

FEAR-RELATED BEHAVIOUR IN TWO MOUSE STRAINS DIFFERING IN LITTER SIZE

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Abstract

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The present experiment compared the fear-related behaviour of a mouse strain selected over 101 generations for high litter size with that of a randomly selected strain. The H-strain, selected for large litter size, has a mean (\pm SD) litter size at birth of 21.5 ± 3.5 pups. The randomly bred C-strain has a mean (\pm SD) litter size of 9.6 ± 2.2 pups. The elevated plus-maze, the light:dark test and a resident:intruder test were used to measure how the mice responded to novelty. In the elevated plus-maze, a well-validated model of animal anxiety, the H-strain was significantly more anxious (having a lower percentage of entries into open arms) than the C-strain at 9 weeks of age. In the light:dark test, in which the light levels were similar to those in the home environment, the H-strain did not differ significantly from the C-strain in its avoidance of the brightest area. In the resident:intruder test, where aggression-trained, older H-strain males were the residents, 11-week-old intruding mice of the C-strain spent a higher percentage of their time in flight and immobility than intruders of the H-strain. There were clear anxiety- and fear-related differences between the strains, which may be related to their selection history. The results illustrate a need for further studies on the consequences of selection for increased production for the ability of animals to adapt to their home environment and cope with environmental changes.

Keywords: *animal welfare, anxiety, behavioural strategy, fear, mouse, selection*

Introduction

A moderate level of fear is assumed to be adaptive, as it leads to functional reactions to actual danger (Marks & Nesse 1994; Jones 1997). Chronic anxiety, on the other hand, which causes animals to overreact to stimuli that pose no actual threat, may lead to stress and reduced welfare under normal farming conditions (Jones 1996). Measurements of anxiety could thus be important for the evaluation of animal welfare. The subject of fear embraces many different phenomena and processes (Jones 1996). As suggested by Boissy (1995) in his review article on fear and fearfulness in animals, 'fear and anxiety have often been considered motivators and are defined here as emotional states that are induced by the perception of any actual danger (fear state) or potential danger (anxiety state) that threatens the well-being of the individual, and which are characterised as a feeling of insecurity'.

Responses to fear stimuli are altered and integrated with one another to provide the most appropriate behavioural strategy for coping with a particular danger. It can be difficult to interpret fear behaviour without considering the strategies which individuals employ in response to danger. Behaviours included in such strategies are fighting, flight, immobility and cautious investigation (Jones 1996).

Benus (1988) practised divergent selection for attack latency in mice. She found that mice with a short attack latency engaged in more flight (active behaviour) when encountering a stronger opponent, while individuals with a long attack latency predominantly showed immobility (passive behaviour) in response to attack. Active individuals responded more slowly to environmental changes and responded more slowly than passive individuals in adapting to continually changing environments. This led Benus (1988) to suggest that active individuals are less dependent on external stimuli and that control of their responses is of a more intrinsic (internally controlled) or habitual nature. She also related these findings to the ecology and social structure in mouse populations and interpreted her results as indicating an individual predisposition to use either predominantly active or passive behaviour in response to social and non-social challenge.

Production efficiency has been improved dramatically during the last 40 years through the development of efficient breeding systems, better nutrition and intensive farming methods. However, new problems have arisen under modern farming conditions, such as abnormal behaviour, reduced reproduction, early death, physical damage and immune suppression, all indicating reduced welfare (Broom 1991; Mills *et al* 1997; Bakken *et al* 1998; Rauw *et al* 1998). Siegel (1989) showed that hens bred for meat and egg production have correlated increases in docility, reduced motor ability, poor immunoresponsiveness and increased appetite.

Genetic variation in behaviour and other characteristics related to welfare are documented in a number of farm animal subspecies, breeds and breed crosses (Hohenboken 1986; Braastad & Katle 1989; Siegel 1989; Luiting 1991; Luiting *et al* 1994). Benus (1988) was able to breed mice, selecting for active *vs* passive behaviour in aggressive social encounters, indicating that the predisposition to use predominantly active or passive behaviour may be partly genetically controlled in rodents. There are many reports of variation in fear- and anxiety-related behaviour in rodents, which may be partly genetically determined (Broadhurst 1974; Beilharz 1975; Beilharz & Beilharz 1975; Gray 1987; Rodgers & Cole 1993; Trullas & Skolnick 1993; Rex *et al* 1996). Plomin *et al* (1997) describe specific quantitative trait loci in the genome of inbred mouse strains influencing several measures of anxiety and activity in the elevated plus-maze and open field test. Hohenboken (1986) suggests that we might use the knowledge of such genetic variation in behaviour to improve animal welfare. Artificial selection in an experimental environment may prove to be a powerful tool for investigating the relationships between selection for production traits, including litter size and welfare-related behavioural characteristics such as fear and anxiety.

The aim of the present experiment was to compare the fear- and anxiety-related behaviour of a mouse strain selected for high litter size with that of a strain selected randomly in the same environment, to illuminate differences in their responses to three novel environments: the elevated plus-maze, the light:dark test and the resident:intruder test. The results are discussed in relation to the animals' ability to cope with environmental changes, with coping defined here as maintenance of mental and bodily stability (Broom & Johnson 1993).

