RESPONSE TO DIVERGENT SELECTION FOR NESTING BEHAVIOR IN *MUS MUSCULUS*

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ABSTRACT

Replicated bidirectional selection (with control lines) for nest-building behavior in *Mus musculus*, where nesting scores consisted of the total weight of cotton pulled through the cage lid during four days of testing, yielded an eight-fold difference between high and low lines after 15 generations of selection. The overall realized heritability pooled across lines and replicates was 0.18 ± 0.02 (0.15 ± 0.03 for high nesting scores and 0.23 ± 0.04 for low nesting scores), or 0.28 ± 0.05 when adjusted for within-family selection. Across the 15 generations and the entire experiment, average body weight and number of infertile matings increased, while average litter size decreased, although these changes were not consistent across lines. Inbreeding could account for average decreases in the fertility traits, but there was also a correlated response to selection, since both high lines showed increased litter size and decreased infertile matings.

PREVIOUS studies of nesting (individual or thermoregulatory) in the house mouse have provided information concerning environmental influences (BAR-NETT 1956; LYNCH and HEGMANN 1973; LYNCH *et al.* 1976), genetic influences (BARNETT and SCOTT 1964; LYNCH and HEGMANN 1972; LEE 1973; LYNCH, STURGIS and POSSIDENTE 1977; LYNCH 1977) and genotype-environment interactions (LEE and WONG 1970; LYNCH and HEGMANN 1973; LYNCH and SANCHEZ 1975). Taken together, these studies strongly suggest that building large nests is an important adaptation to cold ambient temperature in this species. Mice build larger nests in the cold than at room (warm) temperatures, large nests are associated with a metabolic advantage (manifested as decreased food consumption) in the cold and mice that build large nests at room temperature show a greater increase in nest size in the cold than do mice from genotypically "low nesting" strains or lines. Prior genetic analyses, based primarily on inbred strains and derived generations, have demonstrated relatively low heritability but substantial dominance variance for nest size measured at room temperatures.

This paper describes a study of replicated bidirectional selection for nest-building in the house mouse. There were two primary goals to this experiment: (1) to obtain more accurate estimates of genetic parameters influencing nest size, and (2) to produce animals with highly divergent phenotypes that could then be used to investigate genetically correlated traits.

MATERIALS AND METHODS

Selection for nesting: The base population for this experiment was a heterogeneous stock of laboratory mice (*Mus musculus*) originally derived from an 8-way cross among inbred strains. McClearn, Wilson and Meredith (1970) gave a description of the origin of the stock, designated HS/Ibg. Each of 2 replicates was established by choosing 8 litters at random from the base population, and selecting from each litter the male and female that built the largest nests (see below) and those that built the smallest nests. Within each replicate, the high-nesting males and females were mated at random (except that sibs were never mated) to establish a high-selected line, and the low-nesting males and females used similarly to establish a low-selected line. Thereafter, the lines were closed, with parents for subsequent generations chosen as the extreme-scoring male and female from each litter of the line. Rather than propagate the control lines from the residual mice with intermediate scores, a third group of 8 litters was taken from the base population, with 2 males and 2 females chosen at random from each litter. The animals thus chosen were divided into 2 groups of 8 males and 8 females and mated at random within these groups to provide 2 control lines. The controls were assigned at random, 1 to each replicate. In this manner, 2 replicates were formed of the high, low and control lines, with 8 litters per line. Within-family selection was employed to reduce both the extent of inbreeding and the influence of environmental variance common to families. In each line, 1 extra mating was always set up to replace any that did not produce offspring. The theoretical rate of inbreeding (Wright 1931), where effective population size is doubled in the case of within-family selection (Falconer 1960), was 1.6% per generation.

The work was begun in Boulder, Colorado, and for the first 5 generations of selection, animals were raised and tested under a 12 light:12 dark photoperiod at 25 ± 1°. Stainless steel cages were provided with sawdust bedding, and those used in testing were 24 × 10 × 12 cm. Parents of the sixth selected generation were shipped to Connecticut, and problems in transfer resulted in there being 36 rather than 48 matings at this generation, with the most severe bottleneck (2 matings) occurring in 1 of the high lines (H_2). From the sixth generation, animals have been raised and tested under a 16 light:8 dark photoperiod at 21 ± 1°, in polypropylene cages (27 × 17 × 12 cm) provided with wood shavings for bedding. All mice were weaned at 25 ± 1 days of age and housed with like-sexed littermates until testing at 50 ± 3 days of age.

