A STUDY OF THE w MULTIPLE ALLELOMORPHIC SERIES IN DROSOPHILA MELANOGASTER

being a thesis presented by
MARGARET ELAINE MACKENDRICK, B.Sc.
for the degree of Doctor of Philosophy of the
University of Glasgow.

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CONTAINS

PULLOUTS

ME. McKENDRICK

SUMMARY.

In recent years more and more attention has been focussed on the detailed architecture of the hereditary material. Increased evidence has become available of the divisibility, by genetic recombination tests, of what had been considered the ultimate unit of heredity, viz., the gene. The gene, in other words, now appeared to be divisible into smaller units of mutation and of crossing over.

Since, to attack this problem, multiple allelic series have been studied, and since many such series show a range of visible and physiological differences, it was considered possible that different sub-units of the gene might control slightly different but related or sequential biochemical processes. The first indication of such a division of function was the cis-trans position effect, noted in all allelic series divisible by crossing over, where it is necessary for all wild type sub-units of the gene to be present in at least one chromosome for the wild type phenotype to be expressed. The origin of this division of function is considered by some to be tandem duplication of a gene at some earlier point in the evolution of the organism with subsequent differentiation of function.

The work described in this thesis was designed to investigate the crossover separability of the w series of alleles in Drosophila melanogaster. This series was chosen because it was not associated with a cytological repeat. It was also hoped to throw some light on the kind of differences which might exist between these sub-units; in other words to find out whether there is a positive correlation between position and function.

Again, as a corollary to the evidence from the cis-trans position effect that different combinations of the genetic material in the heterozygote give rise to different phenotypes, it was thought that new phenotypes might arise by combining in a different way the sub-units already identified.

Fourteen visibly different alleles of the w series, of independent origin by spontaneous mutation, were classified in three groups according to physiological differences other than eye colours. In order to detect recombination between any two w alleles, the progeny from heterozygotes for these two alleles, with suitable markers closely linked on either side, were examined for eye colours differing from those of the expected segregants.

In seven crosses, involving eight different alleles, twenty seven unexpected segregants were isolated. The distribution of markers in these segregants indicated that crossing over had taken place between the alleles.

One of the alleles appears to be mutant at two sites. Evidence is provided in support of the double nature of this allele as opposed to the results in question being due to unequal crossing over. It also appears that one of these two alleles acts as a partial suppressor of the other.

In a further thirteen crosses involving twelve alleles (six of which gave positive results in other combinations) no unexpected segregants were detected. In the majority of these thirteen crosses, however, the number of individuals examined was rather small and the negative results cannot be taken to indicate that crossing over between these alleles does not occur.

The evidence from all the crossing over data suggests that there are at least three sites of mutation in the w region. It is possible that more exhaustive tests would identify more.

New phenotypes have been shown to result from recombination between two phenotypically identical alleles, and at least one phenotype, viz. white, is associated with at least three different genotypes.

The correlation sought between spatial arrangement and function is not considered proven although the evidence weighs in its favour. Emphasis on this point, however, is no longer so important, the real problem being the lack of refinement to the same degree of resolving power of recombination and of function.

In conclusion, it seems more feasible to consider the phenomenon of cross-over separability of alleles as due, not to duplications of existing genetic material, but to the apparently universal property of divisibility of the genetic material into the order of millions of mutational sites separable by crossing over irrespective of whether these sites belong to one field of physiological co-operation (one gene) or to different fields.

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1. INTRODUCTION

The definition of the gene, as the Mendelian "factor" came to be known, has undergone considerable modification since the early part of this century. Mendel's conception of the inheritable material was of a number of pairs of factors segregating, one of each pair into each germ cell and each pair segregating thus, independently of all other pairs.

It became evident, however, (Sutton 1902), that the chromosomes in the cells which cytologists had studied were the carriers of the genes and, moreover, that the genes were arranged in a linear fashion along the chromosomes rather like a string of beads. (Morgan 1911). Apart from occasional aberrations the order of the genes on the chromosome was found to be constant and the location of any gene could be plotted relative to other genes and maps of the chromosomes made. Of course it was possible to identify a gene and study its segregation only when two alternative forms with different developmental effects were available. These forms were termed alleles and some insight was sought into the nature of the differences between the normal or wild type allele and the changed or mutant allele. Bateson in 1914 discussed at length the "Presence-absence" theory of the gene which allowed for only two alternative states; the wild type allele and loss of the wild type allele giving the mutant phenotype. This theory, however, became inadequate following the discovery of multiple allelomorphic series in Drosophila melanogaster in which already by 1919, 9 different members had been found.

Morgan (1919) suggested that alleles might be very closely linked genes but discarded the idea since alleles were not complementary and, at that time, no crossing over had been demonstrated between them.
[Non - complementarity is still considered, for most purposes, as a useful criterion for allelism. (Beadle 1957)]
Morgan therefore defined allelism as being due to modifications of the same gene and beyond the limit of recombination.

The gene was now assumed to be a unit of function, of mutation and of crossing over and this definition was widely accepted till Dubinin (1929) introduced his Step-allelomorphism theory to account for the distribution of bristle patterns due to the scute alleles of Drosophila melanogaster. Briefly this theory stated that the scute gene was made up of a number of sub-genes each of which controlled the development of a particular bristle or set of bristles. Progressive mutation of the scute gene therefore gave rise to a progressively more extreme phenotype depending on the number of sub-genes affected. The bristle pattern was in turn dependent on the arrangement of the mutant sites. The work of Serebrovsky and of Agol in the following year provided experimental evidence in support of Dubinin's theory.

Agol's work, in particular, was criticised by Sturtevant and Schultz (1931) who preferred a developmental to a sub-gene interpretation of the scute data. By now, however, evidence from various lines of research, particularly on position effect, was beginning to point to the compound nature of the gene. In 1940 Raffell and Muller reviewed the state of the gene theory in the light

of the chromosome breakage studies - again in the scute region of Drosophila melanogaster. They obtained evidence that the 13 breaks studied fell into only 4 groups with respect to location of the break. The implication was that there were certain weak points in the scute gene and that these points delineated sub-genes. Moreover they did not consider as coincident the currently accepted definitions of the gene, viz. a unit of crossing over; of breakage; of mutation and function; of reproduction and finally of auto-attraction. However at that time sufficiently sensitive genetic tests had not been devised to substantiate their hypothesis on all points.

On the question of cross-over, mutational and functional separability of alleles, with which the present investigation is mainly concerned, some of the earliest evidence was furnished by the work of Lewis. Between 1942 and 1948 he noted several cases of recombination between what he considered to be very closely linked genes with similar effect and exhibiting a new type of position effect: namely that the trans heterozygote is mutant while the cis heterozygote is wild type or less mutant in phenotype. Green and Green (1949), following up the discovery by Oliver and Green (1939) of a lz^{+} (lz = lozenge) segregant from the lz^{3}/lz^{6} compound, studied in more detail the lozenge multiple allelomorphic series in Drosophila melanogaster and demonstrated recombination between alleles by recovering both the products of crossing over. Here again the cis - trans position effect was noted and it seemed likely that both Lewis's and the Greens' results demonstrated the same phenomenon.

Following the above discoveries Lewis and Green reviewed

their conception of genes and alleles and considered as separate genes, alleles (by previous definition) which were shown to be separable by crossing over. Furthermore Lewis considered crossover separability to be a special property of duplicated genes occupying tandem repeats in the chromatin material and biased his selection of experimental material in this direction.

In 1950, however, Pontecorvo emphasised that a more rational approach to the question of the relationship between chromosomal architecture and function was necessary. On the basis of some considerations by MacIlwain (1947) he suggested that one line of reasoning might be as follows. A chain of reactions involving, at each step, millemicromolar quantities might require an assembly line organisation of the appropiate enzymes. This might be reflected in the very close linkage and relative positions of the genes controlling these reactions. The first deliberate search for this type of relationship was made by Roper (1950) on 3 biotin requiring strains of Aspergillus nidulans. Very close linkage was found between what he considered to be 3 separate bi (biotinless) genes and interpreted according to Pontecorvo's hypothesis.

The question now arose; how widespread was this phenomenon and what classes of allelic series, in the classical sense, could be expected to be so divisible? On both Lewis's and Pontecorvo's hypotheses, allelic series in which crossover separability might be demonstrated would be expected to be limited to certain classes and much more rare on Lewis's hypothesis.

For the investigation to be described here it was decided to test an allelic series in Drosophila melanogaster which, from cytological evidence, appeared not to be associated with a tandem repeat and which had several phenotypically different alleles. The "white" multiple allelic series was thus chosen as it was the classical and one of the largest series in D.melanogaster, it was not obviously associated with a cytological repeat and the alleles showed well-marked visible and physiological differences. It was also hoped, because of the good visual separability of the alleles, to detect new alleles as a result of recombination.

The preliminary results of this work (MacKendrick and Pontecorvo 1952) demonstrated crossing over between white alleles. It was emphasised however that the white alleles represented only one of 13 series of alleles, randomly chosen with respect to repeats, in which crossing over had been demonstrated. There were in fact, at that time, only 3 cases, viz. ras (Ives and Noyes 1951) in D. melanogaster, R (Stadler 1952) in maize, and S (D. Lewis 1949) in Oenothera, where crossing over had not been demonstrated.

Thus, on the basis of the 13 series above showing the cis - trans effect and of further work on the bi alleles which could not be distinguished from one another by available biochemical tests, Pontecorvo (1952) modified his earlier hypothesis, visualising the gene as a short section of the chromosome acting as a unit of function but separable into a number of sites of mutation and of crossing over.

There was not yet enough evidence in the preliminary work on white alleles referred to above, to throw any light on the relationship of position and function. The following account represents an attempt to resolve this and other problems.

2. MATERIALS AND METHODS

(i) Description of stocks.

white ($\underline{w}$; 1 - 1.5), the first known sex-linked recessive mutant of *Drosophila melanogaster*, was discovered in a wild type laboratory culture by T.H.Morgan in 1910. (Morgan 1910). Flies of this genotype have nearly snow white eyes and colourless Malpighian tubules and, in the male, testis sheath. Mutation to $\underline{w}$ occurs frequently and to the numerous alleles intermediate between $\underline{w}$ and the wild type red only occasionally. The white series is in fact the classical multiple allelomorphic series in *Drosophila melanogaster*, representing more than twelve alleles all phenotypically different and mostly discovered before 1920. A list of some of the alleles in chronological order of discovery is as follows:- $\underline{w}$: white 1910; $\underline{w}^{e}$: white-eosin 1911; $\underline{w}^{ch}$: white-cherry 1912; $\underline{w}^{bl}$: white-blood and $\underline{w}^{t}$: white-tinged 1914; $\underline{w}^{bf}$: white-buff 1915; $\underline{w}^{co}$: white-coral 1917; $\underline{w}^{ec}$: white-ecru and $\underline{w}^{i}$: white-ivory 1918; $\underline{w}^{a}$: white-apricot 1923; $\underline{w}^{am}$: white-apricot of Muller 1928; $\underline{w}^{a2}$: white-apricot 2 and $\underline{w}^{bf2}$: white-buff 2 1932; $\underline{w}^{sat}$: white-satsuma 1933; $\underline{w}^{a3}$: White-apricot 3 and $\underline{w}^{h}$: white-honey 1934. Of course coloured alleles of the series are still being accumulated and unfortunately designated by the above symbols. Thus it is no longer possible to trace the origin of many stocks.

For this study a number of alleles of the white series was collected from various laboratories. They are mainly of spontaneous origin but a few were induced by X-irradiation or by formaldehyde treatment of male larvae.

In the following work, with one exception, only those alleles which arose spontaneously were used for tests of recombination. They would thus have less probability of being associated with minute chromosomal rearrangements, a very necessary precaution when investigating a short region of the chromosome.

Apart from the obvious colour differences between the phenotypes determined by these alleles there are other morphological and physiological properties not shared by all members of the series. Since it was hoped to find some relationship between the location of an allele and its phenotype, the alleles used for tests of recombination were grouped according to two of these properties, viz. dosage compensation and interaction with zeste. Dosage compensation defines a state where a single dose of a sex-linked allele in the male determines almost the same phenotype as a double dose in the female. (Muller 1948, Goldschmidt 1955). Zeste: z (1 - 1.0) is a sex-linked recessive mutation giving, in the female, a lemon yellow eye colour but in the male the wild type red eye colour. (Gans 1953). The action of z in reducing the amount of eye pigment in the female is in some way dependent on the presence of at least one wild type allele of white. Some of the alleles in the white series however have a dominant suppressor effect on z, i.e. a female homozygous for z and heterozygous for one of these alleles has a wild type eye colour. (see Table 1).

Table I.

Genotype	Phenotype
$\frac{zw^+}{zw^+}$	zeste
$\frac{zw^x}{zw^+}$	zeste
$\frac{zw^y}{zw^+}$	wild type

w^x denotes white alleles which do not suppress z.

w^y denotes white alleles which suppress z.

All the alleles used in the present investigation are grouped below according to these two properties. Those marked (P) were obtained from Gans of Paris. Tests for interaction with zeste were carried out by Gans on the alleles listed including those obtained from other sources.

