

## Selection against stereotypic behaviour may have contradictory consequences for the welfare of farm mink (*Mustela vison*)

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### Abstract

The present study aimed to examine if divergent selection for stereotypic behaviour in mink influences the welfare of the animals. Two breeding lines were used, a high stereotyping line (HSL,  $N = 139$ ) and a low stereotyping line (LSL,  $N = 132$ ). Their welfare was assessed on the basis of adrenocortical activity (faecal cortisol metabolites, FCM), confident versus fearful temperament, growth and reproduction. The results showed that the LSL performed less stereotypic behaviour and were less active than the HSL. Furthermore, the results demonstrated that: (1) the LSL had lower concentrations of FCM, indicative of better welfare; (2) there was no clear difference in temperament between the lines, however, within the HSL a positive correlation was found between the frequency of stereotypy and the frequency of confident temperament; (3) there was no difference in reproduction; (4) there was no failure to grow in any line, but on average the body weight in the LSL was higher than that in the HSL. The correlation between stereotypy and confidence in the present study supports earlier findings showing a higher proportion of fearful animals in groups of low stereotyping mink. So, although the selection against stereotypic behaviour clearly reduced the FCM it may have contradictory consequences for the welfare of the mink.

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## 1. Introduction

Stereotypies are found in captive animals and are rare in nature (Mason, 1991). The occurrence of stereotypies in farm animals seems to increase during periods of feed restriction (Rushen et al., 1993) and if environmental conditions are barren or otherwise poor (Mason, 1993a,b). However, individuals that develop stereotypies in a certain environment may in some instances experience better welfare, either because the repetitive behaviour has a calming effect or because the behaviour substitutes effectively for natural behaviour patterns that the animals are motivated to perform (Mason and Latham, 2004). These general trends are also seen in mink. They perform more stereotypic behaviour during and after feed restriction (Bildsøe et al., 1991) and they develop more fear, pelt gnawing and stereotypic behaviour in barren environments as compared to enriched environments (Jeppesen, 2004). However, individual mink that perform high levels of stereotypic behaviour may experience better welfare as evidenced by lower baseline urinary cortisol concentrations (Bildsøe et al., 1991), better reproduction (Jeppesen et al., 2004) and less fearful behaviour (Hansen and Jeppesen, 2006).

The use of genetic selection to improve animal welfare has been recommended by the Council of Europe (1999). Selection against stereotypy should be possible in mink (Hansen, 1993; Jeppesen et al., 2004) as well as in other species (Schwaibold and Pillay, 2001; Schoenecker and Heller, 2000). However, it is not clear whether such selection will improve welfare. Since high stereotyping mink may experience better welfare than low stereotyping ones, selection against stereotypy might lead to mink that experience poorer welfare, due to an impaired ability to do something that they can consider relevant (Mason and Latham, 2004). On the other hand, selection against stereotypy could also lead to mink that perform fewer stereotypies because they are less sensitive to possible negative effects of their cage environment. In that case, selection against stereotypy should be expected to benefit welfare. Examples of improved welfare through genetic selection exist in mink (reduced fearfulness; Malmkvist and Hansen, 2001), as well as in other species, such as laying hens (Muir and Craig, 1998) and sheep (Scobie et al., 1999).

In the present experiment it is examined if selection for or against stereotypic behaviour influences the welfare of the animals. The selection was started in the year 2001 (Jeppesen et al., 2003). In accordance with Broom and Johnson (1993) and with the necessary reservations, good welfare is implied by low cortisol, confident as opposed to fearful temperament, good reproduction and absence of failure to grow. At present, the F3 generation of adult female mink are used to examine if the selection leads to changes in the frequency of stereotypic behaviour or other activities, adrenocortical activity (measured non-invasively via faecal cortisol metabolites; Möstl and Palme, 2002), reproduction, weight and temperament.

