LINKAGE OF A DILUTE COLOR FACTOR AND DARK-EYE IN MICE¹

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Received December 24, 1923

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INTRODUCTION

In a recent paper, the senior writer described the occurrence of an extremely dilute mutant in the house mouse, which proved to be allelomorphic to both color and albinism (DETLEFSEN 1921). Multiple color-allelomorphs had been described in the guinea-pig (wild or intense color, light dilution, ruby-eyed dilution and Himalayan albinism); in the rat, (wild color, ruby-eyed dilution and albinism); and in the rabbit (wild color, ruby-eyed dilution or so-called chinchilla, Himalayan albinism and complete albinism). WRIGHT (1923) recently added a fifth color mutant (dark dilution) in the guinea-pig series. The paucity of such color allelomorphs in the house mouse stood out in contrast to the other rodents. Although it had been bred by fanciers and investigators for many years, color and albinism were the only two members of the series known. *A priori*, there was no apparent reason why mutations similar to those discovered in other rodents should not occur in mice; and it was therefore of some interest to discover this additional form in this series. Curiously enough, a second

¹ The data were collected by both authors at the College of Agriculture, UNIVERSITY OF ILLINOIS. The senior author is alone responsible for this manuscript and any views or shortcomings it may contain.

additional member of this series in the house mouse appeared at about the same time in the cultures of one of my students (FELDMAN 1923), this last form resembling the ruby-eyed dilute guinea-pig and rat, and the chin-chilla rabbit rather more closely than my mutant. The four forms discovered up to the present time in mice represent a set of quadruple allelomorphs and are arranged in the following scale of dominance: wild or intense color (C), ruby-eyed dilution (c^r), extreme dilution (c_d), and complete albinism (c^a). The first is dominant to the other forms, but incomplete dominance is found in connection with the interrelationships among the last three. The experiments described in this paper deal with the third form, extreme dilution (c^d) in relation to intense color (C), albinism (c^a), dark-eye (P), and pink-eye (p). Since it is allelomorphic to intense color, it should likewise show linkage to dark-eye (P). This mutation $\frac{c^d P}{c^d P}$ occupies, in a scale of dominance, an intermediate position between color and albinism, and therefore gives a coupling series when mated to albinos carrying pink-eye ($F_1 = \frac{c^d P}{c^a p}$), but a repulsion series when mated to pink-eyed colored individuals ($F_1 = \frac{c^d P}{C p}$).

The original mutant, ♂ 1, and two of his male mutant grandsons, ♂ 3 and ♂ 46, gave rise to 1950 descendants in three types of matings as follows:

TYPE 1. *Matings to pink-eyed brown-spotted females.* From such matings, ♂ 1 produced 8 intense offspring (all wild gray). His grandson, mutant ♂ 3, produced 39 brown agoutis and 36 pink-eyed brown agoutis, and was therefore homozygous in agouti and brown, and heterozygous in dark-eye. His color was fully as dark as that of ♂ 1, thus indicating that the brown and black forms of the mutant may be indistinguishable. The other grandson, ♂ 46, produced 4 wild grays and 4 brown agoutis. The total of 91 intense-colored offspring from these matings proved conclusively that intense color was completely dominant over the extremely dilute color of the mutant. These offspring entered into the experiments on repulsion, for their zygotic formula was obviously $\frac{c^d P}{C p}$.

TYPE 2. *Matings to albinos carrying pink-eye.* The original mutant ♂ 1, was used in a number of such matings, and produced 57 mutant offspring. Such offspring showed pigmented eyes at birth, the pigment being collected largely in a ring around the inner margin of the iris. The

hair was at first white or almost white, but assumed a faint trace of color as the animals grew older. However, dominance was not complete, and the young were as a rule more lightly pigmented than the original homozygous mutants. Their zygotic formula was obviously $\frac{c^d P}{c^a p}$ and they were used in later experiments on coupling.

TYPE 3. *Matings to homozygous blacks.* The original mutant, ♂ 1, produced from such matings 13 agoutis of intense color. Their formula was $AaCc^d$, and when mated *inter se* gave 40 offspring classified at birth as 29 intense to 11 dilute,—well within reasonable limits of error for a monohybrid ratio. At the age of two weeks, 36 survivors were classified as 17 agoutis : 9 blacks : 10 mutants, where 20.25 : 6.75 : 9.00 is the calculated result on the basis of a 9 : 3 : 4 ratio. The experimental results were therefore consistent with the hypothesis.