Group I. Alleles which exhibit dosage compensation and do not suppress zeste.

w^a: white apricot. Pasadena; designated w^{aC} in this paper.

w^{bf2}: white-buff 2.	Pasadena	(P)
w^{bl}: white-blood.	Pallanza	(P)
w^{co}: white-coral.	Edinburgh; designated w^{coE} in this paper.	
w^{co}: white-coral.	Paris; designated w^{coP} in this paper.	(P)
w^{e}: White-eosin.	Stockholm; origin unknown but not the same allele as used by Muller (1948) Designated w^{eS}.	
w^{t}: white-tinged.	Pallanza	(P)
w^{sat}: white-satsuma.	Cold Spring Harbor	(P)

Group 11. Alleles which exhibit dosage compensation and have a dominant suppressor effect on zeste.

w^{a}: white-apricot.	Edinburgh; designated w^{aE} in this paper.	
w^{h}: white-honey.	Pasadena	(P)
w: white.	Glasgow; origin unknown.	
w^{11E4}: white 11E4.	Paris; by irradiation of $\underline{z}$ males	(P)

Group 111. Alleles showing no dosage compensation and no interaction with zeste.

w^{i}: white-ivory.	Pallanza	(P)

During the course of the work described here nomenclature of white alleles from different laboratories has been found not to be reliable. Thus, although according to Muller's work (Muller 1948), w^{e} should be

included in Group III subsequent testing of the "w^e" allele used in the present work indicated that it did not exhibit dosage compensation and cannot therefore be the allele used by Muller in his experiments. This test had not been carried out until much of the work described below had been completed and throughout this time w^e was assumed to be in Group III. In the following, this stock will be designated white-eosin of Stockholm or w^{eS}. w^i, the only allele in Group III, the females having eyes lighter in colour than the males, is of interest also because of the unexpected effect it produces in heterozygous condition with other members of the series. Heterozygotes for any two coloured alleles of the series are intermediate in eye colour between the two homozygotes and darker than either heterozygote with w.

A heterozygote for any of the coloured alleles and w^i is also intermediate in eye colour between the homozygotes but is lighter than the heterozygote of that allele with w, i.e. w^x/w^i is lighter than w^x/w where w^x is any other coloured allele. This lighter eye colour appears to be due not to a change in quality or reduction in quantity of the eye pigments, but to a clumping of the eye pigment granules (Castiglioni 1951, 1956); the effect is the same as in the case of "dilution" in the coat colour of mammals. (Russell, 1949).

The markers used in the experiments to be described are as follows.

y - yellow: 1 - 0.0: body and bristles yellow.

sc - scute: 1 - 0.0 :post-scutellar bristles absent.

z - zeste ; 1-1.0 approximately: females lemon yellow eye colour; males normal.

w - white and other alleles ; 1-1.5: reduction in eye pigmentation.

ec - echinus ; 1-5.5: roughened eye surface.

cv - cross-veinless ; 1-13.7: absence of cross-veins in the wings.

(ii) Culture method.

For all the experiments described, unless otherwise stated, flies were cultured in ½ pint milk bottles containing about 60ml yeast-agar-meal-malt medium and kept at 25°C. This method gave very satisfactory results, 20 pairs in such a culture producing an average of 900 - 1,000 progeny. The formula, given below, is that used for Drosophila medium for most purposes in the Department of Genetics in Glasgow.

Formula for 1 litre medium

Agar	7.5 gm.
Malt	1½ tablespoons
Meal	½ cornmeal, ½ bran. 112gm.
Yeast (dried)	12 gm.
Shirlan (anti-mould)	1 teaspoon
Water	1 litre

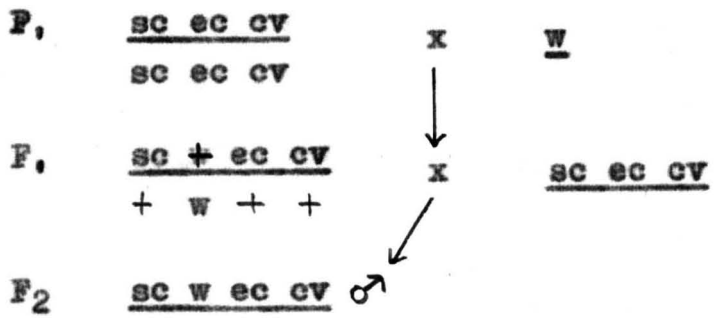
Hatching commenced on the 9th day after the cultures were set up and the flies were examined every day until

the 9th day after the beginning of hatching. After that an overlap of the generations was possible.

(iii) Description of crosses

As the purpose of this work was to determine whether crossing over can occur between alleles of the white multiple allelomorphic series, the progeny of females heterozygous for any two of the alleles listed was examined for individuals different from either grandparental type or from the heterozygote. In order to check whether unexpected types might have arisen by spontaneous mutation, suitable markers on either side of and closely linked to white were employed. For this purpose scute, echinus and cross-veinless were chosen as their phenotypes are sufficiently distinct for ready classification, they do not greatly reduce viability and they are reasonably close to w. This last condition is most important as it ensures that, assuming there is no negative interference in the white region, further crossing over between w and the linked markers would be rare. A sc ec cv stock was obtained from the John Innes Horticultural Institute and w was interposed between sc and ec as shown in Fig.1.

Fig.1 Method of synthesis of sc w ec cv stock.



It was expected that the sc w ec cv stock would have to be synthesised in two steps by adding first the sc marker and, in the following generation, ec and cv. Fortunately a single bottle, cultured for the F_2 and giving about 1,000 progeny, yielded a sc w ec cv male, a double crossover between sc and ec. As there are 1.5 units between sc and w, and 4 units between w and ec this event would be expected to occur with the frequency of $0.015 \times 0.04 = 6 \times 10^{-4}$ assuming no interference. This male was mated in turn to yellow vermilion forked attached-X females (symbol $\frac{yvf}{\ }$) to obtain patroclinous males in the next generation, and to wild type females. The heterozygous sc w ec cv / + + ++ females from the latter cross were mated to sc w ec cv males from the former and virgin sc w ec cv females and sc w ec cv males isolated from the progeny and a tester stock built up.

The working hypothesis was that, if crossing over does occur between members of the white series, the grouping of alleles according to position on the X-chromosome

might correspond with physiological activity. For this reason two mutants, one exhibiting and one not exhibiting dosage compensation were chosen as standards against which as many as possible of the mutants listed could be tested. These were $\underline{w}$, of unknown origin, which had been in culture for some time in Glasgow and which is placed in Group 11, and $\underline{w^{eS}}$ which at that time was considered not to exhibit dosage compensation, placing it in Group 111, and which was already in combination with yellow and echinus when obtained from Stockholm. A further advantage in choosing $\underline{w^{eS}}$ as a standard is that, should crossing over occur between it and any of the alleles intermediate in eye colour between white and wild type red, the double mutant $\underline{w^{eS}w^{x}}$ may be white, or at least paler than $\underline{w^{eS}}$ or $\underline{w^{x}}$ and therefore distinguishable among the progeny, cf. Green's work on the lozenge series (1949) where a double mutant has the extreme mutant phenotype.

The scheme of crossing is shown in Fig.2.

Fig.2. Design of experiment to test whether $\underline{w}$ recombines with $\underline{w^{x}}$, $\underline{w^{x}}$ being any member of the series other then $\underline{w}$.

$$P_1 \quad \frac{sc\ w\ ec\ cv}{sc\ w\ ec\ cv} \times \underline{w^{x}}$$

$$\downarrow$$

$$F_1 \quad \frac{sc\ w\ ec\ cv}{+\ w^{x}\ +\ +} \times \underline{sc\ w\ ec\ cv}$$

$$\downarrow$$

$$Bx \quad \underline{w}/\underline{w}\,♀ \ ; \ \underline{w}/\underline{w^{x}}\,♀ \ ; \ \underline{w}\,♂ \ ; \ \underline{w^{x}}\,♂$$

With all possible combinations of $\underline{sc}$, $\underline{ec}$ and $\underline{cv}$.

These progeny are the expected products of a back-cross if $\underline{w}$ and $\underline{w}^x$ do not recombine. If however $\underline{w}$ and $\underline{w}^x$ occupy closely linked but separable sites on the chromosome the products of the cross will be as shown in Figure 3.

Fig.3. Interpretation of products of crossing $\underline{w}$ and $\underline{w}^x$ where progeny of unexpected phenotype indicate that crossing over occurs between $\underline{w}$ and $\underline{w}^x$.

a) $\underline{w}$ occupying a position to the left of $\underline{w}^x$ on the chromosome.

P_1 $\frac{sc\ w+\ ec\ cv}{sc\ w+\ ec\ cv}$ x w^x

F_1 $\frac{sc\ w-\ ec\ cv}{+\ +w^x+\ +}$ x sc w+ ec cv

Bx

	Non-crossovers	Crossovers
♀	$\frac{scw+ec\ cv}{scw+ec\ cv}$	$\frac{sc\ ww^x+\ +}{sc\ w+ec\ cv}$
	$\frac{+\ +\ w^x+\ +}{sc\ w+ec\ cv}$	$\frac{+\ +\ +ec\ cv}{sc\ w+ec\ cv}$
♂	sc w+ec cv + +w^x+ +	sc w w^x+ + + + +ec cv

b) $\underline{w}$ occupying a position to the right of $\underline{w}^x$ on the chromosome.

P_1 $\frac{sc+\ w\ ec\ cv}{sc+\ w\ ec\ cv}$ x w^x

F_1 $\frac{sc+\ w\ ec\ cv}{+\ w^x+\ +\ +}$ x sc+ w ec cv

Bx

	Non-crossovers	Crossovers
♀	$\frac{sc+w\ ec\ cv}{sc+w\ ec\ cv}$	$\frac{sc+\ +\ +\ +}{sc+w\ ec\ cv}$
	$\frac{+\ w^x+\ +\ +}{sc+w\ ec\ cv}$	$\frac{+\ w^x w\ ec\ cv}{sc+w\ ec\ cv}$
♂	sc+w ec cv + w^x + ++	sc+ + + + + w^x w + +

The markers sc and ec on either side of w thus indicate not only whether the unexpected types have arisen as a result of unidirectional crossing over in that region but also determine the order of w and w^x on the chromosome with respect to the other markers.

This technique also rules out unequal crossing over of the Bar type (Sturtevant 1925) as a possible source of the unexpected recombinants as, in such a case, recombinants of any type with respect to Bar can be produced by crossing over in either direction.

3. EXPERIMENTAL DATA.

(i) PRELIMINARY CROSSES

A preliminary series of experiments was run in order to test as many combinations as possible. A limit had to be set to the number of progeny examined in each cross in order to avoid wasting time on one which might give only a negative result. The limit was set at 5,000 flies. If in this sample no rare recombinants were detected, the cross was abandoned and a new combination tested.

Examination of such large numbers of individuals was effected rather rapidly as it was necessary to detect only those with an eye colour markedly different from the expected segregants, i.e. to detect a red or wild type eye colour and, in the crosses involving two alleles intermediate in the series, to detect a very pale or completely colourless eye. Unless a fairly sharp difference from the expected segregants was observed in any individual it was considered useless to make

further investigations on it as detectable changes in colour are produced by aging, condition of food, etc. As will be described later there were, in several crosses, flies with eyes paler than expected, but when analysed further the cause was found to be dilutors of white. Attention was paid to the segregation of the markers sc or y and ec only among unexpected flies. In addition, any bottle from which such a fly was isolated was marked to detect clustering which could be due to contamination. In no case, however, although about 1,000 progeny hatched from each bottle, did more than one unexpected fly per bottle occur.

It will be noted in Table II, which summarises the results of the preliminary crosses, that the limiting number of 5,000 was not strictly observed. The experimental procedure was not yet standardised however so it was not easy to assess the number of bottles required to yield 5,000 progeny. Where many more flies were hatching they were included in the examination.

The cross involving w^{bl} and w gave the first positive result with one female which was scute and red eyed. A progeny test revealed that she carried also the sc w ec cv chromosome introduced by the F_1 male and was not therefore likely to be due to contamination, although a sc stock did exist in the laboratory. A sc stock was built up from this female and maintained in the homozygous condition. A further 33,225 progeny were examined from the same cross to confirm that crossing over occurs between w and w^{bl}. Of these, 4 more sc recombinants were recovered, 3 sc males and 1 $\frac{sc + + +}{sc\ w\ ec\ cv}$ female. It would have been reasonable

Table II. Backcross results from preliminary crosses.

Genotype of F_1 female.	No. of progeny examined.	No. and genotype of presumed crossover.
$\frac{y\ w^{eS}\ ec}{w^{aE}}$	5,000	0
$\frac{y\ w^{eS}\ ec}{z\ w^{11E4}}$	6,715	0
$\frac{y\ w^{eS}\ ec}{w^{h}}$	4,874	0
$\frac{y\ w^{eS}\ ec}{w^{sat}}$	5,000	0
$\frac{sc\ w\ \ ec\ cv}{w^{bf2}}$	5,000	0
$\frac{sc\ w\ \ ec\ cv}{w^{coE}}$	9,278	0
$\frac{y\ w^{eS}\ ec}{w^{bl}}$	4,881	0
$\frac{y\ w^{eS}\ ec}{w^{t}}$	9,875	0
$\frac{sc\ w\ \ ec\ cv}{w^{sat}}$	5,000	0
$\frac{sc\ w\ \ ec\ cv}{w^{bl}}$	5,000	1 $\frac{sc\ +\ +\ +}{sc\ w\ ec\ cv}$
Totals	60,596	1

to suppose that the double mutant $\underline{w^{bl}w}$ would give a white phenotype. Thus any such double mutant recombinants would not be distinguishable from the parental type segregants.

