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Analysis of quantitative inheritance of body size in mice

V. Effects of small numbers of polygenes on similar genetic backgrounds*

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1. INTRODUCTION

Conventional methods of obtaining statistical parameters of additive and non-additive genetic variances, such as the crossbreeding of strains or of breeds differing in a given trait, are practical for quantitative genetic studies but not sensitive enough where individual polygenes are concerned, especially when interactions of various orders are present and counterbalanced. From both the practical and academic points of view, experimental designs for obtaining knowledge of the individual effects of polygenes are greatly needed, since such knowledge is essential to quantitative genetics. The recent work of Rendel & Sheldon (1960) showed interaction of the Scute locus with polygenes affecting bristle number in *Drosophila*. Thoday (1961) located polygenes in short chromosome segments between genetic markers and studied their individual effects. As experimental approaches in polygenic analysis these methods are effective, giving clear-cut results.

Our design for studying the properties of polygenes affecting body size in mice comprised a breeding scheme (Fig. 1) divided into three major stages: conventional crossbreeding between two strains (*SM* and *LG*, highly contrasted in body size) (See Chai, 1956*a* for the history of development); repeated backcrossing with directional selection; and inbreeding with selection, the object being to introduce a small number of genes from one strain into another, to genetically fix them in the lines established, and then to study their effects individually.

The F_2 and first and second backcrosses to each parental strain indicated that differences between *LG* and *SM* mice were attributable to a large number of polygenes (Chai, 1956*b*). We proceeded therefore with repeated backcrossing to the seventh generation in the *SM* backcross line, and to the fifth in the *LG*. In the last two to three generations of *SM* backcrosses the line selected for large body size exceeded that selected for small body size by a mean difference of less than one gramme in body weight at 60 days. In the *LG* backcrosses practically no difference appeared between the selected lines in the last two generations (Chai,

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1961). These results raised some questions. Is there a real genetic difference in body size between the two backcrossed *SM* lines? Did some genetic mechanism prevent a response to selection in the *LG* backcrosses?

The data in this paper were obtained from the third stage of our design, that is, from the sublines produced by selection and inbreeding beginning from the last generation of each backcross; and although they may not furnish complete answers to the above questions, we are nevertheless confident that they show definite effects of individual polygenes and their order of magnitude.

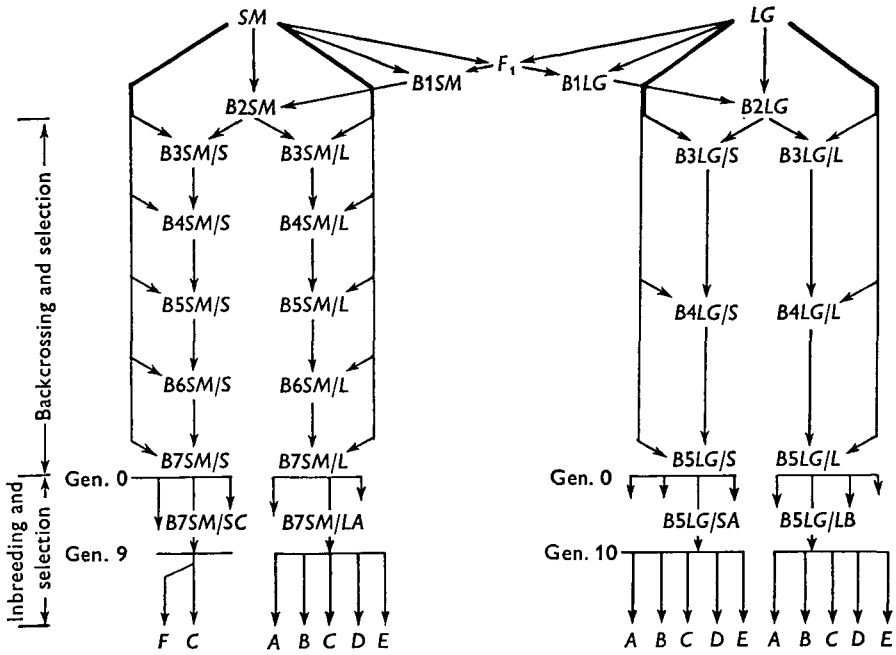

Fig. 1. The breeding scheme beginning by crossbreeding of *LG* and *SM* continuing with repeated backcrossing and selection, and then inbreeding with selection. See text for detailed explanation.

