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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^1/y^1 gt^{E6} *cv* v^1 *f* and y^1 *col* gt^{E6} *cv* v^1 *f*/v^1 females. The data from both crosses exhibit the following features: (1) recovery of reciprocal recombinants between y^1 and *col*; (2) recovery of reciprocal recombinants between *col* and gt^{E6}; 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 $su(s)^+ \cdot col$, and that recombination in $su(s)^+ \cdot col/su(s)^+$ (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^1 (*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, 1971*b*). 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*^{r}, *e14*^{r}, *e14*^{v}, F_3♂*2* and *e*♀*6*. 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, F_2 and F_3 generations. In the F_4 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 *e5·4*^{r} was derived from the same single pair mating of F_4 coloured flies that yielded *e5·6*, but descends from different F_5 parents. The stock *e*♀*6* descends from a different single pair mating of F_4 coloured flies.

The stocks *e14*^{r}, *e14*^{v}, and F_3♂*2* all descend from the mating of one F_4 coloured male with *yf: = ; bw* (attached-*X*) females. Mass matings of the progeny of this cross gave rise to a stock designated *e14*. The males in this stock, possessing a common *e14 v*^{1} 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 *e14*^{r} (reddish) and *e14*^{v} (yellowish); the results of preliminary analysis suggest that one or more autosomal modifiers are responsible for this difference.

The stock F_3♂*2* arose from *e14*^{r} 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 F_3♂*2* stock. Its eye colour is somewhat more yellow than that of *e14*^{r}.

Details of these derivations are available upon request.

(iii) *Previous mapping experiments*

Among 1400 male progeny of *e5·6 v*^{1}*/y*^{2} *cv v*^{1} *f ; bw/bw* females, one *cv v f* recombinant was observed (Fox *et al.* 1971*b*). Assuming that this individual arose from a single crossover between *y*^{2} 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 *v*^{1}, by restoring partial tryptophan pyrrolase activity (Baglioni, 1960; Kaufman, 1962): other *v* mutants, like *v*^{36f} and *v*^{48a}, are not suppressed (Lindsley & Grell, 1968). The phenotype of *su(s) v*^{1}*; bw* resembles that of *col v*^{1}*; 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/e♀6 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)ˢ*. (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 v^36f; bw* and *col v^48a; bw* are white-eyed, as are the corresponding *su(s)* genotypes.

(v) *Genetic experiments*

The map positions and salivary chromosome locations of the *X*-chromosal 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*		
	y	*col su(s)*	*gt*	*cv*	*v*	*f*
Map position ...	0·0	0·04	0·3	13·7	33·0	56·7

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 2*a*, are close to the tip of the chromosome. The mutants y^1 or y^2 were used as markers to the left of *col*, while the mutants *cv* or gt^{E6} served as right-hand markers. Lindsley & Grell (1968) give descriptions of all mutants used except gt^{E6} and gt^{13Z}; the latter are described by Judd, Shen & Kaufman (1972) and Kaufman (1973).

In all crosses, both v^1 and the autosomal mutant *bw* were homozygous. Since some phenotypic overlap occurs between *col* v^1; *bw* and v^1; *bw* (Fox & Yoon, 1970; Fox *et al.* 1975) progeny testing of a small number of flies was necessary. Penetrance 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 gt^{13Z}, since gt^{E6}/gt^{13Z} is almost completely lethal while gt^{+}/gt^{13Z} is viable. A unique diagnosis could be made for each tested chromosome.

