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LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS)

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The mutated genes of the rat now on record are as follows:

A, a	agouti, non-agouti
B, b	black, chocolate
C, c^r, c	fully colored, ruby-eyed, true albino
Cu, cu	curly, normal coat
Cu_2, cu_2	curly, normal coat
D, d	intense, dilute
H, h^I, h	self, Irish, hooded
Hr, hr	normal coat, hairless
K, k	normal coat, kinky
P, p	fully colored, pink-eyed yellow
R, r	fully colored, red-eyed yellow

Of these A, C and H were found by Castle to be independent of each other, but P, R and C were found by Castle and Wright, Dunn and Wachter to lie in a common linkage system. Subsequently Roberts discovered genes d and hr and showed that they are probably independent of the genes previously known (A C H P and R). More recently King has discovered the dominant mutation, curly (Cu), and the recessive mutation, brown or chocolate (b). Linkage studies on these newly discovered genes will be reported in this paper. Gregory has recently described a second dominant curly mutation apparently distinct from the curly described by King; and Feldman (unpublished data) has found a recessive mutation called *kinky*, which even more strongly than the "curly" mutations has a tendency to shorten the hair and make it curly. We are indebted to Dr. Feldman for an opportunity to study the kinky mutation in advance of his forthcoming publication concerning it.

The increasing number of mutated genes increases the probability that additional genetic linkages will be discovered, if search is made for them,

since the haploid number of chromosomes of the rat is known to be twenty-one, and at least five of these are already tagged with identifying genes, viz., (1) *A*, (2) *CPR*, (3) *D*, (4) *H* and (5) *Hr*. The required experimental matings have been made by King, while Castle has coöperated in the genetic analysis of the experimental data and has written this report.

1. First we may report an experiment to discover whether the new genes, brown and curly, are linked with each other. A cross was made between cinnamon females (*b cu*) and curly males (*B Cu*). The F_1 animals were gray and curly (*Bb Cu cu*). A backcross was made of the F_1 animals with the cinnamon parent race, the double recessive combination. It is expected that, if there is no linkage, four classes of offspring will be produced, all equally numerous. The observed result is as follows:

Gray curly (*B Cu*)	Gray straight (*B cu*)	Cinnamon curly (*b Cu*)	Cinnamon straight (*b cu*)
190	125	128	182

The two middle groups are the crossover classes. They are both smaller than the non-crossover classes. Together they number 253, whereas the non-crossovers total 372. This is a clear indication of linkage, for we expect half of the young to be crossovers if there is no linkage. The observed departure from expectation is 59.5 ± 8.43, a deviation clearly significant, since it is more than 7 times the probable error. A similar indication of linkage is obtained irrespective of whether the F_1 parent is a male or a female as is shown in table 1.

TABLE 1

CLASSIFICATION OF THE YOUNG PRODUCED IN A TEST FOR LINKAGE BETWEEN CURLY AND BROWN

	GRAY CURLY	GRAY STRAIGHT	CINNAMON CURLY	CINNAMON STRAIGHT	TOTAL	PER CENT CROSSOVERS
Hybrid females backcrossed to cinnamon males	122	67	76	106	371	38.54 ± 1.75
Hybrid males backcrossed to cinnamon females	68	58	52	76	254	43.30 ± 2.12
Total	190	125	128	182	625	40.48 ± 1.35

In certain experiments with rats and mice, it has been found that crossing-over occurs more frequently in females than in males, but the present experiment lends no support to this idea, as is shown by table 1. Linkage between curly and brown is shown in both sorts of backcross matings, but the indicated crossover percentage is actually greater in the case of hybrid males than in the case of hybrid females, though on account of smaller numbers, the probable error is greater. Nevertheless this discordant result

rests on too slender a statistical basis to disprove the generalization drawn by Castle and Wachter from very extensive observations.

2. A mating between curly males and inbred albino females affords tests for linkage between curly (Cu) and the three genes a, c and h. The parents were, respectively, $A\ C\ Cu\ H$ and $a\ c\ cu\ h$ in formula. Consequently the F_1 animals were heterozygous for all four genes. Since the albino stock was homozygous for the four recessive genes, a backcross was made to this race. A population of 136 backcross young has been recorded, 72 individuals being curly and 64 straight-haired, expectation being 68 ± 3.9 of each. The ten expected phenotypes are all present but not exactly in the expected proportions, the hooded classes being small, possibly because of the classification of certain wide striped hooded animals as non-hooded. Nevertheless the relation of curly to each of the other genes (including h) shows that no linkage is involved.

