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Unusual recombination values and the mapping of the lethal miniature in the house mouse

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SUMMARY

Data are presented from a balanced backcross experiment involving three linkage group VI markers in the house mouse. These establish the order *N–Ca–bt* (naked–caracul–belted), and confirm the unusual recombination values reported earlier; the values for male heterozygotes are about 4 times those for the female ones. The linkage group is extended by the linkage of miniature, *mn*, with *N* and *Ca*, here reported. Data are given which establish the order *mn–N–Ca–bt*; they indicate that the recombination values for males may be greater than those for females for some way to the left of the *N–Ca–bt* segment.

1. INTRODUCTION

Linkage group VI has been until recently one of the smaller groups in the house mouse; nineteen of a possible twenty groups are now established in this species. This paper presents: (i) Complete data from a balanced linkage backcross experiment for the markers – naked, *N*; caracul, *Ca*; and belted, *bt*; for which provisional figures have been published (Mallyon, 1951). (ii) New (unpublished) data for linkage between these two dominants and miniature, *mn* (Bennett, 1961).

The order of the main markers for the linkage map for group VI is: *N*–2 %–*Ca*–8 %–*bt*–40 %–*uw*, where *uw* is underwhite (Dickie, 1968). Miniature lies to the left of the two dominants, and has a recombination value with them of about 24 %; the linkage thus considerably extends the length mapped for this group. The bulk of the breeding work was done by the late S. A. Mallyon, and the analysis mainly by M. E. Wallace.

2. MATERIALS AND METHODS

Belted, *bt*, is a spotting gene with imperfect penetrance. For accurate linkage work therefore, caracul *Ca* (a hair waving gene) and naked, *N* (which causes large bare areas to appear after the first moult), were backcrossed several times to a selected stock of belted with strong expression and 100 % penetrance. The four types of heterozygote were made up and backcrossed to the belted triple recessive, over 2000 progeny being raised from the total of female heterozygotes, and another 2000 from the male heterozygotes. This design of experiment (Fisher & Bailey, 1949) was used, and this large number was obtained, in view of the remarkable difference in recombination between the sexes earlier reported (Mallyon, 1951); there the male values for the two adjacent segments *N–Ca* and *Ca–bt* exceeded the female, in

contrast to the situation for practically all other segments in the mouse, where there is either no sex difference or the female value exceeds the male (Green, 1966). It was desired to have decisive confirmation of these values.

In an attempt to find a linkage for the gene miniature, *mn*, (Bennett, 1961), a known heterozygote for *mn* was crossed with animals mutant at the nine independent loci *Re* (VII), *T* (IX), *N* (VI), *Mi*^{wh} (XI), *s* (III), *b* (VIII), *sh*-1 (I), *se* (II) and *a* (V). (Bracketed numbers are linkage groups.) F1 were then intercrossed, their progeny segregating for ten markers when sex is included. Miniature is usually lethal shortly after birth, few living to 3 months, so that death occurs mainly before other markers can be classified. No homozygotes have been fertile; the gene is, however, fully penetrant. For these reasons, evidence for linkage had to be based upon intercross data in which, due to lethality of *mnmn*, a marker linked in repulsion segregated in excess of the Mendelian expectation; this excess is clearly associated with the segregation of *mnmn* only if some of the latter are viable at the time of classification of the marker. Of the ten markers used, naked, *N*, segregated in excess in some of the intercrosses, practically no *mnmn* surviving until *N* was classifiable. Linkage with *mn* was suggested (Wallace, 1963).

In order to investigate the relation between *mn* and the other three linkage group VI markers, *mn* was introduced into the belted stock. However, it proved extremely difficult to select for both penetrance of belted and viability of miniature, so the latter aim was given priority. Belted became too difficult to classify with certainty and none of the single intercross data obtained on its joint segregation with *mn*, *N* and *Ca* is worth presenting. However, indirect evidence on the position of *mn* in relation to *Ca* and *bt* is discussed below; it concerns the genotypes of four female offspring of the first male heterozygous for *mn*, *Ca* and *bt*. These females were noticed, from their progeny segregation, to be recombinant for *Ca–mn* and/or *Ca–bt* and so able to throw light on gene order.

To improve the viability of *mnmn* and to accumulate at the same time putative linkage data, a closed maintenance colony was established consisting of successive matings between caraculs and normals, and between caraculs and nakeds, choosing in every generation the sibs of the more viable miniatures. The first double heterozygotes after the introduction of *mn* would be of genotype *Ca* + / + *mn* or *N* + / + *mn*; after a crossover they would be *Camn*/ + + or *Nmn*/ + +, and the colony would consist of alternating runs of coupling and repulsion single intercrosses.

It should be noted that in these circumstances it is not possible to make up one set of heterozygotes with markers and *mn* foreknown to be in coupling, and another set with markers and *mn* foreknown to be in repulsion. Linkage phase of heterozygotes can be deduced only from their progeny segregations.

