LINKAGE IN MICE AND RATS*

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INTRODUCTION

The important generalizations concerned with the mechanism of heredity have until recently been supported almost wholly by evidence drawn from breeding experiments with insects. The reasons for this are obvious, for the proof and application of the chromosome theory of heredity depends primarily on linkage. The study of linkage can only be prosecuted where animals or plants exhibiting well defined unit variations can be bred in large numbers. Critical evidence must hence be accumulated by strictly experimental methods. These conditions were at first satisfied only by the vinegar-fly, *Drosophila melanogaster,* which still remains in many ways the most ideal material for experimentation in heredity. However, generalizations so important as to affect considerably our ideas of evolution, should be tested for other organisms, and the last few years have witnessed an extension and confirmation of the chromosome theory by investigators working with many kinds of animals and plants.

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Most recently, perhaps, the mammals have been added to the list of animals exhibiting linkage. Investigation in the higher animals must of necessity proceed more slowly than in either plants or insects. The mammals as compared with the insects or plants, are slow breeders. Their period of gestation is relatively long, the numbers born in each litter are small, and the difficulties regarding space, maintenance and disease are many. The number of known variations is small, and since most of the species available for laboratory study are characterized by many chromosomes, the probability of more than one character being located in each chromosome is small.

Nevertheless, it is important that search should be made in the mammals for data bearing on the chromosome theory. Critical evidence is that derived from the study of the linkage relations between genes, and the establishment of linkage has as its object the localization of the hereditary determiners. Some evidence of the localization of genes in mammals is now available. That linkage has not often been found is a not unexpected or entirely negative result, for it may often be shown that the genes for certain characters are not linked (independent). This fact localizes the genes in different chromosomes, which is in many ways as definite a result as the establishment of linkage which localizes them in the same chromosome.

In the paragraphs which follow there are presented the results of experimental studies of two cases of linkage in rodents, one in some detail. Later it is planned to summarize the results of investigations of heredity in mice, which indicate that other genes for color variations are not linked but independent.

ESTABLISHED CASES OF LINKAGE IN MICE AND RATS

Previous investigations

There are now four well established cases of linked genes in rats and mice, and these are also the only known cases of linkage (aside from sex-linkage) in mammals. CASTLE and WRIGHT (1915) reported the first of these cases. They found evidence of linkage between the genes for two new color variations of the Norway rat, red-eyed yellow and pink-eyed yellow, which had been described previously by CASTLE (1914). Subsequently CASTLE (1916, 1919) investigated this linkage in greater detail and found the crossover value between these two genes to be about 18.3 percent. This value was calculated from a total of 4476 young and probably describes quite accurately the relation of the gene

for red-eye to that for pink-eye. Crossing over in both sexes was indicated.

The publication of the first report on this linkage was soon followed by a preliminary report by HALDANE, SPRUNT and HALDANE (1915) on the linkage between the genes for pink-eye and albinism in house mice. The authors preferred, however, to explain the results of their experiments in terms of the reduplication hypothesis. They found the gametic series 1:3:3:1 to be characteristic of the relation between these two genes, as compared with the gametic series 1:1:1:1, normally expected from hybrids between two independently segregating characters.

Data for this cross had been extant since the work of DARBISHIRE (1904) and of CUÉNOT (1907). Both of these authors had crossed pink-eyed mice with albinos. Their data showed that the two characters were distinct, for in the first generation dark-eyed, full-colored mice were produced. DARBISHIRE's data comprised fairly large numbers, and a serious departure from normal Mendelian expectation was apparent in his second generation, which indicated linkage between the two genes involved. His data, however, were not suitable for a study of this point, and it went unnoticed. In the summer of 1916 the writer, under the direction of Professor CASTLE, undertook the investigation of the relationship between pink-eye and albinism in mice. At that time we were not aware of the work of HALDANE *et al.*, but were familiar with DARBISHIRE's data. This investigation was conducted by the writer until April 1917, and was then taken over by Professor CASTLE, who carried it on until my demobilization in March 1919. A report by CASTLE gave the crossover value between pink-eye and albinism as approximately 14 percent.

The linkage between albinism, pink-eye and red-eye in rats was first noted by CASTLE (1916). It was later established by increased data (CASTLE 1919) from which was calculated a crossover value of 21.1 percent between pink-eye and albinism. This rested on a total of 90 gametes. Only one cross-over was detected between red-eye and albinism in 434 tested gametes. The crossover value between these genes was hence but a fraction of one percent and a very close linkage between them was indicated.

In confirmation of this linkage WHITING and KING (1915) have reported crosses between red-eyed and ruby-eyed-dilute rats. Such crosses produced full-colored gray rats in F_1 (87) and 59 grays, 19 ruby-eyed dilutes and 23 red-eyed yellows in F_2. No double recessives, viz., ruby-

reds, appeared in F_2, indicating that no crossing over occurred between ruby and red, and hence that these two genes are closely linked. Ruby-eyed dilution is an allelomorph of albinism and is therefore determined at the same locus as albinism; it should exhibit the same linkage with red-eye as with albinism. The results obtained indicated that this was the case.

