Recombination of recessive v+ transformants in Drosophila melanogaster

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SUMMARY

Recessive transformants (col) obtained from v (vermilion) embryos treated with v+ DNA are shown to map at 1-0-02, a position not distinguishable from that of su(s) (suppressor-of-sable) and in agreement with observations indicating phenotypic allelism of col with su(s) mutants. Recombination in the y-col-gt segment of the X chromosome, over a total map length of 0-3 units, was studied among the progeny of col v+y1 gtE6 cv v1 f and y1 col gtE6 cv v1 f/v1 females. The data from both crosses exhibit the following features: (1) recovery of reciprocal recombinants between y1 and col; (2) recovery of reciprocal recombinants between col and gtE6; and (3) striking negative interference in the y-col-gt segment. These results allow three alternative interpretations: (1) that recombination in the y-col-gt segment results from conventional crossing-over, with high coincidence of crossovers in the two subsegments; (2) that it results from symmetrical gene conversion at the col site (col to col+, and col+ to col), which may be accompanied by single cross-overs in either of the adjacent regions; (3) that col behaves like a transposable element, formally symbolized su(s)+·col, and that recombination in su(s)+·col/su(s)+ (i.e. col/col+) heterozygotes results from transposition of the col element from homolog to homolog, accompanied sometimes by crossing-over, either in the y-col subsegment or in the col-gt subsegment.

1. INTRODUCTION

Genetically altered stocks of Drosophila melanogaster obtained by the treatment of v+ (vermilion) embryos with v+ (wild-type) DNA fall into two groups: dominant transformants, in which the genetic alteration maps at 33-0 on the X chromosome, and recessive transformants, in which the genetic alteration maps at 0-0 on the X (Fox, Yoon & Gelbart, 1971b). The first of these positions corresponds to that of the vermilion (v) locus (Lindsley & Grell, 1968), which includes the structural gene for tryptophan pyrrolase (Tartof, 1969), while the latter is in a region including the suppressor-of-sable [su(s)] locus (Lindsley & Grell, 1968).

In the present report we examine recombination between the genetic alterations in recessive transformants (col) and the y and gt loci (map positions 0-0 and 0-3, respectively).
2. MATERIALS AND METHODS

(i) General characteristics of recessive \( v^+ \) transformants

The transformed stocks used in this work resulted from the treatment of \( v^1; bw \) embryos with DNA prepared from wild-type adults (Fox & Yoon, 1970). Further details regarding the methods of DNA preparation and embryo treatment are given in Fox et al. (1975). In contrast to the tinged, white-eyed phenotype of \( v^1; bw \) these stocks exhibit coloured eyes; this results from the presence of variable, but less than wild-type amounts of ommochrome demonstrable by chromatography of extracted pigments (N. Slepekis, unpublished). The eye colour of the stock designated \( e5-6 \) has been described objectively in terms of the Munsell colour system (Fox et al. 1975); the remaining stocks used here are closely similar. Their larval fat bodies exhibit kynurenine-fluorescence (T. M. Rizki, personal communication), and extracts of adults exhibit tryptophan pyrrolase activity resembling that exhibited by \( su(s)^2 v^1; bw \) flies, namely about 2–3 % of wild type (Tobler, 1975). They are all homozygous for \( bw \) (2–104-5) which, because it eliminates the red eye pigments, allows detection of small amounts of ommochromes. They are regarded as recessive transformants because females heterozygous for an \( X \) chromosome carrying \( v^1 \), but homozygous for \( bw \) on the second chromosome, exhibit off-white eyes like those of \( v^1; bw \). In this paper their genotypes will collectively be designated \( col v^1; bw \), where \( col \) symbolizes the presumptive DNA-induced alteration. Where appropriate the specific stock designation will be substituted for the general symbol, as for example \( e5-6 v^1; bw \).

(ii) Derivation of individual transformed stocks

Six recessive transformed stocks were used in this work: \( e5-6, e5-4^c, e14^c, e14^v, F_332 \) and \( e26 \). The derivation of these stocks dates to an experiment performed during 1968 (Fox & Yoon, 1970). In the original experiment, \( v^1; bw \) embryos were treated with \( v^+ \) DNA to yield a \( P \) generation consisting of 178 white-eyed flies. Mass matings of these yielded nothing but white-eyed progeny in the \( F_1 \) and \( F_2 \) generations. In the \( F_3 \) generation, 42 coloured flies were observed out of a total of 206 progeny. Since analysis of the transformed stocks subsequently derived from these coloured flies disclosed that their genetic alterations (\( col \)) were recessive and sex-linked, these results presented an enigma; \( F_4 \) coloured females would be presumed to be homozygous for \( col \), in which case they should have received an \( X \) chromosome carrying \( col \) from their fathers. The hemizygous \( F_3 \) fathers, therefore, should have been coloured, but were white-eyed.

Seven single pair matings were performed among \( F_4 \) coloured flies. These should have produced nothing but coloured progeny, but all produced white-eyed progeny in variable proportions (Table 5; Fox & Yoon, 1970). Progeny tests of the \( F_5 \) flies showed, however, that they transmitted the \( col \) alteration in a perfectly Mendelian fashion in all cases. Thus, for three generations after DNA treatment the \( col \) alterations failed to manifest themselves, and for two additional generations they...
were transmitted in an irregular fashion. Their early behaviour, therefore, differs markedly from that of conventional mutations.

