

Mutation rates at a new set of specific loci in the mouse

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1. INTRODUCTION

The 'specific-locus' method of measuring mutation rates has been of great value in investigating the mutagenic effects of radiation in mice. The outstanding workers in this field have been W. L. Russell and his colleagues, whose studies have included the relation of mutagenic effect to radiation dose, dose-rate, germ-cell stage and various other factors (Russell, 1951, 1963; Russell & Russell, 1959*a*). Contributions from Harwell have also covered dose-rate (Carter, Lyon & Phillips, 1958; Phillips, 1961) and germ-cell stage (Carter, Lyon & Phillips, 1960) and more recently the mutagenic effect of fast neutrons (Batchelor, Phillips & Searle, 1964).

One limitation of all this work, however, is that the same seven specific loci, those of *a*, *b*, *c*, *d*, *p*, *s* and *se*, have been used throughout, and there is no means of knowing how representative these are of mouse loci as a whole. The seven loci vary considerably among themselves, Russell & Russell (1959*b*) having found a 35-fold range of difference in sensitivity from the most to the least sensitive locus after a dose of 600 r. of X-rays to spermatogonia. The specific locus mutation rates usually quoted are the average rates for the seven loci, but in view of their wide range of variation there is room for doubt whether any other group of loci would give a similar figure. This does not, of course, invalidate or detract from the results obtained in the studies mentioned above, since these did not involve extrapolation to other loci. It is important, however, in considering the overall mutagenic effect of radiation in the mouse, and in comparing the mouse with other organisms. In particular, Russell (1956), studying these loci, found the mouse to be fifteen times as sensitive as *Drosophila melanogaster* to the mutagenic effect of an acute X-ray dose. Lyon, Phillips & Searle (1964), on the other hand, studying the overall mutation to dominant and recessive lethal and visible genes in the mouse after a similar X-ray dose, found the mutation rates lower than would have been expected from the specific locus studies. Their figures on recessive lethal mutation suggested that the mouse was only four to five times as sensitive as *Drosophila* and that the average mouse gene locus was less mutable than the seven studied until then. There was in fact some theoretical reason to think that these original seven loci might be above average in mutation rate, since mutant alleles at all except one of them were found among mice bred as pets. This means that these loci must have undergone spontaneous mutation sufficiently frequently to have been discovered before serious mouse genetics was started, in contrast to many other genes with similar effects which are known now.

The present paper describes a specific locus mutation experiment designed to compare the results from a new group of six loci with those obtained with the original group. The radiation dose, dose-rate and strain of mice used were all chosen so as to be closely comparable with those for which the mutation rate of the original seven loci was most accurately known, viz. 600 r. X-rays at 90 r./min. to males of the hybrid stock (C3H \times 101). The loci chosen were those which had easily scorable mutant alleles with good viability and penetrance, and which were conveniently available for use in this laboratory. These loci were agouti (*a*), brachypody (*bp*), fuzzy (*fz*), leaden (*ln*), pallid (*pa*), and pearl (*pe*). The agouti locus was also among the original seven loci but was included here because the allele of brachypody used, *bp^H*, is closely linked with non-agouti, *a*. Leaden, pallid and pearl are all genes affecting coat colour and the mutant alleles result in colours similar to those produced by three genes of the original seven, *d*, *p* and *c^{ch}*. It therefore seems likely that if amateur mouse-breeders had found mice carrying them they would have kept them as pets. None of these genes is, in fact, known among pet mice, however, and so it may be that their spontaneous mutation rates are lower than those of *d*, *p* and *c^{ch}*. Brachypody, which results in short feet, and fuzzy, which results in sparse, fuzzy fur would probably not have been kept among pet mice.

2. MATERIALS AND METHODS

Male mice of the hybrid stock (C3H/HeH \times 101/H) were given a dose of 600 rad. X-rays (250kVcp; 10 mA; HVL 1.2 mmCu; 88.25 rad/min.) to the posterior third of the body at the age of 6–7 weeks. Comparable control males were set aside at the same time but not irradiated. Twelve weeks later each male was mated to two females of the genotype *aa bp^Hbp^H fz fz ln ln pa pa pe^Hpe^H*. The allele *bp^H* was discovered at Harwell among the descendants of a mouse being tested for mutation at another locus, and the allele *pe^H* arose among a stock of CBA mice at the M.R.C. Laboratory Animals Centre, Carshalton, and was later investigated at Harwell. The first known mutant allele of pearl showed a high rate of back-mutation when on a C3H background (Russell & Major, 1956). The allele *pe^H*, however, has never been observed to back-mutate.

