A COMPARISON OF THE RATE OF EXCEPTIONALS RECOVERED AMONG PROGENY OF FEMALES HETEROZYGOUS FOR LOZENGE PSEUDOALLELES IN *TRANS* AND *CIS* ARRANGEMENTS IN *DROSOPHILA*¹

EDGAR ALTENBURG AND LUOLIN S. BROWNING

University of St. Thomas, Houston 6, Texas

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IN *Drosophila*, as is well known, females heterozygous for pseudoalleles in *trans* arrangement $\left(\frac{a+}{+b}\right)$ exceptionally produce offspring with the normal $(++)$ or double mutant (ab) combination of the alleles. These exceptionals are usually due to crossing over, especially when outside markers so indicate. However, the normals $(++)$ might conceivably sometimes be due to conversion or reversion of either one of the mutant genes, and the double mutants to mutation of either normal allele. Exceptionals among progeny of females with the *cis* arrangement $\left(\frac{ab}{++}\right)$ would have received $a+$ or $+b$, and corresponding considerations would apply to them. The results herein reported indicate that in *Drosophila*, the rate of recovered exceptionals (crossovers, most likely) among the progeny of females heterozygous for two lozenge alleles (lz^{BS} and lz^{46}) is significantly greater when the females have the *trans* arrangement of the alleles than the *cis*.

METHODS

The lozenge alleles employed in the present studies were lz^{BS} and lz^{46} (at locus 27.7 in the X), kindly supplied by DR. M. M. GREEN. (Hereafter these alleles will be designated simply as *BS* and *46*.) It has been shown by GREEN and GREEN (1949a) that *BS* is to the left of *46*. Accordingly, if we indicate both of the subloci involved, then the two alleles might be written as *BS* + and + *46*, the double mutant as *BS 46*, and the normal allele as ++. The *trans* arrangement is then $\frac{BS+}{+46}$ and the *cis* $\frac{BS\ 46}{++}$.

The eyes of lozenge mutants are ellipsoid in shape as contrasted to the normal round. Those of the single mutants (*BS* + and + *46*) are not distinguishable from each other phenotypically. The eye of the double mutant (*BS 46*) has a dark rim, not present in either single mutant, and is glossy. It resembles the single lozenge mutant known as spectacle (lz^s). The single mutants ordinarily are red, but when the genetic background is vermilion, they are a bright vermilion. The double mutant is then a very light apricot and is conspicuous among either the single mutants or the non-lozenge. Stocks homozygous for vermilion were therefore employed as parents in most of the tests for exceptionals. The stocks also happened to be singed (*sn*), but singed usually served

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no purpose in the present connection. In some of the crosses *sn* and *v* were used as outside markers and are then indicated in the genotypes, but otherwise not.

Females with the *trans* arrangement were crossed to *BS +* males, thus $\frac{BS +}{+ 46}$ females $\times$ *BS +* male. By crossing over within the lozenge locus the female produces the two recombinant classes of gametes *BS 46* and *++*. Among the male offspring from the cross, both of these crossover classes are phenotypically distinct from one another and from the non-crossover classes, as above indicated. But since female offspring with *BS 46* and *++* receive *BS +* from the male parent, they are *BS 46/BS +* and *++/BS +*. The latter appear normal (for shape of eye) and are readily recognizable as the nonmutant recombinant class, but the former (*BS 46/BS +*) appear lozenge and are not distinguishable from either non-crossover lozenge class; i.e., from those receiving either *BS +* or *+ 46* from the mother. It is assumed, however, that the number of female offspring derived from the *BS 46* class of gametes would be about equal to the number of males so derived. The total number of offspring (female and male) derived from both the crossover classes of gametes (*BS 46* and *++*) is therefore taken to be about twice the number of males of class *BS 46* (twice, so as to allow for females of this crossover class as well as males), plus males and females of the nonmutant class (*++*).