## 2. Materials and methods

### 2.1. Animals and housing

The animals were 271 wild type adult female farm mink (*Mustela vison*), housed in standard two-row outdoor mink sheds at the Danish Institute of Agricultural Sciences in Foulum, Denmark (56°29'N and 9°34'E). They were housed individually in standard-sized cages (W, 30 cm; H, 45 cm; L, 90 cm) connected to straw covered wooden nest boxes (W, 28 cm; H, 20 cm; L, 23 cm). Straw was continuously provided at the top of the nest box and from there it was pulled into the box by the mink. Food was available close to ad libitum, according to Danish standard farm feeding routines. The amount of leftovers from the day before was controlled every morning and the amount of feed delivered the same day was regulated so that about

80% of the animals could be expected to have leftovers on the following day. Every morning the leftovers were distributed among the animals that had no leftovers. Water was available ad libitum.

The animals belonged to the F3 generation of two breeding lines, a high stereotyping (HSL;  $N = 139$ ) and a low stereotyping (LSL;  $N = 132$ ), established by controlled selection of breeding mink since 2001. The selection criteria was the frequency of stereotypic behaviour in adult female mink, measured in about 50 scanning observations performed over 3 days in mid October. All of the females that were scanned delivered a litter in the summer before the scanning, and new breeding animals for the lines were selected from the offspring of the high stereotyping, respectively, the low stereotyping adult females within each line. This procedure was necessary since mink usually do not perform stereotypic behaviour at high frequencies earlier than their second year (for further description of selection criteria see Jeppesen et al., 2003; Hansen et al., 2005). The animals used in the present study were born in 2003 and delivered a litter around May 1, 2004.

## 2.2. Behavioural observations

In total, 56 scanning observations of each of the mink were conducted between 09:00 and 15:00 h during 5 consecutive days starting October 13, 2004, 11 or 12 scanings per day. Feeding, usually took place at 11:00 h, but was postponed until 15:00 h on observation days. This was done in order to maximize the frequency of stereotypic behaviour (which usually decreases after feeding; Mason, 1993a). The same procedure was followed during the scanning observations performed to establish the selection criterion.

During observations, the observer walked slowly and quietly along the feed gangway and observed the cage sections (one section = six cages) one by one. During the observation of one section the observer always stood in front of the neighbouring section. This was done in order to keep a distance to the animals, to allow habituation to the observer, and to keep disturbance at a minimum. Following 30 s of observation, the behaviour of the six animals in the section was scanned and classified to one of the behaviour elements described in Table 1. It took about 30 min to scan all of the mink once. The interval between successive scanning observations of any individual was between 30 and 60 min. The scanning observations were equally distributed over days and observations hours.

## 2.3. Sampling and analysis of cortisol metabolites

Faecal samples were collected 14 days before the first scanning observation. The feed was removed from the animals at 07:00 h and they were fed fresh feed at 11:00 h. Sample collection began at 12:00 h and ended at 17:00 h; the samples were collected from wire nettings that were placed below the cages around 10:00 h and kept free of faeces until 12:00 h. Thereafter the nettings were inspected every 15 min and faeces that were delivered in that interval were sampled. A small part of the sample ( $0.5 \pm 0.01$  g) was frozen at  $-20$  °C. The period from the defecation (with an accuracy of 15 min) until faeces were frozen, the faeces

Table 1  
Ethogram of the behavioral elements used in the scanning observations

Elements	Description
Active	The animal was moving in the cage, exploring, eating, drinking or performing other normal behaviours
Inactive	The animal was lying in the cage without moving
Nest box	One-half or more of the body of the animal was in the nest box
Stereotypy	The animal repeated fixed movements at least five times within a period of 10 s in exactly the same invariable, repetitive way. All of the movement stereotypies described by Bildsøe et al. (1990) were included in the element, e.g. running back and forth the length of the cage, sometimes with a somersault in the back of the cage, moving the head in circles, or repeatedly jumping back and forth with the head, forelimbs and upper part of the body without moving the hind part

collecting period, was registered. After thawing and extracting the faeces with 5 ml methanol (80%), concentrations of faecal cortisol metabolites (FCM) were analysed in an aliquot of the supernatant with two different enzyme-immunoassays (EIAs) previously described in detail (11-oxoetiocholanolone-EIA, *Palme and Möstl, 1997*; 11 $\beta$ -hydroxyetiocholanolone-EIA, *Frigerio et al., 2004*). The interassay coefficient for a high and low quality control was 12.3% versus 14.5% for the first assay and 10.4% versus 10.6% for the second assay. The sensitivity was 24.3 and 21.1 nmol/kg, respectively. Animals that did not defecate during the collection period were not used in the final analysis.

