THE GENETICS OF A DIFFERENCE IN SKELETAL TYPE BETWEEN TWO INBRED STRAINS OF MICE (BalbC AND C57blk) *

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THE axial skeleton of the house mouse is composed in part of various combinations of 12, 13, or 14 thoracic (*i.e.*, rib-bearing) and 5, 6, or 7 lumbar vertebrae. Only the 12/5 and 14/7 combinations have not been observed. Inbred strains of mice differ with respect to the incidence of the various skeletal types. The 13/6 type occurs in about 98 percent of the mice in the C57blk strain. The 14/5 type occurs in about 50 percent of the mice of the BalbC strain, the remaining 50 percent being 13/6, 14/6, and some other types.

The variations in skeletal type invite the study of two problems. The first is an analysis of the cause or causes of variations within a highly inbred strain or within the F_1 between two inbred strains. Such variations must be non-genetic, or at least this appears to be so in the BalbC strain except for a sex difference (GREEN 1941). The second problem is an analysis of the basis for the differences between highly inbred strains. Such differences are presumably genetic, but the kind and number of genes are not known. This paper deals with the second problem. On the basis of the results of matings between the BalbC and C57blk strains, it was found that with some simplifying assumptions at least three pairs of genes are needed to account for the differences in skeletal type. These pairs of genes appear to assort independently of the *A/a* (non-agouti) and *C/c* (albino) loci, but may be linked with the *B/b* (brown) locus.

PLAN OF EXPERIMENT AND DESCRIPTION OF STRAINS

Mating plan. In matings the strain or type of the sire will be given first, as (BalbC × C57)♀ means BalbC ♂ mated with a C57blk ♀. For easier notation, C57 will stand for C57blk.

Matings were made reciprocally between the two strains, there being 4 BalbC sires and 9 C57 dams in matings of the type (BalbC × C57) and 6 C57 sires and 15 BalbC dams in matings of the type (C57 × BalbC). The BalbC sires were brothers of the BalbC dams, and the C57 sires were brothers of the C57 dams. From the (BalbC × C57) mating, 9 male and 43 female offspring in 9 litters were raised to produce an F_2 and two backcross generations. From the (C57 × BalbC) mating, 5 male and 33 female offspring in 9 litters were raised to produce another F_2 and two backcross generations. The backcrosses

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were in each case produced by mating F_1 females to their own fathers or to their uncles, with the exception that three C57 sires had to be replaced by C57 sons in the latter part of the experiment. An effort was made to mate each F_1 female to her brother, her father, and her uncle in random rotation through 6 matings. This was accomplished in general, but there were several exceptions caused by the death of one of the appropriate mates prior to completing the mating tour.

The matings may be summarized as:

To produce P_1: (C57 × C57),
To produce P_2: (BalbC × BalbC),
To produce F_1: (BalbC × C57) and (C57 × BalbC),
To produce F_2: (BalbC × C57) × (BalbC × C57) and (C57 × BalbC) × (C57 × BalbC),
To produce B_1: (C57 × (BalbC × C57)) and (C57 × (C57 × BalbC)),
To produce B_2: (BalbC × (BalbC × C57)) and (BalbC × (C57 × BalbC)).

Duration of experiment. The matings were in progress over a period of 17 months, from May of one year to November of the following year. Records on the BalbC and C57 strains, the F_1, F_2, B_1, and B_2 were taken continuously during this time with the exceptions that the first segregating litters (F_2, B_1, B_2) appeared in March of the second year, and that some litters of both pure strains born prior to this time were used to increase the sample sizes. Upon test, it appeared that no heterogeneity was being introduced into the records by doing so.

The BalbC strain. The BalbC mice used in matings with the C57 mice were in the 49th to 52nd generation of brother × sister inbreeding and were all descended from a single pair in the 45th generation. This is a group designated as BalbC, subline 31, in the OHIO STATE UNIVERSITY colony. It is derived from a single pair obtained from the JACKSON MEMORIAL LABORATORY in 1937. In previous publications, this strain has been referred to as Bagg albino (GREEN 1941, GREEN and GREEN 1946). Following the usage in Mouse Genetics News No. 2 (LAW 1948), the strain is designated BalbC.

The BalbC mice used for comparison with the C57 mice and their hybrids consisted of 67 litters in the 49th to 55th generation of inbreeding. In this sample, which contained 426 mice, 11 percent were 13/6 in skeletal type, 54 percent 14/5, 18 percent 14/6, and 17 percent were of other types (table 1).

The C57blk strain. The C57 mice used in matings with the BalbC mice were in the 33rd to 35th generation of brother × sister inbreeding and were all descended from a single pair in the 30th generation of C57blk, subline 4, obtained from the JACKSON MEMORIAL LABORATORY in 1942.

The C57 mice used for comparison with the BalbC mice and their hybrids consisted of 77 litters in the 31st to 36th generation of inbreeding. Of 463 mice in the sample, about 98 percent were 13/6 in skeletal type (table 1).

Skeletal type. The axial skeleton of the house mouse consists of 7 cervical, 12 to 14 thoracic, 5 to 7 lumbar vertebrae, and 30 to 35 sacral and caudal vertebrae combined. The number of vertebrae between the cranium and the

TABLE 1

Percentage distributions of skeletal types in strains, hybrids, and segregating generations in mating of BalbC × C57blk. Skeletal type is given as the ratio of thoracic to lumbar vertebrae. A_1 and A_2 indicate two types of asymmetry at the lumbosacral border.

