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Genetic instability in *Drosophila melanogaster: De novo* induction of putative insertion mutations

(directed mutation/spontaneous mutation/mutable genes)

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ABSTRACT **The capacity of a so-called male recombination (MR) chromosome in *Drosophila melanogaster* to generate mutations at 14 X-chromosome loci specifying visible phenotypes was investigated. Appreciable increases in mutation were found at three loci *y, sn,* and *ras.* Tests of representative mutants generated at each locus establish all to be putative insertion mutants, as judged by their spontaneous mutability. These facts suggest that insertion mutation production may be a general function of MR chromosomes. The relationship of the MR chromosomes to established insertion elements (IS and Mu) is discussed.**

Documentation is given in an earlier report (1) for the recovery of an array of presumptive insertion mutants at the singed (*sn*) bristle locus on the X chromosome of *Drosophila melanogaster*. These mutants are of particular interest because they represent a systematic recovery of insertion mutants in *Drosophila*. Because the mutants stem from flies caught in the wild, they pose some all-important questions. What is the origin of these mutants? Do they represent the chance isolation of mutants preexisting in a population of wild flies? Or, are the mutants being regularly generated in the flies by some yet to be identified extrinsic or intrinsic mutation induction process?

There are cogent reasons for concluding that segregation of preexisting mutants is in all probability not involved. The *sn* mutants—X-linked and recessive—descend from males caught in the wild, phenotypically wild type and crossed to homozygous *Basc* females. (See ref. 2 for an explanation of symbols used.) On the basis of phenotype the wild males carried no X chromosome mutants. The *sn* mutants were found in the F_2 progeny among males carrying the wild X chromosome. Thus, the mutants arose either in gametes of the parental wild males or in gametes of the F_1 *Basc*/+ females. Furthermore, the *sn* mutants described stem from males collected in two geographically widely separated populations of the U.S.S.R. In a subsequent report, additional *sn* mutants were isolated from wild males collected at yet two additional widely separated populations in the U.S.S.R. (3). Thus, segregation of pre-existing mutants appears to be highly unlikely.

While it is conceivable that some extrinsic mutagen—radioactivity, a chemical mutagen, or an infectious agent such as a bacterium or virus—could be responsible for directly or indirectly inducing the several *sn* mutants, it is not immediately obvious what this agent might be. Such an extrinsic agent would have to be present in geographically widely separated regions. This seems unlikely and, in the absence of any leads as to what the extrinsic agent might be, this hypothesis cannot be easily tested in the laboratory.

Therefore, attention was directed to the possibility that some intrinsic agent—specifically, a mutator gene or genes—might be responsible. Presumably such a mutator gene would be segregating in wild fly populations and, because it lacks a discernible phenotype, except for increasing the mutation rate, it could be unwittingly brought into the laboratory with flies caught in the wild. This idea is plausible because several examples of mutator genes have been described in *D. melanogaster,* some of which were detected in flies caught in the wild (4). There is the even more intriguing report by Ives (5) of a second chromosome mutator, *hi,* isolated from wild caught flies which caused at least one X chromosome locus, folded wing, to mutate at an enormously high rate. The bearing of this observation on the origin of the *sn* mutants is self-evident. Should a mutator gene(s) be involved, it must be of widespread geographic distribution. Here, the relevance of so-called male recombination (MR) second chromosomes originally isolated by Hiraizumi (6) from wild flies becomes apparent. Although initially identified because they increase the frequency of mitotic crossing-over in males, MR second chromosomes also act as mutators (7, 8) and increase the incidence of chromosomal aberrations (9, 10). Furthermore, MR second chromosomes are geographically widespread, having been isolated all over the U.S. and in Japan, Australia, England, Yugoslavia, and Israel. Wherever sought, MR second chromosomes exist in wild populations in an incredibly high frequency, with 20–50% of the isolated second chromosomes exhibiting MR activity. These facts suggested that the MR chromosome is, in all probability, also widespread and in high frequency in the U.S.S.R. and could conceivably be responsible for the reported (1) high incidence of *sn* mutants. The test of this hypothesis represents the subject of this communication.

