# THE GENETICS OF MOUSE BEHAVIOR IN NOVEL SITUATIONS[1]

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Thompson (1953) has made a significant contribution to the study of the genetics of behavior by systematically testing 14 inbred strains and 1 hybrid strain of *Mus musculus* for food drive, emotionality, and exploratory behavior. Food drive was measured by the amount of food consumed following deprivation, emotionality was measured in terms of the tendency to defecate in the food-drive situation, and exploratory behavior was determined by recording the number of squares traversed on a checkerboard-type floor which held barriers at intervals. Large interstrain differences were found on all tests, food drive and emotionality were shown to be negatively correlated, and no relationship was found between food drive and exploratory activity.

This paper presents the results of two further studies on the genetics of exploratory behavior of mice.

## EXPERIMENT I

The principal purpose of the first study was to determine the situational generality of the behavior, i.e., the extent to which the same ordinal relationships among the various strains could be obtained in a variety of situations which presumably measure exploratory behavior. In addition, observations were made on the stability of strain rank orders on retest, and on sex differences within strains.

### *Method*

#### *Subjects*

Six strains of mice were chosen for study: C57Br/cd, C57BL/10, LP, AKR, BALB/c, and A/Jax. The origin, degree of inbreeding, and tumor characteristics of these strains have been described (Committee on Standardized Nomenclature, 1952).

Ten females and five males of each strain were obtained from the Roscoe B. Jackson Memorial Laboratory at approximately 40 days of age. They were approximately 65 days old when the testing program commenced.

#### *Colony Conditions*

Sexes and strains were housed separately. As far as possible, five animals were housed per cage.

The temperature was controlled between 70° and 75° F., and a dehumidifier prevented rapid changes in humidity. An artificial light cycle was maintained, with lights on from 7 A.M. to 7 P.M.

#### *Apparatus*

Four apparatuses were used: Arena, Hole in Wall, Open Field, and Barrier.

*Arena.* This apparatus is a modification of that used by Thompson, differing principally in the extent of control of extra-apparatus stimuli. A 30-in. square floor is subdivided into 36, 5-in. squares. Barriers 3⅞ in. high are erected at staggered intervals on the square borders. This floor is enclosed by walls 36 in. high at the front and 48 in. high at the back. The sloped top contains a one-way mirror. The front wall slides upward in channels, providing access to the inside of the apparatus. Illumination is provided by four 10-w. frosted bulbs, one in each corner 34 in. from the floor. A starting box is mounted outside the apparatus, and a guillotine door permits the animal to enter the corner square from the starting box.

*Hole in Wall.* This apparatus consists of two chambers, approximately 3 in. by 4 in. by 3 in., separated by a partition. A guillotine door covers a hole ⅞ in. in diameter in the partition. When raised to expose the hole, the door activates a microswitch, which starts a timer. The starting chamber is covered by a transparent plastic cover that can be locked in place. The goal chamber is covered by an opaque wooden top and is relatively dark inside. The floor of the goal chamber is hinged, and when depressed by the weight of *S*, activates a second microswitch, which stops the timer.

*Open Field.* This apparatus is an elaboration of one used by Fredericson (1953). A gray unobstructed floor, 30 in. square, is provided to fit within the Arena apparatus, resting above the barriers. Lighting and observation conditions are, therefore, the same as for the Arena. A centered square 21.2 in. by 21.2 in. is outlined on the floor. The area enclosed by the square is thus equal to the total area of the margins surrounding the square. A small 5-in. square is also drawn in the center of the floor. An inverted funnel is placed on a corner of the outer square. A string through the top of the apparatus permits the raising of the funnel.

*Barrier.* In this apparatus, a 12-in.-square floor is subdivided into four 6-in. squares by intersecting barriers. From the center to a point 3 in. out, the barriers are 7 in. high, preventing *S* from climbing diagonally from one square to another. The remaining half of each barrier, next to the wall, is 3 in. high. This floor is placed in a box 12 in. by 12 in. by 36 in.

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high, which contains a one-way mirror in its top. Illumination is provided by four $7\frac{1}{2}$-w. frosted bulbs located in the corners 34 in. from the floor. The front wall of the box is hinged to permit introduction and retrieving of the animal.

Except for the boundaries of the squares in the Arena and Open Field, all apparatuses are painted neutral gray.

