A BALANCED THREE-POINT EXPERIMENT FOR LINKAGE GROUP V OF THE HOUSE MOUSE

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1. INTRODUCTION

SOME fourteen linkage groups are now available in the house mouse, *Mus musculus*. In some of these, as many as seven or eight factors have been located. In many groups, the order of the various loci has been reliably ascertained, and some of the recombination fractions for adjacent loci have been accurately established. However, the chromosomes have not yet been mapped in a thorough and detailed manner: there is much to be done before it is possible to specify the frequencies of the modes of gamete formation of multiply-heterozygous animals—that is, before chromosome maps can be used to predict the outcome of experimental programmes involving many linked loci, such as will no doubt be essential to the fields of applied genetics of the future.

The obstacles retarding progress towards this end appear to be two-fold. Firstly, knowledge about the phenomenon of interference is still elementary. Some preliminary experimental work has been reported, and much theoretical work has been done—in fact, theory is greatly in advance of observation. Little progress appears possible until further data are at hand.

Secondly, experimental material, in its turn, has often been neither sufficiently comprehensive nor sufficiently accurate to allow quantitative rather than merely qualitative conclusions concerning interference to be drawn from it. Such inaccuracy is usually found to be due to one or all of the following causes: poor design, inadequate practical execution, and imperfect analysis of results.

The experiment now to be reported is not free from imperfections, nor does it present any basically new techniques or ideas. It is an attempt to make practical use of relevant theory in design, construction and analysis, and in so doing to make a small but reliable contribution to the study of interference.

2. DESIGN: SOME HISTORICAL NOTES

The need for homogeneity in linkage data was recognised some forty years ago. Bridges (1915) and Muller (1916) devised a very competent method of eliminating differential viability, a method which uses what is now termed “balance”. Further, Bridges and Morgan (1923) listed a number of factors capable of causing heterogeneity in *Drosophila* data: fluctuation of recombination value with age, with the chromosome involved and even with different sections of the same chromosome,

response to temperature and other environmental agents, and "a large number of mutant conditions which decrease, or, more rarely, increase the amount of crossing-over".

It is surprising, therefore, to find that Bridges' and Muller's concept of balance, which can be used to overcome one of these difficulties, was seldom used for two-point work, very rarely for three-point, and not at all for work involving more than three factors.

Bridges in his 1915 paper states: "In obtaining data upon any linkage case it is best to have half the data in the form of 'coupling' and half as 'repulsion' experiments. . . . We may offset . . . disturbance by balancing the viability of a certain class against itself." He then sets out two tables of data balanced in this way. It is quite clear that this method was intended for experiments involving more than two loci, for Bridges continues "To balance the data of an experiment involving three loci requires four sets of data, instead of two, as in the case of two loci," and a further treatise by Bridges and Morgan (1916) states: "For three pairs of genes there are eight classes, and in order that each of them may appear as a non-cross-over, as each single cross-over, and as the double cross-over, four experiments must be made." In fact a three-point experiment using all four kinds of heterozygote is tabled here for vermilion, sable and bar: about 7500 flies were bred in all. Another three-point, with imperfect balance (very unequal numbers of progeny were raised from the four heterozygotes) is reported by Bridges and Morgan (1919), using black, purple and vestigial: about 5000 flies were classified. A further three-point experiment, on a very large scale, is described by the same authors (1923), using *dichaete*, *ski-III* and *spineless*; more than 15,000 flies were bred.

It is in the 1923 paper that a reason for the paucity of good multi-point data becomes apparent. It is there stated that not only does "the combination of the data from these four crosses give a neutralisation of the effect of inviability," but "this method has the further great advantage that since the sources and relations of the chromosomes are varied, cross-over variations are also neutralised or revealed".

The mistaken idea that all possible causes of heterogeneity are cancelled out by balance probably led indirectly to the abandonment of this method: for a workman who expects too much of his tool discards it when it does not fulfil his expectations. Certainly, a scrutiny of the published *Drosophila* three-points reveals a degree of heterogeneity which renders impossible an accurate interpretation of the operation of interference. In some cases there is heterogeneity of output between heterozygous types, in others between matings of the same type; and the source of heterogeneity is sometimes found to be due to (possibly real) fluctuation in recombination values, and sometimes cannot be traced at all, due to inadequate presentation of the data.

Perhaps the most surprising historical feature is that the balanced design has not been used extensively elsewhere. In the house mouse, in particular, it should be expected to be of great practical value; for neither of the main causes of fluctuation in the recombination value in *Drosophila*, namely age and temperature variation, have been established sufficiently strongly in mice to arouse the suspicion that "balanced" experiments may be heterogeneous without standardised temperature and age factors. Moreover, it should be comparatively easy to standardise these factors, if it should become necessary. Certainly the first balanced three-point in mice, reported by Wright (M. E. Wallace) in 1947, is remarkably homogeneous.

One may surmise that a further reason why balance was not generally envisaged in the planning of multi-point experiments is that the conditions for perfect balance were not generally realised—and were not understood even by Bridges. Nowhere does he mention the mathematical process whereby "cancellation" can be effected. This situation is strongly indicated by his insistence, even for two-point work, on equal numbers of classified progeny from each kind of heterozygote, an idea later improved by Fisher (1935-53). Muller (1916)—whose description of the same

method of balancing data is apparently independent, since he gives no reference to Bridges' earlier paper—does introduce some mathematical considerations. His main contribution to the understanding of the problem is his proposal that the best estimate of the recombination value is not obtained from the sum of the recombinants from both coupling and repulsion, but is derived from the square root of the following: the products of non-recombinants of both phases divided by the products of recombinants. His treatment falls short of Fisher's in that he does not consider the members of a complementary pair of genotypes as one unit and uses only half of the data. Neither does he give a method of estimating the standard error. No one among the early *Drosophilists* seems to have applied Muller's treatment to actual data.

It is apparent from a glance at the early *Drosophila* work that the development of statistical methods had not reached a stage when it could be of much use in this problem; and in fact, it is with admiration that one realises the acuity of judgment with which the enormous volume of work done by these pioneers was compiled and edited by them. It was not until the development of the Method of Maximum Likelihood by Fisher (1912 and 1921, 1925-1954), that the statistical foundations for modern linkage work were laid. As an outcome of this, papers by Hutchinson (1929) and Immer (1934) are noteworthy. Fisher (1935-1953), in giving a thorough treatment of two-point work, shows how the totals from each phase of linkage may be adjusted during the course of an experiment so as to secure the greatest precision for a given number of organisms bred. These adjustments result in equality of *zygotic outputs* from each phase; this condition (rather than equality of the totals of classified progeny) allows an accurate estimation of the recombination fraction—that is, unbiased by viability disturbance—to be made by the simple sum of the recombinants from both phases. In addition, Fisher gives an efficient formula for the estimation of the recombination fraction when the zygotic totals are unequal; this is similar to Muller's, but uses the whole data.

The concept of balance as proposed by Fisher can be extended to multi-point data. There is no direct way such as that available for two points, by which one can calculate the numbers required to be bred from each type of heterozygote in three-point work so that the totals of zygotes produced by each are the same. Nor is there a simple formula for the recombination fraction when they are different. If, on the other hand, viability disturbance is not extreme and approximately equal numbers are bred from each type of heterozygote, a good estimate can be made by the addition method. In designing a test for linkage of six factors and sex, Fisher and Mather (1936*b*) listed, for up to eight factors, the minimum number of types of heterozygotes required in order to obtain both coupling and repulsion data for all pairs of factors. These ideas, which were later used by him (Fisher, 1948) in the design of Wright's three-point test and made generally available (Fisher, 1954) are inherent in Bridges' concept of balance.

The analysis of Wright's experiment, which tested the linkage of wavy₂, shaker₂ and sex, was made under his direction, and included methods of detecting viability disturbance, should any be present. No significant disturbance was found, hence efficient estimates of the recombination values could be made by the addition method.

A more sensitive method of disclosing disturbance and for correcting for it was then produced by Fisher (1949*a*): this did not disclose significant disturbance in these data, but the method is now available for use in future work, and has been applied to the three-point data from the experiment involving chromosome V now to be described.

A method of eliminating viability disturbance closely related to that of Fisher has been devised by A. R. G. Owen (unpublished); and its application to *Drosophila* data is demonstrated by P. A. Parsons (1957).

Parsons' paper describes the first balanced experiment in *Drosophila* in which the common environmental sources of heterogeneity inherent in this species, which

are not due to viability disturbance, are eliminated by the design of the experiment. With viability disturbance also eliminated during the analysis of the results, he is thus able to draw reliable conclusions both about linkage relations and about certain unusual fluctuations within them.

Besides Wright's experiment in the house mouse there has been one other series of balanced three-points with sex as one of the markers, that of Carter and Phillips (1953). This paper, and some cytological work by Carter, Lyon and Phillips (1954) and Slizynski (1954) cast doubts on the reality of the apparent linkage of wavy₂ and shaker₂ with sex, demonstrated by Wright. An appraisal of the present evidence on this subject will be published shortly elsewhere: certain discrepancies in Carter and Phillips' data will be discussed, and new evidence in favour of the linkage will be given.

The present experiment with mice was completed in 1952 and received preliminary publication as part of a thesis (Wallace, 1954a). As the first balanced three-point in this species which certainly concerns linked genes, it may thus be used unconditionally as a contribution towards a knowledge of interference. A balanced four-point experiment involving the same chromosome has been completed by Owen and a preliminary report (1953a) given on the results. A further balanced four-point experiment, involving linkage group XIII, is being carried out by Fisher. This is particularly interesting since it involves polydactyly (*py*), a factor which shows imperfect manifestation. The only balanced multiple linkage experiment which has, apparently, been carried out with a species other than *Drosophila* and mice, is that of Anderson (1921) in maize; and here the interference relations are somewhat obscured by misclassification; this the author did much to eliminate from the data but did not consider in the analysis. A balanced five-point, involving linkage group V and using the well-spaced markers Ragged (*Ra*), agouti (*A*), wellhaarig (*we*), fidget (*fi*) and Danforth's short tail (*Sd*), is under construction by the present writer.

