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Genetical studies on the skeleton of the mouse

XXX. A SEARCH FOR CORRELATIONS BETWEEN SOME MINOR VARIANTS

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INTRODUCTION

The C57BL strain of the mouse has a large number of minor skeletal variants which are partly under genetical control. This is also true, to a lesser extent, of other inbred strains like CBA and A. In the course of inbreeding, but apparently following the fixation of the initial genetical variance, the C57BL strain has split up into numerous genetically distinct sublines (Deol, Grüneberg, Searle & Truslove, 1957; Carpenter, Grüneberg & Russell, 1957). The differentiation of these sublines is thus due to freshly arising mutations, but the number of these events involving skeletal characters is unexpectedly high. One possible reason for this is that the same mutation may affect more than one skeletal variant and may, therefore, be noted more than once. The 'subline differentiation method' for the estimation of mutation rates, suggested by Deol *et al.* (1957), can thus be validly applied only if it is known to what extent the minor variants used are phenotypically correlated (i.e. have common causes) within the inbred strain, or are genetically correlated (pleiotropism, linkage) in outcrosses.

MATERIALS AND METHODS

A preliminary search for correlations between minor skeletal variants in the C57BL/Gr strain was carried out on sublines V–VII (263 animals). Those correlations which were found to be significant at the 5 % level were then reinvestigated in sublines I, II, III and IV (a total of 578 animals). A similar search was carried out using the CBA/Gr strain (489 animals), although there were no sublines available. Lastly, the F_2 generation from a cross between C57BL and CBA (401 animals) was investigated to see if there were any genetical correlations.

Table 1 gives a list of the thirty-one skeletal variants, already described in some of the previous papers of this series, with the percentage incidence in C57BL/Gr sublines V–VII, CBA/Gr and the F_2. Those in italics are characters where a significant correlation was found in one of the strains. With the exception of No. 7 where the relevant bones were not collected in CBA or F_2, the characters marked — did not occur in CBA and failed to segregate out in F_2. Two characters (Nos. 24 and 25) were not included in the C57BL analysis for reasons of convenience. All the variants were treated as all-or-none characters and 2×2 tables were produced by suitable pooling of classes (following the convention used by Deol,

Table 1. *Percentage incidence of skeletal variants in C57BL (sublines V–VII), CBA and F_2*

No.	Variant	C57BL	CBA	F_2
1	Interfrontal	85·6	85·3	66·1
2	Lacrimal-maxilla fusion	15·9	0·8	1·3
3	*Parted frontals*	*7·3*	*75·3*	*44·0*
4	Fused frontals	4·2	—	2·5
5	*Frontal fontanelle*	*7·5*	*35·0*	*3·7*
6	Interparietal-occipital fusion	12·5	—	1·5
7	*Squamosal-parietal fusion*	*7·0*	?	?
8	*Periotic-occipital fusion*	*2·8*	—	—
9	Alae palatinae	72·1	23·9	20·3
10	Foramen ovale single	3·0	63·6	23·2
11	*Foramen ovale open posteriorly*	*3·8*	—	*1·0*
12	Foramen sphenoidale medium	25·7	87·9	23·9
13	*Inframaxillary crest*	*9·4*	*0·9*	*23·8*
14	Processus pterygoideus	17·1	89·7	38·7
15	*Presphenoid, preoptic sutures*	*36·2*	*97·4*	*26·3*
16	*Presphenoid, abnormal metoptic roots*	*1·1*	*72·1*	*29·6*
17	*Foramen hypoglossi single*	*24·8*	*16·4*	*15·6*
18	Accessory mental foramen	26·2	23·0	28·0
19	*Atlas-axis dyssymphysis and/or fusion*	*77·3*	—	*2·5*
20	Other cervical fusions	3·4	—	—
21	Foramina transversaria imperfecta, C III	2·1	—	—
22	*Foramina transversaria imperfecta, C IV*	*34·2*	—	*1·0*
23	*Foramina transversaria imperfecta, C V*	*66·2*	—	*8·5*
24	C VI, tuberculum anterius absent or dystopic	?	1·5	0·5
25	C VI, tuberculum anterius inflexum	?	34·3	1·1
26	*Dystopia of processus spinosus of Th II*	*10·6*	—	—
27	Dyssymphysis of Th I	14·2	—	0·5
28	Dyssymphysis of Th II	0·4	—	1·5
29	Size of processus spinosus of Th II	72·2	57·5	82·3
30	Dyssymphysis of Th X	4·5	—	0·5
31	*L VI sacralized*	*5·9*	*98·9*	*53·5*