Full details of the derivation of e5-6 have been published (Fox & Yoon, 1970). The stock e5-6 was derived from the same single pair mating of F4 coloured flies that yielded e5-6, but descends from different F5 parents. The stock e6 descends from a different single pair mating of F4 coloured flies.

The stocks el4, el4v, and F3s2 all descend from the mating of one F4 coloured male with yf: = ; bw (attached-X) females. Mass matings of the progeny of this cross gave rise to a stock designated el4. The males in this stock, possessing a common el4 v1 chromosome, exhibited wide variability in eye colour with two modal classes, reddish and yellowish. Seven generations of divergent selection succeeded in accentuating this difference, giving rise to el4 (reddish) and el4v (yellowish); the results of preliminary analysis suggest that one or more autosomal modifiers are responsible for this difference.

The stock F3s2 arose from el4 flies which were grown for three generations on medium containing acridine orange (5 μg/ml). Flies from recessive transformed stocks are usually white-eyed when grown on acridine orange, but their progeny are invariably coloured when grown on normal medium (Yoon & Fox, unpublished). In the third generation of the experiment in question a single male was mated with yf: = ; bw females on normal medium, to give rise to the F3s2 stock. Its eye colour is somewhat more yellow than that of el4v.

Details of these derivations are available upon request.

(iii) Previous mapping experiments

Among 1400 male progeny of e5-6 v1/y2 cv v1f ; bw/bw females, one cv v f recombinant was observed (Fox et al. 1971b). Assuming that this individual arose from a single crossover between y2 and e5-6, the map position of the latter was tentatively placed to the right of the y locus at 1–0.07 (where the standard position of y is taken as 0.0). Small-scale experiments with a number of other recessive transformants (unpublished) disclosed that they also mapped close to, but probably to the right of, y.

(iv) Functional relationship of col to su(s)

The locus of suppressor-of-sable [su(s)] maps at 1–0.04 (Fox & Kreber, unpublished). Mutants of su(s) suppress some v mutants, including v1, by restoring partial tryptophan pyrrolase activity (Baglioni, 1960; Kaufman, 1962): other v mutants, like v50 and v48a, are not suppressed (Lindsley & Grell, 1968). The phenotype of su(s) v1; bw resembles that of col v1; bw. The mechanism of suppression is not understood. Mutants at loci other than v (s, 1–43.0; pr, 2–54.5; sp, 2–107.0) are also suppressed (Lindsley & Grell, 1968), and the specificity of the restored tryptophan pyrrolase in su(s) v is dictated solely by the identity of the suppressed v allele (Marzluf, 1965; Tartof, 1969). Evidence is accumulating that the su(s) locus is a regulator of tyrosyl iso-tRNA’s (Tswardzik, Grell & Jacobson, 1971; White et al. 1973).
A close functional relationship of the col elements in recessive transformants to the su(s) locus is indicated by the following observations (Yoon, Kreber & Fox, unpublished). (1) Females heterozygous for two different recessive transformed X chromosomes, for example e5-6 v1/e26 v1; bw/bw, are invariably coloured in phenotype. (2) Females of the general genotype col v1/su(s) v1; bw/bw are coloured in phenotype, and darker than su(s)+ v1/su(s) v1; bw/bw. This relationship holds true for all tested recessive transformants, including those used in the present study, with either su(s)2, su(s)3, or su(s)8. (3) The col elements of all recessive transformants tested, which includes all those utilized in the present study, suppress both sp (speck) and s (sable). The extent of suppression varies among col elements, as it does among su(s) mutants. Because pr (purple) is an eye-colour mutant, suppression by col has not been tested. (4) When combined with unsuppressible v alleles, recessive col elements do not produce coloured eyes. Thus, col v38j; bw and col v49a; bw are white-eyed, as are the corresponding su(s) genotypes.

(v) Genetic experiments

The map positions and salivary chromosome locations of the X-chromosomal loci used in this study are given in Table 1. For our purposes the map is divided into four regions:

| Region | 1 | 2 | 3 |
|--------|---|---|---|
|        | a | b |   |

| Map position | 0-0 | 0-04 | 0-3 | 13-7 | 33-0 | 56-7 |
|--------------|-----|------|-----|------|------|------|
| y            |     |      |     |      |      |      |
| su(s)        |     |      |     |      |      |      |
| gt           |     |      |     |      |      |      |
| cv           |     |      |     |      |      |      |
| v            |     |      |     |      |      |      |
| f            |     |      |     |      |      |      |

The map positions given, when not standard (Lindsley & Grell, 1968), are derived either from unpublished data (Fox & Kreber) or from the present study.

The regions of special interest, 1 and 2a, are close to the tip of the chromosome. The mutants y1 or y2 were used as markers to the left of col, while the mutants cv or gtE6 served as right-hand markers. Lindsley & Grell (1968) give descriptions of all mutants used except gtE6 and gt13Z; the latter are described by Judd, Shen & Kaufman (1972) and Kaufman (1973).

