

SOME EVIDENCE CONCERNING THE "MUTATIONAL LOAD" IN INBRED STRAINS OF MICE

MARY F. LYON

M.R.C. Radiobiological Research Unit, Harwell, Berkshire

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1. INTRODUCTION

RADIATION genetics theory postulates that the reproduction of any species of animal is adjusted to the spontaneous mutation rate in that species, and consequently that increases in the mutation rate may, by increasing the "mutational load", jeopardise the survival of the species (Muller, 1954). Hence in studying the radiation genetics of any animal it is essential to know something of the spontaneous mutation rate. The present paper describes an attempt to measure the spontaneous occurrence of recessive lethals in inbred strains of mice.

It is well known that as a result of death of embryos not all the ova shed by a female mouse are represented by living young at birth. McDowell (1924) counted the number of corpora lutea in pregnant mice and the number of young which came to term. He found that the number of living young at birth was only 66 per cent. of the number of corpora lutea. Similarly, Fekete (1947) showed that in the DBA and C57BL strains the mean litter-sizes at birth were only 58 per cent. and 84 per cent. respectively of the mean number of fertilised ova.

The causes of this death must include :

- (i) dominant lethals arising in female gametogenesis ;
- (ii) dominant lethals arising in male gametogenesis ;
- (iii) recessive lethals ;
- (iv) non-genetic agencies.

Of these, deaths due to recessive lethals are only likely to occur with an appreciable frequency when the parents are related, as for instance in an inbred strain. However, it has usually been supposed that the greater part of embryonic death, even in an inbred strain, is due to the fourth cause, *i.e.* to some unfavourable factor of the uterine environment. On the other hand, Hartman (1953), showed that in the opossum defective embryos could be found at a stage so early that their defects were most unlikely to be due to an unfavourable uterine environment.

In the present work crosses were made within and between three inbred strains of mice in all reciprocal combinations, and embryonic death between implantation and $13\frac{1}{2}$ - $15\frac{1}{2}$ days' gestation was measured by opening pregnant females. The results show that, as might be expected, a proportion of the embryonic death in inbred strains is

traceable to the action of recessive lethal genes, and from the observations an estimate has been made of the mutation rate to such recessive lethals in the strains used. Negative observations of visible mutation in the CBA/H strain are also reported.

2. STOCKS AND METHODS

The three inbred strains used were CBA/H, C₃H/HeH, and 101/H. Females were used at the age of six weeks to three months, and males at ages of six weeks to four months. The strains were crossed in the nine possible reciprocal combinations, choosing for the within-strain crosses males and females which were sibs. In crosses involving CBA or C₃H males, boxes of five females were made up and one male was placed in each box on a Monday morning and left there until Friday morning. In crosses using 101 males, which mate less readily, a male was placed with only one female at a time, the female being replaced as soon as she had mated. Mating was detected by examining females for copulation plugs, on the evening of the first day and the morning of subsequent days. All females which were found to have plugs, and any others which were visibly pregnant, were dissected 13-15 days after mating; the numbers of corpora lutea, of uterine implantations with a live embryo, and of implantations with no live embryo were counted. The crosses were made between September 1957 and May 1958.

3. RESULTS

Embryonic loss can be detected in two main ways :

- (i) by the difference between the number of corpora lutea, representing the number of ova shed, and the number of implantations in the uterus ; and
- (ii) by the number of implantations at which there is no living embryo.

Loss between the shedding of the ovum and implantation could be due either to death of the embryo after fertilisation or to failure of fertilisation. Loss after implantation, however, must involve embryonic death. Hence, in considering the results, pre-implantation and post-implantation loss will be kept separate, and more attention will be paid to post-implantation loss.