The genotype of F_1 females should be designated $\frac{sc + w\ ec\ cv}{+ w^{bl} + + +}$ with $\underline{w}^{bl}$ to the left of $\underline{w}$. Crossing over between $\underline{w}^{bl}$ and $\underline{w}$ produces the $\underline{sc + + + +}$ chromosome with a frequency of 5/38,225, i.e. 0.00013. This figure however accounts for only half the recombinants, the $\underline{w^{bl}w}$ class not having been isolated. The recombination fraction for $\underline{w}^{bl}$ and $\underline{w}$ is thus 0.00026.

(ii) Further tests to determine relationships between position and function of w alleles.

Having obtained evidence that recombination between alleles of the white series does occur, more exhaustive tests could now be carried out with the emphasis on determining whether there exists a relationship between location and function. Crosses made in this series of experiments, the results of which are listed in Table 111, will be seen to involve in some cases females heterozygous for alleles from any two of the three groups listed earlier (pp 9-10) and in others for two from the same group.

Since the rate of crossing over between white alleles was much lower than originally allowed for, it was necessary to increase the number of progeny in any particular experiment. From this point at least 20,000 was set as the minimum sample. It must be borne in mind, however, that since the recombinants in these experiments are very rare the fact that they are not found in some samples cannot be taken as evidence that the alleles being tested do not recombine. As Pontecorvo has pointed out (1956b), detection of recombination of the order of 1×10^{-8} is technically possible for micro-organisms and the rate of 1×10^{-6} has been recorded by Forbes in A. nidulans. (Forbes 1955). Thus, were the techniques described for this work not so time consuming, much larger samples could be investigated and the results in consequence would indicate with more certainty which alleles recombine.

No effort was made to increase the rate of crossing

over by introducing chromosomal rearrangements, (inversions or translocations) into the autosomes as had been done by Lewis (1948). The reasons for this decision are first that introduction of chromosomal aberrations tends to lower viability and secondly that although the estimation of the frequency of such a rare event as crossing over between the white alleles is very rough, this technique would reduce the accurracy even more. Furthermore shortly after the results of our preliminary experiments were published (MacKendrick and Pontecorvo 1952, Table 1), Lewis (1952a) published the results of his work on crossing over between white and "apricot" where he used the technique of adding autosomal inversions. Comparison of the two sets of data showed that by his method the estimated rate of recombination had only been doubled.

It will be noted in Table 111 that 14 of the 15 rare wild type segregants were recombinant also for the linked markers and it is reasonable to assume that these rare recombinants resulted from crossing over in the sc - ec or y - ec regions. If these wild type individuals were due to back mutation the chances of crossing over between y or sc and ec occurring simultaneously with mutation would be about 5.5% or 1 in 18, there being 5.5 units between y or sc and ec.

Table III. More fully investigated combinations.

No. of cross.	Genotype of female and corresponding allele group.	No. of progeny examined.	No. and genotype of rare recombinants.	Recombination fraction.
1	$\frac{yw^{eS}ec}{w^{aE}}$ I / II	52,710	3 $\frac{+\ +\ ec}{yw^{eS}\ ec}$ ♀♀	0.00011 ± 0.00005
2	$\frac{yw^{eS}ec}{w^{h}}$ I / II	46,742	0	0
3	$\frac{sc\ w\ ec\ cv}{w^{bl}}$ II / I	38,225	3 sc + + + ♂♂ 2 $\frac{sc\ +\ +\ +}{sc\ w\ ec\ cv}$ ♀♀	0.00026 ± 0.00008
4	$\frac{sc\ w\ ec\ cv}{w^{coP}}$ II / I	21,067	2 sc+ + + ♂♂ 3 $\frac{sc\ +\ +\ +}{sc\ w\ ec\ cv}$ ♀♀	0.00047 ± 0.00015
5	$\frac{sc\ w\ ec\ cv}{w^{h}}$ II / II	22,280	0	0

Table III. (continued.)

No. of cross.	Genotype of female and corresponding allele group.	No. of progeny examined.	No. and genotype of rare recombinants.	Recombination fraction.
6	$\frac{sc\ w\ ec\ cv}{w^i}$ $\frac{II}{III}$	55,509	1 $\frac{sc + + +}{sc\ w\ ec\ cv}$ ♀ 1 $\frac{+ + + +}{sc\ w\ ec\ cv}$ ♀ *	0.00007 ±0.00004
7	$\frac{sc\ w\ ec\ cv}{w^{aE}}$ $\frac{II}{II}$	43,374	0	0
8	$\frac{yw^{eS}ec}{w^{coP}}$ $\frac{I}{I}$	18,266	0	0
Totals.		298,173	15	

* see text.

Group I: dosage compensation; no zeste interaction.

Group II: dosage compensation; zeste suppression.

Group III: no dosage compensation; no zeste suppression.

Standard error = $\pm_N \frac{\text{No. of recombinants}}{\text{Total}}$

The probability would be even less that, with the exception of the $\underline{w}/\underline{w^i}$ cross, the wild type segregants from any cross were always the result of crossing over in only one direction.

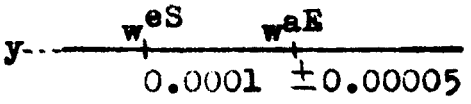
The completely wild type female (marked with an asterisk in Table III) isolated in the $\underline{w}/\underline{w^i}$ cross may have arisen either by back mutation of the $\underline{w^i}$ allele, or by a second crossing over between sc and w. Since back mutation from $\underline{w^i}$ has not previously been reported, and a search through the literature indicates that it is a very rare event among the lower alleles of the white series, it seems more probable that a second crossing over has occurred between sc and w. The chance of such an event occurring among the rare recombinants is about 1 in 70 as there are 1.5 units between sc and w.

Crosses 4 and 6 of Table III can be interpreted in the same way as cross 3, (p.20). Cross 2 is more fully designated thus: $\frac{yw^{eS}+ec}{+\ +\ w^{aE}+}$ with crossing over between $\underline{+^{w^{eS}}}$ and $\underline{+^{w^{aE}}}$ giving rise to the $\underline{+\ +\ +\ ec}$ exceptions.

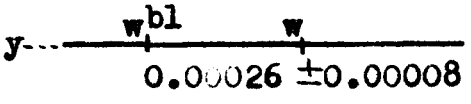
Figure 4. gives a diagrammatic representation of the white region of the X-chromosome based on the results given in Table III. The relationship of position with function will be dealt with in the discussion.

Fig. 4. Diagrammatic representation of the positions of $\underline{w}^{eS}$ and $\underline{w}^{aE}$; $\underline{w}^{bl}$ and $\underline{w}$; $\underline{w}^{coP}$ and $\underline{w}$; $\underline{w}^{i}$ and $\underline{w}$ respectively, and the corresponding recombination fractions.

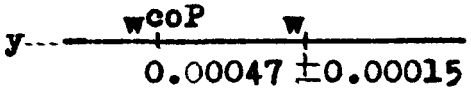
(a) $\underline{w}^{eS}$; $\underline{w}^{aE}$



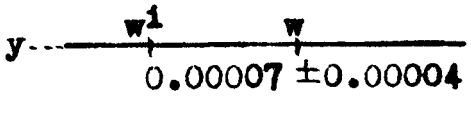
(b) $\underline{w}^{bl}$; $\underline{w}$



(c) $\underline{w}^{coP}$; $\underline{w}$



(d) $\underline{w}^{i}$; $\underline{w}$



In the $\underline{w}^{eS}$/ $\underline{w}^{aE}$ cross, although it should have been possible to detect the $\underline{w^{eS}w^{aE}}$ recombinants had they been considerably paler than $\underline{w}^{eS}$, $\underline{w}^{aE}$, or $\underline{w}^{eS}$/ $\underline{w}^{aE}$, no such types were isolated. This may have been due to the fact that the sample examined was not sufficiently large.

There were, however, in two of the mass cultures, several males with an eye colour paler than expected but not apparently having arisen in connection with crossing over in the $\underline{y}$ - $\underline{ec}$ region, as both paler $\underline{w}^{aE}$ and to a

less extent yw^{eS}ec males were detected. Females of this type were almost certainly present but as they were heterozygous for w^{eS}, the dilution in colour would not have been marked and so would escape detection in a fairly rapid examination. It was possible that this dilution effect was due to mutation of a specific dilutor of white of the type recorded by Bridges (Bridges and Brehme 1944). That this was in fact so will be shown in the appendix, (see pp.61,62).

(iii) **Analysis of relative positions of alleles which recombine with w and w^{eS}.**

Having established that w recombines with w^{bl}, w^{coP} and w^{i}, it was of interest to find out whether w^{bl}, w^{coP} and w^{i} were in turn separable by crossing over and whether w^{eS} and w^{bl}, or w^{aE} and w^{bl} were separable. For this analysis a tester stock was made with w^{bl} by adding the markers sc and ec cv exactly as described for the sc w ec cv stock. Only one bottle was cultured for the backcross and again the sc w^{bl} ec cv male was isolated in one step.

Table VI summarises the results of the crosses using w^{bl} as the tester allele. w^{aC} was included in this group of tests, to determine whether it behaved differently from w^{aE}. w^{h} was also included here as no recombinants had resulted from the crosses with w and w^{eS} although fairly large samples had been examined. w^{i} was not included here as the unexpected results from the w^{aE}/w^{bl} cross were of more immediate interest.

Table VI. Data from experiments carried out to analyse position relationships between $\underline{w}^{bl}$ and $\underline{w}^{coP}$, $\underline{w}^{eS}$, $\underline{w}^{h}$, $\underline{w}^{aC}$ and $\underline{w}^{aE}$.

Cross No.	Maternal genotype	No. of progeny examined.	No. and genotype of rare recombinants.	Recombination fraction.
1	$\frac{sc\ w^{bl}\ ec\ cv}{w^{coP}}$	48,826	0	<0.00002
2	$\frac{sc\ w^{bl}\ ec\ cv}{y\quad w^{eS}}$	45,313	0	<0.00002
3	$\frac{sc\ w^{bl}\ ec\ cv}{w^{h}}$	41,800	1 $\frac{+\ +\ ec\ cv}{sc\ w^{bl}\ ec\ cv}$ ♀ 1 $+w^{?}\ +\ +$ ♂ *	0.000048 ± 0.000034
4	$\frac{sc\ w^{bl}\ ec\ cv}{w^{aC}}$	27,654	0	<0.00004

(cont. on following page).

Table VI. (cont.)

Cross No.	Maternal genotype.	No. of progeny examined.	No. and genotype of rare recombinants.	Recombination fraction.
5	$\frac{sc\ w^{bl}\ ec\ cv}{w^{aE}}$	54,878	2 $\frac{+\ +\ ec\ cv}{sc\ w^{bl}\ ec\ cv}$ ♀♀ 1 + + ec cv ♂ 2 sc |w| + + ♂♂ 3 + |w| ec cv ♂♂ 2 + |w| + + ♂♂	0.00016 ± 0.00005

* See text.

\|w\| denotes a white phenotype. The full genotype will be discussed in the text.

The results in Table VI indicate that $\underline{w}^{bl}$ did not recombine with $\underline{w}^{eS}$, $\underline{w}^{coP}$ or $\underline{w}^{aC}$ and thus, within the resolving power of the present experiment, these four alleles occupy indistinguishable positions on the X-chromosome. $\underline{w}^{bl}$ and $\underline{w}^{h}$ recombine, the recombination fraction being 5×10^{-5}. In this cross there appeared a white male, marked in Table VI with an asterisk, with neither the sc or ec cv markers. As the eye colour of this male was not pure white but somewhere between white and honey, the presence of a modifier of spontaneous origin similar to that in the $\underline{w}^{eS}$/ $\underline{w}^{aE}$ cross was suspected. Males of this type were therefore mated to wild type females and the backcross progeny examined for the possible segregation of a dilutor. The results are presented in Table VII.

Table VII. Backcross results from "white"/+ x "white".

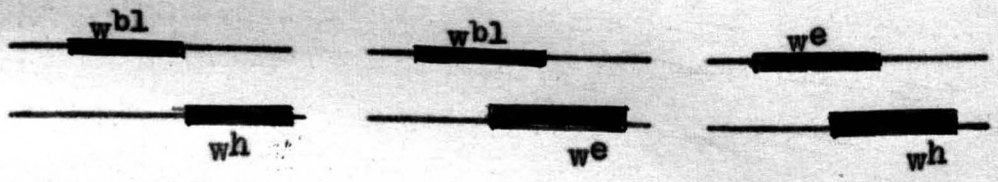
Phenotype			
Wild type		"White"	
♀	♂	♀	♂
118	115	110	103

"white" denotes pale eye colour.

