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Further evidence for the theory that crossover interference in *Drosophila melanogaster* is dependent on genetic rather than physical distance between adjacent crossover points*

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ABSTRACT

The effect of heat shock on certain meiotic parameters in *Drosophila melanogaster* was studied in the *cv – v – f* region of the X chromosome of females homozygous for the *mus*309 mutation, deficient in DNA double-strand break repair, or those of wild type. The heat shock in the wild females caused the frequencies of the single crossovers and double crossovers and all the map lengths to decrease while crossover interference remained unchanged. In the *mus*309 mutants all parameters, crossover interference included, remained unchanged despite the heat shock treatment. However, the *mus*309 mutation had a significant effect on all meiotic parameters both in the females not given the heat shock and in the heat shocked females with the exception that the recombination frequency of the *v* and *f* markers was the same in both genotypes in the females not given the heat shock. It seems that the heat shock treatment has an effect on crossing over which is independent on the *mus*309 gene and affecting the occurrence of crossing over itself. On the other hand, the *mus*309 gene has an effect on crossing over which is independent of the heat shock treatment and affects some precondition of crossing over. This precondition is probably the choice between two routes of the repair of double-strand DNA breaks known to be controlled by the *mus*309 gene. As explained in the discussion, the results are in accordance with the genetic models of interference in which interference depends on genetic distance between the crossover points, but in contradiction with physical models where interference is dependent on physical distance between the crossover points.

Keywords: Chiasma; Chromosome; Map Length; Meiosis

1. INTRODUCTION

1.1. General Introduction

Meiotic crossing over, the exchange of genetic material between homologous chromosomes during the generation of gametes in animals and sexual spores in plants and fungi, leads to recombination of genes and formation of chiasmata. A chiasma is a sufficient condition for the segregation of homologous chromosomes, which leads to the reduction of the chromosome number from diploid to haploid.

An important phenomenon, which has recently garnered considerable attention, associated with crossing over is crossover interference, *i.e.* the fact that multiple crossovers in each pair of homologous chromosomes are less frequent than would be expected on the basis of random coincidence of single crossovers [1-3]. The phenomenon of crossover interference is very likely responsible for the occurrence of obligate crossovers, and thus for the formation of obligate chiasmata.

The term “obligate crossover” refers to the fact that, in most species, it is rare to find chromosomes that do not undergo crossing over. For example, in *Drosophila*, there is usually one chiasma per chromosome arm. The feature of the obligate chiasma is biologically sensible because it ensures the disjunction of homologous chromosomes.

1.2. Models of Crossover Interference and the Purpose of the Present Study

In principle, there are two different categories of models

*Unfortunately the first printing of this paper, published in Open Journal of Genetics 2, 155-162 (2012) contained several mistakes in the statistical analysis of the data and errors in some tables. The mistakes in statistics resulted in changes in the content of the paper. Therefore the whole paper will here be published anew as a corrected version. The main conclusions of the paper concerning the mechanism of crossing over, however, remain unaltered.

of crossover interference. The first of these categories of models is called the genetic models and assumes that interference depends on the genetic (*i.e.* linkage map) distance, measured in Morgans, between adjacent crossovers [4]. To my knowledge, currently only one model, called the "counting model", falls into this category [4,5].

The second category of models, "physical models", hypothesizes that crossover interference is dependent on the physical distance (microns or base pairs) between the adjacent crossovers. In general, these models, which are many, suggest that some kind of physical signal travels along the bivalent and determines the distribution of crossovers.

Recently I presented evidence for the genetic models of crossover interference in *Drosophila melanogaster* [6]. The aim of the present study was to obtain further evidence for the theory that crossover interference is dependent on genetic rather than physical distances between adjacent crossover points. Crossing-over frequencies, crossover interference, recombination frequencies and map distances were compared in the *cv – v – f* region of the X chromosome of *D. melanogaster* in females bearing either wild type 3rd chromosomes (control) or having the DNA double-strand break repair deficient *mus*309^{D2}/*mus*309^{D3} mutant constitution in the 3rd chromosomes (experiment) and either treated or untreated with a heat shock of 24 hours in 35˚C.

