ON A BACK CROSS IN MICE INVOLVING THREE ALLELO-
MORPHIC PAIRS OF CHARACTERS¹

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INTRODUCTION

According to current conceptions in genetics, any two Mendelian factors may bear to each other one of the following relationships: allelomorphic, independent, or linked. The relationship presumably depends upon the locus of the factors in a particular pair of chromosomes. Numerous instances of the first two types of relationship have been found in mammals. Many data bearing on linkage in *Drosophila*, silkworms, sweet peas, snapdragons and stocks, have prompted the search for linkage in mammals. Up to the present time, the following cases of linkage in mammals have been shown or suggested: the red-eye and pink-eye factors in rats (CASTLE and WRIGHT 1915, 1916), the red-eye and color factors in rats, the pink-eye and color factors in rats (CASTLE and

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WRIGHT 1916), and the pink-eye and color factors in mice (DARBI-SHIRE 1904; HALDANE, SPRUNT and HALDANE (1916).

There are several reasons why linkage has not been abundantly observed in mammals. Unless linkage is of a rather pronounced type, the numbers available in dihybrid or multihybrid crosses are usually inadequate to disclose the fact. Whenever linkage between two characters is weak, positive association easily escapes our attention; for, as the $n:1:1:n$ gametic ratio of coupling approaches the $1:1:1:1$ gametic ratio of simple Mendelian segregation, it becomes increasingly difficult to distinguish between these two cases. Furthermore, the usual plan of mating the F_1 individuals *inter se* tends to magnify disparity in the size of segregating classes and to obscure linkage, so that it is not seen directly but must be calculated. However, back-crossing the F_1 to the ultimate recessive gives a zygotic ratio which is an immediate index of the gametic ratio, and does not magnify disparity in the size of segregating classes. The former type of mating often gives F_2 classes so extremely large and small that one hesitates to apply any measure of goodness of fit. The advantages of back-crossing are seen in the ease with which coupling is disclosed in sex-linked inheritance in *Drosophila* because requisite back-crossing gives F_2 males which immediately indicate the gametic ratio of heterozygous F_1 females. In the absence of any apparent linkage, we look for Mendelian inheritance,—segregation of allelomorphs with chance recombination of independent factors in gametogenesis and chance fertilization of gametes, subject to fluctuations of sampling. To be sure, we may fail to obtain either clear evidence of coupling or satisfactory Mendelian ratios, and we may then account for the discrepancy on the assumption of selective elimination of certain zygotic classes, although not always altogether convincing.

The purpose of the present study is: (1) to apply a more sensitive test for linkage in the relation of agouti, dark eye, and black to each other by the use of back crosses; (2) to study the effect of elimination upon F_2 ratios involving these characters; and (3) to study fluctuations of sampling. The first two studies are embodied in the present paper, but the third will be dealt with in a subsequent paper.

Three wild gray male mice were bred to 24 pink-eyed intense brown females producing 324 F_1 individuals, of which 268 (126 males + 142 females) lived to be classified as wild gray. The F_1 generation was bred back to the triple recessive parent type, the pink-eyed intense brown. All of the F_1 females were examined at five-day intervals and each pregnant female was removed to a separate pen; hence each litter was born in a

separate pen and there is no doubt as to parentage. Each pregnancy pen was examined daily until the birth record was taken. The young were classified when about two weeks old, and reclassified a week later. Whenever pink-eyed black agouti or pink-eyed brown agouti types were involved, they were reclassified several times to avoid confusing two varieties which may appear similar at an early age but are clearly differentiated at four weeks. All litters from F_1 females can therefore be classified as undepleted or depleted. The undepleted are those in which the total number recorded at birth were classified; but in the depleted, deaths occurred between birth record and classification. The number of full litters is greatly increased by isolating pregnant females. Of 531 litters only 170 showed any depletion. We have made no attempt to subdivide the depleted litters according to the causes of elimination. When elimination is due to destruction by mothers, it is undoubtedly unselective and should have no effect upon the F_2 ratios. But when elimination is due to poor care, or neglect by the dam, or to disease and the like, elimination may be selective with regard to Mendelian classes. Since poor ratios have been attributed to elimination, we made an effort to distinguish between these two types of litters. The possibility exists that some elimination may have occurred between the actual birth and the time of birth record, for it might be supposed that young died and were entirely eaten during this interval. We are inclined to put little weight on this possibility for each pregnant female was nested in clean white paper excelsior, and when young died or were eaten, very evident remains disclosed this fact. The interim between actual birth and birth record was not more than 24 hours, and was usually much less. The short time between actual birth and the time of birth record, the excellent conditions under which births occurred, and the absence of traces of elimination, lead us to believe that depletion is readily detected and undepleted litters are easily recognized. We have been dealing entirely with post-natal elimination. Prenatal elimination also occurs in mice as is shown by the presence of atrophic fetuses in the uterus of pregnant females. Whether or not selection is involved here, we do not know; but the results of our experiment indicate that there is no prenatal selection with regard to the Mendelian classes studied.

In Mendelian symbols the parents are wild gray, *AADD BB*, and pink-eyed brown, *aadd bb*. The F_1 hybrids, wild gray in appearance, are *AaDd Bb*. The symbols have the following significance:

A, the agouti factor, which restricts black or brown pigment in the subapical yellow band in the individual hairs, producing the

ticked or agouti pattern. Its allelomorph, the absence of ticking, is designated by *a*.

D, the factor for the dark or pigmented eye. Its allelomorph is the pink-eyed condition, *d*. A reduction in the amount of black and brown in the hair goes with the pink-eyed condition.

B, the factor for black pigment. Its allelomorph is brown, *b*.

The F_2 generation obtained by back-crossing the F_1 hybrids to the triple recessive should give in equal numbers the following classes:

<i>AaDdBb</i> , black agouti, dark-eyed	<i>AaddBb</i> , black agouti, pink-eyed
<i>AaDdbb</i> , brown agouti " "	<i>Aaddbb</i> , brown agouti, " "
<i>aaDdBb</i> , black, " "	<i>aaddBb</i> , black, " "
<i>aaDdbb</i> , brown, " "	<i>aaddbb</i> , brown, " "

The cross, taken as a whole, is a trihybrid cross, and is dealt with as such in table 3. It is convenient, however, to discuss the results in the following order: monohybrid ratios (table 1), dihybrid ratios (table 2), trihybrid ratios (table 3). Tables 1 and 2 are derived entirely from the ultimate class frequencies of table 3, the data being grouped into monohybrid and dihybrid ratios, a perfectly legitimate procedure inasmuch as we are making the ratios a test against Mendelian theory. Each table contains several ratios, for the class frequencies from four distinct sources are tabulated separately. The four sources of data are indicated in each table and are always given in the following order:

F_1 females giving F_2 young in undepleted litters.

