

Factors affecting Vertebral Variation in Mice

1. Variation within an Inbred Strain

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WITH ONE PLATE

INTRODUCTION

A SURVEY by Green (1941) of skeletal types in different strains of mice revealed that whereas the C57BL strain has 6 lumbar vertebrae in over 95 per cent. of mice, there are substrains of the C3H strain which show an equally strong tendency in favour of 5 lumbar vertebrae. Russell & Green (1943) and Green & Russell (1951) have since demonstrated a marked difference between the reciprocal hybrids when the two strains are crossed, the deviation being in the maternal direction. For distinguishing between two alternative interpretations—the effects of the egg cytoplasm and the effects of the uterine environment—Green & Russell suggest that the transplantation of eggs or of ovaries may prove to be of value.

As a preliminary to attempting such transplantations we have been making a survey of vertebral type in samples of various substrains of the C3H strain. Recently Green (1953) has published a report of a similar survey which shows that two distinct vertebral types of C3H substrain exist. One of the three main substrains, C3H/He, has predominantly 6 lumbar vertebrae. (For the nomenclature of inbred strains and substrains of mice see the Committee on Standardized Nomenclature for Inbred Strains of Mice, *Cancer Research*, 1952, **12**: 602–13.) The other two—the C3H/St and C3H/Bi substrains—have predominantly 5 lumbar vertebrae.

The same conclusion has emerged from our own survey, the results of which we publish here. It will be seen that these results confirm and amplify Green's findings, and also indicate some of the factors affecting vertebral variation within C3H substrains.

MATERIALS AND METHODS

The first task was to test whether X-ray photography *in vivo* could be used to determine vertebral type with a reliability comparable to the method used in

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the previous surveys—namely the preparation of alizarin transparencies of dead mice. Twelve C57BL and seventeen C3H/He mice, belonging to five distinct vertebral types, were classified by X-ray photography and then made into alizarin preparations and again classified. The two classifications were identical. Thenceforth all material, whether living or dead, was classified solely by X-ray photography, since this method permits the survival of classified mice for subsequent breeding.

The number of lumbar vertebrae can vary as a consequence of variation either in the number of vertebrae classified as thoracic by their articulation with ribs, or in the number classified as sacral by their articulation with the sacrum. A defect of X-ray photography is that unless great care is taken in placing the mouse on the film the ribs cannot always be counted so as to allow this distinction to be made. But the C3H strain is so constant in number of ribs, as shown by Green's (1953) survey, that this defect is assumed to be of no consequence in the present work.

We have sometimes been in doubt as to whether a transverse process should be classified as lumbar or sacral in type. In such cases we have been guided by the shape of the process and the presence of an articular surface, rather than by whether or not a functional articulation appears to exist. This decision was taken because the former criterion is less affected than the latter by slight variations in the angle at which the mouse is placed relative to the X-ray tube. Plate 1, fig. D, shows a marginal case which was actually classified as sacral although a clear space can be seen between the sacrum and the articular surface of the transverse process on the left-hand side (right side of mouse). Photographs of 6/6, 5/5, and 6/5 vertebral types are shown in Plate 1, figs. A, B, and C, for comparison (see explanation of Plate 1 for notation). The mice were placed on their backs directly on the film envelope. For live mice the procedure eventually adopted was to strap the anaesthetized mouse down with two transverse strips of cellulose tape 2 in. in width, covering the whole mouse apart from the nose and tail. The mouse will then usually remain motionless even if it recovers from the anaesthetic while still imprisoned. By this method we have photographed as many as a dozen live mice at a single exposure.

Some of our material was bred by ourselves from foundation stocks supplied by other workers, some of it was sent to us preserved; in some instances we were able to X-ray samples of living mice in the laboratories in which they were maintained, and in one case finished X-ray photographs were sent to us.

RESULTS

The new data are given in Table 1, and Green's (1953) data in Table 2. In Text-fig. 1 are set out the genealogical relationships of the various colonies from which the samples in the two bodies of data were taken. The genealogical information was elicited by personal correspondence and some of the dates given for the transfer of stocks may be subject to small errors.

In addition, data on one colony of the C3H/He substrain are set out *in extenso* in Table 3 for reasons which will be explained in the course of the discussion.