Tables 1 and 2 show the ratio of live implants to the total number of implants in each cross. The three lowest ratios are those for the three within-strain crosses, in agreement with the hypothesis that death due to recessive lethals is occurring in these crosses but not, to any appreciable extent, in crosses between strains. When the within-strain and between-strain crosses are summed there is a difference of 9.6 per cent. between the proportions of live implants in the two types, and χ^2 for heterogeneity is 57.5 for 1 degree of freedom. The marginal totals for each type of female and male are given to show that there is no indication of any strain tending to yield a different proportion of dead implants. Moreover, a χ^2 test shows that there is no heterogeneity among the between-strain crosses. Thus there is no indication of any factor other than that being investigated having disturbed the ratios. χ^2 for heterogeneity among the within-strain

crosses is significant at the 1 per cent. level, suggesting that the frequencies of deaths due to recessive effects in the three strains differ. Table 3 shows the values for this excess death in the three strains.

TABLE 1
Ratio of implantations at which a live embryo was present to the total number of implantations

Male parent	Female parent						Total	
	CBA		C ₃ H		101			
	Nos.	Per cent.	Nos.	Per cent.	Nos.	Per cent.	Nos.	Per cent.
CBA .	292/336	86.9	312/341	91.5	232/254	91.3	836/933	89.6
C ₃ H .	270/306	88.2	306/364	84.1	256/276	92.8	832/946	87.9
101 .	263/285	92.3	329/350	94.0	231/309	74.8	823/944	87.2
Total .	825/927	89.0	947/1055	89.8	719/841	85.5	2491/2823	...

Total within-strain 829/1009, 82.16 per cent.
Total between-strain 1662/1812, 91.72 per cent.

TABLE 2
χ² for heterogeneity

	χ ²	Degrees of freedom	
Between within-strain and between-strain crosses	57.5	1	P < 0.001
Among within-strain crosses . . .	17.7	2	0.001 < P < 0.01
Among between-strain crosses . . .	8.5	5	0.1 < P < 0.2

TABLE 3
Excess embryonic death in the within-strain crosses for the three strains used

Cross	Per cent. survival	Per cent. excess death
Between-strain . . .	91.7	...
CBA	86.9	4.8
C ₃ H	84.1	7.6
101	74.8	16.9

Of all the implants examined 332 had no live embryo. Of these 310 were of the type known as "small moles", which remain when embryonic death has occurred before the formation of the placenta, 8 were "large moles" in which death occurred after the formation of the placenta, but left no recognisable embryo, and at 14 there were

recognisable dead embryos. Four of these 14 occurred in a CBA × CBA cross of one male with his two sisters ; there were 17 implants, 12 with live embryos, 4 with embryos dead at 12-13 days and one small mole. This suggests that in this particular cross a recessive lethal gene causing embryonic death at 12-13 days was segregating. The remaining 18 of the 22 late deaths were distributed more or less randomly.

If the excess deaths were in fact due to recessive lethals, as suggested, rather than to some non-specific effect of inbreeding one would expect variation between females in their numbers of dead embryos according to whether or not a lethal was segregating in that particular mating. Qualitatively, such variation was found, being particularly noticeable

TABLE 4
Ratios of numbers of implantations to numbers of corpora lutea

Male parent	Female parent						Total	
	CBA		C ₃ H		101			
	Nos.	Per cent.	Nos.	Per cent.	Nos.	Per cent.	Nos.	Per cent.
CBA .	336/454	74.0	341/444	76.8	254/444	57.2	931/1342	69.4
C ₃ H .	306/377	81.2	364/445	81.8	276/384	71.9	946/1206	78.4
101 .	285/338	84.3	350/408	85.8	303/427	71.0	938/1173	80.0
	927/1169	79.3	1055/1297	81.3	833/1255	66.4		

Total within-strain 1003/1326, 75.64 per cent.

Total between-strain 1812/2395, 75.66 per cent.

χ^2 for heterogeneity of within-strain and between-strain crosses is very small.

in the 101 strain, where the amount of death in a few uteri was consistent with the simultaneous segregation of two lethals, whereas in other uteri there were no dead embryos. Statistically, however, the data are not sufficiently extensive for a comparison of the observed and expected distributions of deaths.