For assessment of nesting, animals were weighed, housed individually and provided with food and water *ad libitum*. A preweighed amount of cotton batting (Mountain Mist) was provided in the food hopper of each cage lid, and the cotton remaining in the hopper was weighed on each of 4 consecutive days. Nests were removed daily, and additional preweighed cotton added to the food hopper if necessary. The weight of cotton which the mice removed from the hopper was taken as an index of nest building. Animals were selected for mating on the basis of the total weight of cotton used during the 4 test days (hereafter referred to as total nesting score).

Data analysis: The distribution of nesting scores in the base population was skewed toward the direction of low scores, and there was a substantial correlation between the means and variances of the selected lines. The square-root of the total scores both normalized the distribution and reduced the mean-variance correlation. Therefore, all analyses were done on square-root transformed data (although graphs are presented as raw scores for illustrative value).

Selection differentials were calculated as the average difference between the scores of selected parents and the mean of their same-sex littermates, weighted by the total number of offspring each parent contributed. Response to selection was calculated as the deviation of the means of selected lines from their respective control values. Calculations of selection differential and response were performed separately for males and females, and then averaged.

The regression of response on cumulative selection differential was used to obtain estimates of realized heritability. Standard errors of the heritability estimates were calculated according to Hill's (1972) method.

Pooled heritability estimates were obtained by pooling the regression coefficients. Likewise, standard errors were obtained from the pooled variances, where the variances used were those calculated according to Hill (1972). The realized heritability from the total experiment, pooled

across line and replicate, was adjusted for the within-family selection index, using the following formula: $h^2 = h^2{}_R(1\text{-}t)/1\text{-}r)$, where h^2 is the overall heritability expected from mass selection or a randomly mating population, $h^2{}_R$ is the realized heritability obtained for this particular index, r is the coefficient of relationship of full sibs (0.5) and t is the intraclass correlation of full sibs for nesting scores (see below).

Parameters of the HS/Ibg stock: Inbreeding usually occurs in selection experiments that have small effective population sizes. To assess the effects of inbreeding on nest size, body weight and litter size, these traits were measured on the HS/Ibg base population for the selection experiment in Colorado and on several later generations of HS mice that were reared and tested in Connecticut. This stock is maintained by a minimum of 40 matings per generation, and was expanded to over 100 pairs for several generations used in comparisons with the selected lines, so that the rate of inbreeding over 7 HS generations was negligible relative to that over 15 selected generations. The intraclass correlation of full-sibs for total nesting scores, required to adjust the realized heritability estimate for the within-family selection index was estimated from both the base population (24 litters, all offspring measured) and 2 later generations of HS mice (251 litters randomly culled to 2 females and 2 males per litter). The later generations were also used to obtain an estimate of heritability of nesting from parent-offspring regression to compare with realized heritability estimated from the selection experiment. Details of the procedure for the parent-offspring regression experiment are given in LACY and LYNCH (1979).

RESULTS

Response to selection: The change in nesting scores across 15 generations of selection is shown in Figure 1. Variations in control scores between generations illustrate the extent to which this measure was influenced by intergenerational environmental fluctuations, as both control lines followed the same general pattern. By generation S_{15}, an eight-fold difference had been achieved between the scores of high and low selected mice.

Response to selection plotted as deviations from controls, relative to the cumulative selection differential for transformed data, is shown in Figure 2. Selection differentials were greater in the high than the low lines, although the total responses to selection were similar in both directions. Relative to the selection differential, then, the responses were asymmetrical, being greater in the low direction. After 15 generations of selection, there was no evidence of a plateau in the response in either direction (Figure 2).

Realized heritability estimates and their standard errors are listed in Table 1. The regressions were calculated over the entire 15 generations, since there was no marked change in slope as the experiment progressed. In both replicates, heritability was greater in the low lines, and the regression lines were significantly different. However, the standard errors of the estimate calculated by HILL's (1972) method are too large to demonstrate significant differences between heritabilities (as opposed to the actual regression) in the upward and downward direction. Nevertheless, the asymmetry is probably real, since both analysis of between-replicate *versus* between-line variance and a z test for the the variance of the difference between heritabilities in the high and low direction resulted in marginal significance at the 0.10 level.