No $\underline{w}^{h}$ - like individuals segregated. Therefore if a modifier was present it must have been very closely linked to $\underline{w}^{h}$. The origin of this male could have been due to spontaneous mutation of the $\underline{w}^{h}$ allele or to double crossing over in the white region producing a $\frac{+ w^{bl} w^{h} + +}{}$ individual. Testing of the hypothesis that this male

was $\underline{w}^{bl}$ $\underline{w}^{h}$ was not carried out, due to lack of time.

Still in connection with the $\underline{w}^{bl}$/ $\underline{w}^{h}$ results, although $\underline{w}^{bl}$ and $\underline{w}^{eS}$ appear to occupy the same position and $\underline{w}^{bl}$ and $\underline{w}^{h}$ different positions, among the 46,742 progeny examined in the $\underline{w}^{eS}$/ $\underline{w}^{h}$ cross there were no recombinants between $\underline{w}^{h}$ and $\underline{w}^{eS}$. However it seems reasonable to suppose that, since the frequency of such crossovers is so low, the fact that they did not appear in the sample taken is not significant. A further important point to be borne in mind when trying to interpret these results is that mutant sites on the chromosome may vary in extent and so a heterozygote may be mutant for overlapping sections. The situation involving the $\underline{w}^{bl}$, $\underline{w}^{h}$ and $\underline{w}^{eS}$ alleles could therefore be summarised by the following diagrammatic representation:-



Thus although these 3 alleles occupy 3 different sites crossing over giving rise to a wild type segregant could result from the $\underline{w}^{bl}/\underline{w}^{h}$ combination only.

Since no recombination was observed between the group of alleles consisting of $\underline{w}^{bl}$, $\underline{w}^{eS}$, $\underline{w}^{coP}$ and $\underline{w}^{aC}$ they must be placed, as a first approximation, at the same position. $\underline{w}^{i}$, having been shown to be to the left of $\underline{w}$ (Fig.4(d)), will also be placed in this group

although further tests may indicate that it occupies a third site in the white region. $\underline{w}$, $\underline{w}^{h}$ and $\underline{w}^{aE}$ may be placed in a second group, no crossing over having been detected between $\underline{w}$ and $\underline{w}^{h}$ and between $\underline{w}$ and $\underline{w}^{aE}$. Of course all these assumptions are also subject to the above reservation that mutant sites may overlap.

Table VIII. Summary of data from crosses which gave positive results, excluding $\underline{w}^{bl}/\underline{w}^{aE}$ cross.

Genotype of F_1 female	No. of progeny examined	No. of rare recombinants	Recombination fraction
$\frac{y\ w^{eS}\ ec}{w^{aE}}$	52,710	3	0.00011 ±0.00005
$\frac{sc\ w\ ec\ cv}{w^{bl}}$	38,225	5	0.00026 ±0.00008
$\frac{sc\ w\ ec\ cv}{w^{coP}}$	21,067	5	0.00047 ±0.00015
$\frac{sc\ w\ ec\ cv}{w^{i}}$	55,509	2	0.00007 ±0.00004
$\frac{sc\ w^{bl}\ ec\ cv}{w^{h}}$	41,800	2	0.00005 ±0.00003
Totals.	209,311	17	

(iv) Analysis of the w^{bl}/w^{aE} data.

The $\underline{w}^{bl}/\underline{w}^{aE}$ cross was the first from which the assumed reciprocal products of crossing over were obtained, i.e. both the wild type and the white eyed recombinants. These results are not however as straight-forward as outlined in Fig.3, p.16. The white eyed recombinants being of 3 different types (Table VI). The simplest interpretation of this situation is that at least 3 sites are involved, $\underline{w}^{aE}$ being a compound of two, $\underline{w'}$ and $\underline{w''}$, thus

$$\frac{sc\ w^{bl}\ +\ +\ ec\ cv}{+\ +\ w'\ w''\ +\ +} \rightarrow \begin{array}{l} 3\ +\ +\ +\ +\ ec\ cv\ \text{♀♀ and ♂} \\ 2\ sc\ w^{bl}\ w'\ w''\ +\ +\ \text{♂♂} \\ 3\ +\ +\ w'\ +\ ec\ cv\ \text{♂♂} \\ 2\ +\ +\ w\ +\ +\ +\ \text{♂♂} \end{array} \left.\begin{array}{l} \\ \\ \\ \\ \end{array}\right\} \begin{array}{l} \\ \\ \text{white} \\ \text{eyed.} \end{array}$$

Crossing over between $+^{wbl}$ and $+^{w'}$ gives rise to the $\underline{+\ +\ +\ +\ ec\ cv}$ females and male, i.e. the red-eyed echinus crossveinless individuals. The reciprocal products of crossing over in this interval are the $\underline{sc\ w^{bl}\ w'\ w''\ +\ +}$ males, i.e. scute white males. A $\frac{sc\ w^{bl}\ w'\ w''\ +\ +}{sc\ w^{bl}\ +\ +\ ec\ cv}$ female would not be sufficiently different from the $\underline{w}^{bl}/\underline{w}^{aE}$ females to be able to distinguish it among the progeny. Crossing over between $\underline{w'}$ and $\underline{w''}$ produces the $\underline{+\ +\ w'\ +\ ec\ cv}$ males. Again females of this type would not be distinguishable. The reciprocal crossover $\underline{sc\ w^{bl}\ +\ w''\ +\ +}$ would perhaps be scute white in phenotype. However later tests with the 2 scute white types indicate that it is unlikely that

this recombinant has been recovered.

The only drawback to this hypothesis is that the white echinus crossveinless crossovers, i.e. the + +w'+ec cv genotype, supposedly being mutant for only one of the 3 sites in the white region, is white eyed in contrast to w^{aE} which is apricot eyed and is a double mutant. A possible interpretation of this phenomenon is that there is some kind of interaction between w' and w", w" being a partial suppressor of w'. It is not possible at this stage to identify the genotypes of the two non-scute, white, non-echinus non-crossveinless males. They may be the result of mutation of $w^{aE} \longrightarrow w$; of spontaneous mutation of a modifier; or a double crossing over between w^{bl} and w", or between sc and w^{bl}, and w" and ec.

In order to determine the possible origin of these two white males they were tested separately against sc+ec females and the backcross male scored for segregation of eye colours which were non-red or non-white.

In the first of these two tests 57,900 males were scored and no rare recombinants noted. Hence it is unlikely that this white male was either a $w^{bl}w^{aE}$ recombinant, in which case w^{bl} and w^{aE} would segregate; or mutant also for a modifier of w^{aE}, in which case w^{aE} would segregate. This male thus appears to have been due to mutation of w^{aE} to w.

In the cross involving the second white male, which appeared to be very slightly pigmented, only 509 males were scored as among these there were 5 w^{aE} and 8 w^{aE}ec

males indicating that there has been a mutation to a dilutor of $\underline{w}^{aE}$ of a type detected in the $\underline{w}^{aE}/\underline{w}^{eS}$ cross. In this case the dilutor was only 4.3 units approximately from $\underline{w}^{aE}$ and therefore not the same as the one from the $\underline{w}^{aE}/\underline{w}^{eS}$ cross.

Returning to the $\underline{|w|\ ec\ cv}$ recombinants in the $\underline{w}^{bl}/\underline{w}^{aE}$ cross an alternative hypothesis to account for their origin is that unequal crossing over has taken place giving rise to the white $\underline{ec\ cv}$ males thus:

$$\frac{sc\ w^{bl}+\ ec\ cv}{+\ +\ w^{aE}\ +\ +} \quad \text{or} \quad \frac{sc\ w^{bl}+\ ec\ \ cv}{+\ +\ w^{aE}\ +\ +}$$

. Crossing over as indicated results in $\underline{+(w^{bl})^{Df}+\ ec\ cv}$ or $\underline{+\ +(w^{aE})^{Df}\ ec\ cv}$ males which are deficient for the $\underline{w}^{bl}$ and $\underline{w}^{aE}$ sites respectively, and may be phenotypically white. $(w^{bl})^{Df}$ denotes deficiency for $\underline{w}^{bl}$, and $(w^{aE})^{Df}$ deficiency for $\underline{w}^{aE}$.

A series of crosses was carried out to test these two hypotheses. The first step was to cross both types of white recombinant with wild type in an attempt to recover the $\underline{w}^{bl}$ and $\underline{w}^{aE}$ components. At this stage it became obvious that in the crosses to be analysed, the rate of recombination might be of the order of 1×10^{-4} and thus estimates to the nearest hundred of the number of progeny examined would give a sufficient degree of accurracy in calculating this value. Having already counted about 558,000 flies in groups of about 50 - 300 the ability to make such an estimation was assumed and hence the investigation was considerably speeded up.

Table IX. Data from crosses of wild type with the two white recombinants resulting from crossing over in opposite directions in the $\underline{w}^{bl}/\underline{w}^{aE}$ cross.

No.of cross	Genotype of F_1 female.	No. of progeny examined	No.and genotype of rare recombinants.	Recombination fraction.
1	$\frac{sc\ +\ +\ +}{+\ \vert w\vert^{\dagger}\ ec\ cv}$ x sc w ec cv	36,400	0	< 0.00003
2	$\frac{sc\ \vert w\vert^{\ddagger}\ +\ +}{+\ +\ ec\ cv}$ x sc w ec cv	34,800	2 $+\vert w^{a}\vert^{*}\ +\ +$ ♂♂ 1 $\frac{+\vert w^{a}\vert\ +\ +}{sc\ w\ ec\ cv}$ ♀ 1 $\frac{sc\ w^{bl}\ ec\ cv}{sc\ w\ \ ec\ cv}$ ♀ 1 sc w^{bl} ec cv ♂	0.00014 ±0.00006

† In cross No.1 the symbol |w| signifies a white phenotype assumed to be due to the w' genotype or to $(w^{aE})^{Df}$ or $(w^{bl})^{Df}$.

‡ In cross No.2 the symbol |w| signifies a white phenotype assumed to be due to the $w^{bl}w'w^{a}$ or $w^{bl}w^{aE}$ genotypes.

* In cross No.2 $|w^{a}|$ signifies an apricot phenotype which is assumed to be genotypically $w'w^{a}$ or w^{aE}.

Fig.6. Interpretation, according to the two different hypotheses, of the results noted in Table IX.

Hypothesis	No. of cross. cf. Table IX.	Genotype of mothers.	Chromosomes resulting from crossing over between w alleles.			
			Expected.		Observed.	
			Genotype.	Phenotype with respect to eye colour.	Genotype	Phenotype with respect to eye colour.
Unequal crossing over	1	$\frac{+\ (w^{bl})Df+\ ec\ cv}{sc\ +\ \ +\ \ +\ \ +}$ or $\frac{+\ +(w^{a})Df\ ec\ cv}{sc\ +\ \ +\ \ +\ \ +}$	None		None	
	1		None			
	2	$\frac{sc\ w^{bl}w^{aE}+\ +}{+\ \ +\ \ +\ ec\ cv}$	sc w^{bl} + ec cv + + w^{aE} + +	blood apricot	sc $\lvert w^{bl} \rvert$ eccv + $\lvert w^{a} \rvert$ + +	blood apricot

(cont. on next page).

Fig.6. (continued)

Hypothesis	No. of cross. cf. Table IX.	Genotype of mothers.	Chromosomes resulting from crossing over between w alleles.			
			Expected.		Observed.	
			Genotype	Phenotype with respect to eye colour.	Genotype	Phenotype with respect to eye colour.
3-site	1	$\frac{+\ +\ w'\ +\ ec\ cv}{sc\ +\ +\ +\ +\ +\ +}$	None		None	
	2	$\frac{sc\ w^{bl}\ w'\ w''\ +\ +}{+\ +\ +\ +\ ec\ cv}$	sc w^{bl} + + ec cv + + w'w" + + sc w^{bl}w'+ ec cv + + + w" + +	blood apricot ? } ? } *	sc $\lvert w^{bl} \rvert$ ec cv + $\lvert w^{a} \rvert$ + +	blood apricot

* The phenotypes of these two were not yet known and could have been indistinguishable from other segregants.

Straightforward crossing over, but taking into account the deficiencies, is assumed throughout Figure 6. Cross No.2 is treated both ways, i.e. as involving 2 sites w^{bl} and w^{aE} and as 3 sites w^{bl}, w' and w" where w' and w" are the postulated components of w^{aE}.

$\lvert w^{bl} \rvert$ denotes blood phenotype, $\lvert w^{a} \rvert$ apricot phenotype.

Table IX gives the results of crosses of the two different types of white recombinant with wild type and Figure 6 a diagrammatic interpretation of the attempt to distinguish between the two hypotheses.

The results of these two crosses strongly suggest that two genotypically different white recombinants have been isolated from the original $\underline{w}^{bl}/\underline{w}^{aE}$ cross, but they do not in any way help to choose between the two hypotheses on their possible mode of origin. In all cases in Fig.6 (apart from those recombinants marked with an asterisk) the observed and expected results on either hypothesis tie up with each other.

