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The rate of genetic divergence of sublines in the C57BL strain of mice

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INTRODUCTION

Sublines of inbred strains of animals are reproductively as isolated from each other as are species and will thus tend to diverge genetically as the result of freshly arising mutations. This fact has been studied particularly with the aid of skeletal variants (C3H strain: Green, 1953; McLaren & Michie, 1954, 1955; Hamer, 1955; C57BL strain: Grüneberg, 1954; Deol, Grüneberg, Searle & Truslove, 1957; Carpenter, Grüneberg & Russell, 1957; A strain: Searle, 1954*a*). The C57BL strain has proved very suitable for such studies on account of the large number of quasi-continuous variants (Grüneberg, 1952) which occur in it; these are discontinuous variants with a multifactorial background; similar entities have been described by other authors as 'threshold variants' or as 'phenodeviants'. The divergence of seven British sublines of C57BL has been studied by Deol *et al.* (1957) and that of an additional three American sublines by Carpenter *et al.* (1957).

An additional substrain of C57BL, not included in the above studies, is being maintained by Professor P. C. Koller at the Chester Beatty Research Institute, London. This subline may have been separated from the main stream of American lines before the British lines were separated in 1932. Unfortunately, the exact relationships of the C57BL/Ko subline to its American origin can no longer be traced with confidence. Professor Koller obtained the foundation animals of his subline from Professor J. J. Bittner (University of Minnesota) in 1952. Professor Bittner, in a letter to Professor Grüneberg, said that his stock was descended from mice obtained from Dr H. B. Andervont in 1947, and that these animals were of the thirtieth inbred generation. Dr Andervont (1954) states that he had obtained the mice of that line from Dr W. S. Murray (Bar Harbor, Maine, U.S.A.) in 1939. Further information about this subline is not available since all the records were lost in the fire at the Jackson Laboratory, Bar Harbor, in 1947. Although unfortunately the exact pedigree of the C57BL/Ko subline must thus remain in doubt, a study of this material was of interest as this subline is probably farther removed from the British sublines than any other subline hitherto studied. As will be described below, it did in fact turn out to be the 'odd-man-out'. Moreover, the analysis of the new material with the aid of new statistical methods led to a reinvestigation of the rate of divergence between the sublines, which gave more

precise results than the methods previously used by Deol *et al.* (1957) and by Carpenter *et al.* (1957), and which poses new problems.

MATERIAL AND METHODS

This study is based on the skeletons of 100 adult mice of the C57BL/Ko subline (32 ♂♂ and 68 ♀♀) which were bred at the Chester Beatty Research Institute; the specimens were prepared by the papain maceration technique between December 1958 and October 1959. It is now well known (Searle, 1954*c*; Deol & Truslove, 1957) that the development of the mouse skeleton may be powerfully influenced by unbalanced maternal diets. However, as has been discussed by Deol *et al.* (1957) and by Carpenter *et al.* (1957), it is improbable that differences between near-optimal diets will have major effects on the development of the skeleton. The skeletons were classified for the same twenty-seven skeletal variants used in previous investigations. They are enumerated, together with key references, in the following list. To ensure continuity with the earlier work on the subject, the criteria of classification were discussed with other workers in this laboratory who are familiar with these variants.

