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MASTER

TECHNICAL PROGRESS REPORT

TOTAL RADIATION-INDUCED GENETIC DAMAGE IN
ENTIRE GENOMES OF DROSOPHILA MELANOGASTER⁽¹⁾

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I. Total Genetic Damage

A manuscript has been prepared for publication of the data obtained from treatment of mature spermatozoa with 4000r of X-rays. This manuscript is enclosed with this report, since it presents in detail the background of this project, the methods employed, the data accumulated in the first series and the manner in which the data is handled.

At present, as well as during the past grant year, the experimental effort has been toward the accumulation of similar data with 2000r. The status of this work is as follows.

Materials and Methods

The stocks used and the procedure are very similar to those used in the 4000r series (for which see the enclosed manuscript). A few changes have been made, however, which simplify and improve the experiment. The males which are irradiated are of the constitution $y/y^+ sc^8 \cdot Y$; red; ci. The marker "chocolate" was removed from the X, since it interfered with the identification of "red", and its only usefulness was in crosses for salivary analysis of lethal or o^7 sterile X chromosomes. "Stubbloid" was removed from chromosome 3, since it weakened the chromosome, and its only usefulness was to aid in identification of chromosome 3 when "cho" was present. The Y chromosome is now $y^+ sc^8 \cdot Y$ rather than $B^S \cdot Y$ simply because each irradiation is carried out using oo^7 from a control stock of the immediately preceding irradiation, and these have acquired a $y^+ sc^8 \cdot Y$. This change can make no difference, since only X-bearing genomes are being collected at present.

The P1 ♀♀ are the same as before, but without an extra Y chromosome. The F1 individual ♀♀ are mated to ♂♂ with the same autosomal markers and balancers as before, but with FM6/y⁺ sc⁸.Y. This is the crossing scheme which was originally planned but which had to be abandoned because these ♂♂ were too poor. Now we are managing to use this cross when and if all culture conditions are optimum. It is the "bottleneck" of the experiment, however, and often causes whole irradiations to be discarded (see below).

It has also been possible in this series to follow the original plan of mating the irradiate ♂♂ for 24 hours only, so as to sample only the spermatozoa which were fully mature at the time of irradiation. In addition, 7-day-old ♂♂ are being used whereas younger ♂♂ (1-3 days old) were used in the previous series. Thus the present sperm sample should be more homogenous for full maturity.

Care is taken to collect and account for every F1 ♀ of the appropriate genotype which emerges.

The status of the genome collecting is shown in the following table.

Genomes collected from 2000r treatment

	No. F1 ♀♀ mated	♀ died	♀ sterile	No stock obtained	Stock lost	Partial genomes	Whole genomes
From 6 successful irradiations	212	3	5	3	1	9	191
From 3 un- acceptable irradiations - discarded	213	?	?	--	--	--	--

The unsuccessful irradiations are included, since they represent time and effort expended and also because they demonstrate the exercise of one of the most important criteria in this type of work. It is essential that each irradiated genome should have as equal a chance as possible to be examined in later generations. Any adverse circumstance which results in an increased proportion of F1 $\phi\phi$ which fail to produce progeny will select against the more heavily damaged genomes. These would all be counted as genomes with non-heritable damage when many of them could have transmitted genetic damage. The decision as to whether a particular group of crosses from a particular irradiation is as successful as can be expected or whether it is unusually poor is largely a subjective decision. The 3 irradiations discarded this year were quite obviously adversely affected by as yet unidentified culture problems. It is hoped that a change of medium will avoid further difficulty.

It would be unrealistic to attempt to establish frequencies or relationships of the different types of damage from this small sample of 191 genomes, even if all the analyses were finished, which they are not. It is possible, and may have sufficient meaning, to at least classify most of them as damaged or undamaged. Even if a genome is not completely studied, if one alteration of any kind has been found, it can be classified as damaged. The amount and exact nature of the damage must await completion of the study. A genome in which all chromosomes are fully viable and fertile can almost certainly be classified as undamaged. Of the 191, only 5 cannot be thus placed. The results, then, are:

