

Behaviour in Potentially Fear-eliciting Situations

C-mink were more explorative and less fearful towards humans than F-mink (Figs 1, 3). This is in agreement with earlier reports (Hansen 1996; Malmkvist & Hansen 2001). In both human tests, F-mink kept much further away from the humans. The Trapezov's hand test was regarded as more threatening, since the distance to the human was greater than in the Stick test. More than 75% of the C-mink were given scores +1 and +2 in the Trapezov's hand test, meaning that they did not flee but showed exploration towards the human intruder, and could often be touched without moving away (Fig. 1). In contrast, only 1.0% of F-mink (two animals) reacted with a positive score towards a human. Since more C-mink were aggressive towards the human, selection for reduced fear seems to induce aggression. This is not, however, a problem in the selection line of confident mink. First, only a few mink were aggressive (2.6–3.1%), with only two being aggressive in both tests. Second, no attempts were made to handle fleeing mink in the Trapezov's hand test, limiting the possibility of aggression. In practice, defensive aggression during handling may be prevalent in fearful mink.

C- and F-mink interacted differently with a novel object in their home cage (Fig. 2, Table 5) and when exposed to an unknown male mink (Fig. 2, Table 6). In all measures, offspring from the confident line were evaluated as less fearful than offspring from the fearful line towards these stimuli. The unknown male mink seemed to elicit fear more than the wooden cube placed in the home cage, based on latency to approach and make contact. The novel object also stimulated more playful behaviour, as shown in raids and manipulations and slightly fewer conflicts. Contact with the object and the social mink was investigative, characterized by repeated bouts of sniffing.

Hansen (1996) found that aggression towards humans emerged in October to November when kits born the same year were tested in the Stick test. MacLennan & Bailey (1969) observed aggressive threats in 17-week-old mink and at about 20 weeks of age all performed adult aggressive patterns towards other mink, when tested repeatedly with full physical contact in a neutral cage. Young mink by the age of 20 weeks have already established the species-specific adult-like sensory and motor ability and general behaviour that enable them to live alone in the wild (Kruska 1996). Our animals were 20–26 weeks old and should therefore have been able to express aggression towards another mink; however, no aggressive interactions were observed between the test mink and the opponent in the Social test.

Compared with C-mink, F-mink had the most visits in all parts (other than the tubes) of the X-maze (Table 7). From the locomotory response alone it is difficult to distinguish between exploration and other behaviours involving locomotion, such as attempts to escape, in an open-field situation (for discussion see Birke & Archer 1983; Russell 1983; Munksgaard & Jensen 1996; Hughes 1997; Weiss et al. 1998). To reduce this problem of interpretation, we added tubes to earlier designs of an open-field apparatus for mink (Malmkvist 1998). C-mink entered the tubes more quickly (Fig. 2), and were more likely to visit them than F-mink. For all mink, the latency to visit a clear or a dark tube was the same. Therefore, the a priori assumption of the dark tubes being a refuge into which mink seek shelter in the novel environment was not confirmed, at least not when mink were placed in the X-maze once for 10 min.

In nature, the mink is an opportunistic carnivore (Wise et al. 1981) feeding on a wide range of food items (e.g. Day & Linn 1972; Lodé 1993; Maran et al. 1998). On farms, however, the food is rather uniform. J. Malmkvist & M. S. Herskin (unpublished data) showed that the novelty of canned cat food evokes a range of reactions in farm mink: a significantly increased latency to enter the cage from the nestbox, to get close, sniff and eat, compared with a delivery of the usual mink food. In the present experiment, F-mink showed more behavioural shifts than C-mink, indicating a higher degree of conflict in the novel situation. Furthermore, C-mink were less hesitant in approaching the unknown food and they began to eat sooner than F-mink (Fig. 2). Food novelty induced more stereotypical activity than any other test situation. After feeding with novel food, stereotypical activity was observed in more than one-third of all mink, whereas this behaviour was infrequent in the Trapezov's hand test (0.5%) and Novel Object test (2.2%) and absent in the Stick, Social and X-maze tests. More F-mink (44.2%) than C-mink (29.5%) performed stereotypies after receiving novel food, delivered as usual. Hunger is a known releasing factor for stereotypical behaviour in mink (Mason 1993). C- and F-mink do not, however, differ in food consumption (Malmkvist 2001), and no evidence exists for a difference between the lines in motivation to eat. Therefore, the breeding experiment affecting fearfulness may have affected stereotypical activity.

Sex Differences in Behaviour

In all test situations, except the Trapezov's hand test, the sexes reacted differently in a few of the registered variables. In the Stick test, C-males were more explorative than C-females. Females had more visits in the middle and back part of the cage, which may indicate that they more actively sought to avoid the human. Manipulation in the Novel Object test indicates that males were more exploratory than females. Males in the C-group also showed less conflict behaviour than females during exposure to a novel object and an unknown mink. In F-mink, this gender effect was reversed (in the Novel Object test) or not present (Social test). Within each genetic line males made contact sooner than females with an unknown male mink. In the Novel Food test, males had a shorter latency to sniff, and had fewer bouts of stereotypies and fewer behavioural shifts than females. If number of visits in the X-maze, as indicated earlier, reflects flight attempts rather than exploratory activity, F-females may be more fearful than F-males in the novel environment. However, some of the observed differences may be explained partly by the sexual dimorphism in mink, with males being about twice as big as females. Based upon their size, males may find it easier to push and tilt the object, a cube weighing about 360 g. Furthermore, the two sexes did not differ in total contacts (including manipulations) with the object. The sex difference observed in the Novel Food test (Table 8) may not be exclusively linked to fear. Even though we controlled hunger by removing refused food, males are likely to be more motivated to eat than females, because of their size and thus greater demand for food.