It was observed that the heat shock in the wild control females caused the frequencies of the single crossovers as well as double crossovers and all the map lengths to decrease while crossover interference remained unchanged. In contrast to this, in the experimental *mus*309 mutant females all meiotic parameters studied, crossover interference included, remained unchanged after the heat shock treatment. However, the *mus*309 mutation had a significant effect on all meiotic parameters both in females not given a heat shock and in the heat shocked females with the exception that the recombination frequency of the *v* and *f* markers was the same in both genotypes in the non-heat-shocked females. Thus, it appears that the heat shock has an effect on crossing over which is independent of the *mus*309 gene and affects the occurrence of crossing over itself. On the other hand, the *mus*309 gene has an effect on crossing over which is independent of the heat shock treatment and affects some precondition of crossing over. It is suggested that this precondition of crossing over is the repair of double-strand DNA breaks known to be controlled by the *mus*309 gene. It should also be noted that the effect of the heat shock on the mutant females was generally speaking the opposite of its effect on the wild type females. As will be explained in the discussion chapter, these results are in accordance with the genetic models of interference, particularly the counting number model, in which interference depends on the genetic distance between the adjacent crossover points. However, the results are in contradiction with any physical model of interference where interference is dependent on physical distance between the adjacent crossover points.

1.3. The *mus*309 Gene and Molecular Models of Crossing over

Molecular models of meiotic crossing over suggest that crossing over is initiated by the formation of meiosis-specific double-strand breaks (DSBs) of DNA, catalyzed eventually in all eukaryotes by the topoisomerase-like Spo11 protein, encoded in *Drosophila* by the *mei-W*68 gene [7], in co-operation with other enzymes. The birth of DSBs is followed by formation of heteroduplex DNA and rejoining of the ends created in the breakage involving a single-end-invasion intermediate. Following this, a physical structure called the displacement loop will be formed. Subsequent DNA synthesis and second end capture form a structure known as the double Holliday junction (dHJ), which is then resolved to form either crossovers or non-crossovers [8,9].

Two alternative pathways for the repair of the DSBs are known: the synthesis-dependent strand annealing (SDSA) pathway and the double-strand-break repair (DSBR) pathway. The former pathway leads exclusively to non-crossover products and the latter to both crossover and non-crossover products [10,11].

In *D. melanogaster*, the *mus*309 gene located on the right arm of chromosome three (86F4) encodes, in a manner similar to its orthologues in other organisms, a RecQ helicase [12-15] and, accordingly, is involved in DSB repair [10,11,16]. In particular, it is known that the product of the *mus*309 gene is involved in the SDSA pathway of the repair of the DSBs [17,18]. More specifically, in the *mus*309 mutants the SDSA pathway is blocked, while the DSBR pathway remains functional [19]. Thus, the *mus*309 gene seems to control the choice made by the oocyte between the two alternative pathways of DSB repair. The same is also true for the *Sgs*1 gene, the *mus*309 orthologue of yeast [20]. Consequently, if in *mus*309 mutants more DSBs are repaired as crossovers by the DSBR pathway, a change in the crossover/non-crossover ratio can be expected, since fewer non-crossovers are produced.

2. MATERIAL AND METHODS

2.1. Experimental Procedures

Crossing over frequency and interference in the X chromosome in the regions between the crossveinless (*cv*, 1 - 13.7), vermilion (*v*, 1 - 33.0) and forked (*f*, 1 - 56.7) markers in four different experimental procedures were studied. In each procedure, six daily broods of progeny

were derived after a certain treatment of virgin females before they were mated with males. The progeny was collected as daily broods in order to obtain the best yield of progeny flies. In the analysis of the results, however, the materials of the broods were pooled. The females were isolated and the treatment started not later than twelve hours after their hatching from the pupa. In the control crosses, *cv v f*/+ + +; +/+ females were crossed with *cv v f*/*Y* males, and in the experimental crosses, *cv v f*/+ + +; *mus*309^{D2}/*mus*309^{D3} females were crossed with *cv v f*/*Y* males. The experimental females were derived from the following preliminary cross: *cv v f*; *mus*309^{D3}/*TM*6, *Tb* females crossed with + + +/*Y*; *mus*309^{D2}/*TM*6, *Tb* males (*Tb*; Tubby 3 - 90.6) and identified on the basis of their non-Tubby phenotype. The treatments in both the control crosses and in the experimental crosses were as follows: The virgin females were either given a heat shock of 24 hours in 35˚C ± 0.5˚C or they were kept in 25˚C ± 1˚C for 24 hours.