The stock homozygous for these six genes was constructed at Harwell and known as the HT stock. At the beginning of the experiment the supply of females homozygous for all six genes was not good enough and some were used which were not homozygous for *pe^H*. The data from these are tabulated separately, and are regarded as giving evidence on only five loci. The males were left breeding for the whole of their reproductive life (up to 2 years) and females were replaced as necessary. Young were scored for mutations at birth and 2½ weeks. Any with phenotypic abnormalities were kept and genetically tested; the remainder were discarded. The identity numbers of the control and irradiated parents were coded, so that it was not obvious to workers scoring mutations from which series the young came.

3. RESULTS

At the six specific loci there were a total of three mutations, two at the *bp* locus and one at the *fz* locus (Table 1). All were in the irradiated series, and all were shown by allelism tests to be mutations at the loci indicated. In addition, there were three dominant mutations in the irradiated series, and one sex-linked mutation in the controls allelic with and indistinguishable from tabby, *Ta*. This must have occurred in the female parent, as one of the two affected offspring was a *Ta* male. The dominant mutations were phenotypically similar to and possibly allelic with genes at the loci: *Xt*, *W*, and *Hk*.

Table 1. *Numbers of mutants and total offspring in the control and irradiated series, with data from females homozygous at five and six loci tabulated separately*

Series	Total offspring	Mutants						Dominant or sex-linked
		Specific locus						
		<i>a</i>	<i>bp</i>	<i>fz</i>	<i>ln</i>	<i>pa</i>	<i>pe</i>	
Control								
Five loci	1,714	0	0	0	0	0	—	1
Six loci	7,614	0	0	0	0	0	0	
Irradiated								
Five loci	3,436	0	1	0	0	0	—	3
Six loci	13,299	0	1	1	0	0	0	

These results were compared with those that might have been expected if the original seven loci had been used. Russell (1963) reported a total of 111 specific locus mutants in 119,326 young after a comparable X-ray dose to spermatogonia, which corresponds to a mutation rate (spontaneous plus induced) of 13.3×10^{-5} per locus. If both the spontaneous and the induced mutation rates at the two sets of loci were the same, then the expected number of specific locus mutations in the irradiated series of the present experiment would be 12.9 whereas only three were observed, giving a mutation rate of 3.0×10^{-5} per locus.

On a hypothesis of no difference between these and Russell's data in the 'true' mutation rate per locus, there are three sets of data to be compared: this experiment with five loci (*pe* excluded), this experiment with six loci, and Russell's data on seven loci. The expected numbers of mutants in the three sets of data should be in the ratio of the numbers of loci and total young in each case, i.e., $(5 \times 3436) : (6 \times 13,299) : (7 \times 119,326)$. This gives the expected figures shown in Table 2, and a χ^2 , comparing the observed and expected values, of 7.52 for 2 degrees of freedom, which is significant at the 2½% level. The 95% fiducial limits for the mutation rate per locus (Table 2) show that the data for five loci in the present experiment are consistent with both Russell's data and those for six loci, whereas the latter indicate a mutation rate significantly lower than that in Russell's experiments.

The numbers of dominant mutations, on the other hand, were in good agreement with those found in other work, e.g. Phillips (1961) found two dominant

Table 2. Comparison of observed numbers of mutations and mutation rates per locus in the irradiated series with those expected on the basis of Russell's (1963) results

Series	Observed		Expected		95% fiducial limits of mutation rate per locus $\times 10^5$
	Mutant	Non-mutant	Mutant	Non-mutant	
Present					
Five loci	1	3,435	2.10	3,433.90	0.15, 32.4
Six loci	2	13,297	9.76	13,289.24	0.30, 9.0
Russell's					
Seven loci	111	119,215	102.14	119,223.86	10.9, 16.1

mutations among 10,761 mice after a 600 rad X-ray dose, which would correspond to an expected number of 3.1 in the irradiated series of the present experiment.