Materials and methods

Animals

The two mouse strains tested were from the 101st generation of a Norwegian selection experiment (Joakimsen & Baker 1977; Vangen 1993) and were, therefore, housed under identical conditions during their selection history. The H-strain has been bred for high litter size since 1972, reaching a plateau of 21.5 ± 3.5 (mean \pm SD) pups per litter. Males of the H-strain weighed 51.3 ± 2.7 g and females, 43.5 ± 2.4 g at 11 weeks of age. The C-strain has been bred randomly for the same number of generations, and had a (mean \pm SD) litter size of 9.6 ± 2.2 pups. The (mean \pm SD) weight of C-strain males at 11 weeks was 37.0 ± 4.1 g and 30.9 ± 3.6 g for females. Both strains came in a variety of colours and were not albino. Within 24h of birth, the litters were standardized to four females and four males per litter. (Animals removed from the litters for standardization were euthanased by exposure to CO₂ for 20min in an airtight container. This is a standard method for euthanasia of laboratory rodents under Norwegian legislation. Approval for the euthanasia was obtained from the Animal Control Officer at the Agricultural University of Norway.)

All mice were housed with 24h lighting in generations 1-101, in standard open-topped, non-transparent cages measuring 30x12.5x12.5 cm, and were provided with wood shavings for bedding. The (mean \pm SD) light intensity was measured at 449 ± 84 lux in the mouse laboratory and 25 ± 12 lux in the cages. The (mean \pm SD) temperature was 21 ± 1 °C. Standard laboratory pellets (R3 pellets; Laktamin Specialfoderföretaget, Stockholm, Sweden) and water were available *ad libitum*.

One male from each of 14 litters in each strain was randomly selected and tested in the elevated plus-maze at the age of 9 weeks, followed by testing in the light:dark test at 10 weeks of age and the resident:intruder test at 11 weeks of age. The mice were divided by sex and housed in pairs after weaning at 3 weeks of age until 2 weeks before testing, at which time they were separated, living singly thereafter. Ten, 20-week-old males from the 100th generation were used as residents in the resident:intruder test. These mice weighed ≥ 51 g and lived singly after the age of 11 weeks.

Before testing, the mice were handled only at birth, weaning, separation into individual cages before testing and when cages were cleaned every second week. Handling at weaning, separation and cage-cleaning constituted lifting the mouse by the tail into the clean cage. Mice were tested in the same order in the three behavioural tests.

General procedures

For the three tests of behaviour, all testing was conducted between 0900h and 1500h in a room adjacent to the mouse laboratory. All mice were put into the test room 1h before testing commenced. After placing the mouse onto the test apparatus, the laboratory technician left the test room. Between subjects, the elevated plus-maze and light:dark test apparatus were thoroughly cleaned, using damp and dry cloths.

All test sessions were recorded by a video camera linked to a monitor and VCR (Panasonic Time Lapse Cassette Recorder, AG-6720; Securitas, Oslo, Norway) in an adjacent room. Behaviour was scored off videotape, always by the same observer who was blind to treatment conditions. An animal's behaviour was taped for 6min from the time it was placed in the test apparatus. Behaviour was recorded after testing. The tape ran at normal speed for taping and recording of behaviour in the elevated plus-maze and the light:dark tests. In the resident:intruder test, behaviour was taped at normal speed (3h/tape) but

recorded with a slower playback (14h/tape) to facilitate observation of behavioural elements of short duration.

The elevated plus-maze

The elevated plus-maze was a copy of the apparatus described by Lister (1987). This test has been well validated for measuring anxiety in rodents, using physiological, pharmaceutical and behavioural methods (Pellow *et al* 1985; Lister 1987; Cruz *et al* 1994; File *et al* 1994). The apparatus is shaped like a plus sign, having two opposite arms with walls and two opposite arms without walls, elevated 45cm above the floor by a single central support. It was illuminated (mean \pm SD of 32 ± 4 lux) by a single 150W red light, elevated 120cm above the centre of the maze floor. Rodgers and Cole (1994) describe the maze in detail. The elevated plus-maze creates a conflict between the motivation to explore open arms and the aversion caused by an elevated open space (Hogg 1996). The traditional measures of anxiety in the elevated plus-maze are based on the observation that less anxious mice spend a higher percentage of time on the open arms and have a higher percentage of entries into open arms than do more anxious mice (Hogg 1996). The purest measure of locomotor activity is the number of entries into the closed arms (Hogg 1996). Some authors suggest that the use of 'risk assessment' measures may increase the sensitivity of the elevated plus-maze (Rodgers & Johnson 1995; Rodgers & Dalvi 1996; Rodgers 1997), but their data indicate that the traditional measures are adequate for measuring 'anxiety' in this animal model. The simple traditional measures were, therefore, used in the present experiment.

The test commenced with placing a mouse on the central platform of the maze facing an open arm, and lasted 5min. As in the validated test, the number of open and closed arm entries and the time spent in the various sections of the maze were measured. An arm entry was defined as all four paws being over a line separating the central platform from a maze arm. Derived measures were: the total number of arm entries; the percentage of entries into open arms (ie [number of open arm entries/total number of arm entries] x 100); and the time spent in the different sections of the maze, expressed as a percentage of the test duration (% time in arm = [time in arm(s)/300s] x 100).