	Mating		Presacral vertebrae and skeletal types										Number
			25	A_1		26		A_2		27	Other	
	Sire Dam		13/5	13/$\frac{5}{6}$	13/$\frac{6}{5}$	13/6	14/5	14/$\frac{5}{6}$	14/$\frac{6}{5}$	14/6		
P_1:	C57 × C57	♂♂	0.8	1.5	1.1	96.6	....	....	....	....	....	262
	(77 litters)	♀♀	....	0.5	....	99.5	....	....	....	....	....	201
		Tot.	0.4	1.1	0.6	97.8						463
P_2:	BalbC × BalbC	♂♂	....	....	....	14.9	56.2	7.2	9.3	11.3	1.0[a]	194
	(67 litters)	♀♀	....	....	....	7.8	52.2	6.0	10.3	23.7	....	232
		Tot.				11.0	54.0	6.6	9.9	18.1	0.4	426
	BalbC × C57	♂♂	....	....	....	99.6	0.4	....	....	....	....	273
	(67 litters)	♀♀	....	....	....	100.0	....	....	....	....	....	249
F_1:		Tot.				99.8	0.2					522
	C57 × BalbC	♂♂	....	....	....	100.0	....	....	....	....	....	176
	(50 litters)	♀♀	....	....	....	100.0	....	....	....	....	....	193
		Tot.				100.0						369
	(BalbC × C57) ×	♂♂	0.5	....	....	96.3	2.7	....	....	0.3	....	374
	(BalbC × C57)	♀♀	0.3	....	....	95.3	3.3	0.3	0.3	0.3	0.3[b]	362
	(90 litters)	Tot.	0.4			95.9	3.0	0.1	0.1	0.3	0.1	736
F_2:	(C57 × BalbC) ×	♂♂	....	0.4	....	95.1	3.8	....	0.4	....	0.4[c]	263
	(C57 × BalbC)	♀♀	....	....	....	96.0	2.8	0.4	....	0.4	0.4[d]	249
	(63 litters)	Tot.		0.2		95.5	3.3	0.2	0.2	0.2	0.4	512
	C57 ×	♂♂	1.1	5.4	1.1	92.5	....	....	....	....	....	93
	(BalbC × C57)	♀♀	1.9	0.9	....	97.2	....	....	....	....	....	107
	(25 litters)	Tot.	1.5	3.0	0.5	95.0						200
B_1:	C57 ×	♂♂	1.8	0.6	....	97.1	....	....	....	0.6	....	171
	(C57 × BalbC)	♀♀	0.6	0.6	0.6	97.6	....	....	....	....	0.6[f]	169
	(37 litters)	Tot.	1.2	0.6	0.3	97.4				0.3	0.3	340
	BalbC ×	♂♂	....	....	....	81.1	16.8	0.3	0.4	1.4	....	285
	(BalbC × C57)	♀♀	....	....	....	80.6	13.5	1.0	1.0	3.9	....	310
	(76 litters)	Tot.				80.8	15.1	0.7	0.7	2.7		595
B_2:	BalbC ×	♂♂	....	....	....	69.3	19.9	3.0	3.0	3.6	1.2[e]	166
	(C57 × BalbC)	♀♀	....	....	....	70.5	20.7	2.1	2.6	4.1	....	193
	(44 litters)	Tot.				69.9	20.3	2.5	2.8	3.9	0.6	359

a: 13/$\frac{6}{7}$, 13/$\frac{7}{6}$ b: 13/$\frac{6}{7}$. c: 13/$\frac{6}{7}$. d: 13/$\frac{7}{6}$. e: two 13/$\frac{6}{7}$. f: 12/7.

sacrum (presacral vertebrae = psv) has been observed to vary from 25 to 27, inclusive.

At the thoraco-lumbar border, the last thoracic vertebra may bear small, intermediate, or large sized ribs on either or both sides. A fully developed last rib is given the grade of 4. Incompletely developed ribs are graded as 3, 2, or 1. By combining the grades for right and left ribs, the amount or grade of rib borne by the last thoracic vertebra varies on a scale from 1 to 8. This scaling neglects asymmetries which frequently occur. A mouse may have 13 ribs on one side and 14 ribs on the other, with the last rib completely or incompletely developed. In the tables, a mouse is classified as having 14 ribs if it has any evidence of a fourteenth rib, irrespective of degree of development. In BalbC, 11.5 percent of the mice had 13 pairs of ribs, the last pair always completely developed; 27 percent had 14 pairs in grades 1 to 4; 62 percent had 14 pairs in grades 5 to 8. In C57, all mice had 13 pairs of ribs, the last pair being completely developed in 74 percent.

At the lumbo-sacral border, an asymmetrical vertebra may occur such that if viewed from one side it appears to be a lumbar vertebra, if viewed from the other it appears to be a sacral vertebra. The notation $13/_6{}^5$ in the tables refers to mice with 13 pairs of ribs and 5 lumbar vertebrae if viewed from the *right* and 6 lumbar vertebrae if viewed from the *left*. The notations $13/_5{}^6$, $14/_6{}^5$, $14/_5{}^6$ may be interpreted similarly.

The skeletal type, except for breeding stock, was observed when the mice were 8 to 10 days of age, after which the type does not change. All skeletons were prepared and stained with alizarin by a modification of the method of CUMLEY, CROW and GRIFFIN (1939).

SKELETAL TYPES IN STRAINS AND HYBRIDS

The distributions of skeletal types in P_1, P_2, F_1, F_2, B_1, and B_2 from the matings of BalbC and C57 are shown in table 1. The mice in F_1 are much more uniform in skeletal type than either of the parental strains. Only one male in a total of 891 in the two F_1 generations combined showed any departure from 13/6. This one male had a fourteenth rib of the lowest possible grade of expression on one side only.

The F_2 generations are similar to each other. Combined they consist chiefly of 13/6, but also contain some intermediate and extreme types from 13/5 to 14/6.

The two backcrosses to the BalbC strain are somewhat dissimilar, but each varies from 13/6 to 14/6 in skeletal type. In contrast, the mice produced in backcrosses to the C57 strain vary from 13/5 to 13/6, with the exception of one 14/6 male in C57 × (C57 × BalbC).

Sex. In the BalbC strain, extra thoracic and lumbar vertebrae occur significantly more often in females than in males. A similar tendency appears in almost all segregating generations, but not to such a marked degree.

Asymmetry. There are four asymmetrical skeletal types, $13/_6{}^5$, $13/_5{}^6$, $14/_6{}^5$, $14/_5{}^6$. Of the first two types, $13/_6{}^5$ appears about twice as often as $13/_5{}^6$.

Of the last two types, there is a tendency for $14/_6{}^5$ to appear less often than $14/_5{}^6$. These tendencies are noted because similar and sometimes more definite inequalities have appeared in other strains (GREEN 1941) and crosses not recorded here.

Correlation between rib-number and psv-number. The number of ribs and of presacral vertebrae tend to increase and decrease together as may be seen in strain BalbC, F_2, and B_2 where variations in both occur. The evidence is summarized in table 2. This is, of course, a "part-with-whole" correlation, since the presacral vertebrae include the thoracic vertebrae.

NUMBER OF PRESACRAL VERTEBRAE

As a convenience, the variation at the lumbo-sacral border may be analyzed separately from the variation at the thoraco-lumbar border, neglecting thereby the correlation between the number of presacral vertebrae and the number of ribs.

TABLE 2

Two-way distribution of number of presacral vertebrae (psv) and number of pairs of ribs in BalbC strain, F_2, and B_2. 14L = 14 ribs in low grades of expression, 1-4. 14H = 14 ribs in high grades of expression, 5-8. A_1 = $13/_6{}^5$ and $13/_5{}^6$. A_2 = $14/_6{}^5$ and $14/_5{}^6$. Sexes combined.

