Genetic Analyses of Nest-Building Behavior in Laboratory Mice *(Mus musculus)*[1]

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A Mendelian genetic analysis and a diallel cross study were conducted to investigate nest-building behavior of inbred mice. The amount of cotton used to build nests was observed for 4 days when the mice were 8 weeks of age. Results indicate (1) a sex difference in the Mendelian and diallel cross populations, (2) a significant genotypic difference, and (3) heterosis in the F_1 hybrid populations. The broad-sense heritability, obtained from the Mendelian analysis, was 0.418 for males, whereas that of females was not estimated because of a negative estimate of genetic variance. The narrow-sense heritabilities were 0.14 and 0.21 for males and females, respectively. The broad-sense heritabilities obtained by the diallel cross method were 0.931 and 0.623 for males and females, respectively, and the narrow-sense heritabilities were 0.068 and 0.166 for males and females. The low narrow-sense heritability and the prevalent heterotic mode of inheritance suggest that nesting behavior has been under selection pressure and possesses adaptive significance.

INTRODUCTION

The maintenance of a thermodynamic equilibrium with the environment for a reasonable length of time is essential to the survival of animals (Porter and Gates,

1969). Animals may die in an extremely hot or cold environment; thus they constantly adjust themselves to seek optimal thermal spaces. Behavior is a very sensitive mechanism in the regulation of the thermal equilibrium. Experimental evidence shows that rodents are capable of adjusting to environmental temperature changes by operant behavior. Stricker *et al.* (1968) reported that mice and rats tended to stay near water, splash water on themselves, or take baths at an ambient temperature of 40 C. Epstein and Milestone (1968) demonstrated that rats learned to press bars to obtain a water shower in hot temperature. Weiss and Laties (1961) observed that rats were able to learn a bar-press response to get a stream of heat in a cool testing chamber. Mice are also observed to select a preferred temperature when placed in a situation with thermal gradients. Ogilvie and Stinson (1966) reported that *Peromyscus maniculatus gracilis, P. m. bairdii,* and *P. leucopus* showed a mean temperature preference of 29.1, 25.8, and 32.4 C, respectively. Herter (See Hall, 1951) found a differential thermal preference in albino and wild mice. Furthermore, rodents are observed to build nests with wool (Barnett and Manly, 1954), paper (Kinder, 1927; van Oortmerssen, 1970), hay (Koller, 1956), and cotton (Lee and Wong, 1970; Lee and Estep, 1971; Lee, 1969). In fact, Kinder (1927), Koller (1956), and Lee and Wong (1970) demonstrated that the amount of nest-building behavior of rats and mice was negatively correlated with temperature. Barnett and Manly (1954) even reported that mice were able to survive and breed at −3 C, provided that a plentiful amount of nesting material was available. The mice built nests so well constructed that the temperature inside the nests was approximately 26 C, sufficient for individual survival and normal growth of the young.

It is reasonable to assume that thermoregulatory behavior, because of its adaptive significance, should be genetically determined to some extent and should have been subjected to natural selection. There is some support for these hypotheses. Heter's (Hall, 1951) Mendelian analysis of the temperature selection in wild and albino mice indicated that the preference for a cooler environment was genetically dominant and that the results might be accounted for by a single gene. Further evidence suggested that hair density and skin thickness were morphological factors closely related to the thermal selection. The thick-skinned, dense-haired mice tended to prefer a cooler environment. Ogilvie and Stinson's (1966) observation of subspecies differences in *Peromyscus* for thermal preference further suggests a genetic origin of differential preferences resulting from natural selection. Species and subspecies differences in *Peromyscus* were further demonstrated for nesting behavior by King *et al.* (1964) and Layne (1969). *Peromyscus* from different latitudes showed differences in nesting activities under laboratory situations. King *et al.* (1964) have reported that the northerly distributed *P. m. gracilis* used more cotton and built larger nests than its southern relative *P. m. bairdii,* and both of these built larger nests than the southernmost relative *P. floridanus.* However, the nest size of *P. floridanus* was

similar to that of a sympatric group of *P. polionotus* which belonged to a different subgenus, *podomys.* Layne (1969) additionally demonstrated that *P. floridanus* used less cotton to build nests then its northern relatives *P. gossypinus* and *P. leucopus.*