The light:dark test

The light:dark test box constructed for the present experiment was a copy of the apparatus used by Cheng *et al* (1994) and was constructed of plexiglas. The apparatus consisted of a white-walled compartment (30x27x27 cm) joined by a small opening (7.5x7.5 cm) to a black-walled compartment (15x27x27 cm). The dark compartment of the light:dark test apparatus was dimly illuminated (mean \pm SD of 42 ± 7 lux) by a single 150W red light bulb, elevated 120cm above the floor at the centre of the illuminated area. The light compartment, in addition to being illuminated by the red light, was brightly illuminated (472 ± 46 lux) by a white 25W light bulb located 40cm above the floor at the centre of the light compartment. The floor of the apparatus was divided by lines into 9x9 cm squares.

In the light:dark test, the mice are faced with a conflict between their desire to explore a novel area and their aversion to bright light (File 1992). Increases in the proportion of activity and time in the light compartment (Costall *et al* 1987) and increases in the frequency of crossings between the compartments (Crawley 1981; Crawley & Davis 1982) have been used to indicate an anxiolytic action. As the validity of these measures as indicators of anxiety is controversial (File 1992), the present experiment used the percentage of time in the light compartment to measure the degree of avoidance of the brightly-lit compartment. Line crossings were used as a measure of locomotion or activity. Mice were placed into one

corner of the white, brightly-lit area for a 5min test. The time that the mouse spent in each compartment and the total number of line crossings were noted from videotape (see, *General procedures*). A line crossing was defined as all four paws being over a line. From these measurements, the percentage of time in the light compartment was derived.

The resident:intruder test

A resident:intruder test was used to evaluate behavioural elements related to active or passive coping strategies of the mice in response to attack in a novel environment, and was a modification of the defeat test used by Benus (1988). The test procedure validated by Benus (1988) was copied exactly in this experiment to allow direct comparison of her findings with those in the present experiment. Choice of a validated method avoided exposing the mice to further (pilot) tests which would have been necessary to validate alternative methodologies. We believe that these tests, combined with the ongoing observational studies on aggression after cage cleaning, were justified as they provide information enabling improvements to be made to the welfare of each strain.

The test box (Figure 1) was divided into three compartments (A, B and C) by plexiglas sliding doors (1 and 2). The overall dimensions of the box were 88x20x20 cm. The floor (88x20 cm) and one wall (88x20 cm) of the box were made of plywood, while the remainder of the test box, including internal partitions, was constructed of transparent plexiglas. Compartment A (40x20x20 cm) functioned as the home-cage of the resident and Compartment B (40x20x20 cm) as the border area of the territory. Compartment C (8x20x20 cm) functioned as a holding room for the territory-holder in the seconds before the test start. The test apparatus was illuminated (mean \pm SD) by 77 ± 10 lux lights mounted on the ceiling of the test room, 200cm above the test box.

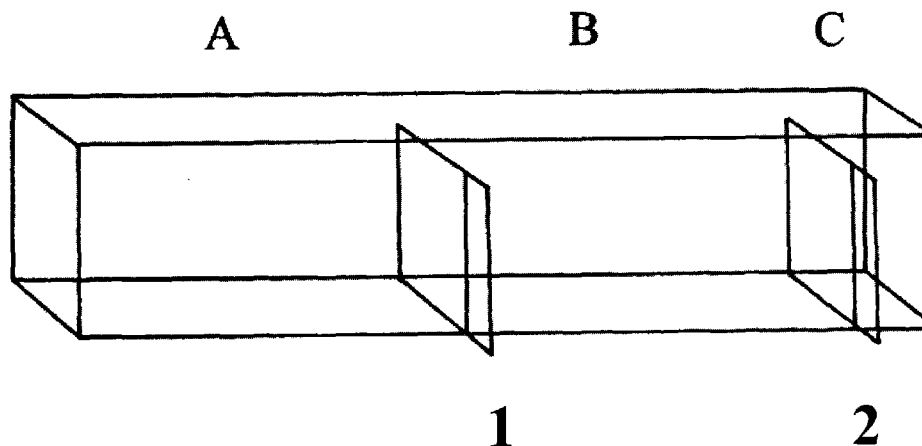


Figure 1 A schematic figure of the resident:intruder apparatus. See text for full description of apparatus and labelling.

The resident mice were 10 trained, aggressive males from the H-strain (≥ 51 g) that had been given numerous brief (duration c 30s) confrontations with younger (6–8-week-old) male opponents in the last month before the test. This training procedure was followed since it is known that terminating a fight after a short time by removing the opponent produces a consistent elevation of aggression in male laboratory mice (Lagerspetz 1961; Leshner &

Nock 1976). Residents were more experienced, usually larger, and always older (mean age 20 weeks) than the intruders (mean age 11 weeks), which gave them an advantage in the aggressive encounters. The residents were placed in observation cages and given 2 weeks to become territorial in Compartment A (Figure 1). The 2-week period was chosen as Benus (1988) showed this interval was adequate for the development of territoriality – observed as consistent attacking of intruder males.

