BIOMETRICAL GENETICS WITH ONE OR TWO LOCI: THE INHERITANCE OF PHYSIOLOGICAL CHARACTERS IN MICE*

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ABSTRACT

Maximum likelihood methods have been used to compare the fit of twenty different genetic models to experimental data on fourteen characters, each measured on two parental strains, F_1 hybrids and both backcrosses. Although variation in all characters was continuous, differentiation between the various models was meaningful, the mean likelihood ratio between the best and worst models for each character being greater than 10^4. Models with only one or two loci were adequate to account for the observed genetic variation in eleven of the fourteen characters. These results indicate that even in species without special genetic advantages, it may be possible to identify individually some of the genes responsible for naturally-occurring variation within the range of normality.

IT is becoming increasingly clear that normal strains of mice may differ markedly from each other in almost any aspect of their physiology. Thus in work which is specifically relevant to the present paper, it has been reported that the strains CBA/FaCam and Peru differ significantly in adrenal structure (BADR, SHIRE and SPICKETT 1968; SHIRE, 1969a) and steroid biosynthesis (BAMBERG, personal communication); in renal structure (SPICKETT, SHIRE and STEWART 1967; DEROUFFIGNAC, STEWART and MOREL 1970) and in water and electrolyte metabolism (STEWART 1968, 1969a); in carbohydrate metabolism (CHARLESWORTH 1969); and in behavior (SHIRE 1968); for summary see SHIRE (1969b). However, if the observation of strain differences is now almost commonplace, hardly any attempts have been made at genetic analysis of such strain differences. One reason for this arises from the fact that environmental variation often causes the parental and F_1 distributions to overlap. This makes it more difficult to

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distinguish the various genotypes resulting from genetic segregation in a second hybrid generation (F_2 or backcross).

The possibilities for genetic analysis in a situation where the parental distributions overlap have recently been reconsidered by STEWART (1969b,c), and efficient statistical methods developed by ELSTON and STEWART (1973). The purpose of the present paper is to apply these methods to the analysis of a number of differences between the strains CBA/FaCam and Peru. The experimental data on which this paper is based consist of measurements on 36 physiological characters on mice from strain CBA/FaCam, strain Peru, their F_1 hybrid, and backcrosses to both parental strains. Preliminary inspection of the distributions for some of these characters, by the methods of STEWART (1969b), suggested that it might be possible to account for the observed genetic variation in terms of relatively few loci. Those characters which appeared promising in this respect (i.e., with well-separated parental distributions, or backcross distributions markedly characteristic of a particular genetic situation) were selected for analysis in the present paper.

MATERIALS AND METHODS

Mice: The basic stocks of mice used in this work were the inbred strains CBA/FaCam and Peru. F_1 hybrids between these strains were all made with CBA mothers. Experimental measurements were made on ten male mice from each of the three genetically homogeneous groups: CBA, Peru, and F_1. Backcrosses were made to both parental strains, in each case with F_1 mice as mothers. Experimental measurements were made on 35 backcross to CBA male mice, and 33 backcross to Peru male mice. Each of the five groups (CBA, Peru, F_1, Bc CBA, Bc Peru) contained at least five different litters with different mothers.

Litter and maternal effects: Litter and maternal effects are potentially important sources of variation in at least some of the characters measured in this study. In the formulation below, such effects are included as a component of environmental variance σ^2; since each of the five groups contained a number of different litters, with a relatively small number of mice from any one litter, this represents a reasonable approximation. It is an advantage of the likelihood approach that there is no difficulty in principle in introducing specific terms for litter and maternal effects. In the present case this would mean introducing over twenty additional parameters, clearly inappropriate since the total number of parameters would approach the number of individual mice.

Experimental measurements: The experimental characters on which the measurements are based have been described in detail in the literature cited at the beginning of this paper. The characters investigated in this paper, together with summary labels which will subsequently be used to refer to these characters, are given below. Logarithmic transformations were applied to some characters, as specified below, to remove skewness from the distributions in the parental strains and in the F_1.

Five-week body weight (5BW)
Six-week body weight (6BW)
Eight-week body weight (8BW)

Weight of renal cortex (RC) was calculated as paired kidney weight × percentage of kidney occupied by cortex, as estimated from histological sections (STEWART and SPICKETT 1967).

Number of nephrons in the kidney (RN) was estimated from histological sections.

Weight of nephron segments in renal outer medulla (ROM; log transform) was calculated as kidney weight × percentage of kidney occupied by outer medulla ÷ number of nephrons. The nephron segments in this region are the *pars recta* of the proximal tubule, part of the thick ascending loop of Henle, and collecting ducts.

Weight of nephron segments in renal inner medulla (RIM) was calculated as kidney weight × percentage of kidney occupied by inner medulla ÷ number of nephrons. The nephron segments in this region are thin descending loops of Henle of all nephrons, thick ascending loops of Henle, and collecting ducts.

Weight of nephron segments in renal papilla (RP) was calculated as kidney weight × percentage of kidney occupied by papilla ÷ number of nephrons. The nephron segments in this region are thin descending and ascending loops of Henle of a minority of nephrons, and collecting ducts.

Relative testis weight (TW; log transform) was calculated as paired testis weight divided by body weight.

Relative adrenal weight (AW) was calculated as paired adrenal weight divided by body weight.

Zona glomerulosa of the adrenal gland (ZG) was taken as the mean width of this zone in a mid-section of the adrenal.

Interest (INT), *Latency* (LAT; log transform), and *Duration* (DUR; log transform) were three behavioral characters described by SHIRE (1968).

Statistical methods

Graphical representations of the frequency distributions were constructed as suggested by STEWART (1969b). The investigation to determine which genetic models best account for the observed distributions for each of these characters has been carried out by the likelihood methods proposed by ELSTON and STEWART (1973). The principle of these methods is to calculate the likelihood of observing the data on the basis of a number of different genetic models, each likelihood being maximized with respect to those parameters necessary to define completely the theoretical frequency distribution. The models tested are all fully described and discussed by ELSTON and STEWART (1973). They may be summarized as follows: In all models the CBA/FaCam distribution was taken as normal with mean μ_1, and variance σ^2, i.e., $N(\mu_1, \sigma^2)$; Peru as $N(\mu_3, \sigma^2)$; and F_1 as $N(\mu_2, \sigma^2)$, where μ_1, μ_2, μ_3 and σ^2 are unknown parameters. The theoretical backcross distributions, corresponding to the genetic models considered, were:

1) *Single locus:* (A-1) Backcross to CBA is distributed as $\frac{1}{2} N(\mu_1, \sigma^2) + \frac{1}{2} N(\mu_2, \sigma^2)$; backcross to Peru is the same in this and succeeding models, simply replacing μ_1 by μ_3.