3. CONSTRUCTION

The loci concerned in the three-point experiment now to be described are agouti, fidget and Danforth's short tail. A brief resumé of the history of linkage group V will relate these loci to the others in the group.

The loci marked by kreisler (*kr*), agouti (*A*), undulated (*un*), wellhaarig (*we*), and pallid (*pa*), were found by various workers during the interval 1935-49 to be on the same chromosome. Fisher (1949b), reporting on their order, gave approximate map distances as:

$$kr \quad 3 \quad a \quad 5 \quad un \quad 10 \quad we \quad 3 \quad pa$$

The year 1950 saw several developments. Carter and Grüneberg reported a linkage of about 33.5 per cent. between *fi* and *A*, and the present writer a linkage of about 25 per cent. between *Sd* and *fi*. Earlier unpublished evidence collected at the Jackson Memorial Laboratory, Bar Harbor, and at Cambridge by myself, had shown indications of a loose linkage—about 40 per cent., between *A* and *Sd*. Thus the order

$$A \quad \text{---} \quad fi \quad \text{---} \quad Sd$$

was strongly indicated.

The relation between this series, and the one known earlier, namely *kr*—*a*—*un*—*we*—*pa*, was settled by Borger in the same year

(1950), using *A*, *pa* and *Sd*; he gave the order *kr—A—un—we—pa—fi—Sd*. Complementary evidence that *fi* is beyond the segment *A—pa* and nearest to *pa* was furnished by Carter the following year (1951).

The shorter intervals in the segment *kr—pa* have been carefully mapped: Fisher (1949*b*) has given very full data on the interval *A—un*; and Fisher and Landauer (1953) and Owen (1953*a*) have given interim reports on the four-point involving *A*, *un*, *we*, and *pa*, already mentioned. It seemed profitable to map accurately the unexplored *fi—Sd* region, and to investigate interference relations over a wider interval than that examined by Owen: accordingly agouti was chosen as the third locus.

A further reason for the choice of these loci was their suitability for demonstrating the advantage of attaining balanced backcross data in eliminating viability disturbance. It was essential that at least two of the mutants used could be expected to show poor viability. On the other hand, the factors chosen could not be so deleterious that one could not expect to breed from them.

Extensive work involving the agouti locus had been done in Cambridge, of which Fisher's (1949*b*) experiment with the *A—un* interval was specifically concerned with the five agouti alleles yellow (*A^Y*), light-bellied agouti (*A^L*), dark-bellied agouti (*A*), tan belly (*a'*) and non-agouti (*a*). These investigations showed that in general no significant inviability of any of the genotypes involving these alleles need be expected. Accordingly, no attempt was made to eliminate any of the four alleles (*A^Y*, *A*, *a'*, *a*) available in the initial stocks used, although, by chance, most of the material involved *a'* and *a*. In the single instance in which an *Aa'* heterozygote (*a'fiSd/A*) was available, in which either *A* or *a'* may be regarded as dominant, *A* was treated as dominant and the heterozygote classed as "*Sd-out*". This was a matter of convenience, these heterozygotes being in short supply at that time. The decision to breed from this mating was fortunate since the progeny threw some light on the nature of the agouti locus (Wallace, 1954*b*).

Fidget was first described by Grüneberg (1943). To judge by his description, it is suitable in exhibiting impaired viability, but practically impossible to breed from homozygously. Grüneberg's more recent work (1952) re-states his earlier view on its infertility: "Some males are fairly reliable breeders; females often fail to breed; those that do have so far failed to rear their litters." Apparently no attempt was made to improve the performance of fidget in the intervening nine years, nor is it clear that this possibility has been realised by others working with him on this gene (Carter and Grüneberg, 1950, and Truslove, 1956). It is common experience that when a mutation first appears, it is usually deleterious in one or more respects: further, the variability of the effects of single genes is also well known, and the possibility of altering or increasing such effects by selection has been amply demonstrated. It seemed reasonable, therefore, to

suppose that a fixed degree of infertility and inviability of fidget was not necessarily characteristic of it. Accordingly it was introduced to several different stocks in Cambridge. The ratios then obtained confirmed the view that not only its viability and fertility, but also its expression are very variable and an attempt was made to improve its performance by selection.

Response was rapid, and a stock was maintained by homozygous and backcross matings by the time the three-point experiment was planned. Viability of fidgets in the three-point is such that, while in the whole experiment there is, as desired, a significant deficiency of fidgets, this deficiency is not too marked to make the gene impossible to work with, and there are several matings which produced large progenies with no shortage of fidgets: three, with progenies exceeding 30, produced a slight excess. (There is, however, no heterogeneity in the overall segregation of fidgets.)

Further, the fertility of both sexes became quite reliable, though still, as a whole, inferior to that of normals. The production of 549 progeny from male triple heterozygotes, all of which were mated to female fidgets, did not lag more than a few months behind the production of an equal number (550) from female heterozygotes and their male fidget mates. Of the 28 female fidgets used, 9 produced over 30 fully classified young, two of these over 50. (The calculated average output of 19.6 progeny per fidget mother is unduly low since many of them were not allowed to breed for more than a few weeks once the data for their particular type of mating were almost complete.)

A further doubtful point was clarified. Grüneberg (1943) had reported a relation between fidget and polydactylism which could have several explanations. This relation was shown not to be linkage, which might have complicated the three-point experiment, but rather the specific modification by fidget of the gene polydactyly (*py*). The work on fidget will be published in due course.

That Danforth's short tail (*Sd*) shows lowered viability was found by Dunn, Gluecksohn-Schoenheimer and Bryson (1940), who also established its genetic basis as a single major gene producing three distinct phenotypes. The heterozygotes have short tails of variable length, and the homozygotes are almost or completely tailless, and die shortly before or after birth. Variability of tail length and viability of both heterozygotes and homozygotes was established by Dunn *et alii* and by Fisher and Holt (1944); the latter also demonstrated rapid response to selection for large and small tail length in heterozygotes, accompanied by correlated changes in viability.

Experience in the manipulation of this factor was gained while conducting a continuation of Fisher's experiment. One of the aims of this experiment was to make *Sd* as completely recessive as possible, and this end had been largely achieved in the production in some matings of tails as long in the heterozygote as in the normal, and in the production of two proved homozygotes which lived, one for

7 days and the other for 22 days. The present writer produced in addition an animal, which, in the stock selected for long tails, was judged by reason of its very short tail, to be probably a homozygote : it lived for 40 days.

Experience showed that, while the expression of *Sd* is variable, it should not be difficult to classify on a comparatively unselected background ; and that, while its viability is also variable, it is controllable. One could be confident that, given a fairly favourable background, the mere inevitable selection of future matings from viable and fertile heterozygotes would ensure a sufficiently high and stable degree of viability.

Danforth's short tail and the four agouti alleles were derived from several sources, and the fidget mice from the investigation of its association with polydactyly. From these stocks, appropriate preparation matings were made to produce the four types of heterozygote of each sex :

(i) Tricoupling	.	.	.	$\frac{a^t + Sd}{a\ fi +}$
(ii) a^t -out	.	.	.	$\frac{a + Sd}{a^t\ fi +}$
(iii) <i>Sd</i> -out	.	.	.	$\frac{a^t + +}{a\ fi\ Sd}$
(iv) <i>fi</i> -out	.	.	.	$\frac{a^t\ fi\ Sd}{a + +}$

The first, tricoupling, is so named because each of the three factors is in coupling with each of the others. The other three heterozygotes have only one pair in coupling and two in repulsion ; each has a different pair in coupling, so exhausting all possible arrangements of two alleles at each of three loci. A convenient nomenclature is to use for the identification of each of these three heterozygotes a symbol for that pair of alleles which is displaced, *i.e.* "out", as compared with tricoupling.

The four types of heterozygote were mated to triple recessives *a fi* (or $a^t\ fi$ where stated below) and 1099 progeny were bred ; half of these were from male heterozygotes and half from female, and they comprised about 135 from each type of heterozygote of each sex.

4. ANALYSIS

The full data are tabulated by genotypes in table 1 (Appendix).

(i) Viability ratios

It has been pointed out that both *fi* and *Sd* were expected, when the experiment was started, to show unequal survival of the various genotypes which are formed from them ; and that the members of

the agouti series were expected to have about equal viability. A summary of the three single-factor ratios (table 2, appendix) shows that these expectations are fulfilled, the ratios being as follows :

	$a^+ : a$	$fi : +$	$Sd : +$
from female heterozygotes . . .	272 : 278	235 : 315	241 : 309
from male heterozygotes . . .	269 : 280	226 : 323	246 : 303

Sd and fi show significant losses : Sd has a survival rate approximating to 80 per cent., and fi to 72 per cent. The segregation of the agouti alleles is very insignificantly unequal.

There is clearly no significant difference between these ratios according to whether the offspring are derived from female or from male heterozygotes.

There is, however, significant heterogeneity in the ratios $+$: fi and Sd : $+$ within the progeny from the four types of female heterozygote, but no heterogeneity within the progeny from male heterozygotes. (χ^2 values for females are 9.9271 and 14.8115, each for 3 d.f., and for males 5.2813 and 5.8819, also for 3 d.f.) This suggests the possibility of a maternal effect. However, further analysis (Wallace, 1954*a*) does not give more than subsignificant support for this hypothesis. It merely suggests two possibilities : a difference in the performance of different heterozygous types corresponding to differences in the gene arrangement at the loci studied here, which constitutes a kind of position effect, and the confinement of these differences to the performance of female as against male heterozygotes, which in turn constitutes a kind of maternal effect.