At the time of the experiment, the resident was placed in Compartment C, an opponent was introduced into B, sliding door 2 was opened, and the confrontation time began within Compartments B and C. Twenty seconds thereafter, the behaviour of the intruder was observed for a period of 5min, using the video. The intruders showed fighting, flight, immobility and exploration. The number of times these behaviours were observed and their durations were measured to indicate the degree to which mice of the two strains expressed these behaviours. To analyse the behavioural response to attack, the same behavioural elements which Benus (1988) used were observed (Table 1). The frequency of the behavioural elements as well as the percentage of time mice spent using these behaviours were determined. The frequency of behaviour X was defined as: (the total number of times X was observed/the sum of all observations of behaviour) x 100.

Table 1 Behavioural categories used in the resident:intruder test (as defined by Benus [1988]).

Fighting	
fighting	the behaviour shown by each of the contestants when locked together in violent kicking, biting and wrestling behaviour.
approach	directional locomotion towards the resident.
attack	rushing and leaping at the resident with kicks and bites.
chasing	chasing a fleeing resident.
side display	approaching the resident in a sideways stance accompanied by intention movements of boxing and biting.
boxing	altered kicking with the forepaws, combined with intention movements of the body toward the resident, the intruder remaining in its place.
Flight	
flight	rapid movement away from the resident, generally accompanied by squeaks, leaps and sudden changes in direction.
withdrawal	locomotion directed away from the resident.
Immobility	
submissive upright	sitting upright, head into the air, forepaws rigidly stretched out forward.
immobility	absence of any movement.
Exploration	
upright	standing or sitting on hind legs, mostly making sniffing movements with the nose up into the air.
investigation	sniffing any parts of the resident's body.
sniffing	standing still with the nose in substrate.
locomotion	diagonal and quadrupedal locomotion, no high speed, no apparent direction.
Other behaviours	
other behaviour	any other behavioural element.

As the resident:intruder test appeared to be aversive to test animals, a minimum sample size ($n = 14$) was used and test mice were exposed to the test situation for the shortest possible time to allow adequate scoring of behaviour. However, the actual level of damage to test animals was minimal (including cuts to the tails of two mice) and the level of aggression was similar to that observed between familiar mice during normal cage cleaning.

Statistical analysis

To analyse results, the SAS system (SAS 1986) was used. A Wilcoxon signed ranks test was used to test for differences in location between the two samples, and Spearman rank order correlation coefficients were used to measure the relationship between different behavioural elements within and between tests. These non-parametric methods were used as some of the data did not conform to the assumption of parametric statistics. All tests were two-tailed.

Results

The elevated plus-maze for 9-week-old male mice

Figure 2 illustrates the results. The H-strain recorded a lower percentage of entries into open arms ($z = 2.05$, $P < 0.05$; Figure 2a) and spent a smaller percentage of time on these arms ($z = 2.33$, $P < 0.05$; Figure 2b). There was, however, no significant difference between the strains in the (mean \pm SEM) number of entries into closed arms: C-strain, 11.7 ± 0.8 and H-strain, 12.1 ± 1.4 respectively; $z = 0.19$, ns. Thus, although the H-strain was more anxious in the elevated plus-maze, the strains did not differ in activity.

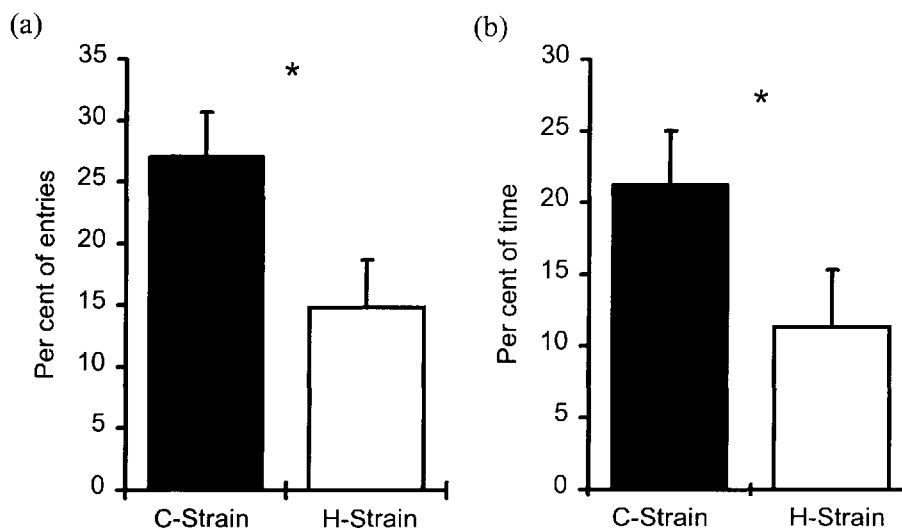


Figure 2 a) The (mean + SEM) percentage of entries into open arms in the elevated plus-maze; b) the (mean + SEM) percentage of time spent in the open arms in the elevated plus-maze. C-strain (dark columns), H-strain (clear columns). * $P < 0.05$.

The light:dark test for 10-week-old male mice

The results are illustrated in Figure 3. The strains did not differ in the per cent of time which the mice spent in the light compartment ($z = 1.81$, ns; Figure 3a), but the total number of

lines crossed ($z = 3.0$, $P < 0.01$; Figure 3b) was greater for the H-strain. The H-strain was more active, but the strains did not differ significantly in their avoidance of the light compartment.