The ten phenotypes recorded, and their frequencies, were as follows:

Albino curly	28
Albino straight	21
Gray curly	19
Gray straight	20
Black curly	16
Black straight	15
Gray hooded curly	7
Gray hooded straight	2
Black hooded curly	2
Black hooded straight	6
Total	136

The relation of curly to each of the genes, A, C and H can be shown as in table 2.

Curly, in relation to albinism, shows slightly more crossovers (the middle classes) than non-crossovers (the outside classes). Hence there is no indication of linkage in that category. In relation to hooding, crossovers

TABLE 2

RECOMBINATIONS IN THE BACKCROSS

$A\ Cu$	$A\ cu$	$a\ Cu$	$a\ cu$	CROSSOVERS	NON-CROSSOVERS
26	22	18	21	40	47
$C\ Cu$	$C\ cu$	$c\ Cu$	$c\ cu$		
44	43	28	21	71	65
$H\ Cu$	$H\ cu$	$h\ Cu$	$h\ cu$		
35	35	9	8	44	43

and non-crossovers are as nearly equal as possible. In relation to agouti, non-crossovers are in excess. This would suggest linkage if the deviation

were of significant magnitude.   But it is only 3.5 ± 3.1.   In other words, it is scarcely greater than the probable error and so no greater than random sampling might cause.

3.   A cross between cinnamon males and albino females produced F_1 gray offspring which were bred *inter se* to produce an F_2 population consisting of 121 individuals.   The parents were, in formula, *A b c H* and *a B c h*, respectively, and the F_1 individuals consequently would be heterozygous for all four differential genes.   If there is no linkage between any of these, we expect classes to result, with the frequencies indicated, for nine different phenotypes, as follows:

	OBSERVED	EXPECTED
Albino	34	30.2
Gray	38	36.7
Cinnamon	11	12.2
Black	14	12.2
Brown	8	4.0
Gray hooded	8	12.2
Cinnamon hooded	4	4.0
Black hooded	3	4.0
Brown hooded	1	1.3
Total, colored	87	86.6

Agreement between observed and expected numbers is excellent, making it clear that no linkage exists between gene *B* and any one of the three genes *A*, *C* and *H*.   For, if we consider the three cases separately, it will be seen that if linkage (repulsion) existed between albinism and brown, there should be an excess of brown pigmented individuals among the colored. The number expected if no linkage occurs is 21.75, the number recorded is 24, an insignificant excess of 2.25, the probable error being 2.72.   If linkage existed between agouti and brown, we should expect an excess of cinnamons and a deficiency of browns.   Instead, we observe the opposite, a slight deficiency of cinnamons (15 where 16.2 are expected) and an excess of browns (9 where 5.3 are expected).   If linkage existed between brown and hooding, we should expect a deficiency of brown hooded and an excess of browns (not-hooded).   We have recorded 5 brown hooded where the expectation is 5.44, and 19 browns (not-hooded) where the expectation is 16.2.   These deviations are without statistical significance, the excess of browns being only 2.8 where the probable error is 2.46.

Accordingly, we find no indication of linkage between the brown gene and either of the three genes, *A*, *C* and *H*.   This supports the conclusions derived from the curly × albino cross, for if curly is linked with brown, all genes which show no linkage with curly should also show no linkage with brown.

4.   In order to make a test for linkage between curly and dilution, curly coated individuals were crossed with blues.   The F_1 animals were then backcrossed with double recessives, blue straight.   A population of 312 young was thus obtained, distributed as follows:

Intense curly	Intense straight	Dilute curly	Dilute straight
71	91	65	85

The crossover classes, intense straight and dilute curly, together number 156.   The non-crossover classes, intense curly and dilute straight, also number 156, indicating that no linkage exists.