1. Lacrimal-maxilla fusion	(Deol, 1955)
2. Parted frontals	(Truslove, 1952)
3. Fused frontals	(Deol & Truslove, 1957)
4. Interfrontal-frontal fusion	(Truslove, 1952)
5. Squamosal-parietal fusion	(Searle, 1954*c*)
6. Periotic-occipital fusion	(Searle, 1954*c*)
7. Foramen ovale single	(Deol, 1955)
8. Foramen ovale open posteriorly	(Deol, 1955)
9. Foramen hypoglossi single	(Deol, 1955)
10. Atlas-axis fusion	(Grüneberg, 1950)
11. Tuberculum anterius inflexum of C VI	(Grüneberg, 1950)
12. Dyssymphysis of Th I	(Searle, 1954*b*)
13. Dyssymphysis of proc. spinosus of Th II	(Searle, 1954*b*)
14. Interfrontal	(Truslove, 1952)
15. Interparietal-occipital fusion	(Deol & Truslove, 1957)
16. Inframaxillary crest	(Deol, 1955)
17.*Alae palatinae	(Deol, 1955)
18. Foramen sphenoidale medium	(Deol, 1955)
19. Processus pterygoideus	(Deol, 1955)
20. Presphenoid, preoptic sutures	(Truslove, 1954)
21. Accessory mental foramen	(Deol, 1955)
22. Cervical fusions	(Grüneberg, 1950)
23. Absence of tuberculum anterius of C VI	(Grüneberg, 1950)
24. Foramina transversaria imperfecta C VI	(Grüneberg, 1950)
25. Dystopia of proc. spinosus of Th II	(Grüneberg, 1950)
26. Dyssymphysis of Th X	(Deol & Truslove, 1957)
27. Sacralisation of L VI	(Searle, 1954*a*, *c*)

RESULTS

The percentage incidence of these twenty-seven skeletal characters in the eleven sublines of the C57BL strain is given in Table 1. In the case of bilateral characters, the frequency is given by sides of animals rather than by animals. That Koller's

* This character was originally called 'posterior border of the palatine'.

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Table 1. *Percentage incidence of twenty-seven skeletal variants in eleven sublines of the C57BL inbred strain. The figure in brackets is the number of animals used*

		Grüneberg's sublines							U.S. sublines			Koller's
		I	II	III	IV	V	VI	VII	6JAX	6p	10Sc	subline
No.	Variant	(163)	(188)	(134)	(95)	(30)	(146)	(87)	(53)	(50)	(24)	(100)
1	Lacrimal-maxilla fusion	12·6	15·2	11·9	11·1	11·7	17·8	14·9	5·8	11·0	20·8	22·5
2	Parted frontals	10·4	8·6	3·0	9·5	6·7	5·5	13·8	25·0	6·0	0	53·0
3	Fused frontals	4·3	5·3	6·7	3·2	0	4·8	4·6	7·7	2·0	4·2	25·0
4	Interfrontal-frontal fusion (visible dorsally)	14·8	13·7	15·3	27·6	12·5	13·9	10·3	27·3	12·0	0	0
5	Squamosal-parietal fusion	6·9	6·1	6·0	10·0	16·7	4·6	4·6	15·4	41·0	37·5	38·5
6	Periotic-occipital fusion	3·7	5·3	3·7	4·2	6·7	2·7	1·7	4·8	2·0	0	0
7	Foramen ovale single	1·8	3·2	4·5	4·2	3·3	3·8	1·7	1·0	14·0	2·1	8·0
8	Foramen ovale open posteriorly	6·1	3·8	2·6	6·3	5·0	4·5	2·3	37·5	10·0	0	2·0
9	Foramen hypoglossi single	22·4	25·9	25·9	22·6	18·3	26·6	24·7	29·8	39·0	47·9	54·5
10	Atlas-axis fusion	8·6	4·8	13·4	6·3	10·0	6·8	16·1	28·3	8·0	4·2	2·0
11	Tuberculum anterius inflexum of C VI	8·7	6·1	4·3	6·7	6·1	4·4	2·5	16·3	21·7	22·5	20·0
12	Dyssymphysis of Th I	16·6	20·9	22·7	26·6	20·0	13·8	12·6	41·5	58·0	4·2	28·0
13	Dyssymphysis of processus spinosus of Th II	1·2	1·0	0	2·1	3·4	0	0	3·9	8·0	0	10·0
14	Interfrontal	57·7	67·4	73·9	84·2	90·0	87·0	81·6	98·1	74·0	75·0	51·0
15	Interparietal-occipital fusion	0	2·1	5·2	4·2	6·7	10·3	18·4	1·9	2·0	0	0
16	Inframaxillary crest	85·4	83·9	95·1	92·6	1·3	7·6	14·4	19·2	26·0	2·1	54·0
17	Alae palatinae	21·6	15·5	7·5	6·5	28·7	28·3	31·1	36·5	48·0	62·5	100·0
18	Foramen sphenoidale medium	44·2	43·9	41·0	42·1	23·3	26·7	25·3	34·6	26·0	20·8	39·0
19	Processus pterygoideus	47·5	48·1	34·3	25·3	11·7	21·2	21·8	41·7	38·1	79·2	25·0
20	Presphenoid, preoptic sutures	30·0	30·9	46·6	33·8	26·7	39·0	33·3	6·7	16·0	27·1	4·0
21	Accessory mental foramen	39·6	42·0	43·2	43·2	28·3	25·7	27·0	26·4	8·2	8·3	17·0
22	Cervical fusions	3·1	4·8	12·7	3·2	3·3	4·8	1·1	0	6·0	8·3	4·0
23	Absence of tuberculum anterius of C VI	15·3	20·6	22·8	21·1	16·7	22·1	29·9	0·9	4·0	6·5	10·0
24	Foramina transversaria imperfecta C V	51·0	68·7	66·4	67·9	66·7	71·6	61·7	8·5	21·0	47·9	10·0
25	Dystopia of processus spinosus of Th II	2·5	4·8	2·2	1·1	13·8	7·5	14·9	0	6·0	8·3	8·0
26	Dyssymphysis of Th X	44·8	52·9	28·4	44·2	23·3	15·1	19·5	24·5	44·0	12·5	0
27	Sacralization of L VI	14·4	11·5	2·4	1·1	8·5	4·5	6·3	1·9	1·0	0	0

