FURTHER LINKAGE DATA ON THE ALBINO CHROMOSOME OF THE HOUSE-MOUSE

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INTRODUCTION

In a previous paper (Grüneberg, 1935) the author described a three-point back-cross experiment in the house-mouse involving the linked factors for shaker$_1$, albinism and pink-eye dilution. It was shown that the three genes are arranged in that order. The presence of interference was shown to be likely, but the total of 1144 animals was too small to allow for a definite answer. On the other hand, the figures, so far as they went, seemed to indicate that interference, if present, was less strong than in *Drosophila*. No definite answer could be given as to whether the cross-over values vary with the age of the parents.

To deal with these open questions, the experiments were continued on a larger scale. The total (including the published data) has now been brought up to 3692 animals. An analysis of all these data will be given in this paper. In view of some unexpected differences between the batches in which the experiments were performed, a more detailed description of the way in which the experiments were prepared seems necessary.

THE EXPERIMENTS

In 1933 a number of pink-eyed shaker mice were given to us by Dr W. H. Gates for further studies. One of the original males (No. Z1) when out-crossed to an extreme dilute ($\mathbf{c^D c^D}$) female produced 10 wild type and 9 black-eyed white offspring. That animal was therefore heterozygous for albinism. The black-eyed whites, being of the constitution $\frac{\mathbf{sh}\ \ \mathbf{c}\ \ \mathbf{p}}{+\ \mathbf{c^D}\ +}$, were inbred. In the F_2 generation, albino shakers were produced. Since in the presence of albinism pink-eye dilution cannot be scored, these animals were tested individually by crossing to pink-eyed ($\mathbf{pp}$) mice. Males found to be of the constitution $\frac{\mathbf{sh\ c\ p}}{\mathbf{sh\ c\ p}}$ were used as the triple recessives in the back-cross published (Exp. I). It proved difficult to establish a pure stock of these animals, since shaker females in the early stages of the experiment were very bad mothers. Later, this has changed fundamentally, and in the last experiments shaker females have proved excellent mothers, the average litter size at recording age being 7·4 young; in one case, as many as 14 young have been born and raised by a shaker mother.

The unsatisfactory behaviour of shaker females at the beginning made an out-cross necessary. One of the original pink-eyed shaker males was therefore out-crossed to commercial females of the constitutions **aaBBDdss** and **aabbddss**. Animals extracted in F_2 and back-crosses have served as a supply of pink-eyed shakers. In later stages of the work the factors **a**, **b**, and **d** have segregated in a number of matings.

The majority of triply heterozygous $\left(\frac{\mathbf{sh}\ +\ \mathbf{p}}{+\ \mathbf{c^D}\ +}\right)$ females for Exp. I were secured by crossing extracted **sh p.sh p** males to $\mathbf{c^Dc^D}$ females. In addition, the few wild-type daughters of Z 1 ♂ were used.

Exp. II was done on a small scale only, since at that time the shaker females were still rather unreliable. The type of cross was

$$\frac{\mathbf{sh}\ +\ \mathbf{p}}{+\ \mathbf{c^D}\ +}♂\times\frac{\mathbf{sh\ c\ p}}{\mathbf{sh\ c\ p}}♀,$$

that is, the reciprocal experiment to Exp. I. The animals were derived from the same sources as in that experiment.

In Exp. I two double cross-overs of the constitution $\frac{\mathbf{sh\ c^D\ p}}{\mathbf{sh\ c\ p}}$ were obtained. One of them, a female, forms the basis of all further experiments. She was out-crossed to an extreme dilute male and produced 16 black-eyed white and 17 extreme dilute offspring. The former were discarded. By inbreeding the extreme dilutes, mice of the constitution $\frac{\mathbf{sh\ c^D\ p}}{\mathbf{sh\ c^D\ p}}$ were obtained. Males of this stock served as triple recessives in Exp. III. The triple heterozygotes for this experiment were obtained by crossing a $\frac{\mathbf{sh\ c\ p}}{\mathbf{sh\ c\ p}}$ ♂ previously used in Exp. I to females of a Black-agouti (**ABD**) pure line. Exp. III was therefore of the type

$$\frac{\mathbf{sh\ c^D\ p}}{\mathbf{sh\ c^D\ p}}♂\times\frac{\mathbf{sh\ \ c\ \ p}}{+\ +\ +}♀,$$

the females being the heterozygous sex as in Exp. I, but all three factors were in coupling.

