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THE GENETIC CONTROL OF MUTATION IN DROSOPHILA

(spontaneous mutation, mutator genes, mitotic recombination)

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SUMMARY

Mitotic recombination (MR) chromosomes are geographically widespread and occur in high frequency in wild Drosophila melanogaster populations. In both sexes, MR chromosomes increase the frequency of mitotic recombination and act as mutators for specific gene loci. Thus, MR chromosomes strongly influence the spontaneous mutation process.

INTRODUCTION

> "The study of spontaneous mutation is laborious, at best, but it seems an indispensible preliminary to the interpretation of mutations induced experimentally, and it may be the only approach now open for the study of gene evolution." L. J. STADLER (1946)

It is especially significant, I submit, to discuss the problem of spontaneous gene mutation at a STADLER MEMORIAL SYMPOSIUM because this was a question to which STADLER devoted much of his scientific career. I shall attempt here to update our knowledge of spontaneous gene mutation in *Drosophila melanogaster*. As a preface to this presentation, I wish to bring to the attention of students of the gene mutation process a paper published by STADLER (1946) on spontaneous mutation in maize. In this publication which now can be classified as a neoclassic paper*--seldom cited and never read--STADLER spelled out in his inimitable, lucid way the specific criteria which must be fulfilled if the unambiguous identification of a spontaneous mutation is to be made. He

*WEINSTEIN (1955) defined a classic paper as "a work that is often referred to and never read."

especially emphasized that spontaneous mutations must be distinguished from small gene deletions. Thus, their genesis may not involve external agents or genetic events (*e.g.*, unequal crossing over) which generate deletions.

STADLER'S incisive genetic analysis was made within the scope of the genetic knowledge of his time. Thus, he could not anticipate the revolution in genetics generated by WATSON-CRICK and the delineation of the mutation process at the molecular level. However, he did clearly anticipate that the rate of spontaneous gene mutation was not invariably an intrinsic gene characteristic but was subject to modification by genetic factors (STADLER 1948, 1949). Such genetic factors, conventionally called mutator genes, which cause an overall increase in the spontaneous mutation rate have been subject to detailed study in a variety of organisms, especially in prokaryote species. The mutational propensities of prokaryote mutator genes have been reviewed *in extenso* elsewhere and need not be repeated here. It suffices to note here that detailed information at the molecular level is known about the mode of action of many prokaryote mutator genes.

The history of mutator genes in eukaryotes is decidedly spotty. More than 40 years ago evidence was adduced for the existence of mutator genes in wild populations of *Drosophila melanogaster*. Yet, the detailed study of putative mutator genes in this species has been quiescent because mutation rate as a phenotype does not lend itself readily to genetic analysis.

However, within the past decade, prompted by two findings, there has occurred a renewed interest in *Drosophila* mutator genes. First, if one perseveres, the formal genetics of specific mutator genes can be managed. As a consequence, a third chromosome mutator gene was mapped (GREEN 1970) and shown to act as a mutator by "inducing" deletion mutations (GREEN and LEFEVRE 1972). Second, it is now reasonably clear that there is widespread in wild *D. melanogaster* populations a mutator with unusual properties. It is on this mutator that I shall concentrate my remarks in the narrative which follows.

In 1970 HIRAIZUMI isolated a seemingly unique second chromosome from wild *D. melanogaster* collected in south Texas. This second chromosome, designated T-007, generated an inordinately high rate of mitotic recombination when present in males (HIRAIZUMI 1971). This observation was followed by a spate of research from a number of laboratories on male recombination (*MR*) factors in *D. melanogaster* the results of which I shall summarize briefly here. *MR* second chromosomes appear to be cosmopolitan in distribution having been found from coast to coast in the U.S., in Europe, in Asia and in Australia. Such chromosomes occur in an amazingly high frequency of from 20-50% of the second chromosomes in the populations sampled. In addition to causing male mitotic recombination, *MR* chromosomes appear to generate chromosome aberrations (inversions and translocations) and act as mutator genes as measured by the induction of recessive lethal mutations.

