J. Anat. (1976), **121**, 3, pp. 507–513
With 2 figures
Printed in Great Britain

The interfrontal bone and mutant genes in the mouse

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(*Accepted* 24 *June* 1975)

INTRODUCTION

The skeleton of the mouse is highly polymorphic (Grüneberg, 1963) and mutant genes may have considerable effects upon minor skeletal variants (Grüneberg, 1955). In this paper the relationship between one such variant, the interfrontal bone of the skull, and a number of mutant genes is examined.

The presence of an 'os wormien fronto-nasal' or intrafrontal bone was first noted in cattle (Cornevin, 1883); it was described as common (about one in twenty animals sampled). Le Double (1903) mentioned a similar bone as a rare variant in man. The bone was first described in the mouse by Keeler (1933) in two strains bred at the Bussey Institution and in a wild female from a Boston house. Truslove (1952) described the bone again in some detail in the context of a minor skeletal variant (Grüneberg, 1963) with embryological detail and frequency of occurrence in two inbred strains, CBA/Gr and C57BL/Gr. Since then it has been recorded in other laboratory strains and in samples of wild mice (Berry, 1963, 1964; Berry & Searle, 1963; Carpenter, Grüneberg & Russell, 1957; Howe & Parsons, 1967).

The presence of certain mutant genes in mouse stocks may modify the expressivity and penetrance of minor skeletal variants such as the interfrontal. During the course of several years' research on a varied sample of mouse mutants it occurred to the author that the presence of a high frequency of interfrontal bones, or of interfrontal bones of large size, was often linked with the presence of a mutant gene affecting the development of the neural tube, and that such genes often had an effect on skull proportion (Table 1).

The present study comprises a series of measurements of skull proportions in mice carrying mutant genes which affect the interfrontal and neural tube, using as controls mutations which are known not to affect the frequency or size of the interfrontal bone. The situation in an inbred strain C57BL/Gr is also considered.

MATERIALS AND METHODS

Measurements were made on skulls cleaned with the proteolytic enzyme papain, using a binocular dissecting microscope with camera lucida attachment. Following Grüneberg & Truslove (1960), skull width (measurement A, Fig. 1) was taken across the maxillary–frontal suture, and skull length (measurement B, Fig. 1) from the posterior margin of the basisphenoid to the nasal spine.

Skulls of mice carrying genes known to affect the interfrontal and of mice carrying

Table 1. *Genes influencing the occurrence of the interfrontal bone in the mouse*

Name	Chromosome	Genotype(s) affected	Source
Brain hernia	7	*bh/bh*	Bennett (1959)
Bent-tail	X	*Bn/+*, *Bn/Bn*	Grüneberg (1955)
Fidget	2	*fi/fi*	Grüneberg (1955) Truslove (1956)
Strong's luxoid	2	*+/lst*, *lst/lst*	Forsthoefel (1962)
Patch	5	*Ph/+*	Grüneberg & Truslove (1960)
Tail-short	—	*Ts/+*	Deol (1971)
Urogenital	—	*ur/ur*	Fitch (1957)
Extra-toes, brachyphalangy	13	*Xt/+*, $Xt^{bph}/+$	Johnson (1967, 1969)

