

'Grizzled', a mutant in linkage group X of the mouse

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1. INTRODUCTION

The mutant described here appeared in 1948, and was discovered by Mrs Susan Sobey, who also obtained much of the segregation data. The gene was soon proved to be recessive and fully penetrant, but there was a substantial, though variable, deficiency of homozygotes from backcross and intercross matings. The mutant was described in *Mouse News Letter*, 2, No. 3 (1950) and named 'grizzled' with the symbol *gr*, but the anomalous segregation had not been recognized then, and no segregation data were given. Recent studies, however, have shown that the deficiency of homozygotes is due to increased mortality both pre- and post-natally. Later work also assigned grizzled to linkage group X. This paper gives the segregation and linkage data, and describes the investigations of differential mortality.

2. DESCRIPTION

The only constant visible effect of grizzled is to dilute the yellow pigment in the sub-terminal band of the hairs. Grizzled mice therefore look like chinchillas but the dilution is usually more pronounced and, unlike chinchilla, grizzled does not noticeably affect the black pigment. Grizzled and chinchilla can therefore be distinguished phenotypically, though not easily. Grizzled can readily be recognized in the presence of non-agouti (*aa*) by the hairs on the ears and round the genitalia being white instead of yellow. In combination with yellow (*A^y*) grizzled produces a less pale colour than does chinchilla, because any black pigment remaining as 'sootiness' of the yellow is not further diluted by grizzled. Grizzled, however, has more profound effects than are manifest in the dilution of yellow pigment. Grizzled mice are smaller than their normal litter mates, the reduction of size amounting to about 20–30% at birth and a little less in the adult. They are also less viable than their normal litter mates, both pre-natally and post-natally. The mortality is higher among males than among females. Some grizzled mice have kinky tails, the degree of kinkiness being very variable. The reduced growth rate and viability will be described more fully after the genetic data have been presented.

3. GENETICS

(i) *Origin*

Grizzled was first recognized as a mutant by the white, instead of yellow hair, in the ears of non-agouti brown (*aa bb*) mice. Its origin, however, can be traced

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one generation further back in the records, to an albino (*cc*) female with a kinky tail in a family of thirty-one. The parents of this family were litter-mates from a cross between a stock carrying fuzzy (*fz*), obtained from R. A. Fisher in Cambridge, and the A inbred strain, and were both *aa bb +c +fz* in genotype. Presumably they were both also heterozygous for grizzled, though all the *gr gr* offspring they may have produced escaped notice except the one with a kinky tail. The kinky-tailed female was investigated as a possible mutant on account of its abnormal tail, and was backcrossed to her father. Animals with white hair in the ears were first noted in her progeny. They were outcrossed to other stocks, both inbred and non-inbred, and these matings, with the subsequent matings derived from them, provided the segregation data given below. The association between the colour and the kinky tail was not immediately recognized and the investigation of the kinky tail was continued independently till it was abandoned on account of the poor segregation ratios obtained.

(ii) *Tests of allelism*

Tests of allelic identity with chinchilla (*c^{ch}*) and grey-lethal (*gl*), the only two previously known mutants which grizzled resembles at all closely, were both negative. The test with chinchilla was made with the albino allele (*c*) rather than *c^{ch}*. A grizzled homozygote mated to an albino homozygote produced twelve classifiable offspring which were all wild-type. The test with grey-lethal was very kindly carried out by Professor H. Grüneberg. A grizzled homozygote female mated to a proved (*+gl*) heterozygote produced thirty-three agouti offspring none of which resembled phenotypically either grizzled or grey-lethal.

(iii) *Segregation*

The segregation of grizzled from different types of mating is given in Table 1. All the outcross matings (*gr gr* × + +) produced only normal offspring, and all the matings of grizzled by grizzled (*gr gr* × *gr gr*) produced only grizzled offspring. This proves that grizzled is recessive and fully penetrant. The backcross (*gr gr* × + *gr*) and intercross (+ *gr* × + *gr*) matings did not, however, produce the proportion of grizzled offspring expected for a single recessive gene; there was a deficiency

Table 1. *Segregation of grizzled*

Type of mating	No. of matings	Numbers of offspring		$\chi^2_{(1)}$
		Grizzled	Non-grizzled	
<i>gr gr</i> × + +	32	0	444	—
<i>gr gr</i> × <i>gr gr</i>	25	257	0	—
<i>gr gr</i> × + <i>gr</i>	30	189	337	41.6
+ <i>gr</i> × + <i>gr</i>	35	205	1082	56.5

of grizzled offspring from both types of mating and the discrepancies were highly significant. Since incomplete penetrance has been ruled out, the deficiency of grizzled homozygotes must be attributed to reduced viability. Confirmatory evidence of reduced viability was obtained and will be described in a later section.