F_1 females giving F_2 in depleted litters.

F_1 males giving F_2 young in stock pens.

F_2 females (triple hybrids) giving F_3 young in stock pens.

Ninety F_1 females produced 531 litters in separate pens, of which 361 were undepleted and 170 were depleted. Two F_1 males, and a number of F_2 females were back-crossed in the stock pens, and the young were classified once a week. No account was taken of elimination between birth and classification. The litters may therefore consist of both depleted and undepleted types. The eight types of F_2 female segregates were likewise tested out in stock pens. With the exception of the F_2 trihybrid wild gray females, these results are tabulated in tables 4 and 5. The total number of segregates classified in all of our experiments is 5566.

It may be stated finally that throughout our experiments the pink-eyed brown (ultimate recessive) is one parent in every mating described

in this paper. If independent allelomorphic pairs are involved, segregating classes should theoretically be of equal frequency. This is an advantage in the use of the formula $\sigma = \sqrt{npq}$ in our experiments. This ideal case, when $p = q = \frac{1}{2}$ and n is large, is not open to the objections frequently raised to its use in many Mendelian ratios. Simply stated, we are always making our ratios a test against a binomial distribution which shows perfect symmetry, and we may therefore make use of the probability integral tables to determine odds against observed deviations without hesitation.

It is misleading to pool data, when subgroups show excessively wide deviation but counterbalance each other. We are not aware of this inaccuracy in our data. We have pooled LITTLE and PHILLIPS'S (1913) data for comparison although such a course is not wholly justified in this case.

MONOHYBRID RATIOS

Since there are three allelomorphic pairs involved, there are three sets of monohybrid ratios; agouti: non-agouti, dark eye: pink eye, and black: brown. These ratios are given in table 1, arranged according to the sources of data, and convenient ways of pooling them. We can state directly that 16 of the 18 monohybrid ratios are entirely consistent with Mendelian theory, for the experimental deviation is relatively small in these 16 cases,—less than 1.9 times the probable error of the binomial distribution on the hypothesis of simple sampling. This means that we may expect a deviation as great or greater than the largest of the 16 about once in five times as a fluctuation of sampling. The exceptional cases are the ratio of agouti to non-agouti in depleted litters and possibly the ratio of dark-eyed to pink-eyed young from F_1 males. It is interesting to note in passing that the ratios show a deviation smaller than the probable error in nine cases, and larger in nine; and that the average of

the sum of all eighteen quotients, $\frac{\text{deviation}}{\text{probable error}}$, is 1.25. We expect unity in the absence of any disturbances preventing the operation of pure chance. If we exclude the poorest ratio (with $\frac{\text{deviation}}{\text{error}} = 4.01$) and all totals because they are duplications, then the average of the 11 quotients for 11 distinct subgroups is 0.99.

LITTLE and PHILLIPS (1913) record a cross between a wild gray male and pink-eyed dilute brown females, in which a large F_2 (1180

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TABLE I
Monohybrid ratios.

Allelomorphic pairs	Offspring obtained from	Frequencies		Calculated frequencies	Deviation	Probable error	Deviation error
Agouti vs. non-agouti	F ₁ ♀ ♀, (undepleted litters)	Dominant	Recessive	1129.5 405.5 1535 207 125 1867	11.5 38.5 27.0 7.0 5.0 39.0	16.03 9.60 18.69 6.86 5.33 20.61	0.72 4.01 1.44 1.02 0.94 1.89
	F ₁ ♀ ♀, (depleted litters)	1118	1141				
	F ₁ ♀ ♀, total	444	367				
	F ₁ ♂ ♂	1562	1508				
	F ₂ ♀ ♀	214	200				
Dark eye vs. pink eye	F ₁ ♂ ♂	130	120	1129.5 405.5 1535 207 125 1867	2.5 6.5 4.0 25.0 2.0 31.0	16.03 9.60 18.69 6.86 5.33 20.61	0.16 0.68 0.21 3.64 0.38 1.50
	F ₂ ♀ ♀	1906	1828				
	F ₁ ♀ ♀, (undepleted litters)	1127	1132				
	F ₁ ♀ ♀, (depleted litters)	412	399				
	F ₂ ♀ ♀	1539	1531				
Black vs. brown	F ₁ ♂ ♂	232	182	1129.5 405.5 1535 207 125 1867	3.5 25.0 5.0 5.0 25.0 45.0	9.60 18.69 6.86 5.33 20.61 35.69	0.36 1.34 0.73 0.94 1.21 1.26
	F ₁ ♂ ♂	127	123				
	F ₂ ♀ ♀	1898	1836				
	F ₁ ♀ ♀, (undepleted litters)	1108	1151				
	F ₂ ♀ ♀	402	409				
Total dominants vs. recessives	F ₁ ♀ ♀, total	1510	1560	1129.5 405.5 1535 207 125 1867	207 125 1867	18.69 6.86 5.33 20.61 35.69	1.34 0.73 0.94 1.21 1.26
	F ₁ ♂ ♂	212	202				
	F ₂ ♀ ♀	120	130				
	F ₂ ♂ ♂	1842	1802				
	Grand total	5646	5556				

individuals) was produced by mating the F_1 *inter se*. Their monohybrid ratios are as follows:

	Ratio	Deviation	Deviation
			error
Agouti to non-agouti	894:286	9	0.90
Black to brown.....	923:257	38	3.79
Intense to dilute.....	894:286	9	0.90
Dark eye to pink-eye	915:265	30	2.99
Calculated 8.....	885:295		

Probable error = 10.033

They noted an excess of dominants in all of the four monohybrid ratios, although it may be added that the excess was quite surely not significant in three of them. Seven of the eighteen monohybrid ratios in our data show in contrast a slight but insignificant excess of recessives. There is evidently no necessary and fixed tendency to produce an excess of dominants such as LITTLE and PHILLIPS found. The ratios given for the sum of the depleted and undepleted litters or for the grand total in our data are comparable to the ratios given by LITTLE and PHILLIPS, for their data probably include both types of litters; but in two of these six ratios our data still show an excess of at least one recessive class, brown, whereas LITTLE and PHILLIPS record a striking and possibly significant deficiency of brown.