I became interested in *MR* chromosomes because of their mutator properties and because of a long-standing interest in unstable or mutable genes. The history of mutable genes in *Drosophila* has been reviewed elsewhere (GREEN 1976). It suffices to emphasize here that until recently mutable genes in *Drosophila* occurred sporadically and were not readily subject to systematic study as a preface to understanding the basis of their mutational instability. The impetus for studying *MR* chromosomes came from the puzzling but challenging discovery made by colleagues in the Soviet Union that among the descendants of *D. melanogaster* collected in the wild and bred in the laboratory there occurred an unexpected high frequency of mutants at the X-chromosome, recessive, singed bristle *(sn)* locus. In addition to occurring frequently, the *sn* mutants were exciting because they were mutationally unstable, reverting the wild type at exceptionally high rates (GOLUBOVSKY *et al.* 1977). They represented the first systematic recovery of mutable genes in *Drosophila* and posed the novel question: what is the origin of the unstable *sn* mutants? Because the unstable *sn* mutants stemmed from wild flies collected at geographically widely separated sites, any explanation concerning their origin must accommodate this fact. The world-wide occurrence of *MR* chromosomes in relatively high frequency plus their demonstrated mutator activity suggested that they could be causally associated with the production of unstable *sn* mutants. This hypothesis was tested by employing an *MR* second chromosome designated *MR-h12*, isolated from wild flies collected in Haifa, Israel and by assaying its mutagenicity for 13 separate X-chromosome gene loci simultaneously. The results, presented in detail elsewhere (GREEN 1977a), can be summarized as follows. Among the 13 loci tested significantly increased mutation rates were found at 4 loci: *sn*, *ras* (raspberry eye color), *y* (yellow body color) and *cm* (carmine eye color). The frequencies compared to a control, taken from SCHALET as collated in GREEN (1977a), are presented in the following table.

Table 1. *MR*-induced mutation at specific X-chromosome loci.

	Mutation rate/10^5/locus			
MR chromosome	*sn*	*ras*	*cm*	*y*
h12	186	34	11	6
control	0.4	0.2	0.8	1

A sample of *sn*, *ras* and *y* mutants was tested further to determine whether or not they were mutationally unstable. Each of six independent *sn* mutants tested proved to be unstable reverting to wild type at inordinately high frequencies. Thus, it appears reasonable to conclude that the original *sn* mutants uncovered in the Soviet Union stem from captured flies which carried *MR* second chromosomes. That such *MR* flies occur in

the Soviet Union in high frequency has been recently demonstrated (GOLUBOVSKY personal communication).

In addition to the unstable *sn* mutants, some of *ras* and *y* mutants were also tested for and found to be mutationally unstable. These findings posed a number of relevant questions. What is the nature of the *MR* induced mutants? Is the mutation propensity characteristic of all *MR* chromosomes? Does *MR* act in females as well as in males?

I have argued at some length elsewhere (GREEN 1977b) that *Drosophila* mutants which are mutationally unstable are analogous to the insertion mutants of *E. coli*. This means they owe their mutated state to the insertion of a segment of "foreign" DNA into the gene. Reversion to wild type represents the occasional excision of the inserted DNA. To be sure the insertion mutation hypothesis poses additional relevant questions. What is the source of the inserted DNA? How does the insertion come about? Why do some loci mutate and not others? I shall defer considering these questions until later.

COMPARATIVE GENETICS OF *MR* CHROMOSOMES

Subsequent to the finding that in males *MR-h12* induces visible X-chromosome mutants at four gene loci, an answer was sought to the question of whether or not *MR-h12* is unique in this property. For this purpose two *MR* second chromosomes were isolated from wild flies collected at two widely separated sites in California (each site appropriately adjacent to a winery). One second chromosome is designated *MR-n1* and the second *MR-s1* where *n* means Napa county and *s* Sonoma county.

The investigation, done collaboratively with D. A. R. SINCLAIR, was divided into two parts. In part one the mutator activity of *MR-n1* and *MR-s1* was determined for the 13 X-chromosome gene loci just as had been done for *MR-h12*. In part two the capacity for inducing crossing over in males, presumably mitotic crossing over, was assayed for each of the three *MR* second chromosomes. Crossing over was studied in both multiply marked second and third chromosomes.