The segregation data are given in more detail in Table 2, where reciprocal mating-types are separated, and the sexes of offspring are classified separately. The chief point of interest is that the deficiency of grizzled homozygotes was greater among male offspring than among female in all types of segregating matings, indicating a higher rate of mortality among male than among female grizzled

Table 2. Segregation of grizzled, and calculated differential viability

Parents		No. of matings	No. of offspring				Viability of <i>gr gr</i> ± s.e.	
			Female		Male		Female	Male
♀	♂		<i>gr</i>	+	<i>gr</i>	+		
<i>gr gr</i>	+	20	0	152	0	152		
+	<i>gr gr</i>	12	0	74	0	66		
<i>gr gr</i>	<i>gr gr</i>	25	164	0	93	0		
<i>gr gr</i>	+ <i>gr</i>	17	45	75	43	84	0.60 ± 0.11	0.51 ± 0.10
+ <i>gr</i>	<i>gr gr</i>	13	65	100	36	78	0.65 ± 0.10	0.46 ± 0.09
+ <i>gr</i>	+ <i>gr</i>	35	112	496	93	586	0.68 ± 0.07	0.48 ± 0.05
Weighted mean viability							0.654 ± 0.052	0.480 ± 0.042

homozygotes. There was also a highly significant deficiency of male offspring from *gr gr* × *gr gr* matings ($\chi^2 = 19.6$ for 1 d.f.) which supports the conclusion that the mortality of males was higher. On the supposition that the deficiency of grizzled offspring from the segregating matings was due to pre-natal mortality, the viability of grizzled homozygotes relative to that of non-grizzled offspring was calculated separately for males and females. The viability (*v*) and its standard error was calculated as follows:

$$\begin{array}{llll}
 \text{Phenotypic class:} & gr & + & \text{Total} \\
 \text{Numbers observed:} & a & b & n \\
 \text{From backcrosses:} & v = a/b; & \sigma_v^2 = v(1+v)^2/n & \\
 \text{From intercrosses:} & v = 3a/b; & \sigma_v^2 = v(3+v)^2/3n &
 \end{array}$$

(The formulae for the sampling variances are from Bailey (1961), adapted in the case of intercrosses to apply to the viability of the recessive instead of the dominant phenotype.)

It is clear that the viabilities do not differ significantly between mating types. The weighted mean estimates of the viability were 65% in females and 48% in males. (Weighting was by the reciprocal of the variance.) The viabilities of males and females differ significantly, the difference being 2.6 times its standard error.

Though the mating types were not heterogeneous in the viabilities of the grizzled

offspring, individual matings were significantly heterogeneous in the segregation of grizzled. This was tested on thirty-five intercross matings and twenty-nine backcross matings by the log-likelihood method of Woolf (1957). Significant heterogeneity in the proportion of grizzled offspring was found among the matings of both types, the significance level being 0.1% for the intercrosses and 5% for the backcrosses. From this it must be concluded that the viability of grizzled homozygotes is influenced by the genetic background.

(iv) *Linkage*

Grizzled was tested for linkage with the markers in the linkage testing stocks maintained in Edinburgh. Linkage was found with waltzer (*v*) in linkage group X. Since the departure from independent segregation in these repulsion intercrosses might possibly have been due to viability interaction, rather than linkage,

Table 3. *Linkage data*

Parents		Progeny				Total	<i>p</i> ± s.e., %
♀	♂	++	+v	gr+	grv		
$\frac{+v}{gr+}$	$\frac{+v}{gr+}$	129	65	37	3	234	25.4 ± 7.1
$\frac{++}{grv}$	$\frac{grv}{grv}$	72	9	9	51	141	12.9 ± 2.8
Combined estimate							14.6 ± 2.6

a grizzled waltzer male segregant was used to set up coupling backcross matings. These confirmed the linkage. The data from both types of mating are given in Table 3. Estimation of the recombination frequency is complicated by the reduced viability of grizzled homozygotes and also, in the backcross matings, of waltzer homozygotes. The recombination frequency (*p*) and its standard error were calculated by the methods given by Bailey (1961), the intercross data being calculated by Bailey's formulae 4.18 and 4.19 and the backcross data by 4.10 and 4.11. The intercross data yielded an estimate of $p = 25 \pm 7\%$ and the backcross data gave $p = 13 \pm 3\%$. These estimates are not significantly different, the difference being 1.6 times its standard error. They were therefore combined by weighting by the reciprocal of the variance, and the combined estimate of $p = 14.6 \pm 2.6\%$ was obtained. As an additional marker in linkage group X grizzled cannot, unfortunately, be recommended on account of its reduced viability and the fact that it is located fairly close to waltzer.

