

COMPARISON OF MUTABILITY INDUCED BY GAMMA RADIATION AND DES IN *Drosophila melanogaster* STRAINS SELECTED FOR RESISTANCE

Elena Diehl-Fleig¹ and Edmundo Kanan Marques²

ABSTRACT

In order to verify whether *Drosophila melanogaster* strains, selected for resistance to induction of recessive lethal mutations of the X chromosome, also are resistant to mutation induction by chemical agents, tests were carried out with gamma rays (4840 R) from ⁶⁰Co, with DES (0.3, 0.4 and 0.5%) and with joint treatments ⁶⁰Co (4840 R) and DES (0.4%).

Tests with gamma rays proved that the strains that were selected for radioresistance still keep such a characteristic, 20 generations after stopping selection and irradiations.

DES induced a significantly lower rate of sex-linked recessive lethals in radioresistant strains, when compared to the controls. These results suggest the existence of common repair mechanisms for damage brought about both by physical and chemical agents.

In the joint ⁶⁰Co and DES treatments, we noticed an increase in the lethal rate, with an almost additive effect in the radioresistant strains; while in the control strain, the lethal rate was similar to that obtained from isolated treatments. Such results suggest that DES partially inhibited or inactivated the repair enzymes, in such a way that pre-mutational injuries induced by radiation and by DES itself became fixed in the genome.

INTRODUCTION

It is likely that during evolution mechanisms have developed through mutations, which control the mutability level of the species (Berg, 1945; Stone *et al.*, 1954;

¹ Laboratório de Genética, Departamento de Biologia, Universidade do Vale do Rio dos Sinos, Caixa Postal 275, 93020 São Leopoldo, RS, Brasil. Send correspondence to E.D.F.

² Departamento de Genética, Universidade Federal do Rio Grande do Sul, Caixa Postal 1953, 90001 Porto Alegre, RS, Brasil.

Sagan, 1961; Zamenhof *et al.*, 1966). Such mechanisms could be cytoplasmatic systems that would either hinder the access of the mutagen into the cell or neutralize its action. They could also consist of mechanisms that would act upon the genes themselves, making them less susceptible to mutagens or, even, by means of processes that would repair damage that took place in the genetic material.

Radioresistance and chemoresistance would make clear, therefore, the existence of mutability control mechanisms. There is much evidence showing that the genetic constitution of an individual can exert influence on the reaction to mutation induction agents (Strömnaes, 1951, 1959; Ogaki and Tanaka, 1963 and 1966; Murakami and Tazima, 1966; Sankaranarayanan and Sobels, 1976; Ramos and Marques, 1978; Andrade and Marques, 1980; Reguly and Marques, 1987 and 1988).

Marques and Wallace (1971) and Marques (1968, 1973) showed that *D. melanogaster* and *D. nebulosa* strains with previous radiation history were less sensitive to the detrimental effects of new radiation doses, thus suggesting the development of radioresistance in these strains. About 25 generations after radiation suspension, in *D. nebulosa* strains selected by Marques (1968), it was noticed by Diehl and Cordeiro (1970), through tests with ^{60}Co , that radioresistance was still present.

Studies with chemical mutagens in radioresistant strains were carried out by Ramos and Marques (1978), Andrade and Marques (1980), Reguly and Marques (1987), showing that these strains are also resistant to chemical agents. Such results suggest the existence of damage repair mechanisms common to physical and chemical agents.

MATERIAL AND METHODS

D. melanogaster males of radioresistant (RAD) and control (CONT) strains aged from 3 to 5 days, were exposed to 4840 R of ^{60}Co gamma radiation, for 20 minutes, at a dose rate of 242 R/minute (^{60}Co source, model 60 RL, Radionics Laboratory). Right after irradiation, they were individually crossed with females of the Muller-5 marker strain.

The diethyl sulfate (Carlo Erba) prepared according to Pelecanos and Alderson (1964), modified in the following way: the wanted concentrations (0.3, 0.4 and 0.5%) were dissolved into 11 ml absolute methyl alcohol to which was added a 5% dextrose solution. Males aged up to 48 h, belonging to RAD and CONT strains were kept in contact with the DES solution for 48 h and, then, individually crossed with the Muller-5 females.

Two tests were carried out with a joint DES and ^{60}Co treatment. During the first treatment, RAD and CONT males aged 48 h were treated with a 0.4% DES solution, for 48 h, and after that irradiated with 4840 R. During the second joint treatment the males were kept on a 22-hour fast, and then subjected to the same

procedure. Following the joint treatment, all males were individually crossed with the marker strain females.

RESULTS

The first ^{60}Co gamma rays test showed that the recessive lethal mutation rate, induced in the X chromosome was significantly lower in radioresistant strains (7.49%) than in control ones (14.00%) ($F < 0.01$). These data show, therefore, that the RAD strains, after twenty generations with no irradiation and no selection for radioresistance, still kept such a characteristic (Table I).

