THE GENETIC DIVERGENCE OF SUBLINES AS ASSESSED BY HISTOCOMPATIBILITY TESTING¹

WILLYS K. SILVERS AND DAVID L. GASSER

*Departments of Human Genetics and Pathology, University of Pennsylvania,
School of Medicine, Philadelphia, Pennsylvania 19174*

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ABSTRACT

The degree of genetic divergence which has occurred between a number of inbred strains of mice and between two sublines of inbred rats was assessed by determining the fate of inter-subline skin grafts. Sublines which had been separated for 29 and 42 generations possessed no detectable incompatibility, while three combinations of sublines judged to have been maintained apart for from 123 to 129 generations showed slight degrees of histoincompatibility. One pair of sublines which had been separated for 119 generations demonstrated a marked degree of incompatibility, and an F_2 test suggested that mutations had occurred at four or five histocompatibility loci.

A subject of considerable interest to the transplantation biologist is that of the mutation rate of histocompatibility genes (BILLINGHAM *et al* 1957; GODFREY and SEARLE 1963; KINDRED 1963; LINDER 1963; BROWN and DINSLEY 1966). BAILEY and KOHN (1965) interchanged tail-skin grafts between 2572 (C57BL/6 $\times$ BALB/c) F_1 hybrids and found 32 animals with H-gene mutations. This apparent mutation rate of more than 1% per zygote per generation so greatly exceeds the spontaneous mutation rates calculated for other determinants that BAILEY (1966) proposed that changes involving transplantation antigens might result from the incorporation of viral genomes into mouse germinal cells. The magnitude of the difference between the observations of BAILEY and KOHN (1965) with respect to histocompatibility mutations and those involving coat-color factors is reflected by the fact that, in the mouse, loci concerned with the latter have been estimated to have an average forward mutation rate of from 7.5×10^{-6} to 10×10^{-6} mutations per locus per gamete (CARTER, LYON and PHILLIPS 1958; RUSSELL 1963; SEARLE and PHILLIPS 1964; SCHLAGER and DICKIE 1967).

The number of incompatibilities reported by BAILEY and KOHN corresponds to a mutation frequency for all H loci of 5.4×10^{-3} /gamete (BAILEY 1970). If the mutation rate for each histocompatibility locus is assumed to be the same as that observed for coat color loci, one is led to conclude that there are 322 to 1500 histocompatibility loci (BAILEY 1970). If on the other hand mice possess a much

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lower number of histocompatibility loci, then the incompatibilities observed by BAILEY and KOHN would suggest a higher rate of mutation than is observed for other loci. Since it is well known that some genetic loci are more mutable than others, it would not be surprising if the mutation rate for histocompatibility genes were higher than that of coat-color genes. However, the magnitude of the disparity suggested by the results noted above appears to be so great that, if substantiated, it would have profound implications.

GRÜNEBERG (1970) has studied subline differentiation of inbred mouse strains and has observed a much greater degree of skeletal variation than would have been predicted according to known rates of spontaneous mutation. He concluded that these variations "might best be explained as consequences of interactions between the mice and extranuclear entities such as viruses" (GRÜNEBERG 1970).

The availability in our laboratory of two inbred lines of mice and one inbred strain of rats which have been separated for a number of years from related lines at the Jackson Laboratory and Microbiological Associates, respectively, prompted us to reexamine this question. We also obtained a subline of mice from Brown University which was compared with a Bar Harbor subline reputed to have originated from the same parental stock.

MATERIALS AND METHODS

In 1957 a breeding nucleus of C57BL/6J mice in the F_{53} generation was obtained from the Jackson Laboratory. These animals were maintained by selected full-sib matings until 1965 at the Wistar Institute and subsequently in the animal quarters of the Department of Human Genetics of the University of Pennsylvania. This line of mice, which was 42 generations removed from the C57BL/6J animals imported from the Jackson Laboratory for comparison, will hereafter be designated C57BL/6JSs.

