INDEPENDENT GENES IN MICE*

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INTRODUCTION

One of the most fruitful hypotheses of recent research in genetics has been that connected with the localization of the hereditary determiners or genes. Under the name of the chromosome hypothesis of heredity this idea has undergone a rapid development. The residence of genes in the chromosomes is now well established, the main data in support of this theory having come from the experimental study of the linkage relations between genes for Mendelian unit characters. The study of the topography of the germinal material of as many species as possible is a necessary forerunner of a unified knowledge of heredity, and to this end it is advisable that the relationships between known unit characters be thoroughly investigated. Several cases of linkage between genes for color variations in rats and mice are now known and consequently we have some positive knowledge of the localization of certain genes in these species. We may widen this knowledge by the investigation of the relationships between other genes. Even if no additional linkages are discovered we may be informed by the evidence thus accumulated that

certain genes are not linked with any others and are therefore presumably determined each in a separate chromosome. The experimental evidence to be presented here is drawn from investigations of this nature on the inheritance of several genes for color variations in mice.

The linkage of the gene for pink-eye with the gene for albinism in mice (CASTLE 1919, DUNN 1920 a) established the identity of one chromosome. Using this as a base it was considered important to find out whether any other color characters were determined in this chromosome. Either pink-eye or albinism could be used as a reference character, and crosses of either of these with other characters should discover whether certain other genes were located in this chromosome or should be referred to other chromosomes. For this purpose it was decided to use pink-eye (p) rather than albinism because its presence as well as the presence of other somatic characters with which its relation was to be tested is immediately discernible, while albinism does not allow the genotypic content to express itself because albinos develop neither color nor pattern.

THE RELATION OF PINK-EYE AND PIEBALD

The first character tested for location in the albino chromosome was piebald or recessive spotting, for which the symbol "s" is used.[1] Its normal dominant allelomorph is self (symbol S). Piebald was selected because there was no significant amount of data on the relations of pink-eye and piebald. Pink-eye had shown no linkage with agouti, yellow, black or brown in the data of LITTLE (1913), LITTLE and PHILLIPS (1913), and DETLEFSEN and ROBERTS (1917), and the numbers involved were large, so that it was not thought necessary to repeat the experiments.

A description of the character piebald in mice may be found in the works of CUÉNOT (1902 to 1911) and LITTLE (1915), and an account of its variability by the author (DUNN 1920 b). A brief description will therefore suffice for our present purposes. Piebald mice are spotted with white on a ground of any color. The white areas vary considerably in extent from a small ventral patch covering 12 percent or more of the belly to a condition in which the dorsal surface is 50 percent white

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[1] The established symbol for piebald spotting has been "sp" (LITTLE 1915). It has been considered better to denote this character by only one letter, following the more general custom of Mendelian nomenclature; especially since the occurrence of "sp" in formulae containing "p" (pink-eye) is likely to be confusing to the reader.

and the belly 85 percent white. The distribution of the white areas follows no such definite pattern as the Dutch markings of belted cattle or the Dutch spotting of rabbits, with which characters the piebald of mice is in other respects quite similar. An approach to a belt of white about the center of the body and a "white face" or "blaze" condition is often seen, due perhaps to modifiers of the central gene for piebald. The gene for piebald determines only the spotted condition as contrasted with self, while the amount and distribution of the spotting appears to be determined by other modifying genes not yet analysed (LITTLE 1917, DUNN 1920 b). When piebald is crossed with self only self mice result, with an occasional very small spot of white. Such mice interbred, produce on the average three self mice to one piebald, showing that piebald differs from self in one cleanly segregating character.

The stocks available at the beginning of the experiment were dark-eyed piebalds ($PPss$) and pink-eyed self mice ($ppSS$). The genes p and s therefore entered the experiment separately, in different parents. Matings were made between dark-eyed spotted males and pink-eyed self females and an equal number of matings was made between dark-eyed spotted females and pink-eyed self males. The first generation from these reciprocal crosses was the same in both cases. It consisted of 557 self mice, all dark-eyed with the exception of 52 which came from dark-eyed spotted parents heterozygous in the gene for pink-eye ($Ppss$). The dark-eyed self F_1's were presumably of genotype $PpSs$ and these heterozygotes were mated *inter se* to produce the second generation. The second generation consisted of a total of 774 animals whose distribution is given below compared with the distribution expected if the genes p and s assort independently in the heterozygote.

