Analysis of quantitative inheritance of body size in mice

IV. AN ATTEMPT TO ISOLATE POLYGENES*

BY C. K. CHAI†

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Large numbers and the weak action of individual units of polygenes usually involved in the inheritance of a quantitative genetic trait constitute basic difficulties in studying their effects. For these reasons the physical and biological properties of polygenes are poorly understood. In studying the effects of polygenes on body size we crossbred the Large and Small strains of mice which differ greatly in body size. Segregation of a rather large number of genetic factors was indicated in the F_2 generation (Chai, 1956). For a better understanding of the effects of polygenes, an attempt was then made to isolate a small number of them, introducing inheritance units from the Large to the Small mice and vice versa by repeated backcrossing and selection in two directions. The methods used and results obtained are the subject of this report.

MATERIALS AND METHODS

The data analysed in this paper are the body weights of 60-day-old mice from the Large and Small strains and their backcross generations. The development of these two strains has been reported elsewhere (Chai, 1956). At the time of the last backcross generations, mice of the parental strains, Small and Large, were at the twenty-fifth and fifteenth generations, respectively, of brother–sister mating. Selection for body size during the early development of these strains may have resulted in some increase in homozygosity in the loci determining body size. It is not likely that there may still be some residual heterozygosity in these loci in the Large mice as the H^2 locus does show some heterozygosity. Agglutination tests of blood cells with respect to the H^2 locus were made.‡ All the Small mice chosen for the test gave the same reaction while a very small portion of the Large mice reacted differently from the rest of them.

Backcrossing to the Large strain was carried to the fifth generation and to the Small strain to the seventh generation. During the course of backcrossing starting with the second generations selection was concurrently carried on by mating large-body-sized mice in one line and small-sized mice in another line in each backcross

to both Large and Small strains. Selection was started with the second backcross generation to the Large strain as well as to the Small strain. Therefore in the backcrosses to each parental strain, two 'paired' lines were formed. The breeding
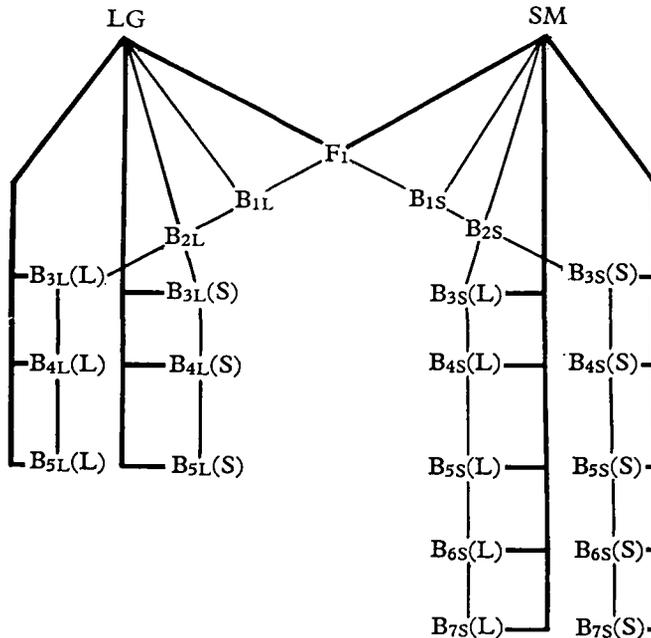

Fig. 1. Diagram of backcrossing and selection (see text for notations).

scheme is diagrammed in Fig. 1. The notations used in the diagram are given below:

LG = Large strain
SM = Small strain
$B_{1L}, B_{2L}, ..., B_{5L}$ = 1st, 2nd, ... 5th backcross generations to the Large strain
$B_{1S}, B_{2S}, ..., B_{7S}$ = 1st, 2nd, ... 7th backcross generations to the Small strain
(L) = mice in a generation selected for large body size
(S) = mice in a generation selected for small body size

For example, $B_{3L}(L)$ refers to the group of mice in the third backcross generation to Large, selected for large body size. This breeding scheme is designed to introduce single inheritance units determining body size from one strain to the other. Each unit may contain one polygene or several which are closely linked. By repeated backcrossing to the Small strain and selecting for large body size, single factors with plus effects might be introduced from the Large mice to the Small mice. Thus it might be possible to observe the effect of single inheritance units from the Large on the Small background. Backcrossing to the Small strain, combined with selection for small body size, tests whether there are factors with minus effects which are present in the Large but not in the Small strain.