5.   In March, 1934, we received from Dr. H. W. Feldman, of the University of Michigan, some stock of a new rat mutation resembling curly, but unlike it, being a recessive rather than a dominant in heredity.   An exchange of animals between our laboratories had been arranged in order that breeding tests might be made to ascertain whether the mutation discovered by Feldman was (1) an allelomorph of curly or (2) linked with it or (3) wholly free from it.   The tests which have now been made show that the last named hypothesis is the correct one.   Kinky (as the Feldman mutation will hereafter be called, to distinguish it from curly, which it closely resembles phenotypically) does not lie in the same chromosome as curly and cinnamon.

A cross was made between a black hooded kinky male (*aa hh kk*) received from Dr. Feldman and gray curly coated females (*AA Cu Cu*).   The F_1 young were all gray coated but bore a white belly spot, indicating that they were heterozygous for the hooded pattern.   Their genetic formula accordingly would be *Aa Cu cu Kk Hh*.   They were backcrossed reciprocally to black kinky mates heterozygous for hooding (*aa kk Hh*).   The groups of young produced by the reciprocal backcrosses were not qualitatively different, hence there is no indication of sex linkage, and the two series may be combined (see table 3).

TABLE 3

RESULTS OF RECIPROCALLY BACKCROSSING (TO THE BLACK KINKY PARENT RACE) F_1
HYBRIDS BETWEEN GRAY CURLY FEMALES AND A BLACK HOODED KINKY MALE

TYPES OF YOUNG	F_1 ♀♀ × *aakk* ♂	F_1 ♂ × *aakk* ♀♀	TOTALS
Gray curly	47	30	77
Gray straight	19	6	25
Gray hooded curly	5	4	9
Gray hooded straight	1	1	2
Black curly	48	25	73
Black straight	18	9	27
Black hooded curly	8	1	9
Black hooded straight	1	1	2
Totals	147	77	224

Since it was known from experiments previously made by Dr. Feldman that kinky is a recessive character, it is certain that the curly character seen in the F_1 young was a manifestation of the curly gene inherited from their curly parent, for the kinky gene would find no expression in the F_1 individuals. Nevertheless they would all be heterozygous for that gene as well as for the curly gene. (1) If Cu and k were allelomorphs, the gametes formed by the F_1 parent would be either Cu or k, and in a backcross with kk animals, all young should be curly coated. In reality 168 were curly and 56 straight, hence Cu and k are not allelomorphs. (2) If Cu and k were borne in the same chromosome, less than half the gametes formed should be crossovers (Cu k or O). It would be impossible to detect in the backcross the presence of a gamete Cu k, since it would produce the curly type of coat, like Cu and k singly. But the O combination (lacking both Cu and k) should produce normal (straight coated) offspring, since there would be in the zygote only a single k gene derived from the kinky parent. Accordingly linkage would be indicated in the backcross by the production of straight coated young *significantly fewer than* 25 per cent of the population. The population raised consists of 224 individuals, of which exactly one-fourth (56) were straight coated. This is conclusive evidence that Cu and k are independent, lie in different chromosomes.

Genes A and k also entered this cross in the repulsion relationship. If they were linked, the crossover combinations ak and AK should be deficient in the backcross, that is, should represent significantly less than half the population. The four types gray curly, gray straight, black curly and black straight occurred in the respective frequencies 86:27:82:29 where the ratio expected if no linkage exists is 3:1:3:1. The second and third of these are crossover combinations, their sum being 109, the noncrossovers totaling 115. The deviation from the expected equality is 3 ± 5.05; hence less than might be expected to occur by random sampling alone. Accordingly we may conclude that no linkage exists between the genes A and k.

Genes h and k entered this cross in the coupling relationship, and should show association significantly oftener than dissociation, if linkage existed between them. In reality 22 hooded young were recorded in the backcross, of which 18 were curly and 4 straight, expectation being 5.5 straight, if no linkage occurs. The departure from expectation is 1.5 ± 1.58, in other words, less than random sampling alone might produce. Hence there is every reason to believe that h and k segregate independently and lie in different chromosomes.

It is therefore clear, from the experiment described, that the kinky gene does not lie in the chromosomes tagged by the genes A, Cu and H. If it does not lie in the Cu chromosome, it should show no linkage with the b gene, which we have shown to be linked with Cu.

