

Is subline differentiation a continuing process in inbred strains of mice?

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SUMMARY

Two new sublines of the C57BL/Gr strain of mice have been studied which were derived from earlier sublines in which no genetic variance could be demonstrated. The incidence of some 31 minor skeletal variants was examined which could thus go up or down. As new subline differences have arisen with about the same frequency and mean magnitude of effect as in the past, there is no doubt that, at least in the C57BL strain, subline differentiation is a continuing process. Its high frequency (about 0.01 changes per character at risk per generation) is difficult to reconcile with spontaneous mutation rates of single genes in the mouse. The possibility must thus be considered that some other self-perpetuating processes or entities of some degree of stability are handed down in the lines of descent in which they have arisen, or perhaps become unmasked. There is no reason to suppose that this type of event is confined to inbred strains (in which it can be demonstrated fairly easily); it presumably occurs similarly in mixed stocks in which it would scarcely be detectable.

1. INTRODUCTION

Mutations in inbred strains happen in a single line of descent. If fixed rather than eliminated by continued inbreeding, a mutation will thus give rise to a genetically distinct subline. The existence of sublines in inbred strains of mice has been known for a long time (Green, 1953; Grüneberg, 1954). Systematic studies (Deol *et al.* 1957; Carpenter, Grüneberg & Russell, 1957; Grewal, 1962) have, however, shown that this happens to an altogether unexpected extent and doubts have arisen as to whether all these changes can be accounted for in terms of conventional mutations. One possibility considered (but regarded as unlikely) is that subline differentiation was largely due to the delayed fixation of residual heterozygosity. If so, that process should now have come to an end as it was found (Deol *et al.* 1960) that the strain studied in most detail, C57BL/Gr (like others), had by then become genetically homogeneous. Hence, if subline differentiation were to continue as it has happened in the past, this would be incontrovertible evidence for its origin *de novo*. At the suggestion of Professor H. Grüneberg, Dr G. M. Truslove therefore branched off, from the existing subline VII, a new line of descent which

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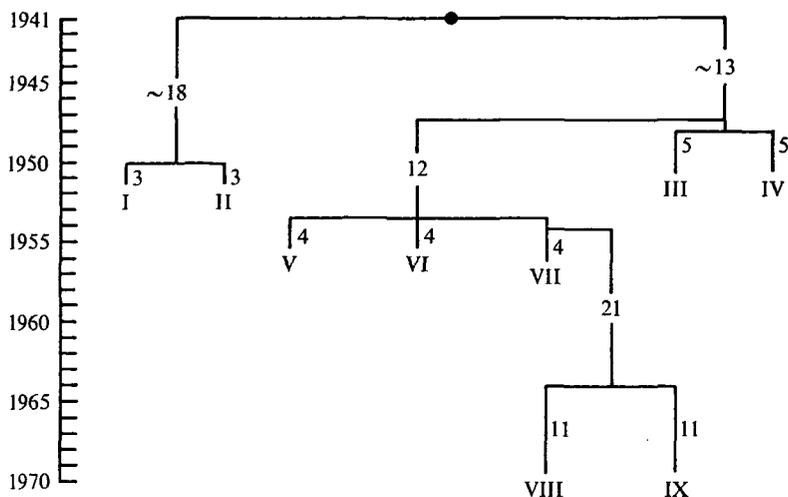


Fig. 1. Pedigree showing the relations of sublines VIII and IX with sublines I-VII.

was carried on for 21 generations and then forked to form two parallel sublines (VIII and IX) which were continued for another 11 generations of brother-sister mating. The present paper is an investigation of these sublines by means of the same array of minor skeletal variants which has been used in previous studies from this laboratory (for a review see Grüneberg, 1963).

2. MATERIALS AND METHODS

The material used includes papain skeleton preparations of two new sublines (VIII and IX) of the C57BL/Gr strain of mice. Their relationship with the other sublines of the C57BL/Gr strain is shown in Fig. 1. The history of sublines I-VII of the C57BL/Gr strain has been described in detail by Deol *et al.* (1957). An offshoot of subline VII was started in 1953, and in 1964 sublines VIII and IX were separated and each was inbred until 1969. About two generations were produced each year. Papain skeleton preparations were made between December 1969 and April 1970 by Miss M. A. Elsmore, all animals being about 60 days of age.