MATERIALS AND METHODS

The MR second chromosome used was isolated from wild flies trapped on Mt. Carmel, Haifa, Israel. Lines were begun with single, wild caught females brought into the laboratory. From 17 such lines, a single second chromosome was isolated by conventional crossing procedures using the second chromosome Curly wing (*Cy*) balancer. Each of the 17 isolated wild second chromosomes was tested for MR activity by testing for male recombination as described by Hiraizumi (6). MR activity was detected in 12 of the 17 chromosomes. In terms of male recombination, the most potent MR chromosome isolated is one designated MR-h12 in which 7 of 12 heterozygous males generated crossovers. Subsequent crosses showed that for the second chromosome genetic intervals *al-dp-b-pr-cn,* MR-h12 induced male crossing-over in a frequency of 0.5%. Genetic tests with MR-h12 showed that it carried a small bristle mutation, which

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Abbreviation: MR chromosome, male recombination chromosome.

Table 1. Mutation frequency at specific X chromosome loci recovered from the cross *Cy/MR-h12* ♂♂ × *Maxy/Balancer* ♀♀

Exp. series*	Locus and no. of mutants recovered														Σ chromosomes scored
	y	*pn*	*w*	*rb*	*cm*	*ct*	*sn*	*ras*	*v*	*m*	*dy*	*g*	*f*	*car*	
I	2	1	1	—†	1	0	—†	5	0	—†	0	0	0	2	45,827‡
II	0	0	1	0	0	0	9	5	0	0	—†	0	0	0	33,338§
Σ I + II	2	1	2	0	1	0	9	10	0	0	0	0	0	2	
III	4	0	1	0	0	0	18	8	0	0	—†	0	0	0	23,299
Control	5	1	5	0	4	9	1	2	0	1	0	5	3	1	490,000

* Series I and II, *Cy/MR-h12* males from stock; series III, from cross *Cy/bw*^{v} ♀♀ × *Cy/MR-h12* ♂♂.
† Not scoreable.
‡ For *y* and *v* loci, 91,654 chromosomes were scored.
§ For *y* and *w* loci, 66,676 chromosomes were scored.
¶ For *y* and *w* loci, 46,998 chromosomes were scored.

renders homozygous females sterile and reduces the viability of both homozygous females and males. Hence, MR-h12 is maintained as a stock balanced to *Cy*. When tested for mutator activity, as measured by the induction of X-linked recessive lethal mutations with the *Basc* method, *Cy/MR-h12* males produced 1.3% lethals among 1567 X chromosomes scored.

The competence of *MR* to induce visible mutants was determined by the specific locus method using a *Maxy* X chromosome of Muller (11). *Maxy* X chromosomes carry 15 different recessive mutants involving eye color, bristle and wing morphology, etc., most more or less easily scoreable. Because *Maxy* is lethal to males, it is maintained intact balanced to a multiply inverted X chromosome marked with dominant *Bar* (*B*) eye. A simple experimental procedure was adopted. Newly eclosed *Cy/MR-h12* males selected from the stock were crossed to *Maxy/In(1)sc*^{S1}*+dl49, y sc*^{S1} *v B* (balancer) females, 4 males and 12 females per cross. Females were allowed to oviposit for 6 days, were transferred to fresh medium for 6 more days, and then were discarded. F_1 females were scored for new mutants. Mutants at the following eleven most easily scored loci were sought: *y pn w cm ct*^{6} *ras*^{2} *v dy g f car*. By chance it was found, after 45,827 X chromosomes were scored, that the *Maxy* chromosome used, which was thought to carry a *sn* mutant, did not. A new *Maxy* chromosome was obtained that carried the easily scored recessive mutants *y pn w rb cm ct*^{6} *sn*^{3} *ras*^{2} *v m g f* balanced to a *Basc* chromosome with a deletion for *y* and marked with *B* and *w*^{a}. Two different experiments were performed. In one, *Cy/MR-h12* males were taken from the balanced stock and mated as described above. In a second, the *Cy/MR-h12* males crossed to *Maxy* females were derived from the cross *Cy/MR-h12* males and *Cy/bw*^{v} females. The latter procedure was adopted because of the peculiar fact that the frequency of male recombination induced in *MR* males is significantly greater if the males inherit their *MR* chromosome from their fathers than from their mothers (8). Among *Cy/MR-h12* males taken from the stock, on the average half their *MR-h12* second chromosomes are paternal and half maternal in origin; all those from the cross are paternal.

