

# SELECTION FOR LITTER SIZE IN MICE IN THE PRESENCE AND ABSENCE OF GONADOTROPIN TREATMENT,

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THE extent to which reproductive characteristics can be modified by selection is of interest in understanding the nature of genetic variation in characteristics closely correlated with fitness. It is also relevant to the question of effecting genetic improvement in an important trait in species producing protein for human food. Different species and races within species have acquired widely different reproductive rates in the course of their evolution, but there is extensive evidence that intrapopulation genetic variability, or at least the component of it providing the basis for response to mass selection, is low. Such evidence has been reported, for example, for *Drosophila* (ROBERTSON 1957; KOJIMA and KELLEHER 1963); mice (FALCONER 1960b; DALTON and BYWATER 1963); swine (BOYLAN, REMPEL and COMSTOCK 1960) and sheep (REEVE and ROBERTSON 1953). This low heritability is commonly attributed to the association of reproductive rate with fitness, i.e., to the fact that natural selection has acted directionally with respect to reproductive capacity, and hence most of the additive genetic variation has been fixed (KOJIMA and KELLEHER 1963).

The experiments to be reported here are part of a series designed to compare the effectiveness of alternative methods of selection for reproductive capacity, using the laboratory mouse as the experimental animal. This paper presents the results of selection for number born in untreated and superovulated lines. Superovulation treatment has been reported (FALCONER *et al.* 1961) to increase phenotypic variability in litter size; selection following such treatment was carried out to determine if this is an effective means of exposing latent genetic variability.

## MATERIALS AND METHODS

Two stocks were used, one a cross of four inbred lines (C57BL/6J, C3H/J, AKR/J and DBA/2J), the other a cross of eight inbred lines (LP/J, SJL/J, BALB/cJ, C57BL/6J, 129/J, CBA/J, RF/J and BDP/J). Thus the only line common to both was C57BL/6J. The 4-way cross was produced by reciprocally crossing two  $F_1$ 's (DBA/2  $\delta$   $\times$  C57BL/6  $\text{f}$  and C3H  $\delta$   $\times$  AKR  $\text{f}$ ) obtained from Simenson Laboratories, Gilroy, California. Since only two nonreciprocal  $F_1$ 's were used, only two of the possible four Y-chromosomes were included in the 4-way cross. The 8-way cross was produced at the Jackson Laboratory by Dr. T. H. Roderick, and carried all eight Y-chromosomes in the original 8-way cross. Representatives of two sublines which had been carried for four generations from the initial 8-way cross by double first-cousin matings were crossed to produce the stock used in the present experiment.

Five closed lines were initiated from the 4-way cross:

1. A randomly selected, untreated control (line C)

2. An untreated line in which selections were made from the largest litters (line S1)
3. An untreated line in which the largest litter produced in each set of littermate full-sisters was selected (intra-litter selection, line S-IL)
4. A randomly selected, superovulated control (line C-T)
5. A superovulated line in which selections were made from the largest litters (line S-T).

An untreated line selected for large litters was initiated from the 8-way cross (line S2). Selection procedure was the same as in line S1. The six lines were kept synchronous throughout the experiment.

The superovulation treatment consisted of 3 international rat units (I.U.) of pregnant mare serum (PMS) followed by 3 I.U. of human chorionic gonadotropin (HCG) 40 hours later (48 hours after generation 7) injected intraperitoneally. This is the procedure described by FOWLER and EDWARDS (1957). The PMS was provided by H. H. COLE, and was assayed periodically throughout the experiment by means of the rat ovarian weight method described by COLE and ERWAY (1941), using the second international standard reported by BANGHAM and WOODWARD (1967). The HCG was a commercial preparation testing 3000 I.U./mg.

Lines C, S1 and S2 were reproduced each generation by mating 18 males to two females each, with the exception that larger numbers of matings were made in line S2 for the first three generations (see Table 1). In lines C-T and S-T, 24 males and 48 females were mated each generation, since the proportion of females producing litters was smaller for superovulated than for untreated groups. Choice of breeding animals in lines C and C-T was at random, with the restriction that, insofar as possible, each sire was replaced by one son and each dam by one daughter. In the selected lines, a minimum of six litters was represented by selected individuals each generation.