Table 4 shows the ratio of implantations to corpora lutea, giving a measure of the pre-implantation loss. In contrast to tables 1 and 2 there is no difference between the two types of cross, within-strain and between-strain. On the other hand the marginal totals for the types of females and males used show large differences. Crosses involving CBA males show more pre-implantation loss than those with C₃H or 101 males, and those involving 101 females show more such loss than those involving CBA or C₃H females. The cross of CBA male with 101 female has the highest pre-implantation loss of all. Thus there is no evidence in these crosses of recessive lethals tending to cause death of embryos before implantation.

Tables 5 and 6 show the mean numbers of corpora lutea and

uterine implants per female in the various crosses. There are considerable differences among the three strains in the numbers of corpora lutea. Females of strain 101 had a high mean number of corpora lutea and also a high variance of corpus luteum count. Their number

TABLE 5
Means and standard errors of numbers of corpora lutea per female

Male parent	Female parent		
	CBA	C ₃ H	101
CBA . . .	9.3 ± 0.25	10.1 ± 0.23	13.9 ± 0.67
C ₃ H . . .	9.7 ± 0.26	10.1 ± 0.25	11.0 ± 0.48
101 . . .	8.6 ± 0.31	10.5 ± 0.28	10.4 ± 0.52
	9.20 ± 0.16	10.21 ± 0.15	11.62 ± 0.35

of implants, on the other hand, was average and it appeared that the high corpus luteum count was the result of some females giving a count roughly twice the number of implants. It is suggested that in 101 females developing follicles may be confused with corpora lutea

TABLE 6
Means and standard errors of number of implants per female

Male parent	Female parent		
	CBA	C ₃ H	101
CBA . . .	6.9 ± 0.31	7.8 ± 0.38	7.9 ± 0.32
C ₃ H . . .	7.8 ± 0.22	8.3 ± 0.30	7.9 ± 0.35
101 . . .	7.3 ± 0.29	9.0 ± 0.32	7.4 ± 0.30
Total . . .	7.30 ± 0.17	8.31 ± 0.20	7.71 ± 0.19

and that this accounts for their high corpus luteum count and consequent apparent high rate of pre-implantation loss. Among the other types of females there is no reason to suppose that variations in the number of corpora lutea have disturbed the results. In 101 females the count appears to vary with the male used, but since the high numbers of corpora lutea in 101 ovaries were very difficult to count the apparent differences may be due to inaccuracy.

4. ESTIMATION OF THE FREQUENCY OF RECESSIVE LETHALS

The increased proportion of dead implants in within-strain crosses could be due to (i) the segregation of clear-cut recessive lethals arising by mutation ; (ii) the segregation of genes with a recessive deleterious

effect arising by mutation and interacting with each other to cause death ; (iii) all embryos of the strain being homozygous for recessive genes which tend to depress their ability to survive in slightly abnormal uterine environments.

If one supposes it to be due to recessive lethals, one can estimate their frequency. If part of it is due to any other cause, the estimate sets an upper limit to the frequency of recessive lethals.

Suppose there are many loci which can mutate to a lethal allele. In some pairs of mice both parents will be heterozygous for one lethal, resulting in the death of one-quarter of their young ; other pairs will be intercross matings for two or more lethals, and others again will not be an intercross for any lethal. Let the sum of the frequencies of intercross matings ($+l \times +l$) for all these lethals be m . Then, if we assume a Poisson distribution, matings which are intercrosses for 0, 1, 2, etc., lethals will occur with frequencies

$$e^{-m} (1, m, \frac{m^2}{2!}, \frac{m^3}{3!}, \dots)$$

Embryos homozygous for any lethal will die. Therefore the frequencies of surviving embryos will be

$$e^{-m} (1, \frac{3}{4}m, \frac{9}{16} \frac{m^2}{2!}, \frac{27}{64} \frac{m^3}{3!}, \dots).$$

In the data of table 1 the excess death attributed to recessive lethals was 9.6 per cent. Therefore the survivors were 90.4 per cent.