If the parent females had been crossed to *BS 46* males instead of *BS +* in the above mating, the female offspring which received *BS 46* from their mother could have been recognized, but at the time the cross was started the *BS 46* males were not yet available, and even if they had been, it would have been necessary to introduce a marker into the chromosome in order to identify nondisjunctionally produced males of class *BS 46*, all of which would have delayed the experiment.

Males of class *BS 46* were derived (as recombinants) from the *trans* cross and later females with the *cis* arrangement $\frac{BS 46}{++}$, the *++* as well as the *BS 46* combination having been derived from the *trans* arrangement. Females with the *cis* arrangement were then crossed to the double recessive males, thus: $\frac{BS 46}{++}$ female $\times$ *BS 46* male. In this cross, the nondisjunctionally produced males would cause only a relatively slight increase in the corresponding non-crossover class, and so it is not necessary to guard against them. The crossover classes of gametes from the *cis* arrangement are *BS +* and *+ 46* and offspring derived from these, both male and female, are vermilion lozenge (vermilion is present in the above cross though not indicated). These crossovers are readily recognized as isolated individuals (exceptionals) among a mass of non-crossovers; that is, among the light spectacle-like double mutants (*BS 46*) and the vermilion non-lozenge (*++*).

In a second series of experiments, outside markers were employed to verify the crossovers within the lozenge locus. In order to do this, either *sn* or *v* was removed by crossing over from the appropriate chromosome used in the first series, so that now the *trans* arrangement was $\frac{+ (BS +) +}{sn (+ 46) v}$ and the *cis* $\frac{+ (BS 46) v}{sn (++) +}$.

In summary, two series of experiments were run to test the apparent rate of crossing over between *BS* and *46* in *trans* and *cis* arrangement (on the assumption that the exceptional progeny were crossovers). In the first, the females were homozygous for *sn* and *v*; in the second, heterozygous, thus:

$$\begin{array}{l} \text{Series I} \quad \frac{sn (BS +) v}{sn (+ 46) v} \text{ (trans) vs. } \frac{sn (BS 46) v}{sn (++) +} \text{ (cis)} \\ \text{Series II} \quad \frac{+ (BS +) +}{sn (+ 46) v} \text{ (trans) vs. } \frac{+ (BS 46) v}{sn (++) +} \text{ (cis).} \end{array}$$

Series I was the larger of the two experiments, but only Series II served as a check on crossing over between *BS* and *46*.

RESULTS

In Series II experiments (marked), the distribution of the outside markers among the progeny from the *trans* arrangement placed *BS* to the left of *46*, thus confirming the order of these alleles as reported by GREEN and GREEN (1949a). Moreover, in this series the outside markers confirmed the exceptionals as cross-overs, for the most part at least, as will be considered later. These will therefore be referred to as definitive crossovers.

In Series I experiments, the observed rate of crossing over in females heterozygous for the *BS* and *46* in *trans* arrangement was about 1.8 times that for the *cis* arrangement. The actual rate for the *trans* arrangement was $33.3 \pm 3.4 \times 10^{-5}$, or about one crossover in 3,000; and for the *cis* arrangement, $18.2 \pm 1.8 \times 10^{-5}$, or about one in 5,500 (Table 1). The difference between the rates is highly significant ($P = .02$).

In Series II, *trans* arrangement, 30 crossovers between *BS* and *46* were recovered among approximately 92,000 progeny, or about one in 3,100 ($32.6 \pm 0.60 \times 10^{-5}$), making the rate about the same as that recovered from the Series I *trans* arrangement (Table 2).