Table I - Effect of 4840 R of ^{60}Co gamma radiation on the induction of sex-linked recessive lethals in radioresistant (RAD) and control (CONT) strains of *Drosophila melanogaster*.

Strain	No. of chromosomes tested	% Lethals	χ^2
RAD	347	7.49	8.04**
CONT	400	14.00	

**Significant at the 1% level.

Table II summarizes the results obtained with 0.3, 0.4 and 0.5% DES concentrations. The data show a higher resistance to DES in the strains which were selected for radioresistance.

Figure 1 represents the straight line regressions that were obtained for each strain. They differ from each other in that for the three DES concentrations, the radioresistant strains show a lower recessive lethal mutation rate of the X chromosome than the control strains.

Figure 2 shows the equivalence between the radiation rate and the DES concentration. According to the sex-linked recessive lethal mutation rate, induced by 0.3, 0.4 and 0.5%, there would be necessary, in radioresistant strains, respectively, about 4065R, 7680 R and 8002 R. For control strains lower radiation doses would be needed, thus, the recessive lethal rates, induced by the same three DES doses, would correspond, respectively, to about ^{60}Co 3353 R, 5566 R and 6880 R.

Table III presents the results for the two joint treatments of 0.4% DES and 4840 R gamma rays. There did not occur, for both treatments, a significant difference in the sex-linked recessive lethal mutation rate, in both radioresistant and control strains. Nevertheless, the lethal rate was lower in both strains in the first joint treatment.

Table II - Effect of diethyl sulphate on the induction of sex linked recessive lethals in two *D. melanogaster* strains.

Strain	Doses (%)	Chromosomes tested	% Lethals	χ^2
RAD	0.3	524	6.30	4.07*
CONT		512	9.69	
RAD	0.4	471	11.89	3.51
CONT		491	16.09	
RAD	0.5	475	12.42	10.04**
CONT		497	19.92	

* Significant at 5% level. **Significant at 1% level.

DISCUSSION

The mutability level of the species must be controlled, thus maintaining genic co-adaptation and ecologic adaptation, as well as, at the same time, providing the necessary genetic variability. Zamenhof *et al.* (1966) suggested that such a control should be a consequence of extragenic (cytoplasmatic) mechanisms and/or of mechanisms acting at the genic level.

According to the first hypothesis, if radioresistance is a consequence of cytoplasmatic controls, peroxidases and catalases that would destroy peroxides, thus hindering them from getting in contact with DNA, it would be expected that the radioresistant strains would not be resistant to chemical mutagens. Similarly, strains that were resistant to a chemical mutagen, would probably not be resistant to other substances, nor to radiation.

Control of the mutability level through a pre-mutation damage enzymatic repair system in DNA would provide the organism with a resistance to mutagenic agents. In this case, the strains which were selected for radioresistance, would show a more active repair mechanism and, therefore, a lower mutation rate, whether treated with physical or chemical agents.

Our results show that the RAD strains that were selected for resistance to sex-linked recessive lethal mutation induction, are also resistant to diethyl sulfate, when compared with those that were not selected for radioresistance. Ramos and Marques (1978) noticed that radioresistant strains were also resistant to intergerrimine,

