A PRELIMINARY LINKAGE TEST WITH *AGOUTI* AND *UNDULATED* MICE

I. THE FIFTH LINKAGE-GROUP

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Received 28.i.49

Of the factors in the fifth linkage-group of the house mouse, the five available in this department are located as shown in the sketch, fig. 1.

Fig. 1.—Five loci in linkage-group V.

The first of these factors to be recognised by geneticists is that for agouti, of which five alleles are now available. These are not related in the simple linear order observable in many allelic series, but exhibit rather exceptional features. The four alleles with viable homozygotes produce phenotypic effects, and display dominance relationships, analogous to two closely-linked factors. The dominance relationships are indicated below (fig. 2) :—

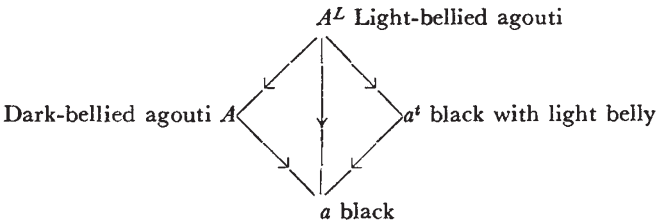

Fig. 2.—The dominance shown by four viable alleles.

That the relationship is more intimate than is usual with two separate loci has been shown by N. R. Bhat in this department by the unquestionably well-ascertained occurrence of a single mutation from a to A^L (1949). The heterozygotes Aa^t and A^La are, however, indistinguishable, as usually are double heterozygotes in repulsion and coupling. The fifth allele, A^Y, for yellow, is evidently of a different kind ; being a lethal it is no more properly described as a dominant than as a recessive. It has numerous pleiotropic effects—yellow mice are small in the nest, nervous and active in the fourth week, large and prolific when mature, liable to obesity in middle age, and are more extensively pigmented than non-yellows in pied types. As no analogous effects are shown by the four other alleles, it would appear that A^Y involves several loci, and is perhaps a short deletion covering the agouti locus.

The first linkage establishing the fifth linkage-group, between agouti and *pallid*, was reported in 1935 by Roberts and Quisenberry (1935). Pallid causes pink eyes, and a considerable dilution of the coat colour. Roberts and Quisenberry found, however, that the agouti alleles could be recognised on pallid mice, most easily if yellow is used. In my own stocks pallid is also a mild "weaver" with gravitational instability, and uncertainty of movement. The oblique posture in the early efforts at crawling is very characteristic. This feature, and some differences in colour make it distinguishable from pink-eye dilution (*p*), of the first linkage-group.

Roberts and Quisenberry found in backcross 49 crossovers out of 231 from females, and 44 out of 224 from males. These numbers indicate 20-21 per cent. recombination, without noticeable sex difference. On the other hand P. Hertwig (1942), with more extensive data, found a large sex difference in the recombination between agouti and wellhaarich (*we*), a hair-waving gene first recognised by A. Bluhm, and indistinguishable from wv_1 and wv_2. For females she found 187/1069, or 17·5 per cent., for males 76/725, or 10·5 per cent.

Early in 1946 *we* was obtained from Professor Hertwig, and as it is now known, from work in this department, to lie between agouti and pallid, and quite near to pallid, the question arises whether recombination of *a* and *pa* is also sensitive to sex, or whether adjacent segments are giving compensating effects. The further work in this department using selections of three or four loci should be able to clear up these points.

In the same paper Hertwig reported a new ray-induced mutant, Circler (*kr*, *kreisler*), also linked with agouti, but apparently so closely as to show no recombination ; this also she was good enough to send me, and it is found to lie to the left of agouti as shown in fig. 1, though the distance indicated is at present very rough, even in comparison with the other indications on that figure.

Shortly after coming to Cambridge in 1943, I was fortunate enough to pick up locally a new mutant, *undulated* (*un*), affecting the tail and spine. This has already been described (1947) by M. E. Wright (Mrs Wallace). From matings made up by Miss Wright with a view to detecting any linkages shown by the new factor, T. C. Carter (1947) has since reported it to be closely linked with agouti. It can now be said to lie between *agouti* and *wellhaarig*, and being the locus most closely linked with agouti, of those so far known, it is the most suitable for making a test, which has long been desirable, as to whether any of the agouti alleles is associated with a chromosomal abnormality. The only abnormality, indeed, to which a recombination test would be at all sensitive would be a fairly long inversion ; however, if four-point tests were to be made in this region, it was an essential preliminary to ascertain whether the different alleles available at the agouti locus were in reality equivalent as markers for accurate mapping. In the interval necessary for the preparation of quadruple recessives,

which took nearly two years, it was therefore decided to test the ten different heterozygotes of agouti, in respect of recombination with undulated ; and incidentally to make an evaluation of this interval sufficiently accurate to allow of the discussion of such effects as those due to sex and age, which too frequently escape examination owing to paucity of data.