In tests involving the *cis* arrangement in Series II, there was one experiment

TABLE 1

Crossover rates in females heterozygous for lz^{BS} and lz^{46} in trans and cis arrangement

Arrangement*	Number of progeny	Number of crossovers				Total	Number per 10 ⁵	Relative numbers
		Females†		Males				
		+ +	<i>lz</i> ^{BS} <i>lz</i> ⁴⁶	+ +	<i>lz</i> ^{BS} <i>lz</i> ⁴⁶			
<i>Trans</i> $\frac{lz^{BS} +}{+ lz^{46}}$	350,000	45	23‡	25	23	116	33.3 ± 3.7	1 in 3000
<hr/>								
<i>Cis</i> $\frac{lz^{BS} lz^{46}}{+ +}$	586,000	<i>lz</i> ^{BS} + and + <i>lz</i> ⁴⁶ §				106	18.2 ± 1.8	1 in 5500
		Females		Males				
		63		43				

* The male parent was $lz^{BS} +$ in the *trans* cross and $lz^{BS} lz^{46}$ in the *cis*.

† Only the X chromosome of maternal origin is shown.

‡ Not phenotypically recognized in the present cross, but taken to be the same as the number of $lz^{BS} lz^{46}$ males.

§ The two crossover classes are phenotypically indistinguishable.

TABLE 2

Crossover rates between lz^{BS} and lz^{46} as based on totals of two series of experiments

Arrangement	Series	No. progeny	Crossovers	Rate $\times 10^{-5}$	Relative rate
<i>Trans</i>	I	350,000	116	33.3 ± 3.7	1 in 3000
	II	92,000	30	32.6 ± 6.0	1 in 3100
	Total	442,000	146	33.3 ± 3.4	1 in 3000
<i>Cis</i>	I	586,000	106	18.2 ± 1.8	1 in 5500
	II	202,500	41	20.3 ± 2.8	1 in 5000
	Total	788,500	147	18.7 ± 1.5	1 in 5370

which was completely out of line with all the rest in that it yielded a highly disproportionate number of exceptionals (32 in a total of 27,000, twelve being lozenge males that were $sn^+ v^+$, but none of the contrary class) and there was one other experiment which yielded a rough mottled eye type of mutant (six in all) considered not ascribable to the lozenge locus. These two experiments have been omitted from the data. If they were included, they would make the cross-over rate in the marked *cis* series about equal to that in the *trans* series, but if not included, the Series II *cis* rate would be about the same as the Series I; namely, 41 in 202,500 ($20.3 \pm 2.8 \times 10^{-5}$), or about 1 in 5,000 (Table 2). If we exclude the aberrant experiments above mentioned, then the crossover rates as based on the total progeny of the first (unmarked) and the second (marked) series of experiments are as follows: *trans*, 146 in 442,000, or 1 in 3,000 ($33.3 \pm 3.4 \times 10^{-5}$); *cis*, 147 in 788,500, or 1 in 5,370 ($18.7 \pm 1.5 \times 10^{-5}$). Thus the *trans* rate is 1.8 times the *cis*, as based on the totals of the two series.

The viabilities of the progeny are pertinent in a study of the present kind since a difference in the viability of the crossovers relative to the non-crossovers in *trans* and *cis* arrangements would lead to a corresponding difference in the recovered crossover rates. In order to determine the viability of the lozenge mutants (*BS*, *46*, and *BS 46*) relative to the normal, backcrosses were made of females heterozygous for each of the mutant types and the normal allele, and the proportion of mutant offspring relative to the normal determined in each case. If the viability of the normal phenotype is rated as 100, that of *BS*, *46*, and *BS 46* was found to be 90, 93, and 80 respectively (Table 3). Thus, the single mutants (*BS* and *46*) are about intermediate in viability between the normal and the double mutant. The viabilities therefore have no significant effect on the relative crossover rates under study, as will be considered later.