Line S-IL was initiated from 18 pair matings, with all females from the resulting 18 litters reared and mated to produce generation two. All females in a litter, where there were four or fewer, were mated to one male; with five or more, they were divided between two males. From generation 2, the female with the largest litter in each full-sib set was selected, and all daughters in such litters were mated to repeat the cycle. Occasionally two females from a set of full sisters were selected, to compensate for litters with fewer than two females or in which all females had the same number of young. The procedure followed in this line is the same as that used throughout the litter size selection experiment reported by FALCONER (1955, 1960b, 1963). The method was chosen by FALCONER in part to circumvent a maternal effect, mediated through body weight, operating counter to the dam's genetic effect on litter size, which he found to be an important source of variation in his material.

Results are presented for 11 generations in lines C, S1, S2 and S-T. The foundation stocks were designated generation one; therefore the number of generations of selection is one less than the generation number. Some additions to and modifications of the experimental design were made at generation 8, as follows:

1. Lines S1 and S2 were crossed reciprocally by mating a random sample of generation 7 females to selected males being used to propagate the parent lines. Thirty-six unselected  $F_1$  females were mated to produce litters contemporary with generation 9 of lines C, S1 and S2, and selection on the same basis as in lines S1 and S2 was initiated from this  $F_2$ . This line was designated line S1.2, and is being selected simultaneously with the two parent lines to determine if the cross of two partially improved lines has a greater response potential than the parent populations.

2. Line S-IL was discontinued.

3. Lines C-T and S-T were reproduced without treatment, to determine if either natural or artificial selection following the gonadotropin treatment had resulted in any genetic change for performance in the normal environment. As an additional check on this point, a random sample of generation 7 females from each of these two lines and from line C, the untreated control, were mated to males from an unrelated 4-way cross line (selected for rapid 3-6 week body weight gain). Females were mated to non-littermate males from their respective crosses to produce litters contemporary with generation 9.

4. Line C-T was discontinued following generation 8. Treatment and selection were resumed

in line S-T, using randomly selected males and females from the untreated litters produced in generation 8. Thirty-six males and 72 females, rather than the usual 24 and 48, were used for this one generation.

Mating in all lines was at random, with the restriction that full and half-sib matings were avoided in all cases, and cousin matings were avoided in most cases also.

Pregnant females were checked twice daily, and all litters were counted the day of birth. The term number born in this paper means total number of young born, including any born dead if fully developed. At two days the litters were sexed, and those with more than ten young were reduced to ten, with a ratio of 3 males to 7 females where possible. No augmentation of litters of less than ten was practiced.

Mice were weaned and the sexes separated at 21 days. They were caged 4 to 7 per cage, usually by litter, from weaning until mating at approximately 9 weeks of age. Males were left in the mating cages 7 or 8 days in untreated lines, and 36 to 48 hours in treated lines. With few exceptions, all matings in the latter occurred within the first 24 hours. The pregnant females were placed in separate cages approximately 17 days after introduction of the males. Pelleted Simenson White Diet was fed *ad libitum* throughout the experiment. Lights in the colony were controlled by a time switch and were on 10 hours daily, from 8:00 a.m. to 6:00 p.m. Temperature was maintained at  $76 \pm 4^\circ\text{F}$ .

*Measurement of selection practiced.* Two selection differentials, intended (I) and realized (R) were calculated for number born for each line in each generation. The intended selection differential was computed as the average difference between the number born in the litter containing the selected individual and the generation mean. The realized differential was obtained by weighting the deviation for each individual by the number of progeny weaned from it in the next generation. In each case the calculations were carried out separately for sires and dams, with the unweighted average of these two providing the values used.

A second set of differentials (I-IL and R-IL) was calculated for line S-IL, based on the deviation of the litter size of the selected female from the average of the set of littermate full sisters including the selected female.

Heritability of intra-litter variation ( $h_w^2 = (V_A/2)/(V_A/2 + V_E)$ ), in contrast to heritability of deviations from the population mean ( $h^2 = (V_A)/(V_T)$ ), where

$V_A$  = additive genetic variance,

$V_C$  = variation common to littermates not included in additive genetic variance,

$V_E$  = environmental variation among littermates,

$V_T = V_A + V_C + V_E$  = total phenotypic variance.

This may be expressed as  $h_w^2 = h^2 (1-r)/(1-t)$  as given by LUSH (1947) and also by FALCONER (1960a), where  $r$  is the within family relationship, .5 in this case, and  $t$  is the phenotypic correlation between littermates  $= (V_A/2 + V_C)/(V_T)$ . For a trait with low heritability and  $V_C = 0$ ,  $(1-r)/(1-t)$  is only slightly greater than 0.5, i.e., intra-litter selection under these conditions is expected to be little more than half as effective as mass selection. On the other hand, with  $V_C$  large relative to  $V_A$ , as expected if maternal effects are important, this fraction becomes much larger than 0.5. In this connection, it appears that in estimating  $h^2$  from  $h_w^2$ , FALCONER (1963, p. 765) assumed  $V_C = 0$ , in material in which maternal effects had been shown to be important.