Such possibilities, which may be explored by the analysis of the single-factor ratios and of ratios within complementary pairs, should not be overlooked. The balanced backcross provides very suitable material for this kind of investigation.

(ii) Viability disturbance

Viability disturbance may be defined as imperfect viability of the complementary pairs of progeny produced by the various modes of gamete formation, which is differential as between one mode and another.

It is clear that if, in a linkage experiment, the members of a series of alleles at only one locus have unequal viability, the whole data will not in this sense be disturbed ; but if the alleles at two or more loci have unequal viability, the survival rates of the different complementary pairs will be unequal.

The single-factor ratios (table 2, appendix) and the ratios within complementary pairs (table 3, appendix) show that fi and Sd are likely to disturb linkage data in this way, although, as will become manifest, further analysis is required to prove whether they actually do so or not.

Two of the single-factor ratios, as already mentioned, demonstrate unequal viability; and, further, they exhibit heterogeneity at least in the progenies from female heterozygotes. The members of the complementary pairs also are unequally viable, the total χ^2 (table 3B) being 43.7271 for 4 d.f., probability <0.001 ; and there is, again, significant heterogeneity within complementary pairs from female heterozygotes, χ^2 for the segregation of $fiSd: +$ (table 3B) being 8.9812 for 3 d.f., probability 0.05 to 0.02. (The a' locus is ignored in table 3B since it is shown in table 2 to have no inequalities: table 3A gives an analysis for female heterozygotes which takes account of the a' segregation.)

TABLE 4
Observed frequencies of complementary pairs (a)

Types of heterozygote	A : Progeny from female heterozygotes					B : Progeny from male heterozygotes				
	Modes of gamete formation					Modes of gamete formation				
	0	1	2	12	Totals	0	1	2	12	Totals
Tricoupling . . .	65	43	21	7	136	71	36	25	3	135
a' -out . . .	69	46	21	7	143	77	25	28	7	137
Sd -out . . .	71	41	15	7	134	71	39	24	5	139
fi -out . . .	66	36	25	10	137	71	27	33	7	138
Totals . . .	271	166	82	31	550	290	127	110	22	549

Modes of gamete formation :

- 0 = no recombination.
 1 = recombination in segment 1 (a' - fi) and not in 2
 2 = recombination in segment 2 (fi - Sd) and not in 1.
 12 = simultaneous recombination in segments 1 and 2.

Complementary pairs form a 4×4 Latin Square :

- A = $a' Sd, a fi$ A B C D
 B = $a Sd, a' fi$ B A D C
 C = $a', a fi Sd$ C D A B
 D = $a, a' fi Sd$ D C B A

Now, recombination estimates based on balanced data are rendered less precise than they would otherwise be if there is an appreciable change in the survival rate of one or more complementary pairs as between one type of mating and another. If single-factor ratios change during the course of the experiment, or if the ratios between members of complementary pairs change, one may in general expect the survival rates of the complementary pairs themselves to change. But the latter changes need not be as large as the former owing to the averaging effect of regarding the complementary pair as one unit. Nor do they necessarily follow at all, since the depletion of one genotype may conceivably be accompanied sometimes by an improvement in the viability of its complementary. Thus, although examination of single-factor ratios and of ratios within complementary pairs has shown such changes (at least in progeny from female heterozygotes), they need not on this account be regarded as proof of disturbance.

If there is differential viability of the complementary pairs, through this or any other cause, there will be non-proportionality within the observed numbers of the complementary pairs as produced by the various modes and the various types of heterozygote. A

TABLE 5
 χ^2 test for 9 d.f. for viability disturbance between complementary pairs

Expected frequencies (m)										
	A : Progeny from female heterozygotes					B : Progeny from male heterozygotes				
	Modes of gamete formation					Modes of gamete formation				
	0	1	2	12	Totals	0	1	2	12	Totals
Tric. .	67.0109	41.0473	20.2764	7.6655	136	71.3115	31.2295	27.0492	5.4098	135
a'-out .	70.4600	43.1600	21.3200	8.0600	143	72.3679	31.6922	27.4499	5.4900	137
Sd-out .	66.0255	40.4436	19.9782	7.5527	134	73.4244	32.1548	27.8506	5.5701	139
fi-out .	67.5036	41.3491	20.4255	7.7218	137	72.8962	31.9235	27.6503	5.5301	138
Totals .	271	166	82	31	550	290	127	110	22	549
$(a-m)^2/m$										
					Total χ^2					Total χ^2
Tric. .	0.0603	0.0929	0.0258	0.0578	0.2368	0.0014	0.7287	0.1552	1.0734	1.9587
a'-out .	0.0303	0.1869	0.0048	0.1394	0.3614	0.2965	1.4131	0.0110	0.4153	2.1359
Sd-out .	0.3748	0.0077	1.2405	0.0404	1.6634	0.0801	1.4572	0.5324	0.0583	2.1280
fi-out .	0.0335	0.6920	1.0245	0.6721	2.4221	0.0493	0.7593	1.0350	0.3907	2.2343
χ^2 sub-totals	0.4989	0.9795	2.2956	0.9097	4.6837	0.4273	4.3583	1.7336	1.9377	8.4569
Degrees of freedom					(9)					(9)

preliminary test for disturbance is, then, the χ^2 test for 9 degrees of freedom ; this should detect gross non-proportionality ; it is made by comparing the observed frequencies of the 16 complementary pairs with those expected on the basis of the marginal totals (tables 4 and 5).

The two χ^2 values, both less than 9, are insignificant for their 9 degrees of freedom.

A more discriminating test for disturbance is obtained by taking out the 3 degrees of freedom relating to the totals of each complementary pair (table 6 ; the observed frequencies are obtained by addition from table 4, and the expected from table 5). These three degrees of freedom relate to overall viability disturbance, leaving six for detecting changes in the survival rates of complementary pairs, *i.e.* variation in differential viability.

These χ^2 values are extremely low and contribute very little to the χ^2 for 9 d.f.

Neither of these tests, however, is specific for viability disturbance, and it may be that some of the variations in numbers of the different pairs of genotypes have been compensated in adjusting the expectations from the row and column totals. An exact and specific treatment is given by Fisher (1949a). When applied to the present data, χ^2 for 3 d.f. for female heterozygotes is 0.23475 and for male heterozygotes is 3.280925. These values are only very slightly greater than those obtained in table 6.

The insignificance of these χ^2 values points to the conclusion that there is no significant viability disturbance in either body of data.

TABLE 6

χ^2 test for 3 d.f. for viability disturbance between complementary pairs

Genotypes	A. Progeny from female heterozygotes			B. Progeny from male heterozygotes		
	Observed (a)	Expected (m)	$(a-m)^2/m$	Observed (a)	Expected (m)	$(a-m)^2/m$
<i>a⁺Sd ; fi</i> . .	136	137.8709	0.0254	127	136.3844	0.6457
<i>Sd ; a⁺fi</i> . .	144	139.4855	0.1461	151	136.8178	1.4701
<i>a⁺ ; fiSd</i> . .	135	135.7110	0.0037	130	137.8871	0.4511
<i>a⁺fiSd ; a</i> . .	135	136.9327	0.0273	141	137.9107	0.0692
Totals . .	550	549.9991	0.2025 (n = 3)	549	549.0000	2.6361 (n = 3)

The remaining values of χ^2 for 6 d.f. (4.4489 for progeny from female heterozygotes and 5.1760 for male) are also insignificant, and indicate that, although there are significant changes in the output of the four kinds of female heterozygote in the single-factor ratios, and in the ratios between members of pairs involving the *fi* and *Sd* loci, these changes have produced no appreciable change in the overall survival rates of the complementary pairs.

The lack of disturbance need not be regarded as rendering the balanced design unnecessary. That the inequality between the survival rates of the two complementary pairs involving *fi* and *Sd* would be insignificant was not known when the experiment was planned. Moreover, this inequality is sufficient to give estimates of the recombination value *fi-Sd* which differ as between coupling data and repulsion, though insignificantly so: clearly the estimate based on the combined data is more accurate than one based on either separately. Further, it could not be certain, when planning the experiment, that the almost complete equality of viability within the agouti alleles which had so far been experienced would be repeated on the genetic background on which this experiment was

carried out ; nor, further, could it be certain that any slight inequality which did exist would not, when combined with the inequalities already observed within the *fi* and *Sd* pairs of alleles, produce significant heterogeneity in the survival rates of the pairs of complementaries made up by all three loci. Hence the balanced design was a necessary safeguard.

In conclusion, analysis of the data from female and from male heterozygotes, treated separately, shows that there is evidence for slight differential viability : hence the high degree of balance attained is justified. The disturbance, however, is insignificant in both sexes : hence the addition formula for the estimation of recombination values is sufficiently accurate. Because of the very mild degree of disturbance, the data are not ideal for a demonstration of Fisher's method of correcting for strong differential viability. The disturbance from

TABLE 7
2xn table of frequencies of modes of gamete formation

	0	1	2	12	Totals
Heterozygous ♀♀ . .	271	166	82	31	550
„ ♂♂ . .	290	127	110	22	549
Totals . . .	561	293	192	53	1099

(Corrected) χ^2 for 3 d.f. = 11.4461
Probability = 0.01 to 0.001

female heterozygotes is less than that from males, despite a sub-significant maternal effect of the heterozygotes on the viability of genotypes involving *fi* and *Sd*.