2. THE TEST OF UNDULATED WITH AGOUTI ALLELES

Early in 1947 matings were made up to produce appropriate double heterozygotes segregating both for undulated and for agouti. As, however, ten different heterozygotes at the agouti locus were required, some of them were not available until the autumn of that year. It was intended to breed 100 from each sex of each genotype, a minimum of 2000 in all, and this programme has now been so nearly completed, with a total classified of well over 3500, that it seems proper now to put it on record.

In discussing the effect of age on recombination frequency it will be necessary to set out the data in detail (table 3) ; at the present stage it is sufficient to use a brief summary, and to express the author's opinion, based on statistical tests, that no differentiation is to be observed between the sexes, and that there is no evidence of an inversion. These points facilitate the discussion of the effect of age, by allowing both sexes and ten genotypes, or twenty bodies of data, to be thrown together for that purpose. Moreover, the four-point crosses can be organised with a selection only of the eighty possible four-fold heterozygotes.

During the course of the experiment sub-significant differences did appear between different genotypes, and these usually began to diminish as the data accumulated. At the same time, the general estimate of recombination fraction steadily fell. Both these effects were, I now believe, due to the age of the heterozygous parent having a rather large effect on recombination. Taking all ages together, however, we have :—

TABLE 1

Recombination fractions observed for twenty classes of heterozygous parents

	$A^{Y}A^{L}$	$A^{Y}A$	$A^{Y}a^{t}$	$A^{Y}a$	$A^{L}A$	
♀	12/194	5/118	12/235	10/146	2/78	
♂	9/128	9/126	6/160	16/243	7/214	
Total	21/322	14/244	18/395	26/389	9/292	
	$A^{L}a^{t}$	$A^{L}a$	Aa^{t}	Aa	$a^{t}a$	Total
♀	9/182	4/210	10/231	8/178	11/159	83/1731
♂	7/213	4/144	13/218	3/159	13/238	87/1843
Total	16/395	8/354	23/449	11/337	24/397	170/3574

An analysis of variance for sex, genotype and remainder can be conveniently carried out by using the angular transformation

$\sin^2 \phi = p = a/n$; for, if this is done all weights are known in advance from the number of animals contributing to each ratio. If any real effects are indicated, such true weights will be needed, since the observed numbers are not proportional for sex, age and genotype.

As a preliminary it will be sufficient to use two direct χ^2 tests of homogeneity. One, for sex-difference within each of the ten genotypes has $\chi^2 = 4{\cdot}827$ for 10 degrees of freedom, nearly on the 90 per cent. point, and indicating no trace of sex differentiation. The second for homogeneity of genotypes, ignoring sex, gives $\chi^2 = 16{\cdot}315$ for 9 d.f., quite near to the 5 per cent. point. This heterogeneity must, therefore, be taken seriously. The pattern of percentage recombination fraction shown in table 2, does not, however, suggest that there is any indication of an inversion.

TABLE 2

Recombination per cent., ignoring sex

	A^L	A	a^t	a
A^V	6·5	5·7	4·6	6·7
A^L		3·1	4·1	2·3
A			5·1	3·3
a^t				6·0

The ten values in table 2, while not suggesting any such relational disturbances as might be due to a chromosomal abnormality, do, however, suggest that possibly the five alleles tested differ *per se* in their effects upon the recombination fraction. We may therefore, seek to divide the 9 degrees of freedom among these ten values, which are already known to show more variability than can easily be ascribed to chance, into a section of 4 degrees of freedom for simple contrast among the five alleles available, and a residue of 5 degrees of freedom not explicable by any such simple contrast.

