SPONTANEOUS MUTATIONS AND MUTATION RATES IN THE HOUSE MOUSE

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THE frequency of occurrence of spontaneous mutations has been under investigation in the extensive mouse breeding colonies of the Jackson Laboratory since the autumn of 1963. The primary goal of this study is to estimate the spontaneous mutation rates, both forward and reverse, at each of five specific coat-color loci (non-agouti, *a*, brown, *b*, albino, *c*, dilute, *d*, and leaden, *ln*). A secondary goal is to estimate an overall rate using all of the mutations found. Forward natural mutation rates based on large numbers of mice have been reported by RUSSELL (1963) and by CARTER, LYON, and PHILLIPS (1958). Preliminary estimates of rates in this study have been reported by GREEN, SCHLAGER and DICKIE (1965, 800,000 mice examined) and by SCHLAGER and DICKIE (1966, 1.5 million mice examined). This report will present estimates based on 3.5 million mice examined, representing ten million gene reproductions at the five specific coat-color loci, and 1.3 to 6.9 million gene reproductions at each of 40 other loci. These data were collected during the period from August 1963, through April, 1966, and include the data previously reported.

MATERIALS AND METHODS

Mice of 18 inbred strains, propagated by brother-sister matings, and of six hybrids, representing crosses of various pairs of strains, were examined weekly between birth and weaning for deviations from the expected normal appearance for the strain. The animal caretakers examined the mice in a prescribed manner which included an inspection of the coat color, body size and shape, head, tail, and appendages. They were also alerted to watch for circling behavior and difficulty in locomotion and movement. Mice that did not conform to the norm for a strain were designated as "deviants" and removed from the breeding colonies, along with their parents and sibs, for further observation. Most of the deviant mice were mated for a genetic study of the trait. Transient characteristics such as moulting, ear infections, broken back-bone or other bones, or ears and appendages damaged by chewing were not tested. Only those traits that were proven to be transmissible to future generations were considered mutations. Since all mice in these colonies are pedigreed, it was possible to count as a single mutational event a recessive mutation that occurred two or more times in collateral lines.

The recovery of deviant mice from the breeding colonies is dependent to a large extent on the attentiveness of approximately 45 animal caretakers. The system virtually precludes the possibility of overlooking a marked deviation in coat, particularly of the five specific coat-color loci, since each mouse is examined three to four times between birth and weaning and then once again after weaning. Generally two or more individuals examine the same litter during this period. The probability of recovery of dominant and recessive visible and semidominant lethal mutations is probably not as high as for mutations at the coat-color loci, but a tabulation of the recovery rate for individual caretakers has shown that during a three-year period 103 proven mutations were

discovered by 46 different people. We are confident that only a very few deviants escaped detection.

The genetic tests to which a deviant was subjected depended upon the type of mutation involved: forward or reverse mutations at the specific coat-color loci, or dominant or recessive mutations at other loci. Forward mutations at the specific loci were usually found in the hybrid mouse and the locus was recognized from the phenotype of the deviant. Since the deviant was heterozygous for the new mutation and the original mutant allele, appropriate matings were set out to produce mice homozygous for the new mutation. The deviant mouse was usually mated with its parent suspected of carrying the new mutant allele, with a mouse of an unrelated strain homozygous for the mimic mutation, and with a nonmutant bearing mouse of an unrelated strain. For example, if a suspected remutation to brown (b) occurred in the B6D2F$_1$ hybrid, a deviant was mated to a mouse of the JB/Di strain (b/b) and to a mouse of the C3H/Di strain ($+/+$ at the b locus). In the cross with the JB/Di strain all offspring were of the brown phenotype, while the F$_2$ of the C3H/Di cross were produced in the expected 3:1 ratio. The summary of all tests indicated that this new mutation was actually a remutation at the b locus.

In addition to the above series, a further test was made when the suspected mutation was to the c allele. The homozygous deviant was mated to a heterozygous ($+/c^a$) mouse of an unrelated stock and if only one class of offspring were produced the new mutation was not a c allele.

Reverse mutations at the specific coat-color loci were usually recognized from the phenotype of the deviant, but it was necessary to test for allelism to exclude the possibility that the phenotype resulted from a mimic mutation at another locus. If the new mutation was presumed to be at the d locus, the deviant was mated to a mouse of the C3H/Di ($+/+$ at the d locus). Twelve matings were established among the offspring of this cross and the new mutation was considered a remutation to dense ($d \rightarrow +$) if approximately one fourth of these matings produced dilute (d/d) offspring. If the mutation was suspected to be to the black and tan allele (a^t), the deviant mouse was mated to a wild-type mouse ($+/+$) and the $+/a^t$ offspring distinguished by their agouti backs and light bellies. Some of the $+/a^t$ mice were intercrossed until about 200 offspring were classified. At least six mice of the a^t/a^t genotype were further tested by crosses to mice with the known genotype a/a. Similar tests were performed for presumed remutations to A^w.