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) 1A8; Lefevre (1974)
su(s), suppressor of sable	0; Lindsley & Grell (1968) 0·2; Stern (1937) 0·04; Fox & Kreber (unpublished)	1B11; E. H. Grell (personal communication)
gt, giant	0·9; Lindsley & Grell (1968) 0·4; Bridges & Gabritschevsky (1928) 0·3; This paper and Fox & Kreber (unpublished)	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)	15F1–3; Lindsley & Grell (1968)

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

	Stocks					
Classes	*e*♀6	*e*5·4^r	*e*14^v	*e*14^r	F_3♂2	Total
Parental						
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 *f*	19	17	19	9	10	74
Triple crossover: regions 1–3						
y^2 *col cv* v^1	0	0	0	0	0	0
v^1 *f*	0	1	1	0	0	2
Total	1607	2510	3887	2182	1288	11474

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Table 3. *Male progeny of* y² e14ʳ cv v¹ f/v¹ *females*

Classes	Number
Parental	
y² e14ʳ cv v¹ f	756
v¹	954
Single crossover: region 1	
e14ᵛ cv v¹ f	0
y² v¹	1
Single crossover: region 2	
y² e14ʳ v¹	136
cv v¹ f	111
Single crossover: region 3	
y² e14ʳ cv v¹	464
v¹ f	452
Double crossover: regions 2 and 3	
y² e14ʳ v¹ f	29
cv v¹	14
Total	2917

Table 4. *Male progeny of* y¹ gtᴱ⁶ cv v¹ f/ col v¹ *females*

	Stocks			
Classes	*e*5·6	*e*♀6	*e14ʳ*	Total
Parental				
col v¹	20217	7339	7367	34923
y¹ cv v¹ f	17097	6581	5313	28991
Single crossover: region 1				
y¹ col v¹	7	2	1	10
cv v¹ f	3	3	0	6
Single crossover: region 2				
col cv v¹ f	2573	1252	1006	4831
y¹ v¹	2216	1000	878	4094
Single crossover: region 3				
col v¹ f	11300	4472	4858	20630
y¹ cv v¹	9382	3967	3499	16848
Double crossover: regions 1 and 2				
y¹ col cv v¹ f	2	0	0	2
v¹	3	0	0	3
Double crossover: regions 1 and 3				
y¹ col v¹ f	1	0	0	1
cv v¹	3	0	0	3
Double crossover: regions 2 and 3				
col cv v¹	580	283	256	1119
y¹ v¹ f	497	226	234	957
Triple crossover: regions 1–3				
y¹ col cv v¹	0	0	0	0
v¹ f	1	0	0	1
Total	63882	25125	23412	112419

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In preliminary mapping experiments (Table 2) several *col* v^1/y^2 *cv* v^1 *f*; *bw/bw* females were mated with several y^2 *cv* v^1 *f*; *bw* males in each vial, and their male progeny were scored for y^2, *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 y^2 *e14*^{r} *cv* v^1 *f*/*Y*; *bw/bw* recombinant male was chosen and maintained in stock by matings to $y^1v^1\cdot$ = ; *bw/bw* (attached-*X*) females. Males from this stock were mated to v^1; *bw* females to yield y^2 *e14*^{r} *cv* v^1 *f*/v^1; *bw/bw* females and v^1/*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	
y^1 *col* v^1	9
gt^{E6} *cv* v^1 *f*	6
Concurrent recombination – region 2*a*	
y^1 *col* gt^{E6} *cv* v^1 *f*	2
v^1	3
Concurrent recombination – region 2*b*	
y^1 *col cv* v^1 *f*	0
gt^{E6} v^1	0
Concurrent recombination – region 3	
y^1 *col* v^1 *f*	1
gt^{E6} *cv* v^1	2
Concurrent recombination – regions 2*a* and 2*b*	
y^1 *col* gt^{E6} v^1	1
cv v^1 *f*	0
Concurrent recombination – regions 2*a* and 3	
y^1 *col* gt^{E6} *cv* v^1	0
v^1 *f*	1
Concurrent recombination – regions 2*b* and 3	
y^1 *col cv* v^1	0
gt^{G6} v^1 *f*	0
Concurrent recombination – regions 2*a*, 2*b* and 3	
y^1 *col* gt^{E6} v^1 *f*	0
cv v^1	1
Total	26

In definitive mapping experiments, single *col* v^1/y^1 gt^{E6} *cv* v^1 *f*; *bw/bw* females were mated with several y^1 gt^{E6} *cv* v^1 *f*/*Y*; *bw/bw* males in each vial, and their male progeny were classified for y^1, *col*, *cv*, and *f* (Table 4). The *X* chromosomes of males exhibiting recombination between y^1 and *col* (Table 5) were maintained in stocks by matings with $y^1v^1\cdot$ = *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 y^1 *e5·6* gt^{E6} *cv* v^1 *f*,

obtained in the previous experiments, was tested further by matings of single *y*^{1} *e5·6 gt*^{E6} *cv v*^{1} *f*/*v*^{1} ; *bw*/*bw* females with several *v*^{1} ; *bw* males. Their male progeny were classified for *y*^{1}, *e5·6*, *cv* and *f*; *y-col* recombinants were characterized for *gt* as described above (Table 6).