Non-cross-overs obtained from Exp. III were used for Exps. IV and V. The constitutions of the animals were

$$\frac{\mathbf{sh\ c^D\ p}}{\mathbf{sh\ \ c\ \ p}}\ ♂ \times \frac{\mathbf{sh\ c^D\ p}}{+\ +\ +}\ ♀ \quad \text{(Exp. IV)}$$

and

$$\frac{\mathbf{sh\ c^D\ p}}{+\ +\ +}\ ♂ \times \frac{\mathbf{sh\ c^D\ p}}{\mathbf{sh\ \ c\ \ p}}\ ♀ \quad \text{(Exp. V)}$$

respectively. As is obvious, $\mathbf{c^Dc^D}$ and $\mathbf{c^Dc}$ young will be expected in equal numbers in animals not carrying the wild-type allelomorph of albinism. In the presence of **pp** these types are indistinguishable. Only in cross-overs between **c** and **p** does the appearance of two different classes become noticeable. Since the discrimination between the two types in the baby coat is sometimes difficult, these two classes are combined in the following tables. Moreover, in the presence of **bb** or **dd** which segregated in some of the matings, the distinction between these types is very unreliable.

In a few cases in Exp. IV males of the $\frac{\mathbf{sh\ c^D\ p}}{\mathbf{sh\ c^D\ p}}$ stock were used as triple recessives. In such matings all the animals in question are $\mathbf{c^Dc^D}$.

RESULTS

The results of these experiments, including the data already published, are given in Tables I and II. For comparison of the relative viabilities of corresponding classes, the totals of Exps. I and II and of Exps. III, IV and V are given. As is obvious from the χ^2's, in none of the cases is there a serious disturbance of the ratios, at least not in the totals. We shall have to come back to that point later. The single-factor ratios in the experiments are given in Table III. They, too, do not show any disturbance in the grand totals, though a closer analysis tells a different story.

The linkage values obtained in the five experiments are given in Table IV. That table also gives the number of double cross-overs observed and the number expected on the assumption that there is no interference. As is seen, altogether 21 double cross-over individuals should have occurred on that assumption, whereas only 5 were actually found.

Arranging the whole of the data in an ordinary fourfold table leads to $\chi^2 = 14{\cdot}439$ for one degree of freedom, while Yates' correction for continuity lowers the value slightly to 13·714. The odds against that happening due to chance alone are about 1 : 5000. The existence of

TABLE I

$$\frac{\mathbf{sh} + \mathbf{p}}{+ \mathbf{c}^{\mathrm{D}} +} \times \frac{\mathbf{sh\ c\ p}}{\mathbf{sh\ c\ p}}$$

Experiment	I	II		
Sex of heterozygous parent ...	♀	♂	Total	χ^2
0 pink-eyed shakers	464	135	599	2·589
0 black-eyed whites	484	172	656	
1 black-eyed white shakers	16	2	18	0·400
1 pink-eyed	18	4	22	
2 black-eyed shakers	85	23	108	0·391
2 pink-eyed whites	75	24	99	
1, 2 pink-eyed white shakers	2	—	2	—
1, 2 wild type	—	—	—	
Total	1144	360	1504	

TABLE II

$$\frac{\mathbf{sh\ \ c\ \ p}}{+\ +\ +} \times \frac{\mathbf{sh\ c^{D}\ p}}{\mathbf{sh\ c^{D}\ p}}*$$

Experiment	III	IV	V		
Sex of heterozygous parent ...	♀	♀	♂	Total	χ^2
0 pink-eyed white shakers	355	260	265	880	0·005
0 wild type	411	239	233	883	
1 black-eyed shakers	21	13	10	44	0·835
1 pink-eyed whites	25	15	13	53	
2 black-eyed white extreme dilute shakers	72	43	31	146	3·351
2 pink-eyed	93	52	34	179	
1, 2 pink-eyed shakers	—	—	—	—	—
1, 2 black-eyed whites extreme dilutes	1	2	—	3	
Total	978	624	586	2188	

* For the differences between the experiments see text.

