Genetica (1963) **34**: 79-94

MOUSE MUTANTS STUDIED BY MEANS OF ETHOLOGICAL METHODS *).

I. ETHOGRAM

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(*Received May* 7, 1963)

In order to determine in how far some known genes will affect the behaviour of mice (*Mus musculus L.*) the author used the ethological method for drawing up an inventory of behavioral elements; this can be considered the phenotype to be investigated.

For this purpose the behaviour was broken down into those elements presented by a solitary male (situation I), by two males placed together (situation II), and by one male and one female together (situation III). The environmental factors for the mice were kept as uniform as was possible. In the present article an outline is given of the methods used in analysing that behaviour; in a later article it will be shown to what ends these methods can be used.

Introduction

Mice are well-suited for research in the field of behaviour genetics. About these animals we possess a good deal of genetic knowledge (GRÜNEBERG, 1952), while in addition they show a particularly rich and varied system of behaviour patterns, as was especially revealed by the work of EIBL-EIBESFELDT (1950, 1958). In fact, new publications on this subject are regularly appearing, but in the greater part of cases research on the behaviour genetics of rodents would seem to have restricted itself to some particular aspect or to the results of their behaviours, e.g. the activity of the animals in running wheels, mazes and arenas (BRODY, 1950; BRUELL, 1962; CARR & WILLIAMS, 1957; MCCLEARN, 1959, 1961; THIESSEN, 1961; THOMPSON, 1953, 1956; WILLIAMS, ZEROF & CARR, 1962), emotionality (BROADHURST, 1957, 1961), audiogenic seizures (FULLER, EASLER & SMITH,

*) I am indebted to Professor S. J. GEERTS, Nijmegen, for suggesting the problem and reading the manuscript.

1950; GINSBURG, 1954), wall-seeking tendency (FREDERICSON, 1953), hoarding (STAMM, 1954, 1956), fighting for food (FREDERICSON & BIRNBAUM, 1954; ROSEN, 1961), latencies and frequencies of some sexual behaviour elements (WHALEN, 1961), and social dominance (GINSBURG & ALLEE, 1942; LINDZEY, WINSTON & MANOSEVITZ, 1961). Further-going analyses of behaviour in genetic work were supplied by BAUER (1956) and SCOTT & FREDERICSON (1951). For a further development of behaviour genetics it seems desirable that behaviour should be studied in all its multiformity; in this respect behaviour genetics may greatly profit from ethological attainments and procedures, among which the drawing up of ethograms, i.e. behaviour inventories, comes first. One might say that the ethogram is, in fact, the phenotype to be studied.

In accordance with ethological views, the stereotype behavioral elements to be described after analysis may be called "innate" (LORENZ, 1950; TINBERGEN, 1951), but this is a controversial point (ANASTASI, 1958; BARNETT, 1958; HEBB, 1953; LEHRMAN, 1953; VERPLANCK, 1955). The difficulty lies in the distinction between "innate" and "learned" behaviour. Although we are on slippery ground here, this need not hamper the course of research in behaviour genetics; on the contrary, it is quite possible. that behaviour genetics should be able to shed some light on this problem. A practical conclusion must be that in first instance numerous precautions have to be taken to ensure that the mice of different genotypes that are to be compared, are reared and examined under circumstances as closely similar as possible. Next, we may vary the environment, in the hope that in doing so more of its *modus operandi* will be revealed.

Within the frame of behaviour genetical research on mice and rats the main stress is now being laid on comparison of strains. Experimental crosses with strains (BRUELL, 1962; MCCLEARN, 1959, 1961; STAMM, 1956; WILLIAMS, ZEROF & CARR, 1962) have as yet been infrequent. In other words, in actual research practice special attention is paid to differences in behaviour, if any, in the strains; starting from this phenotype it is attempted to trace the differences back to the genotype, thus landing us in the field of polygenic inheritance.