The experiments to be reported here are concerned with the linkage relations of pink-eye and albinism in mice and of red-eye and albinism in rats. It is due to the kindness of Professor CASTLE that I am able to include mouse-breeding data gathered by him during my absence from the laboratory.

THE PRESENT INVESTIGATION

Linkage of pink-eye and albinism in mice

a. The characters involved

The albino and pink-eye characters studied by HALDANE *et al.* and by CASTLE in mice, and by CASTLE and WRIGHT in rats, are identical with the characters under consideration in the present report. The Mendelian nature of each has been firmly established by numerous investigators. Albinism has long been known as a unit variation recessive to color in the mouse, rat, guinea-pig and rabbit, to mention only members of the order Rodentia. It consists in complete (or almost complete) absence of color from the hair, skin and eyes, leaving the hair and skin white and the eyes pink. CUÉNOT first attributed this absence of color in the albino to the absence of a chromogen or color base in the presence of which pigment-forming enzymes may produce their respective colors. The gene for color or chromogen has been represented by *"C,"* its absence or albinism by *"c."* The genes for pigment-forming enzymes were shown to be present in albinos but to have no effect in the absence of *C*. This has remained the most convenient theory for genetic experiments and the notation arising from it is still used, although its correctness has been seriously questioned. ONSLOW (1915) has found evidence that absence of color in mammals is due not to absence of chromogen but to enzyme differences, in certain specific cases to the absence of a peroxidase. When tyrosinase was added to preparations from albino animals, the oxidation of tyrosin proceeded and black pigment was formed. This agrees well with recent discoveries of graded series of albinos in guinea-pigs, rabbits, rats, cats, and other animals. All of these grades of albinism possess a common gene which may be present in a series of alterna-

tive forms, each condition allelomorphic with its fellow members of the series. This central gene for albinism is present not only in animals entirely devoid of color, but also in animals capable of developing a certain amount of black or brown pigment, such as the Himalayan rabbit or the Siamese cat, in which the extremities are pigmented. The explanation of albinism is, then, nothing so simple as mere presence or absence of a gene for color. It is rather, as WRIGHT (1916) has suggested, a graded condition due to the varying activity or inhibition of a basic enzyme on a chromogen. Each grade has shown itself to be due to a gene of an allelomorphic series, the more intense grades being dominant to those of less pigmentation. Thus in guinea-pigs WRIGHT (1916) has identified four multiple allelomorphs of the color gene, (1) intense or full color, (2) dark-eyed dilution, (3) red-eyed dilution, and (4) albinism. The form of albinism found in guinea-pigs should be distinguished from the true pure white albinism of rabbits, rats and mice, for the albino guinea-pig has pigmented extremities like the Himalayan albino rabbit. No ordinary albinism has yet been reported in guinea-pigs. In rabbits, CASTLE, PUNNETT and others have shown that a series of multiple allelomorphs exists from (1) intensity through (2) Himalayan albinism to (3) ordinary albinism. In rats, WHITING and KING (1918) have recently found a ruby-eyed dilute variation due to a gene allelomorphic with intensity and albinism. The allelomorphs of the color gene in rats are now, in order of dominance (1) intensity (2) ruby-eyed dilution (3) albinism.

The experimental breeding of mice has disclosed no such alternative conditions of the color gene as are exhibited in guinea-pigs, rabbits and rats. Unit variations in density of color occur, but in each case these have been traced to genes distinct from that for color.

A second unit variation affecting color has been found in rats, guinea-pigs, and mice. The appearance of animals exhibiting this variation is similar in all three species. The eyes are pink, resembling the eyes of albinos, although a slightly darker shade is discernible on close examination, due to microscopic amounts of pigment in retina and iris. The fur and skin of pink-eyed animals are distinctly lighter in color in those portions which in dark-eyed individuals are black or brown, while yellow portions appear similar in both dark-eyed and pink-eyed animals.

The gene for this character appears to be homologous in all three species in which it is found, for it produces homologous results and has shown in the rat and mouse similar relationships with other genes. It was shown by CASTLE and LITTLE (1909) to be distinct from the in-

tensity-dilution pair of allelomorphs (*D* and *d*) and to be the recessive allelomorph of dark-eye (*P*). By them it was given the symbol (*p*). The work of DARBISHIRE had shown it to be distinct also from the gene for color.