TABLE III

Single-factor ratios

Experiment ...	I	II	III	IV	V	Total	Homogeneity test χ^2 $(n=4)$
Sh	577	199	530	308	280	1894	9·907
sh	567	161	448	316	306	1798	
χ^2 $(n=1)$	0·087	4·011	6·875	0·103	1· 54	2·496	
C	567	163	525	304	277	1836	10·561
c ($\mathbf{c}^{\mathrm{D}}$)	577	197	453	320	309	1856	
χ^2 $(n=1)$	0·087	3·211	5·301	0·410	1·747	0·108	
P	585	196	505	297	274	1857	8·229
p	559	164	473	327	312	1835	
χ^2 $(n=1)$	0·591	2·872	1·047	1·442	2·464	0·131	

For 4 degrees of freedom $P_{0\cdot10}=7\cdot779$; $P_{0\cdot05}=9\cdot488$; $P_{0\cdot02}=11\cdot668$.

interference in the mouse can therefore be regarded as being established beyond doubt.

On the other hand, it is not so certain whether that interference is in fact weaker than in *Drosophila*. As pointed out previously, in a similar case in that organism, we should expect a coincidence of 0·1 or less. This would lead to an expectation of 2·1 or less double cross-over individuals, whereas 5 were actually found.

TABLE IV

Experiment	I	II	III	IV	V	
Sex of heterozygous parent ...	♀	♂	♀	♀	♂	Total
Cross-over percentage:						
sh–c	3·1	1·7	4·8	4·8	3·9	3·8
c–p	14·2	13·1	17·0	15·5	11·1	14·5
Double cross-over individuals:						
Expected	5·1	0·8	8·0	4·6	2·5	21·0
Observed	2	—	1	2	—	5

Now, when the frequency of a type in a population is q, the probability that 5 or more will be found in a sample of n individuals is P, where

$$1-P=(1-q)^n+nq\,(1-q)^{n-1}+\frac{n\,(n-1)}{2\,!}\,q^2\,(1-q)^{n-2}$$
$$+\frac{n\,(n-1)\,(n-2)}{3\,!}\,q^3\,(1-q)^{n-3}+\frac{n\,(n-1)\,(n-2)\,(n-3)}{4\,!}\,q^4\,(1-q)^{n-4}.$$

In our case, $n=3692$, $q=0{\cdot}0005688$. So $P=0{\cdot}0577$. It is, therefore, likely but not certain that interference is weaker than in *Drosophila*.

Comparing the cross-over values obtained for females and males, there is a lower value for males in both sections when I is compared with II, or III and IV are compared with V. This corresponds well to the expectation.

What does not correspond to expectation is that the value for **sh–c** in V (♂♂) is higher than that in I (♀♀). The difference is not significant, but is in the wrong direction. As is obvious from Table IV, for the **sh–c** interval the values of III and IV are distinctly higher than that for I, while V is markedly higher than II. In other words, for the **sh–c** section the values obtained from coupling are consistently higher than those obtained from repulsion in both sexes. Significance tests by means of fourfold tables lead to $\chi^2=4{\cdot}659$ in the case of the females and to $\chi^2=3{\cdot}827$ in the case of the males ($n=1$). Adopting the 5 per cent. level of significance, both differences are to be regarded as established. The evidence from both sexes may be combined by adding the respective χ's and ascribing to this sum the standard error $\sqrt{2}$. Then we get

$$2{\cdot}158 + 1{\cdot}956 = 4{\cdot}114 \pm 1{\cdot}414.$$

The odds against so big a deviation being due to chance alone are less than 1 : 250. There can, therefore, be little doubt that these differences are significant, whatever the reason may be.