For purpose of discussion here, a summary of representative data are presented. So far as mutator activity among the 13 X chromosome gene loci, *MR-n1* and *MR-s1* are essentially equivalent to *MR-h12*. Significant increases in mutation frequency were found at only four loci, and precisely those loci prone to *MR-h12* mutator action. Table 2 includes the mutation rates calculated for the *MR-n1* and *MR-s1* chromosomes. Qualitatively, they appear to be equivalent to *MR-h12*, causing the *sn* locus to mutate most frequently followed by the *ras* locus. Whether or not the rates of mutation at the *sn* locus differ significantly is problematical since each rate is based on a sample of 30-40,000 chromosomes scored.

A parallel study was carried out in which the effect of each *MR* chromosome on crossing over in males was assayed in multiply marked second and third chromosomes. For this

Table 2. *MR-n1* and *MR-s1* induced mutation at specific X-chromosome loci.

MR chromosome	Mutation rate/10^5/locus			
	sn	*ras*	*cm*	*y*
n1	93	29	4	4
s1	235	25	14	3.5

discussion only the second chromosome data are presented. The exact details of these experiments will be described elsewhere. It will suffice to note here that crossing over was studied for the genetic interval *al*(0.01)-*dp*(13)-*b*(48)-*pr*(54.5)-*cn*(57.5). In parentheses the genetic map position of each gene is given. The genetic intervals are designated 1*(al-dp)*, 2*(dp-b)*, etc. Interval 4*(pr-cn)* spans the centromere. Individual males, heterozygous for the *MR* chromosome and the marker genes, were brooded by crossing to 3-5 successive harems of females homozygous for the marker genes. As controls heterozygous Oregon wild type males with and without 3000R X-rays were similarly brooded.

The results of these experiments are tabulated in Table 3 which follows. Taken at face value there is a strong suggestion that the pattern of recombination is not the same for all

Table 3. *MR*-induced recombination in males for the interval *al-cn*.

Second chromosome	% recombination per genetic interval					Number progeny
	1	2	3	4	Total	
MR-h12	0	0.28	0.02	0.19	0.49	22,116
MR-n1	0	0.03	0.06	0.18	0.27	7,718
MR-s1	0	0.30	0.08	0.35	0.78	7,896
Oregon-R wild	0	0	0	0	0	11,010
Oregon + 3000R	0	0.04	0.06	0.29	0.39	5,719

MR chromosomes. *MR-n1* appears to mimic X-rays in its effect on male crossing over with recombinants occurring primarily in the interval spanning the centromere. In contrast *MR-h12* and *MR-s1* generate more or less equivalent frequencies of recombination in both the centromere region (interval 4) and the non-centromeric interval 2.

We have attempted to obtain some insight into the male recombination process by answering two questions. The first: what is the effect of combining X-rays and an *MR* chromosome

on male recombination? Without spelling out the details, we found that by combining *MR-h12* and 3000R X-rays, the net effect on male recombination is additive rather than synergistic. This suggests that recombination mechanisms of *MR* and X-rays may be different.

The second question is: does the *MR* exchange event involve homologous or non-homologous regions? If the latter, one would predict that the recombination event would generate duplications and deficiencies, the deficiencies often behaving as recessive lethal mutations. We assayed a sample of recombinant and non-recombinant chromosomes derived from *MR-h12* males and found no difference in the recessive lethal frequency. Thus, the exchange event appears to involve symmetrical rather than asymmetrical (oblique) synapsis of the homologous chromosomes.

GENETIC EFFECTS OF *MR* CHROMOSOMES IN FEMALES

In contrast to the extensive studies of the genetic effects of *MR* chromosomes in males comparatively little information is available on the behavior of *MR* chromosomes in females. By way of rectifying this situation two types of experiments were undertaken (in collaboration with D. A. R. SINCLAIR). In one experiment the effect of *MR-h12* on recombination in females was assessed. In a second, the mutator effect of *MR-h12* in females was determined.

