GENETICAL STUDIES ON THE SKELETON OF THE MOUSE
X. RARER VARIANTS IN THE A AND C57BL PURE LINES

By A. G. SEARLE
*University College London**

(With Ten Text-figures)

The preceding paper of this series (Searle, 1954) analyses causes of variation in twenty-one skeletal anomalies of the A/Gr and C57BL/Gr inbred strains. Some other variants, which were too rare for a full analysis, seem nevertheless of sufficient interest to justify the following brief account of them. The previous paper gives details of how the skeletal material was obtained and prepared for examination.

Description of variants

Bent-nose

In four out of 735 C57BL mice the anterior part of the skull is bent over to one side (Fig. 1) with associated asymmetry of the nasals. Twisting is to the right in two males and one female, to the left in one male. Keeler (1929) has reported a similar twisting of the nose in a black silver strain of mice; the mode of inheritance was uncertain but compatible with the anomaly being due to a dominant with normal overlap. A similar condition is also associated with microphthalmia in myelencephalic blebs (Bagg & Little, 1924). Microphthalmia occurs sporadically in the C57BL strain, but was not observed in any of the four mice with bent-nose. The sporadic appearance of this anomaly may be connected with nutritional factors, as in the Norway rat, where the calcium/phosphorus balance is important; on an unbalanced ratio and with vitamin D deficiency bent-nose may be common, although the incidence varies between inbred strains (Heston, 1938).

Hydrocephalus

As has been known for some time, C57BL mice are occasionally hydrocephalic, with an incidence of about 0·5% in strain C57BL/Gr. The anomaly was studied in one female and two males. The female showed clockwise circling movements; it also had anophthalmia, with a small pigment mass instead of an eye. When killed at 4 months the cerebral hemispheres were almost completely destroyed by the pressure of fluid. Neither of the two males showed any eye defect or circling movements. Celloidin sections of one, killed at 7 weeks, show great distension of the cerebral hemispheres; the swollen lateral ventricles are widely confluent in one area and separated elsewhere only by thin membranes (Fig. 2). There are also abnormalities of the hippocampus, corpus callosum and adjacent structures. The aqueduct of Sylvius is blocked, presumably causing the hydrocephalus. With hydrocephalus-1 in the mouse, obstruction of the aqueduct is secondary (Bonnevie, 1943), but there is dilatation of the whole ventricular system and a defective development of the vermis cerebelli, not found here.

In an outcross of C57BL mice to a short-ear stock, 58 F_1 animals were all normal, but ten out of 233 F_2 progeny were noted as having hydrocephalus, and there were several

* Present address: University of Malaya, Singapore.

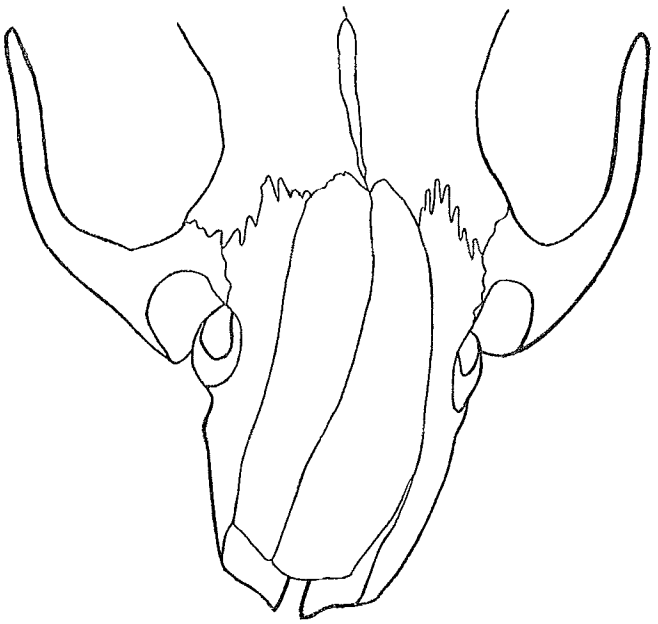

Fig. 1. C57BL skull showing bent-nose. Camera lucida drawing.