The mode of action of the gene for pink-eye is to reduce materially the number of black and brown pigment granules formed in hair, skin and eyes. Its action is selective, for yellow, which occurs as a non-granular ground color, is not affected in amount or intensity. Pink-eyed black rats and mice are a pale cream color, while in pink-eyed black guinea-pigs the black is still less in amount and the animals are sooty white. Pink-eyed brown mice and guinea-pigs are paler in color than pink-eyed blacks and show a tawny or ochraceous tinge. The pink-eyed black agouti and cinnamon varieties of all three animals are taken by the casual observer to be yellow, since the intense yellow tips of the agouti hairs are as fully colored as in dark-eyed agoutis and overlie the usually black or brown portions of the hairs nearer to the body. In pink-eyed agouti mice, these black or brown areas are pale blue-gray and pale brown respectively, while in rats and guinea-pigs the black or brown pigments have almost entirely disappeared from the hair leaving the bases a dirty cream color and the tips clear yellow. In rats such animals are known as pink-eyed yellows, though when crossed with dark-eyed blacks they soon give evidence of their agouti nature by producing dark-eyed agouti young. A similar quantitative reduction of granular pigments is seen in the eyes of pink-eyed colored animals. The ordinary dark eye of rodents is colored by black and brown pigment granules in retina and iris. In pink eyes Miss DURHAM (1908) found that pigment was not entirely lacking as in albinic eyes, but that iris and retina contained very small numbers of black or brown granules. LITTLE found very little pigment in the retina and a moderate amount in the iris. The pinkness of the eye is due to the reflection of light from unobscured blood vessels of the retina through an almost transparent iris.

b. The plan of the experiments

The plan of the experiments designed to determine the linkage relations between pink-eye and albinism in mice does not differ from the general plan already established for testing linkage relations in insects and plants. Albino females of known genotype (*ccPP*) were crossed with pink-eyed colored males of genotype *CCpp*; and similar matings were made between albino males and pink-eyed colored females. The first generation from these crosses consisted entirely of dark-eyed col-

ored animals of which 142 were raised. These F_1 animals were then mated to each other and a second generation of 580 individuals was recorded. The actual distribution of these F_2 animals is given below contrasted with the distribution expected on an hypothesis of independence of the pink-eye and albino genes.

Dark-eyed colored	Pink-eyed colored	Albino	Total	
Actual 295	129	156	580	$\chi^2 = 7.597$[1]
Expected 326.25	*108.75*	*145*	*580*	$P = .0231$

The distribution obtained fits the theoretical distribution very badly and shows a distinct excess of pink-eyed and a paucity of dark-eyed This agrees with the F_2 distribution of DARBISHIRE and of HALDANE, and indicates that repulsion has taken place between (*p*) and (*c*) in the gametes of F_1, for by random, independent assortment of these characters among the F_1 eggs and sperm we should obtain the following array of genotypes:

F_1 parents	*CcPp*	×	*CcPp*	
Gametes	*CP*		*CP*	crossover
	cp		*cp*	crossover
	Cp		*Cp*	non-crossover
	cP		*cP*	non-crossover
F_2 zygotes expected	1 *CCPP*	1 *CCpp*	1 *ccPP*	
	2 *CCPp*	2 *Ccpp*	2 *ccPp*	
	2 *CcPP*		1 *ccpp*	
	4 *CcPp*			
	9 dark	3 pink	4 albino	

Since the F_2 distribution is manifestly altered by linkage, and since the characters supposed to be linked entered the original cross separately

[1] In judging the goodness of fit of the distributions obtained to the theoretical distributions required by independent Mendelian assortment I have used PEARSON'S formula $\chi^2 = \Sigma \frac{(o-c)^2}{c}$ and the values of *P* corresponding to values of χ^2 as calculated by ELDERTON in PEARSON'S "Tables for statisticians and biometricians" (1914). Believing, however, that this method of measuring goodness of fit should not supersede but rather aid the usual method of geneticists in judging by inspection combined with a consideration of the particular biological facts and conditions of the experiment, I have added to each table without comment the values of χ^2 and *P* which I calculated for my own convenience.

(*Cp* × *cP*) these combinations should be relatively more frequent; while the combinations *CP* and *cp* could occur only as a result of crossing over and should be relatively less frequent.

To measure the frequency of crossing over in the F_1 gametes, the F_2 pinks and albinos were saved and tested. The F_2 pinks were crossed with pure albinos (*ccPP*); the F_2 albinos were crossed with pure pinks (*CCpp*). In this way the F_2 pinks and albinos were sorted into their several biotypes. Out of 75 F_2 albinos thoroughly tested by crossing with pure pink, 55 proved to be of genotype *ccPP* and 20 of genotype *ccPp*. Of the 150 F_1 gametes which produced these 75 albinos, 110 (2 × 55) must have been *cP* (non-crossovers); of the remaining 40 gametes, 20 must have been *CP* and 20 *cp* (crossovers). The F_1 crossover gametes were then 20 out of a total of 150 or 13.33 percent.

Similarly, out of 63 F_2 pinks, 45 proved to be of genotype *CCpp*, due to combinations of 90 non-crossover gametes, while 18 proved to be *Ccpp*, referable to 18 *Cp* (non-crossover) and 18 *cp* (crossover) gametes. The crossover gametes totalled 18 out of 126 or 14.28 percent. A combination of the figures for both of these tests shows that out of 276 F_1 gametes tested, 38 or 13.76 percent (± 1.396) were due to crossing over.[2] We may regard this as a preliminary estimate of the strength of the linkage between these characters.