To ensure continuity with earlier work, familiarization with the criteria of classification of the various characters was achieved by reference to previously classified skeletons. Punch cards were used for recording the skeletal variants. In order to randomize subjective errors of classification, approximately equal numbers of skeletons from sublines VIII and IX were examined each day.

The frequency of central variants was expressed as occurrence per animal; in the case of bilateral variants it was based on the number of sides on which the character occurred.

In the case of central characters, χ^2 significance tests were carried out by means of fourfold tables, with Yates's correction where appropriate. For bilateral characters, a χ^2 test devised by C. A. B. Smith (Appendix to Grüneberg, 1955) was

used. Fisher's 'exact' treatment of 2×2 tables was used where appropriate. The difference between sublimes in terms of standard deviations was obtained from probit transformations of the percentage incidences.

3. RESULTS

The incidence of 27 skeletal variants in sublimes I–IX is given in Table 1. In several cases, as a result of damage to specimens, the value is based on a lower total than that recorded for the subline. Table 2 summarizes the incidence of four rare variants for which no data are available for sublimes I–IV and which for that reason are not included in the quantitative treatment.

(i) Comparison between sublimes V–VII and VII + IX

In cases where VIII and IX agree with each other and differ from V–VII, the difference has presumably arisen in the 21 generations before the fork. Ten such differences are listed in Table 3. There are thus 10 'events' in 27 characters at risk in 21 generations, or 0.0176 events per character per generation. This compares with an average value of 0.01 deduced for the earlier sublimes (Grewal, 1962; Grüneberg, 1970). The mean difference is 0.83 standard deviations as compared with the average mean of 0.79 standard deviations for sublimes I–VII (Deol *et al.* 1957).

(ii) Comparison between sublimes VIII and IX

Differences between these sublimes have presumably arisen since they were separated from each other. Four of the 27 variants listed in Table 1 differ significantly between VIII and IX. These are interfrontal–frontal fusion ($\chi_1^2 = 4.98$; $P = 0.025$), foramen ovale single ($P = 0.029$; Fisher's exact test), preoptic sutures of the presphenoid ($\chi_1^2 = 9.18$; $P < 0.01$), and processus pterygoideus absent ($\chi_1^2 = 4.17$; $P = 0.04$). An additional case is probably atlas–axis fusion. Here IX differs significantly from V–VII ($\chi_1^2 = 16.55$; $P < 0.001$), whereas VIII is similar to V–VII (13.9 as compared with 10.2%; $\chi_1^2 = 1.11$; $P \sim 0.3$); although VIII and IX do not differ significantly from each other by conventional standards ($\chi_1^2 = 3.06$; $P \sim 0.08$), there is little doubt that this is a change which occurred subsequent to the separation of VIII and IX. If this is accepted, there are five events in 27 characters at risk in 22 generations, or 0.0084 per character per generation.

Combining all the data, there are 15 events in 27 characters at risk in 43 generations, or 0.013 per character per generation, in satisfactory agreement with the value of 0.01 obtained previously. As, on a pure chance basis, only one or two comparisons out of 27 might be expected to have reached a level of formal significance, most of the events are clearly real. Similarly, the overall average difference in terms of standard deviations is 0.65, again in satisfactory agreement with the estimated value of 0.61 (Deol *et al.* 1957). The difference between sublimes VIII and IX is, however, 0.32 standard deviations.

Table 1. *Percentage incidence of 27 skeletal variants in nine sublines of the C57BL/Gr inbred strain*
 (The figures for sublines I-VII are those of Deol *et al.* (1957) as given by Grewal (1962). The number of animals used for each subline is given in parentheses.)