In May of 1959 a breeding nucleus of Lewis rats was sent to Microbiological Associates from this laboratory for purposes of commercial distribution. In 1970 a group of these rats was obtained from Microbiological Associates (Lewis/Micro) and skin grafts were exchanged between them and our descendants of the original parental stock (Lewis/Ss), which had now gone through an additional 29 generations of brother $\times$ sister matings.

The mouse strain designated A/Ss is descended from animals brought to the United States in 1957 by R. E. BILLINGHAM. They had been maintained in the laboratory of P. B. MEDAWAR, who obtained them from H. GRÜNEBERG. GRÜNEBERG obtained them from J. B. S. HALDANE, who in 1932 obtained them from L. C. DUNN. Assuming 3 mouse generations a year, we estimate that this line of mice is separated from the A/HeJax line by at least 126 generations, and from the A/J subline, also obtained from Bar Harbor, by at least 123 generations. A/HeJ and A/J mice are judged to be separated by 129 generations.

The C57BL/10Ch mice were obtained in 1971 from HERMAN CHASE of Brown University who initiated his subline in 1938 from C57BL/10J animals. The Chase subline was compared with C57BL/10Sn mice, another subline of C57BL/10, which was obtained from the Jackson Laboratory. C57BL/10Ch and C57BL/10Sn animals are estimated to be at least 119 generations removed from one another (STAATS 1972).

Ears were removed from donor animals and split-thickness grafts were placed on the thoracic walls of recipients according to procedures described elsewhere (BILLINGHAM 1961). Inasmuch as there is evidence that when only weak incompatibilities prevail, small grafts are more likely to be rejected than large grafts (HILDEMAN 1967), most of the mice were challenged with small grafts (3 mm $\times$ 3 mm) comparable to the size used by BAILEY and KOHN (1965). In some cases, however, an entire ear served as the donor material. In addition to exchanging skin grafts be-

tween sublines, small grafts were also exchanged among ten members of each subline to confirm its isogenicity. In all instances donors and recipients were of similar sex. Finally, to obtain some estimate of the number of segregating histocompatibility factors, small ear skin grafts were transplanted from C57BL/10Sn females to a population of (C57BL/10Ch $\times$ C57BL/10Sn) F_2 mice.

Primary inspection of all grafts was made on the eighth or ninth postoperative day, and subsequent inspections were carried out daily until the thirtieth day, after which the grafts were inspected weekly. A dissecting microscope was employed to assess the viability of all grafts.

RESULTS

In all instances grafts exchanged between members of each subline healed-in well, displayed no signs of incompatibility, and remained in excellent condition during the 200 days they were followed. It is therefore evident that all the sublines utilized were uniform, at least with respect to their histocompatibility genes.

The data for skin grafts exchanged among the three sublines of Strain A, two sublines of C57BL/6 and two sublines of C57BL/10 mice, as well as the survival of skin grafts exchanged between the two sublines of rats, are shown in Table 1. In no instance did any of the 41 grafts transplanted between the 2 sublines of C57BL/6 mice display any signs of rejection during the 150–200 days they were followed. Similarly, all grafts made between the 2 sublines of rats survived in impeccable condition. On the other hand, some incompatibilities were evident in all the other subline combinations tested. Thus, although all 12 grafts from A/Ss to A/HeJ mice survived for at least 100 days, 2/12 A/HeJ grafts were rejected by A/Ss animals. Moreover, almost all of the accepted grafts in this group initially displayed some cosmetic imperfections before improving. When the two A/Ss recipients which rejected their initial A/HeJ grafts, at 20 and 23 days, respectively, were rechallenged with a second graft of the same origin, these grafts survived for only 10 and 8 days, respectively. Three out of 13 A/Ss grafts were also rejected by A/J recipients, at 20, 45 and 82 days, even though, here again, all 14 grafts in the reciprocal direction were accepted for the 150 day observation period. Some of these grafts likewise initially displayed some signs of cosmetic imperfections, from which they recovered.