Both the *mus*309 alleles used carry mutational changes that could potentially impair or abolish at least the helicase function of the MUS309 protein. In *mus*309^{D2}, there is a stop codon between the sequence motifs encoding the third and fourth helicase motif of the protein. *mus*309^{D3}, for its part, has a glutamic acid to lysine substitution in the conserved helicase II motif, in addition to another amino acid substitution close to the C terminus [21]. It has been demonstrated that the genotype *mus*309^{D2}/*mus*309^{D3} is semi-sterile (Janos Szabad, personal communication; see also [21-23]).

Because of the semi-sterility of the females, the mutant female crosses were carried out in cultures in which three females were mated with 3 - 5 males, whereas the control crosses were single-female cultures. The same number (30) of crosses was made in both the control and the mutant female series. After the initial mating, the parental flies were transferred without etherization into fresh culture bottles every 24th hour for five consecutive days, and discarded after the sixth day of egg laying. The progeny, thus consisting of six daily broods in both the experimental and control procedure, were raised in 25˚C on a standard *Drosophila* medium consisting of semolina, syrup, agar-agar and both dried and fresh yeast.

2.2. Calculation of the Frequency of the True Single Crossovers

Some of the observed single crossovers in the *cv-v* and *v-f* intervals actually result from meioses that have two exchanges, one in each interval. Assuming no chromatid interference, the three classes of double-exchange tetrads, 2-, 3- and 4-strand doubles, occur in a 1:2:1 ratio [24]. Therefore, the true frequency of single crossovers, *i.e.* the number of single crossovers that resulted from meioses with only one exchange in the *cv* – *v* – *f* region, was calculated by subtracting the observed frequency of double crossovers from those of each of the single crossover classes.

2.3. Measurement of Interference

The coefficient of coincidence, *C*, was calculated according to the following formula of Stevens [25], which is a maximum likelihood equation

$$\hat{c} = \frac{wn}{(w+x)(w+y)},$$

where *w* is the number of flies which were double crossovers, *x* and *y* are the numbers of flies which were single crossovers for *cv* and *v*, and *v* and *f*, respectively, and *n* is the total number of flies.

The variance of *C* was calculated according to the following formula, also given by Stevens [25]

$$V(\hat{c}) = \frac{c}{n}\left(\frac{1-ca-cb-cab+2c^2ab}{ab}\right),$$

where *a* and *b* are the recombination frequencies of *cv* and *v*, and *v* and *f*, respectively. This is also a maximum likelihood equation.

2.4. Statistical Methods

In the calculations of the variance of the coefficient of coincidence, the formula of Stevens [25] given above was used. Otherwise, the variance of binomial frequencies, such as recombination frequencies, was calculated according to the usual formula: $s^2 = pq/n$, where *n* is the total number of flies, *p* is the recombination frequency, and *q* is 1 – *p*. The standard deviation (S.D.) of all the binomial frequencies, the coefficient of coincidence included, is the square root of their variances.

In the analysis of the significance of difference of other parameters than the coefficients of coincidence a one-tailed Student's *t*-test was employed. A one-tailed test in these cases is justified because non-crossing over is *a priori* more likely to occur than crossing over. This justification does not, however, apply to coefficients of coincidence since no value of *C* is *a priori* more probable than any other, and consequently the two-tailed Student's *t*-test was employed.

3. RESULTS

The distribution of the progeny into different phenotypic classes in the control crosses is given in **Table 1**, and those in the experimental crosses in **Table 2**.

The effect of the heat shock on the phenomenon of crossing over including crossover interference in the control cross females is given in **Table 3**. It appears that all the parameters studied with the exception of the

Table 1. Results of the control crosses. Distribution of progeny from the crosses in which *cv v f*/+ + +; +/+ females with or without a heat shock of 24 h in 35˚C were crossed with *cv v f*/*Y*; +/+ males.

	Number of progeny								
Phenotype of the progeny	+ + +	*cv v f*	cv + +	+ *v f*	*cv v* +	+ + *f*	*cv* + *f*	+ *v* +	Total number of flies
No heat shock	4690	4311	1197	1243	1499	1577	147	179	14843
Heat shocked	2428	2383	566	570	700	753	70	67	7537

Table 2. Results of the experimental crosses. Distribution of progeny from the crosses in which *cv v f*/+ + +; *mus*309^{D2}/*mus*309^{D3} females with or without a heat shock of 24 h in 35˚C were crossed with *cv v f*/*Y*; +/+ males.