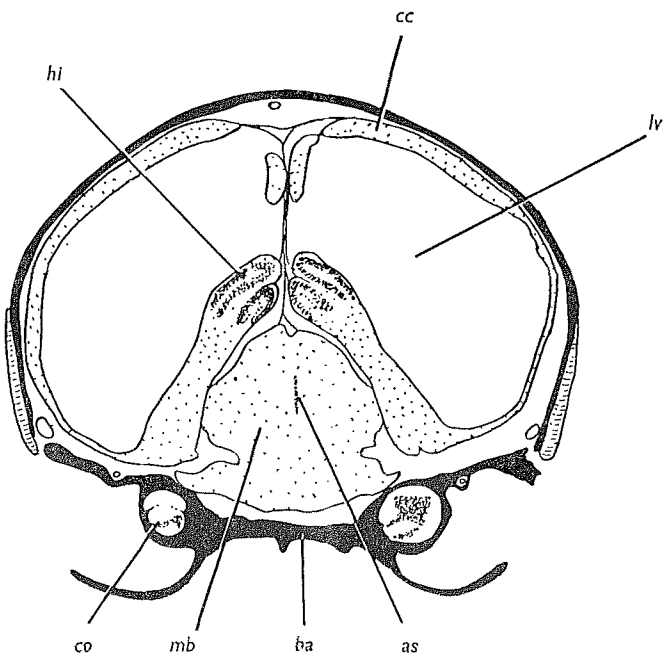

Fig. 2. Transverse section through head of adult C57BL hydrocephalic. *as*, aqueduct of Sylvius (closed); *ba*, basisphenoid; *cc*, cerebral cortex; *co*, cochlea; *hi*, hippocampus; *lv*, lateral ventricles; *mb*, mid-brain. Bone, black; nervous tissue, stippled; muscle, striped. Camera lucida drawing from celloidin section cut at 50 μ.

more in later generations (Grüneberg, unpublished). Two hydrocephalics were also observed in crosses between C57 BL/Gr-d^l and short-ear, but none was seen in several hundred F_2's from matings between the pure line and a stock carrying dominant spotting (W). Thus different genetic backgrounds alter the penetrance of this character.

Dyssymphysis anterior of the atlas

This anomaly is particularly associated with atlas-axis fusion, which often leads to great distortion of the atlas anterior arch, sometimes represented only by an amorphous lump of bone attached to the odontoid process (Fig. 3). Grüneberg (1953*b*) has shown that an essentially similar situation exists in mice with congenital hydrocephalus, the lateral parts of the atlas anterior arch never being formed in cartilage, though the atlas tuberculum anterius may chondrify to some extent. Fig. 3 also shows that this C57 BL dyssymphysis may occur as a separate entity, without any of the bone overgrowth found with atlas-axis dyssymphysis and fusion. There may be merely a failure of fusion in the mid-line, as shown, or there may be a lateral gap. The anomaly was found on its own in four females.

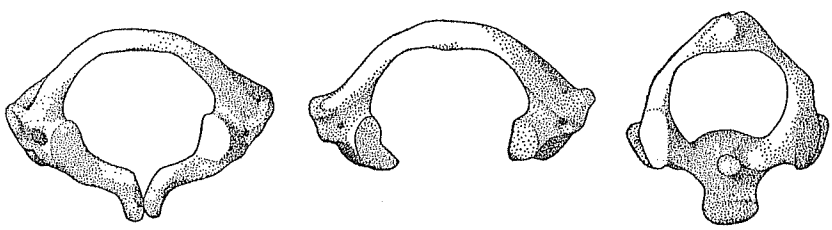

Fig. 3. C57 BL anterior dyssymphysis of the atlas. Left, atlas with failure of fusion in the mid-line; right, atlas and axis in which the atlas anterior arch only remains as a lump on the odontoid process of the axis. Camera lucida drawings.