2) *Two equal additive unlinked loci.* A-2) Backcross to CBA is distributed as $\frac{1}{4} N(\mu_1, \sigma^2) + \frac{1}{2} N(\overline{\mu_1 + \mu_2}/2, \sigma^2) + \frac{1}{4} N(\mu_2, \sigma^2)$; backcross to Peru is similar.

3) *Equal and additive unlinked loci:* If there are l equal and additive loci, in each parent m ($< l - m$) acting in one direction and the remainder ($l - m$) acting in the opposite direction, the backcross to CBA is distributed as

$$\sum_{h=0}^{m} \sum_{k=0}^{l-m} \frac{1}{2^l} \binom{m}{h} \binom{l-m}{k} N(\mu_{1hk}, \sigma^2)$$

where

$$\mu_{1hk} = \frac{1}{l\,(l-2m)} \left[-h\{m\mu_1 - (l-m)\mu_3\} + k\{(l-m)\mu_1 - m\mu_3\} \right] + (l-h-k)\mu_2/l.$$

In general these models may be labelled A-*lm*. The following eight models of this type have been studied: A-30, A-31, A-50, A-51, A-52, A-60, A-61, A-62.

4) *Large number of equal additive unlinked loci:* Backcross to CBA is distributed as $N(\mu_1 + \mu_2/2, \sigma^2_\infty)$, where $\sigma^2_\infty = \sigma^2 + C(\mu_1 - \mu_3)^2$; C is a constant which has the value of 0 if all the alleles tending to increase the character are grouped in one parent, those decreasing it in the other parent (model A-L0); but which can have positive values otherwise (model A-LC).

5) *Two linked loci:* Backcross to CBA is distributed as $\frac{1}{2}(1-r) \cdot N(\mu_1, \sigma^2) + \frac{1}{2} r\, N(\mu_{12}, \sigma^2) + \frac{1}{2} r\, N(\mu_{21}, \sigma^2) + \frac{1}{2}(1-r) \cdot N(\mu_2, \sigma^2)$, where r = recombination frequency between the two loci (i.e., $r \leq 0.5$), and μ_{12} and μ_{21} are unknown means of the "recombinant" genotypes. Backcross to Peru is the same, with 1 exchanged for 3 in all subscripts.

This general two-locus model may have one or both of the following restrictions placed upon it:

'*Additivity*' of the two loci: $\mu_{12} + \mu_{21} = \mu_1 + \mu_2$

'*Symmetry*' (This corresponds to similar dominance ratios at each of the two loci when used in conjunction with the 'additivity' restriction.) This restriction comes in two forms,

a) $(\mu_{12} - \mu_{21})/(\mu_1 - \mu_2) = (\mu_{32} - \mu_{23})/(\mu_3 - \mu_2)$, appropriate when the recombinant means lie between the parental means, and

b) $(\mu_{12} - \mu_{21})^2 - (\mu_1 - \mu_2)^2 = (\mu_{32} - \mu_{23})^2 - (\mu_3 - \mu_2)^2$, which is appropriate when the recombinant means lie outside the parental means.

The model thus has four forms:

with neither restriction (model B-00);

with the 'additivity' restriction alone (model B-A0);

with the 'symmetry' restriction alone (model B-0S); and

with both the 'additivity' and 'symmetry' restrictions (model B-AS).

6) *One major locus and a large number of equal and additive loci:* Backcross to CBA is distributed as $\frac{1}{2} N(\mu_{12}, \sigma^2_\infty) + \frac{1}{2} N(\mu_{21}, \sigma^2_\infty)$; backcross to Peru similarly. This model was always subject to the symmetry condition $(\mu_{12} - \mu_{21})/(\mu_1 - \mu_2) = (\mu_{32} - \mu_{23})/(\mu_3 - \mu_2)$, i.e. the proportion of the parental difference due to the single locus was the same in both backcrosses. As in model A-LC,

$$\sigma^2_\infty = \sigma^2 + C(\mu_1 - \mu_3)^2.$$

This model has four forms:

$C > 0$, and no further restrictions (model C-OC)

$C > 0$, together with the 'additivity' condition. $\mu_{12} + \mu_{21} = \mu_1 + \mu_2$ (model C-AC)

$C = 0$ (i.e., all alleles tending to increase the character grouped in one parent), and no further restriction (model C-OO)

$C = 0$, and 'additivity'; (model C-AO)

Testing Goodness of Fit

Four methods of testing agreement between observed and theoretical distributions have been described by ELSTON and STEWART (1973). Each of these methods results in a χ^2 with five degrees of freedom corresponding to the five groups of data (CBA, Peru, F_1, backcross to CBA, backcross to Peru). Test U_1^2 is sensitive to differences in mean between observed and theoretical distribution; test U_2^2 to differences in variance; and tests $U_2'^2$ and L^2 detect relatively uneven spacings in the observed distribution.

Computation

The computations involved were performed on an IBM 360/75 computer at U.N.C., Chapel Hill, U.S.A. and on the Titan computer at the Mathematical Laboratory, University of Cambridge, England.

RESULTS

Tests for goodness of fit: Since the tests for goodness of fit will be used in the sequel, it is useful at this point to consider several general features of the results obtained using these tests. Values of χ^2_5 from each of the four tests are given for those models with the highest likelihood, for each of the fourteen characters, in Table 3 below.

As shown in Table 3, there are several characters (RP, INT, DUR, and possibly also ROM, TW and AW) where even the model with the highest likelihood fails to fit the data. Inspection of the data showed in each case that the reason for the poor fit was inadequacy of the 'environmental' part of the model (e.g., non-normality or unequal variances in the parental and F_1 groups, or large numbers of tied values). The fact that in each case there was a discernible reason for the high χ^2 is an empirical indication that the tests used do not give rise to falsely

high significance levels. At the same time, the battery of four tests does appear to have some power. Table 4 shows the results of the goodness of fit tests applied to models with relatively low likelihoods. For every character, at least one of the tests shows a rise in x^2_5, and in nearly all cases statistical significance is reached so that the tests exclude the low-likelihood models.

Selection of 'preferred models': The distributions of four of the fourteen characters, selected to illustrate the rangs of types of backcross distributions encountered, are given in Figures 1 through 4.