	Number of progeny								
Phenotype of the progeny	+ + +	*cv v f*	*cv* + +	+ *v f*	*cv v* +	+ + *f*	*cv* + *f*	+ v +	Total number of flies
No heat shock	2545	2035	601	868	589	839	104	180	7761
Heat shocked	1661	1311	373	552	386	577	76	116	5052

Table 3. Effect of heat shock on crossing over in females of wild type regarding the *mus*309 locus. Parameters measured from the results of the crosses in which *cv v f*/+ + +; +/+ females with or without a heat shock of 24 h in 35˚C were crossed with *cv v f*/*Y*; +/+ males.

Parameter		No heat shock	Heat shocked	Significance of the difference	
Total number of flies		14843	7532		
Frequency of true single crossovers in the *cv*-*v* interval;	% ± S.D.	14.24 ± 0.29	13.25 ± 0.39	t = 2.02	P = 0.0217
Frequency of true single crossovers in the *v*-*f* interval;	% ± S.D.	18.53 ± 0.32	17.46 ± 0.44	t = 1.96	P = 0.0250
Frequency of double crossovers;	% ± S.D.	2.20 ± 0.12	1.82 ± 0.15	t = 1.89	P = 0.0294
Recombination frequency of the *cv* and *v* markers;	% ± S.D.	18.64 ± 0.32	16.89 ± 0.43	t = 3.22	P = 0.0006
Recombination frequency of the *v* and *f* markers;	% ± S.D.	22.92 ± 0.34	21.10 ± 0.47	t = 3.09	P = 0.0010
Map distance of the *cv* and *f* markers;	cM ± S.D.	41.55 ± 0.40	37.99 ± 0.56	t = 5.13	P < 0.0001
Coefficient of coincidence;	C ± S.D.	0.5142 ± 0.0253	0.5101 ± 0.0392	t = 0.088	P = 0.9299

coefficient of coincidence changed due to the heat shock treatment. The frequencies of true single crossovers decreased in both intervals studied, and as did the frequency of double crossovers. The recombination frequencies, directly giving the genetic map distances between the markers involved, firstly of *cv* and *v* markers and secondly of *v* and *f* markers decreased, and so did—of course—the map distance of the *cv* and *f* markers.

The respective figures derived from the experimental crosses are given in **Table 4**. The measurement of the parameters studied revealed results that were almost completely opposite to those of the control crosses: All the parameters, the coefficient of coincidence included, remained unaltered after the heat shock treatment.

Comparison of the meiotic parameters between the genotypes studied in non-heat-shocked and in heat shocked females are given in **Tables 5** and **6** respectively. As can be seen from the tables, all parameters apart from the frequency of recombination of *v* and *f* markers in the non-heat-shocked females were different in both sets of data. It should specifically be observed that the frequency of double crossovers and the coefficient of coincidence were higher in the *mus*309 mutant females than in the wild type females. These data indicate that in both series the density of crossovers increased due to the effect of the *mus*309 mutation.

4. DISCUSSION

4.1. The *mus*309 Gene Controls the Choice Made by the Oocyte of the Route of Double Holliday Junction Repair

The first six broods after the initiation of egg laying by virgin females, *i.e.* the broods constituting the material of this study represent oocytes which, for the most part at least, were in the prophase stage of meiosis during the heat shock treatment, and had mainly passed the stage of DNA replication during the premeiotic interphase [26-

Table 4. Effect of heat shock on crossing over in *mus*309 mutant females. Parameters measured from the results of the crosses in which *cv v f*/+ + +; *mus*309^{D2}/*mus*309^{D3} females with or without a heat shock of 24 h in 35˚C were crossed with *cv v f*/*Y*; +/+ males.

Parameter		No heat shock	Heat shocked	Significance of the difference	
Total number of flies		7761	5052		
Frequency of true single crossovers in the *cv*-*v* interval;	% ± S.D.	15.27 ± 0.41	14.51 ± 0.50	t = 1.18	P = 0.1190
Frequency of true single crossovers in the *v*-*f* interval;	% ± S.D.	14.74 ± 0.40	15.26 ± 0.51	t = 0.81	P = 0.2090
Frequency of double crossover ;	% ± S.D.	3.66 ± 0.21	3.80 ± 0.27	t = 0.41	P = 0.3409
Recombination frequency of the *cv* and v markers;	% ± S.D.	22.59 ± 0.47	22.11 ± 0.58	t = 0.64	P = 0.2611
Recombination frequency of the *v* and *f* markers;	% ± S.D.	22.06 ± 0.47	22.86 ± 0.59	t = 1.06	P = 0.1458
Map distance of the *cv* and *f* markers;	cM ± S.D.	44.65 ± 0.56	44.97 ± 0.70	t = 0.36	P = 0.3594
Coefficient of coincidence;	C ± S.D.	0.7344 ± 0.0362	0.7518 ± 0.0448	t = 0.29	P = 0.7742