Having established that the two white recombinants are probably different in genotype, the next step was to cross them together, the possibility being that a distinguishable recombinant could be isolated. The results of this cross are given in Table X and interpreted in Fig.7.

This cross was carried out twice using both scute white phenotypes recovered from the $\underline{w}^{bl}/\underline{w}^{aE}$ cross. Of the 3 white ec cv males isolated from the latter cross only one survived long enough to mate and hence it was possible to test only one of the 3. Both crosses in Table X appeared to give the same result indicating that the 2 scute white combinations are probably identical.

Table X. Data from crosses of white ec cv with each of the two independently arisen sc white recombinants from the w^{bl}/w^{aE} cross.

No.of cross	Genotype of parents.	No. of progeny examined	No.and genotype of rare recombinants.	Recombination fraction.
1	$\frac{sc \mid w \mid + \; +}{+ \mid w \mid ec \; cv}$ x sc w ec cv	54,200	2 $\frac{+ \mid w^a \mid + \; +}{sc \; w \; ec \; cv}$ ♀♀ 3 $+ \mid w^a \mid + \; +$ ♂♂	0.00018 ± 0.00006
2	$\frac{sc \mid w \mid + \; +}{+ \mid w \mid ec \; cv}$ x sc w ec cv	63,400	3 $\frac{+ \mid w^a \mid + \; +}{sc \; w \; ec \; cv}$ ♀♀ 2 $+ \mid w^a \mid + \; +$ ♂♂	0.00019 ± 0.00006

$\mid w \mid$ denotes white phenotype, $\mid w^a \mid$ white-apricot.
Genotypes to be determined.

Figure 7. Interpretation of the results in Table X according to the unequal crossing over hypothesis and the 3-site hypothesis.

Hypothesis	Genotype of parents.	Chromosomes resulting from crossing over between w alleles.			
		Expected		Observed	
		Genotype	Phenotype with respect to eye colour.	Genotype	Phenotype with respect to eye colour.
Unequal crossing over	$\frac{sc\ w^{bl}w^{aE}+\ +}{+(w^{bl})Df+ec\ cv}$ x sc w ec cv	sc w^{bl} + ec cv	blood	+ | w^{a} | + +	apricot
		+ (w^{bl})Df_{waE} + +	white - indis -tinguishable		
	or				
	$\frac{sc\ w^{bl}w^{aE}+\ +}{+\ +(w^{aE})Df\ ec\ cv}$ x sc w ec cv	sc w^{bl}(w^{aE})Df ec cv	white - indis -tinguishable		
		+ + w^{aE} + +	apricot		
3 - site	$\frac{sc\ w^{bl}w'w''+\ +}{+\ +\ w'+\ ec\ cv}$ x sc w ec cv	+ + w'w" + +	apricot ? probably white and hence indis -tinguishable	+ | w^{a} | + +	apricot
		sc w^{bl} w' + ec cv			

It will be seen in Fig.7 that the 3-site hypothesis is favoured by the data, the unequal crossing over hypothesis being supported only if the |w|ec cv chromosome is considered to be deficient for w^{aE} and not w^{bl}. It is clear however that it is not yet possible to choose between these two hypotheses.

A further attempt to do so was made by crossing w^{aE} with wild type in search of the hypothetical w' and w" recombinants. Table XI and Figure 8 summarise and attempt to interpret these results.

Table XI. Data from $w^{aE}/+$ crosses.

Genotype of parents.	No. of progeny examined	No.and genotype of rare recombinants.	Recombination fraction.
$\frac{+\ w^{aE}\ +\ +}{sc\ +\ ec\ cv}$ x sc w ec cv	55,200	0	< 0.00002
$\frac{sc\ w^{aE}\ +\ +}{+\ +\ ec\ cv}$ x $sc\ w^{aE}\ +\ +$	97,800	1 sc |w| ec cv♂	0.00001 ±0.00001
Totals.	153,000	1	0.000007 ±0.000007

The first cross shown in Table XI was made with the standard sc ec cv strain from stock cultures and the F_1 females were crossed with sc w ec cv males.

Figure 8. Interpretation of the results in Table XI according to the unequal crossing over hypothesis and the 3-site hypothesis.

Hypothesis	Material genotype	Chromosomes resulting from crossing over between w^{aE} alleles. Expected. Genotype	Expected. Phenotype with respect to eye colour	Observed. Genotype	Observed. Phenotype with respect to eye colour
Unequal crossing over	$\frac{+\ w^{aE}\ +\ +}{sc\ +\ ec\ cv}$	$sc(w^{aE})^{Df}\ +\ +$	white	None	
		$+\ Dp(w^{aE})+ec\ cv$	red- hence indisting -uishable		
	or				
	$\frac{+\ w^{aE}\ +\ +}{sc\ +ec\ cv}$	$+\ (w^{aE})^{Df}ec\ cv$	white		
		$sc\ +Dp(w^{aE})\ +\ +$	red- hence indisting -uishable		

(cont. on following pages.)

Figure 8. (continued)

Hypothesis	Maternal genotype	Chromosomes resulting from crossing over between w alleles.			
		Expected.		Observed.	
		Genotype	Phenotype with respect to eye colour.	Genotype	Phenotype with respect to eye colour.
Unequal crossing over	sc w^{aE} + + / + + ec cv	sc Dp(w^{aE}) + ec cv	red - hence indistinguishable		
		+ (w^{aE})^{Df} + +	white		
	or			sc | w | ec cv	white
	sc w^{aE} + + / + + ec cv	sc (w^{aE})^{Df} ec cv	white		
		+ + Dp(w^{aE}) + +	red - hence indistinguishable		

(cont. on following page)

Figure 8. (continued.)

Hypothesis	Maternal genotype	Chromosomes resulting from crossing over between w alleles.			
		Expected		Observed	
		Genotype	Phenotype with respect to eye colour	Genotype	Phenotype with respect to eye colour
3-site	$\frac{+\ w'w''+\ +}{sc\ +\ +\ ec\ cv}$	$+w'+ec\ cv$ $sc+w''+\ +$	white ?	None	
	$\frac{sc\ w'w''+\ +}{+\ \ +\ +ec\ cv}$	$sc\ w'+ec\ cv$ $+\ +\ w''+\ +$	white ?	sc |w| ec cv	white

$Dp(w^{aE})$ denotes duplication of the w^{aE} site.
$(w^{aE})^{Df}$ denotes deficiency for the w^{aE} site.
\|w\| denotes white phenotype.

Thus all females in the F_2 would be heterozygous for w (barring non-disjunction) and should a rare recombinant female be produced carrying the white w', or w" which may be white, it would be phenotypically white and therefore distinguishable among the progeny. Any white male recombinant from this cross would be expected to be of the scute white or white echinus cross-veinless phenotypes and would not thus be confused with sc w ec cv patroclinous males which could arise from primary non-disjunction among the female F_1 parents. From this cross no rare recombinants were noted.

The second cross was made using one of the ec cv recombinants from the original w^{bl}/w^{aE} cross. This time the F_1 females were crossed to sc w^{aE} males, i.e. a straightforward F_2 was carried out. For the reasons noted above in connection with non-disjunction, sc w ec cv males could not be used for this F_2. Here although 97,800 flies were examined only one recombinant was isolated. Unfortunately this male was very weak and died before it was possible to mate it and produce a stock for further tests.

There are several points of note in the results from Table XI.

1. The sc | w| ec cv combination is recovered but with the exceedingly low frequency of 1 in 153,000. Going back to the w^{bl}/w^{aE} cross (Table VI, pp.28-29), crossing over, giving rise to the | w| ec cv recombinant due to either of the two mechanisms discussed, occurred with a frequency of 3 in 54,878. As these are the only two crosses which

could give an estimate of this recombination fraction it is impossible to decide which cross gives an estimate nearer to the mean.

2. In the first cross in Table XI, where it would have been possible to detect primary non-disjunction through the presence of sc w ec cv patroclinous males, none was noted among 55,200 progeny. According to Morgan, Bridges and Sturtevant (1925) the rate of this event is approximately 1/2,000, and thus several would have been expected here. However according to Demerec and Farrow (1930) the rate of incidence of primary non-disjunction varies from stock to stock and from experiment to experiment and it can only be assumed that in this particular case an exceedingly low rate was encountered by chance.

3. In neither of the two crosses has the postulated w" recombinant been isolated, although even a slight departure from the eye colours of the normal segregants was searched for.

There are two possible explanations for the failure to isolate the w" recombinant. One is that, due to the exceedingly low recombination rate in the w^{aE} region, insufficient flies were examined to ensure isolation of at least one such crossover. The second possibility is that the phenotype of w" is like either of the grandparental types, i.e. it is apricot or red eyed and would not therefore be detectable among the progeny. The following section gives an account of a further attempt at recovery of w" by use of the attached-X technique and hence of choosing finally between the unequal crossing over and 3-site hypotheses.

(v) ATTACHED - X TECHNIQUE

Lewis (1952) in his investigation of white alleles, and Green (1955) on vermilion alleles made use of L.V.Morgan's technique (1931) employing attached-X females heterozygous for the two alleles to be tested, in order to isolate in one female the reciprocal products of crossing over between these two alleles.

It was decided to produce a stock with sc on one attached-X chromosome and w^{aBec} on the other by using the triploid method (Emerson and Beadle, 1933). As no suitable triploid stock was available in the laboratory, Dr.M.M.Green very kindly offered to constitute a triploid sc w^{bl} female such that substitution of the desired type $\frac{}{Y\ M\text{-}5}$ of X-chromosome was possible. This female was produced as shown in Figure 9. M-5 refers to the Muller-5 chromosome whose genetic formula is sc^{SI} B In·S w^{a} sc^{8} and contains two large inversions which effectively prevent crossing over along the whole length of the X-chromosome. (written communication from Muller quoted by Spencer and Stern, 1948).

Figure 9. Method of synthesis of $\frac{sc\ w^{bl}}{y\ M\text{-}5}$ triploid stock.

P. 3n ♀♀ y ∧ y | y M-5 x sc w^{bl} ec cv

↓

F_1 3n ♀♀ y ∧ y | sc w^{bl}ec cv x y M-5 (= yw^aB)

↓

Select 3n ♀♀ which are phenotypically non-yellow, broad Bar and thus of the y ∧ sc w^{bl} | y w^a B genotype. These 3n ♀♀ may carry on the non-yellow attached-X chromosome <u>sc w^{bl} ec cv</u>, <u>sc w^{bl} ec</u> or <u>sc w^{bl}</u>.

F_2 y ∧ sc w^{bl} | y w^a B x y w^a B

↓

Reciprocal single crossovers in the attached-X chromosomes will give y ∧ y | y w^aB and sc w^{bl} ∧ sc w^{bl} | yw^aB females. Thus select for

F_3 3n ♀♀ sc w^{bl} ∧ sc w^{bl} | y w^aB x y w^aB

These triploid females are kept in stock by mating at each generation with <u>y M-5</u> males. They are distinguishable from intersexes and the diploid segregants as they have very broad Bar eyes due to the fact that they have 2 non-Bar and one Bar carrying chromosomes.

$\frac{sc + +}{+\ w^{aE} cv}$ attached-X females could then be derived by substitution of the <u>sc w^{bl}</u> chromosomes by the <u>sc</u> and <u>w^{aE}cv</u> chromosomes in the triploid females.

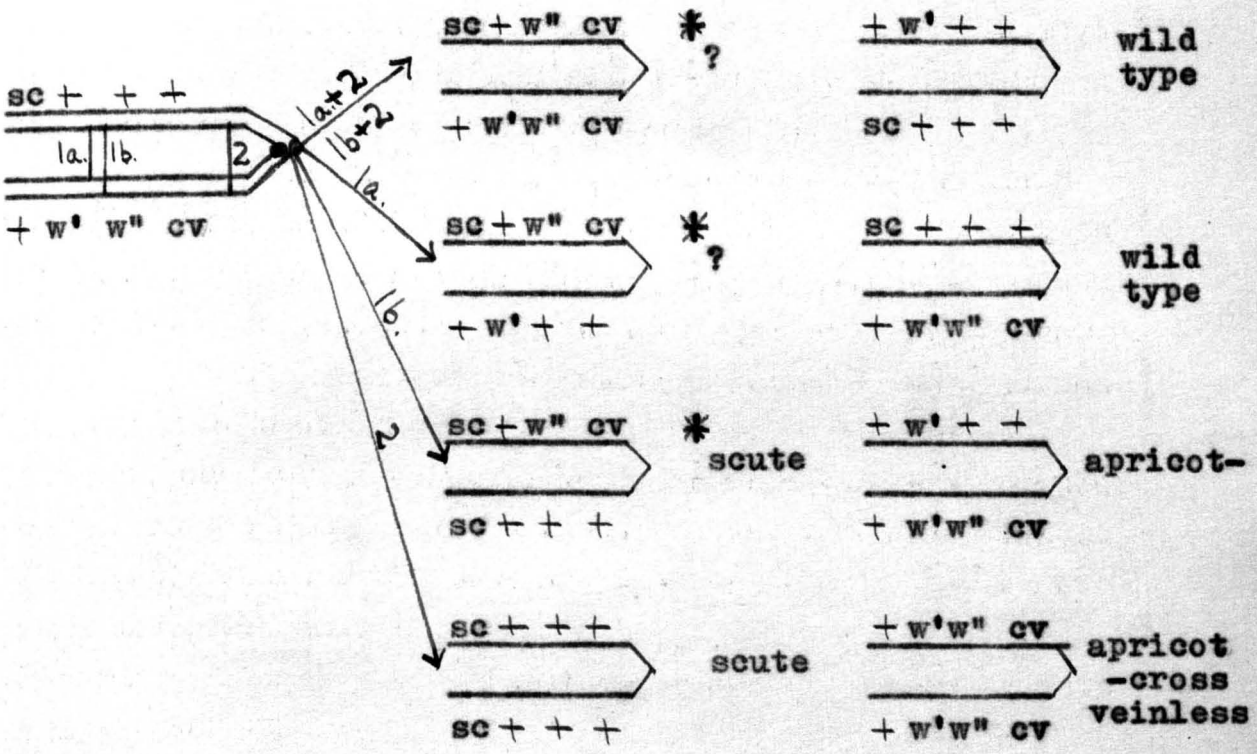
At the first attempt attached-X females of the desired phenotype were isolated. Proof of this genotype is easily obtained by progeny testing the females, as a single reciprocal crossover between cv and the centromere gives rise to females homozygous for the marked loci on either of the two X-chromosomes. The stock was maintained by crossing these females to Bar males, thus enabling detection of detachment of the X-chromosomes (i.e. by production of a heterozygous Bar female or a non-Bar male) and selected at each generation.