Realized heritability was calculated for each selected line by doubling the regression of deviation of generation means from those of the appropriate control (treated or untreated) on cumulative selection differential. This method of estimating heritability was suggested by FALCONER (1955, 1960a). The regression coefficient is doubled in this case since the trait on which selection is based is expressed in females only, and hence with the selection procedure used in this experiment the phenotype on which selection is based is that of the dam rather than of the individual selected.

Daughter-dam regression coefficients were calculated for each generation and pooled over generations, for each of the unselected lines. This regression coefficient is expected to contain half of the additive genetic variance, and fractions of the additive by additive interaction components, transmitted maternal effects, and covariance between genetic deviations in transmitted and direct maternal effects (DICKERSON, 1960). If one assumes that additive genetic variance is

TABLE 1

*Mean and standard deviation for litter size by generation—untreated lines*

Genera- tion*	Selection method, line designation and foundation stock														
	Random			Mass selection						Intra-litter selection					
	C (4-way cross)			S1 (4-way cross)			S2 (8-way cross)			S1-2†			S-IL (4-way cross)		
	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s
1	35	8.23	1.88	34	8.23	1.39	62	9.10	1.82	..	..	..	18	8.17	2.00
2	32	7.31	1.77	36	7.33	1.96	41	9.51	2.06	..	..	..	52	7.08	2.18
3	36	7.42	1.65	32	8.22	1.69	37	9.86	1.84	..	..	..	56	7.70	1.79
4	30	7.23	1.87	32	8.47	2.02	32	10.47	1.52	..	..	..	52	7.90	2.11
5	33	6.85	2.05	32	8.25	2.20	34	10.56	2.45	..	..	..	75	7.19	2.21
6	33	6.79	2.32	31	8.65	1.58	31	9.13	2.20	..	..	..	88	7.38	2.31
7	33	7.18	2.74	33	8.76	2.50	33	11.24	1.52	..	..	..	70	7.99	2.18
8	32	6.15	1.98	33	8.82	2.02	35	10.66	1.88	..	..	..	64	7.50	2.06
9	35	7.57	2.42	34	9.00	2.73	27	11.26	2.73	32	10.69	2.07	..	..	..
10	36	7.39	2.11	35	8.63	1.89	32	11.25	2.34	35	9.77	2.51	..	..	..
11	36	7.72	2.13	34	9.29	2.04	34	12.09	1.71	35	11.14	2.65	..	..	..

\* Selections initiated among generation-1 litters.  
 † Line S1-2 produced by crossing generation-7 individuals from lines S1 and S2.

the most important of these, doubling this regression coefficient provides an estimate of heritability. Data from the selected lines were unsuitable for heritability estimation by this method because of the fact that selection reduced the variation among dams to a very low value.

Analyses of variance using the method of unweighted means were carried out on data from the following pairs of lines: C vs. S1, C vs. S2, S1 vs. S2, using data from generations 2-11 in each case; C vs. S-IL and S1 vs. S-IL, generations 2-8; and C-T vs. S-T, generations 2-7, with tests of significance made for lines, generations and interaction in each. Data from generation

TABLE 2

*Mean and standard deviation for litter size by generation—superovulated lines*

Generation	Line designation and selection method*								
	C-T. Random			S-T. Mass selection			C. Random (untreated)		
	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s
1	23	8.09	3.29	23	8.39	4.05	..	..	..
2	16	6.88	4.16	31	7.71	3.92	..	..	..
3	16	10.31	3.63	17	7.82	4.63	..	..	..
4	19	7.84	4.39	19	7.11	4.43	..	..	..
5	20	8.15	4.31	23	10.04	4.35	..	..	..
6	28	10.21	3.94	18	10.78	4.24	..	..	..
7	22	9.82	4.67	19	6.89	4.63	..	..	..
8†	40	7.05	1.43	35	6.89	1.68	32	6.15	1.98
F <sub>1</sub> ‡‡	31	7.32	2.76	27	7.74	2.18	31	7.55	2.50
9	..	..	..	41	7.41	4.22	..	..	..
10	..	..	..	24	11.17	4.31	..	..	..
11	..	..	..	24	10.92	3.72	..	..	..

