Genet. Res., Camb. (1976), **72**, *pp.* 3–10
Printed in Great Britain

Mapping an invisible lethal in the mouse

By MARGARET E. WALLACE
Department of Genetics, University of Cambridge

(*Received* 14 *July* 1975)

SUMMARY

An effective breeding policy is described for detecting linkage in the mouse, for an ante-natal lethal from crosses of heterozygotes with stocks homozygous for several linked recessive markers. A method of analysing the ensuing data is described, together with a method of estimating the map distance to close markers. A practical illustration is described, involving an ante-natal lethal obtained from a colony of wild mice and a four fold recessive chromosome 2 marker stock.

1. INTRODUCTION

Apart from the deletion in chromosome 2 (Wallace, 1972), no completely recessive antenatal lethal which is not an allele of an existing mutant has been mapped in the mouse without knowledge of the foetal phenotype. A possible exception is a^z (Russell, McDaniel & Woodiel, 1963).

Where the lethal's phenotype is known at the outset, as in congenital hydrocephalus, *ch* (Grüneberg, 1953), amputated, *am*, and pupoid foetus, *pf* (Meredith, 1964*a*; Robinson, 1972, Part B), males heterozygous for the lethal may be identified by mating them to several putatively heterozygous females. Their progeny are then examined by dissection of the females; alternatively the females are made to litter down in grid-bottomed cages through which newborn offspring fall, and mutilation by the mother is thus avoided (Meredith, 1964*b*). Then the mapping is done by identification of non-crossover and crossover chromosomes in the male only.

The genotype of the male's chromosome is easily discerned if he carries the lethal; however, it is less easily discerned if he does not, for non-segregation can be due only to the chance choice of 'tester' females which do not happen to carry the lethal. The method can be tedious where, to obviate misclassification, a large number of 'tester' females for each male is used. It is also tedious if, before mapping can be started, the dissection of a large number of females has to be used merely to identify the phenotype of the lethal foetus and to discern the age at which it is best classified; this may well occur where the existence of a lethal is first suspected from heterogeneity of litter size.

It seems worthwhile, therefore, to consider lethal-mapping without dissection, i.e. from segregation of putatively linked markers only, and appropriate statistical analysis. The exercise is feasible in the case where diminished litter size first indicates the lethal; and also when the first observation is disturbance of ratios for a segregating marker and a closely linked lethal is suspected. It is the method of

choice where the lethal dies at a very early foetal age and certainty of identification by dissection cannot be guaranteed.

Many mouse mutants are good models for medical and veterinary studies. The dearth of antenatal lethal ones can only be surmised to be due to difficulties in methods involving dissection or grid-bottomed cages, and in difficulties of maintenance. A method which indicates linked markers is useful both in identification and in maintenance.

In practice the various methods can often be combined. This paper presents the linked marker method only. It has not been used in the mouse as yet, presumably because of the small progeny size of this species compared with that of other laboratory organisms, or because so many putative mouse marker mutants suffer impairment of viability or of penetrance. It is presented with an illustrative example which shows how the difficulties may be compensated for or taken into account.

2. THEORETICAL CONSIDERATIONS

The first step towards location is to mate a known heterozygote to a well-marked stock. For a particular chromosome there may be only recessive markers; in this case, the existence of linkage will be discerned in the next generation (intercrosses in repulsion) from the single-factor ratios observed for the linked markers: they will be between 3:1 and 2:1 depending on the closeness of linkage. The F_2 should be as large as possible to increase the chance, in the case of loose linkage, of discriminating between the 3:1 expected for independence and the likely $< 3:1$ observed.

The smallest number of F_2 progeny needed to distinguish at the 5% level of probability every possible observation from either 2:1 or 3:1 is approximately 480 (from Mather 1963, pp. 30–31). The number required in order to be sure, at the same level, that an observed 2:1 does not fit a 3:1 expectation, is about 108. This will be called 'a working minimum number' and it refers to the outcome most easily managed, namely when the lethal is close to its marker.

Where the marker is dominant, detection of linkage from the F_2 segregation involves discrimination between ratios deviating much more from each other, so there is no need to follow up the theoretical considerations for this situation. The recessive situation only will be considered further.