From pink-eyed individuals shown by the test matings to be *Ccpp*, albino young were obtained which were recessive in both characters, *viz.* albinos pure for pink-eye (*ccpp*). All animals of type *CcPp*, where *c* and *p* had entered separately, were also saved, and reciprocal matings of *CcPp* and *ccpp* were made in large numbers. Results of these matings gave a direct index of the amount of crossing over in both sexes as may be seen from the possible combinations.

Theoretically, the crossover classes *CcPp* and *ccpp* should be equal, and therefore all dark animals resulting from this mating should be equal to one half of the total number of crossovers. Fortunately dark-eyed

[2] The probable errors of linkage values in this paper have been calculated by use of the formula $E_p = .6745\sqrt{\frac{P(1-P)}{n}}$ as given by HALDANE (1919). *P* is the observed value of the theoretical linkage value *p*, and is calculated from the distribution of gametes formed by the double heterozygote in the proportion $\frac{p}{2}$ *AB*: $\frac{1-p}{2}$ *Ab*: $\frac{1-p}{2}$ *aB*: $\frac{p}{2}$ *ab*. Where *A* and *B* enter separately, the linkage value is *p*; where *A* and *B* enter together the linkage value is 1—*p*.

young may be distinguished at birth from light-eyed (pink-eyed and albino) young by the presence of a distinct ring of black pigment in the position of the eye. This region in pink-eyed and albino young is quite colorless. This fact made it possible to record the numbers of dark-eyed and light-eyed young soon after birth. The young were then killed and the mother bred again. Large numbers were raised by this method in a comparatively short time and in few pens.

These matings produced 3141 young, of which 2919 were pink or albino and 222 were dark. Since the darks represented approximately one-half the crossovers, the total crossovers were presumably 444 or 14.14 percent (± .418) of the total.

Calculations of linkage strength had up to this time been based on the relations between the genes *c* and *p* when they entered the cross separately (repulsion). To complete the experiment crosses were made in which the genes entered the cross together (coupling). Dark-eyed animals (*CCPP*) were mated with albinos recessive in both genes, (*ccpp*).

The first generation consisted entirely of dark-eyed animals (*CcPp*) and these were mated again to the double recessives. These matings should yield the following genotypes:

Parents		*CcPp* × *ccpp*			
Non-crossover gametes	*CP*, *cp*	× *cp* =	*CcPp* (dark), *ccpp* (albino)		Non-crossover zygotes
Crossover gametes	*Cp*, *cP*	× *cp* =	*Ccpp* (pink), *ccPp* (albino)		Crossover zygotes

Here the distinguishable class (dark) represents one-half the non-crossovers. To find the number of crossovers, twice the number of darks produced must be subtracted from the total number of offspring.

The actual number of animals produced from this cross was 3331 of which 1414 were dark and 1917 were light. The calculated number of crossovers was 503, which is 15.1 (± .417) percent of the total produced.

The final value of the linkage between the genes for pink and albinism in mice is calculated by combining the figures for all experiments as in table 1.

For several reasons this final value may be regarded as expressing very closely the relations between these two characters in mice. The number of observations is large, and the agreement between the values in the separate crosses is close. The probable error (± .288) of the

TABLE 1

Experiment	Total	Crossovers	Percent
Repulsion ($Cp \times cP$)	3142	444	14.13 (± .418)
Coupling ($CP \times cp$)	3331	503	15.1 (± .417)
Repulsion (F_2).....	276	38	13.76 (±1.396)
Total	6749	985	14.59 (± .288)

total value is extremely small for linkage determinations. The personal error is small with such easily distinguishable characters, and the method of taking records within twenty-four hours after birth has probably reduced the possible error arising from selective depletion of young after birth. In the latter part of the experiments a study of this last-named source of error was made in order to determine whether it had altered the linkage value by affecting the distribution of light and dark young. A brief discussion of this study will show that it has not seriously altered the results.

c. Depletion of litters

It has been noticed by several experimenters with rodents that the size of the litter is subject to a certain reduction from birth until the young animals are weaned. A quantitative study of such reduction or elimination has been made by DETLEFSEN and ROBERTS (1918) and my results bear out their general conclusion. Although a part of this reduction is due to infant mortality, accident and malnutrition, the more important cause is the destruction of young by older animals in the pen, spoken of in the present paper as depletion. The young are eaten either by the male, by other pregnant females in the pen, or by their own mother because of annoyance or overcrowding by other animals. This is due almost entirely to the usual method of breeding several females, (in these experiments three) to one male, and allowing the young to be born in the breeding-pen. Isolation of pregnant females in separate pens has been found to eliminate almost entirely the loss from depletion. To study its effect in these experiments certain mothers from the coupling series were isolated when pregnant. One-hundred and forty-five litters born of these mice in isolation averaged 6.02 young per litter while 145 control litters born in breeding-pens averaged 4.47 young per litter. Has this depletion altered in any way the distribution of the classes of young expected? The only way in which the distribution could be altered is by discrimination on the part of the mother between light-eyed