Hence to find the frequency of intercrosses for a lethal it is necessary to find a value of m for which

$$e^{-m} (1 + \frac{3}{4}m + \frac{9}{16} \frac{m^2}{2!} + \dots) = 0.904.$$

Iteration shows this value to be $m = 0.4$.

Haldane (1936) has calculated in terms of the mutation rate the frequency of heterozygotes for a lethal which are likely to be present in an inbred strain as a result of spontaneous mutation. By similar reasoning the frequency of matings which are intercrosses for a lethal can be found. The calculations are shown in the appendix. The result obtained was that if matings which are intercrosses for a lethal leave three-quarters as many young in the succeeding generation as matings not segregating for a lethal, then the frequency of intercross matings is $4n\mu$, where μ is the gametic mutation rate and n is the number of loci mutating to a lethal.

From the results it has been calculated that the frequency of intercross matings, m , is 0.4.

$$\text{Therefore } 4n\mu = 0.4$$

$$n\mu = 0.1$$

Thus if the excess death in crosses within inbred strains over that in crosses between them is due to recessive lethal genes then any gamete has a chance of 0.1 of carrying a newly mutated recessive lethal. Haldane showed that the frequency of heterozygotes for a lethal in such a strain is $9.3n\mu$, or in this case 0.93. Hence the results suggest that the frequency of heterozygotes for a lethal in an inbred strain is near unity.

However, this figure is based on the sum of the numbers of live and dead implants over the three strains used, and it has been shown that there is significant heterogeneity among the strains, the CBA strain showing only 4.8 per cent. more dead than in the between-strain crosses and the 101 strain 16.9 per cent. At first sight this seems to imply that in order to continue to accept the hypothesis of recessive lethals one must postulate different mutation rates in the three strains. However, Haldane showed that differences in the method of maintaining the strains could alter the frequency of heterozygotes for a lethal. He considered two possibilities, either that intercross-matings should leave three-quarters as many offspring in the next generation as the other types, or that they should leave an equal number. Another method of stock maintenance, practised in the CBA strain used in this experiment, is to select for production when choosing parents for the next generation. In this strain animals for breeding were chosen from among those whose parents' first two litters were above average in size. The offspring of lethal-intercrosses, which have a reduced litter-size due to death of homozygotes, would thus have a poor chance of being chosen as parents. In the 101 strain, on the other hand, little selection was practised and only a small stock was kept. Since only a few young were kept for breeding from each pair of parents, lethal-intercrosses may have stood an equal chance with the other types of mating of leaving offspring.

The exact chance of a lethal-intercross leaving young in the next generation cannot be decided for either strain but by taking the extreme cases one may find within what limits the observed proportion of dead embryos may vary for a given mutation rate. These extremes are (a) that an intercross leaves no offspring and (b) that an intercross leaves as many offspring as the other two types in the next generation. It can be shown (see appendix) that the frequencies of intercross matings will be in case (a) $2n\mu$ and in case (b) $6n\mu$.

Thus if $n\mu = 0.1$, in the first case m , the frequency of intercross matings, is $2n\mu$

$$\begin{aligned} m &= 2n\mu \\ &= 0.2 \end{aligned}$$

And in the second case

$$\begin{aligned} m &= 6n\mu \\ &= 0.6 \end{aligned}$$

The proportion of surviving animals in the strain is given by

$$S = e^{-m} \left(1 + \frac{3}{2}m + \frac{9}{16} \frac{m^2}{2!} + \frac{27}{64} \frac{m^3}{3!} + \dots \right).$$

$$\begin{array}{ll} \text{If } m = 0.2 & S = 0.951 \\ \text{If } m = 0.6 & S = 0.861 \end{array}$$

Thus, with $n\mu = 0.1$ the proportion of recessive lethal death could range from 4.9 per cent., a value close to that in the CBA stock, to 13.9 per cent., rather less than that in the 101 stock. Therefore, allowing for sampling error, the results are not incompatible with the hypothesis that in all three strains recessive lethal genes arise with a frequency of 0.1 per gamete.