If n_{ij} stand for the number of mice bred from heterozygotes between two alleles designated as i and j, then we may define an angle α_{ij} such that $\sin^2 \alpha_{ij}$ is the fraction of recombinations observed in this group of mice. We may then seek for five angular values θ_i corresponding to the five alleles, such that the sum for all ten classes

$$S\{n_{ij}(\theta_i + \theta_j - \alpha_{ij})^2\}$$

shall be minimised for variations of the five parameters θ_i. If the angles are measured in degrees, sums of squares must be divided by $(90/\pi)^2$, or 820·7, to find the corresponding values of χ^2.

The equations of estimation are then :—

$$\begin{aligned}
1350\theta_1 + 322\theta_2 + 244\theta_3 + 395\theta_4 + 389\theta_5 &= 18826{\cdot}3^\circ \\
322\theta_1 + 1363\theta_2 + 292\theta_3 + 395\theta_4 + 354\theta_5 &= 14946{\cdot}1^\circ \\
244\theta_1 + 292\theta_2 + 1322\theta_3 + 449\theta_4 + 337\theta_5 &= 15703{\cdot}1^\circ \\
395\theta_1 + 395\theta_2 + 449\theta_3 + 1636\theta_4 + 397\theta_5 &= 20564{\cdot}8^\circ \\
389\theta_1 + 354\theta_2 + 337\theta_3 + 397\theta_4 + 1477\theta_5 &= 18021{\cdot}6^\circ
\end{aligned}$$

yielding the solutions :—

A^Y	θ_1	8·26084°
A^L	θ_2	4·36894°
A	θ_3	5·65906°
a^t	θ_4	6·52796°
a	θ_5	5·93282°

with the analysis of variance :—

	D.f.	S.S.	χ^2
Alleles . . .	4	8695·90	9·865
Remainder . .	5	7579·94	9·236

The effect of alleles is formally significant for 4 degrees of freedom, but the large value of χ^2 for the remaining 5 degrees of freedom renders the result unconvincing, since it suggests that there is a considerable amount of variation still not accounted for by simple effects of the five alleles.

3. THE EFFECTS OF AGE OF PARENTAGE

In table 3 the data have been subdivided according to whether the birth interval from the birth of the heterozygous parent is less than three months, three to five months, five to seven, and so on up to fifteen months. It will be noticed that the results from females and males are closely parallel and that in each the apparent percentage of recombinations is high for birth intervals less than three months, and low for birth intervals greater than nine months, being of intermediate value for the intermediate period. *Prima facie*, the recombination percentage would seem to depend largely on the age of the heterozygous parent. Any apparent heterogeneity, however, in respect of age is open to some suspicion, since the litters compared are now not contemporaneous, and an apparent effect of age might be due, for example, to misclassification having occurred with higher frequency among the earlier litters when the observer was less experienced, than among the later litters when he had learned to make a more exact discrimination. With the present data there is scarcely any possibility of error in classification for the agouti locus. The author had had considerable experience with undulated before these tests were begun, but seeing that with a small fraction of about 5 per cent. recombination even 1 per cent. of errors of classification would introduce a considerable bias, the question of accurate classification deserves very careful attention.

In the author's experience undulated may be classified with almost invariable certainty in young aged two to three days. Not infrequently, however, heterozygotes show a noticeable subundulation particularly from about four to ten days of age ; during this period judgment may be aided by the absence of hunchback, which is common though not invariable in undulated mice, and by the tail, though not being

straight, being of full length and lacking the sharp bend at the base, which is characteristic of undulated homozygotes. At all times throughout the test particular care was given to the diagnosis of undulated and, speaking subjectively, accurate discrimination gave rise to no anxiety.

TABLE 3

From heterozygous females

Months	A^LA^V	AA^V	A^Va^t	A^Va	A^LA
0- 3	2/29	1/22	4/30	4/36	1/12
3- 5	3/71	1/18	2/56	1/47	1/27
5- 7	6/56	0/42	4/71	3/38	0/28
7- 9	1/38	3/23	2/43	2/24	0/7
9-11	...	0/13	0/19	0/1	0/4
11-13	...	...	0/16	...	...
13-15	...	...	...	...	...
	12/194	5/118	12/235	10/146	2/78

From heterozygous females (continued)