## *Procedure*

*Arena.* The mouse was placed in the starting box. The guillotine door was raised, and a timer was started. The number of square entries was recorded on a hand tally counter for a period of 5 min. A "square entry" was defined as the placing of both forepaws across a line. At the termination of the trial, the front wall was raised, and *S* was retrieved.

*Hole in Wall.* The animal was placed in the starting chamber, and the plastic cover was locked in place. Fifteen seconds later the guillotine door was lifted, exposing the hole and starting the timer. When *S* had crawled through the hole and placed its weight on the floor, the timer stopped. The time was recorded, and *S* was immediately removed from the apparatus. If *S* did not enter the goal compartment within 180 sec., the trial was terminated.

*Open Field.* The *S* was placed under the inverted funnel, which restrained it until the front door could be lowered. When the funnel was lifted, a timer was started. The time *S* spent within the large square was recorded cumulatively by means of a hand-operated switch which controlled a timer. The number of times *S* crossed the line with both forepaws was recorded on a hand tally counter, and the number of contacts with the small center square was counted. A "contact" required entry with both forepaws.

After 2 min. the trial was terminated and *S* retrieved.

*Barrier.* The mouse was placed in a back compartment, the front door was closed, and a timer started. The total number of barrier crossings was recorded. A "crossing" required that all four feet touch the floor on the other side of the barrier. Each trial lasted 2 min.

## *Testing Schedule*

Two trials were given to each *S* on each apparatus. A six-day rest was given between Trial 1 and Trial 2 of each test, and a three-day rest between Trial 2 of any test and Trial 1 of the next test. Two *E*s conducted the testing, with a different *E* conducting the first and second trials for any given animal. It was not feasible to counterbalance the order of presentation of tests, so all animals were run in the same order: Arena, Hole in Wall, Open Field, and Barrier.

Testing was conducted between 8 A.M. and 10 A.M. in a windowless room. A ventilating fan masked extraneous noises.

Commercial mouse food and tap water were available in the living cages at all times.

# *Results*

Nonparametric statistics were extensively used in the analysis for several reasons. Most important is the uncertainty about equality of intervals of the scales used. It appears likely, for example, that the difference in exploratory behavior represented by the difference between zero and one barrier climbing is greater than that represented by the difference between three and four climbings. In addition, there were indeterminate scores in the Hole in Wall, and inspection revealed that other distributions were skew. All nonparametric tests were computed according to Siegel (1956).

TABLE 1

Rank-Order Correlation Coefficients Between Strain Medians on Trial 1 and Trial 2

| Measure | *Rho* |
|---|---|
| Arena | .94 |
| Hole in Wall | .88 |
| Open-Field crosses | .90 |
| Open-Field seconds | .49 |
| Open-Field contacts | .79 |
| Barrier | .96 |

## *Stability under Repeated Testing*

Because the focus of interest in this study is on behavior in novel situations, it is not essential that the relationships be reproduced on Trial 2, which is no longer novel. However, other things being equal, it would be desirable to have tests on which phenotypic expression is not greatly modified subsequent to one exposure.

Table 1 shows Spearman rank correlation coefficients, which were computed from the ranked strain medians of Trial 1 and of Trial 2 for each test. A value of .829 is required at the .05 level. Only the measures of seconds within square and the contacts with center square on the Open Field test fail to reach this level. Because these items are both related to the number of crosses into square, which shows a significant correlation, the latter is the only exploratory measure of the Open Field test which will be discussed.

## *Strain Differences*

A summary of the Trial 1 results is presented in Table 2. The data have been rounded to the nearest whole numbers. Because of the necessity of committing some *S*s to a breeding program, some observations were made on less

TABLE 2

First-Trial Results for All Strains on All Tests

| Measure | Strain | | | | | |
|---|---|---|---|---|---|---|
| | C57BR/cd | C57BL/10 | LP | AKR | BALB/c | A/Jax |
| Arena | | | | | | |
| Mdn square entries | 311 | 267 | 286 | 207 | 78 | 11 |
| Range | 199–365 | 203–313 | 143–397 | 158–284 | 3–238 | 1–41 |
| Hole in Wall | | | | | | |
| Mdn sec. | 9 | 9 | 17 | 40 | 46 | 149+ |
| Range | 3–28 | 3–48 | 7–180+ | 12–110 | 20–180+ | 19–180+ |
| Open Field | | | | | | |
| Mdn no. of crosses | 9 | 7 | 7 | 2 (10) | 1 | 1 (9) |
| Range | 3–19 | 6–14 | 0–18 | 0–12 | 0–12 | 0–5 |
| Barrier | | | | | | |
| Per cent climbing | 58 (12) | 80 | 6 | 13 | 0 | 0 (7) |
| Range (no. of climbings) | 0–6 | 0–17 | 0–8 | 0–1 | 0 | 0 |

than 15 *S*s. For these instances, the number observed is shown in parentheses.