Table 1. *Distribution of dyssymphysis posterior of the atlas in the A strain*

Subline	Affected	Unaffected	Total
I	2	21	23
II+III+IV	0	449	449

Dyssymphysis posterior of the atlas

Grüneberg (1950) found gaps in the posterior arch of the atlas in the FF stock and in the F_2 between that stock and undulated females. Here it was found only in subline I of the A strain (Table 1). Owing to sterility this subline is small, but genetic differences have arisen between it and the other group of three closely related sublines. Thus Table 1 treated by Fisher's exact method gives $P = 0{\cdot}0023$. But since there is no *a priori* reason to expect this anomaly in subline I rather than the others, this figure multiplied by 472/23 gives a more accurate estimate of the probability of such a distribution occurring by chance (Haldane, quoted by Grüneberg, 1950). The corrected probability is 0·04, which is still significant.

This dyssymphysis, shown in Fig. 4, was found in two females, both of which also had foramina transversaria imperfecta (C III–C V); this anomaly is also confined to subline I in the A strain except for a single example. Here the two anomalies may be associated in development, but although nearly every C 57 BL mouse has foramina transversaria imperfecta (f.t.i.), dyssymphysis posterior of the atlas is unknown in that strain.

Reduction of the odontoid process

One female in my collection of 735 C57 BL mice, and one in Dr Grüneberg's collection of 99, have a much reduced odontoid process of the axis (Fig. 5). In both, the usual three-point articulation between atlas and axis has been replaced by a single horseshoe-shaped joint, just as described by Grüneberg (1953*a*) for Danforth's short-tail (**Sd**/+) mice. These lack the odontoid process and also show shape changes in the centra of cervical and other vertebrae which are not found in the affected C57 BL. In newborn mice with congenital hydrocephalus the odontoid process is also absent or rudimentary (Grüneberg, 1953*b*). In the C57 BL females the missing structure is probably the interventral of the pro-

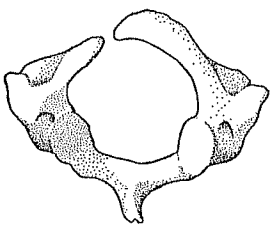

Fig. 4.

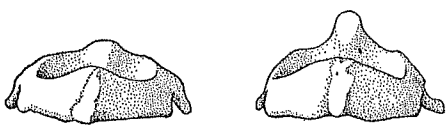

Fig. 5.

Fig. 4. Atlas of A strain female, with dyssymphysis of the posterior arch. Camera lucida drawing.
Fig. 5. Dorsal view of C57 BL axis with a reduced odontoid process, with normal axis on right. Camera lucida drawings.

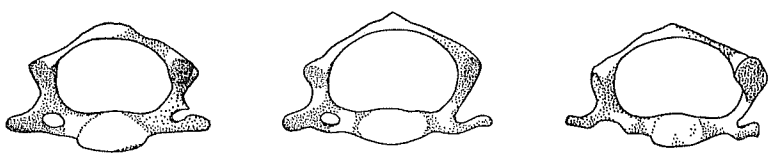

Fig. 6. F.t.i. with dorsal opening in C57 BL mice, with associated defects. Camera lucida drawings.

Table 2. *Distribution of dorsally open foramina transversaria imperfecta in 735 C57 BL mice*

	C III		C IV			
	Left	Right	Left	Right	Total examined	% affected
Males	0	1	1	1	338	0·9
Females	0	1	1	2	397	1·0

atlas, which forms the tip of the normal odontoid process and occasionally occurs in man as a separate bone, the ossiculum terminale (Le Double, 1912).

Dorsal opening of foramina transversaria imperfecta

Out of 2409 f.t.i. found in C57 BL mice, seven open dorsally instead of ventrally (0·34%). Table 2 shows that C V and C VI are never affected; since the usual type of f.t.i. is about four times as common on these two vertebrae combined than on C III + C IV, there is clearly a difference in the distribution of the two types of f.t.i. In two of the affected vertebrae (Fig. 6) there is also reduction of the transverse process and some distortion of the post-zygapophysis on the affected side. It looks as if the anomaly may sometimes be due to one vertebra being in a somewhat skew position relative to the others and sometimes to an abnormal position of the vertebral artery.