In the case of recombination, it was assumed that the frequency of *MR* generated recombination in females approximates that in males and therefore is relatively low compared to normal meiotic recombination in females. Therefore, to minimize meiotic recombination and maximize *MR* generated recombination, females were made homozygous for the third chromosome meiotic mutant *c(3)G* which effectively eliminates almost all meiotic recombination (GOWEN 1933) without affecting mitotic recombination. Crossing over for the genetic interval *al-cn* (as described above) was determined in homozygous *c(3)G* females with and without the *MR-h12* second chromosome. The results are summarized in Table 4. The *MR-h12* female data have been corrected for meiotic recombination by subtracting the recombinaiton percentage observed for each genetic interval in homozygous *c(3)G* females from that of the *MR-h12* homozygous *c(3)G* females. In homozygous *c(3)G* females, the total crossing over for the *al-cn* region amounted to 0.24% of the 13,580 progeny scored. For purposes of comparison the recombination data for *MR-h12* males are included in Table 4.

The results demonstrate that *MR-h12* effects a substantial frequency of recombination, in all probability mitotic, in females. Whether or not the increased recombination frequency in females compared to males is significant is not entirely clear. There are problems in estimating mitotic crossing over, which cannot be discussed here, which leave this question open.

Paralleling *MR-h12* recombination experiments in females, the mutation propensity of *MR-h12* in females was also assayed.

Table 4. *MR*-induced recombination in homozygous *c(3)G* females for the intervals *al-cn*.

	% recombination per genetic interval					Number progeny
	1	2	3	4	Total	
MR-h12 ♀♀	0	0.75	0.23	0.29	1.27	13,580
MR-h12 ♂♂	0	0.28	0.02	0.19	0.49	22,116

Because the *Maxy* chromosome carrying 13 recessive X-chromosome mutants is lethal in males, the mutation experiment was limited to specific loci, the *sn*, *ras* and *y* loci, which are highly mutable in *MR* males. Mutants at the three loci were scored among the progeny of females heterozygous for *MR-h12*; sisters lacking *MR-h12* served as the control. The results of these experiments are given in Table 5.

Table 5. *MR-h12* induced mutation in females.

Parental ♀	Number mutants per locus			Chromosomes scored
	sn	*ras*	*y*	
MR-h12	29(40)*	9(12)*	18(24)*	71,478
Control	0	0	0	33,095

()* = Number of mutants/10^5 chromosomes.

The data given in Table 5 demonstrate that without doubt *MR-h12* acts as a mutator in females as it does in males. A few additional comments are in order. The mutation rate at the *sn* locus in females is distinctly lower than that found in males ($40/10^5$ vs. $186/10^5$). Whether or not this is real is not altogether evident. The design of the mutation experiments was such that appreciably more gametes per individual male were sampled than per individual female. Thus, the discrepancy could be a sampling problem. This quesiton needs further study.

The frequency of *ras* mutants given in Table 5 is in all likelihood an underestimate. This because experience with *ras* mutants generated in *MR* males demonstrates that at least 60% are male lethal. Such mutants induced in females and transmitted to their sons would escape detection. In support of this explanation are the results of an experiment undertaken for quite another purpose where *MR-h12* males were crossed to attached-X females and thus where X-chromosome mutants are scored among their sons. In this experiment the frequency of *sn* mutants recovered was 78/55,864 gametes or $139/10^5$ gametes but *ras* mutants only 5/55,864 or $9/10^5$.

All the mutants at the *y* locus listed in Table 5 were recovered in females. On progeny testing each proved to be

lethal in males, indicative of a deficiency which includes the *y* locus. A cytological study of a sample of these mutants kindly made by GEORGE LEFEVRE demonstrated each *y* mutant to be associated with a cytologically discernible deficiency. This observation is of some interest because all of the *y* mutants generated by *MR* in males have proved to be male viable. The basis for this discrepancy between *MR* in males and females is not immediately obvious and is presently under further study.

UNRESOLVED QUESTIONS

The data presented in the foregoing sections demonstrate that there occur in wild populations of *D. melanogaster MR* second chromosomes which significantly raise the spontaneous mutation rate in both sexes at specific loci. This means that the spontaneous mutation rate is not simply an inherent characteristic of each gene, but is subject to significant modification at specific loci. This finding prompts a number of questions.

What is the nature of *MR*? Is it a conventional gene equivalent to the numerous genes which respond to conventional genetic analysis in *Drosophila* or is it something quite different? Are *MR's* extracted from geographically widely separated populations identical or different? Clearly, for these and other related questions there are presently no clear-cut answers. However, it is reasonable to expect that the formal genetic analysis of *MR* will supply the necessary answers. This analysis is underway.