Months	A^La^t	A^La	Aa^t	Aa	a^ta	Total	Recombination percentage
0- 3	1/42	1/39	3/36	2/33	1/35	20/314	6·37
3- 5	3/73	1/85	1/66	4/45	2/37	19/525	3·62
5- 7	3/56	1/73	5/87	1/37	6/34	29/522	5·56
7- 9	2/11	1/13	1/36	0/48	0/24	12/267	4·49
9-11	...	...	0/6	1/15	2/23	3/81	
11-13	...	...	...	...	0/4	0/20	2·91
13-15	...	...	...	...	0/2	0/2	
	9/182	4/210	10/231	8/178	11/159	83/1731	4·79

From heterozygous males

Months	A^LA^V	AA^V	A^Va^t	A^Va	A^La
0- 3	2/12	1/6	2/32	1/6	1/9
3- 5	2/27	2/30	1/64	7/79	1/37
5- 7	0/35	3/35	3/48	2/28	3/38
7- 9	3/17	3/27	0/8	4/80	1/53
9-11	2/27	0/28	0/8	1/32	1/44
11-13	0/10	...	...	1/18	0/27
13-15	...	...	...	...	0/6
	9/128	9/126	6/160	16/243	7/214

From heterozygous males (continued)

Months	A^La^t	A^La	Aa^t	A^t	a^ta	Total	Recombination percentage
0- 3	1/34	1/24	4/50	0/14	3/41	16/228	7·02
3- 5	3/79	2/22	4/77	1/38	3/53	26/506	5·14
5- 7	1/37	1/65	3/65	0/35	4/48	20/434	4·61
7- 9	1/30	0/25	2/17	2/43	2/42	18/342	5·26
9-11	1/19	0/8	0/9	0/10	0/37	5/222	
11-13	0/14	...	...	0/19	1/17	2/105	2·10
13-15	...	...	...	...	...	0/6	
	7/213	4/144	13/218	3/159	13/238	87/1843	4·72

Had misclassification of undulated been a factor in the apparent change of recombination fraction with birth interval, one would have expected a considerable disturbance in the proportion of

undulated among those judged to be crossovers. Actually, of the 83 recorded crossovers from heterozygous females 39 were undulated and 44 normal. Of the 87 crossovers recorded from males, 45 were undulated and 42 normal. There is evidently no disturbance of the 1 : 1 ratio, such as would have occurred had any considerable fraction of the supposed crossovers been ascribable to the systematic misclassification of the undulated factor. Since such misclassification might particularly be suspected among the early births, it is relevant that of the 36 recorded recombinants with birth intervals less than three months, exactly 18 are recorded as undulated. Examination of the data *a posteriori* thus seems to exclude change of standards of classification as a possible cause of the apparent change of recombination fraction with age.

Using the angular transformation we may now consider the regression of recombination fraction on birth interval. Table 4 gives the data for males and females together in which the 8 individuals born to parents over thirteen months old have been thrown into the penultimate class. The angles (α) in the fifth column correspond with the percentages in the fourth. The last two columns give the values reconstructed by linear regression.

TABLE 4

Frequency of recombination according to the age of the heterozygous parent

Months	t	Number n	Observed recombination percentage	Angular measure α	Reconstructed by linear regression	
					α	percentage
0- 3	−2	542	6·64	14·9°	14·289°	6·092
3- 5	−1	1031	4·36	12·0°	13·303°	5·295
5- 7	0	956	5·13	13·1°	12·317°	4·550
7- 9	1	609	4·93	12·8°	11·331°	3·860
9-11	2	303	2·64	9·3°	10·346°	3·225
11-13	3	133	1·50	7·0°	9·360°	2·645

The weighted mean of α is 12·455372°, and of t is −·14017907 ; for the regression analysis we have

A	$S\ n(t-\bar{t})^2$	6146·77
B	$Sn\alpha(t-\bar{t})$	−6059·46
C	$S\ n(\alpha-\bar{\alpha})^2$	10897·50

giving the rate of change, B/A as 0·9858° per two months. The analysis of variance shows

	D.f.	S.S.	χ^2
Regression . .	1	5973·39	7·278
Remainder . .	4	4924·11	6·000

The values of χ^2 show that the general fall of recombination fraction with increase of birth interval is clearly significant, but that the 4 degrees of freedom representing deviations from the fitted line, though far from significant, have a χ^2 somewhat greater than expectation. As shown by the graph, fig. 3, these deviations might be regarded as indicating that after a sharp fall during adolescence in the first

three months of life, the value remains steady during middle life, from three to nine months of age, after which a second fall sets in associated with senility, and loss of reproductive activity. On the other hand the deviations are not too large to have been due to chance, and the data as so far examined are equally consistent with the view that the recombination percentage has fallen steadily with age. On the first of these views it would be appropriate in reporting recombination fractions for the establishment of accurate maps, to ignore births with birth intervals less than three months or greater than nine, and to base definitive estimates only on those births occurring in the middle period, thus, for the present data, we should have, for females—60/1314 or 4·57 per cent., and for males—64/1282 or 4·99 per cent.