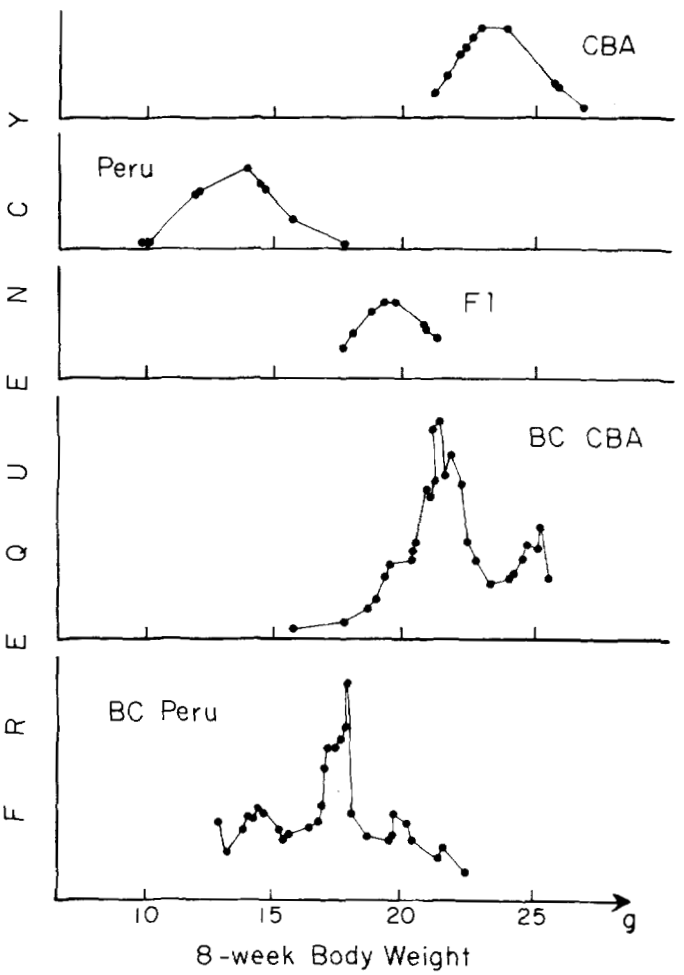

FIGURE 1.—Frequency distributions of "eight-week body weight", in CBA Peru, F_1 hybrids, backcross to CBA and backcross to Peru. Ordinate gives frequencies, at abscissa point x_0, calculated from the midpoint formula

$$\frac{28}{3x_3 + 2x_2 + x_1 - x_{-1} - 2x_{-2} - 3x_{-3}}$$

where $x_{-3}, x_{-2}, \ldots\ldots\ldots x_3$ are seven consecutive points on the abscissa.

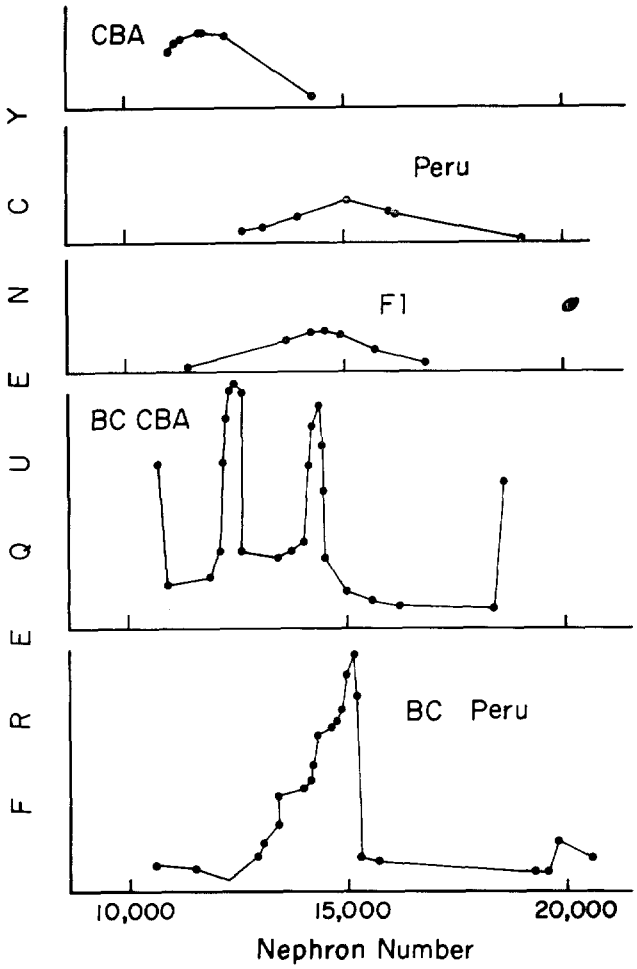

FIGURE 2.—Frequency distributions of "nephron number". Construction and key as for Figure 1.

The $\log_e$ likelihoods for each of the models described above, in each case maximized with respect to all the variable parameters, are given for all fourteen characters in Table 1. In evaluating the significance of relative likelihoods, a difference of less than 1.0 in log likelihood was considered as probably insignificant; between 1.0 and 2.0 as suggestive but not conclusive; and greater than 2.0 as probably significant. This is not to be considered as an exact method of determining significance, but simply a first approximation. It has intuitive appeal when it is realized that, for the simple situation in which one parameter is being estimated and the likelihood function has standard shape, a 95.4% confidence interval for that parameter is given approximately by the two values corresponding to a log likelihood of two less than the maximum (HUDSON 1971). In addition, of course, the goodness of fit tests can also be used when comparing the various

TABLE 1

Log_e likelihoods (plus or minus arbitrary constants) for each of twenty different genetic models, in each case maximized with respect to all the variable model parameters. The key to the fourteen different characters is given in the text.

TABLE 1A

Equal and additive unlinked loci

	Number of loci (number in repulsion)											
Model number:	1 A-1	2 A-2	3(0) A-30	3(1) A-31	5(0) A-50	5(1) A-51	5(2) A-52	6(0) A-60	6(1) A-61	6(2) A-62	∞ (O) A-LO	∞ (C) A-LC
Character												
5BW	—127.9	—127.6	—128.0	—134.8	—128.5	—133.1	—140.2	—128.7	—128.4	—130.2	—129.8	—129.1
6BW	—119.9	—117.9	—117.4	—127.1	—117.1	—118.0	—132.0	—117.0	—117.4	—121.6	—116.8	(—116.7)
8BW	—118.0	—114.2	—113.9	—129.4	—114.0	—114.4	—136.1	—114.1	—114.0	—120.2	—114.8	—114.2
RC	—242.1	—241.3	—241.3	—246.6	—241.4	—241.4	—250.0	—241.4	—241.3	—243.0	—241.7	—241.5
RN	71.7	71.8	71.9	72.7	72.0	72.1	73.0	72.0	72.1	72.6	72.1	73.4
ROM	58.3	58.5	58.6	57.4	58.6	58.5	56.7	58.7	58.6	58.1	58.8	(59.2)
RIM	—69.9	—69.6	—69.5	—72.9	—69.5	—69.6	—75.2	—69.5	—69.5	—70.6	—69.5	(—69.5)
RP	—6.5	—6.6	—6.6	—6.4	—6.6	—6.6	—6.3	—6.6	—6.6	—6.5	—6.6	—6.2
TW	131.4	134.8	136.1	131.5	136.9	136.4	129.3	137.3	136.9	134.7	138.4	(138.8)
AW	280.7	281.0	280.9	278.0	280.8	280.3	276.7	280.7	280.5	279.3	280.3	(281.6)
ZG	114.0	108.9	106.2	98.6	102.6	102.9	94.8	101.6	101.9	101.4	96.3	99.4
INT	—301.8	—308.9	—309.2	—308.7	—309.3	—309.2	—308.8	—309.3	—309.3	—309.1	—309.5	—308.9
LAT	—35.9	—35.9	—35.9	—35.6	35.9	—35.9	—35.3	—35.9	—35.9	—35.7	—35.9	—34.9
DUR	—6.2	—5.9	—5.8	—5.7	—5.7	—5.7	—5.7	—5.7	—5.7	—5.7	—5.7	—5.6