2. MATERIALS AND METHODS

The breeding scheme. To facilitate tracing the derivation of the inbred sublines from the last generation of backcrosses to the parental strains *SM* and *LG*, the breeding scheme is illustrated from the beginning (Fig. 1). Starting with the second backcrosses we selected for small as well as for large body size in each generation, and to eliminate maternal influence selection was applied to male parents only. Thus two lines were developed for each backcross, e.g. in the backcross to *SM*, one line was selected for small body size (*BnSM/S*) and the other large (*BnSM/L*), and similarly in the backcross to *LG* we developed *BnLG/S* and *BnLG/L*, where *n* is the number of generations of repeated backcrossing.

From the last generation of each backcross, that is, *B7SM/S*, *B7SM/L*, *B5LG/S* and *B5LG/L*, we started inbreeding by sib-mating, and continued with con-

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comitant selection for small and large body size of male parents only as during backcrossing; this generation is designated as the zero generation with reference to inbreeding and selection. In *B7SM/S*, for example, we selected for small, and in *B7SM/L* for large body size during inbreeding, thereby developing several sublines in each backcross. Three or four sublines were started at generation zero in each backcross, but all except one had to be discontinued due to various difficulties, including poor production. At the ninth inbred generation of *B7SM/L*, the fourteenth of *B7SM/S*, and the tenth of *B5LG*, sublines were again developed from a single subline. In the illustration each subline is designated by an arrow, with a letter assigned for identification. The number of inbred subline generations here reported varies from 13 to 24; some sublines were again extinct (see Figs. 1–5 for the number of generations in the sublines).

In the early generations five to ten pair-matings were set at the age of 60 days in each generation of each subline, the number being gradually increased to a minimum of ten as difficulties in maintaining the lines were encountered. Four to five litters per productive mating were usually obtained.

In the *B7SM* sublines Old Guilford mouse food was used throughout the generations, but in *B5LG* it was replaced after about the tenth generation with Purina Mouse Laboratory Chow. Percentages of crude protein, crude fat, and crude fibre for Old Guilford were respectively 19, 11, and 2; for Purina 23, 4·5, and 6.

3. RESULTS

Means. The means and variances of body weights at birth, weaning, and 60 days were computed for each generation of each subline, and plotted so that general trends within the sublines can be compared. We did not adjust the individual weights for litter-size difference for reasons discussed elsewhere (Chai, 1957). Sublines derived from the same parental backcross were plotted in the same chart, as, for instance, *B7SM/S* and *B7SM/L* (Figs. 2 and 3); and *B5LG/S* and *B5LG/L* (Figs. 4 and 5). For a few *B5LG* generations the means were not plotted because the numbers of weights available were so small. For example, in the *B5LG/S* three sublines were plotted for the females for the early generations but only two for the males, and also there are broken lines in the late generations for both the female and male plots.

The mean birth, weaning, and 60-day weights of *B7SM/S* mice were smaller than those of *B7SM/L*, and overlapped them in the early generations of selection and inbreeding (Figs. 2 and 3). The differences between the means became greater both as the mice grew older and as inbreeding advanced. There was a general trend toward increased body size at weaning and at 60 days, from the first through the ninth inbred generation.

An unexpected result was the direction taken by mean differences in the *B5LG* sublines: the ones selected for small body size had means larger than those selected for large body size. However, as in the *B7SM* sublines, separation of the means between the two subline groups increased as the mice grew older, and the 60-day

weights showed practically no overlapping at any time. Weaning and 60-day weights decreased after the ninth generation, particularly in the *B5LG/S* sublines.