In P_2: BalbC				In F_2 combined				In B_2 combined			
psv	13	14L	14H	psv	13	14L	14H	psv	13	14L	14H
26	47	94	136	25-A_1	4	0	0	26	732	124	39
A_2	2	17	53	26	1195	33	6	A_2	2	11	16
27	0	3	74	A_2-27	3	3	4	27	2	7	21

Two deductions are possible by inspection of the variation in psv-number in table 1. First, there is considerable non-genetic variation within the pure strains and presumably also in the cross-bred generations. Second, despite the non-genetic variation, there are clearly some genetic causes of variation, since each backcross generation tends to resemble the parent strain used in the backcross and since the F_2 spreads out over a wider range of psv-numbers than the F_1 or either parent strain. The problem is to discover the nature of the genetic variation. Specifically, these questions should be answered: (1) are there one or several gene pairs, (2) is there dominance between alleles, (3) is there epistasis between non-alleles, (4) is there potence of the set of genes in one strain relative to the set in the other, (5) is there linkage between the "skeletal" genes and other known genes and between the "skeletal" genes themselves. Not all parts of this problem can be answered with the present data, partly because the skeletal type is neither clearly a discrete nor clearly a continuous measured variable and partly because not all of the required segregating generations were produced. With some assumptions and restrictions to be noted, the questions that may be answered, at least in part, are (1) how many pairs of genes are responsible for the differences between the BalbC and C57 strains, (2) is dominance apparent on a scale which mini-

mizes factor interaction, (3) what is the potence of the gene sets in the strains, and (4) what linkages, if any, are present between the " skeletal " genes and the coat color genes *A/a, B/b, C/c.*

Threshold concept. Attempts to explain the genetic differences between the strains by the assumption of one or two pairs of genes, acting with or without dominance, seem to be unpromising because no familiar segregation ratios can be recognized in the backcross and F_2 generations. The non-genetic variation modifies or obscures the differences between genotypes.

It seems reasonable to assume that mice of the same genotype, as in the BalbC or C57 strain, may have 25 or 26, or 26 or 27 presacral vertebrae, depending upon the interaction of a large number of non-genetic factors, each with a small effect upon skeletal type. If further the effects are additive and uncorrelated, the entire array of factor combinations will yield a distribution like the normal curve of variation. According to this view, at some point the combination of " plus " effects is sufficient to produce 27 psv. A somewhat smaller number of " plus " effects may produce only 26 psv. A still smaller number of " plus " effects may produce only 25 psv. The asymmetrical types, A_1 and A_2, may be regarded as the consequence of intermediate grades of factor combinations. Thus it is possible to suppose that four thresholds, T, U, V, W are distributed along the scale of factor effects such that 5 psv-types result:

(25) T (A_1) U (26) V (A_2) W (27).

If, in addition to the non-genetic factors, the genetic factors are numerous and have small, additive and uncorrelated effects upon skeletal type, they may be considered as acting upon the same scale as the non-genetic factors. In the BalbC and C57 strains and in the F_1, only the non-genetic factors operate in causing the observable variation. In the F_2 and backcross generations, the non-genetic factors are reinforced by the genetic factors to increase the variation.

These suppositions lead, as a first approximation, to the view that each strain and each crossbred generation is characterized by a frequency distribution of factor combinations which determine skeletal type. Each distribution, assumed to be normal in form, may be uniquely specified by a mean and a variance. The location of the mean of a generation is determined by the combination of, first, the residual genetic constitution common to all mice and, second, the number of effective genetic " skeletal " factors present in the generation. The magnitude of the variance about the mean is determined by the non-genetic factors only in the case of pure strains and F_1, and by the compound of non-genetic and segregating genetic factors in the case of segregating generations.

Means and variances. The computation of numerical values of the mean and variance for each distribution is complicated by the fact that the scale values are unknown. All that is known is the proportion of the distribution that lies above or below a particular scale value or threshold. WRIGHT (1934) has

shown in a similar case that when a distribution is cut by two thresholds it is possible to estimate the mean and variance from the proportions below, between, and above the thresholds.

The method consists of finding the standard normal deviates corresponding to the fractions of the curve below the first and below the second thresholds. If c is the threshold difference in " standard deviation units," the distribution may be transformed to a scale of " threshold units " by dividing all values by c, thus transforming the mean and variance to a scale of " threshold units." The percentages required for the computations are given for each generation in table 3, where it may be seen, for example, that 65.0 percent of P_2 (= BalbC) is below the V-threshold and 81.9 (= 65.0 + 16.9) percent is below the W-threshold.

The computation of the mean and variance for P_2 may be illustrated with the aid of figure 1. Further details about the method may be found in WRIGHT (1934). Let t_V and t_W be standard normal deviates such that

$$p_V = \int_{-\infty}^{t_V} f(t)dt \quad \text{and} \quad p_W = \int_{-\infty}^{t_W} f(t)dt,$$

where $f(t) = \exp(-t^2/2)/\sqrt{2\pi}$, the frequency function of the standard normal curve. From a table of cumulative areas of the standard normal curve for which the mean $m_t = 0$ and the standard deviation $\sigma_t = 1$, it may easily be found that $t_V = 0.386$ and $t_W = 0.912$, corresponding to $p_V = 0.650$ and $p_W = 0.819$. Therefore $t_W - t_V = 0.526$ " standard deviation units." We may now define a new variable y such that $y = (t - t_V)/(t_W - t_V)$. On the scale of y, the difference $y_W - y_V = 1$ becomes the unit of measurement; it may be called a " threshold unit." The origin is at the V-threshold ($y_V = 0$) and the standard deviation on the y-scale becomes $s_y = \sigma_t/(t_W - t_V) = 1.901$ " threshold units." Other corresponding points are:

t:	$t_V = 0.386$	$t_W = 0.912$	$m_t = 0$	$\sigma_t = 1$
y:	$y_V = 0$	$y_W = 1$	$\bar{y} = -0.734$	$s_y = 1.901$

Instead of using the V-threshold as origin, it will be slightly more convenient to avoid negative signs for the means of P_1, P_2, . . . , B_2 by using the U-threshold as origin. By using the F_2 generation, the only one with mice below U and above V, it was found that $y_V - y_U = 16.199$ " threshold units." The final change of scale is therefore to $x = y + (y_V - y_U)$. The same unit of measurement is preserved and the standard deviation is the same, $s_x = s_y$. Only the origin is changed from the V- to the U-threshold. Some corresponding points are:

y:	$y_V = 0$	$y_U = -16.199$	$\bar{y} = -0.734$	$s_y = 1.901$
x:	$x_V = 16.199$	$x_U = 0$	$\bar{x} = 15.465$	$s_x = 1.901$

TABLE 3

Percentage distributions of psv-number and of rib-number in pure strains and segregating generations. Sexes combined. 13H means 13 ribs in grades 5-8; 14L means 14 ribs in grades 1-4; 14H means 14 ribs in grades 5-8. Estimated values in ( ).