For Arena results, a Median test over all strains gave a $p < .0001$.

For the Hole in Wall, the A/Jax median was indeterminate, because only 40% of the animals had entered the goal chamber within the 180-sec. time limit. A Median test over all strains yielded a $p < .001$ for this test.

The results of the Barrier test are best described in terms of the percentage of animals which climbed a barrier one or more times. Median values for the strains with only one or two animals climbing are not very meaningful, but it is instructive to compare the C57BL/10 median of 7.0 with the next highest median, 0.8 for C57BR/cd. It appears that a C57BL/10 animal is not only more likely to be a "climber" but, compared with "climbers" from other strains, will climb more barriers within the 2-min. interval. Because of the large number of expected cell frequencies less than five, an over-all Median test was not possible for the Barrier test.

An over-all Median test on the Open Field results gave a $p < .001$.

A general consistency in order may be seen over all four measures, with C57BL/10, C57BR/cd, and LP generally obtaining higher exploratory scores than AKR, BALB/c, and A/Jax. To evaluate this consistency, the strain medians were ranked from most exploratory to least exploratory on each test, and a Kendall's coefficient of concordance was computed. The resulting $W$ is .90, for which $p < .01$.

TABLE 3

Sex Differences in Performance

| Strain | Measure | ♀ | ♂ | $p$ |
|---|---|---|---|---|
| BALB/c | Arena, Mdn line crossings | 10 | 170 | <.05 |
| C57BR/cd | Hole in Wall, Mdn sec. | 6.5 | 16.0 | <.02 |
| C57BR/cd | Barrier, per cent climbing | 88[a] | 0[b] | <.05 |

[a] $N = 8$.
[b] $N = 4$.

In addition to the strain differences in median scores or percentages described above, substantial differences in variability are apparent in the ranges shown in Table 2. To determine if some strains were consistently more or less variable than others in respect to all measures, a coefficient of concordance was computed on the ranked ranges. The resulting $W$ is .64, which is not significant.

## *Sex Differences*

Sex differences were tested within each strain by use of the Mann-Whitney test, except for a few cases when Fisher's exact procedure was more appropriate. The results of all tests which gave a $p < .05$ are shown in Table 3.

## EXPERIMENT II

The determination of differences in behavior of a number of inbred strains which have been selected for some other, nonbehavioral, characteristic yields the following information: (*a*) the strains are known to differ genotypi-

TABLE 4
First-Trial Results by Groups on All Tests

| Strain | N | Apparatus and Measure | | | |
|---|---|---|---|---|---|
| | | Arena: Mdn entries | Hole in Wall: Mdn sec. | Barrier: per cent climbing | Open Field: Mdn crossings |
| C57BL/10 | 22 | 252 | 15 | 36 | 9 |
| C57BL/10 x A/Jax | 47 | 194 | 18 | 67 | 5 |
| A/Jax x C57BL/10 | 20 | 195 | 10 | 55 | 6 |
| A/Jax | 20 | 22 | 180 | 5 | 1 |

cally, and (*b*) the strains differ in behavior. When rearing and testing have been performed under standardized conditions, the reasonable interpretation may be made that genotypic differences are in some way related to the phenotypic differences. The nature of the relationship cannot, however, be specified in any detail, because there is no systematic way of ordering the genotypic values of the strains. It is possible, however, by mating animals of different strains, to manipulate genotype, and the relationship between behavioral phenotype and the genotypic values of the derived generations may be examined.

The purpose of Experiment II was to apply the tests used in Experiment I to the $F_1$ hybrids of two of the most extremely different inbred strains, C57BL/10 and A/Jax, as an initial step in the description of the genotype-behavior relationship.