In all crosses, both v1 and the autosomal mutant bw were homozygous. Since some phenotypic overlap occurs between col v1; bw and v1; bw (Fox & Yoon, 1970; Fox et al. 1975) progeny testing of a small number of flies was necessary. Penetration of the giant phenotype is low, so that most genetically gt flies are phenotypically normal and require progeny testing for genetic diagnosis. In the present study this was done in two ways: each chromosome to be tested was rendered homozygous and observed for several generations for the occurrence of giant flies; and it was rendered heterozygous with gt13Z, since gtE6/gt13Z is almost completely lethal while gt+/gt13Z is viable. A unique diagnosis could be made for each tested chromosome.
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Table 1. X-chromosomal loci used in present study

| Locus                        | Map position and reference | Salivary chromosome location and reference |
|------------------------------|---------------------------|--------------------------------------------|
| y, yellow                    | 0-0; Lindsley & Grell (1968) | 1A5-8; Lindsley & Grell (1968) |
| su(s), suppressor of sable   | 0; Lindsley & Grell (1968)  | 1B11; E. H. Grell (personal communication) |
| gt, giant                    | 0-9; Lindsley & Grell (1968) | 3A1; Judd et al. (1972) |
| cv, crossveinless            | 13-7; Lindsley & Grell (1968) | 4F9–5D2; Lindsley & Grell (1968) |
| v, vermilion                 | 33-0; Lindsley & Grell (1968) | 10A1; Lefevre (1969) |
| f, forked                    | 56-7; Lindsley & Grell (1968) | 16F1–3; Lindsley & Grell (1968) |

Table 2. Male progeny of $y^2$ cv $v^1$ f/col $v^1$ females

| Classes                       | Stocks                  |
|-------------------------------|-------------------------|
| Parental                      | e96 | e5-4' | e14' | e14' | F3'2 | Total  |
| col $v^1$                     | 477 | 771 | 1302 | 653 | 423 | 3626  |
| $y^2$ cv $v^1$ f              | 491 | 661 | 1132 | 692 | 396 | 3372  |
| Single crossover: region 1    |     |     |     |     |     |       |
| $y^2$ col $v^1$               | 0  | 0  | 0  | 0  | 0  | 0     |
| cv $v^1$ f                    | 0  | 0  | 0  | 0  | 0  | 0     |
| Single crossover: region 2    |     |     |     |     |     |       |
| col cv $v^1$ f                | 59 | 66 | 96 | 53 | 57 | 331   |
| $y^2$ v^1                   | 59 | 110 | 120 | 67 | 50 | 406   |
| Single crossover: region 3    |     |     |     |     |     |       |
| col $v^1$ f                   | 235 | 380 | 601 | 354 | 202 | 1772  |
| $y^2$ cv $v^1$                | 254 | 473 | 598 | 343 | 138 | 1806  |
| Double crossover: regions 1 and 2 |     |     |     |     |     |       |
| $y^2$ col cv $v^1$ f          | 0  | 0  | 0  | 1  | 0  | 1     |
| $v^1$                        | 0  | 0  | 0  | 0  | 0  | 0     |
| Double crossover: regions 1 and 3 |     |     |     |     |     |       |
| $y^2$ col $v^1$ f             | 0  | 0  | 0  | 0  | 0  | 0     |
| cv $v^1$                      | 0  | 0  | 0  | 0  | 0  | 0     |
| Double crossover: regions 2 and 3 |     |     |     |     |     |       |
| col cv $v^1$                  | 13 | 31 | 18 | 10 | 12 | 84    |
| $y^2$ v^1                    | 19 | 17 | 19 | 9  | 10 | 74    |
| Triple crossover: regions 1–3 |     |     |     |     |     |       |
| $y^2$ col cv $v^1$ f          | 0  | 0  | 0  | 0  | 0  | 0     |
| $v^1$ f                        | 0  | 1  | 1  | 0  | 0  | 2     |
| Total                         | 1607 | 2510 | 3887 | 2182 | 1288 | 11474 |
Table 3. *Male progeny of $y^2 e14^r$ cv $v^1 f/v^1$ females*

| Classes                          | Number |
|---------------------------------|--------|
| Parental                        |        |
| $y^2 e14^r$ cv $v^1 f/v^1$       | 756    |
| Single crossover: region 1      |        |
| $e14^r$ cv $v^1 f/y^2 v^1$       | 0      |
| $y^2 v^1$                       | 1      |
| Single crossover: region 2      |        |
| $y^2 e14^r$ cv $v^1$            | 136    |
| $cv v^1 f$                      | 111    |
| Single crossover: region 3      |        |
| $y^2 e14^r$ cv $v^1$            | 464    |
| $v^1 f$                         | 452    |
| Double crossover: regions 2 and 3|        |
| $y^2 e14^r$ cv $v^1 f/v^1$      | 29     |
| Total                           | 2917   |