Grüneberg, Searle & Truslove, 1960). The first material analysed came from sublines V–VII of C57BL and consisted of 406 fourfold tables. Two-hundred-and-eighty-seven of these were eliminated by inspection as not deviating significantly from proportionality. For the remainder the log likelihood ratio test (the G-test, $G = 2\,\Sigma a(\ln a - \ln m)$, where a is the observed number and m the expected number in a typical cell or class) was calculated from the tables given by Woolf (1957). These values of G were treated as χ^2, and any $\sqrt{G} > 1{\cdot}96$ (corresponding to $P < 0{\cdot}05$) was considered significant. However, if any of the cells in the fourfold tables contained observed numbers less than one, P was calculated by Fisher's exact method. Any correlations found to be significant were reinvestigated using the data from sublines I, II, III and IV. The four $\sqrt{G}$ values thus obtained were added together (taking into account whether they were positively or negatively correlated) and divided by $\sqrt{4} = 2$. Similar analyses were carried out on the data from CBA and F_2, but as there were no sublines present in CBA significant correlations could only be compared with F_2.

ANALYSIS

Table 2 gives the twenty-six $\sqrt{G}$ values judged to be significant in sublines V–VII of C57BL. This is not significantly more than expected on a chance basis (20·3), but nevertheless some genuine correlations may be included in this group. Six significant $\sqrt{G}$ values were repeated by sublines I–IV, but one of these (variants 15 and 31, in the centre column of Table 2) shows a significant positive value in one group of sublines and a significant negative value in the other; for this reason it may be considered spurious. But in the F_2 data there is again a

Table 2. $\sqrt{G}$ *values of twenty-six significant correlations in sublines V–VII compared with those from sublines I–IV. The correlations on the left are negative in both groups of sublines, those on the right are both positive, while those in the centre are positive in one and negative in the other*

Variants	V–VII	I–IV	Variants	V–VII	I–IV	Variants	V–VII	I–IV
	−	−		−	+		+	+
				+	−			
2: 3	2·89	0·72	2:30	2·02	0·68	3: 5	*3·85*	*3·52*
2:18	2·28	0·23	3: 8	2·40	0·08	5: 7	*2·11*	*2·66*
12:21	2·17	0·61	5:13	2·66	0·32	5: 8	3·03	0·35
15:26	2·48	0·49	6:26	2·91	0·87	5:11	2·53	0·79
19:26	*2·18*	*3·21*	8:20	2·63	1·27	7: 8	2·73	0·40
19:31	*2·41*	*2·62*	10:22	2·61	0·38	7:10	3·18	0·16
27:31	2·71	0·24	15:18	2·45	0·45	7:12	2·87	0·40
			15:31	2·05	2·18	8:11	*3·69*	*3·88*
						8:14	1·97	1·56
						14:19	3·54	0·81
						22:30	2·05	1·80

high negative correlation which indicates, perhaps, that this is in fact a genuine case and that the difference between the two groups of sublines is due to differences in the frequencies of the two entities. The other five cases (in italics in Table 2) appear to be genuine correlations, but in none of them is the correlation at all close, the correlation coefficients ($r = \sqrt{(\chi^2/N)} \pm 1/\sqrt{N}$) all being in the range of 0·1–0·3 (Table 3).

Table 3. *Correlation coefficients* (r) *for five pairs of C57BL variants*

No.	Variants	V–VII	I–IV	F_2
3 5	Parted frontals Frontal fontanelle	$+0{\cdot}31 \pm 0{\cdot}062$	$+0{\cdot}17 \pm 0{\cdot}042$	$+0{\cdot}12 \pm 0{\cdot}050$
5 7	Frontal fontanelle Squamosal-parietal fusion	$+0{\cdot}15 \pm 0{\cdot}062$	$+0{\cdot}12 \pm 0{\cdot}042$	(—)
8 11	Periotic-occipital fusion Foramen ovale open	$+0{\cdot}30 \pm 0{\cdot}062$	$+0{\cdot}23 \pm 0{\cdot}042$	—
19 26	Atlas-axis fusion Dystopia of p.s. of Th II	$-0{\cdot}14 \pm 0{\cdot}062$	$-0{\cdot}12 \pm 0{\cdot}042$	—
19 31	Atlas-axis fusion L VI sacralized	$-0{\cdot}16 \pm 0{\cdot}062$	$-0{\cdot}12 \pm 0{\cdot}042$	$-0{\cdot}10 \pm 0{\cdot}050$

For two of the five character pairs in Table 3, independent confirmation comes from the F_2 data. As will be discussed below, the reappearance of a correlation in F_2 may be regarded as supporting evidence for conclusions drawn from the study of the inbred strain, but the converse may not be true. In the remaining three cases, the F_2 data give no information, either because one of the variants involved failed to segregate out in the F_2 generation or, in one case (—), because the relevant bones had not been included in the original collections. The anatomical nature of the correlated character-pairs will be discussed below.