TABLE I

F_2 generation from cross of pink-eyed self (pS) × dark-eyed piebald (Ps).

	Dark-eyed self (PS)	Pink-eyed self (pS)	Dark-eyed piebald (Ps)	Pink-eyed piebald (ps)	Total
Observed	436	145	150	43	774
Expected	*435.4*	*145.1*	*145.1*	*48.4*	*774*

$\chi^2 = .14496$ P = approximately I[2]

[2] The mathematical criterion used for judging goodness of fit of an observed to a hypothetical distribution is that of PEARSON ($\chi^2 = \Sigma \dfrac{(o-c)^2}{c}$) aided by ELDERTON'S values of P corresponding to values of χ^2. The "expected" or hypothetical distribution is in each case that required by independent (non-linked) assortment of genes.

The actual distribution is extremely close to the expected and apparently the F_1 heterozygotes formed gametes PS, pS, Ps and ps in numbers so nearly equal that the chance that p and s are linked is practically zero. Nevertheless a further test for even an extremely small amount of linkage was made by mating the F_1 heterozygotes ($PpSs$) to the double recessive pink-eyed piebalds ($ppss$) obtained in the second generation.

If p and s are independent the heterozygote $PpSs$ should form equal numbers of four sorts of gametes, PS, ps, Ps and pS which if united with ps gametes from the double recessive, should produce equal numbers of the four possible combinations of pink and piebald. This cross actually produced 1172 young falling into the following distribution as compared with the distribution expected.

TABLE 2

Back-cross of F_1 [from cross of pink-eyed self (pS) $\times$ dark-eyed piebald (Ps)] to pink-eyed piebald (ps).

	Dark-eyed self (PS)	Pink-eyed self (pS)	Dark-eyed piebald (Ps)	Pink-eyed piebald (ps)	Total	
Observed	291	315	308	258	1172	$\chi^2 = 6.61$
Expected	*293*	*293*	*293*	*293*	*1172*	$P = .087$

The distribution observed deviates widely from that expected if p and s are independent. Its salient features are an excess of self mice (606) over piebald (566); of dark-eyed (599) over pink-eyed (573) and the consequent deficiency of the pink-eyed piebald class. This class was deficient also in the F_2 distribution and it is probable that these departures from expectation are due to one cause, viz., the grading of certain pink-eyed piebalds as selfs, especially since the pink-eyed self class exhibits the greatest excess. The contrast between white and colored spaces is sometimes very slight on young pink-eyed mice, especially when dilution is present. Some piebald mice are spotted only on the belly and as this region is usually lighter in color, the contrast is even less and a few errors would be likely to occur.

The new combinations (crossovers) number 549 while the grandparental combinations (non-crossovers) number 623.[3] One of the non-

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[3] If this inequality be explained by linkage the crossover value may be computed as 46.84 percent which is subject to a probable error of $\pm$ .97 (cf. HALDANE 1919, and DUNN 1920 a). The difference between this crossover value and the normal value of 50 percent, expected if no linkage occurs, is 3.16 which is 3.2 times the probable error. Since the departure from expectation appears to be largely due to errors in grading it is not regarded as sufficient to exclude the hypothesis of independence of p and s.

crossover classes, pink-eyed self, has probably been factitiously enlarged at the expense of the crossover class pink-eyed piebald, while the other non-crossover class is more nearly normal. These facts considered in connection with the normal distribution of the second generation, which according to HALDANE is, in the case of repulsion, nearly as sensitive a test of linkage as the back-cross, make it seem probable that no linkage occurs between the genes for pink-eye and for piebald.

RELATION OF PINK-EYE AND NON-AGOUTI

Another relationship of the gene for pink-eye was tested incidentally in the crosses reported above, and the data may be added to the evidence already obtained on this point by LITTLE and PHILLIPS (1913), and DETLEFSEN and ROBERTS (1917). Certain F_1 mice ($AaPp$) were mated with mice recessive in both of these genes ($aapp$), viz., pink-eyed blacks or browns. From such crosses four types of young are expected, and if a and p are independent these types should be equally numerous. The observed and calculated distributions follow.

TABLE 3

Back-cross of F_1 [from cross of pink-eyed agouti (Ap) × dark-eyed non-agouti (aP)] to pink-eyed non-agouti (ap).