In the Social test, no effect of the identity of the male opponent was found, possibly as a consequence of their training prior to the experiment. When confronted with an unknown male, male mink may be more explorative than females, since males made contact sooner, F-males had more contacts than F-females, and C-males fewer conflicts than C-females. However, males and females within each breeding line did not differ in duration of contacts with the social male. MacLennan & Bailey (1969) found no difference in the latency to encounter a mink male when testing male and female mink, but their results were in contrast to ours based on agonistic interactions in repeated, full contact situations.

There is no conclusive evidence for a general exploratory sex difference in mink in the literature. Trapezov (2000) reported that male 'Sapphire' mink were more explorative than females towards humans, whereas no effect of gender existed in 'Standard' mink, a colour type resembling the one we used. In experiments by MacLennan & Bailey (1969), females showed slightly lower levels of curiosity than males when exposed to an open-field test including human presence, valid only for adults kept visually isolated since weaning. This result was based on only six animals per treatment group. Based on the present large-scale experiment, male mink are more explorative and less fearful than females in the Stick test (in accordance with findings after six generations in Hansen 1996), in the Social test and to a lesser extent also in the Novel Object test. The finding of sex differences in the X-maze and Novel Food test cannot with certainty be linked to fear or exploration.

Effects of Human Exposure and Handling

Human contact was minimized in test situations that did not involve a human as test stimulus and was negligible in the Novel Food test, where wet food was quickly delivered by a man-driven feeding machine. Some human exposure was inevitably included in the preparation of the Novel Object, Social and X-maze tests. Mink use senses other than vision (i.e. hearing, olfactory), to which input are difficult to control. However, the test results showed only minor and sporadic effects of human exposure or handling. Handling duration did not, for example, affect number of/time spent in contacts and conflicts in the Novel Object or the Social test, nor did the use of force affect the latencies and number of visits to different parts of the X-maze. Therefore, mink behaviour observed in the Novel Object, Social and X-maze tests was not an artefact of the exposure to a human handler prior to the tests.

In two cases, waiting time (with the mink restricted in the nestbox prior to the test) affected the results, since number of object contacts increased in F-mink and number of conflicts towards the social mink increased in C-mink with waiting time. One explanation of the first result could be that human exposure depresses the exploratory activity of F-mink (more than in C-mink), so that they show more object contacts the longer the time since the last human exposure. Shors & Wood (1995) found that exposure to a stressor (tailshocks) 2 h prior to testing impaired activity and exploration of rats towards both unfamiliar and familiar conspecifics. However, in the present study no major effects were found of the handling itself (preparatory time), nor were there any corresponding effects of waiting duration in other variables.

Changes in Behaviour During Repeated Testing

We observed a development in the responses of mink, exposed to a different test once a week for 6 weeks. Incidences of screaming, which occurred only in F-mink, declined over the period. In particular, latency to approach/make contact with an object or another mink, to enter arms in the X-maze and to eat novel food decreased with the number of tests (one to six), for either one or both of the genetic lines. This trend, which indicates a reduced level of fearfulness, may be due to habituation, even though the six test sessions were not identical. The influence of experience in repeated tests is well documented in other species (e.g. the elevated plus-maze for rats: File 1993; File & Zangrossi 1993; the four-plate test for mice, *Mus musculus*: Hascoet et al. 1997) as well as in mink (the plus-maze: Malmkvist 1998; the Stick test: Malmkvist & Hansen 2001). Habituation is regarded as a form of learning, a mechanism underlying decreased responsiveness as a result of repeated application of a stimulus, different from sensory adaptation and effector fatigue (McFarland 1991). Evidence exists that habituation and carry-over effects operate even between different tests, for example, rats in an open-field test were more active when the test was early than late in a sequence of four tests, at least 3 days apart (Paré 1994). Paz-Viveros et al. (1997) found similar results, the open-field test as the last of three tests within a day resulting in reduced locomotion and exploration, while immobility increased, an effect they suggested was due to the accumulation of stress with number of tests. In the present experiment we regarded an interval of at least 4 days between tests to be sufficient to allow for recovery from the previous test conditions. The intensity of the six tests was not as severe as in Pare's (1994) study, which included multiple unavoidable shocks and a forced-swim period of 15 min. Nevertheless, it is likely that the experimental mink over the test period became accustomed to being tested in general, in some cases, leading to increased exploration. Hansen's (1996) results indicated that visual contact without aversive consequences to the animals seems sufficient to reduce the mink's fear of humans in the Stick test. Age and seasonal effects may also affect the test responses in mink. For example, time spent grooming in the Novel Food test decreased with test week, possibly because of the change from summer to winter coat, which took place during the experimental period.

Conclusion

We conclude that after 10 generations of divergent selection based on reaction to humans, offspring from a confident breeding line of mink were less fearful than offspring from a fearful breeding line, when confronted with voluntary or forced human contact, a novel object, an unknown mink, novel food in their home cage and when placed in a novel environment. Thus, mink from these breeding lines generalize their fear responses across several social and non social situations. An overall reduced level of fearfulness may result in improved welfare of farmed mink.

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