An approach in which we are not directed towards polygenes is to begin with a mutant as well as with the normal genotype and to

pursue this monogene difference as far as behaviour, i.e. the phenotype. This procedure, which has the advantage that a good many known genes may be screened for their effects on behaviour, has found up to now only limited application: the effects of the mutations *maltese dilution* and *short-ear* on the activity of mice (ASHMAN, 1957), of *yellow* on wing vibrating in *Drosophila* (BASTOCK, 1956), of *brown*-eye and *white* on phototaxis in *Drosophila* (SCOTT, 1943), of some *white*-alleles on the mating success in *Drosophila* (GEER & GREEN, 1962), of some genes, especially coat colour genes, on the temperament of rats (KEELER, 1942, 1948; KEELER & KING, 1942).

Although an investigation that starts from behavioral traits to work its way towards the genotype, is not without value, still the other approach, which studies the influence of single genes on behaviour, would seem to be preferable. This in spite of HIRSCH's contention (1962) that "The available evidence makes it appear unlikely that variations in many behaviors can be explained by simple Mendelian relations". It is, indeed, the starting-point that matters: if we start on the "simple Mendelian relations" we can trace any attendant variations. These variations may be expected to exist (SCOTT & FREDERICSON, 1951; KEELER & KING, 1942; CASPARI, 1958). It is worth while to investigate whether there are differences in behaviour as a result of such pleiotropic gene actions; at first, in view of the time-consuming character of such work, with a limited number of animals; next, if this should lead to positive results, for some particular mutant with more animals.

An Inventory of the Behavioral Elements *)

The problem is how these elements should be grouped. SCOTT (1958) speaks of exploratory, ingestive, eliminative, agonistic, sexual, nest building, epimeletic, etepimeletic and social behaviours. In fact, these terms have a functional or finalistic character, i.e. they are as it were prompted by the result of the behaviour. In the present investigation the author has refrained from such indications, the grouping of elements used here being a purely practical one: first, the behaviours occurring in Situation I where a single male is put in the terrarium

*) Thanks are due to Mr. H. J. M. SPRUIJT for technical assistance in taking the photographs, from which the figures were redrawn.

used (108 × 49 × 49 cm); secondly, the behaviours in Situation II, when two males are put together; thirdly those behaviour elements presenting themselves when one male and one female are put together (Situation III). Apart from these a number of other situations are feasible. The terminology of the elements as used below conforms as much as possible to that used in the literature.

Situation I

If a male is brought into an unfamiliar environment, the testes may be observed to descend into the scrotum (Fig. 8). The behavioral elements observed are:

Staring at observer, STA, figure 1

The animal suddenly 'freezes' and fixes the observer intently, the ears being turned in his direction. As a rule this will last for some seconds, occasionally over a minute. Sometimes it will occur in consequence of a movement made by the observer in writing, sometimes without any demonstrable cause.

Hair fluffing, FLU, figure 11

'Katzenbuckel' (EIBL-EIBESFELDT, 1958). The action tends to occur right at the beginning of an obervation period. Never seen in females.

Tail rattling, RAT, (EIBL-EIBESFELDT, 1958)