All the cases in Table 3 are, presumably, true correlations. Table 2 may contain additional examples, such as variant pairs 8 & 14 or 22 & 30, and the fact that of the remaining twenty $\sqrt{G}$ values, eleven exceed 2·58 (corresponding to $P = 0{\cdot}01$), perhaps shows that some of these are true correlations as the chance expectation of this number is 4·06. However, as the remaining number of 20 deviations significant at the 0·05 level is practically the same as the chance expectation (20·3), perhaps the matter need not be pursued further.

The CBA strain has a far less variable skeleton than C57BL and only seven 'significant' correlations were found (Table 4). These could only be compared with the F_2 data. One of these seven, preoptic sutures and abnormal metoptic roots of the presphenoid, is paralleled highly significantly in the F_2 data (CBA, $r = +0{\cdot}19 \pm 0{\cdot}045$, and F_2, $r = +0{\cdot}18 \pm 0{\cdot}050$).

Table 4. $\sqrt{G}$ *values of seven significant correlations* ($\sqrt{G} > 1{\cdot}96$) *in the CBA strain together with those in the* F_2 *generation*

Variants	CBA	F_2	Variants	CBA	F_2	Variants	CBA	F_2
	−	−		−	+		+	+
				+	−			
16:29	2·36	1·84	5:15	2·10	1·92	15:16	*4·35*	*3·51*
			10:12	3·05	0·52	15:18	2·06	1·11
			14:17	1·99	0·14			
			15:25	1·99	1·51			

From the F_2 data, twenty-three comparisons were found to be significant at the 0·05 level (Table 5), the chance expectation being 16·25. While the excess of 6·75 is not formally significant it probably includes some genuine correlations. Three F_2 deviations have already been judged to be 'true' correlations on the basis of agreement with the two inbred strains (Tables 3 and 4). For the rest, the greater the deviation, the less likely is it to have arisen by accident alone. The best way to pick out the 'true' correlations, therefore, is to base the selection on the size of $\sqrt{G}$ and proceed in descending order. How far this process should be followed is a matter of choice. Five more $\sqrt{G}$ values exceed 3·2 ($P < 0{\cdot}001$), and may be 'true' correlations. Having thus removed eight from the original total of twenty-three deviations, the fifteen remaining is less than the chance expectation. Moreover, this includes only four values larger than 2·58 ($P < 0{\cdot}01$) which is close to the chance expectation (3·25). It is probably advisable to stop selecting at this point.

Table 5. $\sqrt{G}$ *values of twenty-three significant* F_2 *correlations compared with those in CBA and C57BL (sublines V–VII only)*

Variants	F_2	CBA	C57BL
9:24	−2·11	−1·74	—
12:15	−2·67	−0·61	−1·52
15:31	*−4·00*	−0·77	*−2·05*
3: 5	*+2·35*	+0·51	*+3·85*
3: 9	+2·97	+1·11	+1·00
15:16	*+3·51*	*+4·35*	+1·70
17:19	−2·21	—	−0·99
19:31	*−1·98*	—	*−2·41*
22:31	−2·86	—	−0·84
4: 5	+1·99	—	+0·18
5:22	+2·19	—	+0·36
11:23	*+3·24*	—	+0·62
22:23	*+4·00*	—	+0·99
4:17	−2·21	—	+0·70
17:28	−2·04	—	+1·28
1:23	+2·11	—	−1·93
28:31	+2·46	—	−0·43
5:14	−2·35	+0·78	+1·51
9:13	−2·46	−0·19	+0·70
13:15	*+3·92*	−1·94	+1·46
13:31	−2·88	+0·42	+1·89
14:15	+2·45	−0·32	−0·66
16:17	*+3·77*	−0·39	−0·55

Table 6 gives the actual correlation coefficients (other than those already given in Table 3 and in the text); they are all small, like those found in the inbred strains above.

Table 6. *Correlation coefficients* (r) *for five pairs of* F_2 *variants*

No.	Variants	*r*
15 31	Presphenoid, preoptic sutures L VI sacralized	−0·20 ± 0·050
11 23	Foramen ovale open posteriorly Foramina transversaria imperfecta, C V	+0·20 ± 0·050
22 23	Foramina transversaria imperfecta, C IV Foramina transversaria imperfecta, C V	+0·25 ± 0·050
13 15	Inframaxillary crest Presphenoid, preoptic sutures	+0·18 ± 0·050
16 17	Presphenoid, metoptic roots Foramen hypoglossi single	+0·19 ± 0·050

DISCUSSION

The identification of character-pairs believed to be correlated has been based entirely on statistical evidence. We must now consider to what extent the eleven correlations thus identified are understandable in biological terms. The explana-

tions of some of the correlations appear trivial, of others plausible, of still others adequate, whilst there is also another group which, at present, cannot be explained biologically. These are discussed in more detail below.