Table 5. Effect of the *mus*309 genotype on crossing over in females not given a heat shock. Comparison of parameters measured from the results of the crosses in which *cv v f*/+ + +; +/+ (control) and *cv v f*/+ + +; *mus*309^{D2}/*mus*309^{D3} (experimental) females not given a heat shock were crossed with *cv v f*/*Y*; +/+ males.

Parameter		Control	Experimental	Significance of the difference	
Total number of flies		14843	7761		
Frequency of true single crossovers in the *cv*-*v* interval;	% ± S.D.	14.24 ± 0.29	15.27 ± 0.41	t = 2.06	P = 0.0197
Frequency of true single crossovers in the *v*-*f* interval;	% ± S.D.	18.53 ± 0.32	14.74 ± 0.40	t = 7.10	P < 0.0001
Frequency of double crossovers;	% ± S.D.	2.20 ± 0.12	3.66 ± 0.21	t = 6.34	P < 0.0001
Recombination frequency of the *cv* and *v* markers;	% ± S.D.	18.64 ± 0.32	22.59 ± 0.47	t = 6.98	P < 0.0001
Recombination frequency of the *v* and *f* markers;	% ± S.D.	22.92 ± 0.34	22.06 ± 0.47	t = 1.45	P = 0.0735
Map distance of the *cv* and *f* markers;	cM ± S.D.	41.55 ± 0.40	44.65 ± 0.56	t = 4.43	P < 0.0001
Coefficient of coincidence;	C ± S.D.	0.5142 ± 0.0253	0.7344 ± 0.0362	t = 4.43	P < 0.0001

Table 6. Effect of the *mus*309 genotype on crossing over in heat shocked females. Comparison of parameters measured from the results of the crosses in which *cv v f*/+ + +; +/+ (control) and *cv v f*/+ + +; *mus*309^{D2}/*mus*309^{D3} (experimental) females which had received a heat shock of 35˚C for 24 h were crossed with *cv v f*/*Y*; +/+ males.

Parameter		Control	Experimental	Significance of the difference	
Total number of flies		7532	5052		
Frequency of true single crossovers in the *cv*-*v* interval;	% ± S.D.	13.25 ± 0.39	14.51 ± 0.50	t = 2.01	P =0.0223
Frequency of true single crossovers in the *v*-*f* interval;	% ± S.D.	17.46 ± 0.44	15.26 ± 0.51	t = 3.26	P = 0.0006
Frequency of double crossovers;	% ± S.D.	1.82 ± 0.15	3.80 ± 0.27	t = 6.82	P < 0.0001
Recombination frequency of the *cv* and *v* markers;	% ± S.D.	16.89 ± 0.43	22.11 ± 0.58	t = 7.32	P < 0.0001
Recombination frequency of the *v* and *f* markers;	% ± S.D.	21.10 ± 0.47	22.86 ± 0.59	t = 2.34	P = 0.0096
Map distance of the cv and *f* markers;	cM ± S.D.	37.99 ± 0.56	44.97 ± 0.70	t = 7.81	P < 0.0001
Coefficient of coincidence;	C ± S.D.	0.5101 ± 0.0392	0.7518 ± 0.0448	t = 4.06	P < 0.0001

29]. DSB formation occurs only during the earlier stages of meiotic prophase and initiates at a specific time after premeiotic DNA replication [29]. Crossing over in *D. melanogaster* for its part is known to occur during the pachytene stage of the meiotic prophase [29,30], and the progenies in the 3rd brood represent this stage of meiosis [28].

It is convincingly established that those meiotic mu-

tants of *D. melanogaster* affecting crossing over which also affect interference involve preconditions of crossing over, whereas those mutants that affect crossing over without affecting interference involve the crossing over event itself [31]. Consequently, the genes involved are called precondition genes and exchange genes, respectively.