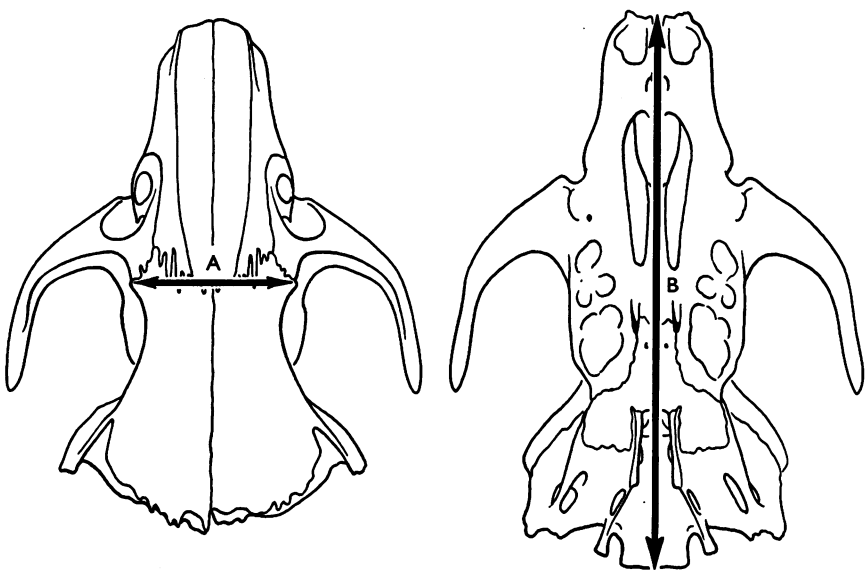

Fig. 1. Dorsal and ventral views of the skull of an adult mouse showing (A) width across maxillary-frontal suture, (B) length from posterior margin of basisphenoid to the nasal spine.

mutant genes known not to affect the interfrontal (Affecting: 20 *Bn/*−♂♂ and *Bn/+*♀♀, Grüneberg, 1955; 20 *fi/fi*, Truslove, 1956; 20 *Ph/+*, Grüneberg & Truslove, 1960; 21 *Xt/+*♀♀, Johnson, 1969. Not affecting: 20 *Sd/+*, 14 *se/se*, 20 *un/un* and 20 *vt/vt*, Grüneberg, 1955) were measured together with an equal number of normal litter mates. One hundred C57BL/Gr skulls were also measured and classified for the presence of an interfrontal bone.

RESULTS

In C57BL and *Ph/+* the ratio of skull width to skull length (A/B, Fig. 1; used to overcome difference in overall size of skulls within samples) was calculated for mice of different interfrontal size. Interfrontal size was graded from 0 to + + + +,