On the second view, however, such values would only be comparable for data in which the age distribution between three and nine months was the same, and it would be better to adopt a standard age, for which six months old seems to be the most suitable. Using the value indicated for that age by the regression line fitted to the whole of the data, in this case, therefore, we should obtain for both sexes together the value 4·55 per cent. at six months old.

It now seems possible that the present data might give an indication as to which of these procedures is preferable, by consideration of the effects upon the apparent observable differences between genotypes, of making allowance for variation in the birth interval. Thus, if the first view were correct, and the recombination fraction remained steady throughout middle life, a re-examination of the comparison between genotypes, using only the 2596 mice of the middle period, should bring out any real genotypic effect more clearly. Whereas, on the second view a better analysis would be obtained by fitting simultaneously a regression coefficient, and, if necessary, five angular constants corresponding with the five alleles. If, as is to be supposed, the large residual variation found in section 2 in analysing the effect of genotype, is in reality due to inequalities in the age distributions, a comparison of the behaviour of χ^2 for these five degrees of freedom would be of particular interest as supplying an indication of the nature of the age effect.

4. ANALYSIS USING AGE AND GENOTYPE SIMULTANEOUSLY

If we take account only of the 2596 mice born at birth intervals of three to nine months, the recombination fractions of the ten genotypes are :—

TABLE 5

Recombination fractions for births in middle life

	A^L	A	a^t	a	
A^Y	15/244	12/175	12/290	19/296	124/2596
A^L	...	6/190	13/286	6/283	
A	...	...	16/348	8/246	
a^t	...	...	...	17/238	

χ^2 is 14·42 for 9 degrees of freedom ; still large though materially smaller than for the entire data. As in the analysis to section 2, the denominators of these fractions supply the coefficients of the unknowns in the equations of estimation, while the right-hand side is found by translating the fractions into corresponding angles, which, multiplied by the weights and added give the values shown in the first column of table 6.

TABLE 6

R.H.S.	Estimates
13917·8°	8·03660°
11346·6°	4·69670°
11471·6°	5·58564°
14915·0°	6·79162°
12975·3°	5·90529°
64626·8°	

The sums of the products of the entries in these two columns is 409827·31, which is less by 5160·83 than the sum of the squares of the values observed, $S(n_{ij}a_{ij}^2)$, and greater by 7610·12 than $2596a^{-2}$; consequently we have the analysis of table 7 :—

TABLE 7

	S.S.	D.f.	χ^2
Between alleles . .	7610·12	4	9·273
Remainder . .	5160·83	5	6·288

The difference between alleles now just falls short of the 5 per cent. point, but stands out more distinctly from the remainder, which contains little more than the expected amount of variation. The omission of mice with birth intervals less than three and greater than nine months has, thus, to some extent had the expected effect of eliminating disturbances due to the variable ages used.

For the simultaneous analysis involving both age and possible genic effects, we require the angular values corresponding with the recombination fractions shown in table 3. With such subdivision, it is necessary to introduce a refinement which has become familiar in the use of probit and angular transformations in biological assay, and consists in using not the empirical angles from each cell, but working angles based upon the corresponding expectations, in which each individual can be scored separately, and the consistency of the analysis is guaranteed by all scores being linear functions of the observed frequencies. Table XIV of *Statistical Tables* (1948) has been prepared with this use in view. Corresponding with the seven provisional angles appropriate to the seven age classes recorded, the table gives minimal working angles and ranges as shown in table 8.

The total assigned to each cell is then the denominator of the recombination fraction multiplied by the minimal angle, added to the numerator multiplied by the range. These total angles, together with the number of mice on which they are based, are shown in table 9.

From these, in which all ten genotypes have been thrown together, the following relevant tests may be made—first we may test whether

TABLE 8

Provisional angle	Minimal angle	Range
14·35°	7·04°	119·410°
13·38°	6·59°	127·394°
12·41°	6·105°	136·718°
11·44°	5·62°	147·620°
10·47°	5·135°	160·638°
9·50°	4·70°	176·450°
8·53°	4·265°	...