* Recombinant means lie outside parental means: appropriate symmetry restriction applied.

A likelihood enclosed in parentheses indicates that the backcross variance σ^2_∞ is less than the within-genotype environmental variance σ^2.

The "preferred" model for each character is underlined twice. The model with the highest likelihood, where different from the preferred model, is underlined once.

TABLE 1B

Two loci

Model number:	Unrestricted B-OO	Additivity alone B-AO	Symmetry alone B-OS	Additivity and symmetry B-AS
Character				
5BW	—123.5	—127.4	—123.6	—127.4
6BW	—114.9	—117.9	—114.9	—117.9
8BW	—114.0	—114.2	—114.0	—114.2
RC	—240.8	—241.3	—241.1	—241.3
RN	80.8	78.5	80.1*	77.3*
ROM	61.4	58.6	61.3	58.5
RIM	—66.2	—69.6	—66.3	—69.6
RP	—3.7	—5.5	—3.9*	—5.5*
TW	141.4	135.0	141.4	134.9
AW	281.8	281.0	281.8	281.0
ZG	115.7	114.1	114.6	114.1
INT	—305.2	—306.0	—305.9*	—306.3*
LAT	—33.1	—34.5	—33.6*	—35.2*
DUR	—3.9	—5.5	—3.9*	—6.0*

TABLE 1C

One major locus in combination with large number of equal additive loci

Model number	C=O Symmetry alone C-OO	C=O Additivity and symmetry C-AO	C>O Symmetry alone C-OC	C>O Additivity and symmetry C-AC
Character				
5BW	—124.8	—127.5	(—124.6)	(—127.5)
6BW	—115.4	—116.8	(—113.3)	(—116.7)
8BW	—114.0	—114.2	(—112.9)	(—113.6)
RC	—241.2	—241.2	(—241.1)	(—241.2)
RN	72.4	72.1	73.9	73.4
ROM	62.5	58.8	(63.0)	(59.1)
RIM	—65.0	—69.5	(—64.8)	(—69.1)
RP	—6.1	—6.4	—5.8	—6.1
TW	145.1	138.4	(145.2)	(138.8)
AW	281.4	281.0	(285.1)	(284.6)
ZG	114.7	114.1	(116.4)	(114.7)
INT	—307.2	—306.6	(—306.0)	(—306.5)
LAT	—35.8	—35.9	—34.8	—34.9
DUR	—5.3	—5.7	—5.2	—5.6

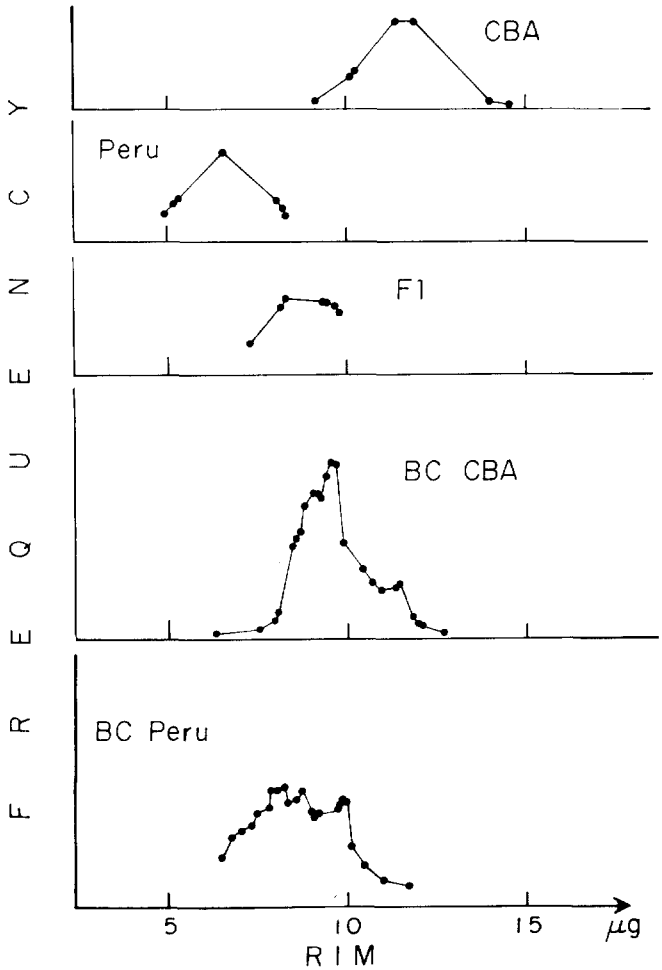

FIGURE 3.—Frequency distributions of "weight of nephron segments in inner medulla". Construction and key as for Figure 1.

genetic models. Except where specifically noted to the contrary, the models with the highest likelihoods were always consistent with the data as judged by each of the four goodness of fit tests. Each character will now be discussed in turn, and the basis on which a 'preferred' model was selected will be explained. The values of the parameters estimated by maximum likelihood, together with standard errors, for each of these 'preferred' solutions (and also, where different, for the models which actually had the highest likelihood) are all given in Table 2.