No.	Variant	I (163)	II (188)	III (134)	IV (95)	V (30)	VI (146)	VII (87)	VIII (115)	IX (120)
1	Lacrimal-maxilla fusion	12.6	15.2	11.9	11.1	11.7	17.8	14.9	13.0	11.7
2	Parted frontals	10.4	8.6	3.0	9.5	6.7	5.5	13.8	0.9	0
3	Fused frontals	4.3	5.3	6.7	3.2	0	4.8	4.6	6.1	11.7
4	Interfrontal-frontal fusion (visible dorsally)	14.8	13.7	15.3	27.6	12.5	13.9	10.3	12.2	23.3
5	Squamosal-parietal fusion	6.9	6.1	6.0	10.0	16.7	4.6	4.6	6.5	8.8
6	Periotic-occipital fusion	3.7	5.3	3.7	4.2	6.7	2.7	1.7	0	0.8
7	Foramen ovale single	1.8	3.2	4.5	4.2	3.3	3.8	1.7	2.6	6.3
8	Foramen ovale open posteriorly	6.1	3.8	2.6	6.3	5.0	4.5	2.3	1.8	3.3
9	Foramen hypoglossi single	22.4	25.9	25.9	22.6	18.3	26.6	24.7	26.5	23.3
10	Atlas-axis fusion	8.6	4.8	13.4	6.3	10.0	6.8	16.1	13.9	23.3
11	Tuberculum anterius in- flexum of CVI	8.7	6.1	4.3	6.7	6.1	4.4	2.5	3.5	2.5
12	Dyssymphysis of Th I	16.6	20.9	22.7	26.6	20.0	13.8	12.6	21.7	20.0
13	Dyssymphysis of pro- cessus spinosus of Th II	1.2	1.0	0	2.1	3.4	0	0	2.6	1.7
14	Interfrontal	57.7	67.4	73.9	84.2	90.0	87.0	81.6	94.8	89.9
15	Interparietal-occipital fusion	0	2.1	5.2	4.2	6.7	10.3	18.4	27.8	23.3
16	Inframaxillary crest	85.4	83.9	95.1	92.6	1.3	7.6	14.4	63.0	53.8
17	Alae palatinae absent	21.6	15.5	7.5	6.5	28.7	28.3	31.1	12.2	14.3
18	Foramen sphenoidale medium	44.2	43.9	41.0	42.1	23.3	26.7	25.3	30.4	38.7
19	Processus pterygoideus absent	47.5	48.1	34.3	25.3	11.7	21.2	21.8	32.2	23.1
20	Presphenoid, preoptic sutures	30.0	30.9	46.6	33.8	26.7	39.0	33.3	37.8	54.6
21	Accessory mental foramen	39.6	42.0	43.2	43.2	28.3	25.7	27.0	7.8	11.3
22	Cervical fusions	3.1	4.8	12.7	3.2	3.3	4.8	1.1	1.7	0.8
23	Absence of tuberculum anterius of C VI	15.3	20.6	22.8	21.1	16.7	22.1	29.9	15.7	18.8
24	Foramina transversaria imperfecta C V	51.0	68.7	66.4	67.9	66.7	71.6	61.7	67.0	71.7
25	Dystopia of processus spinosis of Th II	2.5	4.8	2.2	1.1	13.8	7.5	14.9	17.4	12.5
26	Dyssymphysis of Th X	44.8	52.9	28.4	44.2	23.3	15.1	19.5	0.9	2.5
27	Sacralization of L VI	14.4	11.5	2.4	1.1	8.5	4.5	6.3	1.3	0

Table 2. *Percentage incidence of four rare skeletal variants in sublines V-IX of the C57BL/Gr inbred strain*

Variant	V + VI + VII	VIII	IX
Basisphenoid-presphenoid fusion	1.13	1.74	5.83
Presphenoid, metoptic roots abnormal	1.13	0.87	1.26
Bent nose	0.37	0.87	0
Thoracic fusions	—	6.09	0.83

Table 3. *Ten variants with differences between sublines V-VII and VIII + IX*

(Subline differences are given in terms of standard deviations (from probit transformations). Where sex differences have been demonstrated the two sexes are treated separately.)

