

ANALYSIS OF QUANTITATIVE INHERITANCE OF BODY SIZE IN MICE.
I. HYBRIDIZATION AND MATERNAL INFLUENCE¹

C. K. CHAI

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Received April 14, 1955

ABOUT 40 years ago in his extensive experiments with hooded rats, CASTLE (1916) demonstrated that by selective breeding the average grade of coloration could be carried far beyond the limits of variation of the foundation stock. He was among the first to prove that selection could produce permanent change in quantitative characters. CASTLE's results on quantitative variation have stimulated investigation in different fields of genetics which continue to the present day. Contributions concerning gene action as well as estimation of number of factors affecting quantitative characters have been derived from both *Drosophila* and plant experiments.

A few extensive studies with laboratory mammals have achieved remarkable success, in spite of the difficulties in obtaining a large amount of well controlled genetic material, the longer periods of breeding required and the complicated environmental effects encountered. In three crosses between races of rabbits having different mean body weight, CASTLE (1921) estimated the minimum number of segregating factors as 3, 14 and 22. WRIGHT (1934) found a minimum of 4 segregating units in a cross between a 4-toed and 3-toed strain of guinea pigs. GREEN (1950) reported at least 3 factors involved in a cross between two strains of mice with different number of lumbar vertebrae. All these estimates were minimum and based on rather restricted assumptions.

Selection experiments for large and small body size in mice have been successful. Both GOODALE (1941) and MACARTHUR (1944) have separately established relatively large-bodied strains of mice by continuous selection. MACARTHUR also established a small-bodied strain. From mouse populations (originated from MACARTHUR's stock) of large, medium and small body size, WARWICK and LEWIS (1953) demonstrated that selection for large and small 60-day weight through five generations was effective, both in populations in the process of inbreeding and in outbred. The same authors (1954) reported in a cross-breeding experiment that the body size of most of the progenies were intermediate between the parental types. FALCONER (1952) showed again the effectiveness of selection by starting with entirely different foundation stocks. The latter has estimated at least 19 pairs of genes involved in his experiment and at least 54 in MACARTHUR's. He also concluded that the genes acted on a geometric scheme.

The current study is based on crosses between mouse strains with different body sizes, which ranged from extremely low to extremely high. Only results based on the data of pure strains and their F_1 hybrids are given in this part. Analysis based on the data of the F_2 and the backcrosses is still incomplete and will be given later. The

¹This investigation was supported by a research grant C-1074(C4) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

object of the experiment was to study the relative magnitude of genetic and maternal influence and to locate any effects of hybridization on body size which may occur in mice.

MATERIALS AND METHODS

Five strains of mice were used in this investigation, namely LG (Large, GOODALE), BALB/cAnd, C57BR/cd, DBA/1 and SM (Small, MACARTHUR), and the four F_1 hybrids from the crosses between LG and each of the other four strains. The following symbols were used to designate the different parental strains and F_1 hybrids throughout the paper.

| Parental strains | F_1 hybrids |
|------------------|-----------------------------------|
| L—LG | CL— F_1 from C ♀ × L ♂ |
| C—BALB/cAnd | LC— F_1 from L ♀ × C ♂ |
| B—C57BR/cd | and similarly for the other F_1 |
| D—DBA/1 | hybrids. |
| S—SM | |

The mice in this experiment were bred at or shortly after 60 days of age. Each breeding pair was kept in a separate pen, and was checked for newborn offspring every morning. All young were weaned when they were 28 days old. Female and male offspring were kept in separate pens with a maximum of six in each pen. Body weight was taken at birth, weaning, and 60 days of age. It has been shown that the growth curve of a mouse has reached a plateau after 60 days of age (BUTLER 1950). Variations caused by environmental factors, i.e. changes in health and fat deposition are likely to occur thereafter. Hence, for this study the 60-day body weight is considered as a reliable estimate of adult body size. The matings were started in September 1953 and were ended in June 1954. Both pure bred and cross bred matings were kept parallel in order to make concurrent observations. The 725 mice used in this study include 259 pure strain and 466 F_1 hybrids. The number of animals involved in each strain and cross is given in table 1. The relative body sizes are shown in plate 1.

As far as the history of these pure strains is concerned, strain BALB/cAnd, C57BR/cd and DBA/1 are long established inbred strains and need no description.