When the realized heritability was pooled across the entire experiment and adjusted for within-family selection, the heritability of total nesting scores was 0.28 ± 0.05. This agrees well with the value of 0.33 obtained from parent-
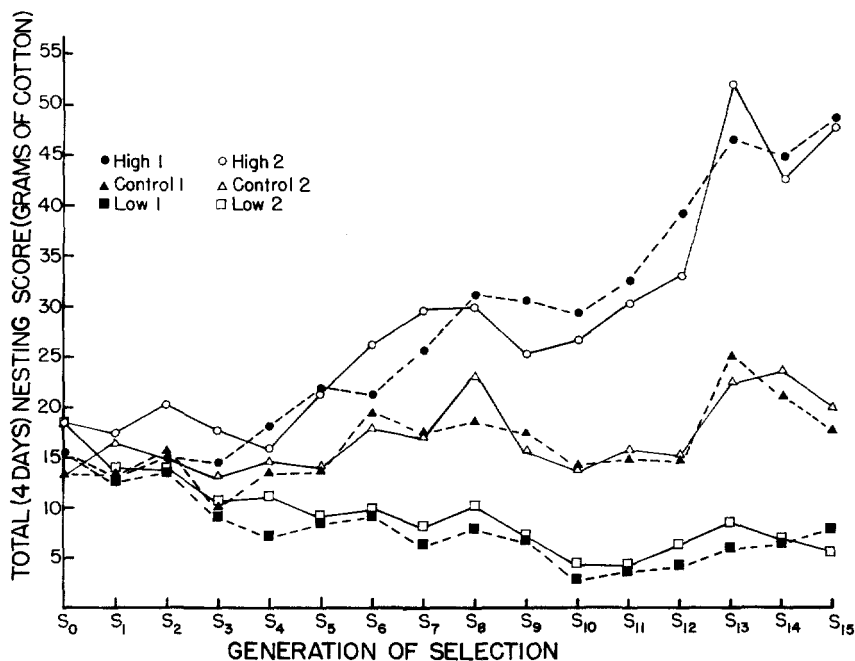
FIGURE 1.—Mean nesting scores across generations of selection for mice from the high, low and control lines of two replicate experiments.
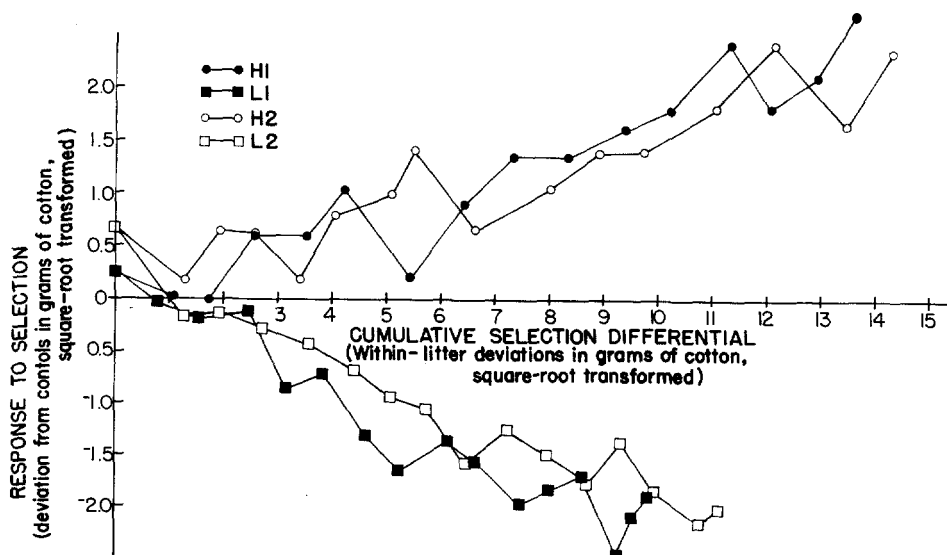

FIGURE 2.—Response to selection, measured as deviation of means of the selected lines from control lines, plotted against the cumulative weighted selection differential for the high and low lines of two replicate experiments.