4. GROWTH

The weights of individual mice in ten litters from backcross matings (*gr gr* × + *gr*) were recorded. The mice were marked at birth and later classified for grizzled. Comparisons between grizzled and normal of the same sex in the same

litter were obtained for 10 *gr gr* and 10 *+gr* females in seven litters, and for 5 *gr gr* and 6 *+gr* males in four litters. The ratio of the mean weight of the *gr gr* mice to the mean weight of the *+gr* litter mates was calculated for each litter. Table 4 gives the unweighted means of these ratios at weekly intervals up to six weeks. The grizzled mice at birth were about three-quarters the weight of their normal litter mates (the ratios in the different litters ranged from 58–90%) in females and from 53–86% in males). The grizzled mice grew relatively a little faster than their normal litter mates in the first 2 weeks, the females reaching about 75–80% of normal weight and the males about 90–95%. These weight differences persisted up to 6 weeks when the weighings were discontinued. (One litter was weighed up to 9 weeks with no further change.)

Table 4. *Post-natal growth. Weights of gr gr as proportion of weights of +gr litter mates*

Age, weeks	Weight ratio of <i>gr gr</i> to <i>+ gr</i>	
	Females	Males
Birth	0.76	0.77
1	0.73	0.86
2	0.81	0.93
3	0.75	0.90
4	0.79	0.94
5	0.80	0.95
6	0.84	0.92

5. MORTALITY

(i) *Post-natal mortality*

The mortality of young mice in the litters from the recording of the births up to the time of classification of grizzled is given in Table 5. These are the same matings and litters that provided the segregation data, so the numbers of mice surviving are as given in Table 2. The matings are arranged in Table 5 in order of increasing proportion of grizzled homozygotes expected. The matings of *+ + ♀ × gr gr ♂* are not strictly comparable with the rest, because most of the females were from highly inbred strains. If these matings are excluded, the mortality increases regularly with the increasing proportion of *gr gr* in the litters; and, except for the intercrosses, the litters expected to include *gr gr* homozygotes have a significantly higher mortality than those without *gr gr*.

The relative viability of *gr gr* homozygotes in the post-natal period can be calculated by a comparison of the matings *gr gr ♀ × gr gr ♂* and *gr gr ♀ × + + ♂*. (Segregating matings cannot be used because the proportion of *gr gr* actually present at birth is unknown.) The relative viability is given by the ratio of the proportion surviving in the two sorts of mating. The viability of both sexes together is thus $v = 0.8771/0.9806 = 0.894$. This is considerably higher than the overall relative viability calculated from the segregation, which was 0.567 averaged

Table 5. *Post-natal mortality, up to the time of classification. The numbers surviving are the same as the numbers classified, as given in Table 2. The 95% confidence limits to the mortality are calculated from Fisher & Yates (1963), Table VIII₁*

Type of mating		Number dead			Mortality %			
		♀	♂	sex ?	♀	♂	All	(95% confidence limits)
♀ <i>gr gr</i>	♂ ++	4	1	1	2.56	0.65	1.94	(0.7- 4.2)
++	♀ <i>gr gr</i>	2	3	0	2.63	4.35	3.45	(1.1- 7.9)
+ <i>gr</i>	+ <i>gr</i>	14	16	3	2.25	2.30	2.50	(1.7- 3.5)
<i>gr gr</i>	+ <i>gr</i>	9	13	8	6.98	9.29	10.83	(7.4-15.2)
+ <i>gr</i>	<i>gr gr</i>	14	9	5	7.82	7.32	9.12	(6.1-13.0)
<i>gr gr</i>	<i>gr gr</i>	11	19	6	6.29	16.96	12.29	(8.7-16.7)

between the sexes. The recorded post-natal mortality is therefore not enough to account for the whole reduction of viability and some further mortality before or soon after birth must be looked for.