It is clear that a small but consistent excess in the dominant classes, such as LITTLE and PHILLIPS found, has a cumulative effect, so that in large numbers the excess of dominants, *irrespective of kind*, eventually becomes significant. Such is the case in the LITTLE and PHILLIPS data, for summing up the total of dominants and recessives, we find 3626 and 1094 respectively, which is a deviation of 86 from the mean value, 3540:1180. This deviation is 4.29 times the probable error, 20.07; hence, we must either regard the observed value as an infrequent fluctuation of chance (in fact too infrequent as it stands to be consistent with Mendelian theory) or we must admit selective elimination of recessives. The odds against a ratio as bad or worse are about 260 to 1. We find no such significant difference between total dominants and recessives in our data in table 1, for the ratio is 5646:5556, which gives a deviation of 45 from the calculated frequency, 5601. This deviation is only 1.26 times the probable error, 35.69; hence, we may conclude that the zygotes are formed in equal numbers and no marked general elimination of re-

cessives, irrespective of kind, has occurred between fertilization and classification, such as the data of LITTLE and PHILLIPS indicate. The only indication of any significant elimination of recessives occurs in the case of non-agouti in depleted litters and possibly in the case of pink-eyed offspring from F_1 males. The numbers in depleted litters are too small to affect the grand total. Nevertheless it would be unjustifiable to argue unreservedly from grand totals which obscure subgroups failing to agree with theory.

In order to compare the ratios of dominants to recessives derived from the various sources, and to study the effects of elimination, table I A has been constructed. Now if we take the totals from F_1 females

TABLE I A
The ratios of dominants to recessives, regardless of kind.

Offspring obtained from	Dominants	Recessives	Calculated	Deviation	Probable error	Deviation error
F_1 ♀ ♀, undepleted litters (1)	3353	3424	3388.5	35.5	27.76	1.28
F_1 ♀ ♀, depleted litters (2)	1258	1175	1216.5	41.5	16.63	2.49
F_1 ♀ ♀, total (3)	4611	4599	4605	6.0	32.36	0.19
F_1 ♂ ♂ (4)	658	584	621	37.0	11.89	3.11
F_2 ♀ ♀ (5)	377	373	375	2.0	9.24	0.22
F_2 ♀ ♀ in tables 4 and 5 (6)	1162	1138	1150	12.0	16.17	0.74
Grand total	6808	6694	6751	57.0	39.19	1.45
Total of 2, 4, 5 and 6	3455	3270	3362.5	92.5	27.66	3.34

giving undepleted litters (no postnatal elimination) we obtain 3353 dominants to 3424 recessives, which is a deviation of 35.5 from the mean or calculated value 3388.5. There is, in fact, an excess of *recessives*, although not significant, for the probable error is 27.76. It is therefore clear that there is no selective prenatal elimination of recessives in the character pairs studied, that the zygotes are formed in accordance with theory, and that the very significant excess of dominants in the LITTLE and PHILLIPS data is probably due to postnatal selection. All of the ratios of table I A and the grand total of 6808 dominants to 6694 recessives are consistent with Mendelian theory, as is shown by the column, deviation

error. We may take the least favorable cases in which the most depletion has occurred by adding the results obtained in depleted litters from F_1 females to the ratios obtained in stock pens (ratios 2, 4, 5, and 6) in which a very large amount of elimination occurred. Even here

we have a ratio of 3455 dominants to 3270 recessives, and $\frac{\text{deviation}}{\text{error}} =$

$$\frac{92.5}{27.66} = 3.34. \quad \text{The results are not a decisive test against a ratio of}$$

equality, although the ratio is not as good as might be desired to prove equality. With the exception of non-agouti, in depleted litters from F_1 females, a general elimination of recessives is not apparent in our experiments, and if it occurs at all, the selective value of a dominant factor is not marked. The excess of dominants in LITTLE and PHILLIPS's data may well have been due to elimination of recessives, for selection was undoubtedly more rigid than under our experimental conditions, where each pregnant F_1 female had a separate pen. A very large part of our total data came from such females as table 1 shows. The total from these F_1 females alone gives 4611 dominants to 4599 recessives,—remarkably close to the calculated value, 4605.

An examination of the results for each allelomorphic pair in table 1 shows the following:

Agouti vs. non-agouti

In all cases but one in our data, the ratios of agouti to its absence agree with the Mendelian expectation, and with the results shown in the experiments of LITTLE (1913) and LITTLE and PHILLIPS (1913). DURHAM (1911) obtained 76 agoutis to 37 blacks, and states: "The results should yield a ratio of 3:1; but my results are not in accordance with this ratio." However, the results are not far from the calculated 84.75:28.25, and may be regarded as a fluctuation due to sampling, when we compare the deviation, 8.75, with the error of a binomial distribution, 3.10.

We found 444 agouti to 367 non-agouti in our depleted litters, the calculated frequency being 405.5. This exceptional case stands out in contrast and suggests postnatal elimination of the non-agouti class. The actual deviation, 38.5, is over four times the probable error, 9.60; the odds against the occurrence of such, or greater deviations, being about 145 to 1. Since the undepleted litters show no excess of agouti (in fact they show an insignificant deficiency of agouti) the excess of agouti in depleted litters suggests selective postnatal elimination. This is also apparent if one examines the second column of table 3, bearing in mind that table 1 is derived entirely from table 3. Each agouti class is larger than the corresponding non-agouti class. Although the agouti factor in

conjunction with other factors gives the dull wild gray coat which is supposed to be of selective value as a protective color pattern, it is difficult to imagine how it can possibly be of any selective value under laboratory conditions unless it has other physiological effects or is closely linked with factors of selective value. However, the fact seems perfectly clear that litters depleted within the first two weeks of life show at that age an excess of agouti that is difficult to attribute to chance. The excess is not so large as to justify an unconditional conclusion, but deserves record. Further experiments will add data on this point. We may take for comparison the total results from all experiments in stock pens to see whether large numbers show agouti to be of selective value under the less favorable conditions in our experiments. There were 681 agouti to 698 non-agouti,—a result agreeing well enough with Mendelian expectation and yet showing an *excess of non-agouti*. Evidently the excess of agouti in depleted litters occurs only under the least favorable conditions where selection is rigid.

If we add to the total of table 1 the results from F_2 segregates bred back to pink-eyed brown males (recorded in tables 4 and 5), our grand total from all experiments shows a ratio of 2243 agouti to 2206 non-agouti, and a deviation of 18.5 from the calculated 2224.5. The deviation is actually less than the probable error, $22.49 \left(\frac{\text{deviation}}{\text{error}} = 0.82 \right)$

As far as we can tell, we are not including in this total any subgroups inconsistent with Mendelian theory except the case of depleted litters from F_1 females already noted.

Dark eye vs. pink eye

These two characters are no doubt alternative in inheritance, but all experimental data have not given results agreeing with a Mendelian hypothesis. The data of DURHAM (1911) and LITTLE and PHILLIPS (1913) agree closely with Mendelian theory. LITTLE (1913) published data showing a marked and significant deficiency of the pink-eyed form, both when the heterozygotes were mated *inter se* and when back-crossed to the recessive pink-eyed form. The results of the former cross were 1673 dark-eyed to 457 pink-eyed, which is a deviation of 75.50 from the mean value, 1597.5 : 532.5. The deviation is 5.60 times the probable error, 13.48. The odds against a ratio as bad or worse are 6200 to 1. LITTLE states that the results are more aberrant when the heterozygotes are mated back to the recessive form, but his data do not seem to justify

this conclusion. The results of this latter cross were 370 dark-eyed to 284 pink-eyed, a deviation of 43 from the mean value, 327.