The spontaneous mutation rate at 7 loci mutating to recessive visible mutations in the mouse is of the order of 1×10^{-5} per locus in male gametogenesis (Russell, 1951; Carter, Lyon and Phillips, 1958). If the average mutation rate to lethal alleles were of the same order

$$\begin{aligned} n\mu &= 0.1 \\ n &= 0.1 \times 10^5 \\ &= 10 \times 10^3 \end{aligned}$$

i.e. the number of loci which mutate to a lethal in the mouse would have an upper limit of the order of ten thousand.

5. VISIBLE MUTATIONS

In the maintenance of the CBA stock in this laboratory during the three years from April 1955 to April 1958 no dominant or recessive visible mutation has been observed. From this negative observation it is possible to find an upper limit to the mutation rate to these classes of genes.

(i) Dominant autosomal visibles

During the three years 25,000 young CBA mice were reared to weaning age and dominant visible mutations producing a clear effect could have been observed in them. In fact none was found. By scanning the Mouse Mutant Gene Symbols list in *Mouse News Letter*, 19, it was found that dominant visible mutations have so far been observed at 32 loci. If the average mutation rate to dominant visibles is 1×10^{-5} and these 32 known loci are potentially mutating, then one would expect to find 32 mutant young per 100,000 tested gametes. Observing 25,000 young is equivalent to observing 50,000 gametes and one would therefore expect 16 mutant young. Clearly the average mutation rate to dominants must be less than 1×10^{-5} . The observed mutation for the 32 loci is 0/50,000, which has an upper fiducial limit at the 5 per cent. level of 3.69/50,000. (Fisher and Yates, 1953, table 8 : 1). This is 7.38×10^{-5} for the 32 loci or 2.3×10^{-6} per locus. Thus if the 32 loci known so far were the only loci in the mouse at which

dominant visible mutations could occur then the maximum compatible average mutation rate to autosomal dominants would be 2.3×10^{-6} . In fact it is unlikely that all loci at which dominant mutations could occur have already been discovered, so the average mutation rate is likely to be correspondingly lower.

(ii) *Recessive autosomal visibles*

Recessive visible mutations will only be detected when both parents are heterozygous. Hence, the calculation of the mutation rate is similar to that for recessive lethals. In this case intercrosses for a visible mutation, if found, would be removed from the stock and not permitted to leave young as parents of the next generation. Hence, the case corresponds to that case in the consideration of recessive lethals in which lethal-intercrosses left no young, *i.e.* the frequency of intercross matings is $2n\mu$.

During the three years under consideration 1500 pairs of CBA mice reared young to weaning age. No pair segregated for a recessive visible. Hence the upper fiducial limit of the frequency of intercrosses is $3.69/1500$ (Fisher and Yates, 1953, table 8 : 1). From *Mouse News Letter*, 19, the number of known loci mutating to recessive visibles is 100.

$$\begin{aligned} 2n\mu &= 3.69/1500 \\ 200\mu &= .00246 \\ \mu &= .0000123 \\ &= \underline{1.23 \times 10^{-5}} \end{aligned}$$

i.e. if there were no more than 100 loci mutating to recessive visibles in the CBA stock the maximum compatible mutation rate per locus would be 1.2×10^{-5} . In fact, as with the dominant visibles, there are likely to be more than 100 loci with the average mutation rate again correspondingly lower. It thus seems probable that the seven loci studied by Russell (1951) and Carter, Lyon and Phillips (1958) and found to have an average mutation rate of 1×10^{-5} are rather more spontaneously mutable than the majority of loci mutating to recessive visibles.