5BW: The model with the greatest likelihood was B-OO, two loci without any restrictions. However, imposing the 'symmetry' restriction B-OS led only to a tiny decrease (0.1) in the log likelihood. All models in which the 'additivity' restriction was imposed (all A-models, B-AO, B-AS, C-AO) had markedly lower likelihoods, indicating that gene interaction was almost certainly occurring. The

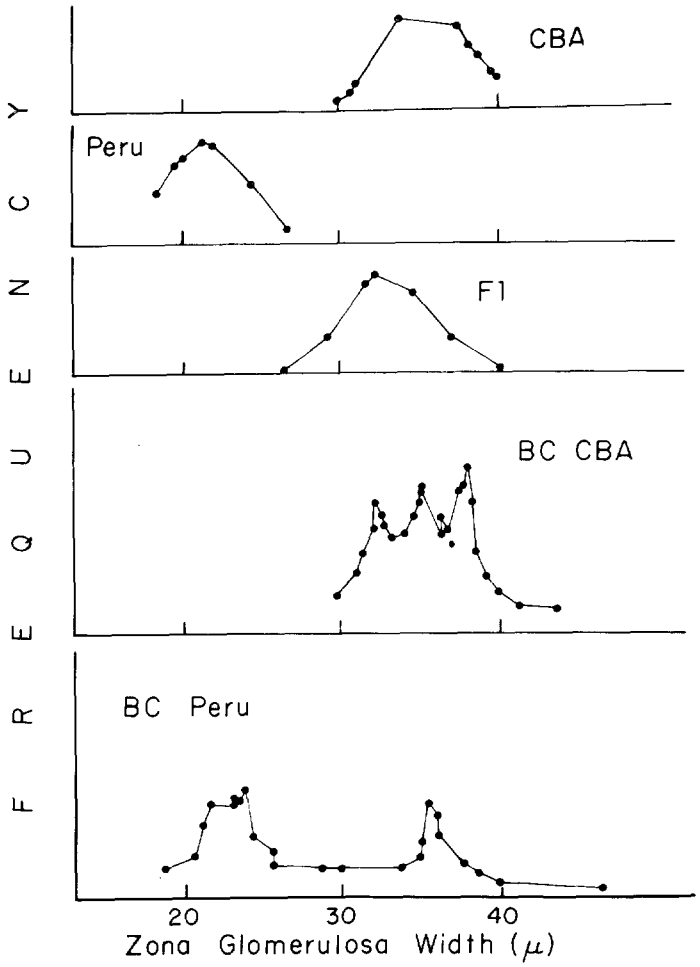

FIGURE 4.—Frequency distributions of "width of zona glomerulosa". Construction and key as for Figure 1.

model with two interacting loci was also superior to that of one major locus interacting with 'polygenes', C-00. The 'preferred' model was thus B-OS. The nature of the gene-gene interaction involved can be seen from Table 2. In both backcrosses, both 'recombinant' means are close to the upper of the two 'parental' means. This implies that CBA alleles at either of the two loci alone have nearly as much effect in increasing body weight as both loci together. The two loci appear to be unlinked.

6BW: The pattern of likelihoods was very similar to that of 5BW (as might be expected on the basis of the similarity in the characters), and similar remarks apply; the 'preferred' model was B-OS.

8BW: In this case a relatively simple model, two equal, additive, unlinked loci (A-2), had a log likelihood which came within 0.3 of that achieved by any of the

more complex models. On the other hand, it differed sharply from the single-locus model which had a log likelihood of 3.8 less (see Figure 1, in which the central peak in both backcross distributions, presumably due to genetic recombination, is clearly visible). Model A-2 was thus 'preferred'. However, it should be noted that model A-LO, many genes all in coupling phase in the parental strain, had a likelihood only 0.6 less than A-2; and model A-LC, many genes with some in repulsion phase, had a likelihood equal to A-2. Thus even though two loci are adequate to account for the observed distributions of 8BW (Table 3), it is not possible to exclude the involvement of more loci.

RC: The pattern of likelihoods is similar to that for 8BW, although less sharply differentiated. Model A-2 was preferred, although again models with more loci cannot be excluded.

RN: The 'two-loci' models (B) have log likelihoods at least 5.0 greater than any other models considered. The reason for the general superiority of two-locus models may be seen by inspection of the frequency distributions (Figure 2). Both backcross distributions contain a number of 'out-lying' points at both upper and lower ends of the distribution. The two-locus models are able to account for these points as recombinants, the loci being linked in repulsion phase in the parental strains. The 'symmetry' restriction, necessary to remove a rather odd placement of recombinant means (see Table 2), caused a reduction of 0.7 in log likelihood. The strong 'additivity' restriction caused a further reduction of 2.2 in log likelihood. The resulting model B-AS was taken as 'preferred', although the maximum likelihood model B-OO was also considered. The placing of recombinant means outside the parental means, suggested by subjective inspection of the frequency distribution, is confirmed by the results shown in Table 2. The recombination frequency between the two postulated loci must presumably be greater than zero (otherwise a one-locus model would fit the data equally well); on the other hand, the maximum likelihood estimate of 16% is significantly less than 50% (Table 2), indicating that the loci are linked.

ROM: Models in which genetic interaction was allowed, B-00, B-OS and C-00, had likelihoods at least 2.5 greater than any others. Among these models, a single major locus interacting with 'polygenes', C-00 had a log likelihood 1.2 greater than the others, and was therefore 'preferred'. This 'single major locus' accounts for an estimated 26% of the difference between the means of the parental strains. It should be noted that goodness of fit tests $U'_2{}^2$ and L^2 indicated statistical significance at the 5% level even for model C-00 (Table 3). Inspection shows that this was due to non-normality in the distribution of the Peru parent (four of seven values clustered very closely at the top of the range).

RIM: The pattern of likelihoods is similar to that for the previous character, and the same general model C-00 is selected. However, in this case, the 'single major locus' accounts only for an estimated 0.3% of the parental difference. Thus in contrast to most of the other characters analyzed in this paper, there is no indication that a limited number of loci (one or two) are responsible for a significant proportion of the observed variation in the character. The frequency distribution for this character is shown in Figure 3.

TABLE 2

Maximum likelihood values of model parameters for "preferred" and, where different, maximum likelihood models for all fourteen characters

Character	Model number	Parameter: μ_1	μ_3	μ_2	μ_{12}	μ_{21}	μ_{32}	μ_{23}	σ^2	r
5BW	B-OS	17.9 ±0.6	10.9 ±0.5	12.8 ±0.6	17.8 ±3.4	17.5 ±3.5	13.2 ±1.5	13.0 ±1.4	3.46 ±0.62	0.5
6BW	B-OS	19.7 ±0.6	12.9 ±0.5	16.4 ±0.5	20.3 ±1.3	20.2 ±1.3	15.3 ±1.3	15.3 ±1.3	3.45 ±0.63	0.5
8BW	A-2	23.6 ±0.5	14.2 ±0.5	19.9 ±0.4	(21.7	21.7	17.0	17.0)	3.26 ±0.68	(0.5)
RC	A-2	155 ±5	91 ±5	115 ±4	(135	135	104	104)	311 ±77	(0.5)
RN ($\times 10^4$)	B-AS	1.29 ±0.04	1.56 ±0.04	1.50 ±0.03	0.88 ±0.07	1.90 ±0.06	1.03 ±0.07	2.03 ±0.06	0.017 ±0.004	0.16 ±0.06
", M.L.	B-OO	1.29 ±0.04	1.55 ±0.05	1.48 ±0.04	1.32 ±0.14	1.92 ±0.12	1.49 ±0.17	2.06 ±0.07	0.0196 ±0.004	0.26 ±0.10
ROM	C-OO	2.54 ±0.08	2.18 ±0.08	2.33 ±0.07	2.67 ±0.10	2.61 ±0.10	2.43 ±0.15	2.39 ±0.15	0.053 ±0.011	(1.0)
RIM	C-OO	11.4 ±0.6	5.7 ±0.6	8.3 ±0.6	9.0 ±0.3	9.0 ±0.3	8.1 ±0.3	8.1 ±0.3	2.56 ±0.44	(1.0)