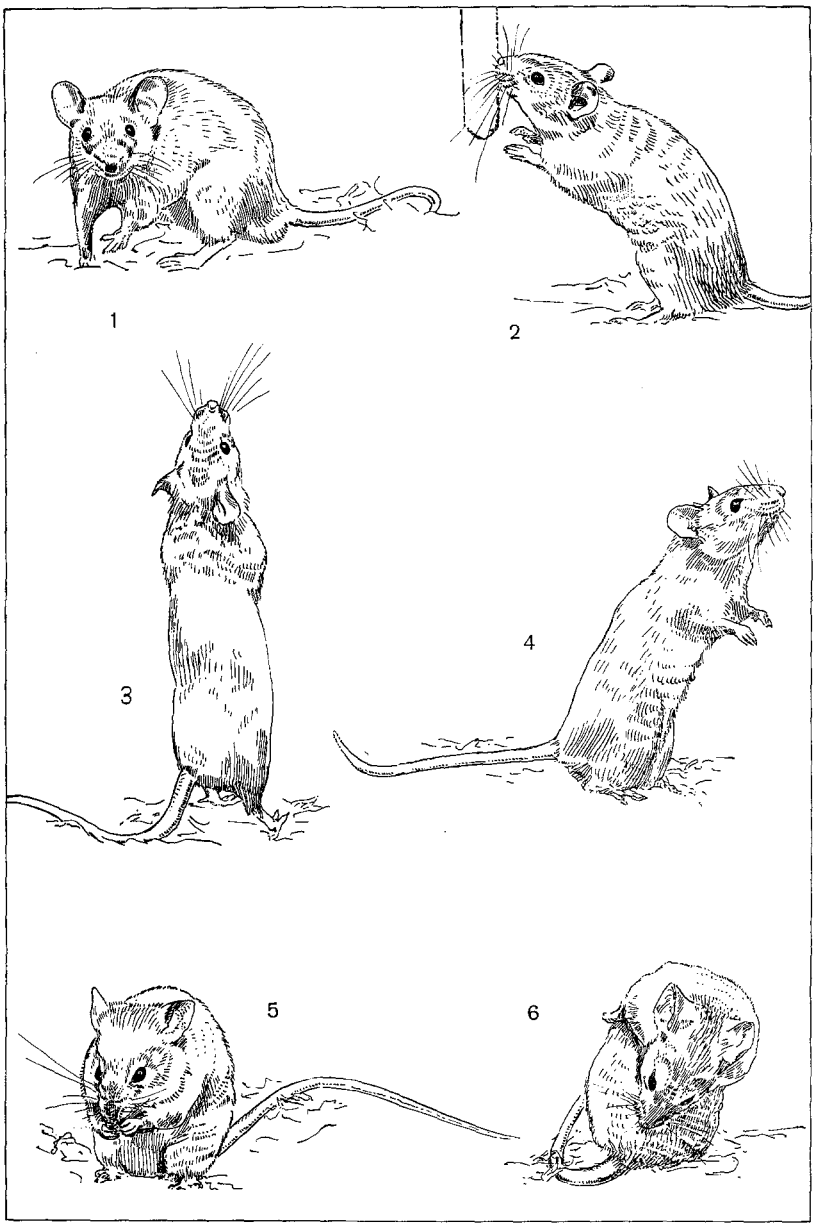
The intensity may vary; occasionally only the tip of the tail will move about a little. Tail rattling would fairly often occur in a mouse that had suddenly caught sight of some object. Solitary females and females in company of a male will occasionally rattle too.

Sniffing at rack, RAC

By this is meant sniffing at the empty rack inside the observation cage, the animal itself still being on the floor. As a rule the mouse will be sitting, bending slightly; the front paws may be placed against the rack. The nose need not touch the rack. During climbing a good deal of rack-sniffing is done, too, but this cannot be registered exactly and is not considered under the present heading.

Sniffing at bottle, BOT, figure 2

Like sniffing at rack.



Figs. 1–6: 1. Staring at observer, lifting one forepaw.
2. Sniffing at bottle. 3. Reconnoitering. 4. Reconnoitering.
5. Eating. 6. Grooming: combing.

Sniffing at peat dust, PEA

The animals start on this as soon as they have been introduced into the terrarium. As a matter of fact, there is constant sniffing at the peat dust covering the floor when the mice move about the cage, but here the action is understood as the sniffing of an animal standing still and clearly investigating one particular spot; occasionally a fragment of the substratum is picked up by the mouth and munched at.

Reconnoitering, REC, figures 3–4

In this the animal may stand upright, on the toes of its hind-legs, the tail being used as support; or the animal may stand on the full feet, the body being slightly bent ('Sichern', EIBL-EIBESFELDT, 1958); also intermediate postures. Its orientation is upwards. Sometimes the animal will repeat the action in a rapid succession of movements.

Lifting one forepaw; LIF, figure 1

The animal is sitting on the haunches, one forepaw is rested on the floor; the other is drawn up. Probably this is intention reconnoitering and it occasionally does pass into real reconnoitering.

Sitting upright, SIT

This activity is uncommon. The animal is sitting on its haunches, the forepaws clear of the floor.

Leaning against wall, LEA

Sniffing, the mouse stems itself on its hind-legs and leans against the wall with its forepaws.

Gnawing at wall, GNA

Now and then the animals will gnaw at a small round cavity in the wall, and occasionally elsewhere.