	Dark-eyed agouti	Pink-eyed agouti	Dark-eyed non-agouti	Pink-eyed non-agouti	Total	
Observed	248	234	240	213	935	$\chi^2 = 2.87$
Expected	*233.75*	*233.75*	*233.75*	*233.75*	*935*	P = .40

The distribution obtained conforms fairly closely to that expected if a and p are independent genes. The number of new combinations (crossovers, AP and ap) is 461 which is very nearly equal to the number of grandparental combinations (non-crossovers, Ap and aP) which is 474. Evidently the distribution as regards linkage only fits the hypothesis of independence. There is however a marked deficiency in the double recessive class, pink-eyed non-agouti. This appears to be due to the fact that the total agoutis (482) outnumbered the total non-agoutis (453) while the total dark-eyed mice obtained (488) outnumbered the total pink-eyed mice (447). This same phenomenon was in evidence in the larger series of young from this cross recorded by DETLEFSEN and ROBERTS. The same cause to which these authors ascribed the distorted ratio appears to have been operative in the present experiment, namely, differential mortality in the litters between birth

and the time of recording young (two weeks) resulting in a greater survival of agouti and of dark-eyed young.

<center>RELATION OF NON-AGOUTI AND PIEBALD</center>

Other incidental data have been extracted from the data dealing with the inheritance of pink-eye and piebald. The original cross was between agouti self (AS) mice and non-agouti piebalds (as). The genes a and s should then show coupling in the F_1 heterozygotes if there is linkage between a and s. Heterozygotes from this cross were agouti and self ($AaSs$). These were mated with non-agouti piebalds ($aass$). From this cross issued the following distribution:

<center>TABLE 4</center>

Back-cross of F_1 [from cross of agouti self (AS) $\times$ non-agouti piebald (as)] to non-agouti piebald (as).

	Agouti self (AS)	Agouti piebald (As)	Non-agouti self (aS)	Non-agouti piebald (as)	Total	
Observed	221	179	183	200	783	$\chi^2 = 5.60$
Expected	195.75	195.75	195.75	195.75	783	P = .13

The fit of the observed distribution to that expected if a and s are not linked is not good. It is not, however, so poor as to exclude the interpretation of the results on the basis of independent random assortment of the two genes concerned. The frequencies of the observed classes deviate from the normal in a definite direction. The original combinations (non-crossovers, agouti self and non-agouti piebald) are in excess of the new combinations (crossovers, agouti piebald and non-agouti self); actually these classes are as 421 : 362. This distortion does not appear to have been due to any serious inequalities between the agouti and non-agouti classes for these are nearly equal 400 : 383 (expected 391.5 : 391.5). Nor is the aberrancy due to serious excess of selfs over piebalds. Actually these classes are as 404 : 379, and the discrepancy is equally distributed between crossover and non-crossover classes. The salient feature is undoubtedly the greater frequency of the original combinations over the new, and if this discrepancy is significant, it indicates linkage between non-agouti and piebald.

To measure the significance we may assume provisionally that a and s are linked, and calculate the crossover value from the distribution observed. The crossover classes As and aS total 362 which is 46.23 percent of the total gametes formed by F_1 (783). This value has a prob-

able error of $\pm$ 1.20. If a and s are not linked the crossover and non-crossover classes should be equal, that is, the crossover value should be 50 percent. The deviation of 46.23 percent from 50 percent is 3.1 times the probable error. Applying YULE's criterion that three times the probable error indicates a significant deviation, the departure from expectation in the present case may be significant. Apart from purely mathematical measures of significance, the following facts point to linkage. The classes agouti and non-agouti occur with equal frequency as expected; an error in grading some piebalds as selfs would in this case increase equally one non-crossover class and one crossover class so that such an error if it occurred would be compensatory. On the other hand, there are considerations which should make us chary of stating from these data that linkage occurs between non-agouti and piebald. The data were gathered for another purpose and not with the primary intention of testing the relationships between non-agouti and piebald. The large class in the distribution is agouti self which may have a greater survival value than other classes. Finally from a mathematical standpoint the number of the animals bred is small and the linkage value is close to 50 percent and has a large probable error, while the method of measuring a significant deviation by thrice the probable error is at best approximate. Therefore it is thought best to hold the conclusion in abeyance until more animals are added to the distribution. All crosses bearing on this point are being continued and it is hoped that additional data will be available soon.