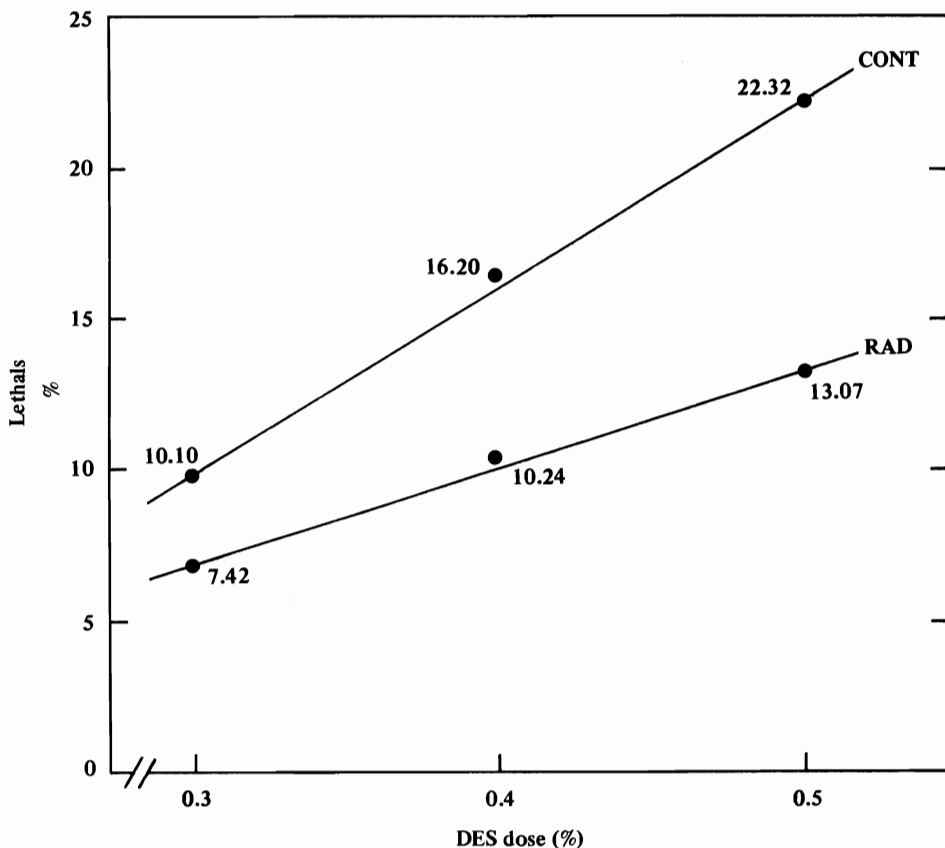


Figure 1 - Regression on mutation rate presented by two strains of *D. melanogaster* treated with diethyl sulphate.

a mutagenic alkaloid, extracted from *Senecio brasiliensis*. Andrade and Marques (1980), in their study of sensitivity differences between two strains, found a lower lethal rate in the radioresistant strain than in the control, exposed to a 0.006 M ethylmethane sulfonate dose. Such a difference in sensitivity was suggested to be a consequence of the repair mechanisms of each strain, not being specific to the mutagen. Similar results were obtained by Reguly and Marques (1987), in strains selected for radioresistance and treated with EMS, when compared with the non-selected strains.

The recessive lethal mutation rates in the X chromosome which were induced in the joint treatment were not significantly different for RAD and CONT strains. In the RAD strain an additive effect appears to have occurred, although the lethal rate was a little lower than the sum of the treatments, taken individually. Nevertheless, in

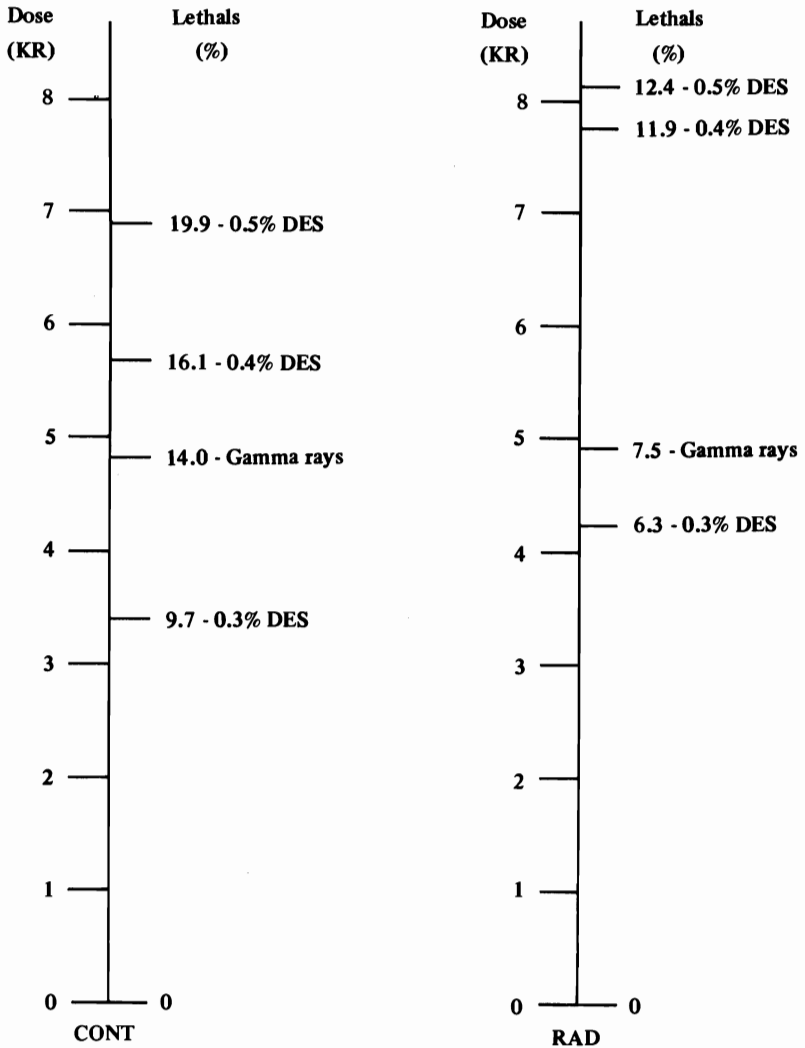


Figure 2 - Equivalence of gamma radiation dose and DES concentration in sex-linked recessive lethal induction in radioresistant (RAD) and control (CONT) strains of *D. melanogaster*.