The hypotheses can further be clarified by behavior genetic analyses. Biometrically, the degree of genetic determination of a behavioral trait may be expressed by the broad-sense heritability (h_B^2), defined as the ratio of the total genetic variance (V_G), which includes the additive genetic variance, the dominance variance, and the epistatic variance, to the phenotypic variance (V_P) (Lush, 1949). Roberts (1967) has attempted to relate, theoretically and empirically, the relation of the fitness of a trait to another behavior genetic parameter, the narrow-sense heritability (h_N^2), defined as the ratio of the additive genetic variance (V_A) to V_P. Theoretically, when a trait is closely related to fitness and has been subjected to extensive natural selection, the V_A approaches zero, and thus h_N^2 also approaches zero. Empirically, it has been found that traits with the lowest h_N^2 are those closely related to reproductive fitness (Falconer, 1960). In addition, Lerner (1954), Bruell (1964), and Robertson (1955) reasoned that behaviors which showed extensive dominance variance were those which had been under the pressure of natural selection or those closely connected with fitness. Recently, "coefficient of genetic determination" and "heritability" have been used to denote h_B^2 and h_N^2, respectively (Falconer, 1960; DeFries, 1967). In the present paper, the terms h_B^2 and h_N^2 will be used.

In the course of investigating the genetic determinants of nesting behavior in inbred mice, Lee (1969) found strain differences in the amount of cotton used and in the characteristics of nests built. The first part of the quantitative data of that study dealing with the developmental aspect of nesting behavior in six inbred strains was further analyzed and has been reported elsewhere (Lee, 1972). The present studies report the second part of Lee's results (1969) dealing with Mendelian and diallel analyses of the nesting behavior. The results allow the estimation of both h_B^2 and h_N^2 and the evaluation of the hypotheses that thermoregulatory behavior should be genetically determined to some extent and that it should have been subjected to selection pressure.

EXPERIMENT 1. MENDELIAN ANALYSIS

Koller (1956) reported that there are two kinds of nests built by mice. The large "brood nest" is built by pregnant females, and the small "sleeping nest" is built by males and nonpregnant females. The "sleeping nest" was studied in this experiment. C57BL/6J (P_1) and BALB/cJ (P_2) strains were selected as parental populations. Two F_1s were obtained, one from $P_1 \times P_2$ and the other from $P_2 \times P_1$. There were no differences between these F_1s; hence only one set of F_1s, F_2s, B_1s, and B_2s were observed and analyzed (see Table I).

Table I. Means and Variances of Six Populations Based on Square Root Transformation

	Male			Female		
	N	$\bar{M}$	V^a	N	$\bar{M}$	V^a
P_1 (C57BL/6J)	10	2.44	0.141	10	1.94	0.118
P_2 (BALB/cJ)	11	2.98	0.350	15	2.71	0.465
F_1 ($P_1 \times P_2$)	17	3.55	0.201	15	3.37	0.232
F_2 ($F_1 \times F_1$)	21	3.10	0.392	17	2.53	0.221
B_1 ($F_1 \times P_1$)	11	3.22	0.356	19	2.23	0.300
B_2 ($F_1 \times P_2$)	9	3.17	0.591	13	2.75	0.293

^{a} Variance.

Method

Subjects

A total of 79 males and 89 females of C57BL/6J, BALB/cJ, F_1, F_2, B_1, and B_2 were tested. Mice of each population were from at least three different mating pairs. They were weaned at 4 weeks of age, and the same-sex littermates were housed together until the day before testing. Mice were tested individually at 8 weeks of age. They were housed in plastic cages with Sanicel as bedding and free access to water and Purina Lab Chow.

Apparatus

Cotton batting with a trade name, Mountain Mist, was used as nesting material. Mice and cotton were weighed to the nearest 0.1 and 0.01 g, respectively. The mice were tested in an environmental chamber with temperatures controlled at 70.5 ± 1 F. The relative humidity of the chamber was approximately 40%. Polypropylene opaque cages measuring 11½ by 7½ by 5 inches and a cage lid with a triangular food and water holder, fabricated of 13-gauge wire bars with 0.3-inch separations, were used to house the animals. The lowest point of the holder was 1½ inch above the cage floor.