Many of the recessive mutations at other loci resembled known mutations. Tests for allelism with all known mimic mutations were made before the mutation was considered a new mutation. The mutations were assumed to be allelic if F$_1$ progeny from a mating of mice homozygous for the new and known alleles were all mutant type. If 12 or more F$_1$ progeny in two or more litters were normal the new and known mutations were considered nonallelic. For most mutations tests for allelism involved only a few test matings, but there are more than 25 coat-color loci and more than 15 loci affecting circling behavior.

Dominant or semidominant mutations at loci other than the five specific coat-color loci were tested for allelism in a variety of ways. Where the mutation was suspected to be at the *Ca*, *Sp*, or *Xt* locus, linkage tests with closely linked markers were made. Mutations resembling *W* and *Sl* were tested for allelism with the mimic mutation. The mutations were considered allelic if mice that were white, black-eyed, and anemic (macrocytic anemia) were produced. Linkage with *Hm* was an additional test made for the *W* mutants. An examination and classification of embryos was an additional criteria for allelism in the case of some semidominant lethal mutations.

The scope of this study can be seen in Table 1 where the strains and hybrids are listed along with the number of mice examined, number of deviant mice, and proven mutations for each strain. The nine albino (c/c) strains yield information on reverse mutations at the c locus only, and their numbers are omitted in all other calculations where the albino coat would interfere with the detection of the mutation (e.g., belly spots, tabby, tortoise, and other coat colors). The seven non-agouti strains yield estimates of reverse mutation rates a to $+$, A^y, A^w, and a^t. The four brown ($a/a\ b/b$), one leaden ($a/a\ b/b\ ln/ln$) and two dilute ($a/a\ b/b\ d/d$) strains yield information about reverse mutations at these loci. Forward mutations at these five loci are detectable in the F$_1$ hybrids with some crosses yielding additional information about reverse mutations for alleles held in common by the two strains crossed.

TABLE 1

The inbred strains and their hybrids used in the study of mutation rates

Strain	Coat-color genotype				Number of mice examined	Number of deviants	Number of mutations
A/HeJ	*a/a*	*b/b*	*c/c*	...	76,915	13	0
A/J	*a/a*	*b/b*	*c/c*	...	206,431	40	3
AKR/J	*a/a*	...	*c/c*	...	309,378	74	11
BALB/cJ	...	*b/b*	*c/c*	...	130,223	23	4
CBA/J	...	...	...	...	86,255	39	6
CE/J	A^w/A^w	...	c^e/c^e	...	1,086	3	0
C3H/HeJ	...	...	...	...	275,991	186	33
C3HeB/FeJ	...	...	...	...	45,680	27	4
C57BL/6J	*a/a*	...	...	...	743,124	284	41
C57BL/10J	*a/a*	...	...	...	24,829	35	1
C57BR/cdJ	*a/a*	*b/b*	...	...	13,409	23	1
C57L/J	*a/a*	*b/b*	...	*ln/ln*	83,500	41	2
C58/J	*a/a*	...	...	...	19,648	9	3
DBA/1J	*a/a*	*b/b*	...	*d/d*	81,704	54	0
DBA/2J	*a/a*	*b/b*	...	*d/d*	534,242	155	9
RF/J	...	...	*c/c*	...	26,150	13	2
SJL/J	...	...	*c/c*	...	18,915	3	0
ST/bJ	*a/a*	*b/b*	*c/c*	...	4,842	9	1
SWR/J	...	...	*c/c*	...	15,575	7	0
129/J	A^w/A^w	...	c^{ch}/c^{ch}	...	31,076	5	0
HRS/J	...	...	*c/c*	...	8,032	8	0
B10.D2/SnJ	*a/a*	*b/+*	...	*d/+*	3,367	3	1
AKD2F_1	*a/a*	*b/+*	*c/+*	*d/+*	36,696	12	5
B6AF_1	*a/a*	*b/+*	*c/+*	...	34,781	8	1
B6D2F_1	*a/a*	*b/+*	...	*d/+*	406,035	34	12
CAF_1	*a/+*	*b/b*	*c/c*	...	70,192	6	1
C3D2F_1	*a/+*	*b/+*	...	*d/+*	33,679	0	0
LAF_1	*a/a*	*b/b*	*c/+*	*ln/+*	125,117	79	5
Total	...	...	...	...	3,446,872	1,193	146

This method differs from the specific-locus method of RUSSELL and of CARTER since in their studies all of the alleles under test are present in all mice. In our study the specific loci occur on different genetic backgrounds, one or more in each of the strains and hybrids. Our method does not have the advantage of similar sample sizes for each locus under test, but does permit a comparison of the spontaneous mutation rate at a given locus between specific genetically fixed strains and hybrids.