CONT strains, the lethal rate was much closer to that obtained in the isolated treatments.

An increase in the mutation rate during the second joint treatment occurred in both strains, though the difference was not significant. This probably occurred due to a higher ingestion of DES, since the flies had been kept in a 22-hour fast, before the treatment.

Table III - Combined effect of 0.4% diethyl sulphate and 4840 R of ^{60}Co gamma radiation on the induction of sex-linked recessive lethal mutants in two *D. melanogaster* strains.

Treatment	Strain	Chromosomes tested	% Lethals	χ^2
I	RAD	272	16.18	0.65
	CONT	388	13.92	
II*	RAD	275	21.45	0.24
	CONT	159	19.50	

* With prior fasting.

Oster (1958) noticed that X-rays, when combined with methane or mustard gas, showed an additive effect in relation to sex-linked recessive lethal induction, in *D. melanogaster*. Nevertheless, Sharma and Prakash-Grover (1970) found a synergistic effect upon recessive lethal induction in X chromosome, with a joint treatment of gamma rays, followed by EMS. They suggested that EMS, besides inducing mutational damage, could also inactivate repair enzymes, in a way that pre-mutational radio-induced damage would be fixed.

Andrade and Marques (1980) found that the CO_3 strain (radioresistant) showed a synergistic effect, whereas the RC_1 strain presented an additive effect for an EMS and radiation joint treatment. Probably, the CO_3 strain has a more efficient repair mechanism but, since the joint treatment could be strongly affected by this kind of inhibition.

Reguly and Marques (1988) noticed that radioresistant strains, pre-treated with caffeine, had their lethal mutation rate increased to the level of the non-pre-treated one, showing no change in the frequency of lethals in the sensitive strain. Caffeine seems to be capable of changing the mutagenesis induced by interfering, with the repair of pre-mutational damage in DNA (Mendelson, 1974, 1976; Nothel and Abdalla, 1982). Reguly and Marques results (1988) suggest that differences in the repair of the pre-mutational damage are due to differences of sensitivity between CO_3 and RC_1 strains. The radioresistant strain which shows a higher or more efficient repair level than the sensitive strain, when pre-treated with caffeine, would have this system inhibited in a way that the mutation rate would increase, whereas the same would not happen to the sensitive strain.

The data that we obtained from the DES treatments show that the strains selected for resistance to sex-linked recessive lethal induction are also resistant to DES, thus suggesting repair mechanisms which are common to both mutagens. During

joint treatments, radioresistant strains showed a lower lethal rate than was expected if there was an additive effect. We suppose that DES has not only partially inactivated the repair enzymes, but also induced mutations. When inactivated, the repair enzymes would not be able to restore pre-mutational damage, induced by radiation and DES itself; thus, the lethal rate reaches the rate of the strains that were not selected for radioresistance. In these, the mutation rate induced by the DES and gamma ray joint treatment neither presented an additive effect, nor a synergistic one, but showed an effect similar to that obtained by isolated treatments. Such a result could be due to a selective cellular mortality, that is, by increasing the harmful charge, more cells one type would be eliminated and, therefore, the increase of the observed mutation rate would be smaller.

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RESUMO

Com o objetivo de verificar se linhagens de *Drosophila melanogaster* selecionadas para resistência à indução de mutações letais recessivas no cromossomo X, também apresentavam resistência à indução de mutações por agentes químicos, foram realizados testes com raios gama de Co^{60} (4840 R), testes com DES (0.3, 0.4 e 0.5%) e testes com tratamentos conjuntos Co^{60} (4840 R) e DES (0.4%).

Os testes com uma dose de 4840 R de Co^{60} comprovaram que as linhagens selecionadas para radiorresistência ainda mantinham esta característica após 20 gerações da suspensão da seleção e das irradiações prévias.

O DES induziu uma taxa de letais recessivos ligados ao sexo significativamente mais baixa nas linhagens radiorresistentes comparativamente aos controles. Estes resultados sugerem a existência de mecanismos comuns de reparo aos danos produzidos por agentes físicos e químicos.

Nos tratamentos conjuntos de Co^{60} e DES observou-se um acréscimo na taxa de letais, com efeito quase aditivo nas linhagens radiorresistentes, enquanto que nas controle, a taxa de letais foi semelhante àquelas obtidas nos tratamentos isolados. Estes resultados sugerem que o DES inibiu ou inativou parcialmente as enzimas do reparo, de forma que lesões pré-mutacionais e induzidas pela radiação e pelo próprio DES foram fixadas.

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