Instead of presenting all the means and variances for all the generations, we summarized in Table 1 the averages for each generation for each line, giving also for the purpose of comparison the weight statistics of parental strains *SM* and *LG*

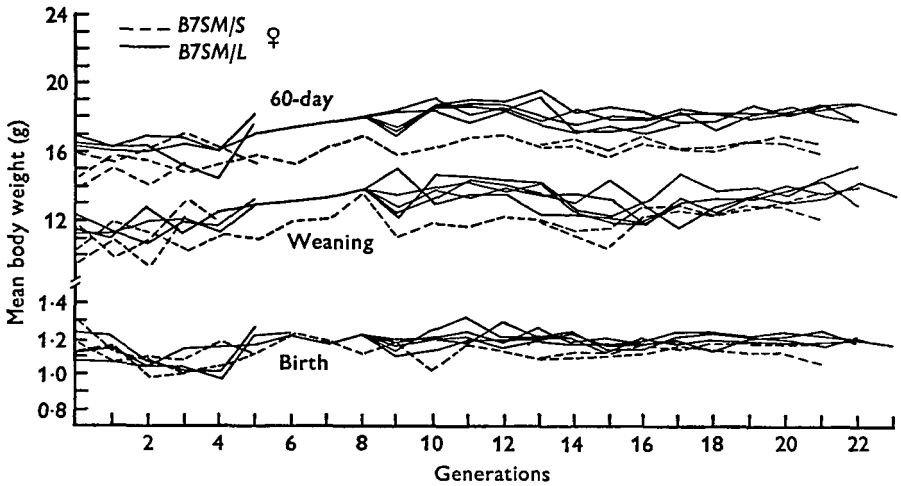

Fig. 2. Plots of the means of birth, weaning, and 60-day body weights in each inbred generation for females of each subline of the *B7SM/S* and *B7SM/L*.

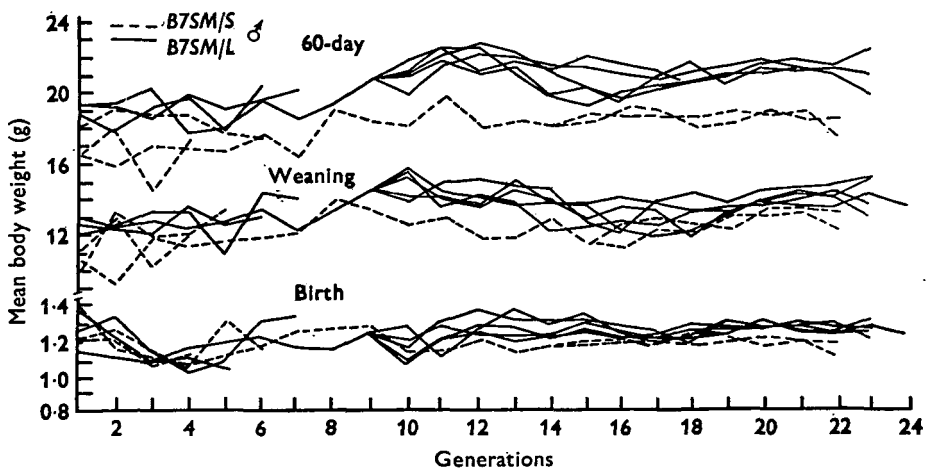

Fig. 3. Plots of the means of birth, weaning, and 60-day body weights in each inbred generation for males of each subline of the *B7SM/S* and *B7SM/L*.

over a period corresponding to that of the late subline generations. It will be noticed that the *SM* and *LG* means are comparable to those of *B7SM/S* and *B5LG/L* respectively, but that the variance in both parental strains are slightly smaller than those in their respective sublines.

4. DISCUSSION

Our query at the outset as to whether the difference between the *B7SM* sublines was truly genetic has been affirmatively answered by the fact that the means for the 60-day body weights of *B7SM/L* subline mice exceeded those of *B7SM/S* through practically all the inbred generations and their differences are significant.