The procedures for calculating the mutation rates were given in detail in an earlier paper (GREEN, SCHLAGER, and DICKIE 1965). In brief, the rate is equal to the number of times the mutation occurred divided by the number of gametes for its occurrence. For reverse mutations at the five specific coat-color loci, and at any other locus where the mutation was detectable in heterozygous condition, the denominator is two times the number of mice examined for that locus since each zygote represents an opportunity for mutation in two gametes. For forward mutations at the five specific coat-color loci the denominator is the number of F_1 mice examined for that locus since only one of the two uniting gametes could carry the mutation. For all other forward mutations we estimated that the breeding system permitted us to detect half of the mutations that occurred (see GREEN, SCHLAGER, and DICKIE 1965) and consequently we doubled the numerator when we calculated the rate at these loci.

The principles of laboratory animal care as promulgated by the National Society for Medical Research are practiced at The Jackson Laboratory.

RESULTS AND DISCUSSION

Breeding tests: The genetic breeding tests were originally performed on every deviant mouse, but time and space limitations did not permit us to continue this practice after the second year. Table 2 tabulates the categories of abnormalities dealt with in this study along with the number of deviant mice discovered in each category and the number genetically tested. This tabulation encompasses a period slightly longer than August 1963 through April 1966 on which the tabulations in Table 1 are based. Approximately 10% of the deviants turned out to be mutations, although only about 8% could be characterized as to the mode of inheritance. The other 2% showed some form of irregular recessive inheritance where the ratios in the genetic tests did not conform to expected Mendelian ratios. For example, only 5% of a large number of F_2 progeny from a recessive tail-mutant had abnormal tails. The trait was transmissible, but the mode of inheritance could not be characterized.

Specific coat-color loci: Table 3 summarizes the forward and reverse spontaneous mutations and their rates at each of the five specific coat-color loci under investigation. The forward rate at the *a* locus is considerably higher than at the other four loci. This is probably a result of the small number of gametes tested

TABLE 2

Categories of presumptive mutations (deviants) and a summary of the number of deviants tested and the results of the genetic tests

Category	Number of deviants	Number tested	Number of mutations	Mode of inheritance		
				Dominant	Recessive	Irregular
Tail deformities	514	176	12	0	3	9
Belly-spots*	195	191	41	16	3	22
Splashes	100	50	0	..	..	..
Neuromuscular	94	94	29	2	27	0
Blebs and tufts	56	20	0	..	..	..
5 specific coat-color loci	56	56	36	24	12	0
W-locus	28	28	19	18	0	1
Ear deformities	23	23	1	0	1	0
To-type	22	22	3	3	0	0
Headspots	21	21	0	..	..	..
Anemics	11	11	0	..	..	..
Wavy hair	8	8	6	1	5	0
Other	32	32	17	2	15	0
Moulting, injuries, and infections	331	0	0	..	..	..
Totals	1,491	732	164†	66	66	32

* Includes *Sl*.

† 101 of these mutations, which could be characterized as being either dominant or recessive alleles, were used to calculate the spontaneous mutation rates in Tables 3, 4, and 5; the balance of these were outside the period of the study reported in this paper.

TABLE 3

Spontaneous mutations and their rates at five specific coat-color loci

Locus	No. of gametes tested	No. of mutations	Mutation rates $\times 10^6$	95% confidence limits $\times 10^6$	Mutations
Forward					
a	33,679	1	29.7	0.8–165.4	$+ \rightarrow A^{iy}$
b	514,558	2	3.9	0.5– 14.0	(2) $+ \rightarrow b$
c	196,594	2	10.2	1.2– 36.7	$+ \rightarrow c^p$, $+ \rightarrow c$
d	479,777	6	12.5	4.6– 27.2	(5) $+ \rightarrow d^l$, $+ \rightarrow d^s$
ln	125,117	1	8.0	0.2– 44.5	$+ \rightarrow ln$
Total	1,349,725	12	8.9	4.6– 15.5	.....
Reverse					
a	4,212,904	20	4.7	2.9– 7.3	(14) $a \rightarrow a^t$, (6) $a \rightarrow A^w$
b	1,675,944	0	0	0 – 2.2	.....
c	1,707,558	0	0	0 – 2.2	.....
d	1,231,892	4	3.3	0.9– 8.3	(4) $d \rightarrow +$
ln	167,000	0	0	0 – 22.1	.....
Total	8,995,298	24	2.7	1.7– 4.0	.....

at the *a* locus. The one mutation from + to A^{iy} occurred very early in this search for mutants and the calculated mutation rate has halved since the last report (SCHLAGER and DICKIE 1966). No other differences in forward mutation rates among the loci are discernible at this time.