TABLE 1

Means (\bar{x}), standard deviations (s), coefficients of variation (s/ \bar{x}) of body weight in the pure strains and the F_1 's, mid-parent means, and the potence ratio of the F_1 's

| Pure strains | | | | | F_1 hybrids | | | | | | Potence | |
|---------------|----|-----------|-----|--------------|---------------|-----|-----------|-------------|-------------|-----|---------|--------------|
| Geno- type | N | \bar{x} | s | s/ \bar{x} | Genotype | N | \bar{x} | \bar{x}_a | \bar{x}_g | s | | s/ \bar{x} |
| S | 41 | 13.6 | 1.4 | 10.3% | LS + SL | 161 | 25.8 | 25.5 | 22.6 | 2.4 | 9.3% | .028 |
| D | 31 | 18.6 | 1.7 | 8.4% | LD + DL | 93 | 28.3 | 28.0 | 26.3 | 2.9 | 10.1% | .038 |
| B | 34 | 20.5 | 1.3 | 6.5% | LB + BL | 102 | 29.4 | 29.0 | 27.7 | 1.5 | 5.2% | .055 |
| C | 88 | 21.0 | 1.5 | 7.2% | LC + CL | 110 | 29.3 | 29.2 | 28.0 | 1.7 | 5.8% | .021 |
| L | 65 | 37.4 | 3.3 | 8.9% | | | | | | | | |

\bar{x}_a — mid-parent (arithmetic).

\bar{x}_g — mid-parent (geometric).

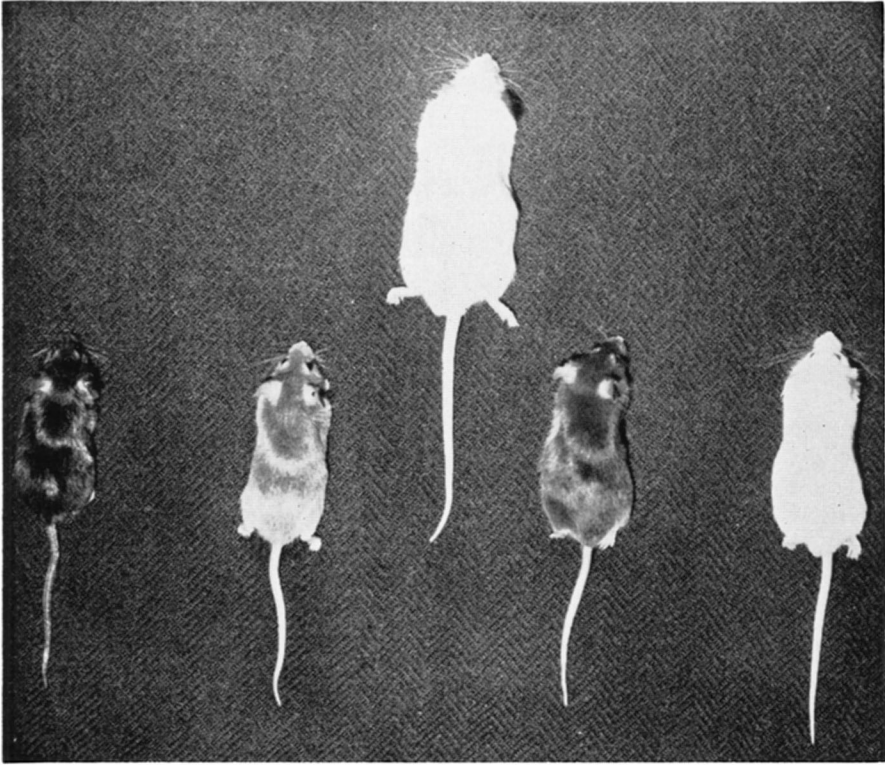


PLATE 1.—Male mice of the five pure strains. One on the top is LG. In the second row from left to right are SM, DBA/1, C57BR/cd and BALB/c.

However, the Large and Small strains are relatively new and up to the start of this experiment have passed through several methods of breeding. The Large strain was originated by DR. H. D. GOODALE before 1937. He selected many generations for large body size. Detailed information is not available concerning the size of the stock subjected to selection and the number of generations involved. In 1948 some of these Large mice were sent to DR. M. N. RUNNER of the Jackson Laboratory. They were sib-bred for four or five generations and then in an attempt to increase vigor interfamily matings were made. Sib breeding was resumed for three generations before the onset of this experiment. Because of lack of detailed information before they entered the Jackson Laboratory it is impossible to calculate the coefficient of inbreeding. It is probable that through the repeated processes of interbreeding, crossing between families and inbreeding a considerable amount of heterozygosity may well have been reduced in these animals.