\* All lines derived from 4-way cross.  
 † Not treated.  
 ‡‡ F<sub>1</sub> females produced by crossing each line (C-T, S-T, C) with the same unrelated line.

8 for lines C, C-T and S-T (all untreated that generation) and from the three contemporary  $F_1$  crosses involving these lines described above, were also analyzed for differences between lines, mating systems (within line vs.  $F_1$  cross) and interaction.

Linear and quadratic regression coefficients of line mean on generation number for each of the four lines C, S1, S2 and S-IL for all generations available beginning with generation 2 were calculated and tested for significance.

RESULTS

Generation means for untreated and treated lines are shown in Tables 1 and 2 and in Figures 1 and 2, respectively. Corresponding selection differentials are

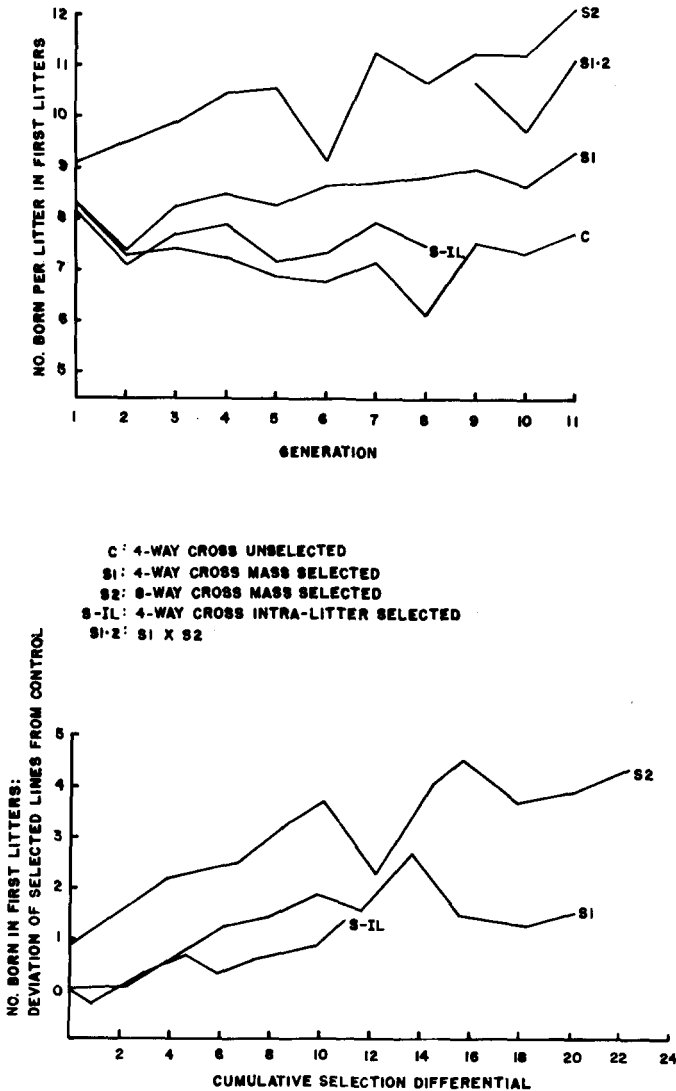


FIGURE 1.—Results of selection for litter size in untreated mice.

given in Tables 3 and 4. Estimated and realized heritability values are given in Table 5.

1. *Amount of selection practiced.* The most striking feature of the selection differentials is that they are nearly twice as large for the selected treated line as for the selected untreated lines. This is a result of the much larger standard deviation in litter size of treated than untreated lines, which may be seen by comparing Table 2 with Table 1. In each case, selection differentials of approximately one phenotypic standard deviation were obtained each generation. This was so in spite of the fact that a higher proportion of available animals had to be selected in the treated line (S-T), because on the average only about one-half of the females mated following treatment produced litters. The shape of the frequency distribution of litter sizes of treated females was quite different from that of untreated females as shown in Figure 3. Data from lines C-T and S-T are pooled in this figure, since there was no evidence that the two distributions differed, i.e., that line S-T had responded to selection, as discussed below.

On the average, small positive selection differentials were found in the unselected, untreated line (C), in spite of the practice of selecting equal numbers of replacements from each litter where possible. In the unselected treated line (C-T), selection differentials were more variable and averaged higher than in line C. The selection differentials for the control lines were subtracted from those of the selected lines in calculating realized heritability.