Both the *a* and *d* loci back-mutate at a significantly higher rate than the *b* and *c* loci. The number of gametes tested at the *ln* locus is too small to make a comparison at this time. It is suggested in the literature that the *a* locus is a complex locus exhibiting pseudoallelism (WALLACE 1965) and has a relatively high recombination frequency (0.5%, RUSSELL, CUPP MCDANIEL, and WOODIEL 1963). The large number of reverse mutations that we found at this locus would also suggest that this is a complex locus. However, the reverse mutation rate at *a* is not significantly different from that at *d*, nor is the forward rate significantly different from that at *c* or *ln*. In addition, the frequency of irradiation-induced forward mutations at the *a* locus is appreciably less than those at the *b*, *c*, and *d* loci (RUSSELL 1965b). In view of these findings the *a* locus will be considered as a single entity and the overall mutation rates at these five coat-color loci will be calculated accordingly.

The 12 forward mutations yielded an overall spontaneous mutation rate of 8.9×10^{-6} mutations per locus per gamete. The 95% confidence limits of this rate (estimated by the method of STEVENS, 1942) encompass the spontaneous forward mutation rates published by RUSSELL (1963) of 7.5×10^{-6}, and by CARTER, LYON, and PHILLIPS (1958) of 10×10^{-6} for seven specific loci, *a*, *b*, *c*, *d*, *se*, *p*, and *s*. RUSSELL's value is for spontaneous mutations in male mice, and he said that the rate in females is lower (RUSSELL 1965a). Our calculated rate is based on mutations that occurred in both males and females. However, 11 of the 12 forward mutations were detected in hybrid mice and the origin of the

mutation was deducible. Eight originated in the female parent and three in the male parent.

The overall spontaneous reverse mutation rate based on nearly nine million tested gametes was 2.7×10^{-6} mutations per locus per gamete. This is identical to the earlier estimate based on about the first half of this number of tested gametes (SCHLAGER and DICKIE 1966). The overall forward rate is three times higher than the overall reverse rate at these five loci and the difference is statistically different at $P < 0.001$. The average per-locus forward rate in Table 3 (12.8×10^{-6}) is eight times higher than the average per-locus reverse rate (1.6×10^{-6}).

Spontaneous mutations involve alterations of the DNA molecule during replication. On the basis of studies of mutations in microorganisms, the evidence is strong that mistakes in replication involve the substitution of noncomplementary base pairs and deletion or insertions of one or more nucleotide pairs in sequence during the synthesis of the progeny chain on its template. The most common type of spontaneous mutation involves changes in only one base pair, generally a transversion involving a substitution in which the purine-pyrimidine orientation is reversed with the replacement of guanine-cytosine by cytosine-guanine or thymine-adenine (KREIG 1963). If a mutation is due to this type of error, the back-mutation to the original allele would involve reverse substitution to the original configuration. Deletions of one or more nucleotide pairs, on the other hand, must be reconstituted by insertions which are dependent to a large extent on the nucleotide pairs adjacent to the deleted area. It is highly unlikely that mutations deleting more than one base pair will revert back to the original form, but those involving one nucleotide pair may do so at a low rate. This theory was supported by evidence from molecular model building which led FRESCO and ALBERTS (1960) to conclude that the substitution of base pairs could produce reversible mutations while the deletion and insertion mechanism could account for the mutational events which have been found to be irreversible. Although it would be premature to make many inferences about what is happening at the biochemical level a few speculations may be made based on the rates in Table 3. A comparison of the rate of forward and reverse mutations to alleles under test may indicate the nature of the mutation, single pair substitution or insertion-deletion. The relatively high reverse mutation rate at the *a* and *d* loci may suggest a single nucleotide pair substitution and the absence of forward mutations to the *a* or *d* allele may suggest that these configurations are very stable. The overall rates of 8.9×10^{-6} and 2.7×10^{-6} mutations per locus per gamete are appreciably higher than those to be presented below for other loci in the mouse, which suggests that the majority of the mutations found at these five specific coat-color loci are due to alterations in a single nucleotide pair in the DNA molecule. However, until a sufficient number of mutations occur to alleles under test, the usefulness of these data for biochemical inferences will be limited.