subline differs considerably from all the other ten sublines is evident by inspection of the percentages in Table 1. Taking these percentages at their face value, Koller's subline lies outside the range covered by the other ten sublines in eleven out of twenty-seven cases.

In order to study the degree of divergence between these sublines in more detail and more critically, the percentage incidences from Table 1 were transformed into angular values as this makes that part of the variance which is due to errors of

Table 2. *'Divergence' of eleven sublines of C57BL as regards twenty-seven minor skeletal variants. The number of generations separating the sublines is given in brackets*

	I	II	III	IV	V	VI	VII	6JAX	6p	10Sc	Ko
I		0·006 (6)	0·051 (40)	0·046 (40)	0·238 (50)	0·199 (50)	0·184 (50)	0·275 (93)	0·197 (93)	0·326 (93)	0·502
II			0·032 (40)	0·024 (40)	0·214 (50)	0·165 (50)	0·159 (50)	0·319 (93)	0·230 (93)	0·330 (93)	0·587
III				0·014 (10)	0·072 (22)	0·208 (22)	0·190 (22)	0·378 (91)	0·298 (91)	0·395 (91)	0·420
IV					0·232 (22)	0·199 (22)	0·190 (22)	0·299 (91)	0·251 (91)	0·432 (91)	0·653
V						0·001 (8)	0·013 (8)	0·201 (101)	0·141 (101)	0·170 (101)	0·519
VI							0·004 (8)	0·245 (101)	0·199 (101)	0·160 (101)	0·515
VII								0·239 (101)	0·205 (101)	0·186 (101)	0·469
6JAX									0·114 (64)	0·342 (96)	0·439
6p										0·167 (96)	0·308
10Sc											0·363
Ko											—

sampling independent of the incidence of the character. The angular value θ corresponding to the percentage p was defined by $\theta = \sin^{-1}(1-2p)$, measured in radians. This appears to have some advantages over Fisher's transformation $\phi = \sin^{-1}\sqrt{p}$, measured in degrees, since whereas the large sample variance for Fisher's ϕ is $820{\cdot}7/n$, where n is the size of the sample, that for θ is simply $1/n$. The two measures are related by the equation $\theta = \pi\left(\frac{1}{2} - \frac{\phi}{90}\right)$. The next step is to construct a measure of the divergence between the sublines. For any pair of sublines this is done by taking the differences between the angular values for each of the twenty-seven characters studied, squaring these differences, adding them together and finally dividing the sum by the number of characters (i.e. 27). This

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raw measure of divergence still contains a part which can be ascribed to random sampling fluctuations. This is allowed for by subtracting $\left(\frac{1}{m_1}+\frac{1}{m_2}\right)$ from the uncorrected measure of divergence between any two sublines M_1 and M_2, where m_1 and m_2 are the numbers of mice in each subline. This measure of divergence between any two sublines shows how far, on an average, the twenty-seven characters have diverged from each other.