$\frac{\text{Deviation}}{\text{Probable error}} = 4.99$. The odds against a ratio as bad or worse are

about 1300 to 1. LITTLE suggests some constitutional weakness in his strain of pink-eyed mice, either directly connected with the pink-eyed modification or closely associated with it, which tends to cause a higher mortality among the pink-eyed than among the dark-eyed young. However, selective elimination of the pink-eyed form is not indicated by our experiments, for the six ratios in table 1 give results agreeing closely with the calculated values. For example, in the 170 depleted litters from F_1 females with a total of 1120 young born, 811 lived to be classified, but the ratio of dark-eyed to pink-eyed shows a deviation from equality even less than the probable error. The widest deviation occurs in the young from F_1 males. In this case the ratio of 232 dark-eyed to 182

pink-eyed shows a deviation of 25. The $\frac{\text{deviation}}{\text{error}} = 3.64$. Although

not a particularly good fit, it can hardly be said to be an impossible ratio. In view of the other ratios from stock pens showing elimination and the ratio from depleted litters, it is probably without special significance and does not indicate postnatal elimination of the pink-eyed form.

We may add to the total of table 1, the results found in tables 4 and 5 and obtain the grand total from all experiments. This gives a ratio of 2250 dark-eyed to 2211 pink-eyed and shows a deviation of 19.5 from the calculated 2230.5. The probable error is 22.52. The $\frac{\text{deviation}}{\text{error}} = 0.87$.

Black vs. brown

The factors for black and brown have been shown to be allelomorphic (LITTLE 1913, LITTLE and PHILLIPS 1913). Of the four monohybrid ratios given in the LITTLE and PHILLIPS data, the case of black and brown shows the greatest deviation from the calculated frequencies. The ratio of black to brown was 923 : 257, the calculated ratio being 885 : 295. The deviation, 38, is in this case 3.79 times the probable error, 10.03. The odds against the occurrence of such and larger deviations are about 92 to 1. While such a result is possible, it would in itself hardly demonstrate Mendelian inheritance in this particular cross as it stands.

All the ratios of black to brown in our subgroups and totals agree well with the calculated. There is no indication of elimination of brown; in fact, table 1 shows a slight excess. Adding to the total result of table 1 the ratios of black to brown in tables 4 and 5, we obtain 2315 : 2277, the calculated value being 2296. The deviation (19) divided by the probable error (22.85) is 0.83.

Summarizing, we may say that all monohybrid ratios for the three allelomorphic pairs studied are wholly consistent with Mendelian expectation, with the sole exception of agouti in depleted litters. With this exception noted, there is no suggestion in the subgroups or totals that postnatal or prenatal elimination vitiates the Mendelian ratios in individuals classified at the age of about two weeks. With respect to each allelomorphic pair, the gametes and zygotes are formed in equal numbers. There is no marked and necessary deficiency of recessives as a class, irrespective of kind, either in undepleted or depleted litters.

DIHYBRID RATIOS

The simplest ratio disclosing partial linkage is the dihybrid ratio; hence our data are arranged in the three possible dihybrid ratios in table 2. The fit of the observed ratios to the calculated is tested by PEARSON'S (1900) formula, $\chi^2 = \sum \frac{(o-c)^2}{c}$. The probable errors of χ^2 and P

(PEARSON 1916) are not given, for we are not dealing with relative goodness of fit. In the original cross all the dominant characters were presented by one parent; and, if linkage exists, the parental combinations in the F_2 should be more frequent unless marked elimination occurs. Numerous dihybrid ratios have been recorded in mice and other mammals, but large numbers are usually obtained with difficulty.

We have rearranged LITTLE and PHILLIPS'S data involving four allelomorphic pairs into the six possible dihybrid ratios in table 2 A. There are always some objections to pooling or subdividing data. In this study we are making the ratios tests against a Mendelian interpretation, and the data should bear dichotomous subdivision or the reverse process, provided no subgroups in the ultimate class frequencies show deviations so large as to exclude the same theory we are applying to the totals. This objection is seen, for example, in the excellent dihybrid ratio for agouti and intensity (table 2 A) taken from LITTLE and PHILLIPS'S data, ($P = 0.818$). We are conscious of including in the 680 agouti intense, 436 wild grays (also agouti intense) which occur as one of the

four subgroups giving the total agouti intense. However this subgroup, wild gray, is so large that it is impossible to regard it as a wide chance deviation. In pooling their data to get the dihybrid ratio for agouti and intensity, we evidently are using wide negative and positive deviations which counterbalance each other. There is always danger of this sort in any Mendelian experiment. When we give totals we assume that, in the absence of more precise data, our subgroups have agreed tolerably well with theory. It is, of course, unjustifiable to manufacture a "good ratio" by the process of adding subgroups, some of which fail to agree with the same theory we apply to the total. We shall have to bear in mind that table 2 A has this objection, but it is interesting to note what we might have concluded had the cross been made for the sole purpose of studying any one of the groups of two allelomorphic pairs, regardless of the other characters involved. Mendelian crosses are often made in which many factors are unknown or disregarded,—our own data may very well be such a case.

There are no empirical probabilities in the cases of these dihybrid ratios, but *a priori* we can suppose each class should occur with equal frequency and we can test our result against this Mendelian hypothesis. The ratios studied are dealt with in the text in the same order as in table 2, as follows:

$$\begin{aligned} AD : Ad : aD : ad \\ AB : Ab : aB : ab \\ BD : Bd : bD : bd \end{aligned}$$

Agouti and dark eye vs. non-agouti and pink eye

The cross, $AADD \times aadd$, should, without linkage, give in the F_2 $AaDd$, $Aadd$, $aaDd$ and $aadd$ in equal numbers. The data from all sources in our experiments agree closely enough with such an expectation to suggest that the sets of deviations observed may well have arisen through sampling, if judged by the values of χ^2 and P . In no one of the 6 cases is the fit so poor as to preclude the hypothesis that the four kinds of gametes and zygotes are formed in equal numbers. There is, however, a peculiar relation in the arrangement of the class frequencies in the undepleted litters. It will be noted that the parental combinations (AD and ad) are nearly equal and yet smaller than the recombinations (Ad and aD) which are likewise nearly equal. The sort of maturation division which gives rise to AD is the same as that giving ad ; i.e., these contrary class frequencies are complementary in a biological sense. Ad

TABLE 2
Dihybrid ratios.