6.   The relationship of gene k to b (and certain other genes) was tested in a mating between cinnamon females homozygous for the A gene ($AA\ bb$) and the same black hooded kinky male ($aa\ hh\ kk$) which was employed in the cross with curly females.   The F_1 gray animals from this cross were thus heterozygous for four genes ($Aa\ Bb\ Hh\ Kk$) and would be expected to form 16 classes of gametes, all equally numerous, if no linkage exists between any of the four genes.   The backcross was made reciprocally to cinnamon kinky individuals obtained as representatives of the double recessive combination ($bb\ kk$) in the F_2 generation.   They were also heterozygous for A, and some of them for H also.   The very fact that the double recessive combination ($bb\ kk$) was readily obtained in F_2 indicates that no

TABLE 4

RESULTS OF RECIPROCALLY BACKCROSSING (TO CINNAMON KINKY) F_1 INDIVIDUALS FROM
A CROSS BETWEEN CINNAMON FEMALES AND A BLACK HOODED KINKY MALE

TYPES OF YOUNG	F_1 ♀♀ × CINNAMON KINKY ♂♂	F_1 ♂♂ × CINNAMON KINKY ♀♀	TOTALS	APPROXIMATE EXPECTATION
Gray straight	19	18	37	40.5
Gray kinky	25	24	49	40.5
Gray hooded straight	5	6	11	13.5
Gray hooded kinky	3	9	12	13.5
Cinnamon straight	25	23	48	40.5
Cinnamon kinky	20	24	44	40.5
Cinnamon hooded straight	10	3	13	13.5
Cinnamon hooded kinky	3	5	8	13.5
Black straight	5	5	10	13.5
Black kinky	6	8	14	13.5
Black hooded straight	1	1	2	4.5
Black hooded kinky	1	0	1	4.5
Brown straight	4	1	5	4.5
Brown kinky	2	9	11	4.5
Brown hooded straight	1	3	4	1.5
Brown hooded kinky	0	1	1	1.5
Totals			270	264.0

linkage exists between B and K; nevertheless it was thought desirable to obtain demonstrative evidence by carrying out the backcross matings. Fifteen of the expected 16 phenotypes were obtained in each of the reciprocal backcrosses, a surprisingly close agreement with expectation, in view of the fact that the total number of young in one backcross was 130 and in the other 140.   Combining the results of the reciprocal backcrosses, which show no qualitative difference and thus give an emphatic negative to the existence of sex linkage on the part of either gene, we have all 16 phenotypes represented by one or more individuals in a total of 270 young.   The most probable expectations are shown in the last column of table 4.   With these the observed frequencies agree excellently.

As a test for linkage between the genes A and k, the data contained in table 4 may be arranged as follows. These genes entered the cross in the repulsion relationship and should recombine freely, if no linkage exists, so as to produce four phenotypes (other differences being disregarded).

	AK	Ak	aK	ak
Observed	109	113	21	27

If all the cinnamon kinky parents had been heterozygous for A, we should expect the four classes to be as 3:3:1:1, but it is probable that some of them were homozygous for A, hence the deficiency of the non-agouti classes below that expectation. Linkage would result in making the AK and ak classes, which are crossovers, less than the non-crossover classes, Ak and aK. The sum of the former is 136, that of the latter 134. Hence no linkage is indicated between A and k.

As a test for linkage between the genes B and K, the data contained in table 4 may be grouped thus:

	BK	Bk	bK	bk
Observed	60	76	70	64

The crossover classes (BK and bk) together include 124 individuals, the non-crossovers (Bk and bK) number 146. The deviation from equality of the two groups is 11 ± 5.54, which is statistically without significance.

As a test for linkage between H and K, the data contained in table 4 may be grouped thus:

	HK	Hk	hK	hk
Observed	100	118	30	22

The number of hooded individuals recorded is possibly less than it should be, for the reason stated elsewhere, but the proportion of kinkys among those classified as hooded is pertinent to the question of linkage. There are 30 hooded straight to 22 hooded kinky, where equality is expected if no linkage occurs. The departure from equality is 4 ± 2.86, which is without statistical significance. Further, the crossover classes (HK and hk) together number 122 individuals, the non-crossovers (Hk and hK) number 148. The deviation from equality is here 13 ± 5.54, which again is devoid of statistical significance.