Climbing, CLI (see EIBL-EIBESFELDT, 1958)

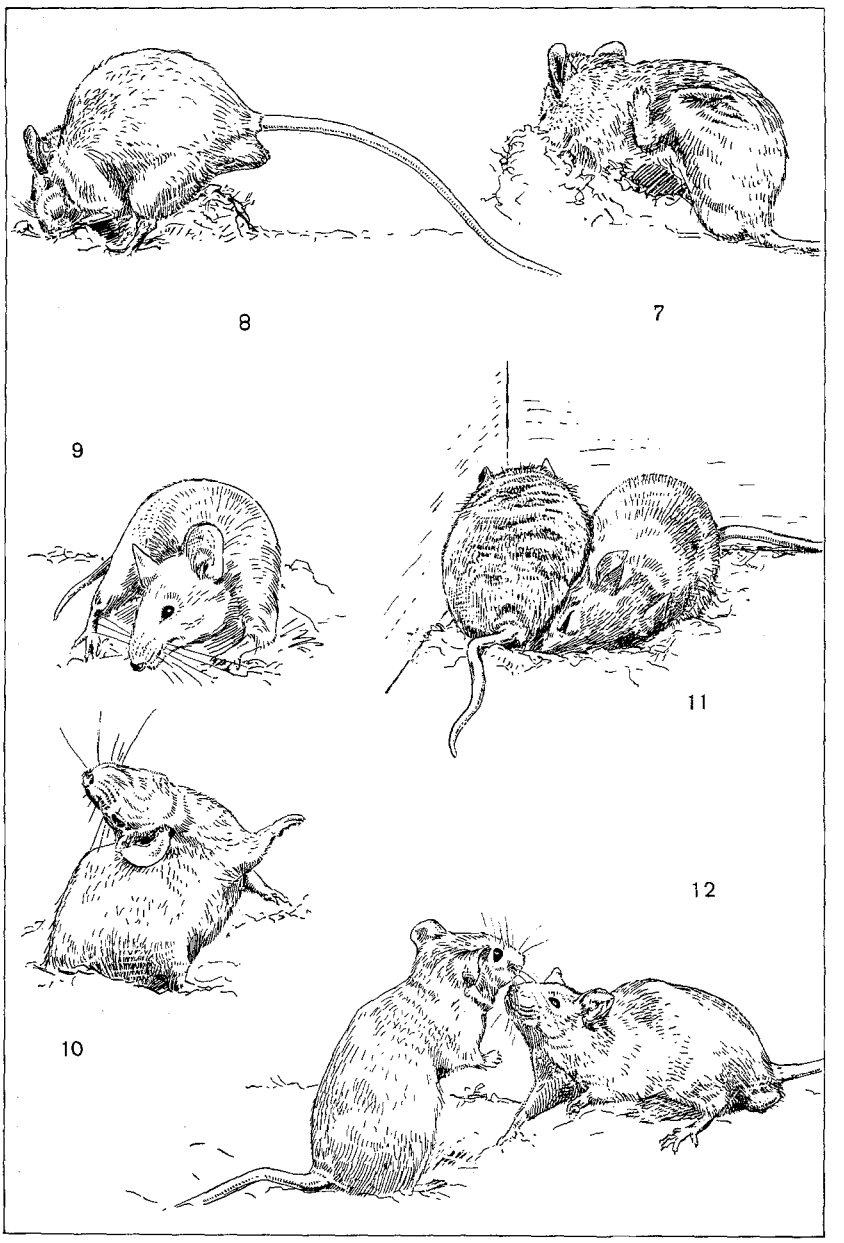
This comprises both climbing the rack and the bottle.

Hanging, HAN

Only when the animal was hanging with one or two paws to the rack this term was used.

Jumping, JUM, figure 15

This only covered jumping upwards, also against the wall; not jumping down from something. In jumping upwards the animal



Figs. 7–12: 7. Grooming: scratching. 8. Digging. 9. Circling. 10. Head shaking, drumming. 11. Hair fluffing, nosing, crowding. 12. Dancing position.

starts from a crouching position. Some mice will jump as high as 25 cm.

Sniffing at food pellet, FOO

Like sniffing at peat dust.

Food carrying, CAR (BROWN, 1953)

As a rule towards a corner of the terrarium (favourite eating spot).

Eating, EAT, figure 5 (see EIBL-EIBESFELDT, 1958)

When eating, the animal will assume a sitting posture, its back very much flexed.

Drinking, DRI

When drinking from a bottle with attached tube the mouse will lap up the water from the opening with its tongue. In doing so the head is kept aslant or backwards.