104 genomes damaged ----- 55.9%

82 genomes undamaged ----- 44.1%

The proportion of the transmitted genomes which are damaged (55.9%) is quite large in comparison with that for 4000r (63.2%). It is thus of special interest to see how the overall and the transmitted damage compares with that for 4000r when considered as a proportion of the irradiated sperm population. At 2000r there are 40.4% induced dominant lethals plus 4.5% control or a total of 44.9% loss through embryonic mortality (Baker and Von Halle, 1953). Thus the 212 genomes collected represent 55.1% of the sperm population, which must have been 385. The fates of these 385 sperm cells becomes

Induced dominant lethals	--	156	
F1 ϕ inviable or sterile	--	12	
F1 ϕ transmitted damage	--	<u>110</u>	-- 28.6%
Total damaged by irradiation	--	<u>278</u>	-- 72.2%
Control egg mortality	--	17	
Genomes undamaged	--	85	
Genomes unclassified	--	<u>5</u>	
		385	

With full respect for the fact that the sample is small and the error undoubtedly large, one can draw certain tentative conclusions as to the relationship between total damage and total transmitted damage induced by 4000r and 2000r. The relationships can best be seen in graphic form in figure 1. The increase in dominant lethals is essentially linear, but at 2000r 28.2% of the sperm transmit genetic damage while at 4000r only 10.2% transmit damage. Thus, even though 4000r inflicts a greater overall damage upon sperm cells, 2000r is a much more dangerous dose, genetically. More than twice as much damage is transmitted to future generations. Furthermore, when the analyses of the genomes treated with 2000r is complete,

Figure 1. Dosage relationships of different kinds of damage

- Dominant lethals (Baker & Von Halle, 1953),
- Total damage
- Inherited damage

the data will probably show that a greater proportion of the damaged genomes will have fewer damaged sites. A tabulation of the damaged genomes which are completely studied at this time yielded the following:

	2000r	4000r
% with 1 site	64	49
% with 2 sites	26	32
% with 3 or more	10	19

It remains to be seen, of course, whether further data from 2000r will agree with these preliminary conclusions.

II. Translocations with the Y chromosome

In the 4000r series of irradiations for the total genetic damage study, both X-bearing and Y-bearing genomes were studied, and it was found that Y; autosome translocations were more frequent than X; autosome translocations. (See enclosed manuscript.) Unfortunately, the number of Y-bearing genomes was quite small (129) and no more are being collected in the present 2000r irradiations, since the scheme has been simplified. It was thought, therefore, of interest to carry out a few irradiations for the purpose of collecting some Y chromosome data at 2000r.

When $\sigma\sigma$ were collected for irradiation for the total damage experiment, extra ones were collected for the Y chromosome experiment and they were irradiated simultaneously. They were mated to attached X qq with no free Y. Individual F1 qq were mated to $\sigma\sigma$ with attached

XY and no free Y ($Y^S X \cdot Y^L$, In(1)EN + dl-49, Y^S car $fvy \cdot Y^L$). Sons from these crosses were mated to females homozygous for the same attached XY, and stocks were established of each irradiated $y^+ sc^8 \cdot Y$. (It is obvious that the same stocks could be obtained in one generation less by mating the irradiated males to homozygous attached XY females and taking individual sons to be mated back to these females. The extra step with the X·X females was thought to improve the chances of recovery of the irradiated Ys, since the $y v f$ car $X \cdot Y/Y^* \text{ } \text{♂♂}$ are somewhat poor in individual matings. In addition, the irradiated Ys in the 4000r series were collected in females.)

After stocks were established, ♂♂ from each were mated to $y; bw; st; pol$ virgin females. Sons from this cross were mated again to $y; bw; st; pol$ virgins and their progeny scored for linkage of the Y with any autosome. Failure of this cross would indicate induced sterility, and in that case, a different scheme was to be followed: $X \cdot Y/Y^+ sc^8 \cdot Y^* \text{ } \text{♂♂} \times C(1)DX, y f; bw; st; pol \text{ } \text{♀♀}$ ---> daughters $X y; bw; st; pol \text{ } \text{♂♂}$ ---> score for translocations.