Strain or generation	Number	psv-number and thresholds					Method 1*		Method 2†		rib-number			Mean§	S.D.
		25 (T)	A_1 (U)	26 (V)	A_2 (W)	27	Mean $\bar{x}$	S.D. s	Mean $\bar{x}$	S.D. s	13H (K)	14L (L)	14H	$\bar{x}$	s
P_1: C57blk	463	0.4	1.7	97.8	....	....	3.38	1.67	3.86	(1.90)	100.0	....	....	....	....
P_2: BalbC	426	....	....	65.0	16.9	18.1	15.46	1.90	15.46	1.90	11.5	26.8	61.7	1.33	1.11
Mid-parent	....	....	....	....	....	....	(9.42)	....	(9.66)	....	....	....	....	....	....
F_1	891	....	....	100.0	....	....	(7.78)	....	(7.54)	(1.90)	99.9	0.1	....	....	....
F_2	1248	0.2	0.1	98.9	0.5	0.3	8.60	3.15	8.60	3.15	96.3	2.9	0.8	-2.90	1.62
B_1	540	1.3	0.8	96.7	....	0.2	5.07	2.72	5.78	3.11	99.8	....	0.2	....	....
B_2	954	....	....	93.8	3.0	3.2	11.39	3.12	11.39	3.12	77.1	14.8	8.1	-1.13	1.52

* The "unit" is the difference between pairs of thresholds such that $x_W - x_V = x_U - x_T = 1$. The means are given with threshold U as origin, where U lies between A_1 and 26.

† The "unit" is the difference $x_W - x_V = 1$. Also, $s_{P1} = s_{P2} = 1.90$. The means are given with threshold U as origin.

§ The "unit" is the difference $x_L - x_K = 1$. The means are given with threshold K as origin, where K lies between 13H and 14L.

Two procedures. At this point two procedures appear for computing the means and variances of the distributions of this experiment. If x is the variable which represents scale value, then x_T, x_U, x_V, and x_W may represent the scale values corresponding to the threshold T, U, V, and W. Also, if s_x is the standard deviation of any distribution on a scale of x, then s_{x1} and s_{x2} may represent the standard deviations of P_1 and P_2 respectively.

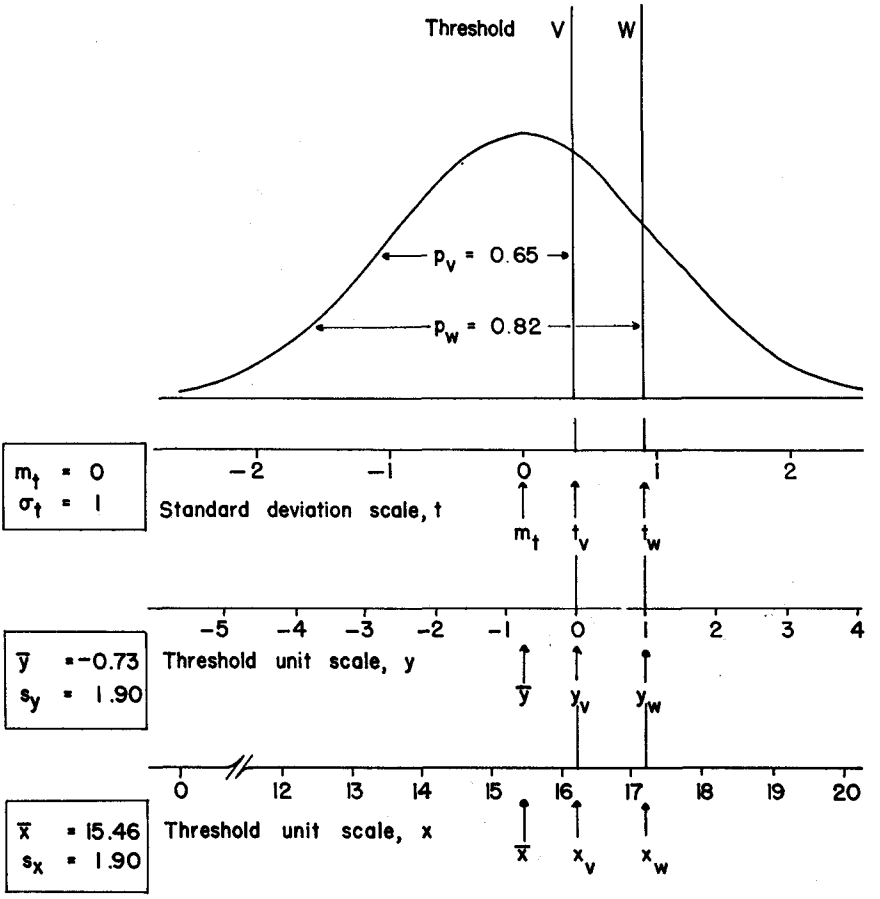

FIGURE 1. Diagram of transformation of percentage occurrence to standard deviates and to threshold units with origin at x_U. Case illustrated is for BalbC strain.

The first procedure consists of choosing the threshold intervals ($x_U - x_T = x_W - x_V = 1$) as equal and of using this common difference as the unit of measurement. The means and standard deviations of P_1, P_2, . . . , B_2 (with the exception of F_1 which passes no threshold) may then be computed with this unit as outlined above. With this procedure the standard deviation of P_1 and P_2 are $s_{x1} = 1.67$ and $s_{x2} = 1.90$ units. The second procedure consists of choosing one of the threshold intervals, say $x_W - x_V$, as the unit and of arbitrarily making $s_{x1} = s_{x2}$. Since s_{x2} may be estimated with greater accuracy than s_{x1} because of the larger percentages below V and above W in P_2, we

may take $s_{x1} = s_{x2} = 1.90$. With this procedure $x_U - x_T = 1.14$ "threshold units," or 1.14 times greater than $x_W - x_V$.

There is no clear *a priori* choice between these two procedures. A choice between them may be based upon the service the selected scale renders in aiding in the analysis of the genetic differences between the pure strains. As MATHER (1949) has stated, an adequate scale should fulfill two criteria. It should be a scale on which genic effects are additive and on which the magnitude of non-genetic effects is uncorrelated with the genotype. Fulfillment of the first criterion (additive genic effects) may be tested by examining the spacing of the *means* of the various generations along the scale. Fulfillment of the second criterion (independence of genetic and non-genetic effects) may be tested by examining the *variances* of the non-segregating generations. The first procedure for computing means and variances in "threshold units" amounts to accepting a correlation between the means and variances of P_1 and P_2. The second procedure amounts to forcing fulfillment of the second criterion by making the variances of P_1 and P_2 equal. However, it is obvious that a scale which is forced to have equal parental variances is not by that fact alone an adequate scale. The choice between the procedures may therefore rest upon their relative fulfillment of the first criterion of additivity.