Intended and realized selection differentials agreed very closely for untreated lines, indicating no tendency to differential reproduction between extreme and less extreme individuals in terms of the size of the litter in which they were born. This result may have been due in part to the fact that the maximum number weaned in any litter was 10. In the treated lines, agreement between intended

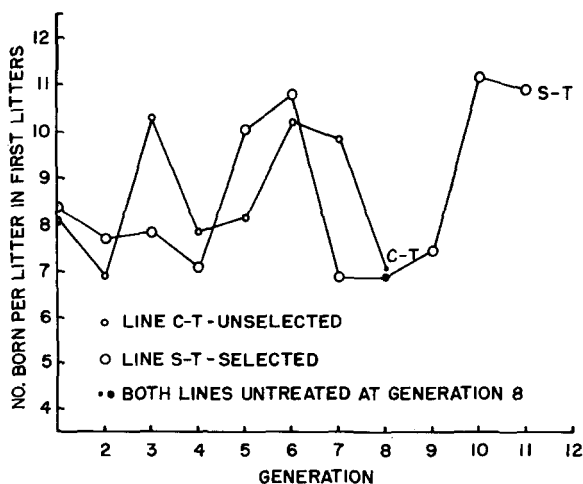


FIGURE 2.—Results of selection for litter size in mice treated with 3 international units (I.U.) of pregnant mare serum (PMS) + 3 I.U. of human chorionic gonadotropin (HCG).

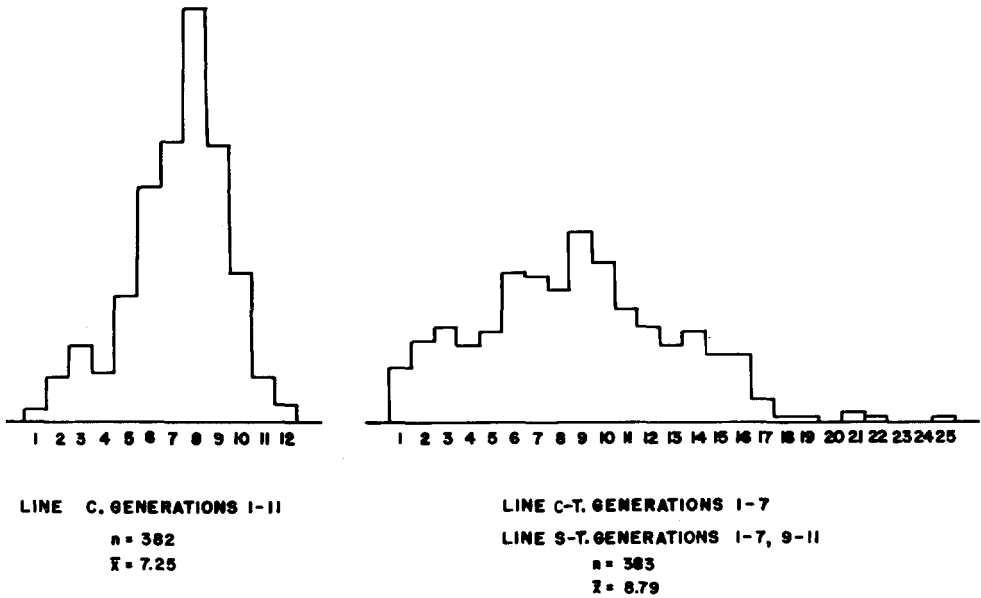


FIGURE 3.—Frequency distributions of litter sizes of untreated mice (line C) and mice superovulated with 3 international units (I.U.) of pregnant mare serum (PMS) and 3 I.U. of human chorionic gonadotropin (HCG). (lines C-T and S-T combined, since the distributions did not differ)

TABLE 3

*Intended (I) and realized (R) selection differentials for total number of young born per litter in untreated lines*

Generation	Line*											
	C		S1		S2		S1-2		S-IL			
	I	R	I	R	I	R	I	R	I	R	I-II $\ddagger$	R-II $\ddagger$
1	0.03	-0.20	1.77	2.05	3.64	3.70	...	...	0.32	0.61	...	...
2	-0.06	-0.03	2.54	2.44	2.82	2.84	...	...	2.10	2.04	1.40	1.42
3	0.06	0.13	1.50	1.57	2.16	1.98	...	...	2.03	1.87	1.64	1.63
4	0.31	0.30	2.13	2.08	1.80	1.79	...	...	1.56	1.55	1.41	1.25
5	0.32	0.47	2.49	2.40	2.83	2.59	...	...	1.94	1.89	1.74	1.78
6	0.30	0.55	2.29	2.30	2.82	2.81	...	...	3.10	3.06	2.63	2.66
7	0.71	0.77	2.98	2.79	2.01	1.95	...	...	1.96	...	1.86	...
8	0.39	0.40	2.32	2.35	2.57	2.64	1.26	1.06	...	...	...	...
9	0.35	0.36	3.14	3.02	2.49	2.53	2.58	2.59	...	...	...	...
10	0.28	...	2.25	...	2.54	...	3.23	...	...	...	...	...
Average	0.27	0.31	2.34	2.33	2.57	2.54	2.36	1.83	1.86	1.84	1.78	1.75

