How pure are our inbred strains of mice?

By M. S. DEOL, H. GRÜNEBERG, A. G. SEARLE* and
G. M. TRUSLOVE

*Medical Research Council Group for Experimental Research in
Inherited Diseases, University College, London*

For many years, the genetic homogeneity of inbred strains of animals and plants has been taken for granted on the strength of the mathematical theory of inbreeding. More recently, evidence has been accumulating that inbred strains tend to break up into genetically differentiated sublines following a course of inbreeding which, according to theory, should have resulted in the fixation of virtually all the initial genetic variance. A systematic study of skeletal variation in a British substrain of the inbred strain C57BL of the mouse by the present authors (1957) showed that in seven sublines which diverged from each other following at least forty generations of brother-sister mating, thirteen out of twenty-seven skeletal variants studied occurred with about the same frequency in all sublines; the remaining fourteen variants occurred in frequencies which differed between sublines or groups of sublines. Some American sublines of the same inbred strain which had been separated from each other for a longer period had drifted apart to a greater extent (Carpenter, Grüneberg & Russell, 1957). For any one variant, subline differences arise by sudden, discontinuous steps. In principle, these may be due to the segregation of residual gene differences dating from the origin of the C57BL strain, it being assumed that physiological advantages inherent in heterozygotes greatly delayed the fixation of genes in homozygous condition; or to mutations which arose following the establishment of a genetically homogeneous strain. All the available evidence led to the conclusion that the subline differences observed are the result of mutation rather than segregation. However, it would still be of interest to know whether a freshly arisen mutation is fixed promptly in an inbred strain according to theory, or whether it tends to linger in heterozygous condition for lengthy periods before it ultimately becomes fixed. In the absence of genetic variance there will be no correlation between parents and offspring for any given character. The existence and magnitude of parent-offspring correlations is a measure of the genetic variance present in an inbred strain.

Three inbred strains of mice were available for investigation, C57BL/Gr, A/Gr and CBA/Gr. Partial pedigrees of the brother-sister pairs whose offspring were examined are given in Figs. 1–3. Much scope for the differentiation of sublines was present in the case of C57BL, less in A, and hardly any at all in CBA. The
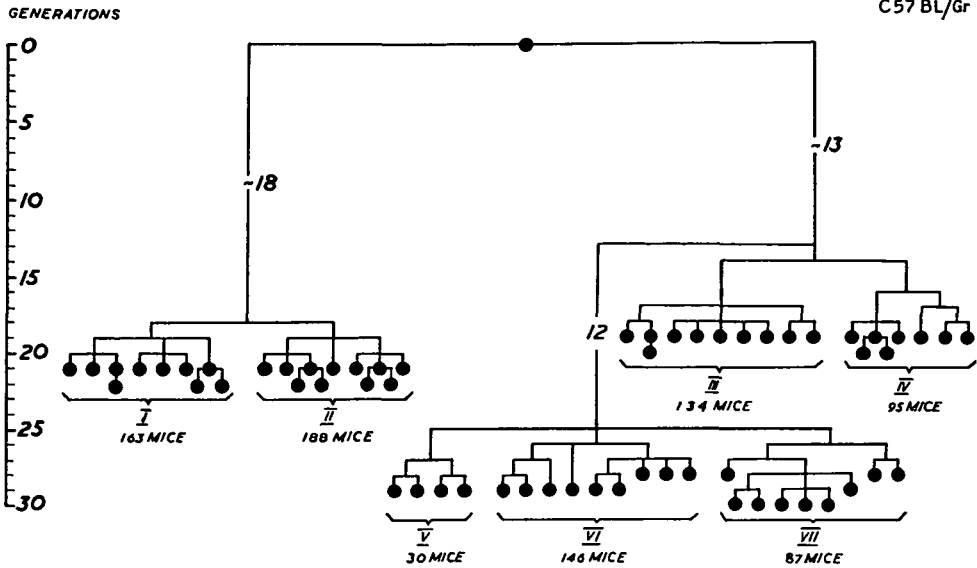

Fig. 1. Partial pedigree of the C57BL/Gr strain. The last common pair of ancestors of the seven sublines (the 'O' generation) lived in 1941 and was preceded by at least forty generations of brother-sister matings. The black circles represent such pairs which produced the young whose skeletons have been examined. From Deol, Grüneberg, Searle & Truslove (1957), by permission of the Wistar Press.