NON-AGOUTI—ALBINO CROSSES

A few crosses were made in repetition of a cross reported by HAGE-DOORN (1914) who bred agoutis heterozygous for agouti and albinism $(AaCc)$ to albinos which lacked the agouti factor $(aacc)$. HAGEDOORN recorded only agoutis $(AaCc)$ and albinos $(aacc)$ from this mating and assumed in explanation that a and c were completely linked showing in this case complete repulsion. LITTLE (1914) in a criticism of HAGE-DOORN's interpretation pointed out that the results obtained appeared, in the light of other experiments, to be anomalous. I have repeated HAGE-DOORN's experiment using mice of the same genetic constitution as those used by him. The double heterozygote $(AaCc)$ mated to the double recessive $(aacc)$ has produced to date 14 agoutis (AC), 10 non-agoutis (aC), and 22 albinos $(Ac$ and $ac)$. The expectation if a and c are independent (11.5 agoutis: 11.5 non-agoutis: 23 albinos) is hence closely approximated, whereas on HAGEDOORN's hypothesis no agoutis should be

produced. This evidence is small in numbers but sufficient to show that *a* and *c* are not completely linked and that HAGEDOORN's hypothesis is not applicable.

THE RELATIONSHIPS OF THE GENE FOR BLACK-EYED-WHITE WITH OTHER GENES IN MICE

The data considered in the ensuing paragraphs deal for the most part with the variety of mice known as black-eyed-whites, in which pigment occurs in very limited amounts including usually only eyes, head, and rump while the rest of the pelt is white. Superficially, some of these mice resemble albinos, for the spotting may be so extreme as to produce an entirely white mouse, but somatically and genetically this variation is distinct from albinism, for the eyes are always colored, while the eyes of albinos are pink and entirely devoid of pigment; further LITTLE has found that the gene for black-eyed-white is not allelomorphic with that for albinism but separate and distinct.

After some preliminary work in this variation by CUÉNOT (1903-1911), Miss DURHAM (1908), and MORGAN (1909), LITTLE (1915) succeeded in analysing it. Black-eyed-white spotting he found to be a distinct variation and not merely the extreme of a continuous series in which piebald and self were the darker members. Crossed with piebald, black-eyed-white produces only black-eyed-white and piebald young in equal numbers; crossed with black-eyed-whites like itself, it produces black-eyed-white and piebald in the ratio of 2 : 1; crossed with self mice it produces two types in equal numbers. One of these types is spotted with white in an irregular and varying distribution; the other type is self or nearly so. The first of these LITTLE has called type "A" and the second is his type "B." From such experimental data LITTLE concluded that the black-eyed-white variation was due to a gene independent of and supplementary to the gene for piebald spotting. In crosses this gene behaved as the dominant allelomorph not of piebald or of self, but of something which can only be defined as not-black-eyed-white. To represent the gene for black-eyed-white LITTLE used the symbol *"W,"* and for its normal allelomorph *"w."* Black-eyed-whites have been found not to breed true. They always give both black-eyed-whites and piebalds. They are "unfixable" heterozygotes, like yellows and must be represented by *Ww.* They are however, homozygous for piebald which LITTLE represented by the symbol *"sp"* ("*s*" in the present notation"). The formula for black-eyed-white is then in full *Wwss.* When mated to-

gether, chance combinations of their gametes should produce some zygotes pure for W. LITTLE (1919) later found evidence that such zygotes were formed but died *in utero* and hence were missing from the animals born. This heterozygous nature of black-eyed-whites explains why two types of young are produced in crosses with self. The spotted young (type A) are due to a union of the gamete containing W with the self gamete and are genetically $WwSs$. Both gametes produced by the black-eyed-white contain s, because type A interbred has produced black-eyed-white, type A, piebald and self, while type B interbred has produced only self and piebald, and has proved to be an ordinary heterozygote between piebald and self (Ss).