These three types of mating also show that the original mutant was homozygous in agouti, black, self-pattern and dark-eye, and was therefore exactly like the wild form from which it arose except that it carried a recessive mutant color gene (c^d) in place of intense color (C).

COUPLING

Male and female double heterozygotes, $\frac{c^d P}{c^a p}$, which had received color dilution and dark-eye from the mutant parent and albinism and pink-eye from the other parent, were back-crossed to double recessives, $\frac{c^a p}{c^a p}$. This type of mating produced individuals of four genotypes and two phenotypes as follows:

$c^d P$	$c^a p$	$c^a P$	$c^d p$
Dark-eyed dilute	Pink-eyed white		
Parental classes		Crossovers	

All pregnant females were isolated; and since the phenotypes could be and were classified at birth, *postnatal* elimination did not enter as a distorting element in these data on crossing over. One parental class, the dilute mutants, showed pigmented eyes at birth. The other parental class and the crossovers were pink-eyed whites and were always indistinguishable except by a breeding test. Since the crossovers could not be counted

directly, their probable number was estimated in the customary way used in this general type of cross, as, e.g., in the linkage of color and dark-eye in mice, or red-eyed yellow and pink-eyed yellow in rats (CASTLE 1919, DUNN 1920). The usual way is to double the visible parental class (the dark-eyed) and subtract this from the total. The difference given by this indirect method is obviously equal to the difference between the dark-eyed and pink-eyed at birth; for one parental class plus all crossovers minus the other parental class leaves crossovers. The difference obtained by either method approximates the number of crossovers, if there has been no marked *prenatal* elimination. While this conventional procedure assumes that the frequencies of the two parental classes are approximately equal, it also implies tacitly that any random deviations of the two parental classes are always in the same direction, as will be shown later; and this exaggerates the calculated crossover value in either a plus or minus direction giving the appearance of wider deviation in crossover value than one might reasonably expect. The random fluctuations of the two parental classes are, to be sure, just as likely to be opposite in direction. If *prenatal* elimination does take place, the pink-eyed phenotype is more likely to suffer, and the net effect is to reduce the calculated number and percentage of crossovers in the total classified.

Table 1 gives the total results of the coupling series. Taken as they stand, the data indicate coupling of color dilution and dark-eye, as we should expect; but they fail to show as high a crossover value in both sexes or the same degree of consistency which DUNN (1919) found in his

TABLE 1

SOURCE OF DATA	DARK-EYED DILUTE	PINK-EYED WHITE	TOTAL	CALCULATED CROSSEVERS	PERCENTAGE OF CROSSEVERS
F ₁ ♂ ♂	329	369	698	40	5.73 ± 2.549
F ₁ ♀ ♀	194	245	439	51	11.62 ± 3.197
Total	523	614	1137	91	8.00 ± 1.994

series for color and dark-eye. Our problem, therefore, narrows down largely to discovering whether the difference is due to a functional difference of mechanism or can be attributed reasonably to external and non-genetic factors peculiar to these crosses, or is perhaps associated with the method of determining the number of crossovers. DUNN's data suggested that coupling in the males (14.28 ± 0.539) and in the females (16.19 ± 0.652) was not significantly different; for the difference (1.91 ± 0.846) was 2.26 times its probable error. If we use in our data the *same*

formula for the probable error of a calculated crossover value, the males (5.73 ± 0.593) and the females (11.62 ± 1.032) appear to differ by 5.89 ± 1.190 with respect to their crossover values;—from which we might conclude that the zygotes classified cannot be regarded as random samples of the same type of population without serious objections and special explanations, for the difference is 4.95 times its probable error. However, our data do not prove a functional difference between the sexes. The routine determination of probable errors of such calculated crossover values by the usual formula, $E = K \sqrt{\frac{pq}{n}}$, must not be taken too seriously,

since the use of this formula in these cases rests on an erroneous assumption. There are two ways of determining whether the results from F_1 males and F_1 females in table 1 are significantly different, namely:

(1) We may disregard the question of hypothetical crossover values, and simply ask ourselves whether the percentages of dark-eyed are significantly different, i.e., whether the ratios of $\frac{329}{698}$ and $\frac{194}{439}$ can be regarded as random samples of the same type of population. The F_1 males gave 47.13 ± 1.27 percent dark-eyed, while the F_1 females gave 44.19 ± 1.60 percent dark-eyed. The difference is 2.943 ± 2.043 . It is clear that there is no significant difference, in spite of the fact that the F_1 males and females appear to differ by 5.89 percent in calculated crossover value (2×2.943 percent for obvious reasons).