RP	B-AS	2.19 ±0.18	1.70 ±0.18	1.91 ±0.16	0.58 ±0.89	3.49 ±0.85	0.35 ±0.89	3.23 ±0.85	0.335 ±0.083	0.07 ±0.11
″, M.L.	B-OO	2.15 ±0.19	1.69 ±0.18	1.78 ±0.17	1.96 ±0.79	3.45 ±0.62	1.58 ±0.72	3.03 ±0.45	0.30 ±0.07	0.23 ±0.20
TW	C-OO	1.54 ±0.04	1.79 ±0.04	1.81 ±0.04	1.66 ±0.09	1.65 ±0.09	1.92 ±0.02	1.92 ±0.02	0.014 ±0.002	(1.0)
AW	B-OO	0.133 ±0.005	0.175 ±0.005	0.136 ±0.004	0.138 ±0.007	0.138 ±0.007	0.143 ±0.004	0.144 ±0.004	4.3×10^{4} $\pm0.7\times10^{4}$	0.5
Z G	A-1	2.20 ±0.03	1.82 ±0.03	2.17 ±0.02	—	—	—	—	0.012 ±0.003	—
″, M.L.	B-OO	2.19 ±0.03	1.70 ±0.03	2.14 ±0.03	2.21 ±0.07	2.21 ±0.07	1.78 ±0.06	2.25 ±0.05	0.010 ±0.002	0.5
INT	B-AS	47 ±6	72 ±7	53 ±6	41 ±9	59 ±9	31 ±9	93 ±9	367 ±80	0.13 ±0.21
″, M.L.	B-OO	46 ±5	72 ±7	60 ±6	44 ±12	44 ±12	87 ±17	24 ±12	349 ±77	0.50 ±0.25
LAT	B-OS	3.90 ±0.20	4.68 ±0.24	4.62 ±0.20	5.68 ±0.68	3.29 ±0.57	2.67 ±0.64	5.00 ±0.89	0.56 ±0.13	0.24 ±0.16
DUR	B-OS	3.16 ±0.14	2.81 ±0.15	2.71 ±0.13	1.49 ±0.55	3.00 ±0.60	1.40 ±0.48	0.62 ±0.62	0.29 ±0.06	0.16 ±0.10

TABLE 3

Four tests for goodness of fit for preferred and, where different, maximum likelihood models for all fourteen characters. The tests are described in detail by ELSTON and STEWART *(accompanying paper). Each test is a χ^2 with five degrees of freedom*

Character	Model number	Goodness-of-fit (χ_5^2) U_1^2	U_2^2	U'^2_2	L^2
5BW	B-OS	0.7	3.4	6.1	2.2
6BW	B-OS	0.8	7.3	7.5	4.0
8BW	A-2	1.0	3.9	7.9	4.4
RC	A-2	0.4	0.2	6.9	3.1
RN (preferred)	B-AS	2.2	4.9	3.9	4.6
RN (M.L.)	B-OO	1.8	3.7	3.6	3.0
ROM	C-OO	0.9	8.6	12.3*	11.0*
RIM	C-OO	0.1	4.2	9.7	6.3
RP (preferred)	B-AS	1.2	0.8	20.1**	20.4**
RP (M.L.)	B-OO	0.7	0.7	20.4**	21.8**
TW	C-OO	0.6	14.1*	3.0	12.6*
AW	B-OO	0.3	26.0**	8.3	14.5*
ZG (preferred)	A-1	2.7	2.7	4.5	1.0
ZG (M.L.)	B-OO	0.7	3.8	4.9	1.5
INT (preferred)	B-AS	1.1	5.5	84.6*	22.4**
INT (M.L.)	B-OO	0.5	7.8	84.4*	21.9*
LAT	B-OS	0.3	9.1	3.6	9.0
DUR	B-OS	1.5	5.8	40.8**	20.1**

* Denotes significance at the 5% level.
** At the 1% level.

RP: The 'two-locus models' (B) have log likelihoods 0.3–2.2 greater than all others. As for the character RN, inspection of the frequency distribution and recombinant means (Table 2) indicates that the reason is the apparent existence of 'recombinant' individuals lying outside the range of the parental distributions at both ends of both backcross distributions. The two loci appear to be linked (maximum likelihood estimate of 7% recombination) in repulsion phase. The significant deviation between the observed distribution and that predicted by models B-OO and B-AS (Table 3) was due to non-normality in the parental and F_1 distributions.

TW: Model C-OO (major locus + interacting polygenes) has much the highest likelihood. However, this 'major locus' accounts for only 4% of the parental difference (Table 2). The significant deviation between the data and model C-OO, indicated by tests U_2^2 and L^2 (Table 3) was due to unequal variances in the parental stocks, that in Peru being much greater than in CBA or F_1.

AW: In general the discrimination between the various models was not very sharp. The two-locus model B-OS, with symmetry restriction alone, and recombinant means lying between parental means, had the highest likelihood. The poor fit of even model B-OO to the data (tests U_2^2 and L^2 in Table 3) was again due to the variance in Peru's being much higher than in CBA or F_1.

ZG: The single-locus model has a markedly higher likelihood than any of the

other 'equal, additive, unlinked loci' models (A). In fact only one other model, B-OO, has an appreciably higher likelihood; and inspection of this model (Table 2) shows that none of the 'recombinant' means is significantly different from the respective 'parental' and 'F_1' means. Thus the single-locus model (A-1) is definitely 'preferred', although model B-00 is also given in Table 2. The distinctive distribution of this character, with two clearly separate peaks in the backcross to the recessive parent, is shown in Figure 4.

INT: The two-locus models (B) have log likelihoods that are about 3 greater than the other models. Inspection of the frequency distributions and 'recombinant' means (Table 2) shows that this is due to outlying 'recombinants'. The 'addivity' restriction is necessary to avoid a rather odd distribution of recombinant means in the backcross to CBA; model B-AS is thus preferred; model B-OO, which actually gives the greatest likelihood, is also given in Table 2. Taking the 'preferred' case, the two loci appear to be linked in repulsion phase (maximum likelihood estimate of recombination frequency 13%, although the standard error is rather large). Tests U'^2_2 and L^2 in Table 3 indicate that even models B-OO and B-AS are inadequate to account for the experimental data. Inspection shows that this was due to a large number of tied values, particularly in the backcross generations.