Since only by examining the progeny of any female can her genotype be determined, single female cultures must be used and where a female is proved to be of the wrong genotype the culture is discarded.

(vi) Application of the attached-X technique to analysis of the w^{aE} complex.

Figure 10. gives a diagrammatic representation of all the products of crossing over within the postulated w^{aE} complex (i.e. between w' and w") and/ or between cv and the centromere.

Fig.10. Products of crossing over in $\frac{sc+\ +}{+\ w^{aE}\ cv}$ females.



Those segregants in Figure 10 marked with an asterisk are heterozygous for w" but as it's phenotype was unknown they must be searched for on the assumption that w" is white, apricot- like or wild type.

Taking these three possibilities in turn, the important types of segregant can be determined.

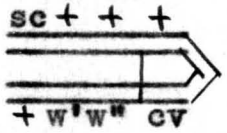
Figure 11 (i) That w" is phenotypically white.

	1a + 2 1b + 2	1a	1b
Genotype	sc + w" cv → + w'w" cv	sc + w" cv → + w' + +	sc + w" cv → sc + + +
Phenotype	Pale apricot crossveinless	white	scute
Remarks	Progeny testing would separate this from + w'w" cv → + w'w" cv from 2	Only white segregant possible	Only scute segregant with white cross-veinless on one chromosome
	Reliable	Very reliable	Reliable

Figure 12 (ii) That w" is phenotypically apricot.

	1a + 2 1b + 2	1a	1b
Genotype	sc + w" cv + w'w" cv	sc + w" cv + w' + +	sc + w" cv sc + + +
Phenotype	apricot cross-veinless	pale apricot	scute
Remarks	Could be confused with 3 strand double crossover thus sc + + + + w'w" cv giving sc w'w" cv + w'w" cv ∴ Not reliable	Separable from + w' + + + w'w" + on progeny testing. Reliable	Separable from sc + + + sc + + + on progeny testing. Could however be confused with the sc w'w" cv sc + + + female which could be produced with a relatively high frequency from the following type of crossing over. sc + + + + w'w" cv Not reliable

Figure 13 (iii) That w" is red eyed.

	1a+2. 1b+2.	1a.	1b.
Genotype	sc+w" cv +w'w" cv	sc+w" cv +w'+ +	sc+w" cv sc+ + +
Phenotype	Cross-veinless	wild type	scute
Remarks	Could be confused with sc+ + cv +w'w" cv which would be produced with a relatively high frequency by the following type of crossing over:  Not reliable.	Progeny testing would separate this from the sc+ + + +w'w" cv parental types which occur with rather a high frequency. However since not many females can be tested simultaneously many wild type segregants are used again as parents. Hence if a Ia segregant occurred there would be a small chance of detecting it. Reliable but small chance.	Easily confused with sc+ +cv sc+ + + which would arise with a relatively high frequency thus:  Not reliable.

Summarising all these possibilities it will be seen that for each of the 3 postulated phenotypes of w", it would be technically possible to isolate at least one of the 3 w" segregants. The females necessary to progeny test are white, pale apricot, pale apricot cross-veinless, scute and as many wild types as possible.

The progeny of 553 wild type attached-X females of the confirmed $\frac{sc + +}{+ w^{aE} cv}$ genotype were examined, in addition to segregants of the above noted types.
The results of this experiment are summarised in Table XII.

Table XII. Data from $\frac{sc + +}{+ w^{aE} cv}$ x B

Maternal genotype.	No.of females tested.	No. of progeny.	Rare recombinants.
$\frac{sc + +}{+ w^{aE} cv}$	553	22,728	0

There were no positive results from this cross. At this point the experiment had to be abandoned for the following reasons.

1. Single pair matings were essential.
2. The progenies had to be classified separately to ensure that the parental female was of the correct genotype.
3. The number of segregants to be progeny tested was rather large,

All these factors made this experiment very time consuming and it was necessary very shortly to terminate this investigation for a period of time.

All that can now be done, therefore, is to review the results of all these experiments on the $\underline{w}^{bl}/\underline{w}^{aE}$ rare recombinants in the light of the two hypotheses and to try to determine on which side the weight of evidence falls.

It will be seen throughout these experiments that the unequal crossing over hypothesis depends entirely on the |w| ec cv recombinant being deficient for $\underline{w}^{aE}$ and not for $\underline{w}^{bl}$. Unfortunately the other 2 |w| ec cv recombinants did not survive for testing and so it is not known whether they were the same as the survivor. The two sc |w| recombinants which, on an unequal crossing over hypothesis, could also be deficient for either $\underline{w}^{bl}$ or $\underline{w}^{aE}$ proved not to be so. They were both straightforward recombinants between $\underline{w}^{bl}$ and $\underline{w}^{aE}$, i.e. $\underline{w^{bl}w^{aE}}$ double mutants, and both different from |w| ec cv with respect to white.

The fact that there could have been unequal crossing over in the first place and later in the $\underline{w}^{aE}/+$ cross is very unexpected, as experiments involving all the other alleles tested, and also $\underline{w}^{aE}$ when tested with $\underline{w}^{eS}$, showed no sign of unequal crossing over. On the contrary, all the evidence from this and other work on recombination between alleles points to very exact pairing.

The only points against the 3-site hypothesis are first that the 3rd. site has not been separately identified and secondly the unexpected interaction between the two sites constituting the $\underline{w}^{aE}$ part of the complex. As already

emphasised, however, failure to isolate a 3rd. site may be due to the difficulty of detecting it phenotypically. The reasons for accepting that there could be interaction between closely linked alleles are best left till the discussion and for the purpose of argument at the moment they will be accepted as feasible.

Thus it appears that the 3-site is a more acceptable, but not yet completely proven, hypothesis.

In the preceeding discussion it has been assumed that w" is the suppressor factor in the w'w" or apricot combination. The possibility that this effect is due to w' cannot however be ruled out. The following test was made to determine what effect w' has with alleles to the left of it. As the attached-X technique was necessary to ensure isolation of the double mutant segregant, and as w^{bl} wasalready present in the attached-X chromosomes of the triploid stock described earlier, it was convenient to test the $w^{bl}w'$ combination.

A $\frac{sc\ w^{bl}+\ +\ +}{+\ +\ w'\ ec\ cv}$ stock was thus synthesised by substituting a w'ec cv chromosome for one of the $sc\ w^{bl}$ chromosomes in the triploid $\frac{sc\ w^{bl}}{y\ M\text{-}5}$ females.

In the following generation attached-X females of the appropiate genotype were isolated. Again the first attached-X females isolated proved, by progeny testing, to be of the desired genotype and were kept in stock by mating to Bar males and selected at each generation. The segregants obtainable by crossing over between w^{bl} and w' and/or cv and the centromere are shown in Figure 14.

Figure 14.

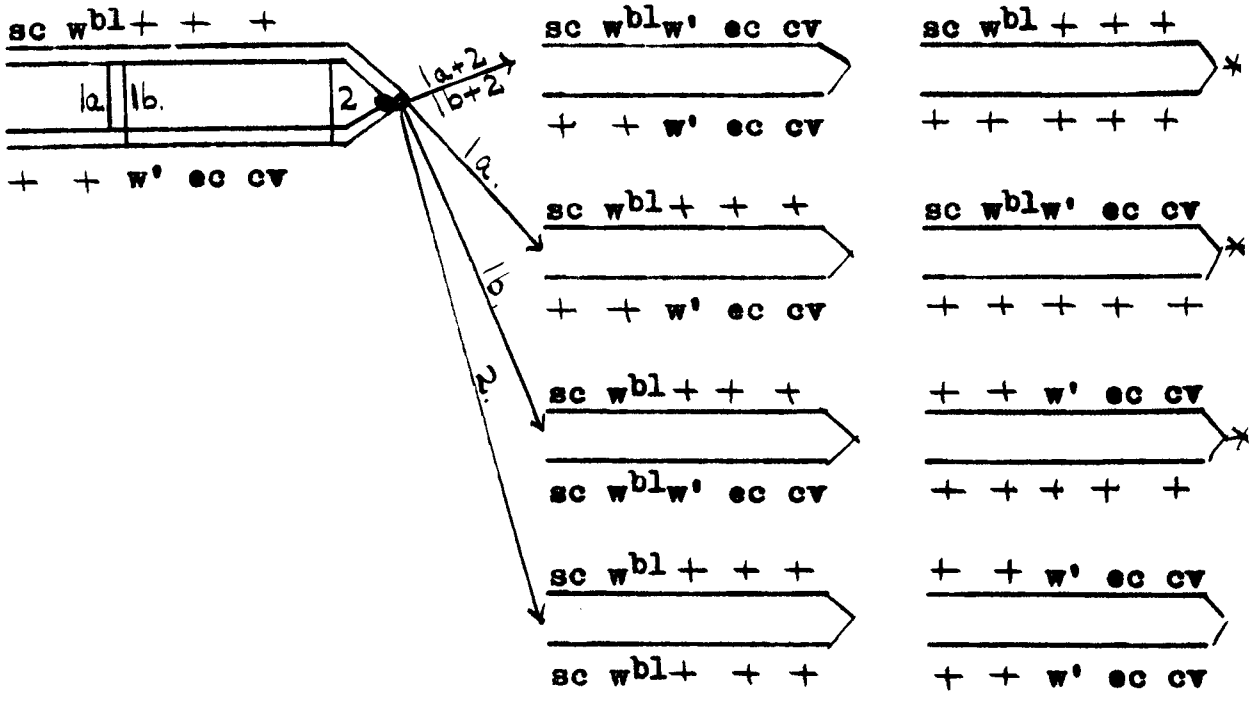


Figure 14. lists the 3 segregants which are wild type (marked with an asterisk). Only that from 1a. is heterozygous for the reciprocal products of crossing over between $\underline{w}^{bl}$ and $\underline{w}'$, i.e. the sc $w^{bl}w'$ ec cv female.

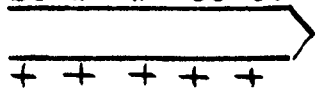


Table XIII. Data from $\frac{sc\ w^{bl}\ +\ +\ +}{+\ +\ w'\ ec\ cv}$ x B

Maternal genotype	No. of progeny examined	No.and genotype of rare recombinants	Recombination fraction
$\frac{sc\ w^{bl}\ +\ +\ +}{+\ +\ w'\ ec\ cv}$	26,787	1 $\frac{sc\ w^{bl}\ w'\ ec\ cv}{+\ +\ +\ +\ +}$	0.000075 ± 0.000052

Table XIII summarises the results from this cross where one wild type female was isolated. Progeny testing of this female indicated that she was $\frac{sc\ |w|\ ec\ cv}{+\ +\ +\ +}$ and was probably derived from recombination in the 1a. region and marked with an asterisk in Fig.14. The fuller designation of this female, where |w| means phenotypically white, would presumably be $\frac{sc\ w^{bl}\ w'\ ec\ cv}{+\ +\ +\ +\ +}$ Thus w' does not suppress w^{bl}, the double mutant $w^{bl}w'$ being white as expected. Thus it seems reasonable tp suppose that w" is the suppressor factor in the w'w" complex.

Quite apart from the attempt to discover the nature of the w^{aE} allele, the data presented in the preceeding pages are of general interest in connection with the production of new phenotypes by recombination.

Table XIV. Summary of facts on different genotypic and phenotypic combinations of $\underline{w}^{bl}$ and $\underline{w}^{aE}$.