(2) If we prefer to discuss the results in terms of linkage values, we cannot use the usual formula for the probable error of simple sampling, $E = K \sqrt{\frac{pq}{n}}$. (HALDANE'S (1919) formula for the probable error of a

linkage value is the same well-known formula expressed in different terms.) The conventional method of arriving at a calculated crossover value in this type of cross has a peculiar consequence which cannot be neglected, for it assumes that random deviations of the two parental classes lie in the same direction. The effect is to exaggerate wide deviations in the calculated crossover value, giving wider deviations than we might at first suspect. If the frequency of each parental class is r and of each crossover class is s , the distribution is theoretically $r+r+s+s=T$, and the calculated crossover value is $\frac{T-2r}{T} = \frac{2s}{T}$. Now, if through

sheer fluctuations of sampling the r dark-eyed become $r \mp n$, then the $r+2s$ pink-eyed become $r+2s \mp n$, since we are dealing with correlated

variables. With a fluctuation of $\pm n$ individuals, therefore, the proportion of calculated crossovers becomes $\frac{2s \mp 2n}{T}$, and the calculated percentage of crossovers is lowered or raised by $\frac{2n}{T} \times 100$. This can perhaps be illustrated best if we assume purely for expositive purposes an arbitrary case as follows: We have an average degree of linkage between *C* and *D* (color and dark-eye) which gives 14.29 percent crossing over (about the value found for dark-eye and color) and the F_1 ($\widehat{CDcd}$) produces 100 offspring in which theory calls for 85.71 of the parental types and 14.29 crossovers distributed in the following proportions:

<i>CD</i>	<i>cd</i>	<i>Cd</i>	<i>cD</i>
6	6	1	1
<hr/>		<hr/>	
Dark-eyed		Pink-eyed	

Since the last three genotypes are indistinguishable we actually classify 42.86 percent as one parental class and 57.14 percent as a common phenotype, if we happen to obtain the most probable expectation; and taking the difference we purport to find 14.29 percent crossing over. Now the probable error in a curve of sampling $(\frac{3}{7} + \frac{4}{7})^{100}$ would be ± 3.34 ; and since it is safe to assume that random deviations of $\pm 3E$ lie within reasonable limits of error in sampling, we might well find ratios of dark-eyed to pink-eyed as low as or as high as $42.86 \pm 3E : 57.14 \mp 3E$; or specifically,

(1) 52.87 : 47.13, i.e., -5.74 percent crossing over

(2) 32.85 : 67.15, i.e., 34.30 percent crossing over.

Thus we might easily arrive at calculated crossover values 20.02 percent

($= \frac{2n}{T} \times 100$, or $\frac{2 \times 3E}{T} \times 100$) below and above the expected 14.29 percent,

or a range of about 40 percent in crossover values, simply because the method assumes that the deviations of the two parental classes lie in the same direction. But if we had actually classified crossovers instead of computing their probable number, we should have expected 14.29 percent with ± 7.08 percent as possible deviations lying within similarly reasonable limits of error. Naturally, as the total classified (*T*) becomes larger, the difference between calculating crossovers and actually classifying them grows less. But it is interesting to note that with numbers even as large as 1000, we might obtain through sheer fluctuations of sampling,

crossover values from 7.95 percent to 20.62 percent, or a range of 12.67 percent, when we calculate the crossover value by this conventional method. Nevertheless, we should expect a range of only 4.46 percent if we were actually able to count the crossovers directly. The method may, therefore, give calculated crossover values which *apparently* lie far outside of reasonable limits of error, if we use the usual formula, which is really inapplicable to cases of this type. Hence, wide deviations do not have the same weight, as if crossovers were counted directly; and we should allow ourselves wider limits of error in dealing with chance deviations in any argument as to what constitutes a significant difference or certainty. If we must allow ourselves wider limits of error in dealing with these calculated crossover values, it follows that the usual formula for the error

of a crossover value, $E_c = K\sqrt{\frac{pq}{n}}$, is inappropriate. The roundabout method of obtaining the calculated crossover value has perhaps obscured its real meaning. To double the number of dark-eyed, subtract this from the total, and then convert the difference into a percentage of the total (see CASTLE 1919, p. 31, and DUNN 1920, p. 333) is clearly equal to subtracting the percentage of dark-eyed from pink-eyed. For, if m = the number of dark-eyed, n = number of pink-eyed, and $S = m + n$, then $\frac{S-2m}{S} \times 100 = \frac{100n}{S} - \frac{100m}{S} = \frac{100(n-m)}{S}$. The calculated crossover value

is then a simple difference and its probable error is that of a difference. It is an ancient theorem that the standard deviation of a variable, d , which is the difference between two component variables, A and B , is

$$\sigma_d = \sqrt{\sigma_A^2 + \sigma_B^2 - 2r_{AB}\sigma_A\sigma_B} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