\* Line C—Unselected control derived from 4-way cross. Line S1—4-way cross mass selected for large litters. Line S2—8-way cross mass selected for large litters. Line S1-2—Cross between lines S1 and S2 at generation 7. Line S-IL—4-way cross selected for large litters using intra-litter selection.

$\ddagger$  Selection differentials computed as deviation from full sister family average. All other selection differentials (I,R) were computed as deviation from the average of the entire line.

TABLE 4

*Intended (I) and realized (R) selection differentials for total number of young born per litter in superovulated lines*

Generation	Line*			
	C-T		S-T	
	I	R	I	R
1	0.13	-0.06	3.30	3.73
2	2.16	2.25	4.83	4.26
3	0.85	0.80	4.27	4.93
4	0.70	0.93	4.17	4.61
5	0.45	0.66	3.55	2.91
6	0.31	-0.68	2.83	3.93
7	0.41	0.21	4.43	4.40
8	...	...	0.18	0.05
9	...	...	5.74	5.63
10	...	...	3.17	...
Average (excluding Gen. 8)	0.72	0.59	4.03	4.30

\* Line C-T—unselected line derived from 4-way cross.  
Line S-T—4-way cross mass selected for large litters.

and realized differentials was not as good, within generations, but the totals again corresponded closely.

## 2. Response to selection.

a) *Untreated lines.* All three lines from the 4-way cross decreased in litter size from generation 1 to generation 2. This is most probably due to inbreeding effects and/or recombination loss. Since the dams of generation 1 litters were original 4-way cross females, they would possess a maximum degree of heterozygosity (for this stock); in the following generation, 25% of the loci on the average would be expected to be homozygous. The fact that the 8-way cross line, which was at  $F_5$ , did not decline, supports this reasoning.

Following the second generation decrease, the unselected control line mean remained relatively constant thereafter, with the exception of generation 8, when it was substantially lower than in the previous and following generations. The generation 8 mean is important to the interpretation of the results, since this was the final generation for the intra-litter selection line. A chance decrease in the control in that generation, which this appears to have been, would produce an upward bias in the estimate of realized heritability in the selected line. To reduce the degree of this bias in the comparison of the two selection methods, realized heritabilities were calculated for lines S1 and S2 to the point contemporary with the final generation of S-IL (Table 5). The estimates at this point may all be too high, but may be compared with each other.

Lines S1 and S2 both responded to selection for larger litters, with line S2 showing the greater increase. Each differed significantly from line C, and in each analysis the line by generation interaction was significant, as expected if response to selection is occurring. Since line S2, derived from the 8-way cross, was com-



TABLE 5

*Estimates of heritability for number of young born per litter*

Line	Method of estimation	Generations	$h^2$
C	Daughter-dam regression	1-10	.39 ± .134
S1	Realized	1-8	.39 ± .058
		1-11	.13 ± .068
S2	Realized	1-8	.34 ± .124
		1-11	.22 ± .064
S-IL	Realized	1-8	.25* ± .050 (.25† ± .076)
C-T	Daughter-dam regression	1-7	.18 ± .160
S-T	Realized	1-7	Negative

\* Based on selection differential calculated as for lines S1 and S2 (R).

† Based on intra-litter selection differential (R-IL).

pared with the 4-way cross control, the initial difference between the two stocks in litter size (.87) and the decline in line C from generation 1 to generation 2 (.92) were subtracted from the line S2 generation means used in calculating realized heritability. Even with this adjustment, line S2 shows a higher realized heritability than line S1. Further evidence for the difference in response between lines S1 and S2 is provided by the results of the analysis of variance, which showed a highly significant difference between the two and a highly significant line  $\times$  generation interaction. Litter size of the cross of these two lines (line S1-2) is quite close to that of the midparent average for all three generations of line S1-2, suggesting little or no inbreeding depression in lines S1 and S2 to this point.