Table 6. *Male progeny of* y^1 e5·6 gt^{E6} cv v^1 f/v^1 *females*

Classes	Number
Parental	
y^1 *e5·6 cv v*^{1} *f*	3071
v^1	4834
Single crossover: region 1	
e5·6 cv v^1 *f*	1*
y^1 *v*^{1}	0
Single crossover: region 2	
y^1 *e5·6 v*^{1}	784
cv v^1 *f*	802
Single crossover: region 3	
y^1 *e5·6 cv v*^{1}	2143
v^1 *f*	3087
Double crossover: regions 1 and 3	
e5·6 cv v^1	0
y^1 *v*^{1} *f*	1†
Double crossover: regions 2 and 3	
y^1 *e5·6 v*^{1} *f*	184
cv v^1	222
Total	15129‡

* Progeny testing demonstrated this recombinant to be *gt*^{E6}.

† 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 *y*^{1} *e5·6 v*^{1}/*e5·6 gt*^{E6} *cv v*^{1} *f* ; *bw*/*bw* were mated with several *sn*^{3} *v*^{1} *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 v*^{1}/*y*^{1} *e5·6 gt*^{E6} *cv v*^{1} *f* females were mated with several *sn*^{3} *v*^{1} *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* v^1/y^2 *cv* v^1 *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:

		y^2	*col*	*cv*	v^1	*f*
Distance	...	0·03	7·83	32·58		
Region	...	1	2	3		

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^1 *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* v^1/y^1 gt^{E6} *cv* v^1 *f* females, not classified for *gt* (Table 4), yield the following map:

		y^1	*col*	*cv*	v^1	*f*
Distance	...	0·02	9·79	35·19		
Region	...	1	2	3		

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.

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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 2*a*) is also consistent with a position for *col* between *y* and *gt*.

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

		y^1	*e5·6*	*cv*	v^1	*f*
Distance	...	0·01	13·17		37·26	
Region	...	1	2		3	

Two recombinants between y^1 and *e5·6* were observed, and both are consistent with a position for the *e5·6* 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 *e5·6* 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.

		y	*col*	gt^{E6}	*cv*	v^1	*f*
Distance	...	0·02		9·99		35·15	
				a *b*			
Region	...	1		2		3	

(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 2*a*). 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 2*a*. 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.

(iii) *Estimate of distance between* col *and* gt

The data considered thus far are inappropriate for estimation of the genetic length of region 2*a*. An approximate estimate of this distance is available from observations of progeny obtained from matings of *e5·6 v*^{1}/*y*^{1} *gt*^{E6} *cv v*^{1} *f* females with *y*^{1} *gt*^{E6} *cv v*^{1} *f* males. 225 *y*$^+$ *e5·6 cv* and 229 *y*^{1} *cv*$^+$ males were collected from these matings; these are generated by single crossovers in region 2, and specification of their genotypes with respect to *gt*^{E6} serves to position the crossover in region 2*a* or 2*b*. Of the 225 *y*$^+$ *e5·6 cv* chromosomes tested, 4 were *e5·6 gt*^{E6} *cv*; of the 229 *y*^{1} *cv*$^+$ chromosomes, 5 were *y*^{1} *gt*$^+$ *cv*$^+$. Thus, of all crossovers in region 2, approximately 2·0 % (9/454) occur in region 2*a*.