#### 2.4. *Reproduction and weight*

The litter size was counted 1 day after delivery and again 8 weeks after delivery, when the dam was removed from the kits (called weaning). The dams were weighed at weaning (20/6–2/7), in September (9/9) and in October (25/10).

#### 2.5. *Temperament*

The temperament of the individual mink subjects was characterized by means of a so-called stick test (*Malmkvist and Hansen, 2002*; *Hansen and Jeppesen, 2006*). The test was repeated 10 times for each subject. The test was performed two or three times a day on 4 consecutive days, starting the day after the last scanning observation was conducted. The animals were tested individually in their wire cage and the tests were evenly distributed between 09:00 and 15:00 h.

A tongue spatula was put through the net in the upper part of the lid of the cage, and the reaction was registered. The reaction was classified to one of four categories: 1, confident (if the animal approached and sniffed the spatula persistently); 2, fearful (if the animal avoided or did not touch the spatula); 3, aggressive (if the animal attacked and bit the spatula); 4, uncertain (if the animal did not react, stayed in the nest box, or showed a mixture of responses and could not be placed in one of the first three categories within 30 s).

The result of the tests are presented as the number of animals being scored one or more times for a certain temperament and as the mean number of times that these animals were scored for that temperament.

#### 2.6. *Statistical analysis*

Non-parametric statistics were used due to non-normal distribution. Differences in the number of animals performing a certain behaviour or exhibiting a certain temperament between the two selection lines were tested statistically using a  $\chi^2$  test. Differences in mean scores of observed behavioural elements, temperament categories and FCM concentrations between the two selection lines were tested statistically with a Mann–Whitney *U*-test. A Spearman rank-order correlation test was used to test for correlations between stereotypic behaviour and the other behavioural elements as well as FCM and reproduction. The relationship between the concentration of FCM and the faeces collecting period was tested with a Spearman rank-order correlation test. The relationship between the FCM concentration and the time of the defecation was examined visually on a scatter diagram that showed maximum levels of FCM in the middle of the sampling period. Based on this distribution it was decided to classify the samples to the five full hour periods between 12:00 and 17:00 h and to test, with a Kruskal–Wallis one-way analysis of variance, whether these 1 h groupings of the samples differed with respect to FCM concentrations. All tests were two-tailed and performed according to *Siegel and Castellan (1988)*. Differences were considered significant if  $P < 0.05$ .

### 3. Results

*Table 2* shows the mean scores of the observed behaviours, the mean concentrations of the FCM, the reproduction results, the body weights and the temperament categories in the LSL and the HSL, as well as the number of animals performing the behaviours.

Table 2

Data for all the measured variables in the LSL and the HSL (The number of animals scored for contributing to a variable measurement with a value greater than zero and the mean scores of these animals for the same variables are shown. Total group sizes are given in bold (*N*) while measured numbers of animals and animals being the base of mean score calculations are given in normal (*N*).  $\chi^2$  test/Mann–Whitney *U*-test)