TABLE 1

Realized heritabilities for total (four-day) nesting scores

Line	Heritability ± S.E.
High 1	0.18 ± 0.04
High 2	0.13 ± 0.04
Low 1	0.24 ± 0.05
Low 2	0.22 ± 0.05
Pooled High	0.15 ± 0.03
Pooled Low	0.23 ± 0.04
Total	0.18 ± 0.02

offspring regression in the HS mice. In contrast, heritability estimates from intraclass correlations of full sibs ranged between 0.4 and 0.6, indicating a substantial amount of nonadditive genetic variance and/or common environmental effects on nesting (see LACY and LYNCH 1979 for further discussion).

Since several of the environmental differences between the Colorado and Connecticut laboratories (*e.g.*, ambient temperature, photoperiod and cage size) had a demonstrable effect on nesting, it was not possible to compare the base and S_{15} generations of control lines to obtain information about the effects of inbreeding on nesting. However, generations S_{12} through S_{14} were measured for nesting during approximately the same time period as HS/Ibg generations 21 through 23, and the average nesting scores for the control lines across those three generations (17.9 g for females and 23.4 g for males) were actually somewhat higher than the overall means of the corresponding HS/Ibg generations (Table 2). Thus, there is no evidence from this experiment that an increase in inbreeding of approximately 20% had any effect on nesting.

TABLE 2

Means (± standard errors) for total nesting scores, body weight, litter size and percentage of infertile matings for HS/Ibg mice

Generation	Total nesting score (g)	Body weight (g)	Litter size	Infertile matings (%)
21 ♀ ♀	13.5 ± 0.6 (139)*	22.4 ± 0.2 (139)*	10.3 ± 0.2 (108)†	17‡
21 ♂ ♂	16.7 ± 0.6 (140)	29.2 ± 0.2 (140)		
22 ♀ ♀	16.8 ± 0.6 (245)	20.8 ± 0.1 (245)	9.5 ± 0.2 (187)	13‡
22 ♂ ♂	22.6 ± 0.6 (257)	25.4 ± 0.2 (257)		
23 ♀ ♀	19.9 ± 0.7 (199)		9.2 ± 0.5 (35)	3
23 ♂ ♂	21.1 ± 0.6 (224)	25.4 ± 0.4 (24)		
24 ♀ ♀			9.7 ± 0.5 (24)	8
Overall means:				
♀ ♀	16.7	21.6	9.7	
♂ ♂	20.1	25.7		

* Numbers of animals.
† Numbers of litters.
‡ These values are inflated because the mothers were subjected to extensive prior testing.

Correlated responses: Body weight, litter size and the number of infertile matings were monitored for correlated responses throughout the course of selection. Traits sensitive to inbreeding might be affected by the approximate 20% increase noted above. Such changes would be detected in the controls and in comparisons with contemporaneous HS generations and would be expected to be similar in the selected lines that were inbred to the same extent. Any additional changes in the selected lines, especially those showing consistent bidirectional change in both replicates, would be attributable to the selection process itself.

A consistent difference in litter size appeared over the course of the experiment, although most of the individual changes were not significant (Table 3). The similar declines in the low lines and controls from an initial level of 9.8 could be due to inbreeding, since contemporaneous HS mice also averaged 9.7, and the average decline of about 0.5 offspring per 10% inbreeding matches previous estimates for both laboratory and wild mice (BOWMAN and FALCONER 1960; ROBERTS 1960; LYNCH 1977). However, both high lines not only failed to show a decline, but actually increased in litter size. A correlated response in litter size, particularly to selection for high nesting scores, is the most plausible explanation.

The number of infertile matings recorded over the entire 15 generations of the experiment showed a similar pattern of high lines being more fertile than control, which was more fertile than low lines in both replicates (Table 3). Although sporadic, infertility tended to increase across generations in the control and low lines, but neither high line suffered any infertility after the experiment was moved to Connecticut, performing better than contemporaneous HS mice (Table 2). The Chi-square value for the difference between control and low lines was not significant, but the high-low difference was significant at $p > 0.005$, providing additional evidence for a positive correlated response in fertility.