TABLE 1

New data on the distribution of vertebral types in samples of C3H mice. For each sample the top row, or rows, of figures gives the actual numbers and the bottom row gives percentages. The samples from Green, Craigie, Wilson, and Howard include descendants of the original samples raised by us

Strain	Origin	Vertebral types				Total
		5/5	6/5	5/6	6/6	
C3H/St	Strong in 1953	24	4	1	9	38
		63.2	10.5	2.6	23.7	100.0
C3H/St	Wilson in 1952	64	1	0	0	65
		98.5	1.5	0.0	0.0	100.0
C3H/Bi	Green in 1952 ♀♀	10	0	0	0	10
		19	0	0	0	19
	♂♂	29	0	0	0	29
		100.0	0.0	0.0	0.0	100.0
C3H/Bi	Dmochowski in 1953	18	3	1	4	26
		69.2	11.5	3.8	15.4	99.9
C3H/Bi	Foulds in 1951	22	0	2	2	26
		84.6	0.0	7.7	7.7	100.0
C3H/Bi	Craigie in 1953 ♀♀	12	6	1	4	23
		41	2	1	0	44
	♂♂	53	8	2	4	67
		79.1	11.9	3.0	6.0	100.0
C3H/Bi	Woodhouse in 1953	23	1	0	0	24
		95.8	4.2	0.0	0.0	100.0
C3H/He	Hewitt in 1951	6	0	0	23	29
		20.7	0.0	0.0	79.3	100.0
C3H/He	Paterson in 1952	0	1	1	28	30
		0.0	3.3	3.3	93.3	99.9
C3H/He	Heston in 1953 ♀♀	0	1	0	14	15
		0	0	1	14	15
	♂♂	0	1	1	28	30
		0.0	3.3	3.3	93.3	99.9
C3H/He	Howard in 1950 ♀♀	6	1	0	54	61
		10	7	1	67	85
	♂♂	16	8	1	121	146
		11.0	5.5	0.7	82.9	100.1

TABLE 2

Green's (1953) data on the distribution of vertebral types in samples of C3H mice. Some alterations from his Table 1 have been made in order to make the terminology consistent with our Table 1. For each sample the top row of figures gives the actual numbers reconstructed from the percentages given in the bottom row

Strain	Origin	Vertebral types			Total
		5/5	6/5 and 5/6	6/6	
C3H/St	Strong in 1937	192	6	2	200
		96	3	1	100
C3H/Bi	Bittner in 1939 (= Bittner's Z)	743	15	8	766
		97	2	1	100
C3H/He	Law in 1948	3	3	170	176
		2	2	96	100
C3H/HeJax	Jackson in 1950	0	1	29	30
		0	3	97	100
C3H/HeR1	Russell in 1950 (Oak Ridge)	1	2	69	72
		1	3	96	100
C3H/Bi	Bittner in 1950 (= Bittner's Z)	134	9	1	144
		93	6	1	100
C3Hf/Bi	Bittner in 1950 (= Bittner's Zb)	66	1	8	75
		88	1	11	100
C3H/StWi	Wilson in 1952	7	0	0	7
		100	0	0	100

DISCUSSION

The family tree shown in Text-fig. 1 makes it clear that in general the new data confirm and consolidate Green's (1953) findings. All samples fall decisively into one or other of the two contrasted types: none occupies an intermediate position. The type to which any sample belongs is related to its history in the manner indicated by Green: all samples appearing in the left-hand sector of Text-fig. 1 have predominantly 6 lumbar vertebrae, while all samples whose ancestors followed the other pathway at the 1930 bifurcation have predominantly 5 lumbar vertebrae.

In Green's (1953) report of his survey he comments:

On the assumption that the difference is genetic, the following are possibilities. (1) The original C3H mice may have been heterozygous for one or more pairs of genes affecting skeletal type. By inbreeding, different genetic combinations may have become fixed in different sublines. The heterozygosity would have had to continue in the line at least until the Andervont subline was established in 1930. (2) One or more mutations

of skeletal genes may have occurred following the separation of the Andervont subline, leading thereby to the establishment of different types in the existing sublines. (3) One of the sublines may have been genetically contaminated by an accidental and unrecorded mating outside the line. (4) Some other strain may erroneously have been labelled as C3H.