The means and standard deviations of each generation with $x_W - x_V = 1$ as the scale unit and with the U-threshold as origin are given in table 3 for both methods of computation.

Tests of the scale. MATHER (1949) has given several statistics which may be used for testing the adequacy of a scale of measurement with respect to additivity when F_1, F_2, B_1, and B_2 generations are available in a cross between two pure lines. In the absence of a direct empirical estimate of the F_1 mean, the only tests available are those which use the means of F_2, B_1, and B_2. On an adequate scale the following equations will hold:

$$m_{F_2} = \tfrac{1}{2}(m_{B_1} + m_{B_2})$$
$$m_{B_1} = \tfrac{1}{2}(m_{P_1} + m_{F_1}) = m_{F_2} + \tfrac{1}{4}(m_{P_1} - m_{P_2})$$
$$m_{B_2} = \tfrac{1}{2}(m_{F_1} + m_{P_2}) = m_{F_2} - \tfrac{1}{4}(m_{P_1} - m_{P_2})$$

where m is the theoretical mean of the generation designated in the subscript. The second and third equations depend upon the further theoretical relationships: $m_M = \tfrac{1}{2}(m_{P1} + m_{P2})$, and $m_{F2} = \tfrac{1}{2}(m_M + m_{F1})$, where M denotes the mid-parent.

The significance of departures from the theoretical equalities may be tested by computing the deviations D_i and their sampling variances s_i^2 from:

$$D_1 = 2\bar{x}_{F_2} - \bar{x}_{B_1} - \bar{x}_{B_2}, \qquad s_1^2 = 4s^2_{\bar{x}F_2} + s^2_{\bar{x}B_1} + s^2_{\bar{x}B_2},$$
$$D_2 = 4\bar{x}_{B_1} - 4\bar{x}_{F_2} - \bar{x}_{P_1} + \bar{x}_{P_2}, \qquad s_2^2 = 16s^2_{\bar{x}B_1} + 16s^2_{\bar{x}F_2} + s^2_{\bar{x}P_1} + s^2_{\bar{x}P_2},$$
$$D_3 = 4\bar{x}_{B_2} - 4\bar{x}_{F_2} + \bar{x}_{P_1} - \bar{x}_{P_2}, \qquad s_3^2 = 16s^2_{\bar{x}B_2} + 16s^2_{\bar{x}F_2} + s^2_{\bar{x}P_1} + s^2_{\bar{x}P_2},$$

where $\bar{x}$ and $s_{\bar{x}}^2$ are the computed mean and the computed sampling variance of the mean, respectively, for the generation designated in the subscript. The

sampling variances of the means $s_{\bar{x}}^2$ have been computed by a method (outlined in the next section) which yields underestimates of the true values. The results of the three tests of an adequate scale for the two methods of computing means and variances are:

	$D_1 \pm s_1$	$D_2 \pm s_2$	$D_3 \pm s_3$
Method 1:	0.74 ± 0.30	−2.06 ± 0.75	−0.92 ± 0.69
Method 2:	0.03 ± 0.31	0.33 ± 0.82	−0.45 ± 0.69

It appears that method 2 has a slight superiority over method 1 for providing an adequate scale for measuring factor combinations, but the evidence is by no means overwhelming. Because the computed variances of the means are likely to be underestimates of the true variances, both methods probably satisfy the criterion of additive genic effects.

The use of method 2 leads to a picture of the distributions of the pure lines and of the segregating generations such as that in figure 2. The location of the mid-parent M is found from $\bar{x}_M = \frac{1}{2}(\bar{x}_{P1} + \bar{x}_{P2})$ and of the F_1 from $\bar{x}_{F1} = \bar{x}_M - 2(\bar{x}_M - \bar{x}_{F2}) = 2\bar{x}_{F2} - \bar{x}_M$.

Variance of a mean. The sampling variance of a standard normal deviate t is given by FISHER (1937) as $s_t^2 = s_p^2(dt/dp)^2 = p(1-p)/nz^2$, where p is the area under the curve to the left of the ordinate z at deviate t and where $dp = z\,dt$. For the mean of a standard normal distribution $\bar{t} = 0$, $p = \frac{1}{2}$, and $z = 0.3989$. Therefore, $s_t^2 = \frac{1}{4}nz^2 = 1.5708/n$. If a change in scale is made such that $\bar{x} = (\bar{t} - t_0)/(t_2 - t_1)$ and if the normal deviates t_0, t_1, t_2 are taken as *constants* corresponding to the locations of the U, V, W thresholds, then

$$s_{\bar{x}}^2 = s_{\bar{t}}^2(d\bar{x}/d\bar{t})^2 = 1.5708/n(t_2 - t_1)^2.$$

For strain BalbC (= P_2) of which 426 (= n) mice were examined, 65 percent below threshold V gives $t_1 = 0.386$, and 81.9 percent below threshold W gives $t_2 = 0.912$. Thus $s_{\bar{x}P2}^2 = 0.0133$ upon substitution. In a similar manner the variances of the means of P_1, F_2, B_1, and B_2 may be found.

This formula for the variance of a mean is far from satisfactory since it will always give an underestimate of the true variance. This is a consequence of regarding t_0, t_1, t_2 as fixed points when in fact they are empirically estimated and so subject to sampling error. Particularly in this experiment, since the interval $t_1 - t_0$ is estimable only from the F_2 generation, which contained very small proportions below the U threshold and above the V threshold, the error is likely to be quite serious. If the errors in t_0, $\bar{t}$, t_1, and t_2 are propagated into the error of $\bar{x}$, the sampling variance of the mean would take the form

$$s_{\bar{x}}^2 = \sum_i \left(\frac{\partial \bar{x}}{\partial t_i}\right)^2 s_{t_i}^2 + 2\sum_{i<j}\left(\frac{\partial \bar{x}}{\partial t_i}\right)\left(\frac{\partial \bar{x}}{\partial t_j}\right) s_{t_i} s_{t_j} r_{t_i t_j}$$

where

$$r_{t_i t_j} = \sqrt{p_i(1-p_j)/p_j(1-p_i)}$$

is the correlation coefficient of t_i with t_j. For P_2, this formula gives $s_{\bar{x}P2}^2 = 0.1082$, or about an 8-fold increase in the sampling variance of the mean. For F_2, the formula gives $s_{\bar{x}F2}^2 = 12.3168$, or a variance of the mean which is about 1.2 times larger than the variance of the F_2 generation! It is apparent, therefore, that the locations of the pure and segregating generations relative to the thresholds T, U, V, W effectively prevent reliable estimations of the sampling variances of the means.