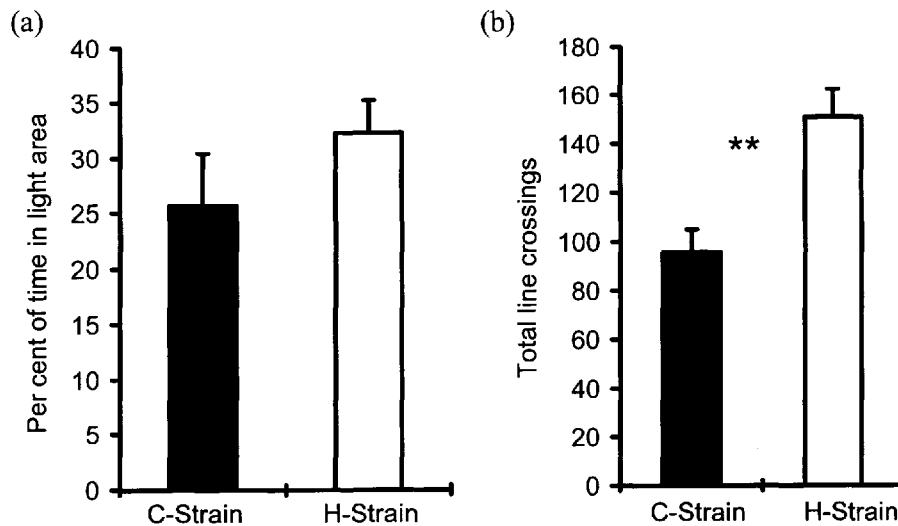


Figure 3 a) The (mean + SEM) per cent of time in the light area in the light:dark test; b) the (mean + SEM) total number of line crossings in the light:dark test. C-strain (dark columns), H-strain (clear columns). ** $P < 0.01$.

The resident:intruder test for 11-week-old male mice

The results are illustrated in Figure 4. The C-strain had the higher frequency ($z = 3.61$, $P < 0.001$; Figure 4a) and per cent of time ($z = 3.10$, $P < 0.01$; Figure 4b) in flight. The C-strain spent the greater percentage of time immobile ($z = 1.98$, $P < 0.05$; Figure 4b) but there was no significant difference between the strains in the frequency of immobility ($z = 1.42$, ns; Figure 4a). The H-strain recorded the higher frequency of exploration ($z = 3.06$, $P < 0.01$; Figure 4a) and had a tendency to spend a larger per cent of time in exploratory activity ($z = 1.93$, $P < 0.05$; Figure 4b). There was no difference between the strains in the (mean \pm SEM) frequency of active behaviour (ie fighting, flight and exploration): C-strain, 67.2 ± 1.3 and H-strain, 65.2 ± 1.5 , respectively; $z = 1.42$, ns; in the (mean \pm SEM) per cent of time spent in active behaviour (C-strain, 32.5 ± 4.7 and H-strain, 43.5 ± 4.8 , respectively; $z = 1.59$, ns); in the frequency of fights ($z = 0.20$, ns; Figure 4a) or the per cent of time spent fighting ($z = 0.34$, ns; Figure 4b). Five H-strain animals continued exploratory activity, at least once, while being bitten by the attacker. This behaviour was never observed for the C-strain in response to bites (Fisher's Exact Test, two-tailed; $P < 0.05$).

Correlations

Measures of immobility in the resident:intruder test (percentage of time in immobility) and elevated plus-maze activity (closed arm entries) were negatively correlated for the C-strain ($r_s = -0.69$, $P < 0.01$), as would be predicted from Benus' (1988) theory, but not for the H-strain ($r_s = -0.41$, ns). Furthermore, there were negative correlations between the frequency