In the Series II experiments, our main object was to determine the distribution of the outside markers among the crossovers between *BS* and *46*. Data pertinent to this aspect of the experiments are listed in Table 4, but before considering the data, it might be mentioned that in a female of genotype $\frac{+ (BS\ 46)\ v}{sn\ (+\ +)\ +}$ (*cis* arrangement), single crossing over between *BS* and *46* would give $+(BS\ +)\ +$ and $sn\ (+\ 46)\ v$, but since *BS* and *46* are phenotypically alike, we are relying on

TABLE 3
Lozenge viabilities

P ₂ Females	F ₂ from backcross						Relative Viabilities (lz ⁺ , 100)
	Males		Females		Total phenotypes		
	lz*	lz ⁺	lz/lz	lz/lz ⁺	lz	lz ⁺	
lz ^{BS} /lz ⁺	945	1040	943	1054	1888	2094	lz ^{BS} , 90
lz ⁴⁶ /lz ⁺	2428	2527	2619	2860	5047	5387	lz ⁴⁶ , 93
lz ^{BS} lz ⁴⁶ /lz ⁺	887	1118	877	1099	1764	2217	lz ^{BS} lz ⁴⁶ , 80

Both males and females carried the outside alleles *sn* and *v*.

* The symbol *lz* stands for *lz^{BS}*, *lz⁴⁶*, or *lz^{BS} lz⁴⁶* as required by the appropriate P₂ female.

TABLE 4

Distribution of outside markers (sn and v) among exceptional progeny from a testcross of females heterozygous for BS and 46 in trans and cis arrangement

<i>Trans</i> arrangement			<i>Cis</i> arrangement			
$\frac{+(BS+)+}{sn(+46)v} \text{ } \varnothing \times sn(BS46)v \text{ } \sigma$			$\frac{+(BS46)v}{sn(++)+) \text{ } \varnothing \times sn(BS46)v \text{ } \sigma$			
Single crossovers		Doubles	Single crossovers		Doubles	
$sn(++)+$	$+(BS46)v$	$sn(++)v$	$+(BS+)+$	$sn(+46)v$	$+(lz) \dagger v$	$sn(lz)+$
9 (4)*	18 (3)	3 (2)	21 (12)	17 (10)	1 (0)	2 (0)

* Total female and male outside parenthesis; female, inside. Thus 9 (4) stands for 4 females, 5 males.

† The symbol *lz* stands for either *BS* or *46*. See text for explanation.

the outside markers to tell us that the first of these two crossover classes is *BS* and the second *46* (the *trans* cross having established the order of *BS* and *46*). However, in the case of double crossovers, the outside markers would remain where they are and it would not be possible to distinguish between the two double crossover classes $+(+46)v$ and $+(BS+)v$, since both would look alike. The same thing would apply to the $sn(BS+)+$ and $sn(+46)+$ doubles. In each instance, the first of the two alternative possibilities would involve a crossover in the segment to the left of the *lz* locus (in addition to a crossover *BS* and *46*); and the second, one to the right. It is therefore possible to recognize the double crossovers in only two phenotypic classes instead of four, and in Table 4 (right) these two are indicated simply as $+(lz)v$, to stand for either $+(+46)v$ or $+(BS+)v$; and $sn(lz)+$ for either $sn(BS+)+$ or $sn(+46)+$.

We shall now consider the data themselves in Table 4. In view of the short distances between the lozenge locus and the outside markers (6.7 units on the left and 5.3 on the right), the number of double crossovers recovered in Series II experiments is surprisingly high. Thus, in the *trans* cross (Table 4) there are 3 double crossovers among a total of 30 offspring (in which one crossover occurred between *BS* and *46*), or 10% doubles, and in the *cis* cross, three among a total of 41 or 7%. These results imply negative interference, since in *Drosophila* there is usually 100% interference over distances as short as those here involved. However, in the present instance, the usual rate of interference would not necessarily apply since the progeny under comparison were selected for crossing over within the lozenge locus, and double crossovers within a short region of effective pairing would therefore be detected. These results (as they bear on negative interference) are suggestive of those reported by MURRAY (1963) in *Neurospora*.