Procedure

On the first day of testing, the mouse was placed in a cage with food on the cage floor and a water bottle on the cage lid. New cotton was placed on the food holder of the cage lid. The weight of cotton and the cage lid was measured. The mouse could pull cotton through the cage lid; hence the difference in the cotton and lid weight between the first day and the subsequent day was taken as the

amount of cotton used per day. The old nest and unused cotton were removed every day. The cotton was given between 8 and 12 A.M., and the unused cotton was measured approximately 24 hr after the initial introduction of the nesting material. Each mouse was tested at approximately the same time every day. Each day the amount of cotton used, characteristics of nest built, and room temperature were recorded. The mice were tested for 4 consecutive days.

Results

Analysis of variance of the mean amount of cotton used indicated significant sex and population differences $(F = 34.01, 12.20;$ df $= 1/156, 5/156;$ $P < 0.01$ in both cases): therefore, data of males and females were treated separately. Since the raw data did not meet Mather's (1949) scaling criteria of removal of effects of nonallelic interactions and genotype x environment interactions, they were transformed with square root, reciprocal, log, and reciprocal of the square root transformations. Among these four transformations, the square root data were the best set satisfying the scaling criteria of both sexes $(t_A, t_B, t_C = 1.22, 0.35, 0.19, F_{max} = 2.14,$ and $P > 0.05$ in all cases for the males; $t_A, t_B, t_C = 1.05, 0.09, 1.08, P > 0.05,$ and $F_{max} = 3.94, P < 0.05,$ for the females). F_{max} of females did not satisfy the criterion, but Mather (1949) indicated that this occurred sometimes. Table I shows the means and variances of the square root transformed data.

Inspection of Table I reveals that F_1's scores are greater than P_1's or P_2's; thus the heterotic mode of inheritance is evident. The h_B^2 may be estimated by V_G/V_P, where V_G is equal to $V_{F_2} - V_E$, V_{F_2} is used to represent V_P, and V_E is estimated from the pooled phenotypic variance of the isogenic generations. For males, the values for V_E, V_{F_2}, and V_G were 0.228, 0.392, and 0.164, respectively, and the estimated h_B^2 was 0.418. However, in the case of the females, the values for V_E and V_{F_2} were 0.291 and 0.221, respectively, and thus V_G was -0.07. Therefore, the estimation of h_B^2 was negative. The result may be caused by a biased sampling procedure. The application of Mather's method for estimating h_N^2, which was further refined by Rasmuson (1961), Parsons (1967), and Whitney *et al.* (1970), showed that V_A values $[V_A = 2V_{F_2} - (V_B + V_{B_2})]$ for both males and females were negative, i.e., -0.163 and -0.151, respectively. Thus h_N^2 was estimated with first-degree statistics. h_N^2 may be estimated as $\frac{1}{2}(a)^2 / [\frac{1}{2}(a)^2 + V_E]$, where $\frac{1}{2}(a)^2 = V_A$ and $(a) = (\bar{B}_1 - \bar{B}_2)$ or $\frac{1}{2}(\bar{P}_1 - \bar{P}_2)$. Since the V_{B_2} of males was unusually large, indicating a biased sampling procedure, the V_A was estimated with mean scores of the parental stocks. The obtained h_N^2 values for males and females were 0.14 and 0.21, respectively. The figures tended to be the upper limits, for the dominance variance was not included in the denominator.

EXPERIMENT 2. DIALLEL CROSS METHOD

The nesting behavior of BALB/cJ, C3HeB/FeJ, C57BL/6J, SJL/J, and F_1s resulting from interbreeding of these four inbred strains was observed and analyzed. Female F_1s of SJL/J x BALB/cJ and SJL/J x C3HeB/FeJ and male F_1s of SJL/J x C3HeB/FeJ were not included.

Method

Subjects

A total of 169 males and 148 females from the interbreeding of the four inbred strains were tested. The data on inbred lines were extracted from the developmental study (Lee, 1972) and the data on F_1s of C57BL/6J and BALB/cJ from Experiment 1.

Apparatus and Procedure

The apparatus and the procedure were the same as in Experiment 1.