(It is realized that in this breeding scheme genes with large additive effects and free segregation as well as highly inbred strains are desirable. Because dominance, genic interaction, linkage or mutations can cause complexity and hinder the progress of isolation, completely satisfactory stocks for this type of investigation may be hard to obtain. Nonetheless, observations on the effects of rather small numbers of, if not single, genetic units acting on relatively homogeneous genetic backgrounds, as in the present study, are considered worth while.)

The management of the mouse colonies remained the same throughout the period of the experiment, and was the same as in the earlier studies (Chai, 1957). Litter sizes larger than eight were reduced to eight. All mice were weaned at 4 weeks of age and were weighed at 60 days. Starting with the third generation, all of the female parents were from the parental strains. This practice made unnecessary the correction for maternal influence, a difficult process which can hardly be carried out adequately.

For convenience of comparison between all backcross generations and the parental strains raised in different years, the means and variances for the mice of the parental strains and the first and second backcross generations previously published (Chai, 1956, 1957) are re-listed.

RESULTS

The means and variances on actual and logarithmic scales for the Large mice and their backcrosses are given in Table 1 and those for the Small mice and their

Table 1. *Means and variances on actual and logarithmic scales for the Large mice and their backcrosses*

Genetic groups	No. of mice	$\bar{x}$	$\overline{\log x}$	s_b^2	s_w^2	$1000s_{\log x, w}$
LG (1954–5)	65	37·35	1·5678	8·36	3·92	0·49
(1956)	116	37·44	1·5707	10·71	6·25	0·86
(1957)	259	36·03	1·5524	16·77	8·96	1·30
(1958)	160	38·50	1·5855	10·68	6·07	0·77
F_1	161	25·82	1·4075	5·47	1·28	0·36
B_{1L}	164	31·59	1·5007	4·15	6·70	1·25
B_{2L}	203	35·30	1·5443	9·02	8·97	1·36
$B_{3L}(L)$	210	35·70	1·5499	9·08	7·33	1·08
$B_{3L}(S)$	98	35·80	1·5514	5·34	9·95	1·48
$B_{4L}(L)$	131	38·38	1·5822	7·78	5·90	0·75
$B_{4L}(S)$	149	36·83	1·5625	13·12	9·91	1·37
$B_{5L}(L)$	212	39·26	1·5926	3·46	6·40	0·79
$B_{5L}(S)$	122	40·32	1·5939	5·34	6·40	0·74

backcrosses in Table 2. With successive backcross generations, the mean body weights of the mice regress toward the mean of their respective parental strain with greater rates of increment and declination in the early backcross generations to Large and to Small mice, respectively (Fig. 2).

The expected means without selection were calculated on the basis of additive

effects of the polygenes, and were plotted in Fig. 2, beginning from the third backcross generation. (The calculations were made by adding the mean of the previous backcross generation to the mean for the parental mice produced in the same year and dividing by 2.) The means for the $B_S(S)$ did not show much difference from the means without selection, while the $B_S(L)$ did. The means of the B_L generations fluctuated.