TABLE 9

Data scored by age and sex

	Females		Males	
Age in months	Number	Total angle	Number	Total angle
0- 3	314	4598·760°	228	3515·680°
3- 5	525	5880·236°	506	6646·784°
5- 7	522	7151·632°	434	5383·930°
7- 9	267	3271·980°	342	4579·200°
9-11	81	897·849°	222	1943·160°
11-13	20	94·000°	105	846·400°
13-15	2	8·530°	6	25·590°
	1731	21902·987°	1843	22940·744°

any significant difference appears between the regressions shown by offspring from the two sexes :—

	D.f.	S.S.	χ^2
Regression lines for each sex	4	570530·0	
Parallel regression lines	3	568647·7	
Difference	1	1882·3	2·2935

The rate of change with age is not significantly different, although that for males is considerably the higher (see fig. 3).

Next, we may test whether there is any significant difference between males and females in the recombination fraction when parallel lines are fitted.

	D.f.	S.S.	χ^2
Parallel regression lines	3	568647·7	
Single regression line	2	568619·8	
Difference	1	27·9	0·0340

The recombination frequency is closely alike in the two sexes, that for males being slightly the higher.

Finally, we have for the test of significance of the observed change of recombination fraction with age :—

	D.f.	S.S.	χ^2
Single regression line	2	568619·8	
No allowance for age	1	562663·8	
Difference	1	5956·0	7·2634

The general effect of age is clearly significant.

The total scores obtained for the ten separate genotypes, without any allowance for age difference between them, are shown in table 10.

TABLE 10

Data scored for genotype of heterozygous parent

Genotype	Number	Total angle	Genotype	Number	Total angle
$A^Y A^L$	322	4831·434°	$A^L a^t$	395	4651·824°
$A^Y A$	244	3391·936°	$A^L a$	354	3287·838°
$A^Y a^t$	395	4805·148°	Aa^t	449	5860·099°
$A^Y a$	389	5890·085°	Aa	337	3514·891°
$A^L A$	292	2920·720°	$a^t a$	397	5689·756°
				3574	44843·731°

The significance of the difference between genotypes after allowance for age may be tested by fitting to these a set of ten parallel regression lines for the ten genotypes. Numerically this gives :—

	D.f.	S.S.	χ^2
Parallel lines for 10 genotypes	11	576818·9	
Single regression line	2	568619·8	
Difference	9	8199·1	9·9904

The observed differences between genotypes, after allowance for age, are just what might be expected from random fluctuations.

It therefore appears that the apparent differences between genotypes previously noted (section 2) may be ascribed wholly to the age difference of the parents used in testing these genotypes, and that, with proper allowances for such age differences, the data from the ten heterozygotes used are completely homogeneous.

Further, since elimination of this disturbance is more complete when allowance is made for the continuous fall of recombination fraction with age over the whole breeding life, than it is merely by selection of young bred between three and nine months of age, we have an indication favouring the view that the fall is in fact continuous.

Taking all genotypes and two sexes together, we have the final estimates of the recombination fraction in angular measure, and as a percentage, shown in table 11.

5. SUMMARY

1. Recombination in the short interval between *agouti* and *undulated* has been tested using heterozygous males and females of each of the ten heterozygotes available by the combination of agouti alleles. About 3600 young were bred before the completion of this test.

2. There is no indication of any significant difference between males and females.

TABLE 11

Recombination fractions at various ages

Months	Angle	Percentage
0- 3	14·372°±0·8296	6·16
3- 5	13·392°±0·5726	5·37
5- 7	12·412°±0·4818	4·62
7- 9	11·432°±0·6332	3·93
9-11	10·453°±0·9133	3·29
11-13	9·473°±1·2377	2·71