Intra-litter selection (line S-IL) was less effective than mass selection when only contemporary results are considered. Line S-IL in fact showed only small deviations from the control, except in the final generation, when, as pointed out earlier, the control was substantially below its usual level. The difference over the 7 generations was not significant, nor was the line by generation interaction. On the other hand, line S1 differed significantly from line S-IL, but again the interaction was not significant. This considered in conjunction with the results of the C vs. S1 analysis confirms the evidence of Figure 1, i.e., that line S-IL was consistently intermediate between lines C and S1. Response in line S-IL was not conclusively demonstrated, but quite possibly would have been had the line been continued longer. The evidence is consistent with the conclusion that response was about half that obtained with mass selection.

The first heritability value given for line S-IL in Table 5 is based on R, and that given in parenthesis on R-IL. The latter, representing  $h_w^2$ , is expected to be less than the former. The fact that the two are the same appears to be due to the fact that the values of R-IL are closer to R than expected. Also, the intra-litter heritability was calculated from 6 generations of data, whereas the first value was based on 7.

The regression analyses revealed a highly significant linear component for lines S1 and S2, but this component was not significant for lines C or S-IL. The quadratic component was not significant for any of the four lines.

Heritability estimated by daughter-dam regression for line C ( $.39 \pm .13$ ) was similar to the realized heritability for lines S1 and S2 through 7 generations of selection, but higher than realized heritability values through 10 generations.

b) *Superovulated lines.* There is no evidence that selection for litter size following superovulation resulted in any increase in genetic merit for litter size, in either the presence or absence of the treatment (Table 2). The regression of the deviation of the selected, treated line (S-T) mean from that of the unselected, treated line (C-T) on either generation number or cumulative selection differential is actually negative. When reproduced without treatment, lines C-T and S-T had litter sizes of 7.05 and 6.89, respectively. These values are slightly above that of the contemporary unselected, untreated control (line C, 6.15), but the latter had mean litter sizes above 7 in the preceding and following generations, suggesting that lines C-T and S-T did not differ from it. Furthermore, females produced by crossing lines C, C-T and S-T with the same unrelated line produced litters of 7.55, 7.32 and 7.74, respectively. Neither the line nor line by mating system interaction mean squares approached significance, substantiating the conclusion of no genetic difference among the three. The average of the  $F_1$  crosses was significantly higher than the average of the three lines mated intra-line; this could be due to heterosis or to genetic superiority of the line with which they were crossed, or both.

Heritability values for litter size following treatment, estimated by daughter-dam regression from line C-T data, were highly variable from generation to generation. The pooled value (.18) was not significantly different from zero.

#### DISCUSSION

The results of experiments on selection for litter size in mice reported to date and those from the present experiment in general support the conclusion that this trait has a low heritability. Nevertheless, substantial changes in mean litter size within a period of 12 generations or less have been effected in at least two cases. FALCONER (1960) reported a realized heritability of  $.08 \pm .02$  for 20 generations of intra-litter selection for large litter size; heritability of divergence between large and small litter size selected lines was  $.14 \pm .02$ . These are equivalent to individual heritabilities of .15 and .24 if one assumes  $V_c = 0$ . DALTON and BYWATER (1963) reported realized heritabilities of  $.06 \pm .08$  and  $.02 \pm .08$  for 15 generations of selection on normal and diluted diets, respectively, whereas BATEMAN (1966) reported a divergence of 5.6 mice between lines selected plus and minus for litter size for 12 generations. No estimate of realized heritability was given, but the divergence of 5.6 may be compared with that reported by FALCONER, 3.3, the latter achieved over a longer period of time. In the present experiment, realized heritabilities were .25 and above for three lines for 7 generations of selection. Heritability has decreased to values of  $.13 \pm .07$  and  $.22 \pm .06$  at the

end of 10 generations in two lines. This last, though only a moderate heritability value, represents an increase of approximately one-third above the base population level, and about the same degree of response as reported by BATEMAN (1966) if one assumes that response in the two directions was symmetrical in his experiment.

In contrast to these two cases, the experiment reported by DALTON and BYWATER (1963) provided no real evidence of any response at all, and in that reported by FALCONER, response was real but not large. We may examine possible explanations for such differences.

1. Number of generations of selection. The two experiments showing greatest response were based on somewhat fewer generations of selection. However, in the two showing lesser response there is no evidence of a curvilinearity of the response curve over the period of the experiment.

2. Foundation stock. The experiments giving greatest and least response were based on crosses of inbred lines, whereas FALCONER's, with an intermediate response, was based on an outbred stock. This factor therefore does not appear to explain the observed differences.