Offspring obtained from	Class frequencies				Total	χ^2	<i>P</i>	Parental combinations	Recombinations	Calculated	Deviation	Probable error	Deviation	Probable error
	<i>Ad</i>	<i>Ab</i>	<i>aD</i>	<i>ad</i>										
<i>F</i> ₁ ♀ ♀, (undepleted litters)	530	588	597	544	2259	5.757	0.126	1074	1185	1129.5	55.5	16.03	3.46	
<i>F</i> ₁ ♀ ♀, (depleted litters)	225	219	187	180	811	7.520	0.058	495	406	405.5	0.5	9.60	0.05	
<i>F</i> ₁ ♀ ♀, total	755	807	784	724	3070	5.057	0.168	1479	1591	1535.0	56.0	18.69	3.00	
<i>F</i> ₁ ♂ ♂	116	98	116	84	414	6.986	0.072	200	214	207.0	7.0	6.86	1.02	
<i>F</i> ₂ ♀ ♀	66	64	61	59	250	0.464	0.908	125	125	125.0	0.0	5.33	0.00	
Grand total	937	969	961	867	3734	6.911	0.075	1804	1930	1867.0	63.0	20.61	3.06	
<i>F</i> ₁ ♀ ♀, (undepleted litters)	<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>										
<i>F</i> ₁ ♀ ♀, (depleted litters)	550	568	558	583	2259	1.074	0.784	1133	1126	1120.5	3.5	16.03	0.22	
<i>F</i> ₁ ♀ ♀, total	223	221	179	188	811	7.520	0.058	411	400	405.5	5.5	9.60	0.57	
<i>F</i> ₁ ♂ ♂	773	789	737	771	3070	1.870	0.602	1544	1526	1535.0	9.0	18.69	0.48	
<i>F</i> ₂ ♀ ♀	105	109	107	93	414	1.498	0.687	198	216	207.0	9.0	6.86	1.31	
Grand total	57	73	63	57	250	2.736	0.439	114	136	125.0	11.0	5.33	2.06	
	935	971	907	921	3734	2.428	0.495	1856	1878	1867.0	11.0	20.61	0.53	
<i>F</i> ₁ ♀ ♀, (undepleted litters)	<i>Bd</i>	<i>Bd</i>	<i>bD</i>	<i>bd</i>										
<i>F</i> ₁ ♀ ♀, (depleted litters)	539	569	588	563	2259	2.169	0.542	1102	1157	1129.5	27.5	16.03	1.72	
<i>F</i> ₁ ♀ ♀, total	215	187	107	212	811	2.549	0.473	427	384	405.5	21.5	9.60	2.24	
<i>F</i> ₁ ♂ ♂	754	756	785	775	3070	0.882	0.825	1529	1541	1535.0	6.0	18.69	0.32	
<i>F</i> ₂ ♀ ♀	122	90	110	92	414	6.754	0.082	214	200	207.0	7.0	6.86	1.02	
Grand total	66	54	61	60	250	2.064	0.501	135	115	125.0	10.0	5.33	1.88	
	942	900	956	936	3734	1.829	0.612	1878	1856	1867.0	11.0	20.61	0.53	

and aD bear the same relationship. The maturation divisions may be looked upon as follows:

$A \mid a$	$A \mid a$
$D \mid d$	$d \mid D$
530 544	588 597
Parental combinations 1074	1185

In the derivation of χ^2 and P , we regard neither the signs nor the order in which frequencies occur; but in biological data the order may at least be suggestive as in this case where the same sort of maturation division gives rise to both members of a pair of complementary classes. We do not know of any precise method of measuring the significance of order in which frequencies or deviations occur. It may be worth while to

point out that in using the formula $\chi^2 = \sum \frac{(o-c)^2}{c}$, we specify at the

outset that a given system of calculated frequencies is to be set over against the observed set. Ordinarily the calculated frequencies are not permutable or only slightly so, but in a back cross of this kind, where theory calls for n equal classes, they are permutable in $n!$ ways. In other words, it is perfectly possible to obtain the same value of χ^2 and P with $4!$ ($= 24$) arrangements of these identical class frequencies. Had we obtained any one of these, we should have come to the same general conclusion in each case as to goodness of fit between theory and observation. Nevertheless, not all of these 24 arrangements prompt the same biological suggestion. The whole scheme happens to assume an order which lends itself to a special explanation in a biological sense, namely, maturation divisions of the various kinds are only approximately equal in this case. Out of the total 24 permutations, only 8 ($= 2^2 \cdot {}_2P_2$) would be as suggestive as the one we found.

There is another method of approach which points to the same conclusion. The total number of maturation divisions giving parental classes is 1074, and recombinations is 1185. There is a deviation of 55.5 from the calculated 1129.5, which is 3.46 times the probable error, 16.03. The odds against such a deviation or wider ones are 51:1. We have always regarded these two allelomorphic pairs as perfectly independent, but when large numbers in undepleted litters are used, the results suggest that this is approximately true and only in a practical sense, but not absolutely. This is repulsion in one sense, and suggests slightly different frequencies in the various kinds of maturation

divisions. It may be added that the class frequencies show association $= -0.10$. The data suggest a gametic ratio of 1:1.1. CAROTHERS (1913) and WENRICH (1916) counted at random the cases where the accessory goes with the larger or smaller dyad of an unequal pair. Both investigators found 51.3 percent of the cases in which the larger went with the accessory and 48.7 percent with the smaller and the accessory together. The deviation from equality is not significant, but it is interesting to note that both investigators found a deviation in the same direction. If continued counts gave the same proportion, the deviation would eventually become significant. It is quite possible that Mendelian recombinations, and hence maturation divisions, do not differ from many other forms of common experience such as coin-tossing, dice-throwing, and the like, in which a slight bias is often found.

All the other subgroups fail to show the relation indicated by the undepleted litters, for elimination probably obscures the small amount of repulsion. If we add to the total data of table 2 the data from brown agouti females in table 4, we have a grand total for all experiments as follows:

<i>AD</i>	<i>Ad</i>	<i>aD</i>	<i>ad</i>	Total	χ^2	<i>P</i>
986	1018	1015	939	3958	4.068	0.255

The LITTLE and PHILLIPS data as they stand (table 2 A) agree with our totals in this case and are no decisive test against a Mendelian hypothesis. $\chi^2 = 5.615$, $P = 0.135$.

TABLE 2 A

The dihybrid ratios obtained by rearranging the ultimate class frequencies in LITTLE and PHILLIPS's data.