and dark-eyed young, although it is possible that certain character combinations have greater survival value than others. Depletion unaccompanied by discrimination or difference in survival values should affect both light- and dark-eyed classes alike. Data on this point are found in the comparative distributions of isolated (undepleted) litters and breeding-pen (depleted) litters. Of 874 young born in isolation pens 40.3 percent were dark- and 59.7 percent were light-eyed (pink or albino). Of 649 young born in breeding-pens 42.8 percent were dark- and 57.2 percent were light-eyed. Although the distributions are quite similar it appears that the smaller (dark-eyed) class has been depleted somewhat less than the larger light-eyed class. If we consider the effect of such depletion on the number of crossovers, it is apparent that in a larger number of litters such differential depletion has not been operative.

For the 649 young born in breeding-pens the percentage of crossovers observed is 14.3 percent; for the 874 young born in isolation this value is 19.04 percent. The effect of depletion seems to be to reduce the number and percentage of crossovers. The crossover value is however a function of the number of dark-eyed animals produced, since the darks constitute in this experiment (coupling) one-half the non-crossovers. Any cause which tends to increase the proportion of darks, therefore, tends also to reduce the proportion of crossovers. If the differences caused by depletion in the two series of litters given above are real and not due to random sampling, the differences should persist when larger numbers are considered. Let us examine the operation of depletion on 3142 young born in the reciprocal experiment (repulsion series). All animals in this experiment were born in breeding-pens and all litters were subject to depletion. According to the previous data the proportion of light-eyed young should be lowered and the proportion of dark-eyed young raised. Since the darks here constituted one-half the crossovers any cause tending to increase the proportion of darks should increase also the proportion of crossovers. Yet the percentage of crossovers for these depleted litters (14.13) (repulsion series) is practically the same as the percentage for the depleted control litters (14.3) of the coupling series and the same cause should operate in opposite directions in the two series. Where the numbers are small, differential depletion of light-eyed young appears significant, yet where the numbers are large, such a significance disappears. It seems probable, then, that differential depletion has not seriously affected the gross result, and that its appearance in small samples was due to random sampling.

d. Crossing over in both sexes

Records were kept throughout the experiments of the number of crossovers produced by heterozygous males and females respectively. These records are summarized briefly in the following table.

TABLE 2

Repulsion experiment ($Cp \times cP$)	Non-crossovers Cp — cP	Crossovers CP — cp	Total	Percentage of crossovers
F_1 ♂♂ ($CcPp$) × cp	1542	230	1772	12.98 (±.612)
F_1 ♀♀ ($CcPp$) × cp	1155	214	1369	15.63 (±.661)
Coupling experiment ($CP \times cp$)	CP — cp	Cp — cP		
F_1 ♂♂ ($CcPp$) × cp	1638	273	1911	14.28 (±.539)
F_1 ♀♀ ($CcPp$) × cp	1190	230	1420	16.19 (±.652)

Here is conclusive evidence that crossing over takes place in both sexes. Mice, therefore, are to be added to the list of animals in which this phenomenon occurs, which now includes the rat (*Mus norvegicus*), the grouse locust (Apotettix) as well as certain plants, e.g., *Primula sinensis* (ALTENBURG 1916).

The evidence for mice as given above seems to indicate that crossing over takes place more frequently in oögenesis than in spermatogenesis, since relatively more crossover gametes are produced by the F_1 females than by the F_1 males. This difference is of doubtful significance, however, for it is only slightly over three times its probable error. This may be seen from a retabulation of the data in table 2.

TABLE 3

	Non-crossovers	Crossovers	Total	Crossover percentage (p)	E_p	Difference	Difference / $E_{\text{Difference}}$
All F_1 ♂♂	3180	503	3683	13.65	±.381	2.26±.593	3.81
All F_1 ♀♀	2345	444	2789	15.91	±.466		

Applying three times the probable error as a test of a significant difference is a rigid criterion and does not exclude the possibility of some significance. It does, however, make the significance appear doubtful.

e. Variations in crossover percentages

In all work on linked genes considerable variation in linkage values has been noted between individuals and families as well as between ani-

mals of different age and sex and those in different environments. In these experiments the environmental conditions and the feeding were to a considerable degree constant and uniform. It is doubtful whether in warm-blooded animals temperature could have an important effect on the behavior of the chromosomes in gametogenesis, beyond an inhibitive effect of very high or very low temperatures on ordinary breeding activities. Concerning the effect of age only very general statements can be made for no record was kept of exact age of parents at the time that litters were born, and the addition of new young parents to the breeding-pens to replace casualties prevented a close study of this effect. However, in general, there was a very slight and probably insignificant increase in the amount of crossing over from the beginning of the experiments to their completion. Animals were discarded after the climax of their breeding capacity had been reached so it is not known whether old age would have brought about any change in amount of crossing over.