RESULTS

The results of the three separate mutation experiments are given in Table 1. For comparative purposes the data of Schalet (ref. 12 and personal communication) on the spontaneous frequency of mutation in laboratory wild type males by the *Maxy* method are included. (In series I, in addition to the *Maxy* chromosome, mutants at the *y* and *v* loci could be scored in females heterozygous for the balancer chromosome. In series II and III, in addition to the *Maxy* chromosome, mutants at the *y* and *w* loci could be scored in females heterozygous for the balancer chromosome.)

A cursory examination of Table 1 reveals that, compared to the controls, substantial increases in numbers of new mutants were effected by *MR-h12* at both the *sn* and *ras* loci. These results, together with those for the *y* and *w* loci, have been recalculated on the basis of mutation frequency per 10^5 chromosomes scored and are given in Table 2. The mutation frequencies are instructive on two points. Irrespective of experiment, the mutation frequency at both the *sn* and *ras* loci has been dramatically increased when compared with the controls. The data also show that appreciably more mutants at the *sn* and *ras* loci were recovered when the *MR-h12* chromosome of males was specifically paternal in origin than when the *MR-h12* chromosome was either paternal or maternal in origin. This suggests that mutation induction, like recombination, is most effective in males who inherited this *MR* chromosome from their fathers. This too appears to be the case for mutations at the *y* locus, where a substantial rise in mutants occurred only in crosses where the male *MR-h12* was paternal in origin. The data also reveal that no increase in mutation occurred at the *w* locus, a frequently mutating locus. Similarly, *MR-h12* appears to have no effect on mutation at the *ct* locus, which is also a frequently mutating locus.

The overwhelming majority of mutants were recovered as single mutants, but a number of exceptions were encountered. Among the *sn* mutants five instances of 2 mutants per cross, one instance of 9 mutants, and one instance of 22 mutants per cross were found. Since the mutants from one cluster could not be separated from one another on the basis of phenotype, they are presumed to be the products of a single premeiotic mutational event. Similarly, the *pn* mutant was recovered as a cluster of three and the *cm* mutant as a cluster of seven. So far as phenotype is concerned, *sn* mutants representative of all possible described phenotypes were found. Among the *ras* mutants, 14 are male lethal and 4 are male viable. Both *car* mutants are male lethal. Thus, by and large the mutants found on phenotypic grounds are typical and represent no obvious departure from the kinds of mutants, spontaneous or mutagen-induced, reported for each locus.

Crucial for the hypothesis submitted is the demonstration that a significant number of the *sn* mutants generated by *MR-h12* are highly mutable and therefore presumptive insertion

Table 2. Frequency of mutation per 10^5 chromosomes at the *y*, *w*, *sn*, and *ras* loci

Source of mutants	Locus			
	y	*w*	*sn*	*ras*
Series I and II	1.3	1.8	27	12.6
Series III	8.5	2.1	77	34
Control	1.0	1.0	0.2	0.4

Table 3. Mutability of *sn* alleles generated by *MR-h12*

sn allele	No. of mutants recovered*			Σ
	sn^+	sn^{su}	sn^{ex}	chromosomes scored
h12-1	7	2	2	18,278
h12-2	0	0	0	15,518
h12-3	3	0	6	17,814
h12-4	4	0	2	8,096
h12-5	3	8	†	14,837
h12-6	9	2	0	7,921

* Phenotypic classes are as described in ref 1; sn^{su} = singed-subliminal, sn^{ex} = extreme singed.
† Not identifiable on basis of phenotype.

mutations. To date the first six *sn* mutants have been tested. The experimental protocol followed is that previously described (1) in which, per cross, two *sn* males were mated to harems of ten y^2 *w spl* sn^3 females and their female progeny were scored. It should be noted that the *sn* mutants tested were representative of the phenotypic spectrum of *sn* mutants described. The results of these crosses are given in Table 3. Five of the six mutants tested proved to be mutable, confirming the notion that the *sn* mutants are in all probability insertion mutants. Evidence that the *sn* mutant *h12-2*, for which no spontaneous mutants were found, is also an insertion mutant came from an experiment in which homozygous *h12-2* females were treated with 4000 R (1.03 C/kg) x rays. [The rationale for this experiment comes from the argument that those mutants that can be reverted to wild type by mutagenic agents known to make deletions, such as x rays, are in fact insertion mutations (13).] Among 21,017 progeny of irradiated homozygous *h12-2* females scored, 5 sn^+ revertants were recovered.