	LSL		HSL	
	<i>N</i>	Mean (S.E.)	<i>N</i>	Mean (S.E.)
Behavioural observations (%)	<b>132</b>		<b>139</b>	
Active	** 111	*** 6.7 (0.5)	132	12.4 (0.8)
Inactive	*** 98	10.7 (1.0)	130	9.4 (0.6)
Nest box	132	*** 85.4 (1.3)	139	68.8 (1.7)
Stereotypy	*** 24	** 6.3 (3.6)	108	13.3 (1.9)
FCM (nmol/kg faeces)	<b>111</b>		<b>115</b>	
11-Oxoetiocholanolone-EIA				
All animals	111	** 185 (13)	115	257 (19)
Stereotyping animals	18	* 167 (24)	88	280 (23)
Non-stereotyping animals	93	189 (15)	27	182 (29)
11 $\beta$ -Hydroxyetiocholanolone-EIA				
All animals	111	*** 235 (43)	115	495 (77)
Stereotyping animals	18	*** 322 (166)	88	549 (98)
Non-stereotyping animals	93	*** 218 (40)	27	316 (69)
Reproduction (kits)				
Litter size day 1	123	7.0 (0.2)	136	7.1 (0.2)
Litter size weaned	123	6.7 (0.2)	136	6.9 (0.2)
Weight (g)	<b>132</b>		<b>139</b>	
Weaning	132	*** 1212 (19)	139	1118 (15)
September	132	*** 1494 (23)	139	1304 (18)
October	132	*** 1596 (22)	139	1497 (18)
Temperament category (%)	<b>132</b>		<b>139</b>	
Confident	109	53.7 (2.9)	113	49.7 (2.6)
Fearful	98	56.3 (3.3)	112	54.1 (3.1)
Aggressive	42	29.3 (1.5)	51	33.7 (1.8)
Uncertain	31	* 19.4 (0.3)	40	12.5 (0.5)

\*  $P < 0.05$ .

\*\*  $P < 0.001$ .

\*\*\*  $P < 0.001$ .

On average, the animals from the LSL were more in the nest box than the animals from the HSL. The number of animals that performed active and stereotypic behaviour was lowest in the LSL, and so was the mean score of these two behaviours when comparing animals that did perform the behaviours. The number of inactive animals was significantly lower in the LSL. However, the mean score of this behaviour did not differ significantly between the LSL and the HSL. In the LSL and in the HSL stereotypic behaviour correlated negatively with being in the nest box and positively with active as well as inactive behaviour (Table 3).

The time from defecation to freezing of the samples ranged from 12 to 185 min. There was no correlation between the time until freezing and the concentrations of FCM ( $r_s < 0.02$ ,  $P > 0.77$ , for both EIAs). The FCM concentrations differed significantly between the five 1 h intervals (11 $\beta$ -hydroxyetiocholanolone-EIA:  $H(4, N = 228) = 15.1$ ,  $P < 0.005$ ; 11-oxoetiocholanolone-EIA:

Table 3

Spearman's rank correlation test between stereotypic behaviour and some of the measured variables (Correlations with behaviour and reproduction based on all animals. Correlations with FCM were based on the subgroup of animals that performed stereotypic behaviour)

	LSL		HSL	
	$r_s$	$P$	$r_s$	$P$
Behaviour element	$N = 132$		$N = 139$	
Active	0.40	<0.001	0.44	<0.001
Inactive	0.37	<0.001	0.30	<0.001
Nest box	-0.48	<0.001	-0.80	<0.001
FCM (nmol/kg faeces)	$N = 18$		$N = 88$	
11-Oxoaetiocholanolone-EIA	0.11	0.67	0.20	0.06
11 $\beta$ -Hydroxyaetiocholanolone-EIA	0.23	0.35	0.14	0.19
Reproduction	$N = 123$		$N = 136$	
Litter size delivered	0.02	0.87	-0.10	0.25
Litter size weaned	0.00	0.98	-0.09	0.32

$H(4, N = 228) = 13.4, P < 0.01$ ). The general picture was an increase in concentrations until 14:00 h followed by a decrease until 17:00 h. This development was most pronounced in the HSL. The initial levels as well as the peak levels were highest in this line.

The HSL differed significantly from the LSL by showing higher mean concentration of FCM (both EIAs). This difference applied when comparing all animals in the two lines and when comparing only the animals that performed stereotypies and (for the 11 $\beta$ -hydroxyaetiocholanolone-EIA) also when comparing only the animals that did not perform stereotypies.

In the LSL and in the HSL there was a non-significant positive correlation between stereotypies and the concentration of the two cortisol metabolites when only the animals that performed stereotypies were included in the analysis (Table 3). In the HSL, this correlation was significant ( $r_s = 0.33, P < 0.001$ , 11-oxoaetiocholanolone-EIA;  $r_s = 0.21, P = 0.023$ , 11 $\beta$ -hydroxyaetiocholanolone-EIA) when all animals in the line were included in the analysis.

