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Variation of the lumbar vertebrae of mice at two environmental temperatures

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The house mouse, *Mus musculus* L., usually has twenty-six presacral vertebrae: these consist of seven cervical, thirteen thoracic and six lumbar vertebrae (Weber, 1950; Deol, 1958; Berry & Searle, 1963; Berry, 1964). The twenty-sixth vertebra is, however, sometimes abnormal, especially among laboratory mice: on one or both sides it may be fused with the sacrum. There are then five typical lumbar vertebrae, instead of six. The proportion of such abnormalities in a population is influenced by genotype, diet and unidentified maternal factors (reviewed by Grüneberg, 1963). Evidence is given below that a low environmental temperature can also increase the incidence of 'sacralization of L6'.

MATERIAL AND METHODS

The mice used included three highly inbred strains: A/Tb, A2G/Tb and C57BL/Tb. A permanent breeding colony of each strain was kept at each of two environmental temperatures, namely, 21 °C and −3 °C. In addition, GFF mice were studied at 21 °C only. F_1 mice were produced by making all possible crosses between the first three inbred strains, in each temperature. GFF mice were crossed with each of the other strains at 21 °C. In the account that follows the designation of the male parent is given first. A mixed stock derived from all four inbred strains was also studied in both environments.

Food was diet 41 (Bruce & Parkes, 1949) in excess. Water was supplied in bottles at 21 °C and as ice blocks at −3 °C. All mice had sawdust and cotton-wool bedding. In each 24 h they had 12 h darkness and 12 h light.

The mice were drawn from first to fifth litters of permanently mated pairs. They were killed as adults, usually at about the age of 16 weeks. All were X-rayed with ventral surface down, after the abdominal viscera had been removed. This procedure allows reliable assessment of the state of the lumbar

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vertebrae (McLaren & Michie, 1954). The lumbar vertebrae were counted and the result was recorded as one of the following:

6/6: six typical lumbar vertebrae;
6/5: transverse process of twenty-sixth vertebra fused with sacrum on right;
5/6: the same on left;
5/5: five typical lumbar vertebrae.

RESULTS

The percentages of mice with abnormal lumbar vertebrae, that is, of those *not* 6/6, are given in Tables 1–4. These findings allow, by construction of contingency tables, assessments of the separate contributions to vertebral variation of (i) sex, (ii) genotype, (iii) maternal and paternal genotype, and (iv) environmental temperature. The influence of heterozygosis (v) was also examined, and the question of laterality (vi). Some results of the statistical analysis are summarized in Table 5.

(i) Males tended to have a higher proportion of abnormal lumbar vertebrae

Table 1. *Incidence of abnormal lumbar vertebrae: males.*

For each class, the number of mice is given on the left, and the percentage of departures from the normal (6/6) on the right.

			♂ parent					
		°C	A		A2G		C57BL	
♀ parent	A	21	35	28·6	29	31·0	17	47·1
		−3	30	43·3	23	65·2	43	48·8
	A2G	21	37	24·3	35	40·0	54	33·3
		−3	50	42·0	57	68·4	68	32·4
	C57BL	21	29	37·9	21	47·6	23	52·2
		−3	53	32·1	18	55·6	36	47·2

Table 2. *As Table 1: females*

			♂ parent					
		°C	A		A2G		C57BL	
♀ parent	A	21	32	18·8	27	25·9	20	30·0
		−3	40	20·0	22	63·6	36	47·2
	A2G	21	35	14·3	29	27·5	65	26·2
		−3	47	34 0	67	62·7	48	43·8
	C57BL	21	24	8·3	21	47·6	29	37·9
		−3	38	39·5	18	38·9	30	36·7

than females. This confirms the observations on C3H mice of Green & Russell (1951) and of McLaren & Michie (1954).

(ii) Of the inbred strains, GFF (at 21 °C) and A/Tb had the lowest incidence of abnormal vertebrae. The mice of substrain A/Tb are atypical in having a tail of reduced length and number of vertebrae (Barnett, 1965*b*), but no other peculiarity of the vertebral column was detected in them. C57BL/Tb had the highest incidence of abnormal lumbar vertebrae at 21 °C, but A2G/Tb had the highest incidence at −3 °C.