P_1 genotype with respect to $\underline{w}$.	P_1 phenotype with respect to eye colour.	Recombinants in F_2	
		Genotype	Phenotype
w^{bl} + +	blood	w^{bl} w' w"	white
x		+ w' +	white
+ w' w"	apricot	+ + +	red
+ w' +	white	None	
x			
+ + +	red		
w^{bl} w' w"	white	w^{bl} + +	blood
x			
+ + +	red	+ w' w"	apricot
+ w' +	white	+ w' w"	apricot
x			
w^{bl} w' w"	white		
+ w' w"	apricot	+ w' +	white
x			
+ + +	red		
w^{bl} +	blood	w^{bl} w'	white
x			
+ w'	white	+ +	red

It will be seen in Table XIV, which summarises these facts, that different combinations of the same genetic material can give rise to different phenotypes, i.e. the <u>cis</u> - <u>trans</u> position effect; and conversely that combinations of different genetical material can have the same phenotypes.

(vii) Appendix

The isolation of pale eyed males was mentioned on p.26 in connection with the w^{aE}/w^{eS} cross. The following represents an attempt to determine whether this paler eye colour is due to mutation of a specific dilutor of white.

In order to test this hypothesis, pale eyed males were crossed to wild type females and the progeny of the backcross examined for segregation of the w^{aE} or w^{eS} gene and the dilutor. The results of this backcross are summarised in Tables IV and V.

Table IV. Backcross results from $w^{aE}\ dil^{w}/++$ x $w^{aE}\ dil^{w}$.

Phenotype	pale apricot		apricot		wild type	
Genotype	$w^{aE}\ dil^{w}$		w^{aE} +		++ and + dil^{w}	
	♀	♂	♀	♂	♀	♂
	74	53	27	29	117	162

dil^{w} denotes "dilutor of w "

Total number of w^{aE} flies = 183

No. of $w^{aE}+$ recombinants = 56

$\therefore$ Distance between w^{aE} and dil^{w} = $\frac{5600}{183}$ units = 30.6 units.

Table V. Backcross results from $\frac{y\ w^{e}\ ec\ dil^{w}}{+\ +\ +\ +}$ x $\frac{y\ w^{e}\ ec\ dil^{w}}{}$

Eye colour Phenotype	Pale eosin		eosin		wild type	
Genotype	y w^{eS} ec dil^{w}		y w^{eS} ec +		+ + + + and + + + dil^{w}	
	♀	♂	♀	♂	♀	♂
	25	31	13	6	47	37

Total number of w^{eS} flies = 75
No. of w^{eS}+ recombinants = 19
∴ Distance between w^{eS} and dil^{w} = $\frac{1900}{75}$ units = 25.3 units.

Segregation was observed for w^{aE} or w^{eS} and dil^{w} among the non-wild-type progeny, the proportions indicating that dil^{w} is sex-linked. As among the wild type male progeny of the two backcrosses described no individuals with a new phenotype were recovered, it may be assumed that dil^{w} by itself has no visible phenotypic effect. Calculating the position of dil^{w} relative to w^{aE} or w^{eS} from Tables IV and V places it at roughly 30 on the X-chromosome. A more accurate localisation could be carried out by testing larger numbers of backcross progeny but it was not considered important in this work to make a more thorough analysis.

4. DISCUSSION

The occurrence of recombination between alleles of the white series in *D. melanogaster* has already been demonstrated (MacKendrick and Pontecorvo 1952) and it remains here to discuss what bearing the fuller set of data has on the question of the organisation of genetic material.

Information has been obtained on the relationships between neighbouring alleles. These appear to be of two different types. First, with the exception of the 2 w^{aE} components, all the alleles separable into 2 groups by crossing over and showing the *cis* - *trans* position effect, are also phenotypically separable on the basis of interaction with zeste. (a closely linked sex-limited recessive affecting eye pigmentation.) There is evidence of a positive correlation between spatial arrangement and zeste interaction. The second type of interaction, for which the evidence is not so conclusive, occurs between the two components of w^{aE}. These two components, or alleles are separable by crossing over and exhibit a suppressor-suppressee relationship not found anywhere else in the white series.

The investigation of a possible relationship between position and function shows interesting results. As noted in the description of stocks used in this study (pp. 9-10) there are 3 groups of alleles classifiable according to dosage compensation, zeste interaction and the absence of both of these properties. The checkerboard in Table XV shows the 91 possible combinations of alleles of which

20 have been tested.

Of these 20 crosses, 8 were between alleles in the same group and of the 8, from which a total of approximately 215,000 flies were examined, no rare recombinants were isolated.

The only cross which tested for recombination between a dosage compensating and a non-dosage compensating allele, viz. $\underline{w}$ of group II in combination with $\underline{w}^{i}$ of group III, gave a positive result. As already pointed out however, (p.11) it was not known until rather late in the course of this work that, contrary to expectation, $\underline{w}^{eS}$ did not belong to the non-dosage compensating Group III. Thus several crosses made to test the relationship between position and this function did not in fact do so.

Table XVI. Checkerboard showing allele grouping, combinations tested and recombination fractions.

(a)

Group	Allele	w^{llE4}	w	w^{i}	w^{t}	w^{bf2}	w^{h}	w^{aE}	w^{aC}	w^{e}	w^{bl}	w^{coE}	w^{coP}	w^{sat}	w^{+}
II	w^{llE4}														
II	w														
III	w^{i}		$\frac{2.2}{10^5}$												
I	w^{t}														
I	w^{bf2}		$\frac{0}{5\times10^3}$												
II	w^{h}		$\frac{0}{2.2\times10^4}$												
II	w^{aE}		$\frac{0}{4.3\times10^4}$												
I	w^{aC}														
I	w^{e}	$\frac{0}{6.7\times10^3}$			$\frac{0}{10^4}$		$\frac{0}{4.6\times10^4}$	$\frac{1.2}{10^4}$							
I	w^{bl}		$\frac{2.6}{10^4}$				$\frac{4.8}{10^5}$	$\frac{9.1}{10^5}$	$\frac{0}{2.8\times10^4}$	$\frac{0}{4.5\times10^4}$					
I	w^{coE}		$\frac{0}{10^4}$												
I	w^{coP}		$\frac{4.7}{10^4}$							$\frac{0}{1.8\times10^4}$	$\frac{0}{4.9\times10^4}$				
I	w^{sat}		$\frac{0}{5\times10^3}$							$\frac{0}{5\times10^3}$					
I	w^{+}							$\frac{1}{10^5}$							

(b)

Group cross	Number of combinations tested	No. +ve	No. −ve
I x I	6	0	6
I x II	11	6	5
I x III	0	0	0
II x II	2	0	2
II x III	1	1	0
III x III	0	0	0
Totals	20	7	13

Testing for correspondence of interaction with zeste with spatial arrangement has indicated a relationship of the type sought. Of the 11 combinations of Groups I and II and a 12th. of Groups II and III (Group II containing the zeste suppressors), 7 yielded recombinants. 4 of the remaining 5 negative results, however, were based on rather small samples of less than 10,000 flies. Only further experiments dealing with larger samples could strengthen the inference that zeste suppressing and non-zeste suppressing alleles occupy neighbouring sites in the white region. Table XVII summarises these results.

Table XVII. Classification of white alleles according to function and position.

Allele	Dosage compensation	Zeste suppression	Position on X-chromosome
w^{coP}	+	−	Distal
w^{bl}	+	−	Distal
w^{aC}	+	−	Distal
w^{i}	−	−	Distal
w^{eS}	+	−	Distal
w^{h}	+	+	Proximal
w^{aE}	+	+	Proximal
w		+	Proximal

Table XVIII. Relationship between position and function in allelic series separable by crossing over.

Organism.	Allelic series.	Nature of physiological differences other than *cis-trans* relationship.	Correlation between physiological properties & spatial arrangement of neighbouring sites.	Latest reference.
Drosophila melanogaster	S/ast	Dominance relationship	+	Lewis 1945
	Sb/sbd	Dominance relationship	+	Lewis 1948
	lz	Morphological	-	Green and Green 1949
		Antigenic	Inconclusive	Chovnick and Fox 1953
	sn	Not investigated	-	Ives and Noyes 1951
		Female fertility	-	Hexter 1955
	w	Dosage compensation	+	Lewis 1952a
		" "	-	Present report
		Zeste interaction	+	Green 1955b
		" "	+	Present report
	bx	Partial suppression of two alleles by a third	+	Lewis 1954a
	v	Response to specific suppressor	+	Green 1954
	f	Response to specific suppressor	-	Green 1956
Aspergillus nidulans	bi	None detected	-	Roper 1950
	paba	None detected	-	Roper 1952 (see Pontecorvo 1952a)
	ad_8	Reaction of two of a group of 5 alleles to a series of specific suppressors	+	Pritchard 1955
	$pro_{1,2}$	None detected	-	Forbes 1955
	$pro_{3,4}$	None detected	-	
	$ad_{15,13}$	None detected	-	Calef 1956
	$ad_{17,13}$	None detected	-	
Phage T_4	rII	Growth characteristics	-	Benzer 1956
Salmonella typhimurium	hiA	Nutritional requirements	+	Hartman 1956 (see Demerec 1956)
	athA	Nutritional requirements	+	Yuma 1956 (see Demerec 1956)
	cysB	Nutritional requirements	+	Clowes (unpubl.) (see Demerec 1956)

Of the numerous multiple allelomorphic series studied, several have been investigated with this relationship in mind. Of those where no such emphasis on correlation of position with function was laid, it is nevertheless possible to judge from the descriptions of the alleles tested and between which crossing-over has taken place, to reach some conclusion regarding such a relationship. All these results are summarised in Table XVIII. It is obvious from these results that agreement between cross-over position and function is not very good, only 11 clear-cut instances out of a possible 24 having been confirmed (see second last column Table XVIII).

There are two possible explanations for these results. First, more exhaustive crossing over tests may split these series into larger numbers of mutational sites. Secondly, some other physiological differences not already detected may differentiate the cross-over groups. In other words the two means of distinguishing between alleles are a) crossing over and b) function. For each of these there is a particular resolving power depending on technical ease of investigation in each particular series.

The examples cited in Table XVIII of spatial and functional correspondence between alleles deals with interactions of members of allelomorphic series with other genes, notably suppressors, which need not be closely linked. The results presented here on the $\underline{w}^{bl}/\underline{w}^{aE}$ cross, pp.33-47) indicate that within an allelic series itself there can be interaction, one allele $\underline{w}^{n}$ acting as a partial suppressor of it's neighbour $\underline{w}'$ in the double mutant genotype. The phenotypic expression of this situation is that the double mutant $\underline{w'w^{n}}$, i.e. $\underline{w}^{aE}$, is less extreme in eye colour than the single mutant $\underline{w'}+$.

The + w" type has unfortunately not been isolated.

A summary of the various genotypic combinations studied and of their observed phenotypes is presented in Table XIX, together with a classification of the phenotypes which would be expected if w' and w" did not have the suppressor-suppressee relationship. Among the identified phenotypes only the cis arrangement agrees with the expected. Whether a position effect or "Lewis effect" would in this case be demonstrated for the cis and trans heterozygotes for w' and w" is difficult to tell. However Lewis (1954a) in his study of the bithorax series of alleles, has found a situation similar to that illustrated here for w^{aE}. In his example the suppressor and suppressed alleles do exhibit the "Lewis effect."

Goldschmidt (1945) found a situation among the svr(silver) alleles in Drosophila which is somewhat analagous to the w' - w" interaction. Among the svr^{poi} alleles he found differences in their qualities as suppressors. Some time later (1955) he pointed out that since the latter situation is possible, there is no reason why reaction to suppressors, as opposed to reaction of suppressors, should not differ among alleles. Carrying this argument one stage further in the case of the w' - w" interaction noted here there seems no reason why a suppressor-suppressee relationship should not exist between alleles of one series.

Table XIX. Observed phenotypes of all possible combinations of w' and w" compared with phenotypes expected if w" did not suppress w'.

No.	Genotype	Phenotype	
		Observed	Expected
1	$\frac{w'\ w''}{w'\ w''}$	apricot	white
2	$\frac{w'\ +}{w'\ +}$	white	coloured
3	$\frac{+\ w''}{+\ w''}$	unidentified	coloured (?)
4	$\frac{w'\ +}{+\ w''}$	unidentified	colour intermediate between 2 and 3.
5	$\frac{w'\ w''}{+\ +}$	wild-type red	wild-type red
6	$\frac{w'\ w''}{w'\ +}$	pale apricot; colour due to w'w".	colour intermediate between 1 and 2 and due to w'+
7	$\frac{w'\ w''}{+\ w''}$	unidentified	colour intermediate between 1 and 3 and due to + w"

Functional differences have however been argued for all cases of alleles separable by crossing over. A working model to explain this situation was first proposed by Pontecorvo (1950) and elaborated on and still maintained by Lewis (1952b, 1955). To quote Lewis (1955, p.75) ".....This model assumes (1) the normal alleles of one of the pseudoallelic genes, a^+, controls a reaction: $S \longrightarrow A$, while the normal allele of a second gene of the series, b^+, controls a reaction: $A \longrightarrow B$; (2) the mutant alleles block or impair these reactions, and (3) the substance A, at least, is produced at, or very close to, the site of the gene in the chromosome more readily than it is transported to the homologous chromosome. As may be seen from the diagram below, the cis arrangement of the wild type alleles (1) is expected on the above assumptions to give a more nearly normal action (production of substrate B) than the trans arrangement (2).