Since, under ordinary conditions there is no correlation between A and B , the formula is usually shortened to

$$\sigma_d = \sqrt{\sigma_A^2 + \sigma_B^2}, \quad . \quad . \quad . \quad . \quad . \quad (2)$$

but it is never safe to assume that $r_{AB} = 0$ in any particular case. In this particular case it happens that the percentage of dark-eyed, $\frac{100m}{S}$, and the pink-eyed, $\frac{100n}{S}$, show perfect negative correlation since they are arithmetical complements. Therefore the probable error of the calculated crossover value in coupling is the error of the difference between $\frac{100n}{S}$

when $2s$ and T are positive and $2s$ is less than T , as is always the case in this type of distribution. In fact, if n becomes greater than $2s$ (when elimination removes more pink-eyed individuals than there are crossovers) the apparent crossover value becomes negative.

A closer analysis of the data and the circumstances involved in collecting them shows why the offspring of F_1 males might be eliminated more readily than the offspring of F_1 females. In testing the F_1 males by back-crosses we are compelled to use the less vigorous double-recessive females as dams; but in testing the F_1 females with double-recessive males, the vigorous F_1 female hybrids were themselves the dams. This difference in vigor between the two types of dams is reflected in the average size per litter,—in the former case 4.78, but in the latter, 5.48. Such differences in vigor and fertility may have included, as a necessary consequence, some prenatal competition for survival in which the dark-eyed mutants had a distinct advantage over the complementary pink-eyed parental class or the crossovers. The data of our repulsion series (table 3) as well as our general experience in handling the stocks used in these experiments support the contention that the dark-eyed are more vigorous, at least in early post-natal life.

Examining the distribution of calculated crossovers in relation to litter size, we find a distinct difference in the percentage of dark-eyed in litters of less than five and in litters of five or more, when the F_1 males were sires and the double recessives were the dams (table 2). Using the conventional method we should conclude that 37 of the total 40 crossovers

TABLE 2

SOURCE OF DATA	SIZE OF LITTER	DARK-EYED DILUTE	PINK-EYED WHITE	TOTAL	CALCULATED CROSSOVERS	PERCENTAGE OF CROSSOVERS
F_1 $\sigma^7 \sigma^7$	Less than 5	71	108	179	37	20.67 ± 4.933
	5 and over	258	261	519	3	0.58 ± 2.961
F_1 $\varphi \varphi$	Less than 5	50	58	108	8	7.41 ± 6.472
	5 and over	144	187	331	43	12.99 ± 3.676
	Less than 6	93	120	213	27	12.68 ± 4.584
	6 and over	101	125	226	24	10.62 ± 4.461

in table 1 occurred in the smaller litters, and that the crossover value was 20.67 ± 4.933 percent. But in litters of five and over the number of crossovers was estimated as 3; i.e., only 0.58 ± 2.961 percent crossing over in a total of 519 offspring. The difference between these two crossover values (or between the ratios of $\frac{71}{179}$ and $\frac{258}{519}$) is 3.492 times the probable error.

We are therefore justified in suspecting some selective prenatal elimination, but perhaps not as much as the crossover values themselves might suggest on a cursory examination. In the litters from F_1 females, however, no such striking inequalities occur (see table 2).