6. DISCUSSION

The results of this investigation into the causes of embryonic death in inbred strains do not show whether the excess death within a strain is due to mutations to recessive lethals or to some other cause covered by the term "inbreeding depression". It is known that tendencies to particular lethal abnormalities can occur in particular strains, *e.g.* the persistent low frequency of harelip and cleft palate in strain A. It is therefore reasonable to suppose that tendencies to early embryonic death may also occur and that not all the excess deaths within strains are due to recessive lethals. On the other hand, as females were opened at $13\frac{1}{2}$ - $15\frac{1}{2}$ days' gestation, lethals acting late in embryonic life or after

birth were not detected. But late embryonic death seems relatively uncommon in the mouse and so it is unlikely that this will have affected the results appreciably. Hence the estimate of 0.1 for the frequency of newly arisen lethal mutations in the mouse can be taken as an approximate upper limit. The incidence of newly arisen autosomal recessive and sex-linked lethals in *Drosophila melanogaster* is of the order of 0.01 (Muller, 1954), hence the frequency of spontaneous lethals in the mouse may be up to ten times that in *Drosophila*. The figure obtained for the number of loci mutating, 10,000, when the average mutation rate to lethals is taken as 1×10^{-5} , is in line with expectation. Carter (1957) suggested, as a result of studying the induction of recessive lethals by γ -irradiation in the mouse, that the number of mouse loci was likely to be not very different from that in *Drosophila*, for which a figure of 5000-10,000 is usually given.

Comparison with the negative observations on dominant and recessive visible mutation suggests that mutation to lethals is likely to occur more frequently than mutation to visibles in the mouse. The upper limit of the average mutation rate to dominants is less than the average mutation rate observed in other experiments to seven recessive visibles. Mutation to dominants is thus probably less common than mutation to recessives. Both these facts are in accordance with findings in *Drosophila*.

7. SUMMARY

Embryonic death has been measured in the nine possible reciprocal crosses within and between three inbred strains of mice, CBA/H, C₃H/HeH and 101/H.

Post-implantation death was higher in the within-strain than in the between-strain crosses, the excess death being 4.8 per cent., 7.6 per cent. and 16.9 per cent. in strains CBA, C₃H and 101 respectively, and 9.6 per cent. when summed over all crosses. Some, but probably not all, of this death is likely to be due to the segregation of recessive lethals arising by mutation in the strain.

Assuming all excess death to be due to recessive lethals an upper limit to the spontaneous mutation rate to recessive lethals in these strains was calculated. This limit is 0.1 per gamete over all loci, giving an estimate of 10,000 loci mutating to lethals if the average mutation rate per locus is 1×10^{-5} .

In the routine maintenance of the CBA stock during a period of three years no dominant or recessive visible mutations were found. From this negative observation it is possible to calculate upper limits to the mutation rates per locus to dominant and recessive visibles. These limits are, for recessive visibles, 1.2×10^{-5} if 100 loci are mutating and, for dominant visibles, 2.3×10^{-6} if 32 loci are mutating. The numbers of loci given are those which are so far known; if as is likely the total numbers of loci are greater than these then the upper limits of the mutation rates will be lower.

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9. APPENDIX

(i) Frequency of intercrosses for a lethal

Let a be a recessive lethal gene and let matings of types $AA \times AA$, $AA \times Aa$, and $Aa \times Aa$ occur with frequencies x_n, y_n , and z_n in the n th generation after the first mating involving a mutant heterozygote. Then, if $Aa \times Aa$ matings leave $\frac{3}{4}$ as many young in the succeeding generation as the other two types, it can be shown that

$$\begin{aligned} x_{n+1} &= x_n + \frac{1}{4}y_n + \frac{1}{12}z_n \\ y_{n+1} &= \frac{1}{2}y_n + \frac{1}{3}z_n \\ z_{n+1} &= \frac{1}{4}y_n + \frac{1}{3}z_n \end{aligned}$$

The first, x_0 , generation will consist of a y -mating with a frequency of 1.