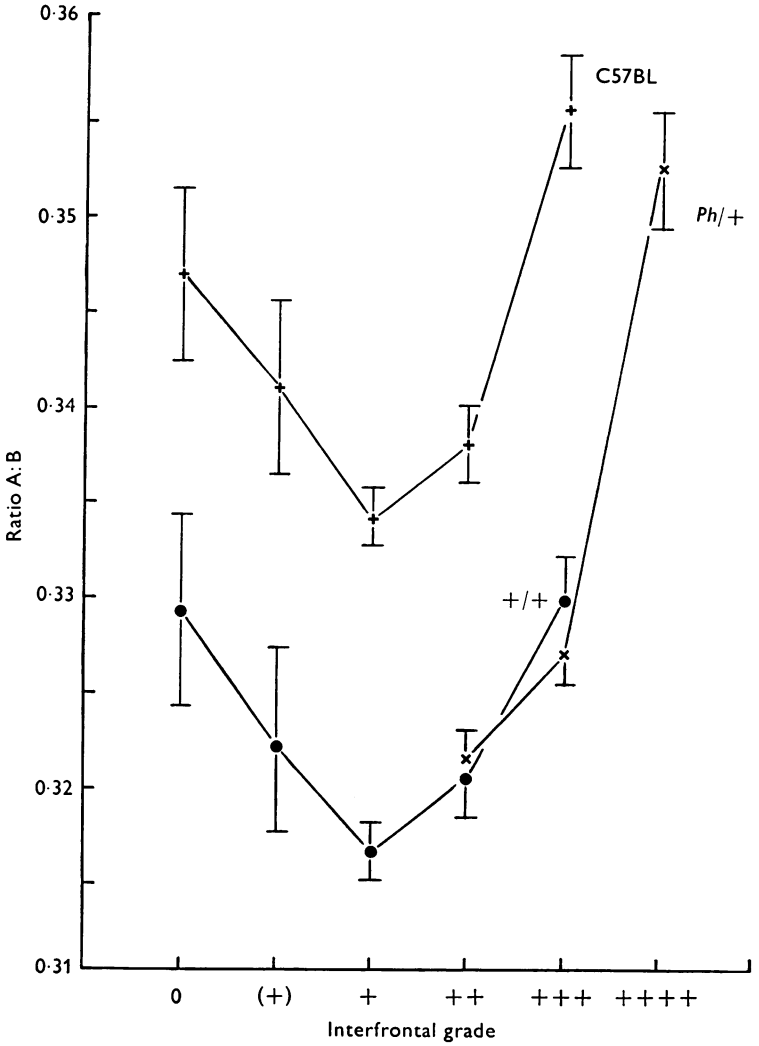

Fig. 2. Relationship between size of interfrontal and width/length ratio of the mouse skull. (Bars indicate S.E.M.)

following the classification of Grüneberg & Truslove (1960); in fact, the *Ph*/+ skulls used had been previously classified by Truslove for this study. On plotting interfrontal size against A/B a consistent pattern emerged in both stocks (Fig. 2). Mice with no interfrontal (0) or no interfrontal visible on the dorsal aspect of the skull (+) had variable A/B values. When the interfrontal became visible on the dorsal surface and thus contributed to the external aspect of the skull its size increased with increasing A/B. The C57BL and *Ph*/+ − +/+ curves are essentially similar in shape but absolute values of A/B are different in the two stocks.

The other mutant genes which affect interfrontal frequency or size are concordant (Table 2). In all cases A/B is increased significantly. Amongst the control group

Table 2. *Width/length ratios of the skulls of mutant mice and their normal litter mates*

Gene symbol	Interfrontal affected	A/B normal (see Fig. 1)	A/B mutant	*n*	*t*	*P*
Bn	Yes	0·310	0·313	20	3·29	< 0.01
fi	Yes	0·314	0·347	20	31·03	≪ 0·01
Xt^{bph}	Yes	0·313	0·339	20	6·96	< 0·01
Sd	No	0·315	0·316	20	0·87	n.s.
se	No	0·326	0·328	14	1·32	n.s.
un	No	0·322	0·342	20	10·96	< 0·01
vt	No	0·323	0·326	20	2·63	~ 0·01

Table 3. *Skull dimensions of mutant individuals and their normal litter mates*

Genotype	Width A (cm)	*t*	*P*	Length B (cm)	*t*	*P*
		Interfrontal affected				
Bn/−, *Bn*/+	0·520	4·36	< 0·01	1·662	0·59	n.s.
+/+	0·513			1·657		
fi/*fi*	0·487	17·03	≪ 0·01	1·402	40·44	≪ 0·01
+/*fi*	0·510			1·625		
Xt^{bph}/+	0·544	3·49	< 0·01	1·608	0·72	n.s.
+/+	0·509			1·629		
		Interfrontal not affected				
Sd/+	0·507	5·88	< 0·01	1·602	6·56	< 0·01
+/+	0·515			1·633		
se/*se*	0·493	0·28	n.s.	1·508	0·13	n.s.
+/*se*	0·492			1·513		
un/*un*	0·500	2·97	~ 0·01	1·463	10·13	< 0·01
+/*un*	0·507			1·558		
vt/*vt*	0·496	1·49	n.s.	1·515	3·29	< 0·01
+/*vt*	0·495			1·533		

Sd and *se* show no significant difference in A/B, but both *un* and *vt* show a significant increase. Reference to Table 3 shows no particular selectivity in the way in which genes affect skull measurements (e.g. *fi*, *Sd* and *un* depress both length and width).

DISCUSSION

The predisposition to an interfrontal bone is clearly present in some stocks of mice (C57BL, A, CBA) and absent in others (BALB/c, Grüneberg, 1963). It seems that we are dealing with another case of quasi-continuous variation (Grüneberg,

1952), with a threshold for the presence of an interfrontal operating at some point in development. That part of the population which exceeds the threshold acquires an interfrontal whose ultimate size depends on the proportions of the adult skull. This hypothesis agrees with the data of Figure 2. Where the threshold has not been exceeded, a wide range of skull proportion is seen; when the interfrontal forms part of the external surface of the skull, a correlation exists between interfrontal size and width/length ratio, and the variability of skull proportions is reduced. Kadam (personal communication to Berry, in Berry, 1964) found no correlation between skull width and the presence of an interfrontal: this is understandable if no correction was made for overall skull size.