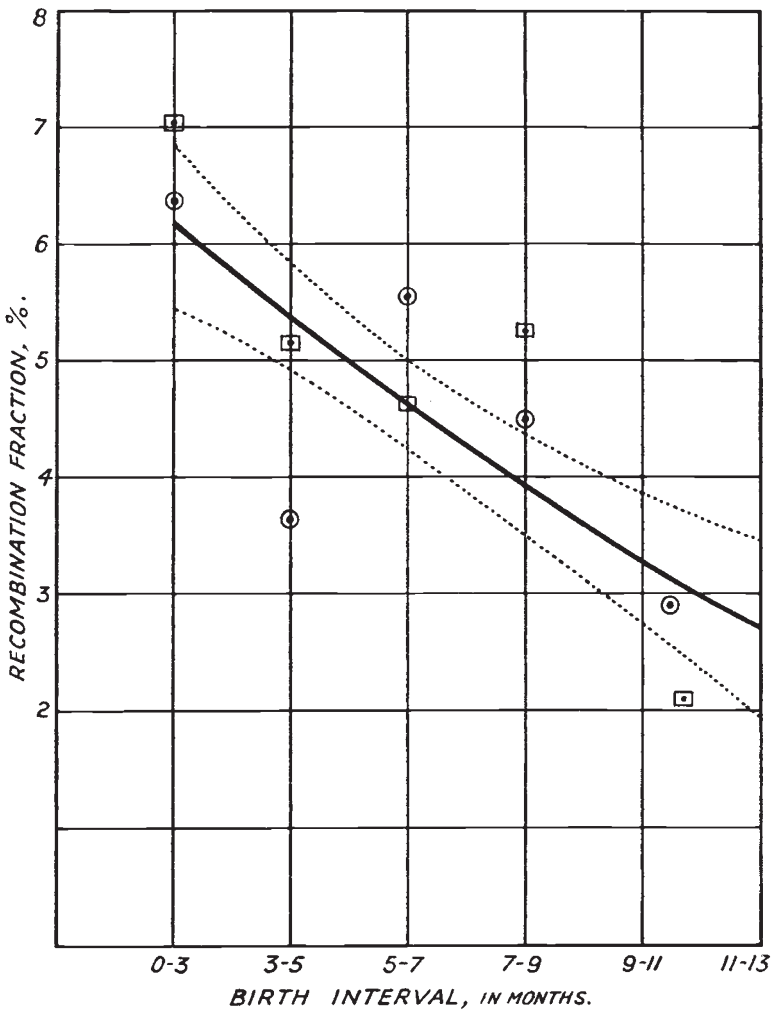

FIG. 3.—Recombination fractions at different ages.

⊙ From heterozygous females.

□ From heterozygous males.

——— Fitted curve.

····· Standard error hyperbola.

3. Differences among the agouti genotypes are large but unsystematic, and suggestive of disturbances due to other causes of variable recombination.

4. In both sexes recombination changes greatly from the earlier to the later litters, from about 7 per cent. to about 2 per cent. (fig. 3).

5. Discarding the records of mice born earlier than three months, and later than nine months, diminishes but does not abolish the apparent differences between genotypes. On the other hand, elimination of the age effect by a continuous curve (linear in the angular measure) leaves no sign of differentiation among the ten genotypes.

6. For mapping, the interval between *agouti* and *undulated* may be given the recombination fraction 4·62, the value for the fitted curve at a birth interval of six months (table 11). Though no significant difference is found between the sexes, the rate of change with age may be higher in males, and such difference as there is in recombination fraction at a given age makes the male value slightly the higher.

7. In the four-point test now in progress with agouti, undulated, wellhaarig and pallid, it will be necessary to obtain adequate comparable material for sex and age, but, in respect to the choice of genotype for the four-fold heterozygotes, it should suffice to balance coupling and repulsion between each pair of the three debilitating recessives.

6. REFERENCES

BHAT, N. R. 1949.
A dominant mutant mosaic house mouse.
Heredity, *3*, 243-248.

CARTER, T. C. 1947.
A new linkage in the house mouse ; undulated and agouti.
Heredity, *1*, 367-372.

FISHER, R. A., AND YATES, F. 1948.
Statistical Tables. 3rd ed. Oliver and Boyd : Edinburgh.

HERTWIG, P. 1942.
Neue Mutationen und Koppelungsgruppen bei der Hausmaus.
Z.I.A.V., 80, 220-246.

ROBERTS, E., AND QUISENBERRY, J. H. 1935.
Linkage of the genes for non-yellow (*y*) and pink-eye in the house mouse.
Amer. Nat., *69*, 181-183.

WRIGHT, M. E. 1947.
Undulated : a new genetic factor in *Mus musculus* affecting the spine and tail.
Heredity, *1*, 137-141.