The Small strain, originated by MACARTHUR by selection for small body size, had undergone 26 generations of selection at the time of its arrival at the Jackson Laboratory. Thereafter, under DR. RUNNER, it has been continuously inbred by brother-sister mating. The Small mice used in this experiment had been brother-sister bred for at least 12 generations. They were all descendants from a single pair of ancestors

at the tenth generation of brother-sister mating. The coefficient of inbreeding was estimated about 1-3% increase each generation under MACARTHUR (1944). Therefore, there is little doubt that the value of inbreeding coefficient of the Small mice was brought not far from 100% at the time when they were assigned to the experiment.

The coat color of the Large mice is albino, and their genotype is *aa cc*. They are relatively long bodied and long tailed and not very fat. The hair of the Large mice is coarse, long and loosely attached. They are susceptible to external parasites, and infections of the skin of the ears and face are frequent. They are tame and slow in action. The coat color of the Small mice is black or agouti, since the strain is maintained by forced heterozygosity (*aa* × *Aa*) at the agouti locus. Small mice are quite active but not timid.

RESULTS AND ANALYSIS

Genetic considerations

The 60-day body weight of mice in each strain and their F_1 hybrids are given in table 1. The average mid-parental body weight for each F_1 hybrid has been calculated on the geometric as well as the arithmetic scale and is given in the same table. The potence ratio was computed for each cross according to MATHER (1949) by applying the following formula:

$$\frac{F_1 - P_m}{|P - P_m|}$$

where P_m refers to mid-parent and P can be either parent. The potence ratio might be interpreted as an estimate of the dominance value for the F_1 involving the two parents under the dubious assumption that loci effects are isodirectional and the dominance increments of genes have the same sign and are constant.

It has been found that there was significant difference in body weight between male and female mice. In order to have equal representation of weights for males and females, the mean body weight of each genotype was obtained by averaging the mean of the males with that of the females. The same procedure was employed for computing the standard deviations. According to the distribution of the means of the pure strains, the Small and Large strains are at the two extremes, while the other strains are intermediate in range. The F_1 means have the same sequence as their parent strains except for the means of LB + BL and LC + CL which are very close in magnitude. This is probably due to the fact that there is no significant difference in body size between the two parent strains, B and C.

A comparison of the F_1 means with their mid-parental means on the arithmetical scale can be made in the graph illustrated in figure 1. In this graph, the intersects of the lines joining the Large and each of the other strains with the center line are the arithmetic means of the mid-parents. The points on the center line are the observed means of the F_1 hybrids, and are slightly above their respective mid-parental values. These results may be interpreted to mean that the net effect of favoring large body

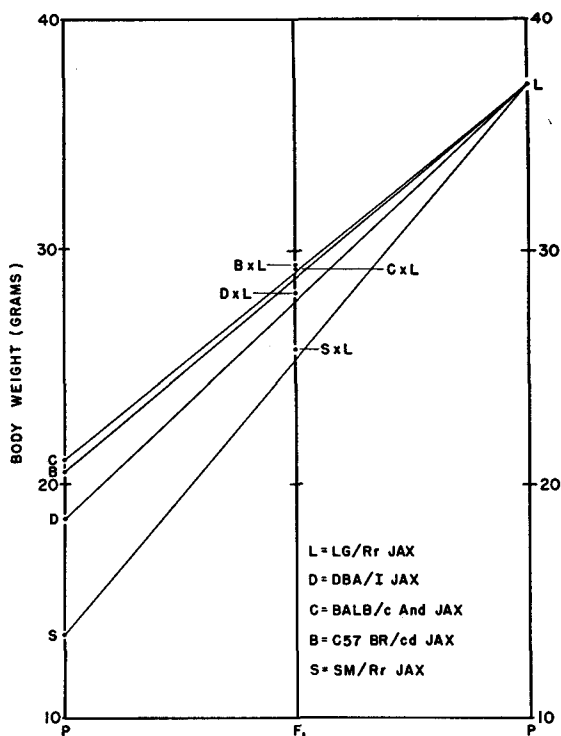


FIGURE 1.—A graph illustrating body weight of the F_1 hybrid in relation to the mid-parent. The intersects of the lines LS, LD, LB and LC with the center line are the mid-parent values, and the points on the center line are the observed means of each of the F_1 hybrids.

size in the Large strain is slightly dominant over the genes in the other strains. This finding may also be explained as due to hybrid vigor in favor of large size.