144 F1 ♀♀ bearing irradiated Y chromosomes were collected and mated, but the results are probably unreliable. The culture medium problem which adversely affected the total genetic damage experiment also affected this one. Of the 144 ♀♀ mated, 11 died, 43 failed to give progeny and 2 failed to produce sons. Only 88 stocks were made. When these were tested, no male sterility was found and no translocations. This data is considered unsuitable and will not be used, but the results did bring to mind the question as to whether the type of marker on the Y might affect its breakability or reunion. It might be interesting to test both a $B^S \cdot Y$ and a $y^+ sc^8 \cdot Y$ in an experiment -- possibly irradiating both in the same male.

III. Insertional translocations and replicating instabilities

It is our belief that lethals which appear in later generations of apparently non-lethal cultures are not due to "instabilities" of a gene but rather can be explained by certain types of chromosome rearrangements, especially insertional translocations or repeats. It was planned to collect some cases of such delayed lethals and observe the salivary chromosomes of the lethal and non-lethal lines in order to establish the cytological basis, if any. Before starting on this, the idea arose that it would perhaps be more efficient to look for insertional translocations and then test them for behavior mimicking delayed lethals.

The means by which a stock carrying an insertional translocation could give rise to a lethal line is of course through segregation at meiosis of the deleted chromosome to one daughter cell and the one bearing the corresponding insertion to the other. Thus a gamete could be formed bearing the deletion only and the deleted chromosome would behave as a lethal. The translocation would have to be viable and fertile when both the deletion and insertion are present in hemizygous or homozygous form in order to give rise to a so-called non-lethal line from which a lethal line would derive. It would also be more convenient to test X chromosome viability than autosomal.

A scheme was devised, therefore, which would screen for viable, fertile translocations between the X and any autosome. The scheme is as follows:

The T(X;A)s will be studied for their cytological nature and if any are insertional, they will be tested for behavior mimicking delayed lethals.

IV. A Search for Translocation Mosaics

Since a small pilot experiment turned up 2 (possibly 3) cases of translocation mosaics, it seemed of interest to do further experiments to attempt to find the frequency of such an event, which might be more frequent than suspected. It also seemed that a study of such cases, especially if induced by different agents and in different stages, might throw some light on the question of chromosome structure.

Unfortunately, neither time nor personnel was available for a serious attack on this problem at this time. Two small experiments were done, one with X-rays and one with EMS. In both cases, 5 day old Oregon R males were treated and mated to bw; e ♀♀. Individual F1 ♂♂ were mated to bw; e ♀♀ and the F2 was scored for translocations. From the non-translocation cases, again 6-10 individual heterozygous males were mated to bw; e ♀♀ and the F3 scored for translocations. The results are shown in the following table:

F2 and F3 translocation results

	X-rays - 4000r	EMS
No. F1 cultures	216	271
F2 translocations	49	1
No. normal F2 cultures tested	106	110
F3 translocations	0	0

Four treatments were done with EMS (0.018M) fed to adult males by the method of Lewis and Bacher (DIS 43). Since this treatment failed to produce T(X:A)s, a series of 3 X-ray treatments was done. The results of both series are shown in the following table.

Translocation test

		EMS (0.0125M)	X-rays, 4000r
No. males treated		107	105
No. F1 males mated		387	493
No. F1 males sterile		71	59
No. giving no F2 males		10	0
Visible mutations		2 ⁽¹⁾	0
Translocations:			
	T(X;2)	0	3
	T(X;3)	0	7
	T(2;3)	14	37

- (1) One wing mutation, which maps in the region of "rudimentary" and one eye color (w^a -like) for which the F1 male was fractional. He was normal but produced eye-color and normal sons.

In order to reap more benefit from the considerable effort which it is necessary to expend in such experiments, it was decided that an additional experimental condition be added to the EMS series, namely to test the effect of storing treated sperm. Watson (1964 and 1966) reported that a storage effect (enhancement of mutagenic action) was found with the bifunctional TEM and DEB but not with monofunctional EO or EI. Since EMS is monofunctional, it was thought of interest to check it for a storage effect. Although only 20 fertile cultures were obtained from the stored sperm, 3 T (2;3)s were found, as compared to 1 T (Y;2) from 271 fertile cultures from unstored sperm. This result certainly indicates a storage effect and therefore EMS should be further tested in this respect.