(ii) *Pre- and peri-natal mortality*

The combined effects of foetal mortality and mortality at or soon after birth can be found in a reduction of average litter size according to the proportion of *gr gr* homozygotes expected in the litters. The average numbers of live young at recording are given in Table 6. Matings were not usually examined daily for litters, so the ages of the litters when recorded ranged up to 4 days and in a few cases up to 6 days. The mean litter sizes are based on variable numbers of litters per mating but litters after the sixth were not included. At the foot of the table, under the heading of 'changed males', are the litter sizes of 12 *gr gr* females which were first mated to ++ males and then remated after their second or third litters to + *gr* males. Litters of these females by the first males are included with the other matings of the same type in the first part of the table, but their litters by the second males are not so included because they were later litters.

Table 6. *Mean litter sizes*

Type of mating		No. of litters	Mean ± s.e.	Viability of <i>gr gr</i>
♀	♂			
++	<i>gr gr</i>	23	7.48 ± 0.47	—
+ <i>gr</i>	+ <i>gr</i>	169	7.15 ± 0.17	—
+ <i>gr</i>	<i>gr gr</i>	46	7.02 ± 0.37	—
<i>gr gr</i>	++	46	6.78 ± 0.36	—
<i>gr gr</i>	+ <i>gr</i>	14	5.29 ± 0.62	0.44 ± 0.20
<i>gr gr</i>	<i>gr gr</i>	71	5.92 ± 0.26	0.87 ± 0.06
(Changed males)				
<i>gr gr</i>	{ ++	26	7.27 ± 0.42	—
	{ + <i>gr</i>	40	4.80 ± 0.34	0.32 ± 0.38

The mean litter sizes, with one exception, show a progressive decline as the proportion of *gr gr* in the litters increases. The differences, however, are rather small in relation to the standard errors and the results by themselves are hardly conclusive. Valid comparisons can be made only between females of the same genotype because, as already pointed out, the + + × *gr gr* matings were of inbred females and because grizzled females, being smaller in body size, would be expected to have smaller litters than +*gr* females. Three estimates of the relative viability of grizzled homozygotes can, however, be calculated from the litters with *gr gr* mothers: these are given in the table. The reduction of litter size in matings of *gr gr* ♀ × *gr gr* ♂ compared with *gr gr* ♀ × + + ♂ leads to an estimate of 0.87 for the relative viability. Matings of *gr gr* ♀ × + *gr* ♂, however, yield much lower estimates: 0.44 in the first set and 0.32 in the matings with changed males. It seems doubtful if this is a real difference of differential viability, especially since no similar difference was seen in the overall differential viabilities calculated from the segregation data. The greater reduction of litter size in *gr gr* ♀ × + *gr* ♂ matings could have resulted from a higher mortality of +*gr* offspring. The differential viability is calculated on the assumption that +*gr* offspring have the same mortality in all types of mating.

The reduced litter sizes, though not fully conclusive, support the supposition of increased pre- or peri-natal mortality. Direct evidence of pre-natal mortality was obtained from dissections.

(iii) Embryonic mortality

Direct evidence of the death of grizzled foetuses was obtained from the dissection of pregnant females. The dissections were made at 16–18 days of gestation. There were three groups of dissected females: (1) + *gr* ♀♀ mated to + + ♂♂ to serve as controls, with no *gr gr* among the embryos; (2) + *gr* ♀♀ mated to *gr gr* ♂♂ to give 50% of *gr gr* embryos; and (3) *gr gr* ♀♀ mated to *gr gr* ♂♂ to give all *gr gr* embryos.

Table 7. Numbers of live and dead embryos in dissected females

Type of mating		Age of embryos (days)	No. of females	Dead embryos		Live embryos		Total No. of implants
♀	♂			Early	Late	Abnormal	Normal	
+ <i>gr</i>	+ +	18	6	2	0	0	53	55
+ <i>gr</i>	<i>gr gr</i>	16	16	10	8	6	129	153
		17	17	9	6	17	129	161
		18	14	12	12	2	106	132
		All	47	31	26	25	364	446
<i>gr gr</i>	<i>gr gr</i>	16	13	5	6	2	92	105
		17	13	13	12	0	81	106
		18	13	4	8	0	95	107
		All	39	22	26	2	268	318

Dead embryos were classified as 'early' or 'late' deaths according to whether they were represented by moles or by recognizable embryos with placentae, i.e. whether they died before or after about 10 days. Some of the living embryos showed external abnormalities and the numbers of these abnormal embryos were recorded. The abnormalities were rather varied. The most frequent were in the shape of the head—shortening of the snout and swellings in the optic and occipital regions. No similar abnormalities were seen after birth and, though the abnormalities did not seem severe enough to be the immediate cause of death, it must be concluded that the embryos recorded as abnormal would have died before or immediately after birth. They are therefore to be included in the total foetal mortality. The results are given in Table 7, where the dissections on different days of gestation are shown separately. The mortality in the three groups of females is summarized in Table 8. Corpora lutea were not counted, so no direct estimate of preimplantation mortality was obtained. The average number of implantations per female, however, was lower in *gr gr* females than in the other groups. Though this could have been due to a higher preimplantation mortality, the cause seems more likely to have been a lower ovulation rate which would be expected from the smaller body size of *gr gr* ♀♀.