(iii) *The effect of sex on recombination*

That there are differences in the frequencies of the modes of gamete formation according to the sex of the heterozygous parent can most readily be seen from the χ^2 value obtained from the *2xn* table above : (table 7 : derived from table 4)

These differences, however, are not distributed uniformly along the whole segment *a^t-Sd*, for the adjacent segments *a^t-fi* and *fi-Sd* behave dissimilarly (table 8).

Since twice the standard error of the difference between the recombination fraction from female and from male heterozygotes does not exceed the difference in the segment *a^t-fi*, this difference is significant at the 5 per cent. level of probability. The difference in the *fi-Sd* segment is in the opposite direction, that is the recombination value is larger in the males here whereas it is larger in the females for *a^t-fi* : but this difference, by the same criterion, is not significant.

(iv) *The effect of age on recombination*

Very little attention has so far been paid in the literature to age effects in the mouse. Nor does it appear to be generally realised that real effects of age and sex on recombination must be taken into account in the treatment of data for mapping purposes and in the study of interference.

The only balanced experiment large enough to show these effects is that reported by Fisher (1949*b*). Here, the interval *a-un* shows a decrease in recombination value with increasing age; a stronger decrease is found in male gametogenesis than in female, but this difference is not found to be significant. The even larger experiment referred to by Fisher and Landauer (1953) and Owen (1953*a*), however, does not confirm this age effect (Owen and Darroch, unpublished).

There is no easy explanation for this inconsistency, and further extensive data are needed. In running the present three-point experiment, it was hoped that some contribution on this subject might be forthcoming, and, towards the end of the work, matings which had already run for several months were allowed to continue as long as possible after the records were closed on completion of "balance". The total number of progeny included in the data therefore exceeds the 1099 bred for "balance", being 766 from female heterozygotes and 591 from male (table 10).

The test of significance used in table 10 is that given by Fisher (1925-54). In addition to the symbolism given in the table, we have :

$$\begin{aligned} q &= S(a')/S(n), \quad p = S(a)/S(n) \\ S(t \times a') &= A'; \quad S(t \times a) = A; \\ S(t - \bar{t})^2 &= S(t^2n) - \frac{\{S(tn)\}^2}{Sn} \end{aligned}$$

then

$$\chi^2 = \frac{(qA - pA')^2}{pq S(t - \bar{t})^2}$$

$(qA - pA')$ is positive if the recombination value increases with maternal age, and negative if it decreases.

It is interesting to note that the only segment which shows a significant response to age ($\chi^2 = 5.2162$ for 1 d.f.) is the one which also shows a significant response to sex, namely *a'-fi*. This segment shows higher recombination in the female than in the male, but, while this value remains almost steady in females with advancing age, it increases in males with advancing age. Since one end of this segment, namely *a-un*, showed in one body of data (Fisher, 1949*b*), if not in the later body, a decrease for both sexes with advancing age, the reality of any age effect is very doubtful.

If the evidence for the segment *a'-fi* is taken at face value it appears possible that there is a real response to age, but because the

TABLE 10

Test for regression of recombination value on age of heterozygous parent

A : Progeny from female heterozygotes										
Age of parent (months) (t)	Total no. of offspring (n)	<i>a</i> ⁺ - <i>fi</i>			<i>fi</i> - <i>Sd</i>			<i>a</i> ⁺ - <i>Sd</i>		
		N (<i>a</i> ⁺)	R (<i>a</i>)	R.f. %	N (<i>a</i> ⁺)	R (<i>a</i>)	R.f. %	N (<i>a</i> ⁺)	R (<i>a</i>)	R.f. %
0-2 . . .	28	14	14	50.00	25	3	10.71	11	17	60.71
2-3 . . .	87	52	35	40.23	65	22	25.29	42	45	51.72
3-4 . . .	88	61	27	30.68	67	21	23.86	46	42	47.73
4-5 . . .	98	66	32	32.65	79	19	19.39	61	37	37.76
5-6 . . .	103	69	34	33.01	78	25	24.27	58	45	43.69
6-7 . . .	69	42	27	39.13	56	13	18.84	39	30	43.48
7-8 . . .	55	39	16	29.09	45	10	18.18	33	22	40.00
8-9 . . .	75	45	30	40.00	61	14	18.67	39	36	48.00
9-10 . . .	56	44	12	21.43	43	13	23.21	35	21	37.50
10-11 . . .	39	26	13	33.33	33	6	15.38	20	19	48.72
11-12 . . .	28	15	13	46.43	22	6	21.43	17	11	39.29
12-13 . . .	16	11	5	31.25	15	1	6.25	10	6	37.50
13+ . . .	24	14	10	41.67	22	2	8.33	14	10	41.67
	766 <i>S</i> (n)	498 <i>S</i> (<i>a</i> ⁺)	268 <i>S</i> (<i>a</i>)		611 <i>S</i> (<i>a</i> ⁺)	155 <i>S</i> (<i>a</i>)		425 <i>S</i> (<i>a</i> ⁺)	341 <i>S</i> (<i>a</i>)	
Deviation χ^2 for 1 d.f.		-18.70	786 0.2109		-54.12	330 2.4876		-63.47	126 2.2356	

All recombination fractions decrease with increase in age. None shows a significant trend.

Note.—N = non-recombinants, R = recombinants.

B. Progeny from male heterozygotes										
Age of parent (months) (t)	Total no. of offspring (n)	<i>a</i> ⁺ - <i>fi</i>			<i>fi</i> - <i>Sd</i>			<i>a</i> ⁺ - <i>Sd</i>		
		N (<i>a</i> ⁺)	R (<i>a</i>)	R.f. %	N (<i>a</i> ⁺)	R (<i>a</i>)	R.f. %	N (<i>a</i> ⁺)	R (<i>a</i>)	R.f. %
0-2 . . .	16	11	5	31.25	11	5	31.25	8	8	50.00
2-3 . . .	72	50	22	30.56	54	18	25.00	34	38	52.78
3-4 . . .	87	68	19	21.84	73	14	16.09	60	27	31.03
4-5 . . .	112	94	18	16.07	77	35	31.25	65	47	41.96
5-6 . . .	78	59	19	24.36	67	11	14.10	48	30	38.46
6-7 . . .	67	48	19	28.36	53	14	20.90	40	27	40.30
7-8 . . .	37	22	15	40.54	26	11	29.73	19	18	48.65
8-9 . . .	38	25	13	34.21	29	9	23.68	18	20	52.63
9-10 . . .	36	21	15	41.67	23	13	36.11	18	18	50.00
10-11 . . .	29	20	9	31.03	20	9	31.03	13	16	55.17
11-12 . . .	12	9	3	25.00	9	3	25.00	8	4	33.33
12-13 . . .	4	3	1	25.00	4	0	...	3	1	25.00
13+ . . .	3	1	2	66.67	2	1	33.33	2	1	33.33
	591 <i>S</i> (n)	431 <i>S</i> (<i>a</i> ⁺)	160 <i>S</i> (<i>a</i>)		448 <i>S</i> (<i>a</i> ⁺)	143 <i>S</i> (<i>a</i>)		336 <i>S</i> (<i>a</i> ⁺)	255 <i>S</i> (<i>a</i>)	
Deviation χ^2 for 1 d.f.		+64.37	839 5.2162		+24.32	828 0.8018		+22.99	621 0.5357	

All recombination fractions increase with increase in age. Only *a*⁺-*fi* shows a significant trend.*Note.*—N = non-recombinants, R = recombinants.

data is not distributed according to age in the same fashion in progeny from females as from males, the whole, or part, of the observed difference in the overall response to sex is only apparent. One interpretation of this latter situation is represented in fig. 1, where straight regression lines have been fitted by eye to the observations given in fig. 2. Support for this latter hypothesis is found in the fact that the mean age of the males is considerably lower than that for females, these being 5.1726 and 5.8399 months respectively (mean age = $\frac{S(t \times n)}{S(n)}$). The expected recombination fractions corresponding to these ages (fig. 1) are 27 and 35 per cent. respectively; that is, the hypothetical lines give at these ages a close approximation to the observed average values.

FIG. 1.—Hypothetical trends of regression of ♂ and ♀ recombination values on age (a^t-fi).

However, an exact fit has not been calculated because it appears unlikely that the relation is, in fact, one of straight line regression. A curved line is often found to give a better fit to biological data of this sort. Moreover, if the relation is assumed to be what the observations more readily suggest, namely a U-shaped curve, some explanation may be found for the conflicting results so far obtained for the $a-un$ segment.

Fig. 2 shows the observed values for the $a-un$ and a^t-fi intervals at corresponding ages as found by Fisher, by Owen and Darroch, and by myself respectively. The data for nine months and over are included, but they are rather scanty in all the bodies of data and may therefore be unreliable. It is striking that while Fisher's observations for $a-un$ give a good agreement with the assumption of a steady decrease, the recombination fractions at six months are higher than those for four months—as they are also in Owen's data for the same segment and my own for the a^t-fi segment. In fact, it is clear that, disregarding the data for nine months and over, a U-shaped curve would give a good fit.

It is possible, therefore, that the whole segment *a-fi* shows a fall in recombination during the first four or five months, and a rise (though probably a shallow one) for the next few months. Conceivably the

FIG. 2.—Observed recombination values plotted against age.

fi-Sd segment also shows a very slight response which might be discernible in more extensive data. In fine, the degree of response of the whole chromosome may be different for different segments; and there may—or may not—be a continuous rather than an abrupt change in

degree throughout its length. At present one cannot say more than that the agouti end appears to respond more strongly than does the *Sd* end.