Analysis of the sn^+, sn^{su}, and sn^{ex} mutants tabulated in Table 3 is in progress. However, a few definitive statements can be made. At least one sn^+ derived from the *sn* mutant *h12-1* behaves, by analogy with prokaryotes, like an insertion sequence that can change its orientation. This sn^+ spontaneously mutates back to what phenotypically appears to be *h12-1* and to an sn^{ex} type that genetically behaves like a deletion (1).

The mutational propensity of the *ras* mutants is similarly under investigation. To date three separate *ras* mutants, all of which are male lethal, have been examined. One such *ras* mutant, designated ras^{j26}, spontaneously reverts to wild type at a high frequency. Among 34 females heterozygous for ras^{j26} and ras^2 *v m* (where ras^2 is a stable allele), 7 produced viable ras^+ males among a total of 2372 ras^{j26} X chromosomes scored. The remaining two *ras* mutants, designated ras^{f2} and ras^{f26}, also appear to be mutationally unstable. However, in contrast to ras^{j26}, ras^{f2} and ras^{f26} appear to mutate to viable males that exhibit a *ras* eye color. Most of these males are sterile because their external genitalia are abnormal. The few viable ras^{f2} males that have been fertile bred as though their X chromosomes carried a male lethal ras^{f2} mutant. Contrariwise, the fertile ras^{f26} males bred as though they transmitted a male viable ras^{f26} mutant. The mutability of these lethal *ras* mutants is under study.

Finally, among the several *y* mutants recovered, the mutability of two has been studied. One, designated y^{d28}, mimics the mutant y^2 in phenotype, exhibiting a yellow body color and black bristles. The second, designated y^{j22}, mimics y^1 in phenotype, with body color and bristles both yellow. In a typical experiment in which revertants were sought among the progeny of y^{d28} males, 5 reversions to wild type were recovered among 5364 chromosomes scored. In a comparable experiment with y^{j22}, 3 reversions were found among 8891 chromosomes scored. Salivary gland chromosome cytology on selected *y*, *sn*, and *ras* mutants described here uncovered no associated cytological abnormalities.

DISCUSSION

The experimental results provide a reasonable and satisfactory explanation for the array of putative *sn* locus insertion mutants reported by Golubovsky *et al.* (1). The widespread geographical distribution of *MR* chromosomes, their high frequency in wild *D. melanogaster* populations, and the demonstration here of their capacity for "inducing" putative *sn* insertion mutations all militate for the conclusion that *MR* is also present in high frequency in the U.S.S.R. Thus, by chance, some of the male wild flies bred in the laboratory carried an *MR* second chromosome which "induced" the putative insertion mutations found by Golubovsky *et al.* (1). This mutator need not be identical to *MR-h12*. Mutation induction experiments now in progress with an independent *MR* second chromosome isolated from flies collected in California suggest it too has mutator activity for the *sn* locus. Therefore, this could be a general property of *MR* chromosomes.

A remarkable feature of the results presented here is that *MR* significantly increases the frequency of mutation not only at the *sn* locus, but at the *ras* and *y* loci as well. Presumptive insertion mutants, as judged by high reversion rates, have been demonstrated at all three loci. The possibility exists, although it has by no means been conclusively established as yet, that the majority or perhaps all of *MR* generated mutations are insertion mutations.

The several genetic properties of *MR* second chromosomes—induction of mitotic recombination of males, potent mutator action, and production of chromosomal aberrations—raise the question: What is the nature of *MR* action? At this juncture a conclusive answer is not possible, but some interesting suggestive facts are on hand. *MR* induces chromosome rearrangements, a property it shares with bacteriophage Mu (14). As a mutator, *MR* appears to be selective since only three of the fourteen loci examined exhibited significant increases in mutation frequency. In this role, *MR* seems to mimic IS whose integration does not appear to be random (P. Starlinger, personal communication). Both IS and Mu excision can lead to deletion mutations, a property also associated with at least some *MR*-generated insertion mutations. The *MR* capacity for inducing male recombination can be transfused to males that lack this capacity (15). This is more consistent with *MR* being associated with a virus like Mu than with an IS element. There is genetic evidence for a nonchromosomal association of *MR* (16). In addition, the effectiveness of *MR* when paternally transmitted does not fit a strict genic effect. However, the precise nature of *MR* remains open. Needless to say, these unique genetic properties prompt further study directed toward the elucidation of the nature and action of *MR*.

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