The variation of individual mice and of different pens in amount of crossing over was considerable but this is inevitable where one individual can furnish in its lifetime so small a sample of the whole result. The amount of crossing over was found to vary for individual fathers from 0 to 50 percent for progenies of over 20 young each. Since crossing over is approximately equally frequent in both sexes this variation is probably similar for individual mothers. The same males which had given low or high crossover percentages with certain females did not maintain this percentage with other females. In this as in other linkage determinations these individual differences are probably due rather to the error of sampling than to genetic differences in amount of crossing over, and the results *in toto* are none the less reliable because of the presence of small individual variations.

Linkage of red-eye and albinism in rats

The discovery of linkage between the genes for red-eyed yellow and albinism in rats by CASTLE and WRIGHT (1915) and CASTLE (1916) has provided the material for the experiments here reported, which are in continuation of the work of the above authors. The rapid spread of a serious infestation of parasites caused the practical discontinuance of the previous experiments with rats and has seriously hampered the present investigation.[3]

[3] The parasite responsible for most of the trouble has now been identified as a minute sarcoptid mite, *Sarcoptis notoedres* (Bourg. and Delaf) var. *muris* (Megnin) which infests the extremities of the rat, chiefly ear, nose and tail. The presence of

In CASTLE's last report (1919) a very close linkage between the genes for red-eye and albinism was indicated and more data on this linkage was sought. The original cross of red-eyed yellow (*CCrr*) × albino (*ccRR*) has been repeated and a large first generation has been raised consisting entirely of dark-eyed, dark-coated animals (*CcRr*). These have been inbred and have produced a second generation of 1494 young distributed as follows:

TABLE 4

	Dark-eyed	Red-eyed	Albino	Total	χ^2	P
Observed ..	737	395	362	1494		
Expected (*c* and *r* independent)	840.38	280.12	373.5	1494	61.09	.0000
Expected (*c* and *r* completely linked) ...	747	373.5	373.5	1494	1.72	.433

The actual distribution is much closer to that expected on an hypothesis of complete linkage than it is to the proportions expected if *c* and *r* are independent. The hypothesis of independence is in fact excluded by the data. If a very small amount of crossing over has taken place, this

this parasite is first noted from the appearance of the pinnae of the ears, near the margin of which may be seen minute swellings in the form of tubercles. These are the nests of eggs which have been laid by the female mite. They soon hatch and the young larvae in enormous numbers begin feeding on the substance of the pinna. They literally eat their way in toward the skull, sucking out the liquid constituents and leaving behind them a mass of dead dry tissue. At this time the ear is characteristically scabby and withered in appearance. The infestation soon spreads to the tail and nose and causes almost entire cessation of breeding activities in the infested rats. The disease is not immediately fatal although it shortens the life of the infested rats and since breeding ceases infested stocks soon die out. Various dips were tested in an attempt to control the spread of the parasite. The most helpful of these was a solution of sodium fluoride (1½ ounces); powdered sulphur (4 ounces); yellow soap (2/3 ounce); warm water (2 gallons). Young rats dipped in this solution at the time of weaning are seldom infested; older rats may sometimes be cured by repeated dippings at intervals of ten days or two weeks, although the older animals are often weakened by the parasite and succumb after dipping. The only satisfactory method was found to be individual applications to the infested parts of warm vaseline containing two percent carbolic acid and enough powdered sulphur to make a workable paste. Although the spread of parasites was never absolutely controlled, the stock was kept in fairly good condition by dipping all rats when weaned and generally once thereafter, killing all cases of bad infestation, sterilizing all pens and using the salve treatment on animals which it was necessary to save.

should result in a slight deficiency in the dark-eyed class and a corresponding excess in the red-eyed class, as explained by CASTLE (1919). Both of these conditions are realized in the data, confirming the previous conclusions from tests of F_2 animals.

The red-eyed F_2 class should normally consist of rats of genotypes *CCrr* and *Ccrr* but if *c* and *r* are linked the genotype *Ccrr* could only be formed by crossing over in the gametes of F_1. Similarly the F_2 albinos should normally consist of genotypes *ccRR, ccRr* and *ccrr,* while if *c* and *r* are linked the last two classes could occur only as a result of crossing over. To measure the frequency of crossing over, F_2 reds were therefore tested by mating them with albinos; and F_2 albinos were tested by mating them with red-eyed yellows. Any F_2 red-eyed animal which, when tested in this way, produces albinos, and any F_2 albino which produces red-eyed young, must have developed from an F_1 crossover gamete. Out of 135 F_2 animals thus tested, 130 produced only dark-eyed young (4 or more) while 5 produced dark-eyed and light-eyed (red-eyed or albino) young. The last named young were due to crossing over in 5 F_1 gametes. The total number of F_1 gametes responsible for the production of the 135 tested animals is 2×135 or 270. The crossover gametes (5) are 1.8 ($\pm$ .54) percent of the total gametes and this may be regarded as a preliminary estimate of the linkage strength between the genes for red-eye and albinism in rats.