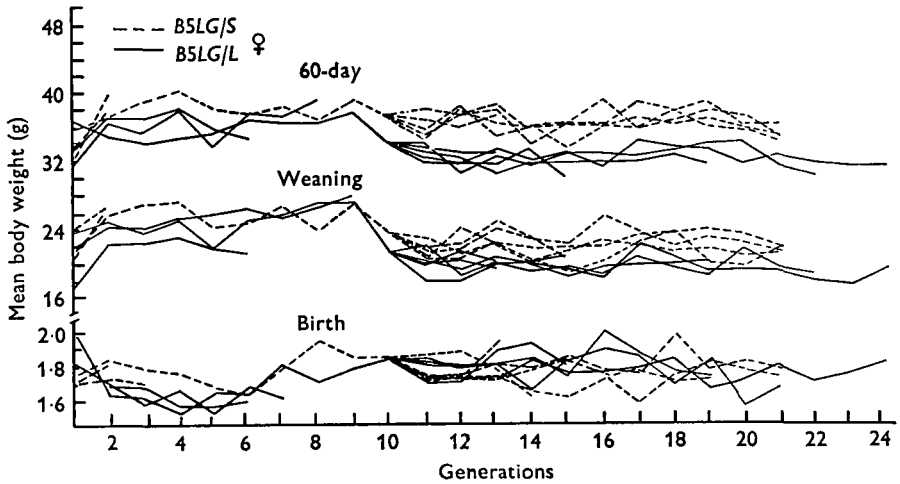

Fig. 4. Plots of the means of birth, weaning, and 60-day body weights in each inbred generation for females of each subline of the *B5LG/S* and *B5LG/L*.

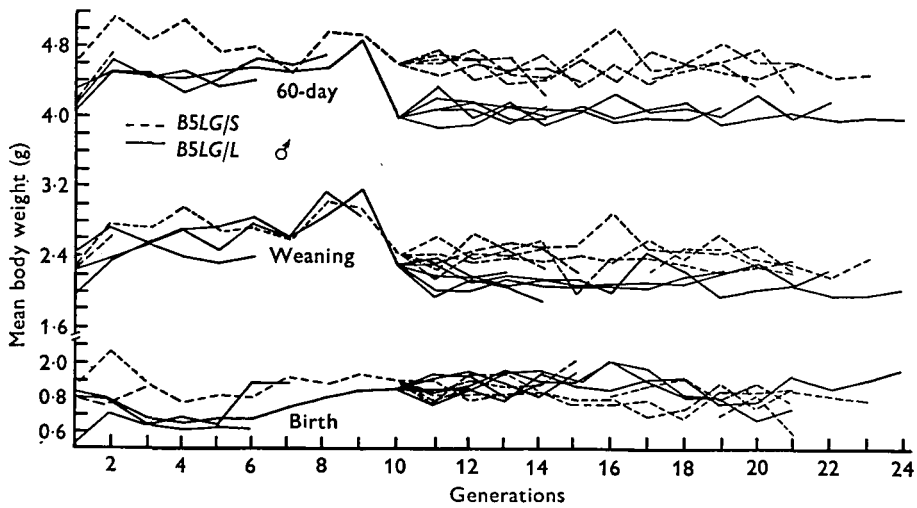

Fig. 5. Plots of the means of birth, weaning, and 60-day body weights in each inbred generation for males of each subline of the *B5LG/S* and *B5LG/L*.

This conclusion raises another question concerning the number of genes that would account for the difference between *B7SM/L* and *B7SM/S* mice. The maximum heterozygosity in the zero generation of *B7SM/L* (that is, in the seventh generation of repeated backcrossing (Chai, 1961)) was 50 % for the gene or genes

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introduced from *LG* mice. The mean difference of 0·7 g should be ascribed to no more than one-fourth of the effects of the *LG* genes and a minimum of $4 \times 0{\cdot}7 = 2{\cdot}8$ g which could be expected as the difference when all mice become homozygous at these loci, and when the gene effects are completely additive. We assume that the

Table 1. *The average means and total variances of birth and* 60*-day body weights in grammes for the early and late generations of the B7SM/S, B7SM/L, B5LG/S and B5LG/L mice*

For the purpose of comparison, the weight statistics of the parental strains *LG* and *SM* corresponding to the late generations of the sublines in time are given.