LAT: The two-locus model B-OO gives the highest likelihood, but imposing the appropriate 'symmetry' restriction caused only a trivial decrease in likelihood. This model B-OS is thus preferred, since imposing 'additivity' caused a decrease in 1.9 in likelihood; there is thus some indication of genetic interaction. This is yet another case where the two loci appear to be linked (estimated recombination frequency of 0.24, although again with rather large standard error), and in repulsion phase, both 'recombinant' means lying outside the parental means in each backcross.

DUR: The two-locus model with 'symmetry' B-OS again has the highest likelihood; and again (Table 2) the loci appear to be linked. An interaction between the two loci results in a low value for one of the recombinant genotypes in each backcross; apart from this, all genotypes appear to have similar values (about 3.0). The high significance levels indicated by tests U'^2_2 and L^2 in Table 3 were due to a large number of tied values in the backcross generations; as for the character INT, this resulted from use of a scale of measurement that was not truly continuous.

DISCUSSION

The goodness of fit tests used in Table 3 indicated that for six characters (ROM, RP, TW, AW, INT and DUR), even the model with the highest likelihood failed to provide an adequate account of the experimental data. However, as discussed in detail above, inspection of the distributions revealed that in each case the reason for the poor fit lay in an inadequacy of the environmental part of the model, i.e., in the general assumption that each genotype has a phenotypic distribution which is normal and has a common variance. In principle this problem could be dealt with by allowing different genotypes to have different phenotypic

distributions; but this approach cannot be recommended since the large number of additional parameters involved would result in methods with very little power. A more satisfactory approach would be to redesign the experimental measurements so that the assumption of normal distributions with common variance would be closer to reality.

In practice, however, it appears that the likelihood methods used here have considerable robustness, even when the assumptions that the environmental variation is normally distributed, and equal for all genotypes, is seriously violated. Even for the six characters ROM, RP, TW, AW, INT, and DUR, the likelihood methods converged to give reasonable parameter estimates for all the twenty genetic models considered; and the differences in likelihood between the various models were still sufficient to permit discrimination among them.

The utility of the likelihood methods is reinforced by the finding that a significant deviation between observations and the 'preferred' model was never due, for any of the fourteen characters studied here, to an inadequacy in the *genetic* part of the model (i.e., numbers, relative frequencies and phenotypic means of backcross genotypes). This is an indication that the range of genetic models considered here, while clearly not exhaustive, may be adequate to approximate most of the cases that are distinguishable in practice. To the extent that these twenty models do provide an approximation to an exhaustive list of all possible models, the use of relative likelihoods to select a 'preferred' model is strengthened. As a summary of the powers of these methods, it may be noted that taking an average for all the characters, the likelihood ratio between the best and the worst models was approximately $e^{9.5}$, or more than 10,000-fold.

A major finding of this paper is that genetic variation in eleven of the fourteen characters studied could be adequately explained in terms of only one or two loci. The fact that for three of the characters (ROM, RIM and TW) a one- or two-locus model did appear relatively inadequate, demonstrates that this general success of one- or two-locus models is not simply an artifact of the statistical methods. This finding is potentially of considerable importance, since it indicates that even in species without the special genetic advantages of *Drosophila*, it may be possible to identify individually at least some of the genes responsible for naturally-occurring variation within the range of 'normality' (cf. THODAY 1961; SPICKETT and THODAY 1966).

It should be noted that neither the pair of strains of mice used in this work, nor the fourteen characters whose inheritance has been investigated in this paper, are completely typical. The fourteen characters studied here were constructed and/or selected from a longer list of 36 characters, specifically with a view to their patterns of inheritance. Moreover, the 36 characters were themselves chosen because previous work had suggested that these two strains of mice might differ with respect to these characters. The strains CBA/FaCam and Peru were also specially chosen because it was suspected that they might differ widely. CBA/FaCam are 'typical' of laboratory strains of mice. The Peru mice, on the other hand, are relatively recent descendants of wild mice, and are still small in size and wild in general behavior. This might be a partial explanation of why two loci appear

TABLE 4

Goodness of fit tests applied to genetic models with relatively low likelihoods. ΔL indicates difference in log likelihood between "preferred" model and that with lower likelihood. Levels of significance as in Table 3

Character	Model number	ΔL	Goodness-of-fit (χ_5^2) U_1^2	U_2^2	U'^2_2	L^2
5BW	A-52	16.6	15.0**	12.6*	8.3	2.8
6BW	A-52	17.1	15.5**	13.3*	9.1	2.9
8BW	A-52	21.9	14.9*	13.0*	9.0	10.6
RC	A-52	8.7	8.6	6.8	3.7	7.0
RN	A-60	5.2	1.1	4.2	9.9	12.1*
ROM	A-52	5.8	7.9	4.8	17.2**	11.9*
RIM	A-52	10.2	8.8	8.5	16.0**	12.7*
RP	A-52	0.8	1.6	1.2	20.6**	20.7**
TW	A-52	15.8	19.4**	20.9**	6.5	17.7**
AW	A-52	5.1	2.5	24.2**	6.0	7.1
ZG	A-52	19.2	7.6	22.2**	3.0	5.8
INT	A-60	3.0	0.9	11.1*	84.4**	20.1**
LAT	A-Lo	1.8	0.5	11.0*	4.3	9.5
DUR	A-52	1.8	1.6	5.7	40.9**	19.2**

adequate to account for the strain difference in body weight. Although single gene loci can cause large differences in body weight in mice (SNELL, 1929; SCHAIBLE and GOWEN 1961), the difference between large and small lines of mice produced by selection from heterogeneous laboratory stocks usually seems to be mediated by many loci (FALCONER 1953, 1960; CHAI 1956; ROBERTS 1966). The body weight difference between Peru and CBA/FaCam mice may thus not be typical of accumulated differences between laboratory stocks.

On the other hand, the atypical nature of these studies should not be exaggerated. It has generally been found that for each of the 36 characters on which this work was based, other laboratory strains of mice differ from CBA/FaCam at least as greatly as the Peru mice, so that the CBA/FaCam *vs.* Peru differences are in no way unique or exceptional (SHIRE 1969b). Also, the characters used in this study were certainly not remarkable for their physiological sophistication or specificity; and in fact even more clear-cut differences can be anticipated when greater physiological specificity is achieved (SPICKETT, SHIRE and STEWART 1967).