Table 3. *As Table 1: mixed stock, both sexes*

°C	♂		♀	
21	48	16·7	55	7·3
−3	59	28·8	41	14·6

Table 4. *Incidence of abnormal lumbar vertebrae among mice with GFF parentage, 21 °C only*

		Other parent							
		A/Tb		A2G/Tb		C57BL/Tb		GFF	
		N	%	N	%	N	%	N	%
♂♂	GFF father	21	42·6	20	0·0	18	11·2	20	0·0
	GFF mother	21	9·5	20	15·0	20	10·0		
♀♀	GFF father	19	10·6	18	0·0	20	20·0	20	10·0
	GFF mother	19	10·6	19	5·3	20	15·0		

Table 5. *Analysis of effects of sex, parentage and temperature*

Factor	χ^2	D.F.	$P <$	Finding
Sex	5·34	1	0·05	More abnormal ♂s than ♀s
♀ parent	0·19	2	—	—
♂ parent	37·02	2	0·001	♂ parent influences vertebral type
Temperature	26·44	1	0·001	More abnormal vertebrae at −3 °C than at 21 °C

(iii) There was no evidence of an effect of female parent on vertebral type. χ^2 was calculated, not only for the whole set of findings taken together (Table 5), but also for each group of matings at each temperature. For example, the results of mating males of strain A/Tb to females of strains A/Tb, A2G/Tb and C57BL/Tb at −3° gave a χ^2 of 1·46 for their male offspring. None of the

twelve groups of crosses gave a statistically significant result. By contrast, there was a marked influence of male parent (Table 5). When the father was A/Tb there tended to be a low incidence of abnormality; A2G/Tb fathers gave a high incidence; C57BL/Tb fathers had an intermediate effect. GFF parentage, both male and female, led to a low incidence of abnormal vertebrae (Table 4).

Table 6. *Incidence of asymmetry: males*

For each class, the percentages of asymmetrical vertebrae are on the left. On the right are the percentages of 'fives' which were on the right side of the mice (6/5)

			♂ parent					
		°C	A		A2G		C57BL	
♀ parent	A	21	0·0	0·0	13·7	75·0	35·3	66·7
		−3	23·3	57·1	17·3	75·0	18·6	37·5
	A2G	21	13·5	40·0	8·6	0·0	16·7	66·7
		−3	22·0	36·4	14·1	87·5	5·8	50·0
	C57BL	21	24·1	42·9	28·6	83·3	26·1	66·7
		−3	13·2	85·7	22·2	50·0	21·0	57·1

Table 7. *As Table 6: females*

			♂ parent					
		°C	A		A2G		C57BL	
♀ parent	A	21	9·4	33·3	18·5	20·0	25·0	60·0
		−3	10·0	75·0	13·6	66·7	16·7	33·3
	A2G	21	8·6	66·7	6·9	0·0	9·2	50·0
		−3	19·2	66·7	26·8	55·6	22·9	63·6
	C57BL	21	8·4	50·0	19·1	75·0	13·7	75·0
		−3	13·1	80·0	16·7	33·3	16·7	40·0

(iv) There was a higher proportion of abnormals at −3 °C than at 21 °C over the whole range of observations shown in Tables 1 and 2 (Table 5). χ^2 was calculated also for six subgroups of matings according to parentage. The effect of temperature was in the same direction in each subgroup, but was statistically significant in only three: when male or female parent was of strain A2G/Tb, and when female parent was of strain A/Tb ($P < 0{\cdot}01$ in each case).

(v) There was no consistent effect of heterozygosis on the incidence of abnormal vertebrae. Out of the 24 possible comparisons from the figures in Tables 1 and 2, the F_1 class had a score intermediate between the inbred parent strains in eleven instances. In eight instances the F_1 had fewer abnormal vertebrae than either inbred strain, and in five the F_1 had more. Out of the twelve possible

comparisons involving GFF mice (Table 4), the F_1 class was intermediate in nine instances.

(vi) McLaren & Michie (1954) found a predominance of structural abnormality, or 'fives', on the right. Our observations tend to confirm this, but with some exceptions. The classes of mice referred to in Tables 1 to 3 are analysed for this purpose in Tables 6 and 7. If the mixed stock are included, and the sexes examined separately, there were 40 classes. Eleven had more 'fives' on the left, in five classes the numbers were equal, and in 24 there were more 'fives' on the right. We did not find, as did Deol & Truslove (1957), a predominance of 'fives' on the left in C57BL mice. The main exceptions came from the mice of which one or both parents were GFF. Of fourteen classes, three had more 'fives' on the left, five had more on the right, and the rest were equal. The numbers of abnormal vertebrae were, however, small. In addition, the mixed stock displayed no consistent effect of laterality.