		Birth						60-day					
		Generations 1–9			Generation 10-up			Generations 1–9			Generation 10-up		
Sublines		No.	Mean	σ^2	No.	Mean	σ^2	No.	Mean	σ^2	No.	Mean	σ^2
B7SM/S	♀	417	1·10	0·015	2042	1·13	0·017	278	15·9	2·8	1239	16·3	3·4
	♂	379	1·15	0·016	2121	1·18	0·017	254	18·3	3·9	1228	18·8	4·8
B7SM/L	♀	484	1·10	0·018	5146	1·20	0·018	316	16·4	2·4	4251	17·9	3·9
	♂	469	1·16	0·020	4859	1·27	0·019	372	19·4	3·2	4196	21·3	6·4
SM	♀	—	—	—	307	1·13	0·11	—	—	—	177	16·2	1·9
	♂	—	—	—	391	1·18	0·010	—	—	—	196	18·4	2·6
		Generations 1–10			Generation 11-up			Generations1–10			Generation 11-up		
B5LG/S	♀	282	1·79	0·056	843	1·76	0·060	303	37·8	11·5	1128	36·4	8·8
	♂	357	1·86	0·053	1055	1·83	0·054	341	47·6	19·1	1327	46·0	14·8
B5LG/L	♀	480	1·65	0·044	1347	1·79	0·061	459	35·9	10·8	1911	32·1	7·8
	♂	550	1·70	0·052	1549	1·88	0·053	495	44·7	15·9	2056	40·6	12·6
LG	♀	—	—	—	276	1·74	0·030	—	—	—	349	33·0	6·9
	♂	—	—	—	307	1·87	0·034	—	—	—	393	41·2	8·1

genes introduced from *LG* after ten generations of inbreeding are close to complete fixation. The observed difference of about 2·1 g between the means of the sublines of *B7SM/L* in the late generations, and the *SM* means over a corresponding period, may be ascribed to the effects of the gene(s) from *LG* when homozygous. This value seems reasonable, considering that genes determining large body size have some dominance over those determining small body size (Chai, 1957). This difference, if not due to only one locus, seems unlikely to be due to much more than one.

An interesting phenomenon occurring in the *B7SM* line was the upward drift of the means to about the 10th generation, despite continual selection for small body size. It appeared to parallel our observations of the parental *SM* strain in the corresponding period (Chai, 1966). Environment may have been a contributing factor, but it seems that the pressures of natural selection outweigh those of artificial selection. A small amount of residual heterozygosity vital to fitness may have been left in the *B7SM/S* mice, with natural selection and inbreeding still .

operating at the involved loci. The high rate of neonatal and preweaning deaths, especially as observed in the *B7SM/S* sublines, may have been the mechanism by which natural selection functioned.

The results of simultaneous selection and inbreeding in the *B5LG* sublines do not fully explain the lack of response to selection during the repeated backcrossing to *LG*. Nevertheless, mice of the *B5LG/S* sublines selected for small body size turned out to be about 4 g heavier than those of the *B5LG/L* sublines selected for large body size, and also larger than those of the *LG* parental strain, thereby suggesting the following possible genetic mechanisms: a gene or a gene combination causing a large increase of body size may have been rapidly fixed by chance in a short period after the start of inbreeding, in view of the fact that the difference apparently was mainly due to increase of body weight in the *B5LG/S* mice during early generations (Figs. 4 and 5). Such a gene or genes, which must have been introduced from the *SM* mice, either merely exert an additive effect, or interact with the *LG* genetic background. Alternatively, the large body size of the *B5LG/S* sublines may be a consequence of heterosis, as the males which were selected for small body size were possibly homozygous like the *LG* males, while most of their mates, which were not selected, may have been heterozygous. Thus under this breeding procedure a large percentage of heterozygotes may have been maintained in each generation. A more satisfactory genetic explanation is being sought through more breeding tests.

As a minor point, a large drop in body weights in the *B5LG/L* sublines, but not in the *B5LG/S*, after the food had been changed from Old Guilford to Purina was evidently a consequence of genetic fixation of a qualitative difference in efficiency of food utilization between these sublines.

SUMMARY

Sublines carrying isolated polygenes determining body size, developed by selection and repeated backcrossing to the *LG* and *SM* strains of mice, were inbred by full-sib matings with concomitant selection to study the inheritance of body size, using birth, weaning (28-day) and 60-day body weights as indices.

Mean body weights in those sublines backcrossed to *SM* and selected for large body size were greater than in those selected for small body size, proving that the difference resulting from seven earlier generations of backcrossing and selection, although small, was genetic and could be fixed by inbreeding. The mean body weights of the sublines selected for small body size drifted upward despite downward selection, a phenomenon thought to be due to the pressure of natural selection outweighing that of artificial selection.

In the sublines developed from backcrosses to the *LG* strain, mice from lines selected for small body size attained a mean body weight greater than that of mice from lines selected for large body size and also than of the parental *LG* strain. These results were contrary to expectation and a genetic interpretation was offered.

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