When a deviation from 3:1 in the right direction is observed, the question arises: What is the best breeding policy? Further breeding should have the minimal aim of ensuring continued segregation of the lethal, and the second (probably overlapping) aim of making those types of mating which make accurate linkage and mapping estimates possible.

The minimal aim would clearly be achieved with new putative intercrosses from the F_2, particularly if two recessive markers segregated with the lethal locus between them – although some matings would not segregate in anything. But the second aim in not efficiently achieved from intercross matings in repulsion, owing to the poor provision of information on crossing-over – backcross segregations are more informative (see Robinson, Part A, 1972, figure 1, p. 14). These can be provided

by mating F_2 crossovers (in terms of markers); sufficient of these can be provided by putting up the requisite number of intercrosses – a number determined by using the known marker crossover values. The question then arises: Is it better to mate like phenotypes, or unlike ones, or both? To find the answer consider the simplest situation:

Situation A

An adjacent pair of recessive closely linked markers, *a* and *b*, with lethal locus, *u*, between them.

The segregating intercross matings will be:

$$\frac{A\ u\ B}{a\ U\ b} \times \frac{A\ u\ B}{a\ U\ b},$$

where *A*-*u* is segment 1 and *u*-*B* is segment 2. The most frequent crossover events will occur in one mate only and will be a crossover in segment 1 only and a crossover in segment 2 only. The viable genotypes, phenotypically recombinant, will be:

recombination in segment 1 only: $\frac{A\ U\ b}{a\ U\ b}$ and $\frac{a\ u\ B}{a\ U\ b}$ in equal numbers.

recombination in segment 2 only: $\frac{A\ u\ b}{a\ U\ b}$ and $\frac{a\ U\ B}{a\ U\ b}$ in equal numbers.

Since the phenotypes which carry *u* and those which do not are indistinguishable, matings between phenotypes alike for their markers, and matings between unlikes, will be at random with respect to *u*; hence, if *u* is exactly in the middle:

$\frac{1}{4}$ of matings between likes will segregate in *u*,

$\frac{1}{4}$ of matings between unlikes will segregate in *u*.

The segregating like matings will do so in respect of only one marker, which will be intercrossed, while the segregating unlike matings will do so for both markers, which will be reciprocal backcrosses.

For the second aim above, the mating of unlikes is more informative than the mating of likes. But if *u* is nearer one marker than the other, say nearer to *A*, hardly any of the unlike matings will segregate owing to the rarity of the appropriate crossovers, here *A*/*u*, and both aims will be frustrated; so the mating of likes is desirable.

Since the whereabouts of *u* is unknown, the plan which will best cover all contingencies is to mate both likes and unlikes. While *u*'s whereabouts remain unknown, the most useful proportion of likes to unlike matings cannot be determined. For a particular crossover phenotype, then, as it reaches breeding age, the best plan is to mate it to a like or to an unlike, whichever is nearer in age: thus it will have the best chance of breeding sufficient progeny to discern the segregation of *u* when *u* does segregate.

This policy can be modified as data accrue from the first few matings of crossovers. For example, if *u* is nearer to *A* than to *b*, those like matings which segregate for *b* will tend to segregate in *u* also, and the like matings segregating for *a* will tend not to segregate in *u*; then the mating of likes of the productive kind will be

indicated as the best policy. Similarly, if the matings of both kinds of unlikes tend to segregate in u, then u is near the middle, and the policy will become that of mating unlikes rather than likes.

However, u may not be between the two adjacent markers. Will its being outside alter the above statement of policy? Consider this situation, taking one of the two possible outside locations of u:

Situation B

An adjacent pair of recessive closely linked markers, a and b, with lethal locus, u, close to the left of a.