Table 4. *Male progeny of $y^1 gt^E6$ cv $v^1 f/col v^1$ females*

| Stocks                          | $e5-6$ | $e5^6$ | $e14^r$ | Total |
|---------------------------------|--------|--------|---------|-------|
| Parental                        |        |        |         |       |
| $col v^1$                       | 20217  | 7339   | 7367    | 34923 |
| $y^1 cv v^1 f$                  | 17097  | 6581   | 5313    | 28991 |
| Single crossover: region 1      |        |        |         |       |
| $y^1 col v^1$                   | 7      | 2      | 1       | 10    |
| $cv v^1 f$                      | 3      | 3      | 0       | 6     |
| Single crossover: region 2      |        |        |         |       |
| $col cv v^1 f$                  | 2573   | 1252   | 1006    | 4831  |
| $y^1 v^1$                       | 2216   | 1000   | 878     | 4094  |
| Single crossover: region 3      |        |        |         |       |
| $col v^1 f$                     | 11300  | 4472   | 4858    | 20630 |
| $y^1 cv v^1$                    | 9382   | 3967   | 3499    | 16848 |
| Double crossover: regions 1 and 2|       |        |         |       |
| $y^1 col cv v^1 f/y^1$          | 2      | 0      | 0       | 2     |
| $v^1$                           | 3      | 0      | 0       | 3     |
| Double crossover: regions 1 and 3|       |        |         |       |
| $y^1 col v^1 f/cv v^1$          | 1      | 0      | 0       | 1     |
| $v^1$                           | 3      | 0      | 0       | 3     |
| Double crossover: regions 2 and 3|       |        |         |       |
| $col cv v^1$                    | 580    | 283    | 256     | 1119  |
| $y^1 v^1 f$                     | 497    | 226    | 234     | 957   |
| Triple crossover: regions 1-3   |        |        |         |       |
| $y^1 col cv v^1$                | 0      | 0      | 0       | 0     |
| $v^1 f$                         | 1      | 0      | 0       | 1     |
| Total                           | 63882  | 25125  | 23412   | 112419|
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In preliminary mapping experiments (Table 2) several col v1[y2 cv v1 f; bw/bw females were mated with several y2 cv v1 f; bw males in each vial, and their male progeny were scored for y2, col, cv, and f; the phenotypic distinction between col and non-col is that between coloured and off-white eyes. From these progeny a single y2 e14 f cv v1 f/Y; bw/bw recombinant male was chosen and maintained in stock by matings to y1v1 =; bw/bw (attached-X) females. Males from this stock were mated to v1; bw females to yield y2 e14 f cv v1 f/v1; bw/bw females and v1/Y; bw/bw males. Matings of several such females to several of their brothers in each vial yielded the data contained in Table 3.

Table 5. Regions exhibiting recombination concurrent with recombination in region 1

| Classes | Number |
|---------|--------|
| No concurrent recombination | |
| y1 col v1 | 9 |
| gtE6 cv v1 f | 6 |
| Concurrent recombination – region 2a | |
| y1 col gtE6 cv v1 f | 2 |
| v1 | 3 |
| Concurrent recombination – region 2b | |
| y1 col cv v1 f | 0 |
| gtE6 v1 | 0 |
| Concurrent recombination – region 3 | |
| y1 col v1 f | 1 |
| gtE6 cv v1 | 2 |
| Concurrent recombination – regions 2a and 2b | |
| y1 col gtE6 v1 | 1 |
| cv v1 f | 0 |
| Concurrent recombination – regions 2a and 3 | |
| y1 col gtE6 cv v1 | 0 |
| v1 f | 1 |
| Concurrent recombination – regions 2b and 3 | |
| y1 col cv v1 | 0 |
| gtE6 v1 f | 0 |
| Concurrent recombination – regions 2a, 2b and 3 | |
| y1 col gtE6 v1 f | 0 |
| cv v1 | 1 |
| **Total** | **26** |

In definitive mapping experiments, single col v1[y1 gtE6 cv v1 f; bw/bw females were mated with several y1 gtE6 cv v1 f/Y; bw/bw males in each vial, and their male progeny were classified for y1, col, cv, and f (Table 4). The X chromosomes of males exhibiting recombination between y1 and col (Table 5) were maintained in stocks by matings with y1v1 = bw/bw females and diagnosed for gt as described above. The method is analogous to that used in prokaryotic genetics, where selected recombinant classes are characterized for non-selective markers.

A single recombinant chromosome of the constitution y1 e5·6 gtE6 cv v1 f,
obtained in the previous experiments, was tested further by matings of single
y₁ e⁵-₆ gtₑ₆ cv v₁ f/v₁; bw/bw females with several v₁; bw males. Their male progeny
were classified for y₁, e⁵-₆, cv and f; y-col recombinants were characterized for gt
as described above (Table 6).

Table 6. Male progeny of y₁ e⁵-₆ gtₑ₆ cv v₁ f/v₁ females

| Classes                                   | Number |
|-------------------------------------------|--------|
| Parental                                  |        |
| y₁ e⁵-₆ cv v₁ f                          | 3071   |
| v₁                                        | 4834   |
| Single crossover: region 1                |        |
| e⁵-₆ cv v₁ f                             | 1*     |
| y₁ v₁                                    | 0      |
| Single crossover: region 2                |        |
| y₁ e⁵-₆ v₁                              | 784    |
| cv v₁ f                                  | 802    |
| Single crossover: region 3                |        |
| y₁ e⁵-₆ v₁ f                            | 2143   |
| v₁ f                                     | 3087   |
| Double crossover: regions 1 and 3         |        |
| e⁵-₆ cv v₁                              | 0      |
| y₁ v₁ f                                 | 1†     |
| Double crossover: regions 2 and 3         |        |
| y₁ e⁵-₆ v₁ f                            | 184    |
| cv v₁                                   | 222    |
| Total                                    | 15129† |

* Progeny testing demonstrated this recombinant to be gtₑ₆.
† Progeny testing demonstrated this recombinant to be gt⁺.
‡ One f male with apparently coloured eyes is omitted from the total. The homozygous
stock established from this fly exhibits highly variable coloured eyes, with a genetic alteration
mapping at or close to the v locus (33-0), rather than at 0-0. Further analysis is in progress.