This was theoretically shown by Sandler *et al*. [32] as follows: Let *a* be the probability of the fulfillment of preconditions of crossing over in one region and only in that region in a three-point crossing-over experiment. Let *b* be the probability of fulfillment of the same in another region and only in that region. Let *d* be the probability of the fulfillment of the preconditions in both regions at the same time, and *x* the probability of exchange, given the preconditions. From this it follows that the coefficient of coincidence, *C*, is

$$C = \frac{dx^2}{x(a+d)x(b+d)} = \frac{d}{(a+d)(b+d)}.$$

Since *C* is independent of *x*, if a mutant that acts on crossing over also affects interference, it must influence the preconditions of crossing over. If, however, interference remains unaltered, the target of the effect is the exchange itself.

What in this respect is true for meiotic mutants is, of course, also true for other factors that affect crossing over, such as the heat shock treatment in the present study.

The heat shock in the control females affected the crossing over frequencies but interference remained unaltered (**Table 3**). Thus, taking the foregoing into account, it can be concluded without any doubt that the heat shock in the control females affected the event of crossing over itself.

In contrast, heat shock in the experimental females affected none of the meiotic parameters studied crossover interference included (**Table 4**).

Interestingly, however, the *mus*309 mutation had a significant effect on all the meiotic parameters studied, crossover interference included, both in the non-heat-shocked and heat-shocked females (**Tables 5** and **6**). The only exception was that the frequency of recombination of *v* and *f* markers in the females not given a heat shock was not significantly different in control and experimental females (**Table 5**).

As indicated by the fact that, while crossing over frequencies were also affected, interference decreased in the experimental *mus*309 mutant females as compared to the control females in both the non-heat-shock-treated and heat shocked females, it can be concluded that, independent of the heat shock, the *mus*309 mutation affected some precondition of crossing over. Therefore *mus*309 belongs to the class of mutations that Baker and Carpenter [33] referred to as “precondition mutants”, meaning that they act prior to the time when crossovers are actually generated.

Thus, it appears that the heat shock has a different effect on the phenomenon of crossing over than the *mus*309 gene. The heat shock affects the occurrence of crossing over itself, while the *mus*309 gene affects some precondition of crossing over. Moreover, these effects seem to be independent of each others.

As indicated in the introduction, the precondition of crossing over, which the *mus*309 gene product affects, is the repair of DSBs—a necessary condition for crossing over. In particular, it is known that the MUS309 protein is involved in the SDSA pathway of the repair of the DSBs. Specifically, it is also known that in the *mus*309 mutants the SDSA pathway is blocked, while the DSBR pathway remains functional [19].

As also indicated in the introduction, of these pathways the SDSA pathway leads exclusively to non-crossover end products of the repairing process, while the DSBR pathway leads to both non-crossover and crossover end products. Therefore, in the *mus*309 mutant females more DSBs are expected to be repaired as crossovers than in the wild type females. In other words, map lengths should be increased in the *mus*309 mutants as compared to the wild type females. This is precisely what was observed in the present study (**Tables 5** and **6**).

Moreover, it should also be noted that, as indicated in the results, the data show that in both the non-heat-shock-treated and the heat shocked females the density of crossovers increased due to the effect of the *mus*309 mutation. This result shows that in the *mus*309 mutant females more DSBs are repaired as crossovers instead of non-crossovers than in the wild type females.

Consequently, it is suggested that the precondition of crossing over which the *mus*309 gene affects is the choice between the two routes of the DSB, or more precisely double Holliday junction, repair.

4.2. Testing the Models of Crossover Interference

This part of the discussion is *mutatis mutandis* similar to the respective discussion of an analogous series of experiments conducted by the present author where the effect of temperature on crossing over and crossover interference in *mus*309 mutants of *D. melanogaster* was investigated [6]. The results of these two studies reciprocally support each other.

As mentioned in the introduction, models of crossover interference can, in principle, be divided into two different categories. The first category of models, called genetic models [4], assumes that interference is dependent on genetic (*i.e*. linkage map) distance (Morgans) between adjacent crossovers. To my knowledge, currently only

one model, called the "counting model" [4,5], falls into this category.

The central feature of the counting model is that recombinational intermediates (C's) have two fates—they can be resolved with crossing over (Cx) or without (Co). The C's are distributed at random with respect to each other, and interference results from constraints on the resolution of C's. The basic constraint is that each pair of neighboring Cx's must have a certain number, *m*, of Co's between them, as if the meiocyte was able to "count" recombination events.