The segregating intercross generation will be:

$$\frac{u\ A\ B}{U\ a\ b} \times \frac{u\ A\ B}{U\ a\ b},$$

where u-A is segment 1 and A-B is segment 2. The most frequent crossover event causing a recombinant phenotype in terms of markers will be a crossover in segment 2 only. The viable genotypes will be:

$$\frac{U\ a\ B}{U\ a\ b} \quad \text{and} \quad \frac{u\ A\ b}{U\ a\ b} \quad \text{in equal numbers.}$$

Clearly, the matings of unlike phenotypes will not segregate in u, so the policy should be to mate likes. Of these, one kind (here $uAb/Uab \times uAb/Uab$) will segregate in u, and not the other. When a particular like does segregate, then, the policy is modified towards favouring that particular like: this will keep U segregating and indicate a position of u nearer to the segregating marker (here A).

However, if u is so close to A that recombination u/A never occurs, this policy will leave one in doubt as to whether u is to the left of A or to the right.

In short, if u is very close to one of its markers, its position (to left or right) may remain uncertain. (However this is not a problem, since the main interest then shifts to continued maintenance, which is easily achieved by mating those likes which segregate in u.) If u is roughly in the middle of two markers, then this will be indicated by the fact that unlike matings segregate.

Hence, at the outset of the breeding programme, the best policy is to mate likes of both kinds in equal numbers, and also to mate unlikes. According to the genotypes of mating which segregate in u, the position of u will become clear and emphasis can then be placed on making matings of the type which most frequently do segregate in u.

If accurate mapping of u, i.e. an estimate of its exact position, is required as well as simply its location relevant to the markers (inside or outside), this may be obtained by considering the proportions of each type of mating involving crossovers, which do, and which do not, segregate in u.

Consideration of a third situation, where three linked markers are used and the lethal is enclosed by a pair of them, is of academic interest: it leads to the conclusions that (i) matings both of likes and of unlikes are desirable, and (ii) the matings are

most informative when the mates are either alike, or complementary, at all three loci.

However, in analysing actual data, it is simpler to consider two loci at a time, as in situations A and B. Indeed, in practice, the more loci involved and the more closely they are linked, the longer will any given animal wait for a mate (since a mate must qualify in so many ways); so that it is better not to aim at this level of sophistication. The practical policy may be stated: mate a given animal to another near in age, which is alike, or unlike, for at least two marker loci, and for more if available.

Consideration of this situation also leads to the conclusion, which intuitively seems right in view of the considerations of situations A and B, that while one may be in doubt as to whether the lethal is outside or inside a well marked segment, one can, more surely than with fewer loci, decide at which end it is. Similarly, one may be in doubt as to which side of a central marker it may be; but this is not a practical problem, since the ensuing data will have disclosed the most useful markers for maintenance, and for eventual use by an investigation wanting the lethal as a veterinary or medical model.

To illustrate the working of the above theory in practice, there follows an account of the location of an actual lethal, using four closely linked markers, mainly recessives, on chromosome 2.

3. A PRACTICAL ILLUSTRATION

Origin of lethal

The trapping of wild mice from a particular location in Peru, and subsequent breeding over several generations, led to the disclosure of an unprecedented incidence of mutants carried by the wild mice (Wallace, 1971). A further trapping and similar breeding led to the disclosure of an even greater incidence in the same population (Wallace, in preparation). The existence of one of the mutants in the second trapping became manifest on outcrossing a wild (agouti) mouse to a stock homozygous for several unlinked markers, and intercrossing the F_1. Only one single-factor segregation appeared disturbed in the F_2, namely that of agouti:non-agouti. The observations (75:45) were significantly deviant from the 3:1 expectation pertinent to all previous work with these alleles ($\chi^2 = 10{\cdot}00$ for 1 d.f., $P < 0{\cdot}01$); they were also close to the 2:1 pertinent to lethality of the agouti homozygote, *AA* ($\chi^2 = 0{\cdot}94$ for 1 d.f., $P > 0{\cdot}3$). Thus it seemed reasonable to postulate a lethal close to the agouti locus.

The number of deaths in litter, before classification of agouti, was insufficient to postulate a post-natal lethal. No work has been done to discover the ante-natal stage at which the lethal dies. It will be symbolized *z*.