Now, turning to the **c–p** section the situation is less clear. The coupling data in females are higher than the repulsion data, thus showing the same trend as the **sh–c** interval. Comparison of I with III+IV leads to $\chi^2 = 2{\cdot}597$ for one degree of freedom which, though it is not significant in itself, is in the "right" direction. In males the (not significant) difference goes in the "wrong" direction; however, that value by itself does not exclude the possibility that the expected difference is obscured by random sampling.

This curious and unexpected situation led to a careful reinvestigation of all the data published about the albino chromosome of the mouse. A complete list of the papers referred to below has been given previously (Grüneberg, 1935).

In the **sh–c** interval Gates' figures (1931) show the same trend as my own data, though to a slightly lesser extent. Comparing coupling and repulsion in females (combined data) leads to $\chi^2 = 3{\cdot}600$ for one degree of freedom which in itself is not significant though suspiciously high. For males we get by the same method $\chi^2 = 3{\cdot}290$, the deviation being in the same direction. Combining the evidence from both sexes as above, the sum of χ's is $3{\cdot}711 \pm 1{\cdot}414$. This corresponds to $P = 0{\cdot}01$ or, in other words, a difference as big or bigger than that observed due to chance alone would occur only once in 100 trials.

TABLE V

sh–c *interval*

	Coupling						Repulsion					
	♀♀			♂♂			♀♀			♂♂		
Author	*n*	Cross-overs	%	*n*	Cross-overs	%	*n*	Cross-overs	%	*n*	Cross-overs	%
Gates (1931)	1920	79	4·1	240	7	2·9	—	—	—	—	—	—
Grüneberg (all data)	1602	77	4·8	586	23	3·9	1144	36	3·1	360	6	1·7
Total	3522	156	4·43	826	30	3·63	1144	36	3·15	360	6	1·67

The calculations for the **c–p** section are complicated by the fact that in the earlier experiments of Dunn (1920), Castle & Wachter (1924) and the coupling data of Detlefsen & Clemente (1924), the bottom recessive **c** was used. The young which were classified immediately after birth fell into two classes only, viz. black-eyed and pink-eyed individuals. In repulsion $\left(\frac{\mathbf{c\,P}}{\mathbf{C\,p}} \times \frac{\mathbf{c\,p}}{\mathbf{c\,p}}\right)$ the dark-eyed young recovered represent one-half of the cross-overs, whereas the corresponding class and both non-cross-over types are pink-eyed. The cross-over percentage is therefore estimated by doubling the number of dark-eyed young and calculating the cross-over value on this basis.

TABLE VI

c–**p** *interval. Coupling. Females*

Type of data	Author	n	Smaller class	Estimate of p	Amount of information
a	Feldman (1924)	1040	208	0·2000	
a	Grüneberg (new data)	1602	263	0·1642	
a	Total	2642	471	0·1783	18,035
c	Dunn (1920)	1420	595	0·1620	
c	Castle & Wachter (1924)	556	225	0·1906	
c	Detlefsen & Clemente (1924)	439	194	0·1162	
c	Total	2415	1014	0·1602	2,479
a+**c**	Total	—	—	0·1761	20,514

Cross-over value $= 17{\cdot}61 \pm 0{\cdot}698$ %.

TABLE VII

c–**p** *interval. Repulsion. Females*

Type of data	Author	n	Smaller class	Estimate of p	Amount of information
a	Detlefsen & Clemente (1924)	98	20	0·2041	
a	Feldman (1924)	313	51	0·1629	
a	Grüneberg (1935)	1144	162	0·1416	
a	Total	1555	233	0·1498	12,207
b	Dunn (1920)	1369	107	0·1563	4,750
a+**b**	Total	—	—	0·1516	16,957

Cross-over value $= 15{\cdot}16 \pm 0{\cdot}768$ %.
Difference between coupling and repulsion in females $2{\cdot}45 \pm 1{\cdot}038$ %.
$D/m = 2{\cdot}360$. $P = 0{\cdot}02$.

In coupling $\left(\frac{\mathbf{C\,P}}{\mathbf{c\,p}} \times \frac{\mathbf{c\,p}}{\mathbf{c\,p}}\right)$ the dark-eyed young represent one non-cross-over class, while the other non-cross-over class and both cross-over types show pink eyes. The cross-over percentage is therefore estimated by using the excess of pink-eyed over black-eyed young as a basis.