There was no significant difference in any of the measures of litter sizes (Table 2). The relationship between stereotypic behaviour and both measures of litter size was low and insignificant (Table 3). The weights increased during the growth period in both selection lines and the LSL had higher body weights than the HSL at all weighings (Table 2).

The confident, fearful and aggressive temperament categories were not markedly influenced by the selection. The only significant difference between the selection lines was a higher level of the category uncertain in the LSL (Table 2). The only significant correlation between stereotypic behaviour and the temperament categories was a low positive correlation in the HSL with confident temperament ( $r_s = 0.17, P = 0.04$ ).

#### 4. Discussion

In the present study the HSL showed a markedly higher level of stereotypic behaviour compared to the LSL. This is in accordance with results obtained from the F1 generation (Jeppesen et al., 2003) and was as expected. It shows that the selection procedure is effective. The percentage of the number of stereotyping animals in the P generation was 71.7%, and the mean percentage of observations of stereotypies, based on all animals in the generation, was 11.3%. The comparable figures for the present data were 77.6% versus 18.4% for the HSL and 18.2%

versus 1.9% for the LSL. It has been shown, that the day-time scannings, being the basis of these results, reliably reflect the 24 h group differences of stereotypy (Svendsen et al., 2005). Based on the first four generations, the heritability of the trait was recently estimated to 0.30 (Hansen et al., 2005).

The divergent selection for stereotypic behaviour produced two lines of animals that differed markedly with respect to stereotypic behaviour as well as a number of other parameters. At present, inadvertent selection for traits other than stereotypic behaviour or even genetic drift cannot be ruled out as possible causes of these differences. This has to be kept in mind in the following, when the results of the selection are considered.

Higher levels of FCM in the HSL was not expected since previous studies have shown cortisol levels to be lower in high stereotyping mink. Zanella et al. (1998) worked with three lines of mink: a randomly bred line and lines bred selectively for high stereotypy versus low stereotypy. They found lower plasma cortisol concentrations in high stereotyping mink from the line selected for high frequency of stereotypies. The blood was collected from previously undisturbed euthanised mink. Bildsøe et al. (1991) showed baseline levels of urinary cortisol metabolites to be lower in high stereotyping mink as compared to low stereotyping mink. However, based on the same animals they also showed that high stereotyping mink tended to react to an acute stressor (immobilisation) by a higher plasma cortisol response than low stereotyping mink, and this may add to the understanding of the apparent discrepancy between the result of the present study and the studies reporting on low cortisol in high stereotyping mink. The animals in the present study might have been stressed by the experimental procedure that differed from every day practice by, the removal of leftovers at 07:00 h, the placement of wire mesh under their cages around 10:00 h, and the presence of people collecting the faeces from the back side of the cages, which is not usually done during everyday handling of the animals. Passage time for the feed gives a rough estimate of expected delay times of excretion of FCM (Palme et al., 2005). As it is reported to be very short in mink (around 4 h, Jørgensen, 1984) FCM showed up in the faeces at maximum concentrations already 2–4 h after an injection of ACTH (Malmkvist et al., 2004). Therefore, one explanation may be that the samples collected and analyzed in the present study, did not reflect baseline levels of cortisol metabolites, but rather reflect responses to the sampling procedure. The observed effect of the defecation time on the FCM may support this interpretation. However, the effect of the defecation time may also depend on a general diurnal rhythm of adrenocortical activity, which has not been examined in mink. A cortisol response or a behavioural reaction to an unusual short lasting experience may in turn reduce cortisol in the hours following the reaction (rebound effect or coping, e.g. Mason and Latham, 2004) and this may be the reason why high stereotyping animals sometimes show lower baseline levels of cortisol than low stereotyping animals. Naturally, such overall baseline levels of cortisol are better reflected in pooled 24 h samples, as it was the case with the urine sampling in Bildsøe et al. (1991). The plasma concentrations measured by Zanella et al. (1998) were based on samples drawn from a few undisturbed mink. They may well represent baseline levels.