As a control, the progeny of females homozygous for col were examined. Two
control experiments were performed. In one, single females of the constitution
y₁ e⁵-₆ v₁|e⁵-₆ gtₑ₆ cv v₁ f; bw/bw were mated with several sn³ v₁ m/Y; bw/bw males.
Their male progeny were classified into two phenotypic groups, y col and col
(other markers were not immediately noted), and were carefully screened for non-
coloured (i.e. white or off-white) phenotypes. In the other control experiment,
single e⁵-₆ v₁|y₁ e⁵-₆ gtₑ₆ cv v₁ f females were mated with several sn³ v₁ m/Y; bw/bw
males, and their male progeny were classified as above with careful screening for
non-coloured exceptions. Exceptional non-coloured individuals obtained in these
two experiments were subjected to progeny testing to confirm their phenotypic
classification with respect to col, and to diagnose their constitution with respect to
the remaining markers.
3. RESULTS

(i) Map position of col elements

(a) Preliminary experiments. Male progeny of \( col^{v1}y^2\ cv\ v^f \) females are recorded in Table 2. No heterogeneity in the results related to the source of the \( col \) element is evident. On the basis of the previous mapping of \( e5\-6 \) (Fox, Yoon & Gelbart, 1971), expected crossover classes are categorized as if \( col \) mapped to the right of \( y \) and yielded the following map:

\[
\begin{array}{cccc}
\text{Distance} & \text{\( y^2 \)} & \text{\( col \)} & \text{\( cv \)} & \text{\( v^f \)} \\
\text{Region} & \text{...} & \text{0.03} & \text{7.83} & \text{32.58} \\
\end{array}
\]

Taken at face value, however, the data in Table 2 would suggest that \( col \) maps to the left of \( y \). Thus, no single crossovers are recorded in Table 2, while double and triple crossovers involving region 1 are observed. Furthermore, the coefficient of coincidence for regions 1 and 2 is 12.8 suggesting negative interference over a region which totals only 7.86 map units in length. These apparent anomalies would be resolved if \( col \) were to the left of \( y \); the apparent double crossovers in regions 1 and 2 would then be regarded as single crossovers between \( col \) and \( y \), the apparent triple crossovers would be regarded as doubles (between \( col \) and \( y \), and in region 3), the absence of expected single crossovers would be attributable to their real origin by double crossing-over (between \( col \) and \( y \), and between \( y \) and \( cv \)), and the coefficient of coincidence (\( col\-y \) and \( y\-cv \)) would be zero as expected.

This easy resolution is contradicted, however, by the results obtained with \( y^2 e14^r\ cv\ v^f/v^1 \) females (Table 3). One recombinant between \( y^2 \) and \( e14^r \) was observed, and its genetic constitution is consistent with the view that the \( col \) element is located to the right of \( y \).

These apparent contradictions made it necessary to perform definitive experiments with a marker closer to \( y \) on the right than \( cv \).

(b) Definitive experiments. The data obtained in experiments utilizing \( gt^{E6} \) as a marker between \( y \) and \( cv \) definitely place the \( col \) elements in all tested recessive transformants immediately to the right of \( y \).

Progeny of \( col^{v1}\ y^1\ gt^{E6}\ cv\ v^1\ f \) females, not classified for \( gt \) (Table 4), yield the following map:

\[
\begin{array}{cccc}
\text{Distance} & \text{\( y^1 \)} & \text{\( col \)} & \text{\( cv \)} & \text{\( v^1 \)} & \text{\( f \)} \\
\text{Region} & \text{...} & \text{0.02} & \text{9.79} & \text{35.19} \\
\end{array}
\]

There is no evidence of heterogeneity related to the source of the \( col \) element, and apparent single crossovers in region 1 are more frequent than multiple crossovers involving that region.
Classification of the 26 \(y\)-\(col\) recombinants contained in Table 4 with respect to \(gt\) yields additional evidence that \(col\) is located between \(y\) and \(gt\) (Table 5). The constitution of 15 of these recombinants is consistent with their origin from single recombination events between \(y\) and \(col\), provided the map order is \(y\)-\(col\)-\(gt\). These would have to be regarded as double recombinants if the order was \(col\)-\(y\)-\(gt\) or \(y\)-\(gt\)-\(col\). The constitution of the next most frequent pair of reciprocal classes (concurrent recombination – region 2a) is also consistent with a position for \(col\) between \(y\) and \(gt\).