As tables 1 and 2 show, the problem of any wide deviation in our ratios from such as DUNN found, is associated largely with litters from F_1 males. The *smaller* litters from the F_1 males may represent either (1) less fertility on the part of the dams, or (2) the residue after rigid prenatal selection, or (3) both in part. If the first condition prevails entirely uncomplicated, there should be no modification of crossover value. Normally small litters would in themselves offer even less occasion for complications due to selective elimination, because, other things being equal, there would be less competition; and we should expect, theoretically perhaps, a fair approximation to the average exchange value found in the case of color and dark-eye. But such smaller litters as represent the residue left after selective prenatal elimination would contain a relatively large number of dark-eyed mutants since these represent the more vigorous zygotes. This would have the effect of apparently lowering the calculated number of crossovers, inasmuch as the difference between the dark-eyed and the pink-eyed at birth is taken as an index of the number of crossovers. The total results found in small litters as a whole would hinge upon the relative frequencies in which these conditions obtain. The *larger* litters may represent (1) those litters which are normally large and have withstood all or almost all elimination by some fortunate sequence of causes; and (2) those litters which remain normally large because they are selected samples containing a larger proportion of the more vigorous and viable dark-eyed mutant zygotes. Had they been samples of the same size but with more pink-eyed zygotes, they would have suffered some elimination of this less viable class. The former conditions would either leave the crossover value unchanged, or lower it according to the amount of elimination. The second condition would lower the crossover value. The net result in larger litters would depend on the balance of these alternative conditions. If our analysis of the conditions which play upon the litter size in these crosses is correct, the result should throw light upon the operation of these conditions. In general, the males gave much more variable results and it will be recalled that they were mated to the weaker double-recessive dams. Dividing their litters into those above and below the average, we find, as stated, no reduction in the smaller litters but a marked lack of calculated crossovers in the larger litters (table 2). It is true that the results in small litters are perceptibly higher than DUNN's

total results; but in view of the method used to calculate crossovers, deviations in crossover value due to sheer sampling can be greater than we might at first expect if we apply the theory of errors to the calculated percentage of crossovers as it stands. The conclusion would be that the smaller litters have suffered at least little or no elimination of pink-eyed zygotes, while the larger litters have undergone some selective process in the form of direct elimination of pink-eyed or the survival of samples with fewer pink-eyed. Either process would lower the calculated crossover value in the larger litters. Somewhere close to the lower limit of relatively large litter size, we should anticipate the cumulative effect of both differential viability and selective sampling, giving the greatest reduction in crossover value. And consistent with this view we found, in litters of six, 103 dark-eyed dilutes to 84 pink-eyed, which gives a calculated crossover value of -10.16 percent, in place of $80 : 107 = 14.29$ percent as we might expect. The result is inconsistent with any present view on crossing over unless elimination or the double effect of two selective processes comes into play at about this point, as hypothesized.

The F_1 females gave more uniform results. The total results (table 1) show a crossover value of 11.62 percent, which is lower than DUNN found for color and dark-eye (16.19 percent). If elimination of the pink-eyed types was the cause of the general decrease in crossover value, it was not as variable a type of elimination as the F_1 males seemed to show (table 2), for the crossover values in litters above and below average litter size do not show such large differences. As stated above, the F_1 dams were more vigorous than the double-recessive females with which the F_1 males were mated, and in this fact we have at least one reason for less intensive elimination.

The variations in calculated crossover values which are due to random sampling are naturally just as likely to occur in excess as in defect; but variations due to direct or differential elimination of the pink-eyed class always give a lowered crossover value. Comparing the two types of modification, it is noteworthy that in any given total (T) of zygotes formed a random fluctuation which gives n pink-eyed less than the calculated $2r+s$ is more effective in reducing the calculated crossover value, than is a direct elimination of n pink-eyed zygotes in the total (T); for $\frac{2s}{T} > \frac{2s-n}{T-n} > \frac{2s-2n}{T}$ under the conditions we find in this type of linkage

experiment. It is therefore difficult to determine in any single experiment whether an individual low crossover value is due to a relatively smaller fluctuation or due to a relatively larger selective elimination. In the light of these observations, we may conclude safely that elimination has played some essential part in reducing our crossover values, because,

- (1) All values in tables 1 and 2, with a single exception, are reduced; and
- (2) Some of the values are so low they fall outside of reasonable limits of error, and thus suggest selective elimination.

REPULSION

Male and female double heterozygotes, $\frac{c^d P}{C p}$, which had received the mutant gene (color dilution) and dark-eye from the mutant parent and intense or wild color with pink-eye from the other parent, were back-crossed to double recessives $\frac{c^a p}{c^a p}$. This type of mating produced individuals of four classes as follows:

$\frac{c^d P}{\text{Dark-eyed dilute}}$	$\frac{C p}{\text{Pink-eyed intense}}$	$\frac{c^d p}{\text{Pink-eyed dilute}}$	$\frac{C P}{\text{Dark-eyed intense}}$
Parental		Crossovers	