Fig. 2. Partial pedigree of the A/Gr strain. The 'O' generation lived in 1948 and was preceded by at least fifty generations of brother-sister matings.
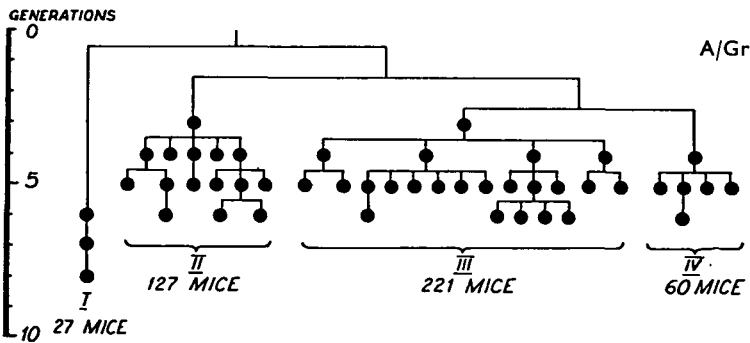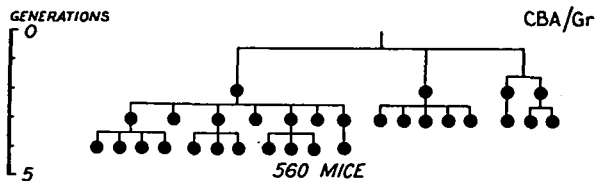

Fig. 3. Partial pedigree of the CBA/Gr strain. The 'O' generation lived in 1948 and was preceded by at least fifty generations of brother-sister matings.

analysis of C57BL has been confined to sublines I–IV; sublines V–VII had to be omitted as the parents of the various sibships were not available for study. The characters studied included some thirty skeletal variants, most of which have been described by the present authors in a series of papers under the general title 'Genetical studies on the skeleton of the mouse' in the *Journal of Genetics*, vols. 50–55, 1950–57. Most variants are all-or-none characters or can be treated as such, giving the possibility of four different types of mating:

	♂	♀
(1)	−	−
(2)	−	+
(3)	+	−
(4)	+	+

Where the variants form a minority of the strain (Nos. 1–12, 15, 18, 20–23 and 25–27 in Table 1), matings of types 2–4 have been pooled, with type 4 often not represented at all. Where the variants form the majority of the strain (Nos. 14, 16, 17, 19 and 29), matings of types 1–3 have been pooled, with type 1 often not represented. With character No. 29 (size of the processus spinosus of Th II), which is a continuous variant, large and medium (+ + + and + +) and small and absent (+ and −) processes have been pooled in C57BL and CBA, but in A, in which the larger processes are virtually absent, the line has been drawn between + and −. For similar reasons the pooling of classes for the same variant often differs from strain to strain (Tables 1–3); the actual frequencies of the characters thus cannot be deduced from these tables, but may be obtained from the original papers. No. 24 (foramina transversaria imperfecta) is a meristic character; on each of the cervical vertebrae C III–C VI, one or both foramina transversaria may be open gutters, so that any one individual may have from 0 to 8 foramina open. In this case, matings in which the combined parental count was 5 or less (average 4·00) were compared with matings in which the combined parental count was 6 or over (average 7·93); in the offspring, individuals with three or less open foramina were classified as '−', while animals with four or more open foramina were classified as '+'. Sublines have been treated separately wherever they differ significantly from each other in the incidence of a variant; to pool such different sublines would, of course, lead to spurious parent-offspring correlations: indeed, the existence of genetically differing sublines was first noticed in our C57BL strain through such a spurious parent-offspring correlation. In Nos. 15, 24, 26 and 27 of Table 1, some sublines have supplied no information, as the parents failed to include affected (or, in the case of No. 24, high-grade) individuals. For the same reason, certain variants (No. 4: interfrontal-frontal fusion; No. 13: dyssymphysis of the processus spinosus of Th II; No. 20*a*: abnormal metoptic roots of the presphenoid; and No. 28: frontal fontanelle) are not included in Table 1, and similarly in Tables 2 and 3.