3. Population size. DALTON and BYWATER selected 13 males and 26 females per line, in contrast to the 18 and 36 used in the present experiment. However, FALCONER used only 10 pairs per generation. Therefore, smaller population size alone does not appear adequate to explain the lack of response in DALTON and BYWATER's experiment.

4. Standardization of litters. FALCONER practiced no standardization of litters, whereas in the present experiment a maximum of 10 young per litter was left on the mother. It appeared that this could be a factor in the greater response obtained in the present experiment, since the practice followed here would tend to reduce maternal effects which are expected to lower heritability. However, BATEMAN compared selected lines with no standardization and with reduction of litters to 6 young, and found no difference in response between the two.

5. Mating age. FALCONER mated selected individuals at 6 weeks, whereas in BATEMAN's experiment and the present one, mating was at 9 weeks of age. Since this would be expected to reduce maternal effects, it seems quite possible that the later mating age was a major factor in explaining the greater response in the latter two experiments. Further, the effect of not standardizing litters might be much greater in mice mated at 6 weeks than at 9. Unfortunately, information on mating age is not given in the report by DALTON and BYWATER. It is probable that too few experiments on this characteristic have been carried out to date to permit generalizations on the pattern of response.

It would be of interest to know if, in the present experiment, the apparent decline in rate of response in lines S1 and S2 after generation 8 represents an incipient plateau or not. The lack of significance of the quadratic component of the regression of generation mean on generation number, found for both lines, is evidence against a plateau. Only further selection will answer this question conclusively; to this end lines S1, S2 and S1.2 are being continued.

Selection for number born following a hormone treatment which markedly

increased phenotypic variation failed to improve the trait, in the presence or absence of the treatment. It might be expected that the use of exogenous gonadotropin would permit more effective selection for ovarian response to gonadotropin, or for the ability of the uterus to gestate a large number of fetuses. Other data obtained on treated females in this laboratory, however, indicate a high loss of embryos, both pre- and post-implantation, even in individuals in which the ovulation rate is not greatly elevated above normal levels. This and the fact that approximately half the treated females failed to become pregnant (or at least to produce litters) suggests that the hormone treatment causes a major disruption of the animal's normal endocrine balance, and that this obscures genetic variation in the characteristics of interest, rather than exposing latent genetic variation as had been anticipated might occur.

A line in which intra-litter selection was practiced was included in this experiment to permit a direct comparison of the effectiveness of this method with that of mass selection. Over seven generations, or six generations of intra-litter selection, this line consistently showed approximately half the response of the mass-selected line from the same stock, as expected in the absence of complicating maternal effects. Since intra-litter selection requires a larger number of matings and more recording than does mass selection, and since the difference between the two seemed fairly clearly established, this line was discarded at generation 8 when space was needed for other purposes. The fact that the mass-selected 4-way cross line made little additional response during three succeeding generations raises a question about the magnitude of the difference between the two lines. The available data for this experiment, however, certainly show no advantages to offset the practical disadvantages of the method. The daughter-dam regression value of  $+0.19$  obtained from the control line indicates much less maternal effect in this material than in that with which FALCONER (1955, 1960b, 1963) has dealt. As already discussed, the main reasons for this difference are probably the later mating age and reduction of large litters to ten in the present experiment. If in fact these (or other) factors have markedly reduced or eliminated maternal effects, then the major reason for using intra-litter selection would no longer exist.

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#### SUMMARY

Mice were selected for number born in first litters, in the presence and absence of a superovulation treatment with pregnant mare serum (PMS) and human chorionic gonadotropin (HCG). Ten generations of mass-selection yielded realized heritability estimates of  $.13 \pm .07$  and  $.22 \pm .06$  in lines derived from 4-way and 8-way cross stocks, respectively. The latter corresponds to an increase in mean litter size of approximately one third, from 9 to 12, for the ten generations. Six generations of intra-litter selection produced a little over half the response

obtained from mass-selection during the same period and from the same 4-way cross stock. This and a daughter-dam regression of  $+ .19 \pm .06$  obtained from the control stock indicate less maternal effect on litter size than reported by FALCONER (1963).

Selection for larger litters following superovulation produced no measurable increase in mean litter size or in proportion of females responding to treatment, in spite of the fact that the treatment permitted selection differentials nearly double those obtained in the absence of treatment. Performance in the absence of treatment of the stock selected for response to treatment was also not improved. It is suggested that the treatment affected the endocrine balance of the animals in such a way as to obscure genetic differences in reproductive characteristics.

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