Progeny of \(y^1\) \(e5\cdot6\) cv \(v^1\) \(f/v^1\) females (Table 6) yield the following map:

| Distance | \(y^1\) | \(e5\cdot6\) | cv | \(v^1\) | \(f\) |
|----------|---------|-------------|----|--------|------|
| Region   |         | 0.01        | 13.17 | 37.26 |      |

Two recombinants between \(y^1\) and \(e5\cdot6\) were observed, and both are consistent with a position for the \(e5\cdot6\) element to the right of \(y\); one would be regarded as a single recombinant in region 1 and the other would be a representative of the most frequently expected class of double recombinants involving region 1. The constitution of these two recombinants with respect to \(gt\) is also consistent with an \(e5\cdot6\) position between \(y\) and \(gt\).

Combination of the data given in Tables 2, 3, 4, and 6 yields the following map which is based on a total of 141939 progeny.

| Distance | \(y\) | \(col\) | \(gt^{Es}\) | cv | \(v^1\) | \(f\) |
|----------|------|-------|-------------|----|--------|------|
| Region   |      | 0.02  | 9.99 | a | 35.15 |      |

\[(ii)\] Evidence for negative interference in the \(y\)-\(col\)-\(gt\) segment

The coefficient of coincidence for regions 1 and 2 is 2.9, indicating negative interference. This estimate is based on 9 coincidences of crossovers in the two regions, among the 141939 progeny. All of this effect, however, is apparently attributable to coincidences in the \(y\)-\(col\)-\(gt\) segment (regions 1 and 2a). Thus, the data contained in Table 4 exhibit 6 coincidences in regions 1 and 2 among 112419 progeny, yielding a coefficient of coincidence of 2.4. Inspection of Table 5, however, discloses that all 6 of these cases involve region 2a. Indeed, of the 26 \(y\)-\(col\) recombinants recorded in Table 4, 8 (31\%) exhibit concurrent recombination between \(col\) and \(gt\) (Table 5). It is apparent that the difficulties encountered in fixing the position of \(col\) are attributable to extraordinary negative interference in the \(y\)-\(col\)-\(gt\) interval.
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(iii) Estimate of distance between col and gt

The data considered thus far are inappropriate for estimation of the genetic length of region 2a. An approximate estimate of this distance is available from observations of progeny obtained from matings of e5·6 v1/y1 gtE6 cv v1 f females with y1 gtE6 cv v1 f males. 225 y+ e5·6 cv and 229 y1 cv+ males were collected from these matings; these are generated by single crossovers in region 2, and specification of their genotypes with respect to gtE6 serves to position the crossover in region 2a or 2b. Of the 225 y+ e5·6 cv chromosomes tested, 4 were e5·6 gtE6 cv; of the 229 y1 cv+ chromosomes, 5 were y1 gt+ cv+. Thus, of all crossovers in region 2, approximately 2.0% (9/454) occur in region 2a.

Utilizing the standard map length of region 2 (13·7 units), the length of region 2a would be estimated at 0·3 units. If the length of region 2 is taken from the data in this paper (9·99 units), region 2a would be approximately 0·20 units long.

(iv) Coefficient of coincidence for the y–col–gt interval

Evidence for negative interference in the y–col–gt interval is summarized above. Quantitative measures of this interference are given by values of the coefficient of coincidence estimated from the data of Tables 4 and 5 and the col–gt distances derived in the previous section.

The data of Tables 4 and 5 contain 8 coincidences for regions 1 and 2a among 112419 progeny. If the y–col distance (region 1) is taken as 0·02 units and the col–gt distance (region 2a) as 0·3 units, the coefficient of coincidence is 119. If the col–gt distance is taken as 0·20 units, the coefficient of coincidence is 176. Thus, remarkably high negative interference is observed in the y–col–gt segment.

(v) Clustering of apparent recombinants

Table 4 omits a cluster of y–col recombinants encountered in a single vial in which one el4r v1/y1 gtE6 cv v1 f; bw/bw female has been mated with two y1 gtE6 cv v1 f/Y; bw/bw males. The cluster consisted of five males; one was y1 el4r gt E6 cv v1 f, three were y1 el4r gtE6 cv v1 f, and one was y1 el4r gtE6 v1 f in constitution.

Such clustering is indicative of a premeiotic (gonial) event, either mutational or recombinational. Since all members of the present cluster are y1 el4r gtE6 v1 f in constitution, the simplest interpretation of its origin is that it resulted from a single gonial event which produced an apparent recombinant chromosome of that constitution. Although it is not possible to specify the constitution of this chromosome with respect to cv and f, the cell in which it arose must have been heterozygous for those loci. After replication during gonial divisions, separate meiotic crossovers in the gtE6–cv and cv–f intervals could give rise to the observed products. It should be noted that among members of the cluster the ratio of cv–f recombinants to gtE6–cv recombinants is 3:1; the ratio of the lengths of these intervals is 3·4:1 (43·0 to 12·8).
A total of 72,576 male progeny of \(y^{1} e^{5-6} v^{1} e^{5-6} gt^{E8} cv v^{1} f; bw/bw\) females were examined. Of these, 32,472 were classified as \(col\), 40,099 were classified as \(y \ col\), and 5 were phenotypically non-coloured (white or near-white). Only one of the latter yielded no coloured progeny, but none of the markers of the two parental chromosomes were present and extensive testing indicated that the fly in question was probably a \(v^{1}; bw\) contaminant. Progeny testing demonstrated that the remaining four exceptions were in reality \(y \ col\) phenotypic overlaps.