Table 2 gives the mean divergence between the eleven sublines of C57BL as regards these twenty-seven minor skeletal variants, fifty-five paired comparisons in all. The figures in brackets give the approximate number of generations separating the sublines where this is known; this is based on the pedigree in Fig. 1,

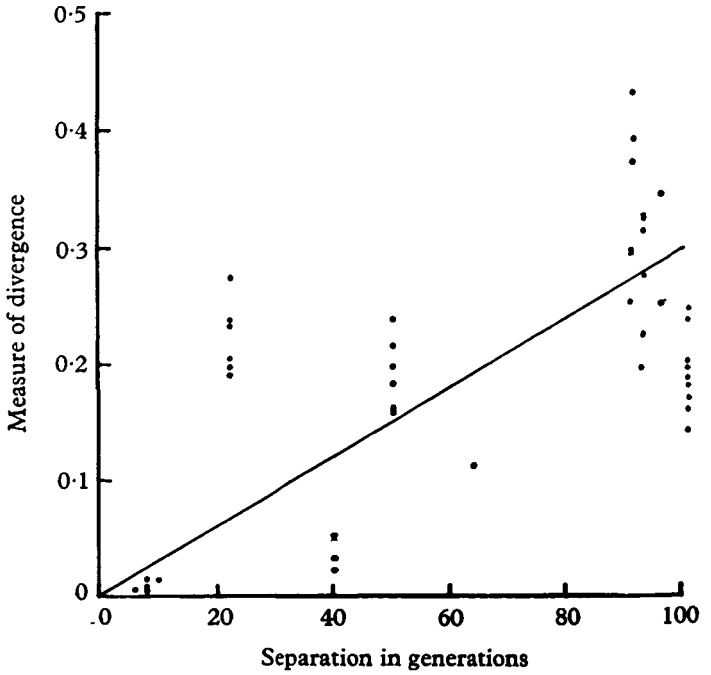

Fig. 1. The forty-five values of the measure of divergence plotted against the separation in generations of the sublines concerned.

p. 378, of Carpenter *et al.* (1957), equating one year of separation to two generations of mice, and adding together both lines of descent from each point of separation. The sum of all the measures of divergence for all sublines (except Koller's) is 8·740; if this is divided by the sum of all separations in generations of all sublines (again except Koller's), i.e. 2901, we obtain as the average divergence per generation per character the value of 0·003. Koller's subline had to be omitted from these calculations as its time of separation from the other sublines is not known.

The forty-five values of the measure of divergence for the paired comparisons between the sublines have been plotted against the separation in generations and the resulting graph (Fig. 1) gives the rate of increase of divergence per character per generation.

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DISCUSSION

A. The relationship of Koller's subline to the C57BL strain

As expected, the divergence between sublines I + II, between III + IV and between V + VI + VII in Table 2 is very small. Taken by themselves the measures of divergence do not deviate significantly from zero. Evidently, the genetic divergence arising in 6–10 generations is too small to be detectable in the present material. It is thus permissible to pool the seven British sublines to form three groups as outlined above. It is less certain whether it is legitimate to do the same with the three American sublines; however, if it is done, the simplified Table 3 is obtained. This table shows that, on the whole, the American sublines differ more from the British ones than the latter differ from each other. This is in agreement with the conclusions reached by Carpenter *et al.* (1957); but, whereas the conclusion reached by these authors was based largely on a somewhat indirect argument, the present values are more objective and moreover convey an estimate of the relative degree of genetic divergence.