If it does prove possible to identify some of the individual genes causing naturally-occurring genetic variation, two further lines of investigation will become possible. The first concerns the mode of action of the individual genes identified by these techniques (SPICKETT and THODAY 1966). Recent work (STEWART 1971) indicates that such studies are both possible and fruitful. The second line of investigation concerns linkage relationships between such genes. MATHER (1943) has proposed that in natural populations these genes will be linked in balanced combinations, so that the potential variability of a population is greater than the variation actually expressed; if the individual genes can be

located, it would be possible to test this hypothesis directly. The linkage relationships between the genes postulated in this paper will be the subject of a subsequent paper. For the moment, it may conceivably be relevant that for five out of the fourteen characters studied in this paper (RN, RP, INT, LAT, DUR), the 'preferred' model consisted of two loci that were both linked (with recombination frequencies significantly less than 50%) and in 'repulsion' phase, i.e., a 'balanced' combination, in the parental strains.

It should be emphasized, however, that the methods described in this paper will rarely be sufficient to 'prove' a particular mode of inheritance. It will often be possible to exclude alternative genetic models, and to select a 'preferred' model which is adequate to account for the observed data; but the 'preferred' model is only a hypothesis which should then be examined further by progeny-testing (WRIGHT 1934). The usefulness of the methods of this paper lies in the construction of meaningful hypotheses from the most readily-available genetic data, i.e., first-generation hybrids and backcrosses. The range of genetic models that can be considered by the likelihood approach adopted here is substantially greater than that assumed in calculations of the number of effective factors (WRIGHT 1934, 1968). Moreover, as pointed out by ELSTON and STEWART (1973), the likelihood methods are statistically more efficient.

Verification of a specific hypothesis by progeny-testing is easiest when the genetic model is simple, in particular when a single locus is involved. The likelihood methods used in this paper have resulted in a 'preferred' model of a single locus in two instances. The first instance is the character 'zona glomurulosa' in the present paper. The hypothesis of a single locus has been tested and confirmed by progeny-testing backcross individuals (SHIRE 1969a). The second instance is the character 'Rate of sodium excretion following saline load', which differs between strains CBA/FaCam and RAP (STEWART and MOWBRAY 1972). The 'preferred' model from the same likelihood methods as used in this paper was that of a single 'major' gene interacting with many small loci, (C-OO); the major locus accounted for an estimated 98% of the parental difference. In this case also progeny-testing confirmed the hypothesis of a single locus (STEWART and MOWBRAY 1972). The likelihood methods suggested here can ultimately only be validated by comparing their results with those of alternative (if more time-consuming) methods of genetic analysis. In the only two instances so far available, agreement between the likelihood and alternative methods is good.

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LITERATURE CITED

BADR, F. M., J. G. M. SHIRE and S. G. SPICKETT, 1968 Genetic variation in adrenal weight: strain differences in the development of the adrenal glands of mice. Acta endocrinologica **58**: 191–201.

CHAI, C. K., 1956 Analysis of quantitative inheritance of body size in mice. II. Gene action and segregation. Genetics **41**: 165–178.

CHARLESWORTH, D., 1969 Small, inherited differences in blood glucose levels in mice. Genet. Res. **14**: 1–7.

ELSTON, R. C. and J. STEWART, 1973 The analysis of quantitative traits for simple genetic models from parental, F_1 and backcross data. Genetics (this volume).

FALCONER, D. S., 1953 Selection for large and small size in mice. J. Genet. **52**: 470–501. ——, 1960 *Introduction to quantitative genetics*. p. 219. Oliver and Boyd, Edinburgh.

HUDSON, D. J., 1971 Interval estimation from the likelihood function. J. Royal Statistical Soc. B **33**: 256–262.

MATHER, K., 1943 Polygenic inheritance and natural selection. Biol. Rev. **18**: 32–64.

ROBERTS, R. C., 1966 The limits to artificial selection for body weight in the mouse. I. The limits attained in earlier experiments. Genet. Res. **8**: 347–360.

DEROUFFIGNAC, C., J. STEWART and F. MOREL, 1970 Étude par microponction de l'élaboration de l'urine. IV. Chez la souris en diurèse saline. Nephron **7**: 350–362.

SCHAIBLE, R. and J. W. GOWEN, 1961 A new dwarf mouse. Genetics **46**: 896.

SHIRE, J. G. M., 1968 'Genes, Hormones and Behavioural Variation'. In: *Genetic and environmental influences on behaviour*. pp. 194–205. Edited by J. M. THODAY and A. S. PARKS. Oliver and Boyd, Edinburgh. ——, 1969a A strain-difference in the adrenal zona glomerulosa determined by one gene-locus. Endrocinology **85**: 415–422. ——, 1969b "Genetics and the Study of Adrenal and Renal Function in Mice". pp. 292–296. In: *Progress in Endocrinology*. Edited by C. GUAL. (Proceedings of the third International Congress of Endocrinology.) Excerpta Medica, New York/Amsterdam.

SNELL, G. D., 1929 Dwarf, a new mendelian recessive character of the house mouse. Proc. Nat. Acad. Sci. U.S. **15**: 733–734.

SPICKETT, S. G. and J. M. THODAY, 1966 Regular responses to selection. III. Interaction between located polygenes. Genet. Res. **7**: 96–121.

SPICKETT, S. G., J. G. M. SHIRE and J. STEWART, 1967 Genetic variation in adrenal and renal structure and function. Mem. Soc. Endocrinol. **15**: 271–288.

STEWART, J., 1968 Diuretic responses to waterload in four strains of mice. J. Physiol. **198**: 355–363. ——, 1969a Diuretic responses to electrolyte loads in four strains of mice. Comp. Biochem. Physiol. **30**: 977–987. ——, 1969b Biometrical genetics with one or two loci: I. The choice of a specific genetic model. Heredity **24**: 211–224. ——, 1969c Biometrical genetics with one or two loci: II. The estimation of linkage. Heredity **24**: 225–238. ——, 1971 Renal concentrating ability in mice: a model for the use of genetic variation in elucidating relationships between structure and function. Pflugers Arch. Eur. J. Physiol. **327**: 1–15.

STEWART, J. and S. MOWBRAY, 1972 Genetical analysis of physiological homeostasis: GFR following saline loading in mice. Genet. Res. **19**: 61–72.

STEWART, J. and S. G. SPICKETT, 1967 Genetic variation in diuretic responses: further and correlated responses to selection. Genet. Res. **10**: 95–106.

THODAY, J. M., 1961 Location of polygenes. Nature **191**: 368–370.

WRIGHT, S., 1934 The results of crosses between inbred strains of guinea pigs differing in number of digits. Genetics **19**: 537–551. ——, 1968 *Evolution and the genetics of populations*. I. Genetic and Biometric Foundations pp. 381–403. University of Chicago Press.