The suggestion that abnormalities of the neural tube can affect the ultimate size of the interfrontal is borne out by the data of Table 2. It cannot be coincidental that all those genes, bar one, which affect interfrontal size also affect the neural tube (Table 1). The brain hernia mutation produces protrusion of the neural tube through a median dorsal opening in the skull (Bennett, 1959). Bent-tail (*Bn*/− ♂♂ and *Bn*/*Bn*♀♀) often has an open neural tube in the sacral region and cranioschisis *in utero* (J. Butler & M. F. Lyon, personal communication). The *Ph*/*Ph* neural tube is wavy from the ninth day, and the 16 day old embryo illustrated by Grüneberg & Truslove (1960) has extensive spina bifida. The neural tube of *Ts*/+ mice is abnormal from the eleventh day in the trunk (Deol, 1971). *Xt* and Xt^{bph} have abnormal neural tubes from the ninth day (Johnson, 1967, 1969). *fi*/*fi* mice show defects of the eyes, ears and cerebellum (the latter probably secondary; Truslove, 1956). Deol (1966) speculates that the eye and ear defects in mouse mutants may be due to a primary neural tube disorder in the early embryo. Strong & Hardy (1956) noted hydrocephalus and occasional cranioschisis in *lst*. Forsthoefel (1962) has described abnormalities in the brain of *lst* which he regards as secondary to the shortening of the basicranium. Only in urinogenital (*ug*) is there no positive evidence for the involvement of the neural tube: the skull is short and wide, but the basis of the syndrome is unknown.

Schowing (1968) found that removal of certain parts of the chick brain caused defects in the overlying bones of the skull. Removal of the prosencephalon reduced the frontal bones; removal of the prosencephalon+mesencephalon resulted in their absence. He suggested that the brain induces the cranium, each region of the brain acting on an adjacent bone anlage.

Clearly a strong case can be made for a derangement of the neural tube being the basis for a change in the frequency or size of the interfrontal, and this by affecting skull proportions. It seems likely that the basis of increased frequency/size of interfrontals in neural tube mutants is simply that the ultimate proportions of the skull are modified by the lesion giving scope for fuller expression of interfrontal potential.

It is equally clear that derangement of adult skull proportions is not in itself sufficient to guarantee a fuller expression of interfrontal size. In two of the four control groups chosen for this study (*un* and *vt*, Table 2) the width/length ratio of the skull is increased with no change in interfrontal incidence. Yet the interfrontal bone was present in both stocks (*un*/*un* 13/16, normal controls 11/16; *vt*/*vt* 1/20, normal controls 1/20, Grüneberg, 1955). The obvious explanation of

these facts is that the overall dimensions of the adult skull must be the end result of the interaction of a whole host of variables acting during pre- and postnatal development: possibly neural tube defects act at a particularly relevant point in time as far as the interfrontal is concerned.

The interfrontal is also common in many inbred strains of mice (CBA, 86 % incidence, C57BL 54–72 %, C57BR 82 %): there is no evidence to suggest neural tube defects in these strains. Berry (1964) remarked on the rarity of the interfrontal in mice from the British mainland: it did not occur once in over 1000 mice sampled. However, it was present (4 %) in mice from Dale (Pembrokeshire) and common (50·3 %) in Skokholm mice. This is presumably a founder effect, and Berry regards Dale mice as the most likely source of the Skokholm population. Island populations are often the result of the isolation of small numbers of mice followed by inbreeding. This state of affairs also applies to the deliberate establishment of an inbred strain. It is possible that the interfrontal in CBA, C57BL and C57BR is also the result of a founder effect.

SUMMARY

The relationship between corrected skull width and the presence and size of an interfrontal bone is discussed with regard to the effect of certain mutant genes in the mouse known to affect the development of the neural tube. All genes reviewed which increase the incidence of the interfrontal bone and affect the neural tube also change the proportions of the adult skull.

