their mates with active sperm, but no larvae develop in eggs laid by these females.

[1] This species is undescribed. Professor J. T. Patterson has furnished the following brief description. A more complete one will be published later.

"*Drosophila micromelanica*, sp. nov.

"A small, very dark melanica-like species with the following general characters: Arista with eight branches; acrostichal hairs somewhat irregular, in six to eight rows; no prescutellars. Spermathecae almost transparent, not chitinized; ventral receptacle emerges from uterus as straight tube and then forms a tangled mass, without definite coiling. The eggs have two filaments. Length of body 2.4 mm.; wing 3 mm. (measurements made on live specimens)."

[2] Mainland, G. B., *Genetics*, **26**, 161 (1941).

LINKAGE STUDIES OF THE RAT (*RATTUS NORVEGICUS*) V

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I. The Albino Chromosome.—The rat chromosome which contains the largest number of known mutant genes is the one which carries the albino gene. Albinism was probably the first observed mutation of the rat and albinos had long been reared in captivity before the Mendelian laws were known. Albinism in the rat, as in other rodents, is a simple recessive in heredity, as are also the four other mutations which are linked with it. The five mutant genes (as we shall presently show) are in the order of their location in the chromosome (1) l, a lethal recently discovered by Grüneberg; (2) c, albino; (3) r, red-eyed yellow; (4) p, pink-eyed yellow; and (5) w, waltzing. The heredity of c, r and p was studied by Castle, Wright, Dunn and Wachter, the first observed case of linkage in the rat having been reported by Castle and Wright as occurring between r and p. Further intensive studies made by Castle, Dunn and Wachter, showed that r was closely linked with c with less than one per cent of crossing-over between them and that p was more loosely linked with c. The fact was also established that crossing-over in this chromosome (as in the albino chromosome of mice) occurs with greater frequency in female than in male individuals. The linkage maps involving the three genes were accordingly drawn thus:

$$\text{For females} \quad \frac{C\ R \qquad\qquad P}{0\ \ 0.5 \qquad\quad 21.9}$$

$$\text{For males} \quad \frac{C\ R \qquad\qquad P}{0\ \ 0.2 \qquad\quad 18.4}$$

After King had discovered the waltzing mutation in a race of albino rats, it was found that a loose linkage exists between waltzing and albinism, with about 45 per cent of crossing-over. This would locate the waltzing gene as far to the right of P in the above maps, as P is to the right of C, unless w lies to the left of c, in which case the total map length of the chromosome would be considerably greater. To decide between these two alternatives the present investigation was made.

Grüneberg on the basis of studies of linkage between l, c and p, concluded that his lethal lies 3.3 units to the left of c, and estimated the total length of the albino chromosome at 49 units.

To ascertain whether w lies to the right or to the left of c it is necessary to have evidence of the linkage strength between w and some third gene of the albino chromosome. For this purpose gene p is most suitable since it lies at a considerable distance from c. If the linkage between p and w is *less* than that between c and w, then w must lie to the right of c; but if the linkage between p and w is *greater* than that between c and w, then w must lie to the left of c. To answer this question King has made a repulsion cross between a normal pink-eyed yellow and a waltzing albino. The resulting F_1 animals were gray non-waltzers of constitution $\dfrac{C \ p \ W}{c \ P \ w}$. They were mated to triple recessive animals, albino waltzers born of pink-eyed yellow parents, and so of constitution $cc \ pp \ ww$, which in every gamete would transmit the three recessive alleles, $c \ p \ w$.

The back-cross population produced in this way consisted of 523 individuals as follows:

	NORMAL	WALTZING	TOTAL
Gray	49	11	60
Pink-eyed yellow	180	42	222
Albino	175	66	241
Total	404	119	523

From these data we desire to estimate the crossover percentage (1) between C and P, (2) between P and W and (3) between C and W. Let us assume first that the order of the genes is $C \ P \ W$. The constitution of the F_1 animals has already been stated. A crossover between loci C and P, will produce gamets $C \ P$ (gray) and $c \ p$ (albino). But since these albinos are not distinguishable from other albinos produced in the experiment, it will be best to disregard them and base the calculation of the crossover percentage solely on the gray class which is distinctive and theoretically should equal half the total number of crossovers in this region. Accordingly $2 \times 60 = 120$ will be the number of crossovers in the population of 523, and this equals 22.9 ± 1.2 per cent. This agrees fairly well with the result obtained by Castle and Wachter in a much larger population which was 21.9 ± 0.4 (for female F_1 parents).

A crossover between loci P and W in the F_1 parent will result in gametes which are $C\,p\,w$ (yellow waltzer) or $c\,P\,W$ (albino normal). Disregarding the albinos, the total crossovers should equal 2×42 (yellow waltzers) = $84 = 16.0$ per cent. There is reason to think this estimate too low, because the waltzers recorded in the entire population fall considerably below the expected fifty per cent. A like deficiency of waltzers was observed in King's earlier experiments with this character. It is undoubtedly due to the low effectiveness ("penetrance") of the waltzer gene, which results in "normal overlaps," animals which though homozygous for the waltzer gene nevertheless do not waltz. The expected number of waltzers (half the total population) is 2.2 times as great as the observed number. Accordingly to correct for the deficiency of waltzers, we should multiply by 2.2 the observed numbers of waltzers, and decrease correspondingly the reported number of normals. If this is done the population statistics become

	NORMALS	WALTZERS	TOTAL
Gray	35.8	24.2	60
Yellow	129.6	92.4	222
Albino	95.8	145.2	241
Total	261.2	261.8	523

From the data thus corrected, the crossovers between loci P and W would be $2 \times 92.4 = 184.8 = 35.3$ per cent of the population 523.