The pairs of dominant characters from male grandparent (<i>XY</i>)	Double dominant, <i>XY</i>	Single dominant, <i>Xy</i>	Single dominant, <i>xY</i>	Double recessive, <i>xy</i>	χ^2	<i>P</i>
Agouti and dark eye	700	194	215	71	5.615	0.135
Agouti and black	707	187	216	70	8.435	0.039
Black and dark eye	730	193	185	72	16.200	0.001
Agouti and intensity	680	214	214	72	0.915	0.818
Black and intensity	701	222	193	64	6.989	0.072
Intensity and dark eye	706	188	209	77	8.508	0.038
<i>Calculated</i>	<i>663.75</i>	<i>221.25</i>	<i>221.25</i>	<i>73.75</i>		

Agouti and black vs. non-agouti and brown

The cross $AABB \times aabb$ should, without linkage, give in the F_2 equal numbers of $AaBb$, $Aabb$, $aABb$ and $aabb$. The total data and all the

subgroups show, on the whole, a rather remarkably close fit to the calculated value, and therefore agree with Mendelian theory. There is no evidence of linkage, for the parental combinations and recombinations are very nearly equal. The depleted litters do not indicate selective elimination in our experiments.

If we add to the total data of table 2 the data from pink-eyed agouti females in table 4, we have a grand total for all of our experiments as follows:

<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>	Total	χ^2	<i>P</i>
1600	1024	973	982	3979	1,527	0.681

LITTLE and PHILLIPS's data as they stand (table 2 A) show a set of deviations which do not exclude a Mendelian hypothesis, but are not a particularly good fit. $\chi^2 = 8.435$, $P = 0.039$.

Black and dark eye vs. brown and pink eye

The cross $BBDD \times bbdd$ should, without linkage, give in the F_2 equal numbers of $BbDd$, $Bbdd$, $bbDd$ and $bbdd$. As in the preceding case, the total data and the subgroups show a close fit to the calculated values and are therefore consistent with the theory that these two pairs of independent Mendelian factors give four classes of gametes and zygotes in equal numbers. There is no indication of linkage in the biological sense for the parental combinations and recombinations are very nearly equal. The differences are very small when compared to the probable error and may well be chance fluctuations.

If we add to the total data of table 2, the data from black females in table 4, we obtain a grand total for all of our experiments as follows:

<i>BD</i>	<i>Bd</i>	<i>bD</i>	<i>bd</i>	Total	χ^2	<i>P</i>
1036	986	1027	1003	4052	1.534	0.679

The LITTLE and PHILLIPS data on this dihybrid cross do not agree with Mendelian hypothesis. The total set of deviations gives a value of $\chi^2 = 16.20$ and $P = 0.001$. There is an excess of the double dominant entirely too large to be attributed to chance. This excess is not due to the elimination of the double recessive, but apparently occurs at the expense of the recombination classes. The total number of double dominants, 730, shows a deviation of 66.25 from the calculated 663.75. The *a priori* probability for the double dominant is 9/16. Since the σ of a binomial distribution resulting from samples of 1180 individuals is 17.04 and the probable error is 11.49, the odds against deviations as wide or

wider than 66.25 are about 10,000 : 1. It is perfectly clear that if this experiment had been conducted to determine the dihybrid ratio for black and dark eye, we would no doubt have called this ratio a very poor fit. Black and dark eye show positive association (+0.19). This association is not due to gametic coupling as the data show, but rather to the survival of the black dark-eyed class and in particular the wild gray class which makes up a large part of this black, dark-eyed total. The wild grays are in great excess and other black dark-eyed classes are at least not markedly deficient in LITTLE and PHILLIPS's ultimate class frequencies.

LITTLE and PHILLIPS's data in table 2 A, as they stand, give the following results:

Agouti and intensity vs. non-agouti and dilution

The data show an excellent agreement with theory, for $\chi^2 = 0.915$ and $P = 0.818$.

Black and intensity vs. brown and dilution

The data show a set of deviations possible on the basis of a Mendelian hypothesis. $\chi^2 = 6.989$, $P = 0.072$.

Dark eye and intensity vs. pink eye and dilution

The data do not exclude Mendelian theory in this case, although the fit is not as good as could be desired. $\chi^2 = 8.508$, $P = 0.038$.

Summarizing, we may say that all three types of dihybrid ratios, regardless of the sources of our data, are consistent with Mendelian theory. The case of agouti and dark eye, however, strongly suggests that a slight repulsion exists between these two factors based upon different frequencies in the two types of maturation division involved. Prenatal elimination does not distort the ratios, but postnatal elimination may have a marked effect as is shown in some of the ratios obtained by rearranging LITTLE and PHILLIPS's data. The factors *B* and *D* (particularly in conjunction with *A* and *I* in the wild gray) are effective for survival in competition.

TRIHYBRID RATIOS

The ultimate class frequencies in our experiments are given in the trihybrid ratios in table 3, arranged according to the sources of data. These ratios describe the cross as a whole and were used to obtain tables 1, 1 A, and 2 by pooling class frequencies. The LITTLE and PHILLIPS data

have been rearranged in the four possible trihybrid ratios in table 3 A for comparison. As in the case of the simpler ratios, their experiment might have been made for the sole purpose of studying any one of these trihybrid ratios, and the conclusions would have varied according to the particular group of three characters chosen for study. We can state directly that the six ratios in our data are consistent with Mendelian expectation if judged entirely by the values of χ^2 and P . Two of the four trihybrid ratios in LITTLE and PHILLIPS's data are not consistent with a Mendelian expectation, for selective elimination has probably distorted the results. We again find that their ratios which group the black, dark-eyed classes together the most unsatisfactory.

TABLE 3
Trihybrid ratios

Color classes	Zygotic constitution	Offspring obtained from					Grand total
		F ₁ ♀♀ (undepleted litters)	F ₁ ♀♀ (depleted litters)	F ₁ ♀♀ total	F ₁ ♀♀	F ₂ ♀♀	
Black agouti	<i>AaDdBb</i>	256	122	378	62	35	475
Brown agouti	<i>AaDdbb</i>	274	103	377	54	31	462
Black	<i>aaDdBb</i>	283	93	376	60	31	467
Brown	<i>aaDdbb</i>	314	94	408	56	30	494
Pink-eyed black agouti	<i>AaddBb</i>	294	101	395	43	22	460
Pink-eyed brown agouti	<i>Aaddbb</i>	294	118	412	55	42	509
Pink-eyed black	<i>aaddBb</i>	275	85	361	47	32	440
Pink-eyed brown	<i>aaddbb</i>	269	94	363	37	27	427
Total		2259	811	3070	414	250	3734
Calculated class frequencies		282.375	101.375	383.75	51.75	31.25	466.75
χ^2		8.039	11.047	6.774	10.116	7.536	10.626
P		0.330	0.137	0.454	0.183	0.377	0.157