This age response may be more or less different as between male and female gametogenesis. If so, it is possible that the difference in mean age for the two sexes at which the three-point data are drawn gives an unreal magnitude to the difference in sex for *a-fi* as calculated without regard to age. The magnitude of the sex difference in the *un-we-pa* segments may also be inflated in this way; but since there is no appreciable sex difference for the *a-un* interval in either Fisher's or Owen's data, it seems likely that there is a real change in response to sex at about the *un* locus.

Finally, it is clear from fig. 2 that the assumption of a U-curve may resolve the apparent conflict in the data for the *a-un* segment: the later data show a rise in the second "arm" of the curve (up to 9 months) more strongly than does the earlier, and thus does not reflect well the latter's overall decrease of recombination with age. Methods of fitting various curvilinear regressions to the observed recombination value, or to its corresponding value as obtained by angular transformation (such as that used by Fisher in 1949), will be elaborated when more extensive data are available.

At present, the significance of the age effect for *a-fi* is not large enough to warrant the labour of correcting for it in the estimation of recombination values and in the treatment of interference in male and female gametogenesis. It will, however, be assumed that there is a real sex difference, and data from male and female heterozygotes must accordingly be treated separately for mapping purposes. Provision is being made in the current five-point experiment for the distribution of data according to age to be as even as possible, and a more thorough examination of the effects of both age and sex on recombination will be made.

(v) *The effect of age on the sex-ratio*

An interesting anomaly emerges from the data compiled for the effect of age on recombination. This is a change according to maternal age in the sex-ratio of offspring produced both by fidget mothers and by the aggregate of the four kinds of heterozygous mothers.

The data are divided into two parts: offspring from females of from two to ten months, and offspring from females of over ten months. This arrangement is more a matter of convenience than of choice, for the data were not compiled specifically for an investigation of this sort. The numbers from mothers over ten months are rather low, and it may be that if there were more data from the later ages and if they were divided according to the ages of the mother in months, a progressive change would be discernible. As they stand, the two bodies of data (A and B, table 11) give a subsignificant χ^2 for disproportionality between the two sex-ratios, the direction of this

disproportionality being the same in both cases ; and these, when combined (C, table 11) give a significant χ^2 value.

Changes in the sex-ratio of inbred lines and of F_1 from reciprocal crosses between them have been observed by Howard, McLaren, Michie, and Sander (1955) ; they found a significant rise in the sex-ratio with increase in litter size ; this is interpreted as due to a greater vulnerability of the female foetus over the male, resulting in a greater depletion of females where competition *in utero* is greatest. They found that parity has no effect.

TABLE 11
*Change in sex-ratio from mothers under 10 months old
compared with those over 10 months*

(Only data from ♀♀ mated at 2 months and living until 10 months old or later are included)

	A. Progeny from fidget females			B. Progeny from heterozygous females			C. Progeny from all females		
	♀	♂	Total	♀	♂	Total	♀	♂	Total
<10 m.	199	164	363	205	179	384	404	343	747
>10 m.	24	29	53	37	50	87	61	79	140
	223	193	416	242	229	471	465	422	887
Contingency $\chi^2_c(1 \text{ d.f.})$	1.3299			2.9262			4.8103		

It seems unlikely that the rise in the sex-ratio of progeny from females over 10 months observed in the present data, is correlated with an increase in litter size ; for general experience, and published work (*cf.* Murray, 1934), indicate that litter size decreases from a maternal age of about eight months onwards : the mean litter size for females over 10 months old is therefore probably lower than that for younger females. The assumption of Howard *et alii* that the female sex is more subject to higher pre-natal mortality is, as they point out, in conflict with the views of several other workers : that the male sex is the more vulnerable is more harmonious with the present observations and with the assumption that intra-uterine competition is an important influence on the natal sex-ratio. On the other hand, the present observations may be more explicable in terms of parity than of litter size, a possibility which will be tested if the larger data from the five-point confirm the present findings.

5. INTERFERENCE AND MAPPING

(i) *Interference: some historical notes*

The term "interference" was first coined by Muller (1916). It may be defined as the influence of one crossover upon others in the

same strand. Both cytological and genetic evidence shows that it is usually positive, *i.e.* the occurrence of one crossover tends to inhibit the occurrence of others near it.

It was evident as early as the second decade that interference varies from organism to organism and even from one segment of a chromosome to another. Several crude measures of the strength of interference were adopted, including that known as "coincidence". Although there was a verbal similarity between the various definitions of this term, it covered, during the next two decades, at least three different quantities. Stevens (1936) was responsible for defining it properly for the first time and for giving it concise mathematical formulation; this can be expressed as follows: the index $C = z/y_1y_2$ where y_1 and y_2 are the observed recombination fractions in segments 1 and 2, and z is the observed frequency of double recombinants, *i.e.* recombinants arising from two simultaneous crossovers in segments 1 and 2. Haldane's early attempts (1919) to include both multiple crossing-over and interference in the general theory of crossing-over, give C an approximate value of $\frac{1}{2}$ for segments of intermediate length, and assume a uniform degree of interference along the chromosome. But "coincidence" is not a very satisfactory measure since it depends on the size of y_1 and y_2 and therefore cannot be constant.

Other theoretical work and practical application was done by several workers, notably by Jennings (1923) and by Anderson and Rhoades (1931). But none of the mapping functions constructed by these authors was found to have more than a limited application, and it was not until 1944 that a relationship of more universal application and practical value was found. Kosambi put forward the functions

$$x = \frac{1}{2} \tanh^{-1} 2y \quad \text{and} \quad y = \frac{1}{2} \tanh 2x,$$

and the corresponding simple addition theorem for y :

$$y_{1+2} = \frac{y_1 + y_2}{1 + 4y_1y_2}.$$

Owen (1950), following a suggestion made by Sir Ronald Fisher, has proposed the term "Kosambi coefficient", for a value K comparable to the coincidence ratio C : unlike C , however, it can, on theoretical grounds, be expected to be constant for segments of any length. Its relation to C may be written: $K = C/2y_{1+2}$, where y_{1+2} is the observed recombination value over segments 1 and 2. The Kosambi relations are satisfied if, and only if, a certain intensity of interference operates uniformly over the total length of the linked segments.

Kosambi's mapping function has been found to give a good fit to many cases of three- and four-point data, including Haldane's 1919 data and Jodon's data (Bhat, 1950). The Kosambi relation is not, however, completely satisfactory. Firstly, as has been pointed out, it assumes a certain uniform intensity of interference over the

length of the chromosome : it takes no account of the position of loci in relation to the centromere and terminus, both of which have been shown to affect the intensity. Secondly, it does not provide a complete theory of recombination, for it does not specify the frequencies of the modes of gamete formation for more than three loci simultaneously. And finally it sets an upper limit of 50 per cent. to recombination between any two linked loci. The importance of the first two defects is obvious : the third could not have become important unless cases of more than 50 per cent. recombination were shown convincingly to occur.

Further theoretical advance was not made until the half-century. It is true that Mather (1933, 1936 and 1937) did much to fill the gaps in the cytological and genetic spheres, and he prepared a theory of serial formation of chiasmata based on Jennings' ideas (1923), which will be remembered as an important phase in the development of recombination theory. But no doubt a more elaborate formal analysis was not an attractive proposition so long as there was no genetical evidence which strikingly conflicted with current thought. Experimental data involving three or more loci, to which Kosambi's formulæ can be applied, have not been abundant nor very free from disturbance, and hence a sufficient number of cases which it has not fitted have been lacking. Moreover, although there have been reports of recombination exceeding 50 per cent.—Clausen (1926) in *Viola*, Wellensiek (1929*a* and *b*) in *Pisum*, and Fisher and Mather (1936*a*, *b*) in mice—contemporary thought has judged them to have been either somewhat biased or not sufficiently significant to be completely convincing. They also lack cytological confirmation. For values exceeding 50 per cent. require the occurrence of chromatid interference ; this involves a non-random relationship between the two strands exchanging material at one point and the two which exchange it at a second point. It alone provides the condition necessary for recombination exceeding 50 per cent., namely that the chance of a single strand being involved in an odd number of chiasmata is in excess of its chance of being involved in an even number. Cytological evidence on this point is very sparse and difficult to obtain ; as far as it goes, it supports only a mild degree of chromatid interference (Newcombe 1941 and Huskins and Newcombe 1941).

It was the experiment with wavy₂, shaker₂ and sex (Wright, 1947) which produced not only strong evidence for more than 50 per cent. recombination, but unusual mutual relationships between the three markers involved ; and this called for a more complex theory of interference than any currently accepted.

These data and the earlier data reported by Fisher and Mather (1936*a*, *b*) were unique in being, through careful design, almost completely free from disturbance through differential viability, and were therefore more convincing than earlier claims. Together they provided a stimulus, and the three-point data provided also a framework, for the further development of recombination theory. Fisher,

influenced to some extent by Mather's concept of chiasma formation as a serial process, produced a new general theory of wide applicability (Fisher *et al.*, 1947, and Fisher, 1948 and 1951). These ideas were developed in greater detail by Owen (1949*a, b*, 1950, 1951, 1952, 1953*a, b*); he showed how finite lengths of chromosome arms may be taken into account, and indicated that recombination fractions exceeding 50 per cent. are characteristic of a wide range of intercept distribution functions provided interference is sufficiently strong.