The second category of models, which may be called physical models, hypothesizes that crossover interference is dependent on physical distance (microns or base pairs) between the adjacent crossovers. In general, these models suggest that some kind of physical signal travels along the bivalent and determines the distribution of crossovers. One of the models belonging to this category, the reaction-diffusion model [34], is quantitative while the other models are qualitative.

According to the reaction-diffusion model, a "random walking" precursor becomes immobilized and matures into a crossover point. The interference is caused by a pair-annihilation of the random walkers, called the A particles, due to their collision together, or by annihilation of a random walker due to its collision with an immobilized point. This model has two parameters—the initial density of the random walkers, α, and the rate, *h*, of their processing into crossover points. It is logical to conclude that interference decreases if the α value increases and/or *h* decreases [34].

It is also quite logical to assume that if the *mus*309 mutations affect the proportion of the double Holliday junctions being resolved as crossovers instead of non-crossovers, the *m* value of the counting model should decrease, and consequently interference should diminish, in the *mus*309 mutants. The results of the present study are consistent with this idea. It is, therefore, very probable that the *mus*309 mutation affects the *Drosophila* counting number, thus being the first mutation of this kind identified. Consequently, the results of the present study support the view that crossover interference in *Drosophila* is tightly tied to genetic distance.

In contrast, however, the results of the present study are not compatible with the reaction-diffusion model. According to this model, interference depends on two factors only, *viz.* the initial density of crossover precursors, *i.e.* DSBs, and the rate of their processing into crossovers. Therefore, it is hard to conceive, in terms of the reaction-diffusion model, how the number of crossovers, *i.e.* the map distances, would change due to the effect of the temperature shock but their distances, *i.e.* interference, would not, as the initial density of DSBs does not change. This seems, however, to be the case in the results of the control crosses of the present study. Namely, because the coefficient of coincidence, *C*, did not change due to the heat shock treatment, it can be concluded that the initial density of the DSBs, *i.e.* the α value did not increase. Therefore, it cannot be assumed that the α value in the experimental crosses would change either.

Thus, if the reaction-diffusion model is correct, *h* in the experimental crosses should decrease due to the heat shock treatment. This means that the coefficient of coincidence, *C*, should decrease. In fact, however, *C* slightly increased.

The results are also in contradiction with any model of crossover interference based on physical distance on the following grounds: The map distances in the experimental and control females are different, and react differently to heat shock, the map distances in the control crosses being heat shock sensitive while those in the experimental crosses are not. However, the crossover interference is independent of the temperature shock treatment in both series of crosses. These results are in contradiction with the models based on physical distance. In fact, if interference was dependent on physical distance, how could it remain unchanged when the genetic map distances change but the physical distances do not? In other words, if interference, the distance between the crossover points, was dependent on physical distance, how could it react in a similar way to the heat shock treatment in the two series of crosses when the map distances, the number of the crossover points, react in a different way?

4.3. A Closer Look at the "Counting Model" of Crossover Interference in Drosophila

The "counting model" of crossover interference assumes that in the wild type of *Drosophila melanogaster* the value of *m* is equal to four, *i.e.* that there are four non-crossover outcomes (Co's) of the recombinational intermediates between each pair of adjacent crossovers (Cx's) [4].

The conclusion of the present study is that *mus*309 has a phenotype similar to that expected for a mutant that reduces the value of *m*. There are two ways in which *m* might be reduced: 1) Most interesting, but less likely, would be a mutant that changed *m* but retained the same rigor. E.g. *m* dropped from 4 to 2 in a manner that adjacent crossovers were now always separated by 2 non-crossover outcomes. 2) A more likely way would be a mutant that has reduced ability to ensure a non-crossover outcome for each of the four non-crossovers that fall between adjacent crossovers. The loss would be more or less random, so that as the mean number of non-crossovers between adjacent crossovers fell, the actual numbers became variable. Distinguishing between these pos-

sibilities is in principle possible but would require immense amounts of data. The test would involve comparing the data with the so called S3 curves in the Figure 2 of Foss *et al*. [4], and equations presented by them.

A further, fundamentally different but at the same time improbable, possibility which, however, cannot be ruled out is that, in individual *mus*309 cells, interference did not change. The *mus*309 mutant may have variable penetrance, such that some meiocytes or flies have better RecQ helicase activity than do others. Meiocytes with the least activity would have the most crossovers, but crossovers would now be non-uniformly distributed in the gamete population so that the crossover interference, as measured, would be reduced.

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