(1) $\underline{S \xrightarrow{a^+} A \xrightarrow{b^+} B}$

$S \xrightarrow[a]{} A \xrightarrow[b]{} B$

(2) $\underline{S \xrightarrow{a^+} A \longrightarrow B}$

$S \xrightarrow[a]{} A \xrightarrow[b^+]{} B$

....." (see also Lewis 1954b).

It will be noted in the above extract that the term "pseudoallelic gene" is used and here a divergence in interpretation becomes apparent. A further assumption, never considered by Pontecorvo but supported mainly by Lewis (1952b, 1955), Green (1955a, 1955b) and Stephens (1952), is that "pseudoalleles" are in fact repeats of a

gene locus which have since differentiated physiologically so that they now control successive steps in a biosynthetic chain of reactions. Each locus may in turn be the site of a series of alleles which are considered to be qualitatively similar but vary quantitatively in efficiency.

The other view, proposed in place of the above hypothesis by Pontecorvo (1952a) and still supported by him (1952b;c,1954, 1956a,1956b), by Benzer (1955, 1956) and by Demerec (1955, 1956a) states that the gene, considered as a functional unit, appears to be a complex structure which may be further divided into units of mutation and crossing over. Thus different allelic states may arise by mutation of different parts of the complex. In the heterozygote for any two such "non-identical" alleles, crossing over is possible, reconstituting the completely wild type gene which is fully functional, and the reciprocal double mutant which in many cases is of the extreme mutant phenotype.

In support of the first hypothesis is the fact that several of the series of multiple alleles so far investigated in D. melanogaster appear to be associated with doublets on the salivary gland chromosomes, e.g. S/ast ; Sb/ sbd.(Lewis 1945, 1948). It should however be emphasised that the selection by Lewis of series of alleles to be investigated for crossing over were chosen because they appeared to be associated with doublets. Such a location for other series of alleles in D. melanogaster separable by crossing over has not been verified, e.g. the lz, v and f alleles (Green and Green 1949, 1956, Green 1954 and 1955a respectively) and sn (Hexter 1955)

As pointed out in the introduction the choice of the white series by Professor Pontecorvo for investigation by the author was influenced, on the advice of Dr.Slizynski (personal communication; Slizynska 1938), by the lack of evidence of a doublet structure. Lewis, on the other hand, on choosing w alleles for study (1952a) supported the view expressed by Panshin (1941) that they are associated with the bands 3C2 and 3C3 on the X-chromosome. It is clear therefore that cytologists disagree as to what is a doublet. Since cytological analysis of fine chromosomal structure is not yet possible in organisms other than the Diptera, corroborative evidence cannot be obtained from work on crossing over between alleles of any other species investigated. It does seem unlikely, however, that the considerable number of doublets visible in the salivary gland chromosomes of D. melanogaster all represent repeats of the type discussed by Lewis. Since then he has shown (Lewis 1954a) that the beadex series comprises at least 5 different crossover units and although cytological evidence of repeats in this region has not been stressed, it seems unlikely that these alleles are associated with a five-fold repeat. Furthermore the present study, where at least 3 sites of mutation are indicated, makes the postulated duplicate nature of the white locus, and hence Lewis's hypothesis, less attractive.

One of the facts arising from this and other work on interallelic crossing over, however, and not generally emphasised, is that pairing during meiosis is very exact. Were it not so, any class of exception could be produced by unequal crossing over in either direction. This would apply particularly to the extreme mutant phenotype which would be characteristic of a deficiency or duplication

for one or more sections. cf. Bar (Sturtevant 1925) and Beadex (Green 1952, 1953) in D.melanogaster. In maize, also, the A series affecting anthocyanin production and the R series affecting seed and plant colour (Laughlan 1955, Stadler 1954 and Stadler and Emmerling 1954) have been proven by crossover tests to be duplications. New types are here produced by unequal crossing over and not as originally thought by interallelic crossing over (Laughlan 1949, Stadler 1952).

This lack of evidence of unequal crossing over in all but 3 of the cases studied, i.e. Beadex, A and R, thus indicates that as far as pairing properties are concerned, crossing over between alleles points not to tandem duplications but to regions of the chromosome differentiated with respect to pairing properties. That they may have originated by a tandem duplication and since differentiated with respect to pairing properties is not however ruled out by the above argument but it is irrelevant to the analysis of the structure and action as we find it today.

An interesting point worth noting at this stage in discussing pairing properties is the negative interference found by Pritchard (1955) and Calef (1956) on crossing over in the ad_8 and ad_9 regions respectively of A.nidulans. With the exception of 3 segregants, all 45 rare recombinants noted in the work reported here are associated with a single crossing over in the white region. As pointed out earlier it is not proven that these 3 segregants were due to double crossing over as opposed to mutation. In view of the fact that the theoretical frequency of double crossovers from these experiments, assuming no interference, would be about 1 in 20, there is no evidence of any interference, either positive or

negative. Nor is there any evidence from this work, or from that on $\underline{ad_8}$ and $\underline{ad_9}$, of "gene conversion" an interpretation suggested by H.K.Mitchell (1956) and by St.Lawrence and Bonner (1956) as an alternative to apparent negative interference in the case of $\underline{ad_8}$.

The evidence from studies of allelic series in micro-organisms weighs far more heavily in favour of the second hypothesis that the gene considered as a physiological unit is divisible by crossing over. Owing to the technical ease of examining in a relatively short time a very large number of segregants, it is possible to detect crossing over between alleles at the rate of 1 in 10^7 to 1 in 10^8. In practice, recombination at the rate of 4 in 10^7 between pro alleles (Forbes 1955), 1 in 10^6 in paba (Roper 1952) and 1 in 10^6 in $\underline{ad_9}$ (Calef 1956) in Aspergillus nidulans has been detected. It also appears from the work of Pritchard (1955) and of Benzer (1956) that within the limits of the scale of their experiments, the majority of mutational events occur at different sites in the gene complex,and the chances of two or more alleles in a series of say 20 alleles being identical is very small.

Wide ranges in crossover frequencies within allelic series have also been noted, e.g. in the $\underline{ad_8}$ series the ratio of the smallest recombination fraction to the total recombination for that region is 1 : 150, in T_4 phage the ratio is 200 : 1 and in this work an w the ratio is 62 : 1. From such data Pontecorvo and Roper (1956) have made attempts to estimate the number of mutational sites in the gene complex and Benzer (1955 and 1956) has even conjectured on the possibility that the sites consist of a number of nucleotide pairs in a Watson-Crick chain

(Watson and Crick 1953).

One would not expect that each of these mutational sites has the same functional properties as others of the same gene complex. If one looks further afield and ceases to regard crossing over between alleles as a special and isolated phenomenon, it will be seen that there are numerous types of relationship existing within gene complexes and between closely linked genes. Referring to Table XX a selection of such phenomena is arranged in order according to the following scheme.

Beginning with cotton we have 2 genes, G and S, similar in their effect on pigmentation but not identical. No recombination has been reported between them but they are complementary in action and it seems reasonable to suppose that they are separable. The T series in the mouse is another example of complementarity. This series is closely linked with another 2 genes, Ki and Fu also affecting tail characteristics (Dunn and Caspari 1945). All 3 lie within 8 map units. The ad_1, and ad_3 genes in A.nidulans also represent a case of complementarity but with the difference that they appear to be identical in phenotype.

From such examples we move via the intermediate situation of the pro alleles to ad_{13} which is allelic with ad_{15} and ad_{17}, i.e. it exhibits the characteristic "Lewis effect". ad_{15} and ad_{17} are in turn complementary. These last 2 cases are the most interesting of all the examples cited and represent perhaps the most fruitful material for investigation of chromosome architecture and its relation to function.

Then follows a number of allelic series showing crossover spparability into only 2 or 3 mutation sites. In the bi series, the 3 alleles studied appear to be phenotypically identical and to occupy 3 different sites. The f series

Table XX. Examples of allelic series and of closely linked genes arranged in order according to functional relationships.

Organism.	Alleles.	Genotypic relationship,	Phenotypic relationship	Latest reference.
Cotton	G and S	Complementary	Morphologically different	Stephens 1952
Mouse	T series	"	" "	Dunn 1956
A.nidulans	ad_1 and ad_3	"	Apparently identical	Pontecorvo 1956 b.
	$pro_{1,2}$ & $pro_{3,4}$	Complementary		
	pro_1 and pro_2	Allelic and separable	" "	Forbes 1955
	pro_3 and pro_4	" " "		
	ad_{15} and ad_{17}	Complementary		
	ad_{15} and ad_{13}	Allelic and separable	" "	Calef 1956
	ad_{17} and ad_{13}	" " "		
	$bi_{1,2,3}$	Allelic and separable: 3 mutation sites	" "	Roper 1950
D.melanogaster	f series	Allelic and separable: 2 mutation sites	2 Physiologically different groups	Green 1956
	v series	Allelic and separable: 2 mutation sites	2 " " "	Green 1955
	w series	Allelic and separable: 3 mutation sites: suppression of one allele by a second.	3 " " "	Present report
Phage T_4	rII series	Allelic and separable: at least 51 mutation sites.	Minor physiological differences	Benzer 1956
Salmonella	hiA series	Allelic and separable by transduction tests.	Physiologically different	Hartman 1956 (see Demerec 1956)
Maize	R series	Unequal crossing over between alleles.	Morphologically different	Stadler 1954
D.melanogaster	sc and ac	Semi-allelic, i.e. *trans* heterozygote slightly mutant; no unequal crossing over.	Slight morphological differences	Komai 1950
Neurospora	pdx and pdxp	Convertible to wild type: crossing over appears not to be essential.	Physiologically different	Mitchell, H.K. 1956
Yeast	MG,CD,HI,an,pr.	Simultaneous conversion of several linked genes.		Lindegren and Lindegren 1956

comprising 6 alleles, is separable into 2 groups on the basis of reaction to a specific suppressor. This grouping however does not correspond with the 2 groups based on crossover data. The v series, on the other hand, does show agreement of position with function. There is also evidence of such a relationship between the w alleles but with the added complication of suppression of one allele by a second.

The rII series in phage, which comes next in the list, provides the best examples so far studied of resolution of one functional region into a large number of mutation sites - in this case at least 51. The hiA alleles in Salmonella also represents a case of resolution (this time by the different technique of transduction) into a number of mutation sites corresponding roughly with the number of alleles. Analysis of several series in Salmonella seems to point to the close juxtaposition on the chromosome of genes having related functions. (Hartman 1957).

Next comes the R series in Maize comprising very closely linked genes with very similar function and pairing properties so that unequal crossing over takes place. Finally the circle is almost complete with very closely linked genes, e.g. sc and ac, which are semi-allelic, the mutants being mutually enhancing rather than suppressive so that the trans heterozygote is slightly mutant.

Also included in this table are the examples of gene conversion which is only loosely associated with crossing over in either direction. Only one class of aberrant segregant is isolated as a result of this event and no complementary types have been recorded. Hence these must be considered to represent quite different phenomena. Not enough is yet known about them to place them in the sequence in Table XX and so they have been tacked on to the end.

In conclusion it seems more feasible to consider the phenomenon of crossover separability of alleles as due, not to duplications of existing genetic material, but to the apparently universal property of divisibility of the genetic material into the order of millions of mutational sites separable by crossing over irrespective of whether these sites belong to one field of physiological co-operation (one gene), or to different fields. (Goldschmidt 1955).

5. SUMMARY

1. Fourteen alleles of the white multiple allelomorphic series of *Drosophila melanogaster* have been studied to investigate the possibility that they are separable by crossing over.

2. These alleles have been classified in three groups according to physiological differences other than eye pigmentation.

3. In order to detect recombination between any two *w* alleles, the progeny from heterozygotes with suitable markers closely linked on either side, were examined for eye colours differing from those of the expected segregants.

4. In seven crosses involving eight different alleles, 27 unexpected segregants were isolated. All but three were associated with crossing over between the markers sandwiching the white region and separated by only 5.5 units. These results are taken to indicate that alleles of the white series are separable by crossing over in the manner already described for other allelic series of *Drosophila* and other organisms.

5. In a further 13 crosses involving 12 alleles (six of which gave positive results in other combinations) no unexpected segregants were detected. In the majority of these 13 crosses however the number of individuals examined was rather small and the negative results cannot be taken to indicate that crossing over between these alleles does not occur.

6. The evidence from crossing over data suggests that there are at least three sites of mutation in the white region. The possibility that there are more is discussed.

7. One of the alleles investigated appears to be mutant at two sites. Evidence is provided in support of the double nature of this allele as opposed to the results in question being due to unequal crossing over. It also appears that one of these two alleles acts as a partial suppressor of the other.

8. New phenotypes have been shown to result from recombination between two phenotypically identical alleles, and at least one phenotype, viz. white, is associated with three different genotypes.

9. The correlation sought between spatial arrangement and function is not considered proven although the evidence weighs in its favour. It was pointed out however that emphasis on this point is no longer so important. The real problem is the lack of refinement to the same degree, of resolving power of recombination and of function.

10. Two spontaneous mutations to dilutors of the higher alleles of the white series were detected and located roughly.

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