Other loci: The spontaneous mutations and estimates of their mutation rates for all loci other than the five specific coat-color loci are shown in Tables 4 and 5. A total of 28 recessive mutations were recovered representing a variety of deviations from the normal genotype of the strains examined. Ten of these mutations involved neuromuscular disorders, eight involved deviations in coat texture or color, three were of ear size or color. An overall spontaneous recessive mutation rate based on these 26 loci was 0.67×10^{-6} mutations per locus per gamete. This rate is 1/13th that calculated for the forward mutations at the five specific coat-color loci. However, the 95% lower confidence limits to the estimates at the *a*, *b*, and *ln* loci do include all of the individual rates in Table 4. On the other hand, none of the individual rates of the five coat-color loci are inside the 95% upper

TABLE 4

Spontaneous recessive mutations and mutation rates at loci other than the five specific coat-color loci

Mutation		Number of mutations found	Number of mice examined*	Mutation rate $\times 10^6$†	95% confidence limits $\times 10^6$
Symbol	Name				
dt	dystonia musculorum	2	3,446,872	1.16	0.32–2.97
ep	pale ear	1	2,553,985	0.78	0.09–2.83
fz	fuzzy	1	3,446,872	0.58	0.07–2.09
le	light ear	1	2,553,985	0.78	0.09–2.83
lt	lustrous‡	1	3,446,872	0.58	0.07–2.09
me	moth-eaten‡	1	3,446,872	0.58	0.07–2.09
mg	mahogany	1	2,553,985	0.78	0.09–2.83
oc	osteosclerotic‡	1	3,446,872	0.58	0.07–2.09
ot	oscillator‡	1	3,446,872	0.58	0.07–2.09
p	pink eye	1	2,553,985	0.78	0.09–2.83
pe	pearl	1	2,553,985	0.78	0.09–2.83
pk	plucked‡	1	3,446,872	0.58	0.07–2.09
pu	pudgy	1	3,446,872	0.58	0.07–2.09
rc	rough-coat‡	1	3,446,872	0.58	0.07–2.09
rg	rotating‡	1	3,446,872	0.58	0.07–2.09
sa	satin	1	3,446,872	0.58	0.07–2.09
se	short ear	1	3,446,872	0.58	0.07–2.09
sh-1	shaker-1	1	3,446,872	0.58	0.07–2.09
sr	spinner	1	3,446,872	0.58	0.07–2.09
un	undulated	1	3,446,872	0.58	0.07–2.09
vl	vacuolated lens‡	1	3,446,872	0.58	0.07–2.09
we	wellhaarig	1	3,446,872	0.58	0.07–2.09
. .	unnamed locus nm(44)	2	3,446,872	1.16	0.32–2.97
. .	unnamed locus nm(62)	1	3,446,872	0.58	0.07–2.09
. .	unnamed locus bs(47)	1	2,553,985	0.78	0.09–2.83
. .	unnamed locus bs(95)	1	2,553,985	0.78	0.09–2.83
	Total	28	83,368,463	0.67	0.51–0.87

* A total of 3,446,872 mice were examined of which 2,553,985 were nonalbino.
† Numerator doubled, see text for details.
‡ Previously unnamed locus.

confidence limits of the calculated individual rates of Table 4. If the overall confidence limits of these two sets of rates were similar, we would have had to find 193 mutations at the nonspecific loci in order to fall within the 95% lower confidence limit of the rate at the five specific loci. It is highly unlikely that we are recovering only 1 out of 7 recessive visible mutations that may occur at these 26 loci. It must be concluded from this comparison that the forward spontaneous mutation rate at the five specific coat-color loci is significantly higher than that at a large sample of other loci. LYON and MORRIS (1966) and others have suggested that some of the specific loci generally used in irradiation studies (*a*, *b*, *c*, *d*, *se*, *p*, and *s*) may have above average spontaneous mutation rates since many of them were found among mice that were bred as pets. They found no spontaneous mutations in 9,328 mice examined for mutations at five or six loci (54,254 gamete-loci) at a new set of specific loci (*a*, *bp*, *fz*, *ln*, *pa*, and *pe*), but the

TABLE 5

Spontaneous dominant mutations and mutation rates at loci other than the five specific coat-color loci

Mutation		Number of mutations found	Number of gametes tested	Mutation rate × 10^6	95% confidence limits × 10^6
Symbol	Name				
Ca	Caracul	1	6,893,744	0.15	0.003–0.81
Mo^{br}	Brindled*	1	1,276,993	0.78	0.020–4.36
Re	Rex	1	6,893,744	0.15	0.003–0.81
Rv	Revolver	1	6,893,744	0.15	0.003–0.81
Sl	Steel	2	5,107,970	0.39	0.047–1.41
Sp	Splotch	4	5,107,970	0.78	0.213–2.00
Ta	Tabby*	1	1,276,993	0.78	0.020–4.36
To	Tortoise*	2	1,276,993	1.57	0.190–5.65
W	Dominant Spotting	18	5,107,970	3.52	2.091–5.57
Xt	Extra toes	1	6,893,744	0.15	0.003–0.81
. .	unnamed locus "mottled"	1	5,107,970	0.20	0.005–1.09
. .	unnamed locus bs(41)	1	5,107,970	0.20	0.005–1.09
. .	unnamed locus bs(45)	1	5,107,970	0.20	0.005–1.09
. .	unnamed locus bs(73)	1	5,107,970	0.20	0.005–1.09
	Total	36	67,161,745	0.54	0.38 –0.74

* Sex-linked loci.

irradiation induced rate was significantly lower in these six loci than in the seven usually used in their studies.