Yet another relevant question bears on the nature of *MR* generated mutations. I have argued at some length on genetic grounds that insertion mutations occur in *Drosophila* analogous to those genetically and physically demonstrated for *E. coli*. By extrapolating from their genetic properties, I have argued further that many, perhaps all, of the *MR* produced mutations are insertion mutations. Assuming this argument is correct, it is proper to ask: What is the source of DNA which is inserted at a specific gene under the influence of *MR*? In the case of *E. coli* the situation is clear. A number of insertion sequences of different sizes appear to be normal constituents of the *coli* genome. Recently biochemical evidence has accrued for the occurrence of insertion sequences in the ribosomal DNA of *D. melanogaster* (GLOVER *et al.* 1975 and others). There is additional evidence that the insertion sequences are not restricted to the rDNA region and occur elsewhere. One analysis suggests the DNA of the insertion sequences represent about 0.2% of the genome (DAWID and BOTCHAN 1977). There is the further suggestion that the insertion sequences occur in 5-kilobase blocks equivalent to about 80 per genome. Thus, it is altogether possible that these insertion sequences are involved in *MR* induced mutation. A possible mutation induction scenario might include the following ingredients. Since *MR* elements generate recombination, they cause frequent chromosome breakage. Insertion sequences are unstable and frequently undergo excision. Excised insertion sequences may become integrated at sites of *MR* induced chromosome breakage leading to

new mutants. Prerequisite to this hypothesis is the demonstration that presumptive insertion mutants do indeed contain an inserted DNA sequence. This prerequisite which on paper appears to be practicable is as yet unfulfilled.

Finally, there remains the intriguing question of how *MR* bearing chromosomes are maintained in *Drosophila* populations in such high frequency in the face of the fact they produce so many deleterious mutations or genetic load. One possible explanation is that the genetic load problem is pure anthropocentrism; for the fly genetic load is not a problem. This I suspect is a trivial explanation and may be dismissed. There is, however, one observation presently in the process of being quantified which may bear on the frequency of *MR* chromosomes. This is the observation, incidental to the genetic experiments reported here, that males bearing an *MR* chromosome manifest an extraordinary mating propensity. Under laboratory conditions *MR* males are far more successful in mating than are males derived from standard wild type stocks. This is true both in a noncompetitive situation where the total frequency of mating was measured and in a competitive situation where *MR* and non*MR* males were competing for the same females. So far as measured to date, geographically widely separated *MR* chromosomes seem to be inseparable in their mating behavior. Whether or not mating propensity serves to maintain *MR* chromosomes in high frequency in wild fly populations is, of course, an open question. The laboratory experiments point in this direction.

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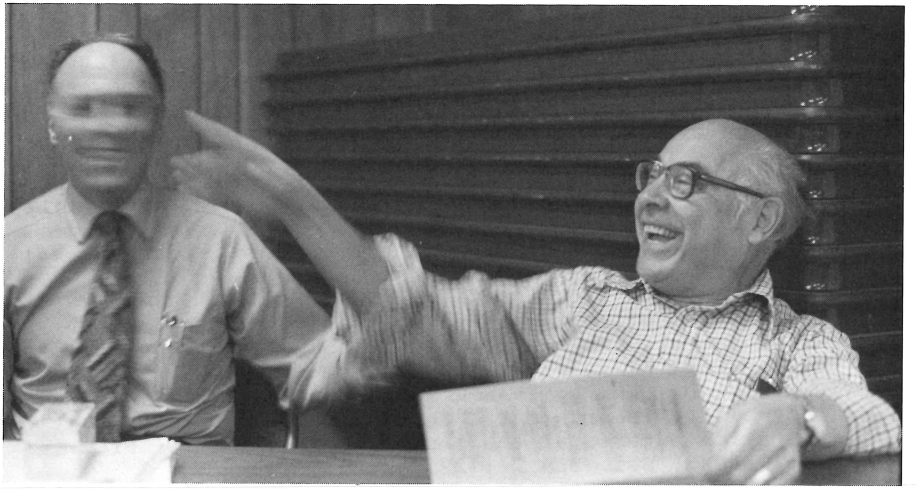

Drs. DeMars and Green at a discussion session