Phenotypic variation

The three vertebral types, 5/5, asymmetrical and 6/6, may be regarded 'as marking threshold values of some underlying continuous distribution' of a developmental process which influences the form of the twenty-sixth vertebra

Table 8. *Effects of three factors on variation*

(Numbers of mice are given in table 1.)

Factor	Class of mice	Variance	Information	$P <$
Sex	Inbred ♂s	6·78	0·70	—
	Inbred ♀s	4·85		
	F_1 ♂s	5·71	0·76	—
	F_1 ♀s	4·41		
Heterozygosis	Inbred	5·74	0·40	—
	F_1	5·11		
Temperature	21 °C	11·02	2·13	0·05
	−3 °C	3·76		

(McLaren & Michie, 1955). These authors describe a method by which the form of this hypothetical distribution (assumed to be Gaussian) can be estimated. We have used this procedure to look for evidence of effects on variance, as distinct from incidence of abnormal vertebrae (Table 8). There is no good evidence that variance was influenced by sex or heterozygosis. The figures indicate an effect of temperature on the inbred strains: surprisingly, those at −3 °C varied less than those at 21 °C ($P < 0{\cdot}05$). This may be due to higher mortality *in utero* or early postnatal life acting differentially on individuals which depart widely from the typical. Hence the percentages given above of abnormal vertebrae in the cold environment may be underestimates.

DISCUSSION

Our principal new observations concern the effects of a cold environment. The mice at −3 °C were fully adapted to the low temperature: they were from stocks breeding permanently in the cold, and their parents too had been reared in the cold environment. Nevertheless, there was a clear tendency towards a higher proportion of abnormal vertebrae in the cold. The genetical and the several environmental sources of variation in the structure of L6 did not obscure this effect. It was expected that heterozygosis, by contrast, would reduce the incidence of abnormal vertebrae: F_1 mice of the types used are more resistant than the inbreds to other effects of cold, just as they are more fertile (reviewed by Barnett, 1965*a*). But there was no evidence that heterozygosis reduced either the incidence of abnormal vertebrae or the variance of any underlying process which determines vertebral development. Hence canalization of the development of this feature was, in our crosses, not correlated with 'hybrid vigour'.

Our strangest observation was of the patroclinous effect: most differences between reciprocal crosses are due to differences between the environments provided for their young by the two types of mother. Our findings were not always cases of F_1 mice tending to resemble their fathers, as were those described by Green & Green (1959). Nor were they due to sex-linkage, since they affected offspring of both sexes. An explanation should perhaps be sought in an interaction between genotype and the cytoplasm or uterine environment provided by females of different genotype from that of their mates.

SUMMARY

Mice of four inbred strains (A/Tb, A2G/Tb, C57BL/Tb and GFF), F_1 offspring produced by crossing these strains, and a mixed stock derived from all four inbred strains were examined for sacralization of the sixth lumbar vertebra. Some of the mice of all classes, except GFF and F_1s derived from GFF, were members of colonies breeding in a room kept at −3 °C; controls were at 21 °C Males had more abnormal vertebrae than females. There were marked differences between inbred strains: in particular, GFF had a low incidence of sacralization. There was no evidence of an effect of female parent, but there was a patroclinous effect: A/Tb males tended to have young with few abnormal vertebrae; A2G/Tb males had the opposite effect. There were more abnormal vertebrae in the cold than in the warm environment. There was no consistent effect of heterozygosis.

RÉSUMÉ

Variation des vertèbres lombaires de souris à deux températures

Des souris de 4 souches (A/Tb, A2G/Tb, C57BL/Tb et GFF), des F_1s issus du croisement de ces souches et d'une souche hybride dérivée des 4 souches

ci-dessus, ont été examinées pour déceler la présence ou l'absence de sacralization de la sixième vertèbre lombaire. Quelques-unes des souris de chaque souche, sauf les GFFs et F_1s dérivées de GFF, ont été maintenues en colonie à -3 °C; les témoins ont été maintenus à 21 °C. Le pourcentage des vertèbres anormales est plus élévé chez les mâles que chez les femelles. Des différences nettes sont apparues entre les différentes souches: en particulier, GFF montre une proportion minime de sacralization. Il n'y a pas d'effet dû à la parenté femelle, mais bien un effet lié à la parenté mâle: le pourcentage de vertèbres anormales est minime chez la progéniture des mâles A/Tb. Chez la progéniture des mâles A2G/Tb, le résultat inverse est observé. Le nombre d'anomalies vertébrales est plus élevé chez les animaux maintenus au froid que chez ceux maintenus à température normale. Aucun effet constant de l'hétérozygosité n'a été constaté.

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