TABLE 3
Data on a colony of the C3H/He substrain

<i>Litter</i>	<i>Not 6/6</i>	<i>X-rayed total</i>	<i>Litter size</i>	<i>Birth order</i>	<i>Maternal age (weeks)</i>	<i>Litter</i>	<i>Not 6/6</i>	<i>X-rayed total</i>	<i>Litter size</i>	<i>Birth order</i>	<i>Maternal age (weeks)</i>
1147-54	3	5	8	1	9	21	0	2	6	1	10
1175-82	2	3	8	1	9	23	0	2	4	1	9
1183-88	0	3	6	1	10	26	0	3	3	1	9
1214-21	0	8	8	1	9	27	0	2	4	1	9
1222-27	0	5	6	1	9	28	0	1	5	1	11
1228-34	0	6	7	1	10	30	0	2	7	1	11
1235-36	1	2	2	1	8	40	0	1	7	1	10
1237-44	0	5	8	1	10	42	0	1	3	1	9
1250-56	0	2	7	1	10	47	0	1	3	1	12
1257-64	0	5	8	1	9	48	0	2	4	2	12
1265-71	1	6	7	1	9	57	1	1	4	1	12
1272-75	0	2	4	1	11	63	0	1	3	2	14
1276-83	0	5	8	1	11	70	0	4	4	1	13
1284-89	1	6	6	1	10	297	0	1	4	1	9
1290-98	0	8	9	1	10	302	0	1	8	2	17
1299-1304	0	1	6	1	9	318	2	3	6	3	20
1305-10	0	1	6	1	10	396	3	4	7	2	19
1311-18	0	1	8	1	10	397	1	4	5	1	19
1319-27	0	5	9	1	11	413	0	2	3	4	23
1328-33	3	6	6	1	9	422	1	4	7	3	26
1334-41	1	3	8	1	12	451	3	3	3	1	14
1342-49	1	6	8	1	9	516	0	5	5	2	25
20	0	1	1	1	9	588	1	1	4	2	14

Effects of birth order: $\chi^2_{(1)} = 2.366$,
 maternal age: $\chi^2_{(1)} = 2.372$,
 litter size: $\chi^2_{(1)} = 1.278$.

The new data can do little towards clarifying this question. They do, however, demonstrate, as is evident from Text-fig. 1, that the difference shows itself in a consistent manner over a wide range of colonies maintained in different laboratories in both England and America, thus confirming that the difference is genetic.

In addition to this major difference, some subsidiary vertebral variation within the C3H strain has shown itself in the present survey. The following three causal factors appear to be involved:

- i. A correlation between litter-mates. Individuals of anomalous vertebral type are not distributed at random among litters within a colony, but tend to be 'clumped' in particular litters.
- ii. A sex difference. The percentage of mice with 5 lumbar vertebrae tends to be higher among males than among females.
- iii. Divergences attendant on the formation of new colonies within substrains.

'Clumping' within litters

In Green & Russell's (1951) data a significant heterogeneity appears between the progenies of different matings within the C3H × C57 and C57 × C3H mating types. The authors did not specifically inquire whether heterogeneity was also present between litters of the same mating, and their data and calculations are not presented in a form appropriate for answering this question. Such heterogeneity (which implies that the anomalous individuals are clumped into particular litters) can arise if the expression of the character is affected by factors of the maternal physiology which change with time—either systematically, as in the case of polydactyly in mice described by Holt (1948), or in a fluctuating manner as in the case of a tooth defect in the CBA strain of mice described by Grüneberg (1951).

Our data, set out in Table 3, on one colony of the C3H/He substrain are extensive enough to test heterogeneity between litters. The χ^2 appropriate to the detection of heterogeneity between the 33 litters containing two or more classified mice each, has 32 degrees of freedom and a value of 62.846. We can take $\sqrt{2\chi^2 - \sqrt{(2n - 1)}}$ as a normal deviate with unit variance (Fisher, 1925–50, Table 3). This expression has a value of 3.27 in the present case, which corresponds to $P \simeq 6 \times 10^{-4}$ using Good's (1950) approximation. This procedure is subject to considerable error in the present case where the expectations in most of the classes are very small. A closer approach to a normal deviate can be obtained by calculating Haldane's (1945) statistic ξ . It has the value 3.90 in the present case, for which Good's approximation gives $P \simeq 5 \times 10^{-5}$. The litter-mate correlation, or clumping tendency, is therefore highly significant.