Potence. WIGAN (1944) has used the term "potence" to refer to the relative strength of the two sets of genes, one in each pure strain, in causing the F_1 generation to deviate from the mid-parent. "Potence" has the same

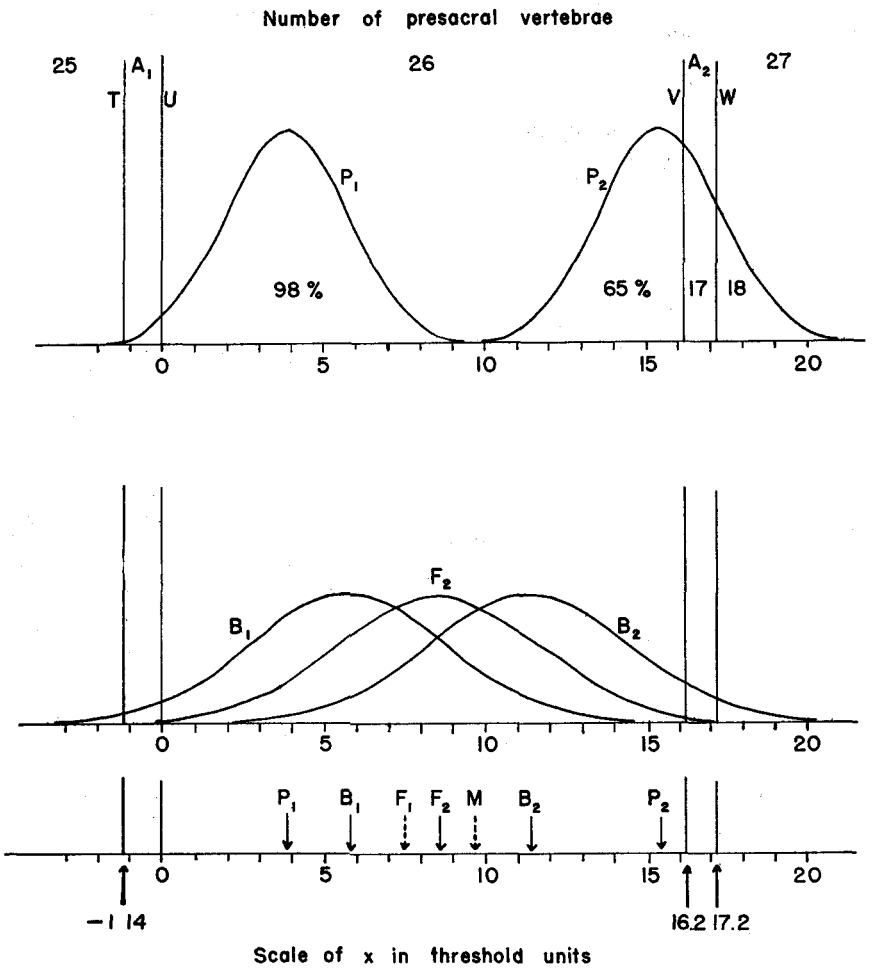

FIGURE 2. Distributions of the pure strains and segregating generations in five categories of psv-number. The hypothetical locations of the F_1 mean and of the midparent M are indicated on the bottom scale. Construction performed using method 1 with $x_W - x_V = 1$ and $s_{P1} = s_{P2} = 1.90$.

meaning for sets of alleles that " dominance " has for a single pair of alleles. The relative potence of the BalbC and C57 strains cannot be evaluated by use of F_1 in this experiment because the F_1 mean cannot be computed directly in " threshold units." However, the F_2 mean, which theoretically on an adequate scale will deviate from the mid-parent by one-half of the deviation of the F_1 mean, may be used. The $\bar{x}_{F2} = 8.60$ deviates from $\bar{x}_M = 9.66$ by -1.06 ± 0.19 units. This deviation may not be significant because of the deflated estimates of variances of the means. If the deviation is significant, it indicates that the set of genes in the C57 strain has more potence than the set of genes in the BalbC strain. The presence of potence implies that there is dominance in the gene pairs involved in the cross and that the dominance is preponderantly in the same direction (toward strain C57), but it does not yield information about the magnitude of the dominance. For this we need estimates of the components of the genetic variance.

Components of variance. The variances of the P_1, P_2, and F_1 generations measure on the chosen scale the effect of non-genetic or environmental factors on the variation in skeletal type. The true environmental variance may be denoted by σ_E^2. The variances of the segregating generations are compounded of the non-genetic variance and the variance due to genetic recombinations. This true genetic variance may be denoted by σ_G^2. The genetic variance may in turn be considered as a compound of the variance due to the differences between the homozygotes of each pair of genes and of the variance due to the difference between the heterozygote and the midparent of each pair of genes. These true variances may be denoted by σ_D^2 and σ_H^2, respectively. A complete exposition of the meaning and use of these variances may be found in FISHER, IMMER and TEDIN (1932) and MATHER (1949).

It has been shown that the variances of the segregating generations are:

$$\sigma_{F_2}^2 = \tfrac{1}{2}\sigma_D^2 + \tfrac{1}{4}\sigma_H^2 + \sigma_E^2,$$

$$\sigma_{B_1}^2 + \sigma_{B_2}^2 = \tfrac{1}{2}\sigma_D^2 + \tfrac{1}{2}\sigma_H^2 + 2\sigma_E^2.$$

By use of the computed values of the standard deviations s of P_2, F_2, B_1, and B_2, given in table 3, it is possible to solve simultaneously for estimates of σ_D^2 and σ_H^2. Thus, $s_E^2 = s_{P2}^2 = (1.90)^2 = 3.61$, $s_{F2}^2 = (3.15)^2 = 9.92$, $s_{B1}^2 + s_{B2}^2 = (3.11)^2 + (3.12)^2 = 19.41$. Upon inserting these values in the equations above, it is found that $s_D^2 = 0.87$ and $s_H^2 = 23.46$, as estimates of σ_D^2 and σ_H^2.

This outcome is quite unexpected. If the estimates of σ_D^2 and σ_H^2 are accepted as reliable, the numerical values imply that the difference between the homozygotes of each pair of genes is almost trivial when compared with the deviation of the heterozygote from the mid-parent for each pair of genes. This suggests two alternative interpretations, both of which are inconsistent in some respect. First, if the dominant genes are concentrated in one parent and the recessive genes in the other, then the F_1 mean should be well outside the interval between the parental means. While the location of the F_1 mean for this experiment is not known, it surely lies between the parental means.