Arrangement of three linked genes in rats

With quantitative data on crosses involving three linked genes in rats now available, we are in a position to represent graphically the relationships of the genes *c* (albinism), *r* (red-eyed yellow), and *p* (pink-eyed yellow). *Cr* (ruby-eyed dilution) has been shown to be an allelomorph of albinism and is evidently to be placed at the same locus with *c*. Assuming that the "distance" between genes is proportional to the amount of crossing over between them, the albino locus evidently occupies the end or zero position in this system of three. Its crossover value with red-eye is 1.8 $\pm$ .54 percent with pink-eye 21.1 $\pm$ 2.92 percent, while the red-pink crossover value is 18.3 $\pm$ .38 percent. Considering the probable errors of the values involved, the mathematical relations of these three characters are such that the "distance" albinism-red (1.8) plus red-pink (18.3) equals approximately the distance albinism-pink (21.1). To satisfy this relation we may assume that the locus *"r"* is between the loci *"c"* and *"p"* and on the same straight line with them.

Graphically the arrangement of the three linked genes in the chromosome may be plotted to scale as follows:

c	*r*		*p*	*p'*
0	1.8		20.1	21.1

The most reliable value, that for *r*—*p* was laid off first; then the next most reliable value (*r*—*c*) was laid off to the left of *r*. Using *c* as a base the value *c*—*p* (the least reliable) was measured along *r*—*p*. This gave the position *p'* which was found to be to the right of *p*, an actual error of 1 unit. But a point can have but one locus. Therefore either *cp'* is too long, or *cr* is too short, or the theory of linear arrangement does not fit the case. The first of these possibilities is the most probable cause of the error since the distance *r*—*p* is subject to but a small probable error and since the linear arrangement is within the probable errors involved.

The above is essentially the arrangement assumed by MORGAN, STURTEVANT, *et al.* (1915) for the linked genes of Drosophila and the present evidence from mammals appears to provide confirmation of the conclusions already drawn from insect data.

More work is necessary before the rat data can be regarded as forming a *critical* test of the linear arrangement hypothesis. In the discussion above I have used only the new data on the amount of crossing over between *c* and *r*. If to these data there are added CASTLE'S (1919) earlier figures for this linkage (one crossover out of 434) the *c*—*r* value becomes .85 ± .23 which on the linear hypothesis increases the actual error of *c*—*p* from 1 to nearly 2. If, then, the three genes *c*, *r* and *p* in rats are linearly arranged, the crossover value *c*—*p*, when larger numbers are obtained, will be found to be less than the present figures.

The occurrence of crossing over now makes possible the production of a race recessive in both red-eye and albinism, and when such rats are crossed with animals heterozygous in these genes (*CcRr*) a method of measuring the amount of crossing over directly and on a large scale will be available. Such a doubly recessive race is now in process of production.

Comparative frequency of crossing over in males and females

No direct data on comparative frequency of crossing over in the two sexes are provided by these results. Dr. CASTLE has, however, kindly given me access to his data on the linkage of pink-eye and red-eye in rats. This evidence is presented in condensed form in table 5.

TABLE 5
Comparative frequency of crossing over in male and female rats.

Non-cross-overs	Cross-overs	Total	Crossover percentage (p)	E_p	Difference	$\frac{\text{Difference}}{E_{\text{Difference}}}$
All F_1 ♂ ♂ 1742	321	2063	15.56	±.538	4.90±.75	6.51
All F_1 ♀ ♀ 2134	549	2683	20.46	±.525		

Applying the probable error of the difference in linkage values as a criterion, the greater frequency of crossing over in the female is significant. In mice where crossing over was also found in both sexes, the frequency was somewhat greater in the female than in the male. In mice, however, the difference was of doubtful significance, since it was only slightly greater than three times its probable error. In rats the difference is greater, and is equivalent to about 6.5 times its probable error. The difference is in the same direction in both rats and mice, for in both animals it is the female which produces the larger proportion of crossover gametes. From this it may follow that oögenesis differs from spermatogenesis in the frequency with which the hypothetical exchange of genes takes place.

The occurrence of crossing over in both males and females is characteristic of the only two mammals in which linkage has been studied. This fact deserves special mention, and one might be tempted to conclude that sexual differences in crossing over vary on a quantitative scale in different animals. Thus in some animals crossing over never occurs in the male; in others it occurs very rarely in the male; while in others it occurs regularly but less frequently than in the female. On the other hand we have examples of its occurrence exclusively in the male, and all of these conditions are met with in fairly closely related orders of insects, Diptera, Orthoptera, and Lepidoptera. The same may prove to be true of mammals. Therefore no wide significance should be attached as yet, to the fact of crossing over in both sexes in mammals.

The important point is the occurrence of differences in crossing over in the two sexes, for the logical *a priori* expectation is similarity. The behavior of the autosomes is apparently similar in both sexes during gametogenesis. This is witnessed by such genetic evidence as the data from the Drosophila gynandromorphs which show that the sexes do not differ in autosomal characters, each sex receiving a similar complement of autosomes by apparently similar methods. No crossing over in one sex appears, then, to be the anomalous condition which needs explanation.