Does this reflect heterogeneity between *matings* within the colony, or between

TABLE 4

Distribution of vertebral types in successive litters from two matings in a colony of C3H/He mice. P is calculated by Fisher's (1925–50; § 21.02) exact method

		Vertebral types		Test of homogeneity between successive litters <i>P</i>
		6/6	Not 6/6	
Mating H506 {	1st litter	0	3	1/56
	2nd litter	5	0	
Mating H454 {	2nd litter	1	3	17/70
	3rd litter	3	1	

litters within the same mating? The sample of mice classified was not chosen with this question in mind, and is unsatisfactory in that the 33 litters were derived from 31 separate matings. However, the two matings from which we have data on more than one litter suggest that the clumping is due at least in part to pre-natal factors which influence all the members of a litter in the same way, but which vary from litter to litter within the same mating (see Table 4).

The variation of these prenatal factors is not related in a systematic way either to birth order, to maternal age, or to litter size. As shown at the foot of Table 3, the χ^2 value testing regression, calculated as in Holt's (1948) paper, is in each case small, having in mind the large χ^2 value for overall heterogeneity. It is concluded that the operative factors of the maternal physiology are probably of the fluctuating rather than the systematic type.

Before leaving the analysis of this sample it is perhaps worth noting that the variation of the two sides is positively correlated ($\chi^2_{(1)} = 78.578$) and that (see Table 1) the 6/5 type significantly predominates over the 5/6 type of asymmetry ($P < 0.02$). This and all other 2×2 χ^2 values have been calculated using Yates's correction for continuity (Fisher & Yates, 1938-53).

The sex difference

Green & Russell (1951) showed that there was a highly significant sex difference in vertebral type among the offspring of their C3H \times C57BL and C57BL \times C3H crosses. The percentage of mice with 5 lumbar vertebrae was higher among the males than among the females. 966 fully classified offspring of C3H matings (which are in fact the same mice as those shown in the first two samples in Green's data given in Table 2) showed a small sex difference in the same direction. This difference, as shown in Table 5, gives a $\chi^2_{(1)}$ of 9.442. We cannot regard

TABLE 5

The sex distribution of vertebral type in two samples of C3H mice

	5/5	Not 5/5	Total
♀♀	466	24	490
♂♂	470	6	476
Total	936	30	966

Green & Russell's pooled sample. $\chi^2_{(1)} = 9.442$

	5/5	Not 5/5	Total
♀♀	12	11	23
♂♂	41	3	44
Total	53	14	67

The sample from Craigie. $\chi^2_{(1)} = 12.986$

this as statistically significant confirmation of a sex difference *within* the C3H strain because the interpretation of χ^2 is obscured by the clumping effect. As we have seen, this effect is liable to inflate the variance to about double the theoretical binomial variance assumed in the construction of tables of χ^2 —since in the sample analysed above, χ^2 divided by the number of degrees of freedom = $62.846/32 = 1.964$. We cannot simply divide χ^2 by this factor in order to get a 'corrected' value, since our estimate of the factor is itself subject to sampling

error; and even if it were not, we cannot expect the variance to be inflated by clumping to the same degree in different colonies and substrains which may differ in their characteristic percentage of anomalies.

But it seems reasonable to regard as formally significant a $\chi^2_{(1)}$ value which is three or four times the conventional 5 per cent. probability level, and as certainly significant any $\chi^2_{(1)}$ value exceeding, say, 20.

With this in mind let us consider the new data. It contains four samples in which the sexes were distinguished—Howard's, Craigie's, Green's, and Heston's (see Table 1). In the first two there is a sex difference, again in the same direction. The χ^2 relating to Howard's mice is not significant, but the value of 12.986 for Craigie's (see Table 5) is sufficient, when taken in conjunction with the data of Green & Russell discussed above, to establish to our satisfaction that there is a real effect of sex on vertebral variation within the C3H/Bi substrain.

Divergences between colonies

Within that part of the C3H strain which remained with Strong in 1930 and which has predominantly 5 lumbar vertebrae, there are three colonies included in the new data which can be shown to differ significantly from the pooled sample of Green & Russell (1951). They are Craigie's and Dmochowski's (C3H/Bi) colonies and Strong's (C3H/St) colony, all of which show a lower percentage of the 5/5 type than Green & Russell's sample.