These latest developments of the theory of recombination and the mathematical models which form an integral part of them now

TABLE 12
Test of significance of interference

	A. In female gametogenesis <i>f_i-S_d</i>			B. In male gametogenesis <i>f_i-S_d</i>			C. ♂ and ♀ combined <i>f_i-S_d</i>		
	Cross- overs	Non- cross- overs	Total	Cross- overs	Non- cross- overs	Total	Cross- overs	Non- cross- overs	Total
<i>aⁱ-f_i</i>									
Crossovers . .	31	166	197	22	127	149	53	293	346
Non-crossovers .	82	271	353	110	290	400	192	561	753
	113	437	550	132	417	549	245	854	1099
Contingency χ^2_e for 1 d.f.	3.9022			8.9558			13.6008		

require suitable genetic material against which they can be tried, tested and perhaps modified. Owen (1948) has tested some of his models, using *Drosophila* data, and found good agreement for chromosomes II and III: the data as a whole give a completely convincing fit despite the failings already mentioned. *Drosophila* has certain genetic features dividing it rather sharply from the rest of the animal kingdom and from plants (such as the suppression of crossing-over in the male), so that there is no *a priori* reason for expecting the same kinds and degrees of interference in other organisms as are found in *Drosophila*. Further experiments of the kind here described, using a large range of organisms, are needed in order to provide the testing material for interference theory.

(ii) *The detection of interference*

A simple test of the significance of interference between any two segments in multi-point data is a χ^2 test based on 2×2 tables of the observed gametic output. In the present data, interference in both male and female gametogenesis is beyond doubt (table 12).

(iii) *The measure of interference*

It has been stated that "coincidence" is a useful measure for obtaining an idea of the order of magnitude of interference; it retains a place among related statistical tools, since it does not assume any theoretical intensity. Table 13 gives estimates of the value of C obtained by the application of the formula $\hat{C} = z/y_1y_2$ to the data as summarised in table 12.

TABLE 13
Values of "coincidence"

A. For female gametogenesis	$\hat{C} = 0.7659 \pm 0.11$
B. For male gametogenesis	$\hat{C} = 0.6141 \pm 0.11$
C. For males and females combined	$\hat{C} = 0.6871 \pm 0.08$

The value of $\hat{C}$ is in all cases less than unity; hence interference is positive.

As Owen (1950) has shown, for short segments C is generally large near the centromere, and small near the terminus. However, it is also dependent on the length of the two segments compared, being lower for long segments: in the present case, therefore, it is unreliable as an indicator of the position of the centromere. Owen (1953*a*) has indicated that the latter is probably "moderately distant" from the smaller segments *a-un-we-pa*. It is reasonable, therefore, to test the hypothesis that the larger segment *a'-fi-Sd* is roughly in mid-arm.

In this position, it may be expected to show interference at the Kosambi level. The measure K , which is analogous to C but is based on the particular degree of interference suggested by Kosambi, is equal to unity in mid-arm, and may be expected to rise near the centromere and fall near the terminus. A convenient way of testing the hypothesis of a Kosambi degree of interference is, therefore, to obtain an estimate of K and of its variance. The variance may be obtained by more than one method, a convenient one being available on testing the fit of a Kosambi map. In the present case, it is reasonable to expect a K value approximately equal to unity, and it is also instructive to obtain as reliable a map as current theory can supply. The procedure followed is, therefore, to fit a map for the observed frequencies of gamete formation assuming that $K = 1$, to test the goodness of fit, and to calculate the exact value and variance of K .

The method of fitting a Kosambi map is an example of Fisher's scoring technique (1946). The present particular application of it has been available for some six years as teaching material in the Cambridge Department of Genetics. Its publication in a practical form may be of general use.

(iv) *Fitting a Kosambi map*

(a) *Female gametogenesis.* Empirical estimates of the three recombination values are made as follows (from table 7) :

$$a'-fi = \frac{(1) + (12)}{N} = 197/550 = 35.8182 \text{ per cent.}$$

$$fi-Sd = \frac{(2) + (12)}{N} = 113/550 = 20.5455 \text{ per cent.}$$

$$a'-Sd = \frac{(1) + (2)}{N} = 248/550 = 45.0909 \text{ per cent.}$$

To fit a Kosambi map, trial recombination estimates are made, based on Kosambi's addition formula; and then adjustments are calculated, using the Maximum Likelihood Method, so that the three

TABLE 14
Provisional expectations for the four recombinant classes, and test of their fit with the observations

Mode	Obs.	Expectation	m_o	$\frac{(\text{Obs.}-\text{Exp.})^2}{\text{Exp.}}$
0	271	$1 - y_1 - y_2 + y_{12} = 1 - \frac{1}{2}(\quad y_1 + y_2 + y_{1+2})$	: 280.5859	0.3275
1	166	$y_1 \quad -y_{12} = \frac{1}{2}(\quad y_1 - y_2 + y_{1+2})$	: 159.4141	0.2721
2	82	$y_2 - y_{12} = \frac{1}{2}(\quad -y_1 + y_2 + y_{1+2})$	: 76.9141	0.3363
12	31	$y_{12} = \frac{1}{2}(\quad y_1 + y_2 - y_{1+2})$	: 33.0859	0.1315
Totals	550	1 1 550		$\chi^2 = 1.0674$

final estimates conjointly fit the empirical values with maximum precision. The latter estimates are then converted into map distances.

It is convenient, in order to shorten the calculation, to use trial estimates as near the final ones as can be judged by inspection. Empirical values for small segments are usually more accurate than those for large ones, so a convenient approximation to these may be taken as trial values. A good trial value for the largest segment is supplied by Kosambi's addition formula. We take

$$a'-fi = \quad \quad \quad = 0.35 \quad \quad = y_1$$

$$fi-Sd = \quad \quad \quad = 0.20 \quad \quad = y_2$$

$$a'-Sd = (y_1 + y_2)/(1 + 4 y_1 y_2) = 0.4296875 = y_{1+2}$$

The adjustments, dy_1 and dy_2 , are calculated from the variances and scores related to changes in y_1 and y_2 , as follows :

Provisional expectations (m_o) for the four recombinant classes are made. These are based on the trial recombination estimates, and are calculated from the identity $y_{12} = \frac{1}{2}(y_1 + y_2 - y_{1+2})$ (table 14).

A preliminary χ^2 test is included. It tests the goodness of fit of the expectations based on Kosambi with the observed numbers. At 1.0674 for 1 degree of freedom, it is not significant. The expectations

based on adjusted values will in general improve the goodness of fit and have a smaller χ^2 .

Next a computation table is made (table 15).

TABLE 15
Computation table

Class	Obs.	Exp. m_o	$\frac{dm_o}{dy_1}$	$\frac{d}{dy_1}(\log m_o)$	$\frac{dm_o}{dy_2}$	$\frac{d}{dy_2}(\log m_o)$
0	271	280.5859	-415.9914	-1.4825813	-360.6020	-1.2851751
1	166	159.4141	415.9914	2.6095017	-189.3980	-1.1880881
2	82	76.9141	-134.0086	-1.7423151	360.6020	4.6883731
12	31	33.0859	134.0086	4.0503247	189.3980	4.7244318

To fill up the computation table, calculate first $d(\log m_o)/dy_1$ for the double crossover frequency (12) as :

$$\frac{1}{y_1} + \frac{1}{y_1 + y_2} - \frac{4y_2}{1 \times 4y_1y_2}$$

Calculated directly, this is :

$$\begin{array}{rcl} 2.85714286 & 1/y_1 & \\ 1.81818182 & 1/(y_1 + y_2) & \\ -0.625 & -4y_2/(1 + 4y_1y_2) & \\ \hline 4.05032468 & \text{(to 8 places).} & \end{array}$$

Similarly $d(\log m_o)/dy_2$ for (12), is calculated from the above equation, interchanging y_1 and y_2 , and is as follows :

$$\begin{array}{rcl} 5.0 & & \\ 1.81818182 & & \\ -1.09375 & & \\ \hline 5.72443182 & & \end{array}$$

The first of these is multiplied by the value of m_o for (12) to give the value of dm_o/dy_1 , i.e. $4.05032468 \times 33.0859 = 134.0086$. The remaining values for dm_o/dy_1 are obtained as follows :

The value for (2) is equal and opposite in sign to that for (12). The value for (1) = total, minus the value for (12),

$$= 550 - 134.0086 = 415.9914.$$

The value for (0) is equal and opposite to that for (1).

The remaining values for $d(\log m_o)/dy_1$ are obtained by dividing the values for dm_o/dy_1 for (0), (1), (2) by the corresponding expected m_o values.

The values of dm_o/dy_2 and $d(\log m_o)/dy_2$ are obtained by the same

process as those for dm_o/dy_1 and $d(\log m_o)/dy_1$; it must be remembered that for dm_o/dy_2 the value for (12) is equal and opposite to that for (1), and that for (2) is equal and opposite to that for (0).

From the computation table the terms of the Information Matrix

$$\begin{pmatrix} \Sigma \left[\frac{dm_o}{dy_1} \left(\frac{d}{dy_1} \log m_o \right) \right] & \Sigma \left[\frac{dm_o}{dy_2} \left(\frac{d}{dy_1} \log m_o \right) \right] \\ \Sigma \left[\frac{dm_o}{dy_2} \left(\frac{d}{dy_1} \log m_o \right) \right] & \Sigma \left[\frac{dm_o}{dy_2} \left(\frac{d}{dy_2} \log m_o \right) \right] \end{pmatrix}$$

which may be abbreviated as

$$\begin{pmatrix} I_{11} & I_{12} \\ I_{12} & I_{22} \end{pmatrix}$$

are obtained by multiplying the entries in the fourth and fifth columns, and adding the products for all rows, and repeating the process for the fifth and sixth columns and the sixth and seventh.

The computation table also provides the scores, S_1 and S_2 ,

$$\Sigma \left[\text{obs} \times \frac{d}{dy_1} \log m_o \right] = S_1 \quad \Sigma \left[\text{obs} \times \frac{d}{dy_2} \log m_o \right] = S_2$$

for the two adjustable variables y_1 and y_2 . These scores are found by multiplying the observations by the fifth and seventh columns.