*Method*

The same apparatus and the same general procedures used in Experiment I were also used in Experiment II. In order to accelerate the testing schedule, only one-day rest periods were given between trials and between problems. Animals were tested between noon and 5 P.M.

*Subjects*

The $F_1$s were obtained by matings of the *S*s previously tested in Experiment I. Twenty-one male and 26 female offspring were obtained from matings of C57BL/10 females with A/Jax males. Twelve male and 8 female offspring were obtained from matings of A/Jax females with C57BL/10 males. These groups will subsequently be designated, respectively, C57BL/10 x A/Jax and A/Jax x C57BL/10, with the strain of the female parent named first.

Because an accelerated testing schedule was used, it was necessary to obtain comparative measures on new *S*s from the parental strains. Ten male and 12 female C57BL/10 and 10 male and 10 female A/Jax were obtained from Jackson Memorial Laboratory. All *S*s were approximately 60 days of age at the beginning of testing.

*Results*

*Reproducibility of Results of Parental Strains*

Table 4 summarizes the data for the first trials of the various tests. With the exception of the Barrier test, the present results on C57BL/10 and A/Jax very closely parallel those of the previous study. The compressing of the testing schedule, or the change in time of day of testing, might have resulted in the greatly diminished percentage of C57BL/10 animals climbing barriers, or the difference might represent sampling fluctuations. It is clear, however, that, for the other tests, the reduced intertrial and intertest rest interval did not affect the behavior.

*Comparison of $F_1$s with Parental Strains*

All differences reported as significant are at the .01 level, unless otherwise specified.

*Arena.* The cumulative percentage curves for all groups are shown in Figure 1. The reciprocal $F_1$s do not differ, and are intermediate between the parental strains, though displaced somewhat toward the C57BL/10. By Mann-Whitney tests, the parental strains differ from each other and also differ from the $F_1$s. The $F_1$ variability appears greater than that of either parental strain.

*Hole in Wall.* Figure 2 presents the cumulative percentage of animals entering the goal chamber within the time interval indicated on the abscissa. In this test, the $F_1$ distributions

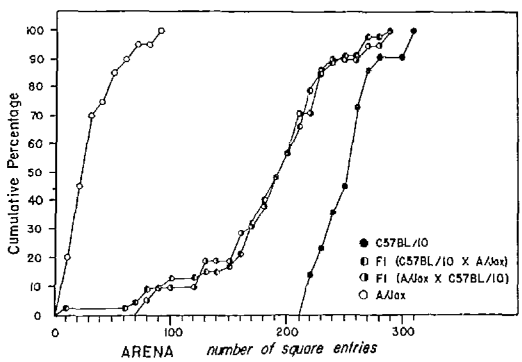


FIG. 1. Cumulative distributions for parental strains and reciprocal $F_1$s for square entries in Arena.

are essentially indistinguishable from that of the C57BL/10, while all of these are clearly different from A/Jax. This is confirmed by Median test.

*Open Field.* The curves of Figure 3, for the Open Field line crossings, are similar to those for the Arena in that the $F_1$s do not differ from each other, are intermediate to the parental strains, and are significantly different from each of them.

*Barrier.* It will be recalled that the C57BL/10 of Experiment II did not climb barriers as frequently as did those of Experiment I. The results of a chi-square test, between C57BL/10 and A/Jax, based on a climb–no-climb dichotomy, gave a $p < .05$. By the same test, both $F_1$s differed from A/Jax with $p < .01$. Comparisons of C57BL/10 with A/Jax x C57BL/10 and with C57BL/10 x A/Jax gave $p$ values of $> .30$ and $< .05$, respectively.

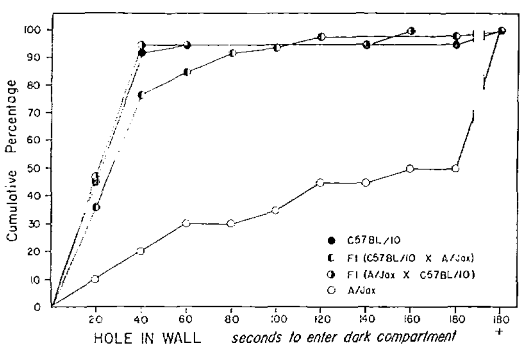


FIG. 2. Cumulative distributions for parental strains and reciprocal $F_1$s for time to enter dark compartment in Hole in Wall.