Defecating, DEF

Especially at the initial stage of an observation period there is a good deal of defecating. BROADHURST (1957, 1961) used it as a parameter of emotionality. No special position is assumed. Every bolus was separately registered.

Shaking the fur, SHA

The fur is forcibly shaken from left to right, the forepaws – occasionally the entire animal – giving a little jump in doing so. It is probable that these are the so-called 'jumpy movements' of SCOTT & FREDERICSON (1951).

Grooming, GRO, figures 6–7

EIBL-EIBESFELDT gives a detailed description. By this term are understood here: wiping, licking, combing and scratching. When grooming, the mouse will assume a sitting position with a high back.

Vibrating with the forepaws, VIB

A very rapid movement. It is probably intention wiping. EISENBERG (1962) describes a similar phenomenon in *Peromyscus:* "washing begins with several rapid motions of the paws under the mouth". Wiping does not always follow.

Digging, DIG, figure 8

This comprises 'Scharren' (EIBL-EIBESFELDT, 1958) and – subsequent or not – 'Auswerfen' (idem).

Sometimes a mouse in digging will move across half the terrarium.

Circling, CIR, figure 9

Moving in circles or 8-shaped figures – including whirling around the animal's own axis – is much seen in neurological mutants, never in normals (see CURTIS, 1956).

Headshaking, figure 10

Not in normal mice. The head is drawn backwards, the animal presenting a half-sitting posture. Frequently the forepaws meanwhile do a rapid beating of the floor ('drumming').

It turned out that gnashing the teeth, scratching at the wall, hopping, running, crawling back, sleeping, resting, urinating, stretching, yawning and pushing are of too sporadic or dubious an occurence to be of any useful value in our type of behaviour genetics research.

Situation II

The two males were always introduced into the terrarium at the same time, so that neither of them might be considered an 'invader' of the other's territory. The observation cage had already been explored by these animals.

Fixing, FIX

Staring motionless at the rival.

Hair fluffing, FLU, figure 11

As described under Situation I.

Tail rattling, RAT

As described under Situation I. Occurs especially when the animals are confronted with each other for the first time. Also done much by male immediately after fleeing.

Dancing position, DAN, figure 12

A sitting position, facing the opponent, with the head thrust forward and the ears folded backwards.

Boxing, BOX

Drumming with the forepaws on the rival. Described in voles by CLARK (1956).

Figs. 13–15: 13. Wrestling. 14. Chasing, fleeing. 15. Jumping, fleeing.

Kicking, KIC

The opponent is dealt a hard kick with a hind-leg, mostly in wrestling.

Nosing, NOS, figure 11

By this is meant all sniffing at the other animal, including the investigation of the anal and genital area. It is common for the most intensive sniffer to start the fight (cf. BANKS, 1957); so nosing is by no means a "friendly" behaviour, as FREDERICSON (1950) contends.

Wrestling, WRE, figure 13

The animals jump at each other and roll over and over; there is also biting, mainly in the flanks. No squealing.

Chasing, CHA, figure 14

One mouse races after the other and bites it in the lower back, the tail or the hind-legs.

Fleeing, FLE, figures 14–15

Rushing away, often at random, from the dominating male. This may happen owing to the rival's chasing or approach. Attended by squealing.

Submission, SUB, figure 18

The typically submissive posture may be described thus: "the chased mouse rears on its hind legs, draws one fore-leg close to the body, extends the other stiffly, remains motionless, and squeals when touched by the other mouse" (GINSBURG & ALLEE, 1942). Not always, however, is the attitude so characteristic; both the fore-legs, either stiff or not, may be held in front. The criterion used in the present case was the erect position of the head; in this respect there was an obvious difference between the submissive posture and the dancing position. An animal in the submissive position may indeed be attacked and bitten, but in most cases the attacker will turn away.

Mounting, MOU

See Situation III.

Grooming, GRO, figures 6–7

In this situation in most cases wiping is done by the dominating males. Displacement grooming seems to occur in mice (EIBL-EIBESFELDT, 1958) and voles (CLARKE, 1956).

Figs. 16–18: 16. Copulation, note lordosis in the female.
17. Lying flat. 18. Submission.