3. ANALYSIS OF RESULTS

1. *Data for* N, Ca *and* bt

The data from the balanced linkage backcross experiment are given in table 1, and confirm beyond doubt the unusual recombination values indicated earlier

Table 1. *Balanced linkage backcross data for* N, Ca *and* bt

Genotype of heterozygote	Female heterozygotes' progeny: Mode of gamete formation* 0	1	2	1, 2	Total	Male heterozygotes' progeny: Mode of gamete formation* 0	1	2	1, 2	Total
$\frac{N\ Ca\ +}{+\ +\ bt}$	478	0	17	0	495	449	12	60	0	521
$\frac{N\ Ca\ bt}{+\ +\ +}$	492	5	22	0	519	444	5	72	1	522
$\frac{N\ +\ bt}{+\ Ca\ +}$	480	3	25	0	508	438	13	49	3	503
$\frac{+\ +\ b}{+\ Ca\ bt}$	491	2	13	0	506	462	13	45	0	520
Totals	1941	10	77	0	2028	1793	43	226	4	2066

*Mode 0 = no recombination.
Mode 1 = recombination in segment 1 (*N–Ca*) and not in segment 2 (*Ca–bt*).
Mode 2 = recombination in segment 2 (*Ca–bt*) and not in segment 1 (*N–Ca*).
Mode 1,2 = recombination in segments 1 and 2 simultaneously.

	Recombination values (%): Female heterozygotes	Male heterozygotes
N–Ca	0·4931 ± 0·1555	2·2749 ± 0·3280
Ca–bt	3·7968 ± 0·4244	11·1326 ± 0·6924
N–bt	4·2899 ± 0·4500	13·0203 ± 0·7404
Kosambi value	0·00	2·94

(Mallyon, 1951), namely that the male values significantly exceed the female, male values being about 4 times the female ones.

The Kosambi values (also given earlier, Mallyon 1962) are recorded here as such values are a routine way of measuring the amount of interference in a chromosomal segment (Kosambi, 1944). However, in the present case they are discrepant as between males and females, probably due to the closeness of the *N–Ca* value in the females, and give no idea of the likely position of the centromere, in contrast to their use in other segments (Owen, 1950, p. 152; Wallace, 1958, p. 245).

2. *Data for* N, Ca *and* mn

Confirmation that *mn* is in linkage group VI comes from a consideration of the proportion of matings in which *mn* segregated, out of all the matings in the maintenance colony. If from intercrosses $+/mn \times +/mn$, non-*mn* are mated, only in $(2/3)^2$ of such matings will both mates be $+/mn$ and so able to segregate. The maintenance régime above selects as mates those non-*mn* which are also caracul or naked. If these markers are linked with the *mn* locus, the régime will increase this fraction; it

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Table 2. *Single backcross data for* Ca *and* mn, *and for* N *and* mn

Heterozygotes mated			*Ca*+,+*mn* × ++,+*mn* Phenotypes of progeny					Heterozygotes mated			*N*+,+*mn* × ++,+*mn* Phenotypes of progeny				
Phase	No.	Sex	*Ca*,+	*Ca*,*mn*	+,+	+,*mn*	Total	Phase	No.	Sex	*N*,+	*N*,*mn*	+,+	+,*mn*	Total
C	11	♀	386	38	251	126	801	C	4	♀	74	7	59	22	162
R	4	♀	59	26	97	13	195	R	1	♀	7	3	15	1	26
Total	15	♀	445	64	348	139	996	Total	5	♀	81	10	74	23	188
C	6	♂	284	25	187	76	572	C	0	♂					
R	3	♂	44	15	74	10	143	R	5	♂	71	25	105	10	211
Total	9	♂	328	40	261	86	715	Total	5	♂	71	25	105	10	211

will do so in proportion to the closeness of the linkage. Sixty-six matings produced enough progeny (20) to discriminate between segregating and non-segregating matings; of these 37 segregated. This fraction, 37/66, exceeds 4/9 (χ^2 for 1 d.f. = 3·64, probability about 0·06). There is thus a case for linkage, and the linkage is loose.

The linkage values *N–mn* and *Ca–mn* can be obtained from the progeny of segregating matings. As these are a mixture of coupling and repulsion single intercrosses, these phases have to be distinguished. The simplest way is to test for heterogeneity in the ratio [*Ca* non-*mn* plus non-*Ca* miniature]:[*Ca* miniature plus non-*Ca* non-*mn*] among the progeny, substituting naked for caracul in the appropriate matings. These classes have in the case of coupling the expected frequencies:

$$[(2-p)+(1-p)]:[p+(1+p)],$$

where p is the recombination fraction; the ingredients of the square brackets are interchanged for replusion. Where $p = \frac{1}{2}$, the classes are expected in equal numbers and there should be no heterogeneity.