In this fashion, the data for all available characters in the three inbred strains are presented in the form of 2 × 2 tables in Tables 1–3. In each case, the number of

Table 1. *C57BL/Gr*

No.	Variant	Subline	Parent(s) – Offspring – (a)	Parent(s) – Offspring + (b)	Parent(s) + Offspring – (c)	Parent(s) + Offspring + (d)	d_{exp}	V_d	P
1	Lacrimal-maxilla fusion		259	88	138	44	45.4	22.40	0.76
2	Parted frontals		401	36	86	5	7.1	5.41	0.37
3	Fused frontals		345	15	157	11	8.3	5.37	0.24
5	Squamosal-parietal fusion		281	45	165	26	26.2	14.20	0.95
6	Periotic-occipital fusion		387	37	98	6	8.5	6.26	0.32
7	Foramen ovale single		441	29	54	4	3.6	3.03	0.71*
8	Foramen ovale open posteriorly		401	37	85	5	7.2	5.48	0.36
9	Foramen hypoglossi single		71	52	236	169	169.5	23.01	0.91
10	Atlas-axis fusion		366	31	128	13	11.5	7.83	0.60
11	Tub. anterius inflexum of CVI		85	6	61	5	4.6	2.51	0.81
12	Dyssymphysis of Th I		367	96	55	18	15.5	10.58	0.45
14	Interfrontal	I, II	108	170	18	36	33.5	10.68	0.45
	„	III, IVA	27	74	13	39	38.4	6.67	0.82
	„	IVB	0	21	2	15	16.1	0.48	0.19*
15	Interparietal-occipital fusion	III, IV	113	8	73	2	3.8	2.25	0.19*
16	Inframaxillary crest	I, II	25	100	51	156	159.6	13.80	0.33
	„	III, IV	14	83	6	94	89.9	4.51	0.050
17	Alae palatinae	I, II	11	21	74	226	223.2	5.52	0.23
	„ „	III, IV	3	39	16	139	140.1	2.89	0.39*
18	Foramen sphenoidale medium	I, II	123	91	169	145	140.4	31.52	0.41
19	Processus pterygoideus	I, II	182	72	49	29	23.7	12.67	0.14
	„	III, IV	32	40	52	72	70.9	11.21	0.73
20	Presphenoid, preoptic sutures	I, II, IV	103	82	124	116	111.8	26.06	0.41
	„	III	19	29	15	38	36.0	5.68	0.23
21	Accessory mental foramen		69	101	115	244	234.1	26.22	0.054
22	Cervical fusions	I, II, IV	400	16	11	0	0.4	0.39	0.65*
	„	III	66	13	28	4	4.9	2.98	0.42*
23	Absence of tub. ant. of CVI	(excl. IA)	157	79	191	110	106.0	30.23	0.46
24	Foramina transversaria imperf.		96	106	138	142	143.9	29.37	0.70
25	Dystopia of proc. spin. of Th II		464	14	57	2	1.8	1.52	0.75*
26	Dyssymphysis of Th X	I, II, IV	198	186	23	20	20.7	9.68	0.81
27	Sacralisation of L VI	I, II	213	43	61	14	12.9	8.29	0.70
29	Size of proc. spin. of Th II		14	80	58	301	301.9	9.98	0.76
	Total					2250	2221.4	358.68	