On the assumption that a coincidence of gene conversion and crossing over within the short distances here involved is a comparatively rare event, it is unlikely that any of the exceptionals listed as crossovers in Table 4 are conversions, with the possible exception of the three of class $sn(++)v$ in Table 4 (left) and the one of class $+(lz)v$ in Table 4 (right). The latter is probably not a conversion, since it is accompanied by two exceptionals of the contrary class. But even if we include the one in question, then (on the assumption above made),

not more than four of the total 71 exceptionals in Table 4 would be conversions.

It would be very difficult completely to dismiss conversion as a significant factor in causing the exceptionals herein reported, since conceivably conversion might often be accompanied by crossing over. However, in the absence of crossing over, conversion is comparatively rare in *Drosophila*. Moreover, evidence is accumulating for double crossing over within short regions of effective pairing, and doubles could account for the relatively few exceptionals in Table 4 (four in the total of 71) which would not require coincident crossing over if they were to be explained as conversions.

DISCUSSION

The difference in the observed crossover rates between the lozenge alleles in *trans* and in *cis* arrangement is probably not due to any great extent to a difference in the viabilities involved. For, in the case of the *trans* arrangement (*BS* +/+ 46) the single mutants (*BS* + and + 46) are the non-crossovers and their viability, since intermediate, is about equal to the average of the crossovers (+ + and *BS* 46). Thus viability is not favoring the average of the crossovers *vs.* the non-crossovers in this case. A corresponding consideration applies to the *cis* arrangement. The viabilities are therefore not causing the difference in the observed *trans* and *cis* rates, since they do not favor the crossover *vs.* the non-crossover rate from either arrangement. Even if there had been some effect of viability on the observed rates, it seems hardly likely that it would have been sufficient to cause the *trans* rate to be as much as 1.8 times as high as the *cis*, in view of the fairly good viability of lozenge in a vermilion background.

In work reported by GREEN and GREEN on crossing over between lozenge alleles (1949b), examination of the data reveals that their crossover rate between *BS* and 46 in *trans* arrangement is about 1.7 times the *cis* rate (1 crossover in 1081 for *trans* *vs.* 1 in 1843 for *cis*). These experiments were done on a numerically smaller scale than ours and not with the stated object of detecting a possible difference in the rates, which if noticed might not have been regarded as statistically significant. However, it happens that our relative rate is in very close agreement with theirs (1.8 *vs.* 1.7). Our absolute rates (i.e., the rate for each arrangement taken by itself) are somewhat lower than theirs, possibly because many of our counts were made of progeny in late broods, the crossover rates in which (recovered from older females) are somewhat lower than those in earlier broods. However, a lowering of both the *trans* and *cis* rate equally would not change their relative rate.

Conversion might conceivably be a factor in causing the difference in the rate of exceptionals recovered from females heterozygous for *BS* and 46 in *trans* and *cis* arrangements, provided it occurred with a frequency comparable to that of the exceptionals and preferentially in females heterozygous for the *trans* arrangement. However, it will be recalled that the data from our marked experiments (Table 4) indicate that very few, if any, of our exceptionals were conversions. Moreover, if we confine ourselves to nonrecombinants for outside markers,

GREEN and GREEN (1956) reported no exceptionals that could be interpreted as conversions of *BS* among approximately 45,000 F_1 from crosses of females heterozygous for *BS* and five pseudoalleles (i.e., five lozenge alleles not in the same column as *BS*), and only one exceptional that might be a conversion among about 100,000 F_1 from crosses involving 46 and ten lozenge pseudoalleles. These figures are to be compared with our presumptive crossover rate of one in 3,000 in the *trans* arrangement. Conversion might at most have increased the apparent *trans* rate by one or two percent, and therefore could hardly have been sufficient to account for an almost twofold higher *trans* than *cis* rate, even if all the conversions had occurred in the *trans* arrangement.