The relation of black-eyed-white spotting and piebald spotting

Interest in the relationships between these alternative conditions of coat patterns in mice leads naturally to the question, If black-eyed-white, piebald and self are not mutually allelomorphic, how are they related? The evidence is conclusive (*vide supra*) that piebald and self are allelomorphic; that is, that these alternative conditions are determined at the same locus in the same chromosome. Where with reference to this locus are we to place black-eyed-white? If at the same locus, W and s (or S) could never enter the same gamete. But since in the cross black-eyed-white $\times$ self, W and s, and W and S are known to have entered the same gamete, this cannot be true. W, therefore, is **determined at** a different locus. If this is in the same chromosome with the S—s locus, then W and s, when entering the cross together, should tend to remain together, and when entering separately, should tend to remain separate. In other words, these two genes should give evidence of being linked. If in different chromosomes, W and s should assort independently and give no evidence of linkage. The critical test of this relationship is the cross of black-eyed-white with self. Here W and s enter the F_1 zygote to form type A, which we may represent as $WwSs$. To test the gametes formed by this zygote it should be mated to the double recessive, piebald ($wwss$). If W and s are independent, F_1's of type A should form four types of gametes in equal numbers, (1) one with both W and s, (2) one with neither W nor s, (3) one with W and lacking s, (4) one with s and lacking W. If W and s are linked, types 3 and 4 can only be formed by crossing over and should then be less numerous than 1 and 2. When this back-cross is made four types of young are produced as follows: black-eyed-whites, type A, piebald and self. Black-eyed-whites and selfs are easily differentiated and form the theoretical

non-crossover classes. Type A and piebald are subject to considerable overlapping, are hard to distinguish, and taken together, should give the total crossovers. My figures for this distribution are as follows:

TABLE 5

Black-eyed-white $(Wwss)$ × *self* $(wwSS)$. *Test cross of* F_1 *type A* $(WwSs)$ × *piebald* $(wwss)$.

	Black-eyed white $(Wwss)$	Piebald $(wwss)$ Type "A" $(WwSs)$	Self $(wwSS)$	Total	
Observed	114	218	117	449	$\chi^2 = .416$
Expected	*112.25*	*224.5*	*112.25*	*449*	P=practically 1

Mathematically, the fit of the actual distribution to that expected if W and s are independent genes, is extremely good, and excludes any other interpretation for the relationship of these genes. It follows that the loci of the genes for these two kinds of spotting in mice are presumably in different chromosomes.[4] This forms an interesting contrast with the conditions obtaining in rabbits, as recently reported by CASTLE (1919). In rabbits the genes for English (dominant) spotting and for Dutch (recessive) spotting appear to be properties of a single locus. CASTLE'S evidence shows them to be either allelomorphic or completely linked. The piebald spotting of mice and the Dutch spotting of rabbits appear to be homologous variations similar in appearance and in genetic cause. The black-eyed-white spotting of mice and the English spotting of rabbits are different in appearance and this difference is reflected in the distinctness of the genes by which they are determined. The fact that both are dominant forms of spotting is thus seen to be an entirely superficial resemblance.

Investigation of types of spotting

Before proceeding to the study of relationships of the gene for black-eyed-white with other genes it was found necessary to study the variability of the various types of spotted mice, in order that the ranges of each type of spotting might be established and that animals resulting from crosses of black-eyed-white with other forms might be classified correctly. This study showed that there was more variability in the expression of the gene for black-eyed-white than had been thought pre-

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[4] These are essentially the conclusions indicated by LITTLE's (1917) data. The present work is not intended merely as supplementary to his but has a distinct object, the investigation of possible linkages in mice.

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viously. It was found, for instance, that black-eyed-white mice may exhibit nearly as much pigment as piebalds, and that the heterozygotes containing the genes for black-eyed-white, piebald and self (type A) are indistinguishable somatically from piebalds and in some cases from selfs. The results of this study have only an indirect bearing on the localization of genes and are therefore presented in a separate report (DUNN 1920 b). All mice bred in the course of the experiments treated hereafter were carefully graded and classified according to the principles established by the study referred to above.

The relation of black-eyed-white spotting and agouti

LITTLE (1917 b) has presented evidence indicating the independence of the genes for black-eyed-white and yellow. This relationship may be tested either by crosses of black-eyed-white with yellow or by crosses involving black-eyed-white and agouti, since agouti , non-agouti and yellow are allelomorphs and should bear the same relationships to other genes. The latter method was chosen for the present study since LITTLE by crosses directly involving yellow and black-eyed-white had established the important fact that the lethal element connected with back-eyed-white was distinct in origin and effect from the lethal of yellow, and that consequently litters in which both yellow and black-eyed-white were present, were subject to reduction from two sources, due to the prenatal mortality from both of these lethals. The cross of non-agouti black-eyed-whites with agouti self introduces only one lethal and should therefore produce larger litters.