The crossover percentage between C and W may also be estimated from the corrected data. Crossover individuals would be colored waltzers and albino normals, but since the former are more distinctive, the calculation becomes 2×116.6 (colored waltzers) $= 233.2 = 42.6$ per cent. This is in fair agreement with the estimate of King and Castle based on a much larger population, which was 45.8 ± 0.7 per cent. That the crossover percentage is less in this experiment than in the earlier one conducted by King may be explained by the known greater frequency of crossing-over in female than in male F_1 parents. In the present experiment all but 49 of the 523 young were sired by F_1 males, whereas in the earlier experiment a larger proportion of the population was derived from F_1 females.

We are now able to ascertain the order of the three genes C, P and W. The indicated map distance $P\,W$ (35.3) is less than $C\,W$ (42.6). Accordingly P lies between C and W and the respective distances between the genes may be indicated thus:

$$\frac{C\ 22.9\ P\ 35.3\ W}{42.6}$$

$C\,P + P\,W = 22.9 + 35.3 = 58.2$ which is greater than $C\,W$ (42.6) because $C\,W$ is made to appear less by double crossing-over (interference).

If instead of the figures for the $C\,P$ and $C\,W$ distances given by this

investigation, we adopt those given by earlier investigations based on larger numbers, and also add to the map the portion discovered by Grüneberg, to the left of C, then the map will become

$$\frac{L \quad C \quad R \quad P \quad W}{0 \; 3.3 \; 3.8 \; 23.3 \; 58.6}$$

II. The Curly-Brown Chromosome.—A second chromosome of the rat in which more than one mutant gene had been found was reported by King and Castle (1935). Here a loose linkage was demonstrated to occur between the dominant gene curly (Cu) and the recessive gene brown (b), the crossover percentage being estimated at 40.48 ± 1.35.

A third gene anemia (an) discovered by Smith and Bogart (1939) has been found to be located in this same second (II) chromosome. Since anemia is a lethal, only animals heterozygous for anemia can be used in linkage studies. Castle has made a cross between such carriers of anemia and curly individuals. F_1 curly individuals, which were found by a breeding test to be carriers of anemia, would carry the two genes in the repulsion relationship. In a backcross of such F_1 animals to non-curly carriers of anemia it was found that very few of the anemic individuals thus produced were also curly coated, an indication of close linkage (repulsion) between the genes for anemia and curly. By suitable crosses (though not without difficulty because of the close linkage between curly and anemia) several triple heterozygotes were finally produced which transmitted all three mutant genes in the same chromosome, their genetic formula being $\frac{Cu \; an \; b}{+ \; + \; B}$. Such animals were now mated with animals homozygous for brown but heterozygous for anemia, this being the nearest approach to a triple recessive combination which would be viable. Such backcross matings produced populations as follows:

	Anemic Phenotypes				
	Cu an B	*Cu an b*	*an B*	*an b*	TOTAL
By F_1 males	129	187	1	6	323
By F_1 females	25	32	0	1	58
Total	154	219	1	7	381
	Normal Phenotypes				
	Cu B	*Cu b*	*B*	*b*	TOTAL
By F_1 males	124	159	257	276	816
By F_1 females	28	47	49	49	173
Total	152	206	306	325	989

Considering first the anemics, it will be observed that only the 7 *an b* individuals and the one *an B* individual have resulted from a crossover in the F_1 parent between Cu and an. They comprise 2.1 ± 0.5 per cent of the anemics.

Crossovers between anemia and brown are represented by the black anemics (whether or not curly), which number 155, or 40.7 ± 1.7 per cent of the anemics.

Crossovers between curly and brown are represented by curly blacks and non-curly browns, which among the anemics number $154 + 7 = 161$, or 42.2 ± 1.7 per cent of the anemics. A diagram expressing these relationships may be drawn thus

$$\frac{Cu\ 2.1\ an\ 40.7\ b}{42.2}$$

Obviously the order of the genes is, as previously assumed, $Cu\ an\ b$.

The non-curly phenotypes give additional evidence of the frequency of crossing-over between the genes Cu and b, since here also curly blacks and non-curly browns will arise from crossover gametes produced by the F_1 parent. They number $152 + 325 = 477$ in a population of 989, or 48.2 ± 1.07 per cent of the population, a somewhat higher value than the anemics alone gave. Combining the evidence from both sets of data, we have 638 crossovers in a population of 1370, which is 46.56 ± 0.92 per cent.

Additional evidence on the crossover percentage between curly and brown is furnished by backcross litters which contained no anemics because the backcross mate of the F_1 animal did not carry anemia. Such matings yielded only normal phenotypes, as follows:

$$\frac{Cu\ B}{86} \qquad \frac{Cu\ b}{97} \qquad \frac{B}{96} \qquad \frac{b}{91}$$

The crossovers $= 86 + 91 = 177$, which is 47.8 ± 1.7 per cent of the population, 370.

The data recorded by King, on which the original discovery of linkage between curly and brown was based, included 253 crossovers in a population of 625, which gives a crossover percentage of 40.48 ± 1.30.

If now we combine all available evidence we obtain a record of 1068 crossovers in aggregate population of 2365, or 45.16 ± 0.69 per cent crossovers.

According to our present knowledge the second (II) chromosome of the rat may be mapped thus:

$$\frac{Cu\ an\ b}{0\ \ 2\ \ 45}$$

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