This first interpretation is therefore not admissible. Second, if the dominant genes are nearly equally distributed between the parental strains but with sufficient inequality of distribution to give the observed difference between the parental means and the observed amount of potence, then the F_2 and backcross generations should exhibit considerable transgressive variation, producing large frequencies of mice below the T-threshold (25-psv) and above the W-threshold (27-psv). The observed percentages among 1248 F_2 mice were 0.2 percent 25-psv and 0.3 percent 27-psv. The observed percentages of 25-psv and 27-psv in B_1 and B_2 were also small. The second interpretation appears therefore to be not admissible.

As another alternative, the estimates of σ_D^2 and σ_H^2 may not be reliable. Since σ_D^2 and σ_H^2 depend upon the variances of P_2, F_2, B_1, and B_2, a faulty estimate of any one of these would be reflected in s_D^2 and s_H^2. Of these, the F_2 variance is the one most likely to be in error since its value depends upon the proportion of the F_2 generation between the V- and W-thresholds and above the W-threshold. These proportions are 0.005 and 0.003, respectively. In comparison with the backcross variances, the F_2 variance seems to be suspiciously small. A shift of one mouse in the F_2 generation from the 26- to the 27-psv category would, for example, increase the F_2 variance to 3.51 and yield 10.46 and 13.92 as the new estimates of σ_D^2 and σ_H^2, respectively. It is obvious, therefore, that no great reliance may be placed upon the computed F_2 variance, nor upon the estimates of σ_D^2 and σ_H^2 actually obtained.

Nevertheless, some degree of dominance is indicated, thus supporting the conclusion based on the deviation of the F_2 mean from the midparent M.

Number of pairs of factors. With only F_2 and backcross data available, any estimate of the number of pairs of genes affecting skeletal type which distinguish the BalbC and C57 strains must necessarily be a coarse approximation. This would be true even if an adequate metric scale were available. With a scale in " threshold units," there is a further loss of precision due to inaccuracies in the estimates of variance.

At least 5 estimates of the number N of pairs of factors are possible with the available data. These estimates are similar (and similarly limited) because for each it is assumed that the effective alleles are concentrated in the " large " parent and the non-effective alleles in the " small " parent, that all of the effective alleles add equal scale amounts to the phenotype, and that the non-alleles assort independently. The estimates are different because different generations and different components of the genetic variance are used. The estimates are given in table 4, along with the contribution of each effective allele in " threshold units."

The estimates, N_1, N_2, N_3, are based upon WRIGHT's (1934) formula for estimating the *minimum* number of pairs of factors. The estimates are minimal because the variance used in the denominator of each is a compound of the estimates of σ_D^2 and σ_H^2, where an estimate of σ_D^2 only is desired. These three estimates suggest that at least three pairs of genes distinguish the BalbC and C57 strains. The remaining two estimates are given by MATHER (1949). The estimate $N_4 = 38$, using s_D^2 only, appears to be an overestimate, since s_D^2

is undoubtedly smaller than the true value. The estimate N_5, using s_H^2 only, is ridiculously small owing to the excessive value of s_H^2 relative to the deviation of the F_2 mean from the mid-parent. If s_D^2 were as large as 10.46, which is by no means unreasonable, the number of pairs of factors would be $N_{4a} = 3$, approximately.

It seems reasonable to suppose that the number of pairs of genes is not less than 3, nor more than 38, and that the true number is nearer to 3 than to 38. No more refined statement seems warranted by the data.

NUMBER OF PAIRS OF RIBS

The variation in rib development is less satisfactory for analysis than the variation in the number of presacral vertebrae. The 13-rib type occurred almost exclusively in strain C57, F_1, and B_1. The 13-rib type occurred in 11.5 percent of strain BalbC, 96.3 percent of F_2, and 77.2 percent of B_2. The grades of rib development may be arbitrarily grouped, as shown in table 3,

TABLE 4

Estimates of the number of pairs of factors which distinguish the C57 strain (= P_1) and the BalbC strain (= P_2) with respect to skeletal type.

Estimate		Definition		Number of pairs of factors	Contribution of each allele in "threshold units"
N_1	=	$(\bar{x}_{P_2} - \bar{x}_{P_1})^2 / 8(s_{F_2}^2 - s_{P_2}^2)$	=	$2.7 \sim 3$	1.9
N_2	=	$(\bar{x}_{P_2} - \bar{x}_{P_1})^2 / 16(s_{B_1}^2 - s_{P_2}^2)$	=	$1.4 \sim 2$	2.9
N_3	=	$(\bar{x}_{P_2} - \bar{x}_{P_1})^2 / 16(s_{B_2}^2 - s_{B_2}^2)$	=	$1.4 \sim 2$	2.9
N_4	=	$(\bar{x}_{P_2} - \bar{x}_{P_1})^2 / 4s_D^2$	=	$38.4 \sim 38$	0.2
N_{4a}	=	$(\bar{x}_{P_2} - \bar{x}_{P_1})^2 / 4s_D^2$	=	$3.2 \sim 3$	1.9
N_5	=	$(\bar{x}_{P_1} - 2\bar{x}_{F_2} + \bar{x}_{P_2})^2 / s_H^2$	=	$0.2 \sim 0$	....

so that low grades 1 to 4 are designated 14L and high grades 5 to 8 are designated 13H or 14H. It is obvious that no simple genetic explanation is possible for the variation in rib number and development in the BalbC and C57 strains. Even the numerous assumptions, such as were made for variation in psv-number, do not help in resolving the question of the number and kind of genetic factors. The analysis is hampered by the absence of variation in strain C57 and in F_1 and B_1.

The means and standard deviations of strain BalbC, F_2, and B_2 do satisfy some of the conditions for multiple factor inheritance. That is, the means relative to a threshold K between 13H and 14L ribs are in the order $F_2 < B_2 < P_2$, and the standard deviations are in the order $P_2 < B_2 < F_2$, as required by the theory of numerous factors with additive effects upon rib number.

The partial construction of figure 3 shows the relationship of the three variable generations to the thresholds K between 13H and 14L, and L between 14L and 14H.