All pregnant females were isolated as in the coupling series, and the young were classified at birth with respect to eye-color. In this repulsion series, however, (unlike CASTLE's and DUNN's repulsion series for dark-eye and albinism *versus* pink-eyed and intense color) our birth records gave no direct or indirect hint of the linkage value. Theoretically, there were two groups of equal frequency, a dark-eyed and a pink-eyed. Each group included a parental and a crossover class. Out of a total of 564 born, 557 were classified at birth as 293 dark-eyed and 264 pink-eyed. The result is not inconsistent with the hypothesis that the two classes are equal at birth, and therefore gives no conclusive evidence of prenatal elimination. If this is so, then our repulsion series differs from our coupling series. The real difference between dark-eyed and pink-eyed may, nevertheless, be greater, and yet partly obscured by a fluctuation of sampling; for, there is always the possibility of a reasonably wide plus deviation in the pink-eyed zygotes at fertilization. The elimination may therefore be greater than is apparent. Furthermore, just as in the case of coupling, the F_1

males were mated to double-recessive females and gave an average of 4.40 per litter (101 litters); but the F_1 females gave 5.43 per litter; although the latter average is based on rather small numbers (21 litters). This difference may again represent prenatal competition and elimination. Of the 557 classified at birth, 83 died within 10 days, of which 61 were pink-eyed and 22 were dark-eyed. Evidently the pink-eyed were in any event less viable in early postnatal life. The remaining 474 were completely identified as to genotype when 10 to 14 days old, and are recorded in table 3.

TABLE 3

SOURCE OF DATA	DARK-EYED DILUTE	PINK-EYED INTENSE	PINK-EYED DILUTE	DARK-EYED INTENSE	CROSS-OVERS	TOTAL	PERCENTAGE OF CROSSOVERS
F_1 ♂ ♂	195	136	22	23	45	376	11.97 ± 1.129
F_1 ♀ ♀	45	33	9	11	20	98	20.41 ± 2.746
Total . . .	240	169	31	34	65	474	13.71 ± 1.066

The data of table 3 show linkage between color dilution and dark-eye. The results in the males (11.97 ± 1.129 percent) and in the total (13.71 ± 1.066 percent) are not significantly different from those of DUNN working with intense color and dark-eye. The number of offspring from F_1 females was small and the crossover value (20.41 ± 2.746 percent) was large; but the result as it stands is not unquestionably different in a statistical sense from DUNN's result, or our results in the F_1 males. The probable errors in this repulsion series were calculated from the usual formula, and not by the formula used in our coupling series. In DUNN's case of dark-eye and intense color, however, it seems to me that the probable errors should be calculated in the same way as in our coupling series, since the number of crossovers is assumed to be twice the number of dark-eyed observed at birth. Here again one implies that the random deviations in the two crossover classes lie in the same direction. In the case of coupling the difference between the dark-eyed and pink-eyed gives the crossovers. In repulsion this difference gives the non-crossovers. Since the probable error of the percentage of non-crossovers is the same as that of the crossovers, the probable error of the percentage of crossovers may also be looked upon as the error of the difference between the percentage of dark-eyed and pink-eyed, always remembering that they show complete negative correlation, or we may simply look upon the percentage of crossovers in repulsion as twice the percentage of dark-eyed, which is tanta-

mount to saying that the percentage of crossovers is the sum of two equal variables completely correlated. In both cases the probable error is twice the error of either attribute, dark-eyed or pink-eyed.

The total results of table 3 show the marked postnatal elimination of the pink-eyed intense. The 409 parentals were divided into 240 dark-eyed dilute and 169 pink-eyed intense, whereas we expect 204.5 of each class. The deviations are much larger than we would reasonably expect. Curiously enough, if the pink-eyed intense were as numerous as the dark-eyed dilute the percentage of crossovers would be *lowered* to 11.93 percent, which, however, is not an unusual deviation from the theoretical value of about 14.29 percent established by DUNN's data.

In passing, it should be recorded that the pink-eyed form of the mutant heterozygous for albinism $\frac{c^d p}{c^a p}$, was indistinguishable from the true albino.

This gives three distinct forms of mice which may have identical somatic characters,—true albinos, the pink-eyed form of black-eyed white (DETLEFSEN 1916), and these pink-eyed dilute-colored.

SUMMARY

Color dilution and dark-eye are linked in mice. The crossover values are in general lower than those found by DUNN for color and dark-eye.

Elimination of the pink-eyed forms takes place, and is probably responsible in part for the reduced crossover values.

A revision of the usual method for calculating probable errors is suggested in cases of this type, where the presumptive total number of crossovers is derived from a single visible parental class or a single visible crossover class.

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