Table 6 shows that for males the difference between Craigie's and Green & Russell's mice is not significant, but that for females the difference is highly significant ($\chi^2_{(1)} = 57.110$), even allowing for any reasonable degree of clumping.

The comparison with Dmochowski's mice is made more difficult by the fact that this material was not classified for sex. But even if we make the assumption which is least favourable to the demonstration of a difference, namely that our sample of Dmochowski's colony consisted entirely of females, the comparison with Green & Russell's females, as shown in Table 6, yields a $\chi^2_{(1)}$ of 24.136.

A similar comparison for Strong's mice, which were also not classified for sex, gives a $\chi^2_{(1)}$ of 49.202.

These results might be interpreted as showing that genetical divergences have occurred within the C3H strain in addition to that between the C3H/He substrain on the one hand and the C3H/St and C3H/Bi substrains on the other. This interpretation should be treated with caution for the following reasons.

The difference demonstrated by Green & Russell between the offspring of reciprocal crosses between the C3H and C57BL strains shows that at least for some genotypes the vertebral character under study is highly sensitive to the uterine environment in which prenatal development occurs. The clumping effect which we have demonstrated in a sample of the C3H/He substrain shows that this sensitivity characterizes pure-strain C3H mice as well as strain-hybrids. It also suggests that the character is sensitive not only to factors of the uterine environment which are determined by the mother's genotype but also to factors

which fluctuate during the mother's lifetime. It is reasonable to assume that some of these factors of the maternal physiology are affected by environmental influences, such as diet, in which some laboratory colonies differ from others. In this connexion we may cite the observation of Searle (1954) that a number of skeletal characters are profoundly affected by changes in the mother's diet. It

TABLE 6

The comparison of the samples from Craigie, Dmochowski, and Strong with Green and Russell's pooled sample

$\sigma\sigma$	5/5	Not 5/5	Total
Craigie	41	3	44
Green & Russell .	470	6	476
Total	511	9	520

$$\chi^2_{(1)} = 4.412$$

♀♀	5/5	Not 5/5	Total
Craigie	12	11	23
Green & Russell .	466	24	490
Total	478	35	513

$$\chi^2_{(1)} = 57.110$$

	5/5	Not 5/5	Total
Dmochowski	18	8	26
Green & Russell ♀♀ .	466	24	490
Total	484	32	516

$$\chi^2_{(1)} = 24.136$$

	5/5	Not 5/5	Total
Strong	24	14	38
Green & Russell ♀♀ .	466	24	490
Total	490	38	528

$$\chi^2_{(1)} = 49.202$$

follows that although the major difference between the two main sections of the C3H line is without doubt mainly genetic, the subsidiary differences which the new data have revealed within one of these two sections might reflect differences in the conditions under which different colonies are maintained rather than differences in genotype.

Since the above was written, evidence has been privately communicated to us by Dr. D. Hamer that some differences between fairly recently separated colonies of the C3H strain may be genetically determined. Dr. Hamer has been working in the laboratories of Dr. D. L. Woodhouse in Birmingham on the activity of

β -glucuronidase in mouse liver. Inbred strains of mice fall into two distinct groups with respect to this character, those with 'high' and those with 'low' activity. Two C3H/He, one C3H/St, and three C3H/Bi colonies have been shown all to belong to the 'low' group. However, Dr. Hamer finds that of the two colonies of C3H/Bi mice maintained in Birmingham (see Text-fig. 1), the one from Leeds is 'low' but the other, from London, is 'high'. These colonies had at the time of testing been maintained in the same laboratory for nearly 4 years, and hence the difference must be attributed to a genetic divergence. This divergence must have arisen between 1941, the year in which they were separated, and 1950, since the colony maintained by Dr. Foulds in London is also 'high'.

SUMMARY

1. By means of X-ray photography the number of lumbar vertebrae was counted in samples of mice from 11 colonies of the C3H inbred strain.

2. The results confirm Green's (1953) finding that, of the three main substrains of the C3H strain, one (C3H/He) has predominantly 6 lumbar vertebrae while the other two (C3H/St and C3H/Bi) have predominantly 5 lumbar vertebrae.

3. In addition, the new data have revealed three factors with significant effects on vertebral variation *within* substrains of the C3H strain:

- i. A fluctuating variable of the mother's physiology which affects the pre-natal development of the offspring.
- ii. A difference between the sexes. The percentage of mice with 5 lumbar vertebrae is higher among males than among females.
- iii. Divergences between colonies of the same substrain. The available data are not competent to distinguish the relative contributions to these divergences of nature and nurture.