(1) In the case of foramina transversaria imperfecta of C IV and C V (Table 6) we are dealing with the same entity manifesting itself in adjacent vertebrae. The information that these two manifestations should be correlated can only be regarded as trivial.

(2) It is plausible that conditions favouring the manifestation of parted frontals will also tend to favour the neighbouring variant of the same suture, the frontal fontanelle (Table 3). Again, it is plausible that the manifestations of preoptic sutures and abnormal metoptic roots of the preshenoid (Table 4) should be correlated, on the grounds of their close proximity, although the exact nature of the common mechanism remains unknown.

(3) The negative correlation between atlas-axis fusion and the dystopia of the processus spinosus of the second thoracic vertebra (Table 3) discovered by Grüneberg (1950) remained inexplicable until it was found by Deol & Truslove (1957) that the former of these two entities is correlated with a low, and the latter with a high, birth weight. The same is true of the other two negative correlations in this series, atlas-axis fusion and sacralization of L VI (Table 3), and preoptic sutures of the presphenoid and sacralization of L VI (Table 6).

(4) While six out of the eleven correlations discussed here are reasonably well understood, the biological meaning of the remaining five is obscure. Nobody familiar with the complexity of biological systems will regard this as an unduly high proportion, nor would it be appropriate to doubt the authenticity of these correlations solely on the grounds that their mechanism is unknown.

Assuming that these eleven correlations are in fact genuine, we have to discuss what they mean in genetical and physiological terms. Correlations between variants within an inbred strain give no information as to whether such correlations are genetical in origin. A positive correlation between two variants A and B may mean that the development of A favours that of B or the reverse, or it may point to a common cause which favours the manifestation of both. A negative correlation between A and B suggests that the circumstances which favour the manifestation of one tend to interfere with that of the other. As shown above, there are six cases which fall into these categories (five in C57BL and one in CBA).

In three of these cases a similar correlation reappears in F_2, indicating that the two variants are either pleiotropic effects of one gene, or that they are the effects of linked genes. While it is impossible to decide which explanation is the correct one from the statistical evidence alone, it may be possible to decide for different reasons. For example f.t.i. in C IV and C V which are correlated in F_2 entered the cross together from C57BL and are the pleiotropic effects of a gene (or combination of genes) with similar effects in adjacent vertebrae. In this particular case the two effects are not significantly correlated in the parent strain, but neither is there a significant correlation between their manifestation on the two sides of the body

(Grüneberg, 1950). In animals genetically capable of showing the character in either vertebra the presence of this phenotype is brought about by local influences which may be described as accidents.

In the remaining three cases, two pairs of the variants which were significantly correlated in C57BL failed to segregate out in F_2. In the last case the relevant bones were not collected in CBA and F_2 and so no further information can be obtained.

The two inbred strains showed 5/406 and 1/136 correlations believed to be genuine, or approximately 1% of all the possible paired comparisons. In the F_2 generation the ratio is a little higher (8/325). The eleven correlations found are all weak, none of them exceeding 0·3. Correlations of less than 0·1 could hardly have been discovered in samples of this size, but some probably exist. The really important fact is that no high correlations between any two entities have been found. This even applies to the F_2 correlation of f.t.i. in C IV and C V, which is an obvious case of pleiotropism. The fact that so many entities should behave independently, or nearly so, is contrary to what one might have expected in an organism or an assembly of closely integrated parts. The reason for this apparent paradox is that these minor skeletal variants are overwhelmingly influenced by chance in their manifestation (Searle, 1954). They constitute the fringe of genetical determination where a slight alteration in the space/time relationships of developing tissues may lead to apparently independent variants.

In any subsequent 'subline differentiation' experiments, the eleven correlations tentatively regarded as genuine, will have to be examined carefully; and differences in sample size taken into account. Conversely correlations found in such subline differentiation experiments may confirm the existence of cases which, on the evidence of the present data alone, have not been established as 'true' correlations.

SUMMARY

A systematic search for correlations between numerous minor skeletal variants in the mouse showed that these are few in number and feeble in extent. This apparent lack of integration is probably due to the fact that these characters are at the extreme limits of genetical determination and so are overwhelmingly influenced by chance in their manifestation.

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