Results

Analyses of variance revealed a significant sex difference ($F = 12.43$, df = 1/306, $P < 0.01$) and a genotypic difference ($F = 11.75$, df = 15/306, $P < 0.01$); thus the data of males and females were analyzed separately with Griffins' analytic method 1 (mode 1) (Griffins, 1956). Tables II and III sum-

Table II. Summary of Analyses GCA and SCA of Males

Sources of variance	SS	df	MS	F	P
GCA[a]	114.24	3	38.08	26.44	< 0.01
SCA[b]	198.47	6	33.08	22.97	< 0.01
RE[c]	13.03	6	2.17	1.51	> 0.05
Error	14.98	176	1.44		

$U = 11.02$
$G_{BALB} = -1.10$
$G_{C3H} = -2.53$
$G_{C57} = 1.68$
$G_{SJL} = 1.96$

$S_{BALB \cdot C3H} = -1.61$
$S_{BALB \cdot C57} = 0.74$
$S_{BALB \cdot SJL} = 0.44$
$S_{C3H \cdot C57} = 2.85$
$S_{C57 \cdot SJL} = 1.36$
$S_{C3H \cdot SJL} = 1.75$

[a] General combining ability (G).
[b] Specific combining ability (S).
[c] Reciprocal effects.

Table III. Summary of Analyses GCA and SCA of Females

Sources of variance	SS	df	MS	F	P
GCA	17.55	3	5.85	2.95	< 0.05
SCA	104.23	6	17.37	8.77	< 0.01
RE	40.09	6	6.68	3.38	< 0.01
Error	17.71	152	1.98		

$U = 9.53$

$G_{BALB} = 0.18$
$G_{C3H} = -0.85$
$G_{C57} = -0.46$
$G_{SJL} = 1.11$

$S_{BALB \cdot C3H} = 0.50$
$S_{BALB \cdot C57} = 1.02$
$S_{BALB \cdot SJL} = 0.87$
$S_{C3H \cdot C57} = 3.22$
$S_{C3H \cdot SJL} = 2.00$
$S_{C57 \cdot SJL} = 0.61$

$R_{BALB \cdot C3H} = 3.50$
$R_{BALB \cdot C57} = 1.38$
$R_{BALB \cdot SJL} = 0$
$R_{C3H \cdot C57} = 1.83$
$R_{C3H \cdot SJL} = 0$
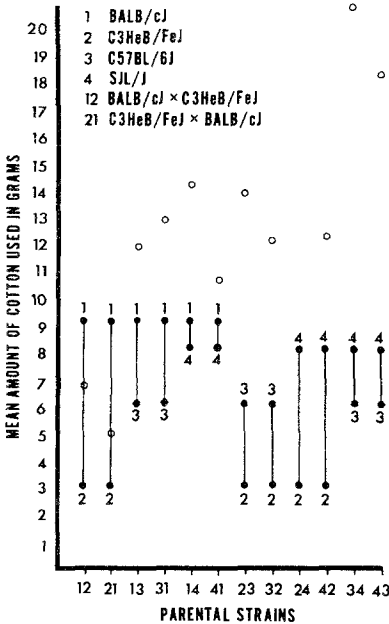$R_{C57 \cdot SJL} = 1.62$

Fig. 1. Mean amount of cotton used by male F_1s and their inbred parents. The open circles are means of F_1s, and the end points of each line are means of the parents. (In the X axis, 12 stands for female BALB/cJ × male C3HeB/FeJ.)
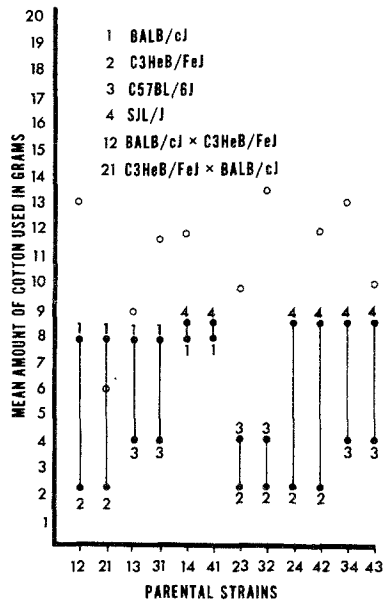

Fig. 2. Mean amount of cotton used by female F_1s and their inbred parents.

marize the results. Both sexes showed significant general and specific combining abilities (GCA and SCA). However, only females showed a reliable reciprocal parental effect (RE). The significant GCA indicated a significant additive genetic component, and the SCA showed the occurrence of dominance, which is demonstrated in Figs. 1 and 2.