Incomplete cervical vertebrae

Some C57 BL mice lack a substantial part of a cervical vertebra; again, only C III and C IV are affected, apart from absence of the tuberculum anterius of C VI, discussed in the previous paper, and the atlas dyssymphysis anterior. There may be no sign of a transverse foramen, the transverse process may be reduced (as shown in Fig. 6) or absent altogether (Fig. 7). On one side, arch, zygapophyses and centrum may also vanish, so that only half the vertebra remains. Although this anomaly is particularly associated with asymmetrical cervical fusions, Fig. 7 shows that absence of transverse process may occur when fusion is fairly symmetrical, and more extreme defects when there are no signs of fusion between the vertebra affected and adjacent ones. But in two of the four affected vertebrae in the last category, articulation with adjoining vertebrae is very asymmetrical; in one of these there are atlas-axis and C V–C VI fusions.

Five out of 261 males and three out of 308 females lack a transverse process or have more extreme defects. One litter has two affected mice, two others are in successive litters. This suggests that maternal influences acting on sibs help to produce the anomalies.

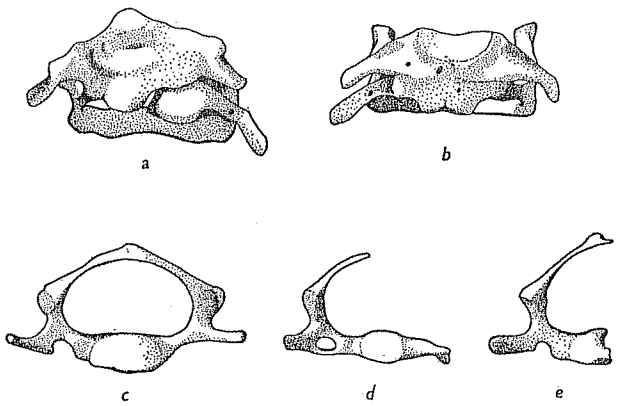

Fig. 7. Incomplete C57 BL cervical vertebrae. *a* and *b*, ventral views of asymmetrical axis-C III and symmetrical C III–C IV fusions with absence of transverse process; *c*, C IV with no right transverse foramen; *d* and *e*, very incomplete C IV vertebrae, with a skew articulation on C V in *e*. Camera lucida drawings.

Dystopia cranialis tuberculi anterioris

Grüneberg (1950) has described the presence of an accessory tuberculum anterius on C V in mice of the A strain, also affected in this experiment. But here the anomaly also turned up in C57 BL mice; three out of 912 tubercula anteriora had shifted cranially on to C V. Unlike the situation in A the dystopia is complete; in all three affected animals the adjoining transverse foramen of C VI is widely open.

Dyssymphysis of the vertebra prominens (Th II)

Dyssymphysis of the arch of Th I, described in the previous paper, is quite common in C57 BL mice. Dyssymphysis of Th II also occurs sporadically in this strain, leading to a central gap in the prominent processus spinosus (Fig. 8). Sometimes there is a proximal gap while the processus spinosus is normal distally. One male out of 338 and six females out of 397 show the anomaly, the overall incidence being 1·0 %. Dyssymphysis of Th I is also commoner in females.

Thoracic and sacro-caudal fusions

One C57BL female has a fusion of Th XI and Th XII, as well as other unique anomalies, namely, caudal fusions and absence of the lower right third molar. Of 472 A strain mice, one male and one female have thoracic fusions (Fig. 9). In the female the whole thoracic region is abnormal, most of the vertebrae being asymmetrical and distorted.

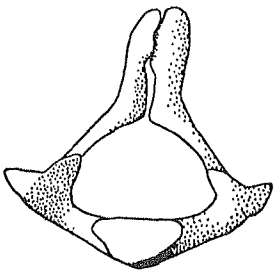

Fig. 8.

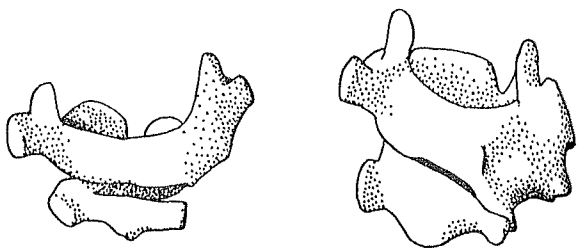

Fig. 9.