Tests of linkage were first done with ragged, *Ra*. This is at the relatively sparsely marked end of chromosome 2 (see Green, 1974) and recombines with the *A* locus with a value of about 22% (Robinson, 1972, Part B, p. 170). But no matings with the required disturbance of the ragged:non-ragged segregation were identified, and it was concluded that *z* is central in the chromosome.

Linkage intercrosses

The best marker stock available for testing a central location was homozygous for the agouti allele tan-belly, a^t, and undulated, *un*, wellhaarig, *we*, and pallid, *pa*, whose recombination values (in percentages) are:

	$a^t - un$	$un - we$	$we - pa$
♀ heterozygotes	4·67 ± 0·04	7·39 ± 0·07	4·20 ± 0·04
♂ heterozygotes	4·60 ± 0·04	4·44 ± 0·04	2·24 ± 0·02

(Fisher & Landauer 1953).

Crosses of wild carriers of the lethal were made with this stock, and 20 intercrosses made from the F_1. The F_1 were very vigorous and fertile, some females producing over 120 progeny.

Crossover F_2 phenotypes could not usefully be mated until the intercrosses segregating in *z* could be identified. Fortunately, with this particular marker stock, use could be made of the lack of dominance of *A* over a^t for identifying the segregation of *z* before the matings had bred the working minimum number 108.

Table 1. *Segregation at the agouti locus in the first ten linkage intercrosses*

Mating no.	Agouti genotypes				χ^2 testing	
	AA	Aa^t	a^ta^t	Total	1:3:1*	d.f.
1	0	20	10	30	8·95	2
2	0	30	3	33	13·81	2
3	11	23	9	43	0·98	2
4	5	17	3	25	1·06	2
5	6	19	5	30	0·23	2
6	3	25	12	40	5·17	2
7	7	24	10	41	0·59	2
8	13	21	12	46	4·00	2
9	18	17	6	41	14·64	2
10	9	14	5	28	0·56	2
Totals	77	222	77	376	49·99	20
Subtract deviation χ^2:					−0·14	−1
Heterogeneity χ^2					49·85	19

* This ratio is an approximation to the observed totals, and is not an error for 1:2:1.

When 25 or more progeny had been bred, from each of the first 10 matings, the segregation of the *A* locus was examined (Table 1). The data are clearly heterogeneous (probability of homogeneity is $< 0{\cdot}001$), and those matings which show a paucity of *AA* are 1, 2, and 6. Crossover phenotypes from these three matings appearing to segregate in *z* were then mated, to like or unlike mates according to age and to what were available. This process continued for further crossovers from these intercrosses, and from another similarly identified, until about 30 pairs had been mated.

Difficulties from recessiveness and inviability

The loss of information for completely recessive markers as against those with no dominance, is best illustrated by two further treatments of the data from the intercross generation.

First, when the four intercrosses segregating in *z* were infertile, the segregations of all intercrosses (21 in number) in terms of the undulated locus only were examined for heterogeneity to see whether this procedure would show up the same four matings as carrying *z*. The heterogeneity χ^2 on these data (not shown) was 20·61 for 19 d.f., $P > 0{\cdot}3$, and no individual χ^2 showed there to be a significant deviation from 3:1. This was despite the fact that a total of 1602 progeny were involved (an average of 80 progeny per mating), and, as shown below, the linkage of *z* to *un* is probably closer than its linkage to *A*.

Secondly, when they had become infertile, the total output of the four matings identified as segregating in *z* were tabulated in terms of all the single-factor ratios, combining the *AA* and Aa^t genotypes. The totals were:

$A:a^t$	$+:un$	$+:we$	$+:pa$	Total
192:56	187:61	190:58	189:59	248

With *z* close to the *un* locus, all four single-factor ratios are expected to be closer to 2:1 than to 3:1. However, they are clearly nearer to 3:1 and some are not far from 4:1. This is due to the high post-natal mortality of the fourfold recessive combination, before full classification, in the context of large litter size and large normal litter-mates. The $A:a^t$ ratio is affected, not because a^ta^t is inviable, but because it is linked to the other three relatively inviable recessives. It is worth noticing also that, due to the differing viabilities of each recessive, and to chance, the observed ratios do not show a gradient in magnitude of deviation from 2:1 as markers further from *z* are considered. Indeed, if inviability is ignored, it appears that *un* is the closest, *pa* the next close, *we* the next and *A* the furthest away – which is incompatible with the known order of the markers.