So, the results suggest that the HSL may have a higher stress response than the LSL. It is interesting that this seems to be independent of whether stereotypies are actually performed or not (based on the  $11\beta$ -hydroxyaetiocholanolone-EIA results). If this is confirmed in future studies, it might be taken to suggest that the selection has worked directly on the brain stress sensitivity. The LSL could, therefore, represent animals that are less sensitive to acute stressors and for that reason are supposed to experience better welfare due to better adaptation to intermittently stressing farm surroundings (e.g. Möstl and Palme, 2002). On the other hand, a cortisol reaction to an unusual event might also be seen as a healthy response to the extent that it

is not prolonged and does not seriously impair biological functioning. On their own, levels of cortisol are difficult to interpret in relation to welfare. Knowledge about a diurnal variation of cortisol levels, as well as levels before, during and after a major disturbance, would be helpful. The separate effects of normal versus stereotypic activity should also be examined.

The temperament categories are supposed to reflect individual mental states, and they are, therefore, more directly related to the welfare concept than cortisol. Unfortunately, the temperament test did not reveal any clear differences between the LSL and the HSL with respect to the most easily interpreted categories, fear versus confidence (Malmkvist and Hansen, 2002). The higher level of animals scored in the category uncertain in the LSL could reflect that these animals simply experienced the test situation to be less disturbing. This result could, therefore, be another sign of low reactivity towards a potentially stressing situation. It could also reflect a tendency, in this line, to hide in the nest box, and in this case it would suggest a lower level of welfare in the LSL. Svendsen et al. (2005) found that low stereotyping mink were more often in the nest box during scanning observations as compared to video observations, and suggested fear of the observer to be the cause of the difference. A comparable difference was not seen in high stereotyping mink.

In the HSL a positive correlation between stereotypic behaviour and confident temperament was found. This relationship is supported by Hansen and Jeppesen (2006), who found a group of high stereotyping mink to be more confident and less fearful than a group of low stereotyping mink. These results indicate that high stereotyping mink experience better welfare than low stereotyping mink, and this is in accordance with the above mentioned suggestion of poorer welfare in the LSL. Taken together, these suggestions support that the measured levels of cortisol do not necessarily reflect poorer welfare in the HSL.

The weight measures increased steadily during the autumn in both lines, so failure of growth, which might be indicative of bad welfare, is not at all implied. The difference in body weights between the LSL and the HSL, with the lowest body weight for the HSL, was maintained throughout the autumn. This is most likely due to the greater level of activity in the HSL. Indications that stereotypies may be the cause rather than the result of low body weight exist (Damgaard et al., 2004; Jeppesen et al., 2004).

Excessive body weight impairs reproduction in mink (Jørgensen, 1984). Previous investigations have demonstrated an indirect positive effect of stereotypic behaviour on reproduction in mink, which seemed to depend on stereotypies lowering the body weight (Jeppesen et al., 2004; Lagerkvist et al., 1994). Although the HSL had a significantly higher level of stereotypy and a lower body weight in October, the present study demonstrates no relationship between stereotypies and fertility in female farm mink. This difference between the studies could depend on the farm management, since they were carried out at two different farms. If the animals in the present study were fed in a way which better prevented the LSL from being too heavy for good reproductive performance, this could have counteracted the negative influence of low activity on weight in relation to reproduction.

Mason and Latham (2004) questioned whether the welfare of stereotypers is definitely worse than that of non-stereotypers. They also cautioned about the consequences of selecting against stereotypy by emphasizing the potential risk of selecting against the ability to express behaviours that might help the mink to accept their environment. The results of the present study may be taken to support the opposite view: that selection favors individuals that are less stress sensitive and better adapted to their environment, and for that reason develop less stereotypy. This suggestion is based on the non-verified assumption that the highly significant results on FCM concentrations reflect baseline levels of adrenocortical activity. The suggestion could not be confirmed or rejected by the reproduction data or by the weight data and it is contradicted by the

possible effect of the selection on fear. Therefore, further research is necessary. Based on the present knowledge, breeding against stereotypy seems to have contradictory consequences for the welfare of the mink.

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