The author is indebted to Mr A. J. Lee for Figure 1, and would also like to take this opportunity of thanking Mr Lee for the excellent illustrations provided by him for many publications during the period 1966–75.

REFERENCES

BENNETT, D. (1959). Brain-hernia, a new recessive mutation in the mouse. *Journal of Heredity* **50**, 264–268.

BERRY, R. J. (1963). Epigenetic polymorphism in wild populations of *Mus musculus*. *Genetical Research* **4**, 193–220.

BERRY, R. J. (1964). The evolution of an island population of the house mouse. *Evolution* **18**, 468–483.

BERRY, R. J. & SEARLE, A. G. (1963). Epigenetic polymorphism of the rodent skeleton. *Proceedings of the Zoological Society of London* **140**, 577–615.

CARPENTER, J. R., GRÜNEBERG, H. & RUSSELL, E. S. (1957). Genetical differentiation involving morphological characters in an inbred strain of mice. II. American branches of the C57BL and C57BR strains. *Journal of Morphology* **100**, 377–388.

CORNEVIN, C. (1883). Étude sur les os wormiens des animaux domestiques. *Revue anthropologique* **6**, 661–670.

DEOL, M. S. (1971). Genetical studies on the skeleton of the mouse. XXXVIII. Tail-short. *Proceedings of the Royal Society* B **155**, 78–95.

DEOL, M. S. (1966). Influence of the neural tube on the differentiation of the inner ear in the mammalian embryo. *Nature* **209**, 219–220.

FITCH, N. (1957). An embryological analysis of two mutants in the house mouse, both producing cleft palate. *Journal of Experimental Zoology* **136**, 329–361.

FORSTHOEFEL, P. F. (1962). Genetics and manifold effects of Strong's luxoid gene in the mouse, including its interactions with Green's luxoid and Carter's luxate genes. *Journal of Morphology* **110**, 391–420.

GRÜNEBERG, H. (1952). Genetical studies on the skeleton of the mouse. IV. Quasi-continuous variations. *Journal of Genetics* **51**, 95–114.

GRÜNEBERG, H. (1955). Genetical studies on the skeleton of the mouse. XV. Relations between major and minor variants. *Journal of Genetics* **53**, 515–535.

GRÜNEBERG, H. (1963). *The Pathology of Development*. Oxford: Blackwell.

GRÜNEBERG, H. & TRUSLOVE, G. M. (1960). Two closely linked genes in the mouse. *Genetical Research* **1**, 69–90.

HOWE, W. L. & PARSONS, P. A. (1967). Genotype and environment in the determination of minor skeletal variants and body weight in the mouse. *Journal of Embryology and Experimental Morphology* **17**, 283–292.

JOHNSON, D. R. (1967). Extra-toes: a new mutant gene causing multiple abnormalities in the mouse. *Journal of Embryology and Experimental Morphology* **17**, 543–581.

JOHNSON, D. R. (1969). Brachyphalangy, an allele of extra-toes in the mouse. *Genetical Research* **13**, 275–280.

KEELER, C. E. (1933). Interfrontal: a heritable cranial variation of the house mouse. *Journal of Mammalogy* **14**, 75.

LE DOUBLE, A.-F. (1903). *Traité des Variations des Os du Crâne de l'Homme*. Paris: Vigot Frères.

SCHOWING, J. (1968). Mise en évidence du rôle inducteur de l'encéphale dans l'ostéogenèse du crâne du poulet. *Journal of Embryology and Experimental Morphology* **19**, 88–93.

STRONG, L. C. & HARDY, L. B. (1956). A new 'luxoid' mutant in mice. *Journal of Heredity* **47**, 277–284.

TRUSLOVE, G. M. (1952). Genetical studies on the skeleton of the mouse. V. 'Interfrontal' and 'parted frontals'. *Journal of Genetics* **51**, 115–122.

TRUSLOVE, G. M. (1956). The anatomy and development of the fidget mouse. *Journal of Genetics* **54**, 64–86.