Fig. 8. Dyssymphysis of Th II through the processus spinosus in a C57BL male aged 454 days. Camera lucida drawing.

Fig. 9. Thoracic fusions in A strain mice, with distortion of vertebrae. Left, Th III–Th IV; right, Th VIII–Th IX. Camera lucida drawings.

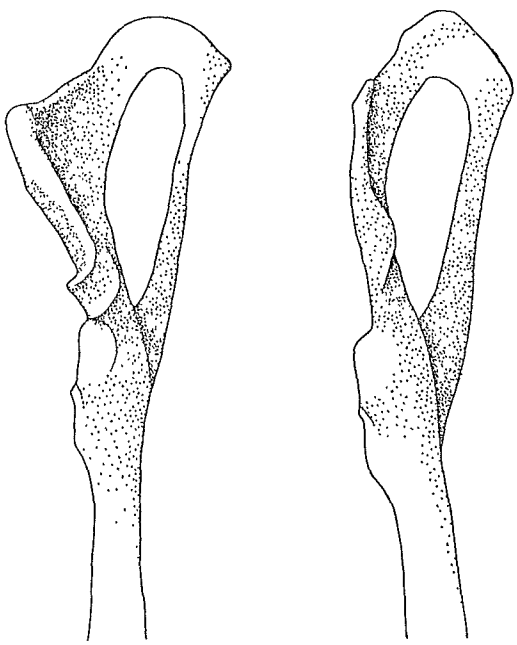

Fig. 10. Dorsal views of a normal (right) and abnormal (left) C57BL female right pelvic girdle. Camera lucida drawings.

with dyssymphysis of the arch, misshapen centra, abnormal articulations and so on. In the affected male, of a different subline, the fused vertebrae are greatly distorted (Th VIII and Th IX) but neighbouring vertebrae are only slightly affected.

One A strain male has a fusion of the fourth sacral and first caudal vertebrae, the neural spines being confluent and the two vertebrae somewhat distorted.

Bent pelvic girdle

In some C57 BL mice the ischium is bent more or less sharply outwards (Fig. 10) instead of being in line with the ilium. The affected bone usually has an abnormal furrow or indentation, due to the action of the tendon of the obturator internus muscle (T. C. Carter, personal communication), which originates on the median face of the ischium and passes over its edge to insert near the head of the femur. Carter (1951) found a similar lateral deviation of the ischium in luxate mice, which he attributed to changes of muscle action due to other abnormalities associated with luxate. Probably the C57 BL anomaly is also due to abnormal muscle action.

All nine affected mice are female; the probability of such a distribution occurring by chance is 0·00375. Thus the condition may be sex-limited in this strain. Two mice are affected on both sides and in two litters a pair of litter-mates have bent pelvic girdles. So it is very likely that some of the factors concerned act on individuals as a whole and some of these factors act alike on litter-mates too.

SUMMARY OF FREQUENCIES

Table 3 summarizes the frequencies in the two strains of the variants discussed.

Table 3. *Percentage frequencies of rare skeletal variants in strains A and C57 BL*

Variant	C57 BL	A
Bent-nose	0·6	0·0
Hydrocephalus	0·5	0·0
Dyssymphysis anterior of the atlas	1·3*	0·0
Dyssymphysis posterior of the atlas	0·0	0·0†
Reduction of the odontoid process	0·1	0·0
Dorsally open f.t.i.	1·0	0·0
Incomplete cervical vertebrae	1·4	0·0
Dystopia cranialis tuberculi anterioris	0·3	0·2
Dyssymphysis of the vertebra prominens (Th II)	1·0	0·0
Thoracic fusions	0·1	0·4
Bent pelvic girdle	1·2‡	0·0

* Of mice without dyssymphysis of atlas and axis.
† Except in subline I, where 8·7%.
‡ 2·3% in females, 0·0% in males.