Attention should be focussed also on the differences of the means from those of the parental strains and between the (L) and (S) groups in each generation of each backcross (Fig. 2). In the backcrosses to the Large, there is practically no difference from the parental strain in the groups either selected for large (L) or

Table 2. *Means and variances of* 60-*day body weights on actual and logarithmic scales for the Small mice and their backcrosses*

Genetic groups	No. of mice	x	$\overline{\log x}$	s_b^2	s_w^2	$1000s^2_{\log x, w}$
SM (1953–4)	39	14·0	1·1434	0·74	0·65	0·62
(1955–6)	144	14·6	1·1623	0·52	1·00	0·89
(1957)	260	15·1	1·1775	1·33	1·14	0·94
(1958)	110	15·9	1·1961	3·12	1·53	1·15
F_1	161	25·0	1·3943	5·47	1·28	0·36
B_{1S}	154	19·4	1·2858	4·25	4·21	1·84
B_{2S}	158	18·6	1·2715	1·02	2·97	1·57
$B_{3S}(L)$	110	17·0	1·2258	2·70	2·70	1·33
$B_{3S}(S)$	57	15·8	1·1971	2·35	0·80	0·60
$B_{4S}(L)$	185	15·7	1·1923	0·81	2·06	1·58
$B_{4S}(S)$	55	15·4	1·1848	0·98	1·05	0·83
$B_{5S}(L)$	253	16·4	1·2119	0·64	1·64	1·15
$B_{5S}(S)$	122	15·4	1·1858	0·65	0·93	0·74
$B_{6S}(L)$	241	16·3	1·2114	1·13	1·24	0·87
$B_{6S}(S)$	228	15·1	1·1778	1·17	1·24	1·02
$B_{7S}(L)$	145	16·8	1·2218	1·30	1·54	1·03
$B_{7S}(S)$	64	16·1	1·2056	0·89	0·89	0·65

selected for small (S) body size after the second backcross generation (B_{2L}). Comparing the means between the (L) group and the (S) group in each backcross generation the differences were not in the expected order with respect to selection. However, in the Small backcrosses the means of the B_{2S}'s are much greater than that of their parental strain, SM. Although the mean of $B_{3S}(S)$ appeared no different from that of SM, the mean of $B_{3S}(L)$ is considerably greater than that of SM. In succeeding backcross generations, the means of the (L) groups continue to be larger (except B_4) than those of the corresponding (S) groups in each generation through the B_7, and also to be larger than that of the parental strain.

The means of the Small mice indicate a gradual, although slight, increase in body weights with lapse of time and advance of inbreeding. This tendency is not shown in the Large strain. The means of both the Large and Small mice for the year 1958 were greater than the respective means for the previous years. This may be considered due to an environmental effect.

Variances between litters and within litters in the parental strains as well as in the backcross generations are given in Tables 1 and 2 and illustrated in Figs. 3*a* and 3*b* on logarithmic scales. The within-litter variance (s^2) is considered to be the better estimate for a comparison of the genetic differences between the backcross generations and the parental strains, and the litter mean variance (s_b^2) is considered to be the better indication of environment effects on animals of the different genotypes, or of genotype-environment interaction between the genetic
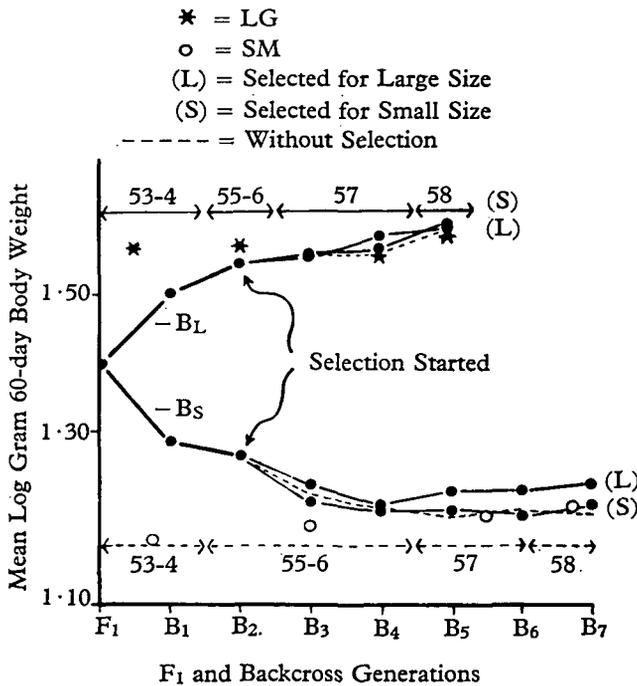