Fourteen different mutations to dominant alleles were recovered at loci other than the five specific coat-color loci (Table 5). Ten involved coat color, and there was one neuromuscular mutant, a polydactylous mutant, and two hair-curling mutants. The overall mutation rates for dominant mutations at the five specific and other loci are also appreciably different, but here the overlap of 95% confidence limits of the individual loci show a greater degree of similarity. The upper confidence limits of loci *b*, *c*, and *ln* cover most of the rates in Table 5, while those of the sex-linked and the *W* loci resemble the *a* and *d* rates. From these data it is evident that the spontaneous mutation rates to dominate alleles at the *a*, *d*, *W*, and *Sp* loci and probably of the sex-linked loci Mo^{br}, *Ta* and *To* are higher than the other 12 loci.

Combining the number of recessive mutations of Table 3 (12 mutations) and Table 4 (56 "expected" mutations) and dividing by the total number of gametes × loci tested, yielded an overall spontaneous forward mutation rate from wild-type to recessive alleles of eight mutations per locus per ten million gametes. This is identical to the overall mutation rate to dominant alleles calculated from the total number of mutations from recessive to dominant alleles (60) divided by the total number of gametes tested. WATSON (1965) estimated that the average probability of changes due to insertions of a new nucleotide pair may be as low as 10^{-8} to 10^{-9}. Our rate of 8×10^{-7} suggests that these mutations are primarily of the insertion type.

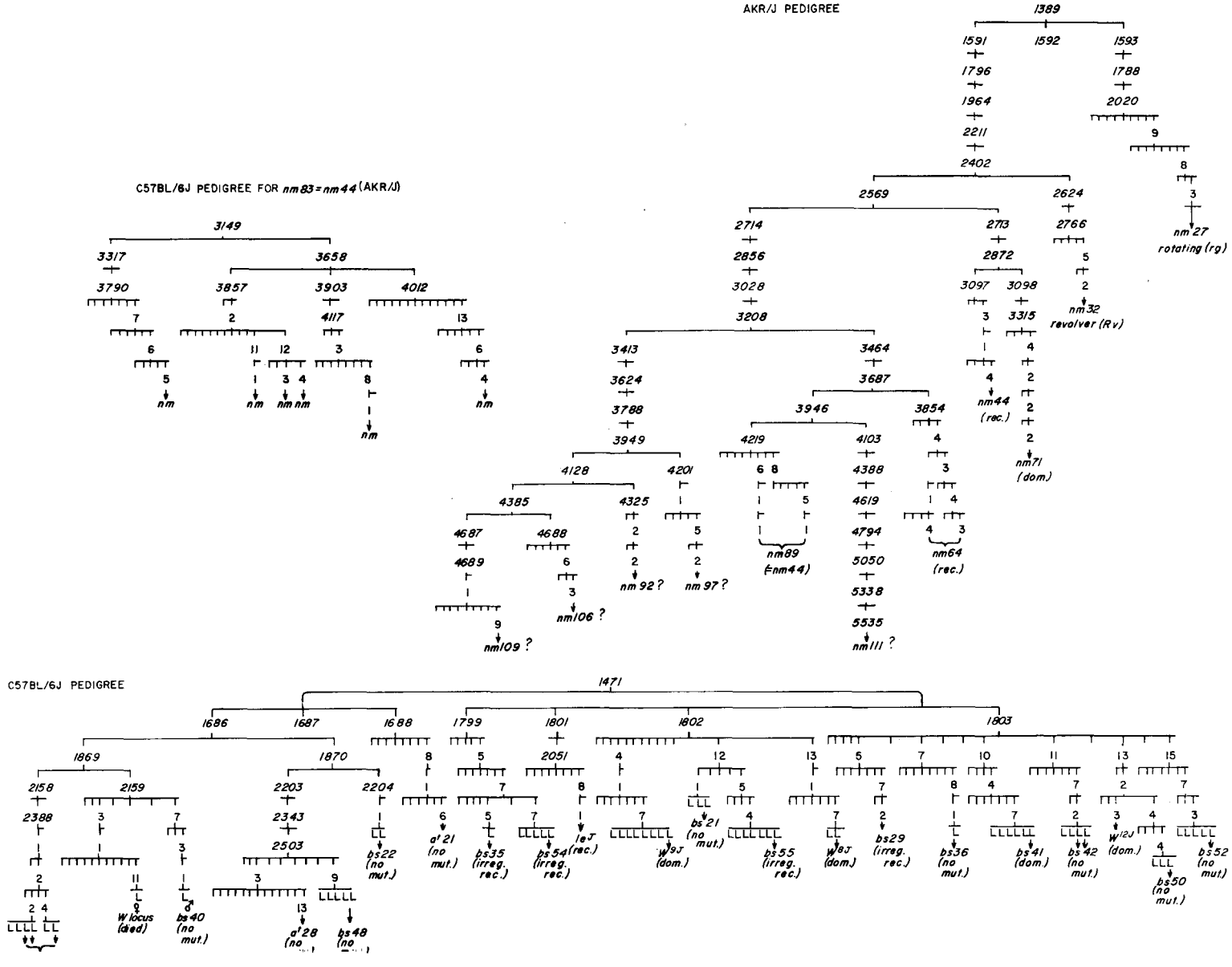
It should be emphasized that we are dealing with only those mutations that