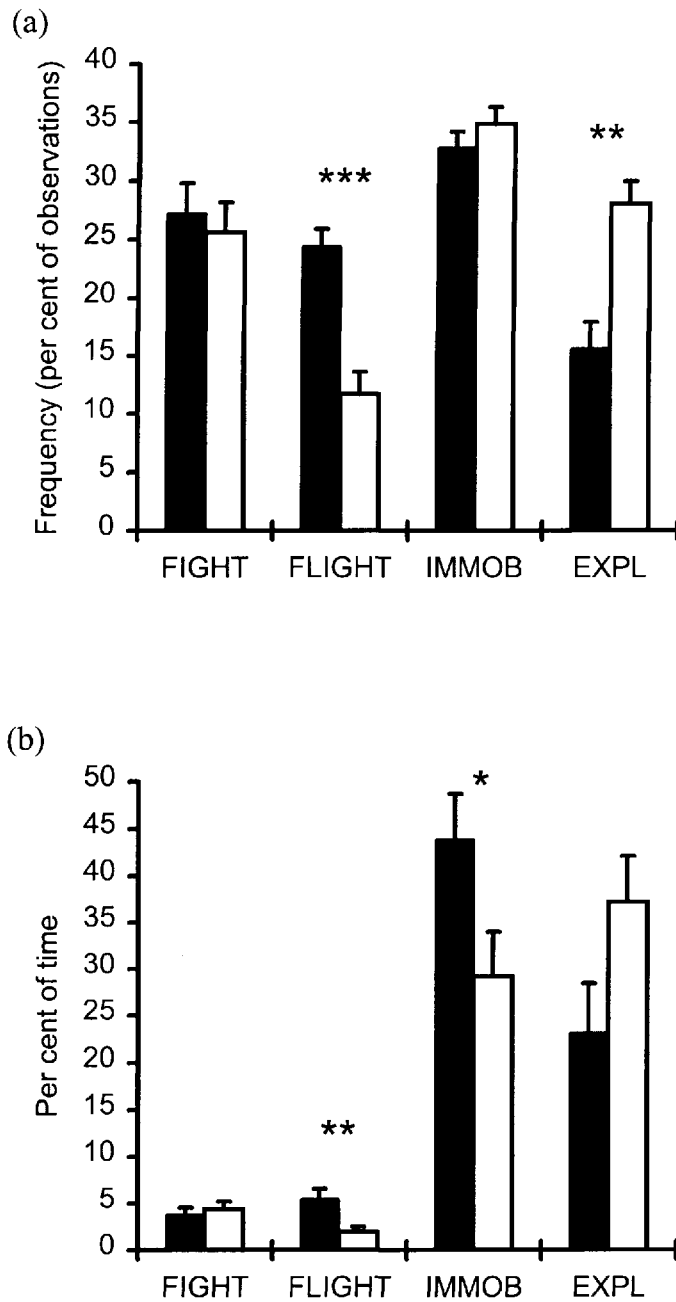


Figure 4 a) The (mean + SEM) frequency (ie percentage of total observations) spent in various behaviours in the resident:intruder test; b) the (mean + SEM) percentage of time spent in various behaviours in the resident:intruder test. C-strain (dark columns), H-strain (clear columns). IMMOB = immobility, EXPL = exploration; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

of immobility and flight for the C-strain ($r_s = -0.60$, $P < 0.05$) but not for the H-strain ($r_s = -0.03$, ns). These two observations indicate limitations in the generality of Benus' (1988) theory.

For both strains, there was a lack of correlation between the measure of anxiety in the elevated plus-maze (per cent open entries) and avoidance of the brightly lit area of the light:dark test measured as the per cent of time which they spent in this compartment (H-strain: $r_s = -0.16$, ns; C-strain: $r_s = -0.03$, ns). Such a correlation would be expected if anxiety was a general, stimulus-independent emotional state.

Discussion

The H-strain was more anxious in the elevated plus-maze but the strains did not differ in their avoidance of the light compartment in the light:dark test. The H-strain was more active in the light:dark test, crossing more lines, but the strains did not differ in activity in the elevated plus-maze or the resident:intruder test. The C-strain spent a higher per cent of time in flight and immobility, and had a higher frequency of flight than the H-strain in response to attack in the resident:intruder test. The H-strain, on the other hand, had a higher frequency of exploration.

The anxiety-invoking stimuli in the elevated plus-maze environment are novelty and elevated open space (Lister 1987; File 1992; Hogg 1996). The H-strain's higher anxiety in the elevated plus-maze environment may indicate that the H-strain had more difficulty than the C-strain in coping with the novel or anxiogenic stimuli in the elevated plus-maze.

In the light:dark test, the novelty of the unknown box and the brightly-lit, white-walled room both function as potentially aversive stimuli for mice (Crawley 1981; Crawley & Davis 1982; Costall *et al* 1987; File 1992; Nasello *et al* 1997; Bilkei-Gorzó *et al* 1998). The H-strain behaved more anxiously in the elevated plus-maze, but did not avoid the light area of the light:dark test to a higher degree than the C-strain. This result was unexpected. Furthermore, within both strains no correlation was found between these two variables. This suggests that states which can be characterized as 'anxiety' may be multi-dimensional and related to specific stimuli for the animals in the present experiment. Marks and Nesse (1994) and Ramos and Mormède (1997) propose a similar view, discussing the evidence for, and selective advantages of, 'subtypes of anxiety'.

The anxiogenic stimuli in the resident:intruder test are novelty and attack by a larger and stronger aggressive male (Benus 1988). This test was used to measure fear reactions and behavioural elements related to active and passive coping strategies, as Benus (1988) proposed. As animals in the H-strain used flight and immobility to a lesser degree than individuals in the C-strain, and appeared to react less to attack, it is possible that the motivational and behavioural systems in the H-strain have been altered by selection. This difference between the strains is well illustrated by the different correlations in the two strains between the behavioural elements in the different tests. The significant negative correlation for the C-strain between immobility and flight in the resident:intruder test supports Benus' (1988) theory of active and passive behaviour as alternative coping strategies for mice. However, the lack of correlations between immobility and flight for the H-strain questions the generality of Benus' theory and indicates that such relationships may be altered through selective processes. The frequency of immobility was the same in both strains, but the duration of immobility was significantly shorter for the H-strain than for the C-strain. It appears that the ability to show immobility responses is the same for the two

strains, but that the functionality of immobility as a behavioural coping response which inhibits active behaviour is reduced in the H-strain.