Then the equations of estimation are (table 16) :

TABLE 16

Equations of estimation

$$\begin{array}{rcl} I_{11} & dy_1 + I_{12} dy_2 & = S_1 \\ I_{12} & dy_1 + I_{22} dy_2 & = S_2 \end{array}$$

of which the calculated values are :

$$\begin{array}{rcl} 2478.5350 & dy_1 + 179.2281 & dy_2 = 14.0880 \\ 179.2281 & dy_1 + 3463.2909 & dy_2 = 16.3989 \end{array}$$

These equations are solved by inverting the information matrix, which then becomes the Covariance matrix (table 17). The value of D is $(I_{11} \times I_{22}) - (I_{12} \times I_{12})$.

TABLE 17

Covariance matrix (per cent.)²

$$\begin{pmatrix} \frac{I_{22}}{D} = V_{11} & \frac{-I_{12}}{D} = V_{12} \\ \frac{-I_{12}}{D} = V_{12} & \frac{I_{11}}{D} = V_{22} \end{pmatrix} \times 10,000. \quad \text{This is } \begin{pmatrix} 4.049797 & -0.209580 \\ -0.209580 & 2.898273 \end{pmatrix}$$

V_{11} and V_{22} are the sampling variances of the trial estimates y_1 and y_2 ; dy_1 and dy_2 are the adjustments, and can be calculated as follows :

$$\begin{array}{rcl} dy_1 & = & V_{11}S_1 + V_{12}S_2 \\ & = & 0.530 \text{ per cent.} \end{array} \quad \begin{array}{rcl} dy_2 & = & V_{22}S_2 + V_{12}S_1 \\ & = & 0.446 \text{ per cent.} \end{array}$$

These are added to their respective trial y values to give corrected estimates, and the standard errors are derived from the square roots of the corresponding variances. The map distances (x) are obtained from the Kosambi formula :

$$x = \frac{1}{4} \log_e \left(\frac{1+2y}{1-2y} \right)$$

Conversion of y into x can readily be made by interpolation in Table VII, p. 46, of Fisher and Yates (1953), using the identities $y = \frac{1}{2}r$ and $x = \frac{1}{2}z$. The corrected estimate of y_{1+2} is obtained by substituting the corrected y_1 and y_2 values in the expression

$$(y_1 + y_2) / (1 + 4y_1y_2).$$

The fitted map is, then, as in table 18 :

TABLE 18
The fitted map

Adjustments (per cent.)	Corrected estimates (per cent.)	Map distances
$dy_1 = 0.530$	$y_1^* = 35.536 \pm 2.012$	44.432 cM (a^t-fi)
$dy_2 = 0.446$	$y_2^* = 20.446 \pm 1.702$	21.718 cM ($fi-Sd$)
	$y_{1+2}^* = 43.376$	66.150 cM (a^t-Sd)

A test for the fit of a Kosambi map is given by χ^2 for the goodness of fit of the expectations (m_1) with the observed values in the classes (0), (1), (2), (12) (using y_1^* and y_2^* in place of y_1 and y_2 in the equations used to obtain m_0), as follows (table 19) :

TABLE 19
Test of Kosambi fit

Exp. m_1	Obs.	(Obs.-Exp.) ² /Exp.
276.7660	271	0.1201
160.7810	166	0.1694
77.7860	82	0.2283
34.6670	31	0.3879
550.0000	550	0.9057 = χ^2 for 1 d.f.

This χ^2 is, as expected, smaller than was obtained in the earlier test (table 14). In both cases it is insignificant.

The value of K is derived from the formula

$$K = (12) N^2 / 2 \{ (2) + (1) \} \{ (2) + (12) \} \{ (1) + (12) \} \\ = 31 \times 550^2 / 2 \times 248 \times 197 \times 113 = 0.8493,$$

and its standard error is $1-K/\chi$. These values are shown below (table 20) :

TABLE 20
K and s.e. of K

χ^2 for 1 d.f.	χ	K	$1-K$	s.e. of K.
0.9057	0.95168	0.8493	0.1507	0.1584

K is thus not significantly different from unity, and a Kosambi degree of interference may be accepted as operating in the segment *a^t-Sd* in female gametogenesis.

(b) *Male gametogenesis*. The tables for male gametogenesis corresponding to tables 18 and 20 are as follows (tables 21 and 22) :

TABLE 21
Kosambi map for males

Adjustments (per cent.)	Corrected estimates (per cent.)	Map distances
$dy_1 = -0.209$	$y_1^* = 26.791 \pm 1.883$	29.92 cM (<i>a^t-fi</i>)
$dy_2 = -0.244$	$y_2^* = 23.756 \pm 1.814$	25.84 cM (<i>fi-Sd</i>)
	$y_1^* + y_2^* = 40.29013$	55.76 cM (<i>a^t-Sd</i>)

TABLE 22
K and its s.e. for males

χ^2 for 1 d.f.	χ	K	$1-K$	s.e. of K
2.7896	1.6702	0.7113	0.2887	0.1729

(c) *Joint map and K value*. It is useful for making overall comparisons with other chromosomes to have a map (table 23) from the combined data from male and female heterozygotes (trial values

TABLE 23
Combined Kosambi map

Adjustments (per cent.)	Corrected estimates (per cent.)	Map distances
$dy_1 = 0.1438$	$y_1^* = 31.1438 \pm 1.3837$	36.41 cM (<i>a^t-fi</i>)
$dy_2 = 0.1067$	$y_2^* = 22.1067 \pm 1.2457$	23.74 cM (<i>fi-Sd</i>)
	$y_1^* + y_2^* = 41.7522$	60.15 cM (<i>a^t-Sd</i>)

taken are $y_1 = 31$ per cent., $y_2 = 22$ per cent., $y_{1+2} = 41.6404553$ per cent.).

That the values of K for male and for female gametogenesis are not significantly different can be seen from the following table (table 24), for twice the standard error of the difference appreciably exceeds the difference :

TABLE 24
Insignificance of difference between K values

K	s.e.	Difference	Twice s.e. of difference
♀ : 0.84930 ± 0.15835		} 0.13804	0.4688036
♂ : 0.71126 ± 0.17288			

It is thus valid, and will be useful, to calculate the combined value of K (table 25) :

TABLE 25
Joint K and its s.e.

$1/(\text{s.e. of } K)^2$	K	Joint K	Variance of K	s.e. of K
♀ $39.880800 \times 0.84930 = 33.8707634$ ♂ $33.458810 \times 0.71126 = 23.7979132$		$\frac{57.6686766}{73.339610} = 0.786324$	$1/73.339610$	0.116769
73.339610	57.6686766			

6. DISCUSSION

Owen (1953a) obtained for the segment *a-un-we-pa* the following K values :

For females : 0.1624 ± 0.1641
 For males : 0.9663 ± 0.6829
 Combined : 0.2092 ± 0.1509

The combined value of K is thus significantly different from unity, "being suggestive of a region towards the chromosome terminus or at least between mid-arm and the terminus. The segment *agouti-pallid* is thus between 30-100 cM from the centromere according to what is assumed for the map length of the arm."

The several and combined values of K for the segment *a'-Sd* are not significantly different from unity. The degree of interference may be said, here, to be on a Kosambi level, in contrast with the smaller portion *a'-pa*. The fact that the actual values are less than unity militates against the opinion that *a'-Sd* encloses the centromere or at least that the centromere is only 30 cM from *agouti*—and favours the view that the centromere is beyond *Sd*.

However, the data are not entirely inconsistent with the location of the centromere between *f* and *Sd*, a position favoured by affinity

studies (Michie, 1953 ; Wallace, 1953), for it is very probable that *a'-Sd* is nearer the centromere than *a-pa*, and a chromosome arm of 60 cM is the average that may be expected on the basis of chiasmata counts. A brief account of the evidence from affinity studies for the position of the centromere in chromosome V has been given (Wallace, 1954c and 1957), and a fuller account will be given shortly with some discussion (Wallace 1958.)

More accurate mapping of the centromere by means other than affinity data may be hoped for from the five-point *Ra-a'-we-fi-Sd*, which should give a K value based on homogeneous data for each of the overlapping triple segments *Ra-we*, *a'-fi* and *we-Sd*.

It is not unlikely that the results of this experiment, and of those with other linkage groups now in progress, together with affinity studies will call for, and make possible, some refinements of the current theory of interference.

7. SUMMARY

Interference theory is in advance of experiment. A balanced linkage backcross was undertaken in 1950 and completed in 1952 as a start in supplying the deficiency of reliable experimental material.

Short notes are given on the development of modern interference theory and of the ideas leading to current methods of designing and analysing linkage experiments.

The loci agouti (*A*), fidget (*fi*), and Danforth's short tail (*Sd*) were chosen to demonstrate the use of balance in eliminating disturbance to linkage due to differential viability. Some 1099 progeny were bred.

An analysis of viability relations shows that, despite the low survival rates of fidget and Danforth's short tail (72 and 80 per cent. respectively), there is no appreciable disturbance of linkage either in the data from male or in that from female heterozygotes. There is subsignificant evidence for a kind of position effect apparent as heterogeneity in the survival ratios from the four types of female heterozygote.

Changes in the recombination values in response to both sex and age are examined. Recombination in the segment *A-fi* is significantly higher in females than in males ; in the segment *fi-Sd*, there is a subsignificant difference in the opposite direction. There is a significant increase in recombination in males in the *A-fi* segment with advancing age, but no appreciable change in females.

These observations, when compared with those for the agouti-undulated-wellhaarig-pallid (*A-un-we-pa*) segments of the same chromosome, reveal a rather complex situation. A simple explanation is offered, but further data, of the kind now being produced with five loci in this linkage-group, are needed before its full applicability may be ascertained.