Note added in proof. An extensive survey by Searle (*J. Genet.* **52**, 68–102, (1954)) of skeletal variation within the A and C57BL strains has just appeared. In the section on lumbo-sacral variation within the C57BL/Gr substrain Searle finds significant effect of all the factors which we have reported here as affecting variation within substrain of C3H mice—namely subline, litter, sex, side and between-side correlation. He also reports an effect of maternal age.

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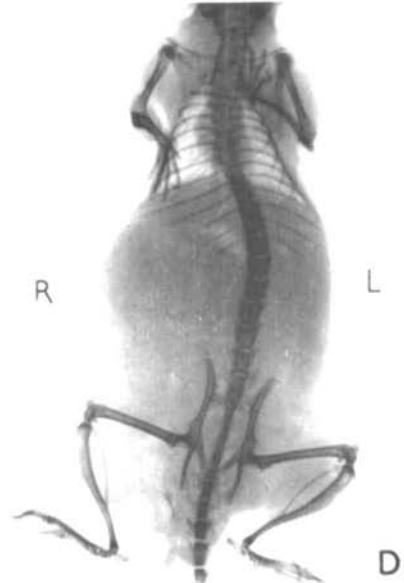
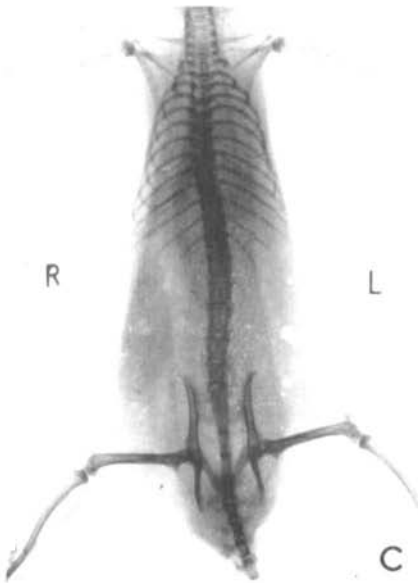
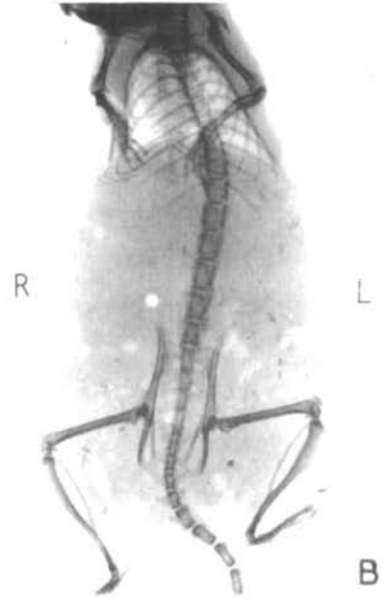
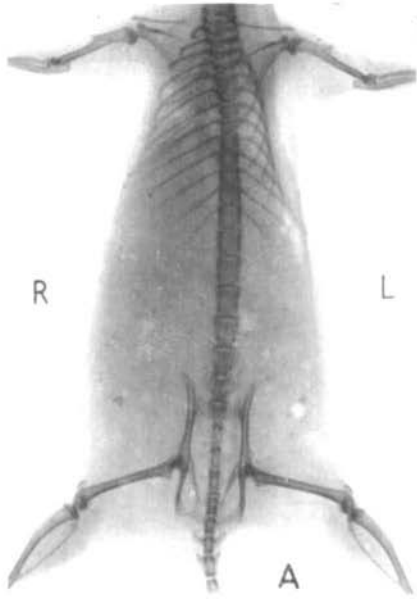
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EXPLANATION OF PLATE

- FIG. A. C3H/He ♀ illustrating 6/6 vertebral type.
 FIG. B. C3H/He ♀ illustrating 5/5 vertebral type.
 FIG. C. C3H/He ♂ illustrating 6/5 vertebral type.
 FIG. D. F_1 ♂ from C57BL ♀ × C3H/Bi ♂ mating, illustrating a marginal case (see text).

The symbol 6/5 means that the mouse has 6 lumbar vertebrae on its left side and 5 on its right, while 5/6 denotes the reverse type of asymmetry.



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