Utilizing the standard map length of region 2 (13·7 units), the length of region 2*a* 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 2*a* 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 2*a* among 112419 progeny. If the *y–col* distance (region 1) is taken as 0·02 units and the *col–gt* distance (region 2*a*) 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 *e14*^{r} *v*^{1}/*y*^{1} *gt*^{E6} *cv v*^{1} *f; bw/bw* female has been mated with two *y*^{1} *gt*^{E6} *cv v*^{1} *f*/*Y; bw/bw* males. The cluster consisted of five males; one was *y*^{1} *e14*^{r} *gt*^{E6} *cv v*^{1} *f*, three were *y*^{1} *e14*^{r} *gt*^{E6} *cv v*^{1}, and one was *y*^{1} *e14*^{r} *gt*^{E6} *v*^{1} in constitution.

Such clustering is indicative of a premeiotic (gonial) event, either mutational or recombinational. Since all members of the present cluster are *y*^{1} *e14*^{r} *gt*^{E6} *v*^{1} 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 *gt*^{E6}*–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 *gt*^{E6}*–cv* recombinants is 3:1; the ratio of the lengths of these intervals is 3·4:1 (43·0 to 12·8).

(vi) *Progeny of* col *homozygotes*

A total of 72576 male progeny of y^1 *e5·6* $v^1/$*e5·6* gt^{E6} *cv* v^1 *f; bw/bw* females were examined. Of these, 32472 were classified as *col*, 40099 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.

e5·6 v^1/y^1 *e5·6* gt^{E6} *cv* v^1 *f; bw/bw* females yielded 14006 male progeny. Of these, 8072 were phenotypical *col*, 5932 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 86581 actual male progeny of *e5·6* 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, 86581 progeny of *e5·6*/*e5·6* homozygotes were screened for *col*$^+$ exceptions and none were found; these would be regarded as mutants if they had occurred. In experiments with *e5·6* heterozygotes (Tables 4, 6) 11 *col*$^+$ exceptions were observed which were apparent *y–col* recombinants, among 40863 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 2×2 contingency table yields $P = 3{\cdot}7 \times 10^{-6}$, 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 *e5·6*) 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 40857 pertinent progeny (including the 5 exceptions plus 34670 y^+ *col* from Table 4 and 6182 *y col* from Table 6). Fisher's exact test of this hypothesis yields $P = 3{\cdot}4 \times 10^{-3}$ so that it too must be rejected.

Thus, the failure to observe non-coloured exceptions among the progeny of *e5·6* homozygotes makes probable two conclusions: (1) the events observed in *e5·6* 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 *e5·6* was a con-

ventional duplication, for non-*col* exceptions should then be produced by homozygotes. 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 conclusions 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:

$$y^1 \quad 0{\cdot}04 \quad su(s)^2 \quad 0{\cdot}2 \quad gt^{E6}$$

The data in the present paper position *col* as follows:

$$y \quad 0{\cdot}02 \quad col \quad 0{\cdot}2 \quad gt^{E6}$$

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: $col/y^1\ gt^{E6}$ and $y^1\ col\ gt^{E6}/+$. The results observed in both cases exhibit the following general features: (1) recovery of reciprocal recombinants between y^1 and *col*, i.e. equal numbers of $y^1\ col$ and $y^+\ col^+$ in the first case and equal numbers of $y^1\ col^+$ and $y^+\ col$ in the second case; (2) recovery of reciprocal recombinants between *col* and gt^{E6}; and (3) striking negative interference in the *y–col–gt* segment.

These results appear to be subject to three possible interpretations. (1) Recombination in the *y–col–gt* interval results from conventional reciprocal crossover events in both subsegments (regions 1 and 2*a*), and there is a higher than expected frequency of coincidental crossovers in the two subsegments (negative interference). (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 recombination 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

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 *bona fide* *su(s)* mutants in $su(s)/y^1\ gt^{E6}$ and $y^1\ su(s)\ gt^{E6}/+$ 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.* 1971*a*; 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 $y^1\ col\ gt^{E6}/+$ 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-

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* chromsome which produces a *col f* phenotype derived from a y^1 *e5·6* gt^{E6} *cv* v^1 f/v^1 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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