Table 3. *Condensed version of Table 2 obtained by pooling sublines I + II, III + IV and V + VI + VII, and by pooling the three American sublines. Mean divergence between groups of sublines, with number of generations separating them in brackets*

	I + II	III + IV	V + VI + VII	American sublines	Koller's subline
Number of animals ...	351	229	263	127	100
I + II	—	0·038 (40)	0·193 (50)	0·280 (93)	0·544
III + IV	—	—	0·182 (22)	0·342 (91)	0·536
V + VI + VII	—	—	—	0·194 (101)	0·501
American sublines	—	—	—	—	0·366
Koller's subline	—	—	—	—	—

It is obvious from Table 3 that Koller's subline differs, on an average, far more from the British or the American sublines than these differ from each other. For the purposes of comparison, it is legitimate to pool all the British sublines. If this is done, the divergence between the British sublines and Koller's amounts to 0·524 in terms of variance per character. It is unknown exactly at what stage the British sublines and Koller's had their last common ancestors. However, if we accept the value of 0·003 for the divergence per character per generation, it would take 0·524/0·003 or 175 generations to produce a divergence as great as that observed. Dividing this value by 2 for the two lines of descent, and allowing two generations per year (as before), it would take about 44 years of brother-sister mating *following* the establishment of a homogeneous strain to produce so great a

divergence by freshly arising mutations.* This would take us back to roughly the year 1912, i.e. a decade before the C57BL strain was started. Even allowing for the uncertainties inherent in some of these admittedly rough calculations, it seems improbable that so great a divergence could have arisen by freshly arising mutations after the elimination of the original heterozygosis. It may therefore be suggested that Koller's subline diverged from the British sublines at a stage prior to the fixation of all the original heterozygosity, and that part, at least, of the present divergence represents gene differences already present in Miss Lathrop's original mice from which the C57BL strain was developed by inbreeding.

It is thus clear that Koller's subline differs considerably from the other sublines of the C57BL strain. The difference is so great that it cannot reasonably be accounted for by mutations which have happened after the C57BL strain had become a homogeneous inbred strain. Now does this mean that Koller's subline has no real claim to be included in the C57BL 'family' of strains, and that it thus represents a different entity altogether? The C57BL skeleton is highly characteristic by virtue of many variants which either do not occur in other strains at all, or which are uncommon elsewhere; the combination of these entities is as unique as a human face. Nobody familiar with C57BL will be in the slightest doubt that Koller's subline belongs to that group after having examined even a small sample of that subline. The characteristic C57BL variants are as follows:

4. Interfrontal-frontal fusion
5. Squamosal-parietal fusion
6. Periotic-occipital fusion
7. Foramen ovale single
8. Foramen ovale open posteriorly
10. Atlas-axis fusion (dyssymphysis of atlas and axis)
13. Dyssymphysis of processus spinosus of Th II
15. Interparietal-occipital fusion
16. Inframaxillary crest
22. Cervical fusions
23. Absence of tuberculum anterius of C VI
24. Foramina transversaria imperfecta
25. Dystopia of processus spinosus of Th II
26. Dyssymphysis of Th X

Ten out of these fourteen variants occur in Koller's subline, generally in frequencies not greatly different from those in the other sublines. The absence of two others (Nos. 6 and 15 respectively) can easily be due to an accident of sampling. In the case of Nos. 4 and 26 the frequency of the variants is undoubtedly reduced, though, of course, not necessarily zero. Taking the evidence as a whole, there cannot be any reasonable doubt that Koller's subline belongs to the C57BL 'family' of strains. It therefore appears that the basic structure of the C57BL skeleton had been fixed before Koller's subline diverged from the rest, but that

* This argument is not influenced by the number of mouse generations per year. If we had taken four generations per year throughout, we would have found the divergence per generation per character to be 0·0015 instead of 0·003. However, 0·524/0·0015 = 350 generations which, in two lines of descent and at the rate of four a year, will still take 44 years to be produced.

genes influencing the frequency of individual variants were still segregating at the time, and that the latter segregation is partly reflected in the sizeable gap which now separates C57BL/Ko from the rest.