It has been demonstrated that in plants (red pepper, KHAMBANONDA 1950) (tomato, POWERS 1941) and animals (mouse, MACARTHUR 1944 and FALCONER 1953) that genes involved in quantitative characters are more likely to act geometrically than arithmetically. Table 1 shows that each F_1 hybrid is above its mid-parental value on either an arithmetic or a geometric scale, with the departure of the F_1 means from the geometric means of the parents further than that from the arithmetic means of the parents. However, the standard deviations showed that the strains of larger size had larger standard deviations. Furthermore, all the standard deviations of the F_1 hybrids, especially in the crosses of L with S and D, were larger than those of their respective parental strains, C, B, D and S. This is true in spite of the greater expected constancy (due to homeostasis) of the F_1 hybrids as compared to that of the pure parental strains. When correlating standard deviations with their means there appear quite large random errors perhaps due to the small sample size. Nevertheless, the values fell approximately in line on a geometric scheme, which was considered as the appropriate scale in the previously cited studies.

The potence ratios, although not identical between different crosses, are probably not significantly different. The average potence for all is 3.7%.

Variation within any of the isogenic populations or their F_1 hybrids is generally regarded as environmental. ROBERTSON and REEVE (1952) demonstrated that in *Drosophila* the variation in the F_1 's was less than in the parental strains. The authors explained this as due to the better adaptability of the F_1 than that of the homozygous individuals. It ought to be mentioned that some environmental factors, causing variation in mammals are quite different from those in *Drosophila*. Factors such as litter size, age of the mother, uterine environment and nursing are important in mammals and almost non-existent in insects. In mice, GRÜNEBERG (1954) suggested that in some polygenic characters the homozygous state tends to be less stable developmentally than the heterozygous one. McLAREN and MICHIE (1954) showed F_1 hybrid mice were less variable in response to bioassay than the inbred lines. Table 1 gives the coefficient of variation in body weight for each F_1 hybrid and each pure strain. Although they vary considerably in the F_1 's and in the pure strains, the average coefficient of variation of the F_1 's appears smaller than that of the pure strains. This seems to be in accord with the findings of the other different investigators.

Maternal influence considerations

As each F_1 hybrid was produced by a mother from one of two different strains, a comparison can be made of the magnitudes of the influence of genetic constitution and maternal effect upon body size. In table 2, the average body weights of the males and the females are given for each F_1 hybrid. The data show that sex, genotype and maternal influence play important roles in determining body size in mice. Analysis of variance was employed in order to find the relative importance of each. If all sources of variation were considered and were carried in one analysis of variance, a three-way classification would be required. In addition, since there would be unequal numbers of animals in the subgroups, the analysis would be a complex nonorthogonal case. However, as maternal and genotypic effects are the essential factors to be considered, the analysis will be made much simpler by separating the data into two parts according to sex. Hence, the analysis has been carried out following the method of disproportionate subclass numbers (outlined by SNEDECOR 1946) in a two-way classification involving maternal parent and genotype only. The complete results of the analysis of variance will not be given to save space. Only the absolute values and the

TABLE 2

Mean body weight of males, females and their average of each F_1 hybrid with different maternal parent

| Mothers other than L | | | | L mothers | | | |
|----------------------|----------|----------|----------|-----------|----------|----------|----------|
| Genotype | Males | Females | Average | Genotype | Males | Females | Average |
| SL | 26.8±2.3 | 22.1±1.7 | 24.5±2.0 | LS | 29.1±2.9 | 25.2±2.9 | 27.2±2.9 |
| DL | 28.8±3.4 | 22.8±3.5 | 25.8±3.4 | LD | 34.1±2.5 | 27.6±2.3 | 30.9±2.4 |
| BL | 31.2±2.1 | 25.4±1.0 | 28.3±1.5 | LB | 34.0±2.5 | 27.1±1.1 | 30.6±1.6 |
| CL | 31.4±1.5 | 25.5±1.4 | 28.4±1.5 | LC | 33.5±2.4 | 26.9±1.7 | 30.2±2.0 |