Table 2. *A/Gr*

No.	Variant	Subline	Parent(s) − Offspring		Parent(s) + Offspring		d_{exp}	V_d	P
			− (a)	+ (b)	− (c)	+ (d)			
1	Lacrimal-maxilla fusion		171	92	111	61	60·5	23·77	0·92
2	Parted frontals	I, II, IV	79	36	71	28	29·6	11·20	0·63
	,, ,,	III	111	17	69	24	17·3	8·18	0·018
3	Fused frontals		299	19	110	7	7·0	4·82	1·00
7	Foramen ovale single	II, III, IV	29	55	94	229	225·4	14·09	0·34
	,, ,, ,,	I	3	1	13	10	9·4	0·85	0·45*
9	Foramen hypoglossi single		122	191	48	69	70·7	20·41	0·70
11	Tub. anterius inflexum of CVI		299	37	58	8	7·4	5·50	0·79
14	Interfrontal		335	36	55	9	6·6	5·07	0·29
17	Alae palatinae		377	8	49	0	0·9	0·79	0·38*
18	Foramen sphenoidale medium		4	62	36	333	335·1	4·69	0·24*
19	Processus pterygoideus		67	219	35	114	114·1	17·63	0·99
20a	Presphenoid, metoptic roots abn.		208	86	89	38	37·4	18·47	0·89
21	Accessory mental foramen		37	37	203	157	160·9	15·21	0·31
29	Size of proc. spin. of Th II		123	82	146	81	85·7	25·37	0·36
—	Dyssymphysis posterior of C II		314	53	63	5	9·1	6·65	0·11
—	Arch foramina in Th V		17	68	67	282	281·5	10·69	0·87
	Total					1455	1458·6	193·39	

Table 3. *CBA/Gr*

No.	Variant	Parent(s) − Offspring		Parent(s) + Offspring		d_{exp}	V_d	P
		− (a)	+ (b)	− (c)	+ (d)			
1	Lacrimal-maxilla fusion	445	8	100	5	2·4	1·94	0·067
2	Parted frontals	46	150	86	276	276·4	23·00	0·94
7	Foramen ovale single	272	177	51	59	46·4	21·59	0·0068
9	Foramen hypoglossi single	87	32	253	114	110·3	18·92	0·39
11	Tub. anterius inflexum of CVI	77	128	139	191	196·8	30·50	0·29
14	Interfrontal	30	169	55	304	304·3	16·56	0·94
17	Alae palatinae	73	48	240	195	190·1	23·33	0·34
18	Foramen sphenoidale medium	7	78	54	421	423·3	7·01	0·40
19	Processus pterygoideus	77	230	46	207	197·4	23·81	0·050
20	Presphenoid, preoptic sutures	8	75	23	450	446·6	3·76	0·080
20a	,, metoptic roots abn.	231	261	38	26	33·0	14·30	0·061
21	Accessory mental foramen	111	67	223	158	153·4	29·23	0·39
27	Sacralisation of L VI	0	41	9	509	509·7	0·60	0·50*
28	Frontal fontanelle	293	146	82	37	39·0	20·67	0·65
29	Size of proc. spin. of Th II	8	135	40	375	379·4	8·38	0·14
—	Absence of third molars	401	99	169	26	35·1	20·72	0·047
	Total				3353	3343·6	264·32	

affected offspring from affected parents (d) is compared with the value expected in the absence of a parent-offspring correlation (d_{exp}), and the variance of the latter (V_d) is also given. This variance is calculated on the basis of the Fisher-Irwin-Yates exact distribution for 2×2 tables. For any one table, with $n = a+b+c+d$ entries, we have

$$d_{exp} = (b+d)(c+d)/n,$$
$$V_d = (a+b)(a+c)(b+d)(c+d)/n^2(n-1).$$

The total expectation for the whole collection of tables is the sum of the expectations in the individual tables, and similarly for the variance V_d, the standard error being the square root of the variance. The P-values given in the last column of Tables 1–3 are based on χ^2 tests or, where appropriate, on Fisher's 'exact' treatment of 2×2 tables; the latter values are marked by an asterisk.