B. Segregation and mutation in subline divergence

We have seen above that the rate of divergence is about 0·003 per generation per character. This value is based on two mouse generations per year, which may be an underestimate. Perhaps two and a half or even three generations is nearer the truth, in which case the rate of divergence would be 0·0024 or 0·002 respectively per generation. It is of interest to compare these values with those of Bailey (1959), who used six metrical characters of the mouse skeleton for his investigation. That author found that in the C57BL/6 strain his measure of divergence (similar to the one used here) ranged from 0 to 0·006, and from 0·002 to 0·013 in the BALB/cAn strain. The agreement with the values presented here is thus very satisfactory. The rate of increase of genetic variance of chaeta number in *Drosophila* estimated by Mather (1956) and others is approximately 0·001 per generation and thus possibly a little smaller than the rate of change in the mouse.

In the British sublines of C57BL investigated by Deol, Grüneberg, Searle & Truslove (1957), twenty-one 'mutations' affecting the twenty-seven variants have occurred; if these were indeed freshly arisen mutations, about seventy-two generations are available during which they could have happened, i.e. about 0·01 mutation per variant per generation, or one mutation in 100 generations. As shown by Grüneberg (1955) and later developed in more detail by Deol *et al.* (1957), the average effect of these mutations amounts to about 0·6 standard deviations or about $0{\cdot}6^2 = 0{\cdot}36$ expressed in terms of variance. Hence this should give a divergence of about $0{\cdot}01 \times 0{\cdot}36 = 0{\cdot}0036$ per character per generation. This value slightly exceeds the rate of divergence actually found; presumably the excess is merely due to the crude nature of the calculation. It thus seems that the total variance introduced by mutations which can be individually recognized is already sufficient to account for the rate of divergence actually found; it seems likely that the contributions from mutations with smaller effects or other sources of variation are comparatively unimportant.

The rate of one mutation per 100 generations per variant is astonishingly high. Actually, it would have been more accurate to speak of one *detected* mutation per 100 generations. In an inbred strain each generation is represented by two animals and hence by four chromosome sets which have given rise to this brother-sister pair. Now if this gene pool *AAAA* by mutation becomes *AAAa*, the final outcome as the result of continued inbreeding will be either *AAAA* or *aaaa*, i.e. the mutant gene *a* will either be eliminated again, or it will be fixed; only the latter result will be ultimately detectable. Supposing that the three possible genotypes have the same fitness in the Darwinian sense, elimination of the new mutant gene *a* will happen in three cases out of four, and its fixation in the population in one-quarter. Hence for every mutant step discovered, there are three which have failed to establish themselves. Hence one *detected* mutation in 100 generations =

400 gametes corresponds to four actual mutations in 400 gametes, or 1%. This is at least 1000 times more than the spontaneous mutation rates of single genes so far measured in the mouse; Russell & Russell (1959) report six spontaneous mutations in an experiment involving seven specific loci in a total of 106,408 mice, or a mutation rate of $0{\cdot}81 \times 10^{-5}$ per locus per gamete, i.e. approximately one mutation per 125,000 gametes.

How can one account for this altogether unexpected number of mutations? In the first instance, the possibility exists that one and the same mutational step might pleiotropically affect more than one variant, so that it would be ascertained more than once. Truslove (1962) has made a systematic search for correlations between these minor skeletal variants and has found that there are few such correlations, and those that exist are all comparatively feeble. Hence there is no prima-facie evidence to support the hypothesis of multiple ascertainment of mutational steps. However, Truslove's results do not exclude such an interpretation. But even if the number of mutations were only one-quarter of the raw figure of twenty-one actually observed, which is almost certainly an exaggerated assumption, the discrepancy would be reduced from a factor of 1000 to one of 250.

Next one may consider that most or all the minor variants here considered are under the control of several genes; there is ample evidence to support this view. If a mutation in any one of these genes would result in a shift in frequency of about 0·6 standard deviations, then if the variants were, on an average, under the control of 1000 such genes, the discrepancy would disappear. The actual number of 'poly' genes in biometrical genetics has always been left discreetly vague. But to create, *ad hoc*, a thousand of them per variant in order to 'explain' the high mutation rate would obviously just replace one improbability by another.

Next, one might consider that the genes responsible for the 'major' gene effects in the specific locus experiments of Russell & Russell (1959) are 1000 times less mutable than those which control the minor skeletal variants. Again, this assumption lacks plausibility.