In the repulsion experiment of Detlefsen & Clemente (1924) and all the experiments of Feldman (1924) and myself, all four possible classes are directly recognizable.

Evidently, the amount of information furnished by the earlier experiments is much less than that of the later ones. Let $100x$ be the cross-over value of **c–p**. Then we have estimates of

(*a*) x from complete back-cross data,

(*b*) $\frac{x}{2}$ from incomplete repulsion data,

(*c*) $\frac{1-x}{2}$ from incomplete coupling data.

In the latter two cases the amount of information (I) about x is $\frac{1}{4}$ that about $\frac{x}{2}$ or $\frac{1-x}{2}$, i.e. $\frac{(a+b)^3}{4ab}$, where a and b are the numbers in the two classes.

TABLE VIII

c–p *interval. Coupling. Males*

Type of data	Author	n	Smaller class	Estimate of p	Amount of information
a	Feldman (1924)	129	15	0·1164	
a	Grüneberg (new data)	586	65	0·1109	
a	Total	715	80	0·1119	7,196
c	Dunn (1920)	1911	819	0·1429	
c	Castle & Wachter (1924)	3324	1431	0·1390	
c	Detlefsen & Clemente (1924)	698	329	0·0573	
c	Total	5933	2579	0·1306	6,036
a + c	Total	—	—	0·1204	13,232

Cross-over value = 12·04 ± 0·869 %.

TABLE IX

c–p *interval. Repulsion. Males*

Type of data	Author	n	Smaller class	Estimate of p	Amount of information
a	Detlefsen & Clemente (1924)	376	45	0·1197	
a	Feldman (1924)	275	32	0·1164	
a	Grüneberg (new data)	360	47	0·1306	
a	Total	1011	124	0·1227	9,395
b	Dunn (1920)	1772	115	0·1298	7,300
a + b	Total	—	—	0·1258	16,695

Cross-over value = 12·58 ± 0·774 %.
Difference between coupling and repulsion in males − 0·54 ± 1·16 %.
$D/m = 0{\cdot}464$. $P = 0{\cdot}62$.

The result of these calculations is given in Tables VI–IX. In females, crossing-over in the **c–p** interval is higher in coupling (17·61 ± 0·698 per cent.) than in repulsion (15·16 ± 0·768 per cent.). The difference of these values is 2·45 ± 1·038 per cent., and therefore 2·36 times its standard error. The odds against so big a deviation being due to chance alone are

1 : 50. In males there is still a very slight and insignificant deviation in the wrong direction. (Coupling 12·04 ± 0·869 per cent.; repulsion 12·58 ± 0·774 per cent. Difference −0·54 ± 1·16 per cent. $D/m = 0·464$. $P = 0·62$.)

The whole of the evidence for both chromosome regions and both sexes is summarized in Table X, where, for sake of convenience, everything is expressed in terms of χ. I am inclined to interpret this table thus. There is strong evidence that in females crossing-over is higher in coupling than in repulsion in both marked intervals, the sum of the χ's being as high as 4·257 ± 1·414, corresponding to $P = 0·0026$. It seems doubtful whether a similar effect exists in males, although there is some indication of it in the **sh–c** interval, for which the sum of χ's for both sexes is 3·711 ± 1·414 ($P = 0·01$).

TABLE X

Difference between coupling and repulsion. χ *table*

	♀♀	♂♂	Both sexes	P
sh–c	+1·897	+1·814	+3·711	0·01
c–p	+2·360	−0·464	+1·896	0·18
Both intervals	+4·257	+1·350	+5·607	
P	0·0026	0·34	—	0·0052

We are left to decide whether in the females the 1 chance in 385 has been a reality in the work with the albino chromosome; or else we must invent an *ad hoc* hypothesis to explain the apparent inconsistency of the data with the classical theory which postulates the same linkage values for coupling and repulsion. The author is inclined to suspend judgement of the matter for the time being, since he is unable to present any plausible hypothesis.