A condensed version of the results is given in Table 4. Treating the three inbred strains separately, the total value of d exceeds its expectation in C57BL and CBA,

Table 4. *Test on sum of* d *values*

Strain	d		Δ	S.E.$_\Delta$	Δ/S.E.$_\Delta$	P
	Observed	Expected				
C57BL	2250	2221·4	+28·6	18·94	1·51	0·13
A	1455	1458·6	−3·6	13·91	0·26	0·79
CBA	3353	3343·6	+9·4	16·22	0·58	0·56
Total	7058	7023·6	+34·4	28·57	1·20	0·23

but falls slightly short of it in A. The difference between observed and expected values is not significant in any single instance; nor is it significant when the data from all the three inbred strains are combined. Considering the sixty-six fourfold tables separately, d is lower than its expectation in thirty-two instances, equals it once, and exceeds it thirty-three times: an embarrassingly close fit.

In Table 5, the fifty-five individual P-values from the χ^2 tests of Tables 1–3 are grouped and compared with their expectations. The observed distribution of

Table 5. *Distribution of fifty-five* P-*values from* χ^2 *tests compared with that expected on a chance basis*

P-value	< 0·01	0·01–0·05	0·051–0·25	0·251–0·50	0·501–0·75	> 75
Observed	1	4	10	18	7	15
Expected	0·55	2·2	11	13·75	13·75	13·75

P-values agrees well with that expected on a chance basis ($\chi^2 = 6·666$; $n = 4$; $P = 0·16$). The table does not include eleven P-values calculated by means of Fisher's 'exact' method as these represent only one tail of the distribution; they range from 0·19 to 0·75.

In Tables 1–3, there are altogether five values of P of 0·05 or less, the expectation in a sample of sixty-six being 3·3. In none of these cases does a study of the respective pedigrees suggest an incipient differentiation of a subline, and presumably most of them can be regarded as the result of random sampling. However, as in four out of five instances the deviation is in the direction of a parent-offspring correlation, there is a suspicion that perhaps one or the other of these bigger deviations may not have been due to chance alone. But even if one or two out of a total of sixty-six tests were to indicate a parent-offspring correlation, this would not alter the conclusion that the three inbred strains examined are essentially homogeneous with regard to the genes which control the array of skeletal variants used for this investigation. As the number of genes involved is undoubtedly large, the test for genetic heterogeneity here reported is clearly a sensitive one.

As all three inbred strains behave alike, and as there is no reason to suppose that the genes responsible for skeletal variation differ systematically from genes in general, it is legitimate to conclude that inbred strains in the mouse are genetically homogeneous in conformity with the theory of inbreeding, except for the formation of genetically distinct sublines. In addition, the essential absence of genetic variance within the inbred strains confirms our previous conclusion (1957) that the formation of sublines is due to mutation rather than segregation, and it shows that such mutations tend to be fixed promptly in homozygous condition.

Our findings probably have a general application to inbred strains of mice, but clearly not to inbred strains of other organisms. To mention only two exceptions, in the rat (Loeb, King & Blumenthal, 1943; see also Billingham & Silvers, 1959) segregation for histocompatibility genes was still found after 102 generations of brother-sister mating, and in the chicken (Shultz & Briles, 1953; Briles, Allen & Millen, 1957; Cock, 1956; Gilmour, 1959) there is evidence for the persistence of genetic variance in the face of close inbreeding; this is evidently due to the greater vigour and consequently selective advantage of certain heterozygotes as compared with either type of homozygote. The genetic structure of inbred strains thus requires special study in each organism.

SUMMARY

Sixty-six individual tests on an array of skeletal variants showed an essential absence of parent-offspring correlations in the inbred strains C57BL, A and CBA in the mouse. It is concluded that these strains, and inbred strains of mice in general, are genetically homogeneous except for the differentiation of genetically distinct sublines as the result of mutations.

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