DISCUSSION

Over thirty skeletal variants occurring in one or both of the A and C57 BL strains have been described in this and previous papers of this series (Grüneberg, 1950; Truslove, 1952; Searle, 1954). Half the variants involve one or more cervical vertebrae, the first four being especially vulnerable. This suggests that the morphogenetic processes giving rise to these particular vertebrae are very complex and the balance between the processes is easily upset by small changes in the local environment. Other variants have been noticed but not yet studied: dyssymphysis of the arch of Th X is quite common in 60-day C57 BL mice, distortion of the manubrium of the sternum has also been seen occasionally in this strain. No thorough examination of soft tissues has been made, yet microphthalmia, anophthalmia, hydronephrosis and absence of pigmentation in from 0% to 50% of the tail are known to occur in C57 BL mice, as well as missing or supernumerary mammae in both strains and various types of tumour. There must be many more clear-cut variants in skeletal and non-skeletal tissues, but enough have been found to emphasize the difference between genetic and phenotypic uniformity.

Of the eleven anomalies described here, only dystopia cranialis tuberculi anterioris and thoracic fusions occur in fairly equal frequencies in the two strains, but even with these there are differences in expression, the dystopia being complete in the C57BL but not in the A strain, while the C57BL thoracic fusion was accompanied by other unique abnormalities which the affected A strain mice do not possess. So there seem to be inter-strain differences with respect to all these characters. But their distribution within the strains is so sporadic as a rule, apart from occasional sex and subline differences, that there can be little doubt their manifestation depends mainly on non-genetic factors. Sometimes maternal influences seem to be at work, as with some of the commoner variants discussed previously, but most variants are probably caused by a rare combination of circumstances affecting an individual mouse, with a physiological threshold involved.

Three or four variants seem to mimic pleiotropic effects of known mouse mutant genes. Perhaps one can look upon the anomalies as being the remote effects of genes the main action of which is not known because they play a part in normal and not in abnormal development. No doubt a study of incompletely penetrant pleiotropic effects of known mutant genes would show that maternal influences are sometimes important as non-genetic causative agents, just as they are with the type of variant discussed here.

Summary

Data are given on eleven uncommon variants found in the A and C57BL strains of mice, frequencies being summarized in Table 3. Nine of these variants are peculiar to one strain. While the tendency of a strain to produce a given anomaly is presumably under some sort of genetic control, the manifestation of these rare variants is mainly due to non-genetic factors.

REFERENCES

Bagg, H. & Little, C. C. (1924). Hereditary structural defects in the descendants of mice exposed to Roentgen ray irradiation. *Amer. J. Anat.* **33**, 119–45.

Bonnevie, K. (1943). Hereditary hydrocephalus in the house mouse. I. Manifestation of the *hy*-mutation after birth and in embryos 12 days old or more. *Skr. norske VidenskAkad.* no. 4, pp. 32.

Carter, T. C. (1951). The genetics of luxate mice. I. Morphological abnormalities of heterozygotes and homozygotes. *J. Genet.* **50**, 277–99.

Grüneberg, H. (1950). Genetical studies on the skeleton of the mouse. I. Minor variations of the vertebral column. *J. Genet.* **50**, 112–41.

Grüneberg, H. (1953*a*). Genetical studies on the skeleton of the mouse. VI. Danforth's short-tail. *J. Genet.* **51**, 317–26.

Grüneberg, H. (1953*b*). Genetical studies on the skeleton of the mouse. VII. Congenital hydrocephalus. *J. Genet.* **51**, 327–58.

Heston, W. E. (1938). Bent-nose in the Norway rat. *J. Hered.* **29**, 437–48.

Keeler, C. E. (1929). The occurrence of heritable twisted nose in the house mouse, *Mus musculus*. *Proc. Nat. Acad. Sci., Wash.*, **15**, 838–9.

Le Double, A.-F. (1912). *Traité des Variations de la Colonne Vertébrale de L'Homme.* Pp. vii + 543. Paris: Vigot Frères.

Searle, A. G. (1954). Genetical studies on the skeleton of the mouse. IX. Causes of skeletal variation within pure lines. *J. Genet.* **52**, 68–102.

Truslove, G. M. (1952). Genetical studies on the skeleton of the mouse. V. 'Interfrontal' and 'parted frontals'. *J. Genet.* **51**, 115–22.