Accordingly, we find that the crosses of kinky with curly and kinky with cinnamon, as recorded in tables 3 and 4, agree in showing no linkage to exist between gene k and the genes A and H. Further table 3 has shown the gene Cu to be free from K, and table 4 has shown B to be free from K. These several findings are all compatible with the previously demonstrated linkage between B and Cu.

7. A report may be made also on a test for linkage between curly and hairless, the results of which indicate free assortment of these genes. A

gray curly female was mated to a pink-eyed yellow hairless male, producing a litter of six gray curly females. These were backcrossed to the yellow hairless male, their father, or to hairless males of similar (double recessive) constitution. Disregarding the segregation for coat color, we may classify the backcross young thus:

Curly normal	Curly hairless	Straight normal	Straight hairless
28	18	24	15

Fewer hairless than normal-haired young were recorded, doubtless owing to the inferior survival ability of hairless individuals; but in relation to curly, hairless obviously segregates freely. The crossover classes, curly hairless and straight normal, together number 42: the non-crossover classes number 43, a result as close to equality as possible in an odd number of young.

The gene for yellow lies in the same chromosome as albinism. The results of this cross show that yellow is independent of curly, thus supporting the result of crosses 2 and 3, which showed albinism to be independent of curly and brown, for the 85 backcross young produced in this experiment may be grouped thus:

Gray curly	Gray straight	Yellow curly	Yellow straight
15	17	31	22

The sum of the crossover classes, gray straight and yellow curly, is 48, exceeding that of the non-crossover classes, 37. Consequently no linkage is indicated.

This same cross shows that hairless is independent of yellow (and so of albinism), confirming the conclusion of Roberts. The 85 backcross young may be grouped thus:

Gray normal	Gray hairless	Yellow normal	Yellow hairless
20	12	32	21

The crossover classes, gray hairless and yellow normal, aggregate 44; the sum of the non-crossover classes is 41. Clearly no linkage exists between yellow and hairless.

Summary.—Linkage studies show that in the common rat, in which eleven genes have been identified, there occur two linkage systems, one containing the three genes C, P and R; the other, the two genes B and Cu. The crossover percentage between B and Cu is 40.48 ± 1.35, which indicates that these two genes lie rather far apart in the same chromosome. They recombine independently of the genes A, C, D, H, Hr and K, and so must lie in a different (sixth) chromosome. It remains to canvass their linkage relations with the gene for curly No. 2.

Incidentally it is shown that the gene K does not lie in the same chromosome as A or H. Its independence is thus established as regards three of

the six chromosomes mentioned, but its relation to the chromosomes which carry genes *C, D* and *Hr* is as yet unknown.

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X-RAY AND ABNORMALITIES: INCREASED ABNORMALITY OF SEGMENTS IN DROSOPHILA DUE TO X-RAYING OF GAMETES

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Communicated May 13, 1935

The study of genetic effects of x-ray can be considered a field rich in resources clarifying much as to the behavior and effect of gene and chromosome constitution. Morgan, Muller,[1] Bridges,[2] Patterson,[3] Painter, Dobzhansky, Schultz and others who worked with Drosophila have already offered explanations of many genetic phenomena. Mutations of genes, translocations and eliminations of chromosome parts with corresponding phenotypic, genetic, lethal and semi-lethal effects all due to x-ray has stimulated interest in this type of research the world over. Some work of this nature was attempted here during the period December, 1933 to September, 1934.

The favorite of genetic workers, Drosophila melanogaster, was chosen as subject, not only because of the simplicity of handling, relatively short life cycle and well-known chromosome composition, but also because reports on similar previous experiments are available.

The stock chosen was the normal unmarked Iowa wild (a stock bred in the Iowa laboratories since June, 1931 from captured Drosophila). To simplify breeding, the experimental results were limited to the consideration of changes on the sex chromosome. To secure results in shortest time, virgin females were x-rayed, and mated, so that any changes produced in the *X*-chromosome would show up in the males of the first generation. Group I, twenty-nine females, was given a dosage of 731 r units. (With the x-ray machine used this was 50 kv., 5 m.a., target distance of 12 cm., Al filter of 1 mm. for 20 min.) Group II, twenty-four females, was given 1462 r units. (Other conditions remaining the same, rayed for 48 min.) Twenty-nine females (Group III) were used as controls for both