In order to estimate the heritability, it was assumed that the strains were randomly chosen from the available inbred strains of mice. Griffins' method 1 (mode 2) (1956), taking the data of the total diallel populations into account, was used for analysis. h_B^2 values of 0.931 and 0.623 for males and females were obtained. In addition, h_N^2 values of 0.068 and 0.166 for males and females, respectively, were obtained.

DISCUSSION

Despite the fact that the estimation of h_B^2 of females with Mendelian analysis was not obtained because of a possible sampling error, the h_B^2 of males observed in Mendelian analysis and the h_B^2 of males and females estimated by Griffins' method substantiate the view that a functionally significant behavior should have a genetic determinant, and possibly a substantial amount. The observation of the predominance of heterosis and the low h_N^2 in the two experiments suggest that nesting behavior has been under selection pressure and has a high degree of fitness, when the rationale of Lerner (1954), Bruell (1964), Robertson (1955), Falconer (1960), and Roberts (1967) is employed. Reports of King et al. (1964) and Layne (1969) further demonstrate that the climatic temperature is a very effective selection force to differentiate genotypes of local populations.

Lynch and Hegmann (1972) studied nest building of inbred mice and reported findings similar to the present experiments. A heterotic mode of inheritance in the F_1s of C3H/FeJ and DBA/1J and in the F_1s of BALB/cJ and C57BL/6J was observed. The reported h_N^2 values estimated from the cross of C3H and DBA using methods suggested by Whitney et al. (1970) were either 0.04 and 0.02 or 0.046 and 0.001 for males and females. These figures are much smaller than the h_N^2 of the present studies. The differences may arise from the facts (1) that different parental strains were employed for analysis and (2) that different testing procedures and housing conditions were used.

In a behavior genetic study of inbred mice, van Oortmerssen (1970) has observed nesting behavior. He found strain differences in nest site selection, nest construction, and responses to nesting materials. A genetic analysis of paper fraying, measured as the degree to which edges of standard strips of paper were roughened, indicated that about one-third of the variability was attributed to additive genetic component. This figure is much greater than results of Lynch and Hegmann (1972) and the present experiments. The difference may be due to

the fact that cotton-pulling and paper-fraying behaviors are two unrelated aspects of nesting behavior. It should be of theoretical importance to study the h_N^2 of various aspects of nesting behavior, such as reaction time to nesting material, time interval required to complete a nest, the type of nests built, and selection of nesting material. The investigation may provide unprecedented information about how natural selection operates on this thermoregulatory behavior.

Results of both experiments showed a prevalent sex difference in nesting activity. Males used more cotton to build nests than females. These results are similar to Lynch and Hegmann's (1972) but are in contrast to those of Lisk *et al.* (1969) and Lee (1972), who reported a lack of sex difference in nesting-material consumption in inbred strains of mice. Lynch and Hegmann tended to take the evidence of the sex difference to question the validity of using nesting behavior as a proper measure of maternal behavior in *Mus musculus,* while the author tends to view nesting behavior as one of the thermoregulatory behaviors as well as one of the components of maternal behavior. Thus the behavior can be subjected to natural selection and also be affected by specific hormonal manipulation common to pregnant females (Lisk *et al.,* 1969). Further more, the observed sex difference may be caused by differences in body weights between males and females. Two observations seem to lend support to this view. First, it was reported that the cotton consumption was positively correlated with age (Lee, 1972). As the mice grew older and became heavier, they used more cotton to build nests. Second, the observational data indicated that mice often mouthed cotton and pulled back vigorously in order to secure nesting material. The whole process gave the observer the impression that the animals were engaged in a task requiring a great deal of strength. Since the male mice were heavier, they might be better equipped to perform the task and thus use more cotton than females. The males of the present studies weighed more than the females. The weight difference was especially prominent in the noninbred populations (males 24.2 g, females 19.5 g). A critical evaluation of results of Lisk *et al.* and Lynch and Hegmann was not possible, since the age and weight of the animals were not specified.

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