There are some additional facts, however, which deserve detailed comment. As in the case of agouti and dark eye in the undepleted litters in table 2 the undepleted litters in our trihybrid ratio show a suggestive order or arrangement of frequencies. The contrary class frequencies which represent biologically complementary classes (e.g., *ADB* and *adb*) formed by the same sort of maturation division show some tendency to equality. It will be noted, for example, that black agouti (*ADB*) and pink-eyed brown (*adb*) are the two smallest classes. They are the result of the same sort of maturation division, for presumably every time an *ADB* egg is produced, an *adb* polar body is produced, and *vice versa*. The

brown agouti (*ADb*) and its complement, pink-eyed black, (*adB*) are the next largest classes and are nearly equal. The same general relationship exists between the remaining complementary classes. If we judge the whole system of deviations by χ^2 and P , we conclude that a fit as bad or worse is obtained about once in three times as a matter of random sampling. Since there is some suggestion of equality between complementary classes, one naturally wonders whether or not the various sorts of maturation divisions are only approximately equal, so that differences are not discovered except with very large numbers. We had noted, in the course of our experiment, as early as January, 1914, that the complementary classes, black agouti and pink-eyed brown, were the smallest and also equal. At that time 263 offspring in undepleted litters were classified, and the frequencies (given in the same order as in table 3) were as follows: 25, 33, 37, 33, 39, 28, 43, 25. We thought there was no special significance to these facts, although we were aware that our results differed from those of LITTLE and PHILLIPS, in whose data the black agouti class gave an excessively large *plus* deviation, whereas our black agouti class showed a *minus* deviation. Continuing the experiment, we had noted the same general tendency whenever a summary of results was made. Just as in the case of agouti and dark eye in the di-hybrid ratios, we find that the order in which the frequencies occur is suggestive in a biological sense, although measuring the goodness of fit by χ^2 does not in itself reveal this fact. The case is made a little more striking if we use the class frequencies found shortly before the end of our experiment (January 1917). Given in the same order as in table 3, they were 256, 273, 283, 313, 294, 291, 275, 266. It is possible, of course, to obtain the same value of χ^2 and P with $8!$ ($= 40320$) permutations of these identical class frequencies. Had we obtained any one of these 40320 permutations, we should have come to exactly the same general conclusions in regard to the agreement between theory and observation. But it must be perfectly clear that not all of the orders would convey the same biological suggestion. In our particular case, the whole scheme happens to assume an order which is highly suggestive and lends itself to a special explanation. Now there are only 384 orders out of a possible 40320 ($2^n \cdot nP_n$, where n = number of contrary pairs, 4 in this case) which would have been equally suggestive. It is what we might expect if the different types of maturation divisions were only approximately equal. We have always regarded these three allelomorphous pairs as absolutely independent, but when larger numbers than are usually available are used and the F_2 is obtained by back cross, the re-

sults suggest that this is only approximately true, but the assumption is probably sufficiently accurate for practical purposes. It may well be that the peculiar arrangement in our trihybrid ratio is due in a large measure to the peculiar relation between *A* and *D*, shown in the undepleted litters in table 2.

In addition to the trihybrid ratio from undepleted litters, the other five trihybrid ratios give sets of frequencies which on the whole agree fairly well with the calculated. These include the depleted litters, the young born in stock pens, and the totals. While these ratios do not preclude a Mendelian hypothesis, it is an open question whether we ought to test our ratios against Mendelian theory alone. We cannot be sure that an *a priori* probability with eight equal classes is a strictly correct assumption as our most reliable experimental data, the undepleted litters, have indicated. Let us replace the *a priori* probabilities of Mendelian theory by the empirical probabilities determined from actual experimental data, the undepleted litters. The numbers are certainly not as large as could be desired for such purposes, but are the best available. In this case the depleted litters now show a very poor fit, as might be expected, for $\chi^2 = 17.286$ and $P = 0.016$. The wild gray class is far too large, since it is the largest class in the depleted and the smallest in the undepleted litters against which we are now making our test.

We thought it worth while to pool our ratios taken from all pens showing elimination, all of which ratios agree well enough individually with Mendelian theory. Hence we added to the depleted litters the young from F_1 males and F_2 females, the latter two having their young in stock pens where considerable elimination occurs. This gives 1475 segregates born under conditions where stringent selection is operative. The distribution thus obtained is about as unsatisfactory from the point of view of Mendelian theory as are the results of LITTLE and PHILLIPS. Our data show $\chi^2 = 19.405$, and $P = 0.007$, while LITTLE and PHILLIPS's data show $\chi^2 = 19.906$, and $P = 0.006$. Now, if we make our distribution a test against the empirical probability based upon our undepleted litters, the fit is even worse; for $\chi^2 = 28.300$ and $P = 0.00016$. In all these cases the wild gray form is the most important contributory element to the poor fit.

It is clear that postnatal elimination distorts Mendelian polyhybrid ratios in mice, particularly ratios including the wild gray class in competition with other classes. The survival of the wild gray class in these experiments has some bearing on our views relative to protective coat color and natural selection. It is far in excess of expectation in pedi-

greed cultures in the laboratory, where coat color is not effective as a protective agent. The corollary is that the wild gray combination in mice (and other rodents?) would have persisted in competition in the wild state regardless of the protective dull gray coat color. We do not doubt that the wild gray coat is a protective color under some conditions, but the effect of the factorial combination, *ADIB*, is more far-reaching

TABLE 3 A

The trihybrid ratios obtained by rearranging the ultimate class frequencies in LITTLE and PHILLIPS's data.

The three dominant characters from male grandparent (<i>XYZ</i>)	Mendelian classes								χ^2	<i>P</i>
	<i>XYZ</i>	<i>XyZ</i>	<i>XYz</i>	<i>xYZ</i>	<i>Xyz</i>	<i>xYz</i>	<i>xyZ</i>	<i>xyz</i>		
Agouti, black, and dark eye	566	134	141	164	53	52	51	19	19.906	0.006
Agouti, black, and intensity	539	141	168	162	46	54	52	18	9.082	0.247
Agouti, dark eye, and intensity	539	141	161	167	53	48	47	24	11.300	0.127
Black, dark eye, and intensity	563	138	167	143	55	42	50	22	20.822	0.004
<i>Calculated</i>	497.81	165.94	165.94	165.94	55.31	55.31	55.31	18.44		

than external appearances indicate. We have no reason to suspect that the combinations *ADB* or *ADIB* are more frequent gametic or zygotic combinations, as our undepleted litters show quite distinctly. In fact, the evidence points to the conclusion that the wild gray is one of the *least* frequent gametic recombinations. In the original LITTLE and PHILLIPS data 436 wild grays (*ADIB*) were observed, the calculated being 373.36. The deviation, 62.64, is 5.81 times the probable error, 10.78. The odds against deviations as wide or wider are about 12500 to 1. We wondered whether their data would show that the selective value of the wild gray class was apparently due to the effect of any combination of the four factors studied. If we consider as a class all individuals with any given two, three, or four factors, regardless of other differences, we find that the classes with *D* and *B* together are always far too large. Thus *DB*, *ADB*, *DIB*, and *ADIB* are all much larger than chance would possibly allow. The excess is due to the fact that any combination of factors with *D* and *B* in the ultimate class frequencies (*ADIB*, *aDIB*, *ADiB*, and *aDIB*) at least holds its own in competition, but the one class which shows the largest positive deviation is the wild gray, *ADIB*.