The changes in 50-day weight (Table 3) are mostly significant, although the average weight of all selected lines at S_{15} (21.5 ± 0.3 and 26.0 ± 0.4 for females and males, respectively) did not differ from the weight of HS/Ibg mice reared in the same laboratory (Table 2). Both control lines increased in body weight during the experiment. The amount of inbreeding in this experiment would not be expected to affect body weight (WHITE 1972; LYNCH 1977), and the reduction

TABLE 3

Correlated responses to selection for nest-building

Line	Regression of litter size on generation number	Percent of infertile matings	Regression of 50-day body weight on generation number
High 1	0.07 ± 0.06	0.8	0.20 ± 0.04
Control 1	-0.11 ± 0.06	8.3	0.11 ± 0.03
Low 1	-0.05 ± 0.05	10.8	0.07 ± 0.04
High 2	0.07 ± 0.05	1.7	0.01 ± 0.04
Control 2	-0.02 ± 0.06	4.2	0.18 ± 0.04
Low 2	-0.15 ± 0.04	5.0	-0.11 ± 0.03

in litter size could account for some of the increase in weight, especially in replicate one. However, in both replicates, the high lines were heavier than the low lines, and, on average, the high lines increased both body weight and litter size, while the low lines decreased in both. Since in the selected lines the changes in body weight were opposite to those expected from the changes in litter size, the data are compatible with a positive association between body weight and nest size.

DISCUSSION

The realized heritability of nesting scores found in this experiment, when adjusted for within-family selection, was in good agreement with estimates obtained from the base population. Both estimates, however, were higher than previously reported values. LYNCH and HEGMANN (1972) found a heritability of only 0.03 from analysis of a cross between two inbred strains. Similarly, LEE (1973) reported values of 0.17 from analysis of a two-strain cross, and 0.11 from diallel analysis of four inbred strains. The higher value of 0.28 found in this study is presumably due to the greater genetic variance in the HS/Ibg population, which was derived from eight inbred strains. HEGMANN, WHITE and KATER (1973) also found that heritabilities in the HS stock for several other traits (body weight, tail length and nerve conduction velocity) were substantially higher than similar estimates from a population derived from a two-strain cross, even though the two strains had been chosen initially for their divergent phenotypes.

Examination of the effects of inbreeding across the 15 generations of selection, utilizing comparisons of control lines with a large outbred sample of the base population, showed no detectable effects on nesting or body weight, which is consistent with results of inbreeding a wild population of *Mus musculus* (LYNCH 1977). The decrease in litter size of about one offspring for approximately 20% inbreeding also agrees with previous studies (ROBERTS 1960; LYNCH 1977).

Beyond effects that can be accounted for by inbreeding, consistent differences between the high and low lines developed during the 15 generations of selection. In both replicates, the high lines showed increases in litter size and decreases in number of infertile pairs, while the control and low lines had opposite trends. Selection favoring heterozygotes could contribute to the observation that mice selected for high nesting scores showed increases in traits known to be subject to inbreeding depression. Previous studies have consistently demonstrated the substantial contribution of nonadditive effects (dominance and epistasis) to variation in nesting (LYNCH and HEGMANN 1972; LEE 1973; LYNCH 1977; LACY and LYNCH 1979), with the most heterozygous mice having the highest nesting scores. The greater response to selection in the low lines also implies that high nesting scores are dominant. Thus, part of the correlated response could be due to greater heterozygosity in the high lines at loci influencing nesting, although this hypothesis is not directly testable with the data presented here.

Body weight was also affected by selection, with the high lines becoming heavier than the low lines. However, this was achieved differently in each replicate: in one, the high line significantly increased weight; in the other, the low

line significantly decreased weight. This difference between replicates indicates that drift may be important as well.

Many studies have demonstrated the value of large nests for increased efficiency and survival of small mammals exposed to cold (PEARSON 1960; MUUL 1968; LYNCH and HEGMANN 1973; LYNCH 1973; GLASER and LUSTICK 1975). Natural selection for increased adaptation to cold environments has presumably resulted in animals that build large nests (KING, MAAS and WEISMAN 1964). LYNCH and POSSIDENTE (1978) demonstrated that selection for high nesting scores results in substantially increased Darwinian fitness (measured as more off-spring weaned) at low ambient temperature (5°). This information, in addition to the evidence for correlated responses in fertility presented here, is consistent with the interpretation that nest-building is a fitness character important for adaptation to cold in *Mus muculus*.

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