To obtain valid results, sources of heterogeneity other than linkage phase must be eliminated. The viability of miniature varied greatly from mating to mating; this source of heterogeneity is reduced by omitting matings with extreme viabilities of *mn*, as also matings with progenies so small that the viability of *mn* can be only roughly determined. When those with less than 10% *mnmn* and, for male heterozygotes, those with less than 30 progeny, are omitted, there remain 29 matings. Five of these were naked × caracul, so their data may be included with those for *Ca* as well as those for *N*, there are thus 34 bodies of data (Table 2). From

15 matings ♀*Ca*+, +*mn* × ♂++, +*mn*, 5 matings ♀*N*+, +*mn* × ♂++, +*mn*,
9 matings ♂*Ca*+, +*mn* × ♀++, +*mn*, 5 matings ♂*N*+, +*mn* × ♀++, +*mn*,

the deviation χ^2 testing equality of the two classes, and their heterogeneity χ^2 values (d.f. bracketed) respectively are:

29·70 (1), 62·70 (14), 2·13 (1), 9·13 (4),
17·86 (1), 32·12 (8), 11·38 (1), 5·71 (4).

Table 3. *Evidence on order for* mn, Ca *and* bt, *from single individuals*

(Each of the four individuals was a female Caracul non-*mn* non-*bt* in phenotype, and came from the same heterogyous male and his females: ♂ + *Ca bt*/*mn* + + × ♀♀ + + *bt*/*mn* + +.)

Individual	Genotype	Evidence for genotype	A single crossover in the ♂ *Ca* parent gives the individual, if the order has as its central locus		
			bt	*mn*	*Ca*
a	♀ *mn Ca* + / + + +	45 progeny segregated as if *Ca–mn* in repulsion; all were non-*bt*	Yes	Yes	No
b	♀ *mn Ca bt*/ + + +	21 progeny segregated as if *Ca–mn* and *Ca–bt* each in repulsion	Yes	No	Yes
c	♀ + *Ca* + /*mn* + +	82 progeny segregated as if *Ca–mn* in coupling; all were non-*bt*	No	Yes	Yes
d	♀ + *Ca* + / + + +	66 progeny included no *mn* and no *bt*	No	Yes	Yes

Probabilities are less than 0·01 except in the cases of the value 2·13 and 5·71 (probability greater than 0·05) and 9·13 (probability about 0·05).

Thus linkage is established beyond doubt; and all but the matings involving ♂ *N* +, + *mn* contain two linkage phases. When matings with the two classes deviating from 1:1 in one direction are separated from those deviating in the opposite direction, the separated bodies of data are homogeneous.

To assign these to coupling and repulsion is to risk some inaccuracy due to chance deviations; this would be suspected if any one mating so assigned did not belong to a run of the same phase. This was not so, and the assignments are therefore likely to be correct. A further check is obtained by testing whether the assigned coupling and repulsion data agree in their contributions to the four classes with expectations $1+p$, $1-p$, $2-p$ and p. In the *Ca* +, + *mn* × + +, + *mn* data where there are several matings assigned to each phase, the heterogenity χ^2 for 3 d.f. testing this is 0·87 where the *Ca* +, + *m* mate is female, and 2·54 where it is male; probabilities in both cases are greater than 0·5. Maximum-likelihood estimates from the combined coupling and repulsion data are therefore likely to be accurate.

The recombination values, obtained by the standard formula (Mather, 1951, ch. 7), are:

	Ca–mn	*N–mn*
Female	24·90 ± 2·45 %	22·87 ± 5·50 %
Male	26·99 ± 2·95 %	26·06 ± 5·30 %

3. *Order of* N, Ca, bt *and* mn

Despite the close linkage between naked and caracul, especially in the male heterozygotes (Table 1), the values for *Ca-mn* exceed those for *N–mn* in both sexes, though not significantly. This suggests the order *mn–N–Ca–bt*. Table 3 presents the only evidence on linkage relations with belted. The sizes of the recombination values between *mn–Ca* and *Ca–bt* preclude an order *Ca–mn–bt*. Interpretation of the table therefore rests on the vote given by the four female progeny on the orders *mn–bt–Ca* and *mn–Ca–bt*. These are: two 'Yes' to two 'No' for the first, and three 'Yes' to one 'No' for the second; so the second is preferred. Further indication that *mn* and *bt* are very loosely, rather than closely, linked, is given by the genotypes of the non-*Ca* chromosomes in the four females of Table 3. These must have come from the *bt/mn* mothers, yet three of the four are crossover genotypes. The order *mn–Ca–bt* is thus confirmed.

4. *Linkage group VI as a whole*

The combination values for male heterozygotes exceed those for female ones, for both *Ca–mn* and *N–mn*, although not significantly. This suggests that the difference between the sexes, so strong for the *N–Ca–bt* segment, may extend some way to the left of it. Linkage group VI, incorporating other recorded linkages (Green, 1966 p. 129; Dickie 1968), and ignoring sex differences, now stands as:

$$\text{mn–24\,\%–}\underline{\text{Sha–1\,\%–N}}\text{–2\,\%–Ca–3\,\%–}\underline{\text{hl–1\,\%–Ht}}\text{–7\,\%–bt–40\,\%–uw}$$

where brackets indicate that the position of the pair concerned, in relation to neighbouring genes, is uncertain. (*Sha* is shaven, *hl* is hairloss and *Ht* is high-tail.)

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