Crossing-over and age of parents

All the data were investigated carefully as to whether there is any change of crossing-over frequency with the age of the parents. The material was subdivided in five age groups (2–4, 5–6, 7–10, 11–14, and over 14 months), the last group being represented by small numbers only. Each experiment was treated separately. The result was completely negative. The values showed a random distribution without any indication of consistent changes with age. It seems unnecessary to give the whole of this negative evidence in detail.

Inhomogeneity of data

As mentioned above, the single-factor ratios in the grand totals (Table III) do not show a serious deviation from the expected equality of corresponding allelomorphs. However, the deviations in the single experiments are markedly greater than expected on the basis of random sampling alone. The sets of data were therefore tested for homogeneity by means of $2 \times n$ tables. This leads to values of χ^2 (for 4 degrees of freedom) of 9·907 for **Sh/sh**, 10·561 for **C/c** and 8·229 for **P/p**.

For 4 degrees of freedom, the chance of a deviation greater than 7·779 is 0·10, of a deviation greater than 9·488 is 0·05 and of a deviation greater than 11·668 is 0·02. Adopting the 5 per cent. level of significance, two out of the three values are to be regarded as significant, while the third one is suspiciously high. The figures, therefore, suggest that the data are probably inhomogeneous, and that causes other than random sampling are responsible for the excess of deviation. Since all the animals were bred under the same conditions, and in Exps. III–IV even largely simultaneously, it seems unlikely that exogenous causes are responsible for this apparent inhomogeneity. The alternative is the assumption of genetical causes. As a suggestion the hypothesis may be put forward that a lethal factor linked with the genes for shaking, albinism and pink-eye dilution segregated in some of the pairs. In coupling with the recessives, such a factor would lead to an excess of the dominants in those matings in which it segregates, while in repulsion an excess of recessives would result. If such a factor is present in coupling and repulsion in about the same frequency, its effect would be blotted out in the grand totals. The material was sufficiently inbred during the experiments to make such a segregation possible.

The investigation of the material according to single paired matings does not allow for a definite decision, since many of the families are too small to be of much value for this question. On the whole the deviations of single families from the total of an experiment are greater than would be expected from chance alone, but this excess is not significant in our material which was not bred for the solution of this question. It seems unprofitable at this stage to discuss all the pros and cons in detail, since new experiments are being undertaken which, it is hoped, will give a firmer basis for such a discussion.

Whatever the result of these experiments may be, it seems clear that even the presence of linked lethals would not account for the difference of crossing-over in coupling and repulsion. In the first place, the good fit in the grand totals of Table III shows that a lethal, if present, was sufficiently evenly distributed over coupling and repulsion as not to influence the cross-over values. Secondly, it seems hard to believe that such a lethal (or several) were present in the experiments of the other authors whose experimental data show the same trend.

SUMMARY

1. A trifactorial back-cross in the house-mouse involving the genes for shaker$_1$, albinism and pink-eye dilution was carried out reciprocally in alternating repulsion and in coupling. Altogether 3692 animals were bred.

2. The existence of interference in the sections marked was conclusively proved. It is likely, though by no means certain, that this interference is weaker than in *Drosophila*.

3. Crossing-over seems to be more frequent in coupling than in repulsion in both marked intervals in females and possibly in males. The same trend is found in the data of previous investigators who worked with this chromosome. No plausible explanation for this unexpected difference could be given.

4. Combining the data from the literature and those presented in this paper leads to the following weighted mean cross-over values:

	Females		Males	
	Coupling	Repulsion	Coupling	Repulsion
sh–c	4·43 ± 0·35	3·13 ± 0·52	3·63 ± 0·65	1·67 ± 0·68
c–p	17·61 ± 0·70	15·16 ± 0·77	12·04 ± 0·87	12·58 ± 0·77

5. No change of crossing-over with the age of the parents was found.

6. Inhomogeneity in the data as regards the single-factor ratios is shown to exist. It is suggested that this may be due to a linked lethal factor segregating sometimes in coupling, sometimes in repulsion.

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A complete list of references to earlier work with the albino chromosome of the mouse is given in:

GRÜNEBERG, HANS (1935). "A three-factor linkage experiment in the mouse." *J. Genet.* **31**, 157–62.