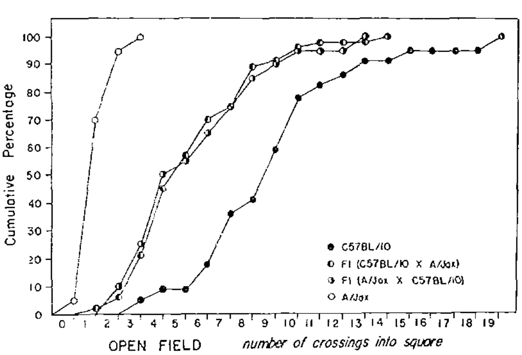


FIG. 3. Cumulative distributions for parental strains and reciprocal $F_1$s for number of crossings into square in Open Field.

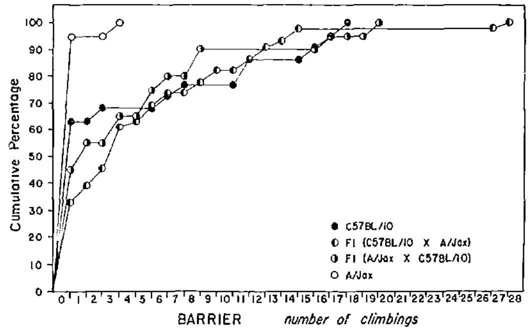


FIG. 4. Cumulative distributions for parental strains and reciprocal $F_1$s for number of climbings in Barrier.

Because of the differences in testing schedule, combining Experiment I and Experiment II data for A/Jax and for C57BL/10 can be, at best, suggestive. Comparisons using the pooled data show that C57BL/10 and A/Jax differ, and that the $F_1$s differ from A/Jax, but not from C57BL/10.

## DISCUSSION

The principal results of Experiment I are, first, the successful general replication of Thompson's results in Arena, and, second, the demonstration that strain differences in activity in novel situations are not specific to any one technique of measurement. These conclusions are supported by further work of Thompson (1956) in which similar strain rankings were found in a Y maze and in an Arenalike situation. The situational generality of the behavior suggests that a fundamental mouse "personality" dimension is being measured.

In view of the essentially continuous array of strain medians in this study, and the similar earlier results, a polygenic (multiple-factor) model appears more adequate than a single-locus model, which would require an unreasonable number of supplementary assumptions. Furthermore, the high coefficient of concordance among the tests indicates that a common genetic system is involved in determining the strain differences on the various tests.

Whereas it is undoubtedly only an approximation, the initial working hypothesis assumes that highly inbred animals are homozygous at all loci. The $F_1$s of Experiment II, therefore, will be heterozygotic at all loci at which the C57BL/10 and A/Jax strains differ, and the

$F_1$ genotypic value will be midway between the genotypic values of the parental strains. The genotypic intermediacy is accompanied by phenotypic intermediacy in Arena and Open Field, but not in Hole in Wall and probably not in Barrier. This outcome suggests that "exploratory behavior," as it is currently conceived, is made up of at least two subcharacters. The postulation of subcharacters is not inconsistent with the above conclusion concerning a common genetic system. Mather (1949, p. 28) has pointed out that "... we must expect that some genes will affect both of any pair of sub-characters while others may affect only one of the pair. The variation of the two will then be correlated but not completely so, and the degree of correlation cannot be predicted. It will depend on the sub-characters in question and on the genes which are contributing to the variation."

Finally, it must be noted that, although it has been convenient to refer to the behavior investigated as "exploratory behavior," it may be equally accurate to consider it to be a measure of timidity, fear, spontaneous activity, sensory acuity, or perhaps even intelligence (running about in a strange situation may prove to be either quite intelligent or quite stupid in terms of natural selection). Further investigations of the genetic mechanism, including study of the physiological link between the genotype and the phenotype, should contribute substantially to the understanding of the biological nature of the behavior dimension.

## SUMMARY

Data were obtained on the behavior of six inbred strains of mice, and of the $F_1$s between two widely differing strains, in four different situations. Two situations involved running, one around barriers, one in an open field. Two situations involved climbing, one through a hole in a wall, one over a barrier.

Wide differences exist among the strains, and there is high consistency in strain rank over the four tests. It is proposed that a polygenic system is common to all tests but that there may also be loci of specific function.

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