Crosses were made between pure wild agouti mice ($AASSww$) and non-agouti black-eyed-whites ($aassWw$). All F_1 young were agouti (83). Approximately half (42) were self ($AaSsww$), and half (41) were spotted type A ($AaSsWw$). The spotted young were heterozygous for agouti and for black-eyed-white, having received one gene from one parent and the other gene from the other parent. The gametes formed by this heterozygote should then be as regards a and W, (1) Aw (2) aW (3) AW (4) aw. If A and W are independent, all four sorts of gametes should be equally numerous. If A and W are linked they should in this case show repulsion, resulting in relatively fewer of sorts (3) and (4), for the latter could only be due to crossing over. To discover which of these contrasted hypotheses is the true one, the F_1 "type A" mice ($AaSsWw$) were crossed with mice recessive in both genes, black or brown piebalds ($aasswv$). There resulted six visibly different classes of young, in the following proportions.

TABLE 6

Cross of non-agouti black-eyed-white (aassWw) × agouti self (AASSww); test cross of F₁ type "A" (AaSsWw) × non-agouti piebald (aassww).

	Non-agouti b.-e.-white aW	Agouti spotted AW and Aw	Non-agouti spotted aW and aw	Agouti self Aw	Non-agouti self aw	Total
Observed	41	41	76	41	43	306
Expected	38.25	38.25	76.5	38.25	38.25	306

The mice classified as "spotted" are all similar in appearance to piebalds and type A in amount of spotting, and consist as is known from experiments on spotting alone, of two genetically distinct classes $WwSs$ and $wwss$. The first of these is type A and the second is piebald, but the somatic expression of these genetic combinations is so similar that they cannot be distinguished directly. Therefore they have been classed together. The distinguishable crossovers are represented by only two classes, agouti black-eyed-white (AW) and non-agouti self (aw). The distinguishable non-crossovers are non-agouti black-eyed-white (aW) and agouti selfs (Aw). The sum of the visible crossover classes (84) is found to be approximately equal to the sum of the visible non-crossovers (82). In each of the spotted classes are contained both crossovers $(AW$ and $aw)$ and non-crossovers $(aW$ and $Aw)$ and it must be supposed that these occur in the same frequency as in the immediately visible classes. The total of the "spotted" is 140, composed presumably of 70 crossovers and 70 non-crossovers. The calculated total crossovers are then 84 plus 70 or 154 and the calculated total non-crossovers are 82 plus 70 or 152.

A few crosses were made between non-agouti black-eyed-whites and agouti selfs which carried the gene for piebald. From such crosses resulted a few agouti black-eyed-whites which were heterozygous for agouti and black-eyed-white $(AassWw)$, A and W having entered separately. These lacked the gene for self and therefore offered better material for testing for linkage between A and W since when crossed with non-agouti piebalds only black-eyed-whites and piebalds resulted, and these classes can be immediately distinguished. The frequency of the combinations Aw, aW and aw in the gametes of the heterozygote can therefore be calculated directly from the somatic ratio in which the young are produced.

Such tests produced the following distribution:

TABLE 7

*Cross of non-agouti black-eyed-white (aassWw) × agouti self carrying pie-
bald (AASsWw). Test cross of F₁ agouti black-eyed-white
(AassWw) × non-agouti piebald (aassww).*

	Agouti b.-e.-white AW	Agouti piebald Aw	Non-agouti b.-e.-white aW	Non-agouti piebald aw	Total
Observed	28	27	25	32	112
Expected	28	28	28	28	112

If linkage occurs between A and W the two genes should in this case show repulsion. On this basis the crossovers (AW and aw) number 60 while the non-crossovers (Aw and aW) number 52.

In all the tests reported above the two genes A and W entered the experiments separately (repulsion). To complete the test, crosses were made in which the two genes entered the experiment together, and if they are linked they should in this case exhibit coupling. From the test crosses of animals heterozygous in agouti and black-eyed-white there were produced a number of agouti black-eyed-whites ($AassWw$) which had received A and W from the same parent. These were tested by mating them with non-agouti piebalds. These matings have produced but few young at present but are being continued.