Fig. 2. Means of 60-day body weights on log scale for mice in the parental strains, F_1 hybrid and the backcross generations. The correspondence in the year of birth between the Small mice and their backcrosses is given by dotted lines and that for the Large mice and their backcrosses by solid lines.

groups (see Chai, 1956). Therefore, for comparison between the genetic groups, s_w^2 is used for evaluation of genetic differences and s_b^2 for variation in response to environment (genotype, environment interaction). (It is realized that there is a genetic component in the s_b^2 which varies with the number of heterozygous loci and differences between loci in each group, or both, but for the data presented here, variation from this source is relatively small in comparison with that from the environment.)

There may be a slight tendency for the within-litter variance to decline with the advance of backcrossing in (L) groups in the B_L generations, but this tendency may be somewhat overshadowed by the great fluctuation of the variances between years, as shown in the LG. In the B_{3L} and B_{4L}, the variances appear to be greater

in the (S) groups than in the (L) groups, but in the B_{5L} generation the variances in the two groups are similar in magnitude. In the B_S generations, the differences in variances between generations and between the (L) and (S) groups are unlike those in the B_L. The variance declines with successive backcrossing in the (L)
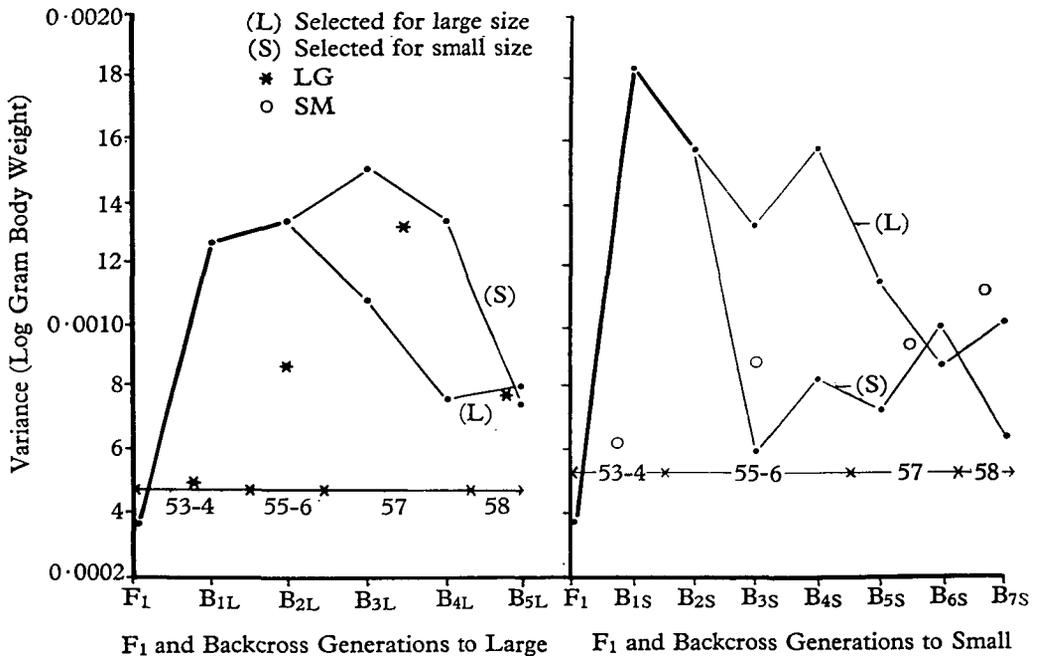

Fig. 3a. Variances of 60-day body weights in logarithm for mice of the Large strain and their hybrid generations. The correspondence in the year of birth between the Large mice and those in their hybrid generations is given by the solid lines with arrows.