are visible on gross examination and these comprise a fraction of the loci at which recessive visible mutations are known to occur. The overall spontaneous mutation rates are then overestimations based on but a sample of visible mutations. Of the named mutants tabulated by GREEN (1966) there are about 160 recessive and 60 dominant mutations that are readily detectable and could be uncovered in our search. A similar estimate of the number of loci tested in our search can be derived from the information in Table 4 and its relationship to the Poisson distribution. The successive terms in the Poisson distribution for the categories with 0, 1, and 2 mutations are 1, m, $m^2/2$ respectively, where m is the mean of the distribution. Since we found 24 loci with one mutation and 2 loci with two mutations the mean m can be estimated from the ratio of the third to second category: $(m^2/2)/m$ or $m/2 = 2/24$, and $m = 1/6$. Since the mean m is the total number of mutations divided by the total number of loci, the number of loci with no mutations is 142 (6×28 less the 26 loci at which mutations were found).

Genetic background and the incidence of mutation: Of the 18 W locus mutations shown in Table 5, ten were recovered from the C57BL/6J strain and three from the C3H/HeJ strain. The mutation rates of the *W* locus in these two strains was 6.7×10^{-6} and 5.4×10^{-6}, respectively, considerably higher than the incidence of this mutation in other non-albino strains. The C57BL/6J strain also showed a high incidence of reverse mutation at the agouti locus, from a to a^t and to A^w. At least 11 of these mutations were found in the C57BL/6J strain for a rate of 7.4×10^{-6}, almost double the rate shown in Table 3 for the a locus. The evidence for a higher frequency of mutations in the C57BL/6J strain may be indicative of the presence of a mutator gene in that strain. Mutator genes are thought to produce a substance that induces transition mutants and evidence for their existence comes from a variety of organisms including maize (MCCLINTOCK 1951), Drosophila (DEMEREC 1937; IVES 1950), and Salmonella (KIRCHNER 1960).

Of the 29 neuromuscular mutants so far recovered in the study, five different mutations, two dominant and three recessive, were discovered in strain AKR/J, four recessive mutations in C57BL/6J, four recessives in A/J, four in C3H/HeJ, three in BALB/cJ, two in CBA/J and one in strains DBA/2J, C57BL/KsJ, C58/J, CAF_1, RF/J, C57L/J and DBA/1J. As of February 1967, six of these mutants are reoccurrences as follows: two remutations to dystonia musculorum (*dt*) in C3H/HeJ; one to spinner (*sr*) in CBA/J; one to shaker-1 (*sh-1*) in C3H/HeJ; and two to *nm44* in C57BL/6J and in A/J (the original mutation occurred in AKR/J). The mutation *nm44* appears to be unrelated to other circling mutants already described. Although no rates are calculated for the occurrence of this type of mutation in various strains, it is interesting to note the preponderance of them in a few strains.