Thus the first few terms of the generation series for each type of mating will be

$$\begin{aligned} x & 0, \frac{1}{4}, \frac{1}{2}, \frac{11}{16}, \dots \\ y & 1, \frac{1}{2}, \frac{1}{3}, \frac{17}{12}, \dots \\ z & 0, \frac{1}{4}, \frac{5}{12}, \frac{11}{12}, \dots \end{aligned}$$

From these terms the recurrence relation for z in successive generations has been calculated using the equations

$$\begin{aligned} u_2 &= bu_1 + cu_0 \\ u_3 &= bu_2 + cu_1 \end{aligned}$$

where $u_0 \dots u_3$ are the first few terms of the series for which the recurrence relation is required, and b and c are the coefficients to be found (Li, 1955, p. 66).

It is shown to be

$$12z_{n+2} - 10z_{n+1} + z_n = 0.$$

From this relation the frequency of z -matings over all generations has been summed to infinity, using the formula given by Haldane (1936). In this case the sum to infinity is 1. Since the chance of occurrence of a y -mating in the first place is $\frac{1}{4}\mu$, where μ is the gametic mutation rate, the frequency of z -matings over the whole inbred strain is $1 \times \frac{1}{4}\mu$, or if there are n loci concerned, $\frac{1}{4}n\mu$.

(ii) *Frequencies of intercrosses under other systems of stock maintenance*

The two cases to be considered are (a) that z -matings leave no offspring in the succeeding generation, and (b) that z -matings leave as many offspring as the other two types.

For the first case the frequencies of mating types will be given by

$$\begin{aligned}x_{n+1} &= x_n + \frac{1}{4}y_n \\y_{n+1} &= \frac{1}{2}y_n \\z_{n+1} &= \frac{1}{4}y_n.\end{aligned}$$

The first few terms will be

$$\begin{aligned}x & 0, \frac{1}{4}, \frac{3}{8}, \frac{7}{16} \\y & 1, \frac{1}{2}, \frac{1}{4}, \frac{1}{8} \\z & 0, \frac{1}{4}, \frac{1}{8}, \frac{1}{16}.\end{aligned}$$

The recurrence relation for z is

$$2z_{n+2} - z_{n+1} = 0$$

and the sum to infinity is $\frac{1}{2}$.

Therefore the frequency of z -matings in the inbred strain is $\frac{1}{2} \times \frac{1}{4}n\mu$ or $\frac{1}{8}n\mu$. Similarly, the frequency of heterozygotes for a lethal, which is equal to $(\frac{1}{2}y+z)$, is $\frac{3}{8}n\mu$.

For the second case the frequencies of matings are given by

$$\begin{aligned}x_{n+1} &= x_n + \frac{1}{4}y_n + \frac{1}{6}z_n \\y_{n+1} &= \frac{1}{2}y_n + \frac{1}{3}z_n \\z_{n+1} &= \frac{1}{4}y_n + \frac{1}{6}z_n.\end{aligned}$$

The first few terms are

$$\begin{aligned}x & 0, \frac{1}{4}, \frac{29}{72}, \frac{673}{1296} \\y & 1, \frac{1}{2}, \frac{13}{36}, \frac{185}{648} \\z & 0, \frac{1}{4}, \frac{17}{72}, \frac{253}{1296}\end{aligned}$$

and the recurrence relation for z -matings is

$$18z_{n+2} - 17z_{n+1} + 2z_n = 0.$$

The sum to infinity is 1.5 and hence the frequency of z -matings in the strain is $\frac{3}{8}n\mu$. Haldane has shown that the frequency of heterozygotes in a strain maintained in this way is $1.25n\mu$.