TABLE 3

Sources of variance in body weight of F₁ hybrid females and males attributed to maternal, genetic, maternal × genetic and individual

| Sources of variation | F ₁ females | | | F ₁ males | | | % of total variance |
|----------------------|------------------------|--------------|----------|----------------------|--------------|----------|---------------------|
| | Degrees of freedom | Mean squares | Variance | Degrees of freedom | Mean squares | Variance | |
| Maternal | 1 | 417.9** | 2.6 | 1 | 505.8** | 4.2 | 26.3 |
| Genetic | 3 | 133.2** | 1.5 | 3 | 320.5** | 5.1 | 23.2 |
| G × M | 3 | 27.6** | 0.7 | 3 | 26.3** | 0.7 | 5.6 |
| Individual | 224 | 4.7 | 4.7 | 218 | 6.7 | 6.7 | 44.9 |

** $P < .01$.

percentages of the variances attributed to each component, maternal, genetic, maternal × genetic and individual sources, are given in table 3.

The maternal and genetic effects are both significant with probability below 1%. The source of interaction between genetic and maternal is significant with probability at a level about 1%. The variances attributed to each source take the following sequence in regard to magnitude:

Individual > Maternal > Genetic > Maternal × Genetic

The variance attributed to individual is due to environment other than maternal. It is interesting to see that when the sexes are averaged the maternal influence contributes more than one quarter of the total variation, slightly more than does the genetic constitution.

SUMMARY

A scheme for cross breeding an extremely large bodied strain of mouse with four other strains ranging from a medium to extremely small body size was carried out.

The mean body weight of each F₁ hybrid was shown to be slightly above the arithmetic mean of the mid-parent, and more above the geometric mean of the mid-parent. The calculated potence ratio (3.7%) was very low. In general, the standard deviation of both the pure strains and F₁ hybrids tended to be proportional to the means. Therefore multiplicative gene action is suggested.

The maternal influence on body size in the F₁ hybrids was estimated to contribute more than one quarter of the total variation, a larger source of variation than was the genetic constitution of the hybrids.

LITERATURE CITED

- BUTLER, L., and J. D. METRAKOS, 1950 A study of size inheritance in the house mouse. I. The effect of milk source. *Can. J. Research* **28**: 16-34.
- CASTLE, W. E., 1916 Carnegie Inst. Washington, Publ. No. 241, pp. 163-187.
- 1921 An improved method of estimating the number of genetic factors concerned in case of blending inheritance. *Science* **54**: 223.
- FALCONER, D. S., 1953 Selection for large and small size in mice. *J. Genet.* **51**: 470-501.
- GOODAL, H. D., 1941 Progress report on possibilities in progeny test breeding. *Science* **94**: 442-3.

- GREEN, E. L., 1951 The genetics of a difference in skeletal type between two inbred strains of mice. *Genetics* **36**: 391-409.
- GRÜNEBERG, H., 1954 Variation within inbred strains of mice. *Nature* **173**: 674.
- KHAMBANONDA, IAN, 1950 Quantitative inheritance of fruit size in red pepper (*Capsicum frutescens* L.). *Genetics* **35**: 311-343.
- LEWIS, W. L., and E. J. WARWICK, 1953 Effectiveness of selection for body weight in mice. *J. Heredity* **44**: 233-238.
- MACARTHUR, J. W., 1944 Genetics of body size and related characters. I. Selection small and large races of the laboratory mouse. *Am. Naturalist* **78**: 142-157.
- MATHER, K., 1949 *Biometrical Genetics*. Dover Publications, Inc.
- MCLAREN, A., and D. MICHIE, 1954 Are inbred strains suitable for bioassay. *Nature* **173**: 686.
- POWERS, L., 1941 Inheritance of quantitative characters in crosses involving two species of *Lycopersicon*. *J. Agr. Research* **63**: 149-174.
- ROBERTSON, F. W., and E. C. R. REEVE, 1950 Heterozygosity, environmental variation and heterosis. *Nature* **170**: 290-293.
- SNEDECOR, G. W., 1946 *Statistical Methods*. The Iowa State College Press.
- WARWICK, E. J., and W. L. LEWIS, 1954 Growth and reproductive rates of mice. *J. Heredity* **45**: 35-38.
- WRIGHT, S., 1934 An analysis of variability in number of digits in an inbred strain of guinea pigs. *Genetics* **19**: 506-536.