LITTLE and PHILLIPS classified their segregates at the age of about 4

weeks, while we classified ours at about two weeks, hence selection had been operative for a longer period in their experiments. Nevertheless our depleted litters show the same tendency as theirs. In our depleted litters the wild gray is the most frequent class, and shows the largest deviation from the calculated value. The deviation 20.625 is 3.25 times the probable error, 6.35. We cannot be sure that a test against Mendelian expectation is as justifiable as a test against the results of our undepleted litters. If thus tested the deviation is 31.09 which is 5.11 times the probable error, 6.09. If we add to the undepleted litters from F_1 females the results obtained in table 3 from F_1 males and F_2 females, which had young in stock pens where rigid selective conditions were present, we obtain 219 wild gray segregates out of a total of 1475. Tested against the Mendelian expectation, 184.375, we find a deviation of 34.625 which is 4.04 times the probable error, 8.57. Tested against our undepleted litters the deviation is 51.85, which is 6.31 times the probable error, 8.21. It is clear from the foregoing facts that the wild gray combination survives in competition even when the coat color and pelage are hardly developed; hence it is probable that it would have an advantage in natural selection regardless of protective coloration, although protective color would be an added advantage.

Summarizing the data on trihybrid ratios we may conclude that our ratios involving simultaneously agouti, black, and dark eye do not exclude a Mendelian hypothesis as they stand. There is, however, a peculiar order or arrangement in the undepleted litters which suggests strongly that complementary classes tend to be equal and that the four types of maturation division are only approximately equal. Grouping all experiments with depleted litters, we find a very poor agreement with the Mendelian expectation, and an extremely poor fit when tested against the empirical probabilities established by undepleted litters. Postnatal elimination distorts the ratios markedly, the wild gray class being the chief contributory element. The wild gray class survives in competition under laboratory conditions even at an early age and under conditions in which its protective coat color cannot function as such.

BREEDING TEST OF F_2 FEMALES

There were eight classes of F_2 segregates in our experiment. All classes were tested by breeding the females back to pink-eyed brown males, *aaddbb*, in stock pens. Of these eight classes, one is a trihybrid, three are dihybrids, three are monohybrids, and one is pure. The trihybrid, *AaDdBb*, has already been noted in the foregoing discussion and

TABLE 4
Breeding tests of F_2 dihybrid segregates from F_1 females in table 3, mated back to pink-eyed brown, *aaddbb*.

Class tested	Zygotic formula	F ₃ classes expected						Total	χ^2	P	Parental combinations	Recombinations	Calculated	Deviation	Probable error	Deviation error
		<i>ADb</i>	<i>aDb</i>	<i>AdB</i>	<i>adb</i>	<i>adb</i>	<i>adb</i>									
Brown agouti	<i>AaDdbb</i>	49	54	49	72			224	6.393	0.096	121	103	112	9.0	5.05	1.78
	<i>aaDdBb</i>	94	71	86	67			318	6.050	0.110	161	157	159	2.0	6.01	0.33
Pink-eyed agouti	<i>AaddBb</i>	65	53	66	61			245	1.710	0.639	126	119	122.5	3.5	5.28	0.66

the results agreed qualitatively and quantitatively with Mendelian expectation.

The dihybrids (table 4) were brown agouti (*AaDdbb*), black (*aaDdBb*), and pink-eyed black agouti (*AaddBb*). Each dihybrid class gave 4 classes of F_2 segregates in expected proportions, for the results do not exclude a Mendelian interpretation, and do not suggest linkage.

The monohybrids (table 5) were brown (*aaDdbb*), pink-eyed brown agouti (*Aaddbb*), and pink-eyed black (*aaddBb*). Each monohybrid class gave two classes of F_2 segregates in the expected proportions.

TABLE 5

Breeding tests of F_2 monohybrid segregates from F_1 females in table 3, mated back to pink-eyed brown, aaddbb.

Class tested	Zygotic formula	F_2 classes observed		Calculated frequencies	Deviation	Probable error	Deviation error
Brown	<i>aaDdbb</i>	<i>aDb</i> 84	<i>adb</i> 101	92.5	8.5	4.59	1.85
Pink-eyed brown agouti	<i>Aaddbb</i>	<i>Adb</i> 121	<i>adb</i> 125	123.	2.0	5.29	0.38
Pink-eyed black	<i>aaddBb</i>	<i>adB</i> 162	<i>adb</i> 133	147.5	14.5	5.79	2.50

The pure class, pink-eyed brown (*aaddbb*), bred true, giving 319 offspring.

LITERATURE CITED

- CAROTHERS, E. E., 1913 The Mendelian ratio in relation to certain orthopteran chromosomes. Jour. Morph. **24**: 487-506, 3 pl.
- CASTLE, W. E., and WRIGHT, S., 1915 Two color mutations of rats which show partial coupling. Science N. S. **42**: 193-195.
- 1916 Studies of inheritance in guinea-pigs and rats. Carnegie Inst. Wash. Publ. 241, pp. 1-192, 7 pls.
- DARBISHIRE, A. D., 1904 On the results of crossing Japanese waltzing with albino mice. Biometrika **3**: 1-51.
- DURHAM, F. M., 1911 Further experiments on the inheritance of coat colour in mice. Jour. Genetics **1**: 159-179.
- HALDANE, J. B. S., SPRUNT, A. D., and HALDANE, N. M., 1915 Reduplication in mice (preliminary communication). Jour. Genetics **5**: 133-135.
- LITTLE, C. C., 1913 Experimental studies of the inheritance of color in mice. Carnegie Inst. Washington Publ. 179, pp. 11-102, 5 pls.
- LITTLE, C. C., and PHILLIPS, J. C., 1913 A cross involving four pairs of Mendelian characters in mice. Amer. Nat. **47**: 760-762.

- PEARSON, K., 1900 On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. *Phil. Mag.* **50**⁵: 157-175.
- 1916 On a brief proof of the fundamental formula for testing the goodness of fit of frequency distributions, and on the probable error of *P*. *Phil. Mag.* **31**⁶: 369-378.
- WENRICH, D. H., 1916 The spermatogenesis of *Phrynotettix magnus*, with special reference to synapsis and the individuality of the chromosomes. *Bull. Mus. Comp. Zool.* **60**: 55-135, 10 pls.