\(e^{5-6} v^{1} y^{1} e^{5-6} gt^{E8} cv v^{1} f; bw/bw\) females yielded 14,006 male progeny. Of these, 8,072 were phenotypical \(col\), 5,932 were \(y \ col\), and 2 were non-coloured. One of the latter was sterile and could not be subjected to progeny testing; the other proved to be a \(col\) phenotypic overlap.

Thus, among 86,581 actual male progeny of \(e^{5-6}\) homozygotes, no verifiable non-coloured progeny were encountered.

4. DISCUSSION

The first question to be answered in evaluating the results of the present study is whether the observed events are recombinational or mutational in origin. The operational distinction between these two possibilities is that the occurrence of recombinational events is confined to heterozygotes, while mutational events occur in homozygotes as well. The method used here has been to screen the progeny of \(col/col^{+}\) heterozygotes for apparent recombination between \(y\) and \(col\). The question, therefore, is whether these are actual recombinants or if they are mutational in origin.

In control experiments, 86,581 progeny of \(e^{5-6}/e^{5-6}\) homozygotes were screened for \(col^{+}\) exceptions and none were found; these would be regarded as mutants if they had occurred. In experiments with \(e^{5-6}\) heterozygotes (Tables 4, 6) 11 \(col^{+}\) exceptions were observed which were apparent \(y\)-\(col\) recombinants, among 40,863 progeny of pertinent genotypes (\(y^{+}\) in Table 4 and \(y\) in Table 6). The appropriate null hypothesis is that these 11 exceptions are mutational in origin. A test of this hypothesis by application of Fisher’s exact test to a 2 x 2 contingency table yields \(P = 3.7 \times 10^{-6}\), requiring rejection of the hypothesis. A more conservative hypothesis is that only the \(col^{+}\) exceptions failing to exhibit recombination of flanking markers are mutants, i.e. \(y^{+} col^{+} gt^{+}\) in Table 4 (for \(e^{5-6}\)) and \(y \ col^{+} gt\) in Table 6. There were 5 of these, all from the experiment in Table 4. These were found among a total of 40,857 pertinent progeny (including the 5 exceptions plus 34,670 \(y^{+} col\) from Table 4 and 6,182 \(y \ col\) from Table 6). Fisher’s exact test of this hypothesis yields \(P = 3.4 \times 10^{-3}\) so that it too must be rejected.

Thus, the failure to observe non-coloured exceptions among the progeny of \(e^{5-6}\) homozygotes makes probable two conclusions: (1) the events observed in \(e^{5-6}\) heterozygotes are recombinational, rather than simply mutational in origin, even where recombination of flanking markers is not observed; and (2) they are not a consequence of unequal exchanges which would be expected if \(e^{5-6}\) was a con-
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ventional duplication, for non-col exceptions should then be produced by homo-
zygotes. Since there are no indications that the genetic behaviour of other col
transformants differs from that of e5-6, it seems justifiable to extend these con-
clusions to them as well.

We have summarized above the observations indicating that the v+ DNA-
induced alterations in recessive transformants are localized at the site of the su(s)
locus; these consist of preliminary mapping data, and of evidence of phenotypic
allelism. The more extensive and refined data reported here confirm this conclusion.
Unpublished data (Fox & Kreber) position su(s)2 as follows:

\[
y^1 \ 0.04 \ su(s)^2 \ 0.2 \ gtE6
\]

The data in the present paper position col as follows:

\[
y \ 0.02 \ col \ 0.2 \ gtE6
\]

The difference between these two maps is not significant.

With respect to the y-col-gt interval, two types of col heterozygotes have been
studied: col/y+ gtE6 and y+ col gtE6/+ . The results observed in both cases exhibit the
following general features: (1) recovery of reciprocal recombinants between y1 and
col, i.e. equal numbers of y1 col and y+ col+ in the first case and equal numbers of
y+ col+ and y+ col in the second case; (2) recovery of reciprocal recombinants
between col and gtE6; and (3) striking negative interference in the y-col-gt segment.

These results appear to be subject to three possible interpretations. (1) Recom-
bination in the y-col-gt interval results from conventional reciprocal crossover
events in both subsegments (regions 1 and 2a), and there is a higher than expected
frequency of coincidental crossovers in the two subsegments (negative inter-
ference). (2) Recombination in the y-col-gt interval results from symmetrical gene
conversion at the col site; i.e. col is converted to wild type and wild type is
converted to col. As in other cases of gene conversion (Hurst, Fogel & Mortimer
1972), these events are non-reciprocal and are not always accompanied by recom-
bination of the outside markers, thus exhibiting apparent negative interference.
(3) The genetic elements designated col behave essentially like transposable
elements in maize (Fincham & Sastry, 1974). Recombination in the y-col-gt
interval results from transposition of the col element from one homolog to the
corresponding site on the other. Such transposition may be accompanied by
crossing-over in one or the other of the adjacent subsegments, but this occurs
infrequently enough to result in apparent negative interference. Transposition
yields reciprocal products; within a heterozygous tetrad one chromatid suffers a
loss of the col element, while a non-sister chromatid acquires the transferred
element.