In view of these unresolved difficulties, it is appropriate to re-examine the evidence that the abrupt changes in the frequency of minor variants observed in the sublines of C57BL and other inbred strains of mice are in fact mutations, as suggested by Grüneberg (Deol *et al.*, 1957; Carpenter *et al.*, 1957). Having disposed of the possibility of trivial mistakes such as unknown outcrosses of the C57BL strain to other mice, these authors had to decide between the possibilities of segregation and mutation. The persistence of residual heterozygosis in so many different entities after so many generations of brother-sister mating could only be accounted for if heterozygotes possessed a marked selective advantage. It has recently been shown (Hayman & Mather, 1953; Reeve & Gower, 1960) that a comparatively moderate advantage of heterozygotes over homozygotes can greatly retard, and indeed arrest, the progress towards the fixation of genes by inbreeding. But this advantage can only express itself if many homozygotes are eliminated either by death or sterility. In view of the normal litter size and fertility of the C57BL strain, the persistence of simultaneous heterozygosis in many genes seemed

impossible. Moreover, it has since been shown (Deol, Grüneberg, Searle & Truslove, 1960) that in C57BL/Gr, sublines I–IV, there is no evidence at all for the existence of genetic variance other than that differentiating the sublines from each other, and the same applied to two other inbred strains of mice (A/Gr and CBA/Gr). These arguments still appear to be valid. But it is perhaps permissible to ask whether all the premises on which the argument rests are valid beyond question. Is it, for instance, certain that the C57BL strain, in its earlier history, was invariably maintained by brother-sister mating? When was it first realized that an occasional cross *between sublines* might largely undo the work of inbreeding? The exact history of the C57BL strain has never been published, nor can it ever be published now as all the earlier records were lost in the fire at Bar Harbor in 1947. If it might be assumed that in the earlier history of the strain occasional crosses between sublines have occurred (at a time when the very existence of genetic differentiation between sublines was not yet realized), then perhaps the C57BL mice which diverged in the year 1941 to form the British sublines I–VII did not have 'at least 40 generations of brother-sister mating' behind them, as was supposed, and perhaps they contained some residual heterozygosis which has since been fixed by more rigorous inbreeding. Referring as it does to happenings in the past which cannot now be verified, this is obviously no more than a conjecture. But it seems not quite so improbable as some of the other hypotheses discussed above to explain this enormously high 'mutation rate'. Moreover, the suggestion can, in principle, be tested. If, in fact, the divergence between sublines I-VII is partly due to the segregation of residual heterozygosis and partly to mutations of recent origin, then subline differentiation in future should happen at a slower rate, as residual heterozygosity has now definitely disappeared from the strain. It would thus be of considerable interest to create deliberately two or more separate sublines now, allow them to proceed for a number of generations, and then compare the divergence which develops with that which emerged in the unplanned separation of sublines in the past.

It should be pointed out that one feature is not in favour of the hypothesis that part of the subline differentiation observed is due to the segregation of residual heterozygosis. As pointed out by Deol *et al.* (1957), the branch of the pedigree which leads to sublines V + VI + VII seems to have given rise to a disproportionately large number of subline differences, considering the number of generations involved. Now this branch diverges from the line of descent which leads to sublines III + IV so late that the persistence of an appreciable residual heterozygosity at such a late stage is rather improbable. If this is accepted, and if subline differentiation in C57BL continues at a similar rate in the future as it has done in the past, the magnitude of the effect presents a major problem in mutation research.

SUMMARY

The ancestory of Koller's subline of the C57BL strain is not known. A study of the minor skeletal variants in this subline showed that it differs from the other ten sublines of C57BL more than they differ from each other. It appears to have

separated from the rest before the genotypes of the sublines had been fixed by inbreeding. There is enough evidence to show that it is indeed a branch of C57BL strain.

The rate of divergence of the sublines of C57BL is surprisingly high. It is reconsidered whether the whole of this divergence can be explained by recent mutations or whether residual heterozygosis has also played a part in it.

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