The H-strain may, therefore, have a reduced ability to use functional defensive strategies. This possibility is illustrated by the observations that individuals in the H-strain did not appear to respond to being bitten by an attacker in the same way as those in the C-strain. In the elevated plus-maze, where the expression of fear is less clearly related to the behavioural coping strategies fight/flight and immobility, the H-strain behaved more anxiously, indicating that their anxiety in this novel environment is high, but their ability to express some elements of functional behavioural strategies – flight and immobility – in social encounters is reduced in relation to the C-strain. This inability to react may be a disadvantage for individuals in the H-strain in environments where social competition is important for survival. The work of several authors (Siegel 1989; Luxford *et al* 1990; Beilharz *et al* 1993; Beilharz & Mitpaiboon 1994) has promoted the view that there is a negative relationship between high production and the ability to cope with environmental changes and other novel challenges. The present experiment cannot provide data illuminating a relationship between high litter size and coping ability, as only males were tested, but the possibility of such a relationship should be investigated in further experiments using females.

An important criticism that could be raised in relation to these interpretations is the fact that only males of the H-strain were used as residents in the resident:intruder test. The residents were, therefore, much larger than the intruders of the C-strain, but not much larger than the intruders of the H-strain. This might be the reason that C-strain intruders fled more frequently than H-strain intruders and had a longer duration of flight and immobility. On the other hand, the different correlations in the two strains and the low level of response specifically to bites in the H-strain are important for the interpretation presented here. It is difficult to see how the size difference between the strains might reduce the legitimacy of the different correlations in the two strains and the low level of response to bites in the H-strain. The results based on the resident:intruder test should be interpreted with caution and understood as pertaining to the experimental design which was used in the present experiment.

Animal welfare implications

When animals are transported from one environment to another, or animal housing is designed, it would be useful to know how well particular animal strains adapt to different environments. Vandenheede (1996) suggests that knowledge of the factors which affect fear reactions would make it possible to avoid the reductions in animal welfare and production caused by exposing animals to stimuli with which they have trouble coping. The present experiments have shown that selection may indeed change the ability of animals to cope with certain novel environments. This should be further investigated in specific laboratory and farm animal breeds by studying genetic and physiological mechanisms in relation to behaviour both in the home and in novel environments.

Conclusion

During exposure to the two non-social novel environments there were clear differences in fear, anxiety and activity-related behaviours between two mouse strains selected in identical environments, one selected for high litter size (H-strain) and one randomly selected (C-strain). The behavioural reactions to anxiety- and fear-inducing stimuli appeared to have been altered in the course of selection. There were dissimilarities between the strains in their

use of coping behaviour in response to attack, the C-strain spending more time in flight and immobility. The behavioural differences between the strains could be attributed to genetic drift, other random genetic effects, or selection for high litter size. These hypotheses must be tested by further studies.

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References

- Bakken M, Vangen O and Rauw W M** 1998 Biological limits to selection and animal welfare. In: Haworth L, Little M and Schmidt I (eds) *Proceedings of the 6th World Congress of Genetics Applied to Livestock Production 27* pp 381-389. World Congress on Genetics and Livestock Production: Armidale, Australia
- Beilharz R G** 1975 The aggressive response of male mice (*Mus musculus* L.) to a variety of stimulus animals. *Zeitschrift für Tierpsychologie* 39: 141-149
- Beilharz R G and Beilharz V C** 1975 Observations on fighting behavior of male mice (*Mus musculus* L.) *Zeitschrift für Tierpsychologie* 39: 126-140
- Beilharz R G, Luxford B G and Wilkinson J L** 1993 Quantitative genetics and evolution: Is our understanding of genetics sufficient to explain evolution? *Journal of Animal Breeding and Genetics* 110: 161-170
- Beilharz R G and Mitpaiboon K** 1994 Environmental limitation on fitness: reproduction of laboratory mice in benign and stressful ('tropical') conditions. *Journal of Animal Breeding and Genetics* 111: 14-26
- Benus I** 1988 *Aggression and coping-differences in behavioral strategies between aggressive and non-aggressive male mice*. Published PhD thesis, University of Groningen, The Netherlands.
- Bilkei-Gorzó A, Gyertyán I and Lévy G** 1998 mCPP-induced anxiety in the light-dark box in rats: a new method for screening anxiolytic activity. *Psychopharmacology* 136: 291-298
- Boissy A** 1995 Fear and fearfulness in animals. *The Quarterly Review of Biology* 70: 165-191
- Braastad B O and Kettle J** 1989 Behavioural differences between laying hen populations selected for high and low efficiency of food utilisation. *British Poultry Science* 30: 533-544
- Broadhurst P L** 1974 The Maudsley reactive and nonreactive strains of rats: a survey. *Behavior and Genetics* 5: 299-319
- Broom D M** 1991 Animal welfare: concepts and measurement. *Journal of Animal Science* 69: 4167-4175
- Broom D M and Johnson K G** 1993 *Stress and Animal Welfare*. Chapman and Hall: London, UK
- Cheng C, Costall B, Kelly M E and Naylor J R** 1994 Actions of 5-hydroxytryptophan to inhibit and disinhibit mouse behaviour in the light:dark test. *European Journal of Pharmacology* 225: 39-49
- Costall B, Hendrie C A, Kelly M E and Naylor R J** 1987 Actions of sulpiride and tiapride in a simple model of anxiety in mice. *Neuropharmacology* 26: 195-200
- Crawley J N** 1981 Neuropharmacological specificity of a simple animal model for the behavioral actions of benzodiazepines. *Pharmacology Biochemistry and Behavior* 15: 695-699
- Crawley J N and Davis L G** 1982 Baseline exploratory activity predicts anxiolytic responsiveness to Diazepam in five mouse strains. *Brain Research Bulletin* 8: 609-612
- Cruz A P M, Frei F and Graeff F G** 1994 Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacology Biochemistry and Behavior* 49: 171-176
- File S E** 1992 Behavioural detection of anxiolytic action. In: Elliot J M, Heal D J and Marsden C A (eds) *Experimental Approaches to Anxiety and Depression* pp 25-44. Wiley: Chichester, UK
- File S E, Zangrossi H Jr, Sanders F L and Mabbutt P S** 1994 Raised corticosterone in the rat after exposure to the elevated plus-maze. *Psychopharmacology* 113: 543-546