The reversion rates of *BS* and 46 as reported by GREEN and GREEN (1956) were also comparatively low. Among approximately 234,000 F_1 from crosses of females heterozygous for *BS* and lozenge alleles in the same column as *BS* (these females could not yield conversions of *BS* to +) there was only one reversion, and there was one among approximately 24,000 F_1 from crosses involving 46 and other alleles in the same column as 46. It is therefore unlikely that either conversion or reversion contribute significantly to the difference in the rate of exceptionals among the progeny of females heterozygous for *BS* and 46 in *trans* and *cis* arrangements herein reported. Even if they did contribute, they would suggest no simpler model than would crossing over in explanation of the differences in the *trans* and *cis* rates.

It is conceivable that the alleles employed in our *trans* and *cis* tests might not have been of identical kinds, or that the genetic backgrounds in the two arrangements might have been different and that the recovered rate of exceptionals might have been influenced correspondingly. However, it will be recalled that in the case of the *cis* arrangement (*BS* 46/++) the mutant alleles (*BS* 46) as well as the normal (++) were derived from the *trans* arrangement (*BS* +/+ 46). There can therefore be no reasonable doubt as to the identity of the alleles in the two arrangements. Moreover, since one arrangement gave rise to the other, any factors having highly specific effects on the *trans* or *cis* crossover rate would have been randomly distributed in stocks with the two arrangements and it was therefore considered unnecessary to make them co-isogenic.

In an earlier series of experiments, the crossover rate between apricot (w^a) and white (w) was determined in attached-X females heterozygous for the two pseudoalleles in *trans* and in *cis* arrangement (ALTENBURG and BROWNING 1959). The observed *trans* rate was significantly higher than the *cis* (about 2.2 times as high), though viabilities did not account for the difference. However, it has been realized since our preliminary report of the experiments (recently confirmed by more extensive unpublished data) that attached X's have a disadvantage for studies of the present kind, in that one X of an attached pair sometimes obscures the genotype of the other one because of dominance, and this dominance effect is greater in obscuring crossovers in the *cis* arrangement than the *trans*. The difference between the actual *trans* and *cis* rates is therefore less than the observed. The calculation of the actual rates on the basis of the observed is somewhat involved and is not yet completed, but at present it appears that the

trans rate will still be higher than the *cis* after the correction for the dominance effect is made.

If the *trans* rate should turn out to be significantly higher than the *cis* at the white locus, it will be of interest to see if the *trans* rate is also higher at other loci in addition to the lozenge and white.

No definite model is forthcoming in explanation of the difference in the cross-over rates recovered from the *trans* and *cis* arrangement of the lozenge alleles herein reported. The difference is somewhat suggestive of a position effect, but it is not clear as to how such an effect would operate in the present instance, unless we assumed that the mutant genes had a localized effect on crossing over and that the effect was not the same when the two were separate as when they were together.

SUMMARY

In *Drosophila melanogaster*, the rate of recovered "exceptionals" resulting for the most part at least from crossing over between two lozenge alleles, lz^{BS} and lz^{46} , was 1.8 times greater in females heterozygous for the two alleles in *trans* arrangement ($lz^{BS} +/+ lz^{46}$) than in *cis* ($lz^{BS} lz^{46}/++$). The relative viabilities probably do not account for the difference in the observed rates, for the single mutants were found to be about intermediate in viability between the double mutant and the nonmutant. Therefore, when the female parent has the *trans* arrangement ($BS +/+ 46$), the average viability of the crossover classes of offspring ($++$ and $BS 46$) is about the same as that of the non-crossover classes ($BS +$ and $+ 46$), and correspondingly for the *cis* arrangement. Accordingly, viability differences are not causing the difference between the observed *trans* and *cis* rates; since they are not favoring the crossover *vs.* the non-crossover classes in either case. Linkage data also rule out gene conversion or reverse mutation as significant factors in causing the difference. The higher *trans* rate as compared with the *cis* is somewhat suggestive of a position effect, though it is not evident just how such an effect would operate in the present instance. Further work will be required to establish the cause of difference in the rates.

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