Digging, DIG, figure 8

It was obvious that in this situation displacement digging occurred repeatedly (for discussions of the concept 'displacement' see ARMSTRONG, 1950; BASTOCK, MORRIS & MOYNIHAN, 1953; MORRIS, 1956; TINBERGEN, 1940, 1951). Dominating males would begin to dig fervently in a corner of the terrarium when the animal they had been chasing suddenly disappeared by jumping onto the rack or when it assumed the submissive position. CLARKE (1956) and ZIMMERMANN (1952) mention displacement digging in voles.

It turned out that approaching, following, deserting, mincing (BAUER, 1956; BEEMAN & ALLEE, 1945; SCOTT & FREDERICSON, 1951), crowding (Fig. 11, BEVAN, BEVAN & WILLIAMS, 1958; FREDERICSON, 1950), biting, squealing, touching, social grooming and snatching food (BROWN, 1953; EISENBERG, 1962) are of too sporadic or dubious an occurence to be of any useful value in such behaviour genetic research as undertaken here.

It is evident that in Situation II there also occur several actions mentioned *sub* Situation I.

Situation III

The male and the female were introduced into the terrarium at the same time, having explored this before.

Nosing genitals, GEN

This covered only sniffing at the partner's perineum; males will do this more than females.

Creeping in front of partner, CRE

Crawling slowly, the animal passes immediately in front of the other mouse, its body sometimes being kept so low as to touch the floor. HAGEMANN & SCHMIDT (1960) speak of 'Aufforderung', especially performed by females. In the present investigation it was especially exhibited by the males.

Touching, TOU

With the mouth the partner is nudged underneath the head and in the neck. Presumably a stimulation for social grooming.

Mounting, MOU

The male places his forepaws on the back of the female and tries to bring the lower part of his abdomen towards the female's genitals. Mounting at the other side, near the head, also occurs.

Copulation, COP, figure 16, and:

Falling over, FAL

Together the copulating male and female fall on their sides and remain in that position for some moments. This is the point at which ejaculation occurs (see CALHOUN, 1956; MCGILL, 1962; LIPKOW, 1960).

Lying flat, FLA, figure 17

Only seen in males, especially when the female does a good deal of parrying. It has some resemblance to a prolonged stretching of the trunk and lasts for about half a minute. EISENBERG (1962) describes a similar phenomenon in *Peromyscus:* '... dragging the perineal region along the floor of the cage after fighting and during the investigation of new areas'. It is, however, questionable whether we have the same thing here. LIPKOW (1960) calls it an 'Erschöpfungsstellung' (this name seems inappropriate): '... es streckt sich immer flacher aus'.

Licking the genitals, LIC

Especially after copulation. The female will usually lift a hind-leg while doing so.

Parrying, PAR

During attempts at approach and mounting by the male the other mouse will make sidelong movements, with the forepaws as well as with the hind-legs, keeping the male at bay.

Submission, SUB, figure 18 (by the female)

See Situation II.

Tail rattling, RAT; chasing, CHA (by the male); grooming, GRO, and digging, DIG, have been described *sub* Situation II. Creeping under partner occurs very sporadically. In Situation III there occur, of course, also various actions of Situation I.

Within the framework of the present inventory of behaviour the author also tried for some time to make a study of the behaviours

related to nest building, birth and care of the young; he attempted to promote nest building by supplying the animals with paper snippets. However, such behaviour presents great difficulties of quantification, because it cannot be evoked at will. For these matters the reader is referred to DIETERLEN (1962), EIBL-EIBESFELDT (1958) and KOLLER (1952).

As regards the development of behaviour in mice: ten-day old animals will already demonstrate leaning against the wall, lifting one forepaw, wiping, scratching – in which the hind-leg really touches the flank –, shaking the fur and tail rattling. On its twentieth day a normal mouse can perform all actions mentioned *sub* Situation I (except circling and head shaking), as well as social grooming. At that time the deviant behaviour of some neurological mutants can easily be ascertained. See further WILLIAMS & SCOTT (1954).

In comparing the behaviour of mutants with that of non-mutants use was made of the ethogram as presented above by registering and tabulating the frequencies of the behavioral elements that occurred.

The findings of the investigation, as well as the way in which the mice were kept and handled before they were observed, will be published at a later date.

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