It will be evident that the gene conversion hypothesis could in principle be
distinguished from the crossover and transposition hypotheses by means of tetrad
analysis, since it postulates non-reciprocal events at the col site while the other two
suggestions postulate reciprocal events. Unfortunately only half-tetrad analysis is
possible in Drosophila, and this would not yield the required information in the

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present case, since the demonstration of gene conversion would require the observation of 3:1 segregation ratios in whole tetrads. Half-tetrads are useful in the demonstration of non-reciprocal recombination between heteroalleles, where attached homologs are employed to recover two exceptional chromatids which could not be produced by reciprocal exchange (cf. Ballantyne & Chovnick, 1971), but such unique exceptions are not recoverable from tetrads which are heterozygous for only one site, as in the present case.

The hypothesis of conventional, reciprocal crossing-over could not be distinguished by tetrad analysis from that of transposition, since both hypotheses postulate reciprocal physical exchange of the \( col \) element between homologs. A critical distinction could be made with a system which would completely suppress crossing-over while still permitting transposition. Conventional crossing-over, however, seems an unlikely explanation of the present results. Evidence from tetrad analysis in fungi (Stadler & Towe, 1963; Fogel & Hurst, 1967) and Drosophila (Ballantyne & Chovnick, 1971) has shown that intracistronic recombinants carrying parental combinations of flanking markers arise from gene conversion rather than from reciprocal crossing-over. Thus, the strikingly high negative interference observed in the very short \( y-col-gt \) segment seems unlikely to be the consequence of highly frequent double crossovers.

The present data, therefore, do not distinguish among the three possibilities, but external considerations confer a degree of preference on the hypotheses of gene conversion or transposition. These two may be distinguishable on the basis of studies of the recombinational properties of \( \text{bona fide} \ su(s) \) mutants in \( su(s)/y^1 \ gt^{E6} \) and \( y^1 \ su(s) \ gt^{E8}/+ \) heterozygotes. Such studies are nearing completion and will be published in the near future (Fox & Kreber).

In the meantime, further specification of the transposition hypothesis seems attractive. In conformity with the conventions established in studies of transposable elements in maize, the constitution of the \( su(s) \) region in such transformants may be represented as consisting of an \( su(s)^+ \) gene associated with a controlling element (\( col \)) introduced by DNA treatment; i.e. \( su(s)^+ \cdot col \). The exosome model (Fox et al. 1971a; Fox & Valencia, 1975; Fox, 1976) suggests that \( col \) is a \( v^+ \) segment which is not integrated into the linear continuity of the chromosome, but neither its genetic constitution nor the nature of its association with \( su(s)^+ \) need be specified for present purposes. As in the case of controlling elements in maize, \( col \) may suffer transpositions, in this case from homolog to homolog.

In heterozygous tetrads of the genotype \( col/y^1 \ gt^{E6} \), transposition yields reciprocal recombinants, \( y^+ \ su(s)^+ \gt^+ \) and \( y^1 \ su(s)^+ \cdot col \ gt^{E6} \) (i.e. \( y^1 \ col \ gt^{E6} \) in our previous symbolism), and crossing-over in either the \( y-col \) or the \( col-gt \) interval yields recombination of outside markers. In tetrads of the genotype \( y^1 \ col \ gt^{E8}/+ \) transposition once again yields reciprocal recombinants, \( y^1 \ su(s)^+ \ gt^{E6} \) and \( y^+ \ su(s)^+ \cdot col \ gt^+ \) (or \( y^+ \ col \ gt^+ \) ), and crossing-over in either flanking segment may occur as before.

In addition to the recombination data with which this hypothesis agrees, there are two other observations for which it provides rationalization. These obser-
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vations consist of the recovery of two putative transpositions of the *col* element from the *su(s)* site to the site of the *vermilion* locus. The first such case has not yet been described in detail (Yoon & Fox, in preparation), but is described briefly in Fox & Valencia (1975). It consists of an X chromosome derived from the recessive transformant *e$6* which exhibits a dominant phenotypic effect, and which on genetic analysis is demonstrated to possess *col* elements both at 1–0–0 and 1–33–0. Correspondingly, cytological examination discloses salivary chromosome anomalies in both the 1B11 and 10A1-2 regions (Fox & Valencia, 1975). This derivative, designated *e$6dom*, may have arisen from an event similar to the ‘replication transpositions’ observed for *Modulator* (MP) in maize (Greenblatt & Brink, 1962; Greenblatt, 1968). The other case is entered as a footnote to Table 6 of this publication. It consists of an X chromosome which produces a *col f* phenotype derived from a *y$1 e5-6 gtE6 cv v1 f/v1* female, but in which the *col* element maps at or close to 1–33–0 rather than at 1–0–0, while *f* maps in its normal position. Although its origin probably included crossing-over between *cv* and *f*, the position of its *col* element could not have arisen by normal recombination. It is possible that this change in position resembles the conventional kind of transposition of controlling elements observed in maize (Fincham & Sastry, 1974). Thus, evidence exists suggesting the transposability of *col* elements in addition to the results reported in this paper.

It remains to be seen if critical evidence distinguishing between transposition and more conventional recombination mechanisms (gene conversion and crossing-over) can be adduced.

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