- Gray J A 1987 *The Psychology of Fear and Stress*. Cambridge University Press: Cambridge, UK
- Hogg S 1996 A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior* 54: 21-30
- Hohenboken W D 1986 Inheritance of behavioural characteristics in livestock, a review. *Animal Breeding Abstracts* 54: 623-639
- Joakimsen Ø and Baker R L 1977 Selection for litter size in mice. *Acta Agriculturae Scandinavica* 27: 301-318
- Jones A 1996 Fear and adaptability in poultry: insights, implications and imperatives. *World's Poultry Science Journal* 52: 132-174
- Jones R B 1997 Fear and distress. In: Appleby M C and Hughes B O (eds) *Animal Welfare* pp 75-87. CAB International: Wallingford, UK
- Lagerspetz K 1961 Genetic and social causes of aggressive behavior in mice. *Scandinavian Journal of Psychology* 2: 167-173
- Leshner A I and Nock B L 1976 The effects of experience on agonistic responding: an expectancy theory interpretation. *Behavioral Biology* 17: 561-566
- Lister R G 1987 The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 92: 80-185
- Luiting P 1991 *The Value of Feed Consumption Data for Breeding in Laying Hens*. Published PhD thesis, Department of Animal Breeding, Wageningen Agricultural University, The Netherlands
- Luiting P, Decuypere E, de Groot P N, Buyse J and Room G 1994 Selection for feed efficiency and consequences for stress susceptibility. In: *45th Annual Meeting of the European Association for Animal Production* pp G5.7. European Association for Animal Production: Rome, Italy
- Luxford B G, Buis R C and Beilharz R G 1990 Lifetime reproductive performance of lines of mice after long term selection for first parity litter size at birth. *Journal of Animal Breeding and Genetics* 107: 188-195
- Marks I M and Nesse R M 1994 Fear and fitness: an evolutionary analysis of anxiety disorders. *Ethology and Sociobiology* 15: 247-261
- Mills A D, Beilharz R G and Hocking P M 1997 Genetic selection. In: Appleby M C and Hughes B O (eds) *Animal Welfare* pp 219-231. CAB International: Wallingford, UK
- Nasello A G, Machado C, Bastos J F and Felicio F 1997 Sudden darkness induces a high activity-low anxiety state in male and female rats. *Physiology & Behavior* 63: 451-454
- Pellow S, Chopin P, File S E and Briley M 1985 Validation of open/closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods* 14: 149-167
- Plomin R, DeFries J C, McClearn G E and Rutter M 1997 *Behavioral Genetics, 3rd Edition*. Freeman: New York, USA
- Ramos A and Mormède P 1997 Stress and emotionality: a multidimensional and genetic approach. *Neuroscience and Biobehavioral Reviews* 22: 33-57
- Rauw W M, Noordhuizen-Stassen E N and Grommers F J 1998 Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science* 56: 15-33
- Rex A, Sondern J P, Voigt S, Franck S and Fink H 1996 Strain differences in fear-motivated behavior of rats. *Pharmacology Biochemistry and Behavior* 54: 107-111
- Rodgers R J 1997 Animal models of 'anxiety': where next? *Behavioural Pharmacology* 8: 477-496
- Rodgers R J and Cole J C 1993 Influence of social isolation, gender, strain, and prior novelty on plus-maze behavior in mice. *Physiology & Behavior* 54: 729-736
- Rodgers R J and Cole J C 1994 The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper S J and Hendrie C A (eds) *Ethology and Psychopharmacology* pp 9-43. Wiley: New York, USA
- Rodgers R J and Dalvi A 1996 Anxiety, defence and the elevated plus-maze. *Neuroscience and Biobehavioral Reviews* 21: 801-810
- Rodgers R J and Johnson N J T 1995 Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacology Biochemistry and Behavior* 52: 297-303

SAS 1986 *SAS User's Guide: Statistics, Version 6 Edition*. SAS Institute Inc: Cary, USA

Siegel P B 1989 The genetic-behaviour interface and well-being of poultry. *British Poultry Science* 30: 3-13

Trullas R and Skolnick P 1993 Differences in fear motivated behaviours among inbred mouse strains. *Psychopharmacology* 111: 323-331

Vandenheede M 1996 Fear reactions in farm animals: assessment, factors of variation and effects on welfare and productivity. *Annales de Médecine Vétérinaire* 140: 423-432

Vangen O 1993 Results from 40 generations of divergent selection for litter size in mice. *Livestock Production Science* 37: 197-211