An incidental anomaly is briefly discussed, namely a rise in the sex-ratio with increase in maternal age.

The three-point analysis discloses significant interference. The Kosambi values (K) are : 0.7113 ± 0.1729 for male gametogenesis, and 0.8493 ± 0.1584 for female. As these values are not significantly less than unity, it may be supposed that interference is at a Kosambi level, and a map is fitted accordingly for the separate and combined data. A method of fitting Kosambi maps, based on Fisher's scoring technique, is demonstrated.

A K value of unity may be expected for segments in mid-arm. The three-point values thus agree with the supposition that the whole segment *A-Sd* excludes both centromere and terminus. They do not, however, preclude the possibility, for which other data for the smaller sections of this region provide some support, that the centromere is between fidget and short tail. This hypothesis is favoured by "affinity" studies.

Acknowledgment—It is a pleasure to acknowledge the guidance I have received from Professor Sir Ronald Fisher during the course of this research.

APPENDIX

TABLE 1

The frequencies of all genotypes from the three-point backcross

In the description of each genotype, which runs vertically, the symbol a^t stands for the agouti allele used as the dominant and a for that used as the recessive : the particular alleles used in each case can be seen from the genotype of the heterozygous parent given in the left-hand margin.

	a^t <i>Sd</i>	<i>fi</i>	<i>Sd</i>	a^t <i>fi</i>	<i>fi</i> <i>Sd</i>	a^t	a^t <i>fi</i> <i>Sd</i>	<i>a</i>	Totals			
A. From female heterozygotes												
Tricoupling :	$\left\{ \begin{array}{l} a^t Sd/a fi \\ A^v Sd/a fi \end{array} \right.$	.	.	28	18	13	16	6	8	3	4	96
a^t -out :	$\left\{ \begin{array}{l} a Sd/a^t fi \\ A^v/a fi Sd \end{array} \right.$	.	.	11	8	7	7	1	6	...	...	40
	$a Sd/a^t fi$	.	.	19	27	33	36	5	2	10	11	143
	$A^v/a fi Sd$	.	.	7	3	3	1	18	28	8	20	88
<i>Sd</i> -out :	$\left\{ \begin{array}{l} A^v/a fi Sd \\ A/a fi Sd^* \end{array} \right.$	.	.	2	1	1	...	7	6	3	4	24
	$A/a fi Sd^*$	.	.	...	...	...	1	3	2	1	1	8
	$A/a^t fi Sd^\dagger$	.	.	1	1	...	1	4	3	2	2	14
<i>fi</i> -out :	$\left\{ \begin{array}{l} a^t fi Sd \\ a^t fi Sd/a \end{array} \right.$	.	.	6	4	12	13	11	25	16	50	137
				74	62	69	75	55	80	43	92	550
B. From male heterozygotes												
Tricoupling :	$\left\{ \begin{array}{l} a^t Sd/a fi \\ A^v Sd/a fi \end{array} \right.$	.	.	19	28	15	6	5	11	3	...	87
	$A^v Sd/a fi$	.	.	10	14	9	6	5	4	...	...	48
a^t -out :	$\left\{ \begin{array}{l} a Sd/a^t fi \\ a fi Sd/a^t \end{array} \right.$	.	.	17	8	42	35	1	6	7	21	137
<i>Sd</i> -out :	$a Sd/a^t fi$	.	.	16	8	1	4	30	41	15	24	139
	$a fi Sd/a^t$	.	.	6	1	14	19	7	20	24	47	138
<i>fi</i> -out :	$a^t fi Sd/a$	.	.									
				68	59	81	70	48	82	49	92	549

* mated to $a^t a fi fi$.

† mated to $a^t a fi fi$ and $aa fi fi$.

TABLE 2
Single-factor ratios

A. From female heterozygotes						
	$Sd : +$	χ^2	$f : +$	χ^2	$a^t : a$	χ^2
Tricoupling	69 67	0.02941	59 77	2.38235	$a^t : a$ 55 41	2.04167
a^t -out					$A^v : a$ 24 16	1.60000
Sd -out	67 76 60 74	0.56643 1.46269	78 65 54 80	1.18182 5.04478	$a^t : a$ 67 76	0.56643
					$a^t : a$ 44 44	0.00000
					$A^v : a$ 11 13	0.16667
f -out	45 92	16.12409	44 93	17.52555	$A : a^t, a$ 11 11	0.00000
Total χ^2	241 : 309	18.18262	235 : 315	26.13450	$a^t : a$ 60 77	2.10949
Deviation χ^2		8.40727		11.63636		6.48426
Corrections		9.77535		14.49814		0.06545
		1.0155232		1.02161432		6.41881
Heterogeneity χ^2		9.92709 (3 d.f.)		14.81151 (3 d.f.)		1.00011902
Probability		0.02-0.01		0.01-0.001		6.41957 (6 d.f.) 0.5-0.3
B. From male heterozygotes						
	$Sd : +$	χ^2	$f : +$	χ^2	$a^t : a$	χ^2
Tricoupling	66 69	0.06667	67 68	0.00741	$a^t : a$ 39 48	0.93103
a^t -out					$A^v : a$ 20 28	1.33333
Sd -out	67 70 62 77	0.06569 1.61871	51 86 57 82	8.94161 4.49640	$a^t : a$ 65 72	0.35706
f -out	51 87	9.39130	51 87	9.39130	$a^t : a$ 76 63	1.21583
Total χ^2	246 : 303	11.14237	226 : 323	22.83672	$a^t : a$ 69 69	0.00000
Deviation χ^2		5.91803		17.13843		3.83785
		5.22434		5.69829		0.22040
Corrections		1.01089713		1.03222349		3.61745
Heterogeneity χ^2		5.28127 (3 d.f.)		5.88191 (3 d.f.)		1.00040162
Probability		0.2-0.1		0.2-0.1		3.61890 (4 d.f.) 0.5-0.3

N.B.—(i) The heterogeneity χ^2 in this and subsequent tables is obtained as follows. It is assumed that each expected two-class ratio is 1 : 1. The χ^2 derived by subtraction of the deviation χ^2 from the total χ^2 is then multiplied by $N/4AB$ where A = the total observed number in one class, B = the total observed number in the other, and $A+B = N$. (In the $Sd : +$ series above, $A = 246$, $B = 303$, $N = 549$.) This gives the corrected heterogeneity χ^2 . It has the same value as that calculable by taking the observed ratio $A : B$ as the expected.

(ii) The contingency χ^2 values throughout the paper are calculated with Yates' correction for discontinuity.

TABLE 3
Heterogeneity in genotypic ratios within complementary pairs

A. Analysis for three loci simultaneously : Progeny from female heterozygotes (<i>A^v</i> , <i>A</i> , <i>a^t</i> lumped as “ <i>a^t</i> ” in contrast to “ <i>a</i> ”)												
	<i>a^t</i> <i>fi</i> <i>Sd</i>	<i>a</i>	χ^2	<i>fi</i> <i>Sd</i>	<i>a^t</i>	χ^2	<i>a^t</i> <i>fi</i> <i>Sd</i>	χ^2	<i>a^t</i> <i>Sd</i>	<i>fi</i>	χ^2	
Tricoupling . . .	3	4	0.1429	7	14	2.3333	23	20	0.2093	39	26	2.6000
<i>a^t</i> -out . . .	10	11	0.0476	5	2	1.2857	36	33	0.1304	19	27	1.3913
<i>Sd</i> -out . . .	14	27	4.1220	32	39	0.6901	3	4	0.1429	10	5	1.6667
<i>fi</i> -out . . .	16	50	17.5152	11	25	5.4444	13	12	0.0400	6	4	0.4000
Total χ^2 . . .			21.8277			9.7535			0.5226			6.0580
Deviation χ^2 . .	43	92	17.7852	55	80	4.6296	75	69	0.2500	74	62	1.0588
Corrections . . .			4.0425 1.1517			5.1239 1.0355			1.2726			4.9992
Corrected heterogeneity χ^2 for 3 d.f.			4.6557			5.3058						

Total heterogeneity $\chi^2 = 16.2333$ (12 d.f.).
Probability is 0.30-0.20.

B. Analysis for fi and Sd loci— a^t locus disregarded : Progeny from male and female heterozygotes									
	(i) Female heterozygotes				(ii) Male heterozygotes				
	$\begin{smallmatrix} fi \\ Sd \end{smallmatrix} : +$	χ^2	$fi : Sd$	χ^2	$\begin{smallmatrix} fi \\ Sd \end{smallmatrix} : +$	χ^2	$fi : Sd$	χ^2	
Tricoupling . . .	10 18	2.2857	49 59	0.9259	13 15	0.1429	54 53	0.0093	
a^t -out . . .	15 13	0.1429	63 52	1.0522	8 27	10.3143	43 59	2.5098	
Sd -out . . .	46 66	3.5714	8 14	1.6364	45 65	3.6364	12 17	0.8621	
fi -out . . .	27 75	22.5882	17 18	0.0286	31 67	13.2245	20 20	0.0000	
Total χ^2 . . .		28.5882		3.6431		27.3181		3.3812	
Deviation χ^2 . .	98 172	20.2815	137 143	0.1286	97 174	21.8782	129 149	1.4388	
Corrections . . .		8.3067 1.0812		3.5145		5.4399 1.0878		1.9424	
Het. χ^2 for 3 d.f. Probabilities . .		8.9812 0.05-0.02		0.50-0.30		5.9175 0.2-0.1		0.7-0.5	

The total χ^2 for unequal viability within complementary pairs is the sum of the deviation χ^2 values and is 43.7271 for 4 d.f. Probability is <0.001.

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