Fig. 3b. Variances of 60-day body weights in logarithm for mice of the Small strain and their backcross generations. The correspondence in the year of birth between the Small mice and mice in their hybrid generations is given by the solid lines with arrows.

groups but not in the (S) groups. Furthermore, in comparison with SM, the variances in the (L) groups are fairly large, while those in the (S) groups are small and distributed within the lower range of the variance for the SM.

DISCUSSION

The results with respect to the distribution of means and variances in the backcross generations seem to support the earlier interpretation that genes responsible for large body size are dominant over those for small body size (Chai, 1957). In the means, a differential remained between SM and the advanced generations of $B_S(L)$, but not between LG and $B_L(S)$, and a differential persisted between the (L) and (S) groups in the B_S but not in the B_L groups. It is evident that selection had no effect in the backcrosses to LG. This can fit with the dominance hypothesis

in that the heterozygotes are hardly distinguishable phenotypically from homozygous dominants but are distinguishable from homozygous recessives.

The decrease in variation following the advance of backcrossing in the $B_S(L)$ indicates a gradual reduction in the number of the genes for large body size introduced from the Large strain. In the $B_S(S)$ the variance did not decrease beyond the third generation. This may be easily explained by the fact that from the third generation the $B_S(S)$ mice were genetically very similar, as far as body size was concerned, to the parental Small mice, indicated by the similarity of means. The variances of the Large backcrosses were entirely different. There was no definite trend, other than random variation, which is probably a reflection of environmental effects and possibly of sampling errors. Although the number of minus genes from the Small strain was expected to decline proportionately in each backcross generation, the variances did not indicate this tendency. This could be explained by the effect of dominance of the plus genes over the minus genes and selection, even if no other factors were involved.

The interpretation of dominance of genes with plus over those with minus effects is in agreement with the results obtained by others (MacArthur, 1944; Falconer & King, 1953). It is, however, not fully justified in view of the fact that the mean of the F_1 hybrids was not much greater than the mid-parent value. The overall situation seems to suggest that the relative effects of dominance and additivity of the polygenes may not be constant within the range of genotypes. In other words, the interactions between genes are not additive with respect to the number of participating genes. It may also be that the Large genes have a different magnitude of dominance.

Clear-cut conclusions certainly cannot be made with respect to isolation of genetic determinants on the genic level on the basis of the data presented. It may be tentatively stated, however, that single, or rather small numbers of, inheritance units have been introduced from the large mice to the small mice as shown by the larger size of $B_7 {}_S L$ over SM. More definite conclusions possibly will be reached by establishing inbred lines from the last backcross generations, crossing between these lines and transplanting tissues between and within them; such studies are in progress.

SUMMARY

The isolation of single inheritance units affecting body size in mice has been attempted. By using Large and Small mice as the parental strains, a breeding scheme has been carried out by repeated backcrossing to each strain with selection for both large and small body sizes in each backcross. The selections were started from the second backcross generations. The Small backcross was carried to the seventh generation and the Large to the fifth. Based on analysis of the means and variances for the parental strains and the backcross generations, it is tentatively concluded that a small number of, if not single, inheritance units may have been introduced from the Large to the Small mice. The 'large' genes appear to be dominant over the 'small' genes.

REFERENCES

Chai, C. K. (1956). Analysis of quantitative inheritance of body size in mice. I. Hybridization and maternal influence. II. Gene action and segregation. *Genetics,* **41,** 157–178.

Chai, C. K. (1957). Analysis of quantitative inheritance of body size in mice. III. Dominance. *Genetics,* **42,** 602–607.

Falconer, D. S. & King, J. W. B. (1953). A study of selection limits in the mouse. *J. Genet.* **51,** 561–581.

MacArthur, J. W. (1944). Genetics of body size and related characters. I. Selection of small and large races of the laboratory mouse. *Amer. Nat.* **78,** 142–157.