The greatest degree of incompatibility between strain A sublines occurred when grafts were exchanged between A/J and A/HeJ mice. Three out of 14 A/HeJ males rejected skin grafts (at 30, 67 and 88 days) as did 2/10 A/J males (at 13 and 34 days). Signs of epithelial weakness were also initially evident in many of the grafts of this group which survived for more than 150 days and which were in excellent condition at the conclusion of the experiment.

The greatest degree of incompatibility which we observed in this study was between the two reputed sublines of C57BL/10 mice. Indeed, not only were all grafts exchanged between C57BL/10Ch and C57BL/10Sn animals rejected, but they were rejected relatively promptly when the C57BL/10Ch animals served as the recipients. Because of this uniform rejection an F_2 population of animals, derived from crossing the two sublines, was challenged with C57BL/10Sn skin as a means of obtaining some estimate of the number of segregating histocom-

Table 1
Fate of Skin Grafts Exchanged Between Various Sublines of Mice and Rats

Donor		Recipient		# Generations Separated	Graft Size	# rejected # grafted	Survival Times (days)
A/HeJ	♂	A/Ss	♂	126	9 mm ²	0/6	6 x >100
A/HeJ	♀	A/Ss	♀	-	9 mm ²	2/6	20, 23, 2 x >100, 2 x >200
A/Ss	♂	A/HeJ	♂	-	9 mm ²	0/6	2 x >100, 4 x >200
A/Ss	♀	A/HeJ	♀	-	9 mm ²	0/6	6 x >100
A/J	♂	A/Ss	♂	123	9 mm ²	0/14	14 x >150
A/Ss	♂	A/J	♂	-	9 mm ²	3/13	20, 45, 82, 10 x >150
A/J	♂	A/HeJ	♂	129	9 mm ²	3/14	30, 67, 88, 11 x >150
A/HeJ	♂	A/J	♂	-	9 mm ²	2/10	13, 34, 8 x >150
C57BL/6J	♀	C57BL/6JSs	♀	42	Whole ear	0/6	6 x >150
C57BL/6J	♀	C57BL/6JSs	♀	-	9 mm ²	0/10	10 x >150
C57BL/6J	♂	C57BL/6JSs	♂	-	Whole ear	0/4	4 x >200
C57BL/6J	♂	C57BL/6JSs	♂	-	9 mm ²	0/5	5 x >200
C57BL/6JSs	♂	C57BL/6J	♂	-	Whole ear	0/3	3 x >200
C57BL/6JSs	♂	C57BL/6J	♂	-	9 mm ²	0/4	4 x >200
C57BL/6JSs	♀	C57BL/6J	♀	-	9 mm ²	0/9	9 x >200
C57BL/10Ch	♀	C57BL/10Sn	♀	119	9 mm ²	10/10	19, 2 x 20, 2 x 27, 28, 29, 37, 2 x 52
C57BL/10Ch	♂	C57BL/10Sn	♂	-	9 mm ²	9/9	18, 2 x 19, 23, 2 x 35, 54, 67, 69
C57BL/10Sn	♀	C57BL/10Ch	♀	-	9 mm ²	8/8	3 x 17, 2 x 21, 24, 2 x 27
C57BL/10Sn	♂	C57BL/10Ch	♂	-	9 mm ²	5/5	4 x 16, 21
Lewis/Ss	♀	Lewis/Micro	♀	29	Whole ear	0/12	12 x >200
Lewis/Micro	♀	Lewis/Ss	♀	-	Whole ear	0/12	12 x >200

TABLE 2

Summary of F_2 test conducted with C57BL/10Ch and C57BL/10Sn mice*

Sex of F_2 recipients	Number	Survival times of grafts (days)	Percent grafts surviving 300 days	Histo- compatibility gene estimate
Female	33	3 x 14, 2 x 15, 2 x 16, 2 x 17, 18, 19, 20, 3 x 21, 2 x 24, 25, 30, 34, 43, 3 x 44, 58, 96, 7 x > 300	21.2	5
Male	39	2 x 14, 2 x 15, 2 x 16, 19, 20, 3 x 21, 22, 24, 25, 29, 30, 33, 34, 2 x 43, 69, 118, 2 x 144, 145, 14 x > 300	35.9	3-4
Male & Female	72		29.2	4

* All F_2 recipients challenged with a C57BL/10Sn female skin graft.

patibility factors. The results of this F_2 test, summarized in Table 2, suggest the two sublines bear different alleles at at least 4 or 5 histocompatibility loci. Our finding that more female than male F_2 recipients rejected C57BL/10Sn skin grafts is consistent with the well established fact that, in general, females manifest a stronger immune response than males (GRAFF, LAPPE and SNELL 1969).