TABLE 8

*Test cross of agouti black-eyed-white (AassWw, A and W enter
together) × non-agouti piebald.*

	Agouti b.-e.-white (AW)	Agouti piebald (Aw)	Non-agouti b.-e.-white (aW)	Non-agouti piebald (aw)	Total
Observed	36	22	22	34	114
Expected	28.5	28.5	28.5	28.5	114

The crossovers in this case are Aw and aW of which there are 44. while the non-crossovers (AW and aw) number 70. In the light of the evidence from the preceding tests the excess of the non-crossovers in the last cross is probably due to the dearth of numbers.

Combining the figures from all test crosses involving both A and W we have the following totals: non-crossovers 274; crossovers 258; total 532. If A and W are not linked these classes should be equal, 266:266. The closeness with which the observations approximate this last expectation indicates that probably no linkage occurs between A and W and that they are hence located in different chromosomes.

The relation of black-eyed-white spotting and pink-eye

Late in the experiments it was decided to test the relationship of the gene for black-eyed-white with that for pink-eye, to determine whether these two genes were linked or independent. DETLEFSEN (1916) had shown that these two genes were distinct and that in the second generation from a cross between them there were produced "pink-eyed-white" mice which carried the color factor. Somatically some of them resembled albinos, but the likeness ended with the pink eyes, since pigment characteristic of pink-eyed mice was present in the same parts which are pigmented in black-eyed-white mice. Genetically these mice were *ppssWw*, while full-colored dark-eyed black-eyed-whites are *PPssWw*. The possible production of such pink-eyed animals resembling albinos or with very small amounts of pale color, required a modification in the plan of the experiments and a test was sought whereby the presence of *W* in a pink-eyed animal might be manifested by some condition other than the black-eyed-white pattern. The cross of a dark-spotted type (*SSWw*) in which *W* produces a small amount of white because acting on self, with piebald (*wwss*) produces two types of offspring. One is type A spotted (*SsWw*) and the other is self (*Ssww*) The inheritance of *W* is hence traceable somatically with a smaller chance of error in classification since only two types are produced. Consequently dark-spotted animals which were also heterozygous for the gene for pink eye (*PpSSWw*) were crossed with pink-eyed piebalds recessive in these three genes (*ppssww*). The genes *P* and *W* had entered the heterozygote together and should hence show coupling if they are linked. This cross should in that case produce relatively more of the combinations *PW* and *pw* (dark-eyed spotted and pink-eyed self) and relatively fewer of the combinations *Pw* and *pW* (dark-eyed self and pink-eyed spotted). If *P* and *W* are independent, equality of these four sorts is expected. The cross has produced young as follows:

TABLE 9

Test cross of mice heterozygous in black-eyed-white and pink-eye
(PpSSWw) × pink-eyed piebalds (ppssww).

	Dark-eyed spotted (*PW*)	Dark-eyed self (*Pw*)	Pink-eyed spotted (*pW*)	Pink-eyed self (*pw*)	Total
Observed	16	21	22	23	82
Expected	*20.5*	*20.5*	*20.5*	*20.5*	*82*

Equality of the four sorts of gametes and hence independence of W and P are indicated. The numbers are however too small to be significant. The total crossovers (wP and Wp) are 43; the total non-crossovers (WP and wp) are 39.

Although it is believed that the cross, the results of which have just been presented, is the proper and most accurate method of testing the relation between black-eyed-white and pink-eye, the results of other tests show that there is less danger of confusion between "pink-eyed whites" and albinos than had been feared. As a matter of fact no albinos appeared in the experiments concerned with this linkage, all the young having a discernible amount of pigment in the fur. Therefore the results of other crosses involving W and P are sufficiently accurate to be included in the totals.

Dark-eyed animals of type A ($PpSsWw$) had resulted from crosses of pink-eyed white ($ppssWw$) with dark-eyed self ($PPSSww$). The genes W and P had then entered the heterozygote separately and if linked should show repulsion. The young produced by matings of such heterozygotes with the double recessive, pink piebald ($ppssww$) are given in the first line of the following table. Similar tests were made using as the double heterozygote type A's which had received W and P from the same parent (coupling). The results of this cross are given in the third line of the table.

TABLE 10

Tests of type "A" mice heterozygous in W and P ($PpSsWw$) $\times$ pink-eyed piebalds ($ppssww$).