It was formerly thought that no crossing over was a peculiarity of that sex which was heterozygous for the sex-chromosome. In Orthoptera, however, the cytological evidence shows that the male is the heterozygous sex and recently crossing over has been reported in male locusts (NABOURS 1919). Likewise in rats and mice the male is apparently heterozygous for the sex element, and the genetic evidence shows that crossing over is independent of this fact. It is more or less apparent why crossing over of sex-linked genes does not occur in the heterozygous sex, for here the sex-chromosome has probably no exact homologue, though even here it may be found that the Y or zero chromosome may carry characters and take part in synapsis. But the fact remains that no crossing over or less crossing over in the autosome cannot be explained by reference to the sex chromosome alone.

The explanation of such puzzling differences is probably attainable only by cytological methods and evidence. The chromosomes appear to be so intimately concerned with linkage and with crossing over that one would naturally look for sexual differences in the structure or functioning of these bodies to explain such sexual differences in crossing over. Such a comparison provides an interesting and difficult problem for cytologists.

SUMMARY

1. The localization of genes in mammals is discussed and possible reasons are given for the comparative infrequency with which linkage has been found in this class.

2. The crossover value between the genes for pink-eye and albinism in mice is estimated to be approximately 14.5 percent based on 6749 observations.

3. Post-natal depletion of litters appears not to have seriously affected the crossover value.

4. Crossing over is found to occur in both sexes in mice, with approximately equal frequency, although there is some evidence that it takes place slightly oftener in oögenesis than in spermatogenesis.

5. The crossover value between red-eyed yellow and albinism in rats is found to be approximately 1.8 percent based on 270 tested gametes. When the present figures are added to those obtained earlier by CASTLE, this crossover value is found to be about .8 percent on 704 tested gametes.

6. The linkage relations of the genes for albinism, red-eye and pink-

eye in rats indicate that these genes are arranged in a linear order in one chromosome.

7. Evidence from a large series of observations by CASTLE, is analyzed, and it is found that in rats crossing over occurs in both sexes, but somewhat more frequently in the female. The sexual difference, measured by the probable error, is significant. It is suggested that since this difference appears in both rats and mice as well as in other animals, it may be due to sexual differences not yet discovered in either the structure or functioning of the chromatin.

LITERATURE CITED

ALTENBURG, E., 1916 Linkage in *Primula sinensis*. Genetics **1**: 354-366.

CASTLE, W. E., 1914 Some new varieties of rats and guinea-pigs and their relation to problems of color inheritance. Amer. Nat. **48**: 65-73.

1916 Further studies of piebald rats and selection with observations of gametic coupling. Carnegie Inst. Washington Publ. 241, pp. 175-180.

1919 Studies of heredity in rabbits, rats and mice. Carnegie Inst. Wash. Publ. 288, 56 pp.

CASTLE, W. E., and LITTLE, C. C., 1909 The peculiar inheritance of pink eyes among colored mice. Science N. S. **30**: 313-314.

CASTLE, W. E., and WRIGHT, S., 1915 Two color mutations of rats which show partial coupling. Science, N. S. **42**: 193-195.

CUÉNOT, L., 1907 L'Hérédité de la pigmentation chez les souris. Arch. Zoöl. Exp. et Gén. **6**: 1-13.

DARBISHIRE, A. D., 1904 On the result of crossing Japanese waltzing with albino mice. Biometrika **3**: 1-51.

DETLEFSEN, J. A., and ROBERTS, E., 1918 On a backcross in mice involving three allelomorphic pairs of characters. Genetics **3**: 573-597.

DURHAM, F. M., 1908 A preliminary account of the inheritance of coat color in mice. Rept. Evol. Comm. IV. p. 41.

HALDANE, J. B. S., SPRUNT, A. D., and HALDANE, N. M., 1915 Reduplication in mice. Jour. Genetics **5**: 133-135.

HALDANE, J. B. S., 1919 The probable errors of linkage values and the most accurate method of determining gametic from certain zygotic series. Jour. Genetics **8**: 291-297.

MORGAN, T. H., STURTEVANT, A. H., MULLER, H. J., and BRIDGES, C. B., 1915 The mechanism of Mendelian heredity. 262 pp. New York: Henry Holt & Co.

NABOURS, R. K., 1919 Parthenogenesis and crossing over in the grouse locust, Apotettix. Amer. Nat. **53**: 131-142.

ONSLOW, H., 1915 A contribution to our knowledge of the chemistry of coat colour in animals and of dominant and recessive whiteness. Proc. Roy. Soc. B **89**: 36-58.

WHITING, P. W., and KING, H. D., 1918 Ruby-eyed dilute gray, a third allelomorph in the albino series of the rat. Jour. Exp. Zoöl. **26**: 55-64.

WRIGHT, S., 1916 An intensive study of the inheritance of color and of other coat characters in guinea-pigs, with especial reference to graded variations. Carnegie Inst. Washington Publ. 241, pp. 59-160.