DISCUSSION

All skin grafts exchanged between sublines of animals which had been separated for less than 50 generations were permanently accepted in our experiments. Incompatibilities were observed in all four subline combinations which had been separated for at least 119 generations. In three of these combinations ($A/HeJ \leftrightarrow A/Ss$, $A/J \leftrightarrow A/Ss$, and $A/J \leftrightarrow A/HeJ$), the degree of incompatibility was relatively slight. In one case however ($C57BL/10Ch \leftrightarrow C57BL/10Sn$), all inter-subline skin grafts were rejected and an F_2 test suggested that the two sublines possessed different alleles at four or five histocompatibility loci. We cannot rule out the possibility that the C57BL/10Ch subline is contaminated by genes from some other strain, but if this were the case, one would normally expect differences at more than four or five loci (BILLINGHAM, HODGE and SILVERS 1962; BAILEY and MOBRAATEN 1964). Furthermore, DR. CHASE is not aware of any black nonagouti mice which were ever present in his colony other than the C57BL/10Ch subline (CHASE, personal communication).

From the data presented in this report, it is not possible to confirm or refute the apparently extraordinary rate of mutation for H loci that was reported by BAILEY and KOHN. One can, however, make certain predictions about the rate of subline divergence that would be expected on the basis of their observations, and we are grateful to BAILEY for suggesting the following approach. If the number of H mutations per gamete is 5×10^{-3} , then the probability of a mutation arising in one generation (from four gametes) is $(5 \times 10^{-3}) (4)$. Since the original frequency of the mutant would be $1/4$, only $1/4$ of these mutations would be fixed by inbreeding. Thus the probability of a mutation occurring in any single earlier generation now being genetically fixed in the line would be $(1/4) (5 \times 10^{-3}) (4) = 5 \times 10^{-3}$. The probability of a mutation from any single generation *not* being present would be $1 - (5 \times 10^{-3})$ and from all G generations $[1 - (5 \times 10^{-3})]^G = (.995)^G$. This is a rough estimate because it only considers fixed mutations, but it does provide a simple model by which one can generate expectations as to whether sublines will or will not remain histocompatible after various numbers of generations. In Table 3 are presented calculations based on this model. Several points of interest emerge from these data. Firstly, even at the extraordinary rate of mutation reported by BAILEY and KOHN (1965), after 200 generations of sublining one would expect approximately 13½% of subline pairs to be fully histocompatible. Secondly, at the same rate of mutation, almost 10% of subline pairs would be *incompatible* after only 10 generations of sublining. Thus, the observations discussed in this report are consistent with expectations based on the mutation rate reported by BAILEY and KOHN, but to determine the re-

TABLE 3

*Probability that two sublines will be histocompatible after separation
for varying numbers of generations**

Number generations separated†	Probability that two sublines will be histocompatible
10	.905
20	.818
30	.704
40	.670
50	.606
60	.548
70	.496
80	.448
90	.406
100	.367
110	.332
120	.300
130	.272
140	.246
150	.222
160	.201
170	.182
180	.165
190	.149
200	.135

* These probabilities are based on the formula suggested to us by BAILEY, as discussed in the text.

† In the formula which was utilized, G is equal to twice the number of generations separated since two branches of the inbred line are being considered.

liability of these calculations would require many additional experiments. For the transplantation biologist, the lesson that emerges is that each pair of sublines must be considered unique whether the sublines have been separated for 10 generations or 200 generations.

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