	Black-eyed white (*PW*)	Pink-eyed white (*pW*)	Dark-eyed spotted (*PW* and *Pw*)	Pink-eyed spotted (*pW* and *pw*)	Dark-eyed self (*Pw*)	Pink-eyed self (*pw*)	Total
P and W enter together	9	5	20	11	8	9	62
Expected	*7.75*	*7.75*	*15.5*	*15.5*	*7.75*	*7.75*	*62*
P and W enter separately	11	4	18	9	7	10	59
Expected	*7.37*	*7.37*	*14.76*	*14.76*	*7.37*	*7.37*	*59*

The immediately distinguishable non-crossovers in the first mating are PW and pw, total 18; the crossovers are Pw and pW, total 13. The occurrence of these combinations in the "spotted" classes may be assumed to be in the same proportions (18:13) and the 31 spotted may

then be divided roughly into 18 non-crossovers and 13 crossovers. Similarly in the second mating the observed non-crossovers Wp and wP are 11, and the observed crossovers are 21. The calculated non-crossovers in the "spotted" classes are 9 and the calculated crossovers are 18.

One other series of matings remains which may be added to the above totals. This consisted of test crosses of black-eyed-whites carrying the gene for pink-eye ($PpssWw$, W and P entering separately) with pink-eyed piebalds ($ppssww$).

TABLE 11

Test cross of black-eyed-white heterozygous in P and W (enter separately) $\times$ pink-eyed piebald.

	Black-eyed white (WP)	Pink-eyed white (Wp)	Dark-eyed piebald (wP)	Pink-eyed piebald (wp)	Total
Observed	21	21	17	21	80
Expected	20	20	20	20	80

Here all classes are immediately distinguishable. The non-cross-overs (Wp and wP) number 38 and the crossovers (WP and wp) number 42.

Adding together the numbers of non-crossovers and crossovers from all the matings:

TABLE 12

	Non-crossovers	Crossovers	Total
W and P enter together	39	43	82
	36	26	62
W and P enter separately	20	39	59
	38	42	80
Totals	133	150	283

The totals obtained are too small to afford a final test of the hypotheses of linkage or independence of W and P. The appreciable excess of crossovers nevertheless creates a strong presumption in favor of regarding these two genes as independent.

SUMMARY

The object of the experiments here reported was to determine through an experimental investigation of linkage relations, the loci of certain genes for color variations in mice.

The following results have been obtained.

1. Pink-eye and piebald spotting segregate from the heterozygote at random in a total of 1946 observations. Their genes are probably not linked but are located in different chromosomes.

2. Additional evidence is added to that already extant, confirming the independence of pink-eye and non-agouti.

3. Evidence comprising 783 observations indicates a slight linkage 46.23 percent ± 1.2 between the genes for piebald spotting and non-agouti. The departure of the observed ratio from that expected if these genes are independent is however of doubtful significance.

4. Black-eyed-white spotting and piebald spotting are neither linked nor allelomorphic, but are properties of distinct loci in different chromosomes. This conclusion, following a preliminary and similar one by LITTLE, is based on 449 direct observations.

5. Black-eyed-white and agouti segregate purely at random and show no evidence of linkage in 532 observations.

6. Black-eyed-white and pink-eye in a short series of data (283 observations) appear to be due to genes independent in heredity and probably located in different chromosomes.

7. Positive evidence is presented which indicates that HAGEDOORN's hypothesis of complete linkage between non-agouti and albinism is either erroneous or applicable only to his data as a special case.

CONCLUSIONS

No new cases of linked genes in mice have been added to the one already investigated, viz., albinism and pink-eye. Other genes which the evidence indicates are *not* located in the albino chromosome are (1) non-agouti, and therefore its allelomorphs agouti, yellow and light-bellied agouti; (2) piebald spotting; (3) black-eyed-white spotting. The probabilities are that albinism and pink-eye constitute one group; the agouti allelomorphs are the only certain representatives of a second to which possibly piebald may also belong; while a third is represented only by black-eyed-white spotting, and a fourth by piebald spotting (if it proves not to be linked with agouti). Thus four groups of genes are provisionally established, representing four of the 19 autosomes of mice. The work of other investigators (LITTLE, PHILLIPS, DETLEFSEN and ROBERTS) showed that black and brown may constitute a fifth group and that dilution is probably not linked with agouti, black or pink-eye. The position of dilution with reference to other genes, and the position of waltzing are still to be determined.

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