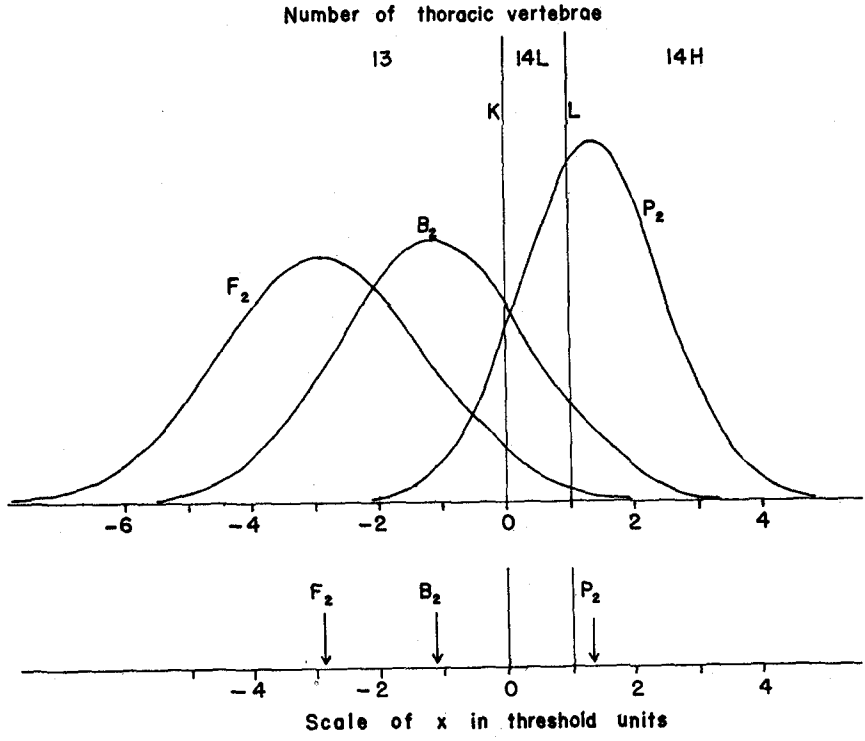

FIGURE 3. Distributions of three generations in three categories of rib number.

TEST FOR ASSOCIATION OF COAT COLOR AND SKELETAL TYPE

The BalbC and C57 strains differ with respect to three pairs of coat-color genes. Strain BalbC is albino with genotype *ccAAbb* and strain C57 is non-agouti black with genotype *CCaaBB*. The F_1 mice were all wild type *CcAaBb*. There were 5 coat color types of F_2 mice, 2 types of B_1 mice, and 3 types of B_2 mice. The observed and expected frequencies of the coat-color types in each segregating generation are shown in table 5. The segregation in each of

TABLE 5

Percentage distribution of coat color types in pure and cross-bred generations of mating of BalbC × C57. Genotype of BalbC is ccAAbb; genotype of C57 is CCaaBB.

Coat color	Black agouti *C-A-B-*	Brown agouti *C-A-bb*	Black *C-aaB-*	Brown *C-aabb*	Albino *cc----*	Total
F_2 Obs.	42.7	14.1	13.8	3.5	25.9	1248
Exp.	42.2	14.1	14.1	4.7	25.0	
B_1 Obs.	48.0	....	52.0	....	....	540
Exp.	50.0	....	50.0	....	....	
B_2 Obs.	26.1	25.2	....	....	48.7	954
Exp.	25.0	25.0	....	....	50.0	

TABLE 6

Tests for association of psv-number and rib-number with coat color genes C/c, A/a, B/b. Expected frequencies are those expected on hypothesis of no association. Entries in table are observed frequency (expected frequency).

Generation		psv-number*						rib-number*							
		25 + A_1		26		A_2 + 27		χ^2 df	13H		14L		14H		χ^2 df
F_2	*C–*	3	(3)	914	(915)	8	(7)	0.18	893	(891)	26	(27)	6	(7)	1.12
	cc	1	(1)	320	(319)	2	(3)	2	309	(311)	10	(9)	4	(3)	2
B_2	*Cc*	0		462	(459)	27	(30)	0.76	385	(377)	74	(73)	30	(39)	4.59
	cc	0		433	(436)	32	(29)	1	351	(359)	68	(69)	46	(37)	2
F_2	*A–*	2	(2)	702	(701)	5	(6)	1.07	683	(684)	22	(20)	4	(5)	1.27
	aa	1	(1)	212	(213)	3	(2)	2	210	(209)	4	(6)	2	(1)	2
B_1	*Aa*	6	(8)	252	(251)	1	(1)	2.19	258		0		1		....
	aa	11	(9)	270	(272)	0	(0)	2	281		0		0		....
F_2	*B–*	3	(2)	700	(697)	2	(6)	13.61	686	(681)	15	(20)	4	(5)	5.39
	bb	0	(1)	214	(217)	6	(2)	2	207	(212)	11	(6)	2	(1)	2
B_2	*Bb*	0		246	(235)	3	(14)	24.01	214	(196)	25	(37)	10	(16)	15.79
	bb	0		216	(227)	24	(13)	1	171	(189)	48	(36)	21	(15)	2

*Deviations of observed from expected frequencies are judged significant or non-significant by means of the χ^2 criterion with the critical value at the 5 percent level of significance. $\chi^2(0.05, 1) = 3.84$, $\chi^2(0.05, 2) = 5.99$.

the three pairs of alleles and the assortment of non-alleles may be seen to conform very satisfactorily with mendelian theory for unlinked genes.

To test for the association of skeletal type with coat color, the psv-numbers and the rib-numbers were each grouped into three categories. This made it possible to construct, in most cases, 2×3 contingency tables with a coat-color gene on one border and psv- or rib-number on the other. The observed numbers of mice and the numbers expected on the hypothesis of no association are shown in table 6. Neither psv-number nor rib-number appears to be associated with genes *C/c* and *A/a*. Gene *b*, on the other hand, is associated with an increase in the number of presacral vertebrae, the more convincing evidence being in B_2. The same gene may also tend to increase the rib development, but the evidence is not so convincing.

This association, if accepted as a fact, may be due either to linkage of the *B/b* locus with one or more loci carrying " skeletal " genes, or to the physiological effect of the *b* gene itself. No discrimination between these two possibilities can be made with the present experiment.

SUMMARY

Matings were made between two strains of mice, BalbC and C57blk, differing in the number of thoracic and lumbar vertebrae. The BalbC strain consists of mice with 14 thoracic/5 lumbar (54 percent), 13/6 (11 percent), 14/6 (18 percent) and other combinations (17 percent). The C57blk strain consists of mice with 13/6 (98 percent) and other combinations (2 percent).

The F_1 and F_2 generations were intermediate between the parental strains and the backcrosses, B_1 and B_2, to the two parents gave distributions located between the midparent and the parent used in the backcross.

With respect to the number of presacral vertebrae (vertebrae between the cranium and sacrum), the two strains appear to be distinguished by at least three pairs of genes. There is a dominant-recessive relationship between the alleles but it is not clear whether the dominant alleles are concentrated in one of the parents or are nearly equally divided between the parents. One or more of the " skeletal " gene pairs may be linked with the coat color genes *B/b*.

A transformation of percentage occurrence to a " threshold unit " scale, which may be made with some simplifying assumptions, appears to yield an adequate scale for measuring combinations of genetic and non-genetic factors.

With respect to the number of pairs of ribs (thoracic vertebrae) the observed variation in the pure strains and segregating generations is not sufficient to permit an analysis of genetic differences.

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