Table 8. *Foetal mortality, %*, with (in brackets) 95% confidence limits, calculated for Fisher & Yates (1963) Table VIII₁

Mating		Early deaths	Late deaths	Abnormal embryos	Late deaths + abnormal embryos
♀	♂				
+ <i>gr</i>	+ +	3.64 (0.4-12.5)	0 (0 - 6.7)	0 (0 - 6.7)	0 (0 - 6.7)
+ <i>gr</i>	<i>gr gr</i>	6.95 (4.7- 9.8)	6.27 (4.1- 9.1)	6.43 (4.1-9.4)	12.30 (9.1-15.9)
<i>gr gr</i>	<i>gr gr</i>	6.92 (4.3-10.3)	8.78 (5.6-12.7)	0.74 (0.1-2.7)	9.46 (6.3-13.4)

The mortality of implanted embryos was higher at all stages among litters expected to contain *gr gr* embryos than among the control series. The early deaths, however, are not significantly different and the early mortality in the grizzled series is not above what is normally found in non-inbred mouse stocks. (See, for example, McLaren & Michie, 1959; Lyon, Phillips & Searle, 1964; McCarthy, 1965.) The higher frequency of late deaths and abnormal embryos in litters expected to contain *gr gr* embryos than in the control series is clearly significant, particularly when the late deaths and abnormal embryos are added together. This provides proof of the foetal mortality of some grizzled homozygotes and shows that the mortality occurs at all stages from about 10 days to after 18 days of gestation.

There is, however, an anomalous difference between the two series of dissections with *gr gr* homozygotes among the embryos, for which there is no obvious explanation. The mortality in the litters from *gr gr* mothers should be about twice as great as that in litters from +*gr* mothers, in which only half the embryos are expected to be *gr gr*. If the late deaths together with the abnormal embryos a

taken to represent the mortality of *gr gr* embryos then this is in fact lower in *gr gr* than in *+gr* mothers. The difference is highly significant when tested against the expectation that the mortality in *gr gr* mothers should be twice that in *+gr* mothers ($\chi^2_{(1)} = 20.0$; $P < 0.001$). The difference between the two series in the numbers of late deaths agrees well with expectation ($\chi^2_{(1)} = 1.8$), so the discrepancy arises entirely from the number of abnormal embryos which is much lower in *gr gr* than in *+gr* mothers. The segregation data showed no difference between *gr gr* and *+gr* mothers in the relative viability of *gr gr* homozygotes up to the time of classification, which suggests that the mortality was taking place later in litters of *gr gr* mothers and escaped detection in the dissections.

Though there is this unresolved anomaly, the results of the dissections prove conclusively that the differential mortality of *gr gr* homozygotes extends back into the pre-natal period, starting at about 10 days of gestation.

6. CONCLUSION

The observations on growth and mortality show that the grizzled gene has more far-reaching effects than are evident in the dilution of yellow pigment or the occasional kinky tail. The extended period over which mortality occurs shows that the defect is not primarily associated with a developmental event, which would be expected to lead to death at a particular stage. The fact that the reduction of body weight is greatest at birth and that post-natal growth rate is not reduced suggests that the defect or disadvantage from which grizzled homozygotes suffer acts mainly in the pre-natal period.

SUMMARY

Grizzled (*gr*) is a recessive gene with complete penetrance but reduced viability. It is linked with waltzer (*v*) in linkage group X, the recombination frequency being $14.6 \pm 2.6\%$. Its visible effect is to dilute the yellow pigment of the hairs. Body weight is reduced by about 25% at birth and remains at about 10–20% below that of non-grizzled litter mates. Viability up to the time of classification is about 50–60% of that of non-grizzled litter mates. The mortality of grizzled homozygotes occurs at all stages from about 10 days of gestation up to the time of classification. No specific cause of the mortality was evident.

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