Variant	Percentage incidence		χ^2_1	P value (exact test)	Subline difference
	V-VII	VIII + IX			
Parted frontals	7.2	0.4	—	0.000041	1.19
Periotic-occipital fusion	2.8	0.4	—	0.0019	0.74
Interfrontal	85.6	92.3	5.65	—	0.36
Interparietal-occipital fusion	12.5	25.5	13.78	—	0.49
Inframaxillary crest, ♂♂	11.5	72.3	121.98	—	1.79
Inframaxillary crest, ♀♀	7.0	45.5	61.76	—	1.36
Alae palatinae absent, ♂♂	20.3	10.7	6.12	—	0.41
Alae palatinae absent, ♀♀	37.8	15.6	19.42	—	0.70
Foramen sphenoidale medium	25.9	34.6	4.53	—	0.25
Accessory mental foramen	26.5	9.6	40.35	—	0.68
Dyssymphysis of Th X	17.5	1.7	32.53	—	1.19
Sacralization of L VI, ♂♂	8.8	0.9	—	0.000019	1.01
Sacralization of L VI, ♀♀	2.2	0.4	—	0.08	0.64

Table 4. *Mean body weights in grammes of sublines VIII, IX and V-VII*

(Weights for sublines V-VII are from Deol & Truslove (1957).)

Subline	Birth weight	21-day weight	60 ± 2-day weight
V-VII	1.36	8.03	22.26
VIII	1.39 ± 0.022	8.15 ± 0.118	22.22 ± 0.32
IX	1.35 ± 0.015	7.63 ± 0.156	21.10 ± 0.31

In addition, thoracic fusions (Table 2) occur with a significantly higher frequency in VIII as compared with IX ($P = 0.026$).

4. DISCUSSION

When the skeletal material of sublines V-VII was collected in 1954-55, the animals lived in the old animal house in Chemistry Yard, University College London, which had neither temperature nor humidity control; they lived in wooden cages which could not be sterilized and hence they were plagued by ectoparasites. By contrast, since 1967 the mice have lived in the greatly improved animal rooms of Wolfson House in plastic cages and are free of ectoparasites.

(Diet 86 of the Rowett Research Institute has been used throughout since 1951.) In view of the drastic improvement of the environment, the possibility must be considered that the differences between V-VII versus VIII+IX might not be wholly genetic. Deol & Truslove (1957) found that a reduction in body weight due to an unbalanced diet can influence the incidence of many skeletal variants in the C57BL strain; conceivably, a change of body weight due to other causes may have a similar effect (a point presently under investigation by Dr G. M. Truslove). However, the weights of V-VII differ little from those of VIII+IX (Table 4); hence there is no *prima facie* case that sublimes V-VII were seriously underprivileged as compared with VIII+IX, the shortcomings of the old animal house notwithstanding. Moreover, whereas subline differences between V-VII and VIII+IX could conceivably be environmental in origin, this cannot be the case for the differences between VIII and IX which have lived contemporaneously under identical conditions throughout. Hence, as in the case of the earlier subline differences (Deol *et al.* 1957), there is no reason to incriminate the environment. Furthermore, Grewal (1962) calculated a mean divergence (measures of distinctiveness) of 0.003 by angular transformation of the percentage incidence. The present results following Grewal's method give a value of $4.586/1394 = 0.0033$ per character per generation. This compares satisfactorily with Grewal's as well as the estimated $0.01 \times 0.61^2 = 0.0037$.

As mentioned above, in the new experiment subline differences have arisen at about the same rate as in the past (0.013 per variant per generation as compared with 0.01), and the average shift (in terms of standard deviations) is also about the same. Hence there is no doubt that, in the C57BL strain at any rate, subline differentiation is a continuing process. And what is fairly easily detectable in inbred strains presumably also happens in genetically heterogeneous material where such events would be very difficult to demonstrate.

Subline differentiation happens at the rate of about 0.01 events per variant per generation and is thus about 1000 times as common as the average spontaneous mutation rate of a number of genes with major effects in the mouse (Schlager & Dickie, 1967). This discrepancy has been discussed by Grewal (1962) and by Grüneberg (1970) who felt that it cannot easily be accounted for by the multifactorial basis of the skeletal variants (i.e. that one and the same variant may be affected by mutations at several loci) and/or by pleiotropism (i.e. that one and the same mutation may affect several skeletal variants and thus be ascertained more than once). If indeed subline differentiation is too frequent to be explicable in terms of conventional mutations (which has been called into question by Beardmore, 1970) it might include changes in other self-replicating entities such as latent viruses which might indirectly influence the incidence of the minor skeletal variants. As experiments to test this suggestion are now in train, there is no point in discussing this question at present.

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