4. DISCUSSION

The results show that the specific-locus mutation rate measured by using the new set of loci (HT) was significantly lower than that given by the original loci. The possibility must be excluded that this was due to some factor other than the difference in loci. One question is whether the male mouse stocks or irradiation techniques used here differed from those used by Russell in some way that would affect the mutation rate. In fact, both these factors had been carefully chosen so as to be comparable to those of Russell and a previous experiment by Phillips (1961) had shown that under such circumstances the specific-locus mutation rate obtained with the original seven loci was the same in this laboratory as in Russell's. Further, there were no grounds for fearing that the mice had for any reason not responded normally to the radiation in this particular experiment, since the mutation to non-specific dominants was up to the expectation and, in addition, the number of translocations found among the progeny of a small number of the males which were mated soon after irradiation was quite typical (Lyon & Meredith, unpublished). It was therefore concluded that the lower specific-locus mutation rate observed among the offspring of these males was indeed due to the new group of loci having a lower average mutation rate than the original seven.

It might be objected that the experiment was biased by the inclusion of the agouti locus among the HT loci when it was already known from Russell's experiments to have a low mutation rate. One can, however, compare the mutation rates of the two groups without the agouti locus. Russell & Russell (1959*b*) reported that only two out of 174 specific locus mutations were at the agouti locus. Therefore, the average mutation rate at the other six loci after 600 r. X-rays to spermatogonia must have been 15.3×10^{-5} , and in the present experiment the expected number of mutations at the five loci other than agouti would have been 12.3. Thus, the general conclusions would not have been altered.

The number of mutations observed in the present experiment is much too small to give any clear idea of the spectrum of mutations at the six loci. The occurrence

of two mutations at the *bp* locus, however, makes it seem likely that individually the HT loci overlap the original loci in mutation rate. Two *bp* mutations out of 16,735 mice gave a mutation rate of 12×10^{-5} at this locus, whereas the mutation rates at the *b*, *c*, *d* and *p* loci after a similar X-ray dose are 19, 8, 15 and 13×10^{-5} respectively (Russell & Russell, 1959*b*; Russell, 1963).

The three mutations observed in the present experiment gave a mutation rate of 3.0×10^{-5} per locus with fiducial limits (Stevens, in Fisher & Yates, 1953) as wide apart as 0.6×10^{-5} and 8.8×10^{-5} . This included spontaneous mutation as well as induced, and the value of the spontaneous mutation rate was not known. No specific-locus mutations were observed in the control series and this was consistent with a spontaneous mutation rate equal to that of the original seven loci, but also consistent with a much lower figure. Therefore, one must take the observed induced mutation rate as lying between 2.25×10^{-5} , if the spontaneous mutation rate was the same as that for the original loci, and 3.0×10^{-5} if the rate was zero.

Since the induced mutation rate for the original loci was 13.3×10^{-5} the new group appear to be about four to five times less sensitive to the mutagenic effect of a spermatogonial dose of 600 rad of X-rays. Alexander (1954, 1960) found the average mutation rate at eight specific autosomal loci in *Drosophila melanogaster* after a dose of X-rays to spermatogonia to be 1.25×10^{-8} per locus per roentgen. The rate per rad for the HT loci was about 5.0×10^{-8} . Thus, if mouse loci in general had a similar sensitivity then the mouse would be three to four times as sensitive as *Drosophila* to the mutagenic effects of radiation. This agrees well with the estimate, mentioned earlier (Lyon, Phillips & Searle, 1964), that the mouse was four to five times as sensitive as *Drosophila* to the induction of recessive lethals, and also with the results of Lüning (1964), again studying recessive lethals but with different stocks and irradiation techniques, who estimated the mouse to be two to four times as sensitive as *Drosophila*. However, it cannot be assumed that the new loci are accurately representative of mouse loci as a whole, and this apparent agreement of results from lethal and specific locus mutation could be quite fortuitous. The point that is important is that these experiments should agree in suggesting that the mouse is less sensitive to the genetic hazards of radiation than had been supposed, although still more sensitive than *Drosophila*.

Further work is to be done with the HT loci in order to estimate their mutation rates more accurately. In general, however, the original loci will remain more useful for the study of mutagenesis in the mouse as their higher mutation rate makes them easier to work with.

SUMMARY

Previous measurements of specific locus mutation rates in mice had all involved the seven loci, *a*, *b*, *c*, *d*, *p*, *s* and *se*. An experiment was performed with the same mouse stock (C3H \times 101) and the same radiation dose (600 rad) to spermatogonia as had been used previously, but employing a new group of six loci, *a*, *bp*, *fz*, *ln*,

pa and *pe*. The observed mutation rate, 5.0×10^{-8} per locus per rad, was significantly lower than that for the original seven loci, but was three to four times higher than the corresponding mutation rate in *Drosophila melanogaster*.

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