In addition to finding a higher incidence of specific mutations in particular strains, individual pedigrees also showed a large number of deviant mice and mutations. The AKR/J pedigree in Figure 1 shows the origin of 11 neuromuscular deviants, six of which were shown to be five different mutations. The genetic status of the other five is still in question. All of these mutations were in a pedigree from one pair of mice (No. 1389). The *nm44* and *nm89* mutations are now


AKR/J PEDIGREE
1389
1591
1592
1593
1796
1788
1964
2020
2211
9
2402
8
2569
2624
3
2714
2713
2766
nm 27
rotating (rg)
2856
2872
5
3028
3097
3098
2
3208
3
3315
nm32
revolver (Rv)
3413
3464
1
4
3624
3687
4
nm44
(rec.)
3788
3946
3854
2
2
3949
4219
4103
4
2
nm71
(dom.)
4128
4201
6 8
4388
3
4385
4325
1 5
4619
1 4
4687
4688
1 5
4794
4 3
2
5
1
1
5050
nm89
(=nm44)
nm64
(rec.)
4689
6
2
2
5338
3
nm 92?
nm 97?
1
5535
nm106?
9
nm111?
nm109?
C57BL/6J PEDIGREE FOR nm83=nm44 (AKR/J)
3149
3317
3658
3790
3857
3903
4012
7
2
4117
13
6
11
12
3
6
5
1
3
4
8
4
nm
nm
nm nm
nm
nm
C57BL/6J PEDIGREE
1471
1686
1687
1688
1799
1801
1802
1803
1869
1870
8
5
2051
4
12
13
5
7
10
11
13
15
2158
2159
2203
2204
7
8
7
2388
3
7
2343
1
4
7
2
7
2
3
7
2503
bs22
(no mut.)
a' 21
(no mut.)
bs35
(irreg. rec.)
bs54
(irreg. rec.)
leJ
(rec.)
W9J
(dom.)
bs21
(no mut.)
bs55
(irreg. rec.)
W8J
(dom.)
bs29
(irreg. rec.)
bs36
(no mut.)
bs41
(dom.)
bs42
(no mut.)
W12J
(dom.)
bs52
(no mut.)
bs50
(no mut.)
W locus
(died)
bs40
(no mut.)
a' 28
(no
bs48
(no

known to be allelic and probably identical. This mutation, temporarily designated by its acquisition number *nm44*, seems to be identical to one that arose in the C57BL/6J strain, from which six deviants exhibiting the trait have been recovered (Figure 1). Another pedigree showing a large number of mutations is shown at the bottom of the figure. Twenty-one deviants were recovered from this pedigree originating from a C57BL/6J pair numbered 1471. Ten of these deviants were shown to be at least five different mutations by subsequent genetic tests. Both of the extensive pedigrees may represent the operation of mutator genes affecting specific loci, those resulting in neuromuscular disorders in the AKR/J strain and in coat-color deviations in the C57BL/6J strain.

We are grateful for the collaboration of DR. E. L. GREEN during the early part of this study and his continuing encouragement throughout the course of the entire study. More than 50 individuals are involved in searching for the deviants, completing the breeding tests, and summarizing the data; we are indebted to each of them for their part in this study. The study is supported in part by Contract AT(30-1)-3249 with the U.S. Atomic Energy Commission and made use of computing facilities provided by grant G-19389 of the National Science Foundation.

SUMMARY

The spontaneous mutation rate of five specific coat-color loci (*a*, *b*, *c*, *d*, and *ln*) based on the examination of 3.5 million mice representing ten million gene reproductions were 2.7×10^{-6} per locus per gamete for reverse mutations and 8.9×10^{-6} for forward mutations. The nonagouti (*a*) and dilute (*d*) alleles back-mutate at a significantly higher rate than the brown (*b*) and albino (*c*) alleles. The overall spontaneous mutation rate to recessive alleles based on 26 other loci was 0.67×10^{-6} per locus per gamete, while the rate to dominant alleles based on 14 loci was 0.54×10^{-6}. The forward mutation rate of the five specific coat-color loci was significantly higher than at the nonspecific loci. Combining the data for all loci, the rate of mutation from dominant to recessive alleles and from recessive to dominant alleles were both eight mutations per locus per ten million gametes. Evidence is presented to show that the genetic background can influence the incidence of mutations. Mutations at the *a* and *W* loci occur at a much higher frequency in the C57BL/6J strain. Pedigrees are presented which show higher incidences of various mutations suggesting the presence of mutator genes in some strains.

FIGURE 1.—Pedigrees of lines within strains showing an abundance of particular types of mutations. Numbers in italics represent pedigree number of the pairs of mice. Vertical lines below mouse number represents pairs of mice used for further matings, the ones numbered being the origin of further matings in the line to the deviant or mutant. Other designations: *L* = litter, *rec* = recessive inheritance, *dom* = dominant inheritance, *irreg. rec* = irregular recessive inheritance, i.e., ratio in offspring does not conform to an expected Mendelian ratio but mutation is definitely not a dominant, *no mut.* = trait not transmitted to progeny. Acquisition number (e.g., *nm109*) followed by a question mark means that genetic breeding tests are not completed. Other abbreviations designate name of mutation or temporary name, e.g., *nm* = neuromuscular mutant, *bs* = belly spot mutant.

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