Matings between F_2 *crossovers*

The viability of the recessives from these matings improved, but not all of them bred enough to specify with a fair degree of certainty all chromosomes in terms of *z* genotype. However, 20 matings bred enough, identifying 20 fully specifiable recombinant chromosomes. This was carried out by considering the segregations of pairs of adjacent markers. The data are too bulky for presentation in full, but it suffices to say that, for *un* and *we* and for *we* and *pa*, there were only two matings of unlikes, of which one (for the *un-we* segment) segregated, whereas there were three matings unlike for a^t and *un*, all of which segregated; this places *z* in the latter segment.

The genotypes of the 20 chromosomes, as discerned from the segregations of a^t and *un*, are given below, so that the proportions of the different genotypes of crossover can be discerned:

https://doi.org/10.1017/S0016672300016189 Published online by Cambridge University Press

Chromosome types	a^t++	$a^tz\ un$	a^tz+	$A+un$
Observed number	7	1	4	8
Segments where crossovers occurred in F_1 parent (segment 1 is a^t-z, and segment 2 is $z-un$)	2	1 and 2	1	1

It may easily be verified that orders *z-A-un* and *A-un-z* require respectively 12 and 7 of the 20 chromosome genotypes to be double crossovers, which effectively shows their improbability. It will be noticed that the acceptable order *A-z-un* requires one chromosome to be a double crossover, and that it is 'non-recombinant' in terms of the markers. It may be wondered how such a chromosome came to be tested; it is in fact the right hand chromosome of one of the following F_1 pairs: $A\ z+/a^t+un \times A\ z+/a^t+un$ and is thus the 'non-recombinant' chromosome of the F_2 crossover genotype: $A+un/a^tz\ un$; and so it arises by a double crossover.

The recombinational events in segment 1, a^t-*z*, are now $8+4+1=13$, and the recombinational events in segment 2, *z-un*, are now $7+1=8$, each out of 20 chromosomes. The segment a^t-*un*, which may be taken as having a 4·6 % recombination value (a compromise between the two figures published, one for the female heterozygous parent and one for the male), must therefore be divided into a portion $13/20 \times 4{\cdot}6\,\%$ and $8/20 \times 4{\cdot}6\,\%$, which gives approximately 2·8 % for a^t-*z* and 1·8 % for *z-un*.

REFERENCES

FISHER, R. A. & LANDAUER, W. (1953). Sex differences of crossing-over in close linkage. *American Naturalist* **87**, 116.

GREEN, M. C. (1974). Linkage Map of the Mouse. *Mouse News Letter* **51**, 7.

GRUNEBERG, H. (1953). Genetical studies on the skeleton of the mouse. VII. Congenital hydrocephalus. *Journal of Genetics* **51**, 327–358.

MATHER, K. (1963). *The Measurement of Linkage in Heredity*, 2nd edn. London: Methuen.

MEREDITH, R. (1964*a*). *Mouse News Letter* **31**, 25.

MEREDITH, R. (1964*b*). Detection and care of mouse mutants. *Journal of Animal Technicians Association* **15**, 22–27.

ROBINSON, R. (1972). *Gene Mapping in Laboratory Mammals*, Part B, pp. 358–359. London and New York: Plenum Press.

ROBINSON, R. (1972). *Gene Mapping in Laboratory Mammals*, Part A, p. 14. London and New York: Plenum Press.

RUSSELL, L. B., MCDANIEL, M. N. C. & WOODIEL, F. N. (1963). Crossing-over within the 'a' locus of the mouse. *Genetics* **48**, 907.

WALLACE, M. E. (1971). An unprecedented number of mutants in a colony of wild mice. *Environmental Pollution* **1**, 175–184.

WALLACE, M. E. (1972). Reduction in recombination due to a deletion in a wild colony of mice. *Journal of Heredity* **63**, 297–300.