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An enhancement of the yield of spontaneous *Minute* mutations by the high temperature of 29°C in the *c3G* female-*ywmf-2* male system of *Drosophila melanogaster*

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Summary

The possible effect of high temperature of 29°C on the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system (Miyamoto, 1992) which is proposed to be responsible for the high yield of production of spontaneous *Minute* mutations was investigated. To determine and compare the yield of spontaneous *Minute* mutations exactly, two different temperature series of experiments were done (29°C versus 22°C). The results show that the yield of spontaneous *Minute* mutations recorded in the *c3G* female-*ywmf-2* male system at 29°C is 3.56 times more than that at 22°C. This finding clearly indicates that the yield of these mutations is enhanced at the high temperature of 29°C. This possibly suggests that the *c3G* female-*ywmf-2* male system may have the temperature dependent nature.

Introduction

Mutagenesis studies in prokaryotic organisms have provided the evidence that some enzymatic steps in DNA repair and genetic recombination are under common genic control (for review see Hanawalt and Setlow, 1975). In *Drosophila*, the evidence suggesting the existence of such a relationship has been obtained(Baker et al., 1976; Boyd et al., 1976; Miyamoto, 1983, 1986, 1990, 1992; Smith, 1976; Watson, 1969, 1972).

In an attempt to shed further light on the relationship between repair, recombination and mutagenesis in *Drosophila*, a series of study on mutagenesis in female and male germ cells of a recombination-defective strain of *D.melanogaster*, *c3G*, was planned. The first series of experiments were done with male germ cells. Evidence was obtained that male germ cells at the meiotic stage of the *c3G* strain show an extraordinarily high sensitivity to γ-ray-induction of hyperploid exceptions,

compared to the corresponding germ cells of the wild-type strain, *Oregon-R* (Miyamoto, 1983). This finding was interpreted to suggest that role of *c3G* or *c3G*$^{+}$ gene may be involved in the process or processes of induction of large structural changes by ionizing radiation, especially during the meiotic stages of spermatogenesis. Such an interpretation prompted us to make a mutagenesis research in female germ cells of *c3G* strain as the second series of experiments. In the course of this series the frequency of spontaneous mutations at the *Minute*, *yellow*, *white*, *miniature*, *forked*, and *dumpy* loci was studied as a first step, where the *c3G* strain females were mated with *y w m f; dp* (*ywmf*) strain males. Unexpextedly, an extraordinarily high yield of spontaneous mutations was recorded at the *Minute* locus in their F_1 progeny. To confirm this finding the frequency of spontaneous *Minute* mutations was investigated in the F_1 progeny of interstrain and intrastrain matings between the *c3G* and *ywmf* strains. As expected, a strikingly high frequency of these mutations was detected in the progeny of *c3G* female x *ywmf* male crosses. No such high yield of *Minute* mutations was recorded in the other three crosses tested (Miyamoto, 1986). These findings were further confirmed in the subsequent study where the frequency of spontaneous *Minute* mutations was elucidated in the F_1 progeny from *c3G* female x *y w m f*/*sc*^{8}(*y*$^{+}$) Y *B*^{s}; *dp* (*ywmf-2*) male crosses (Miyamoto, 1990). To monitor the extent of meiotic defects of the *c3G* females exactly, this *ywmf-2* strain was newly established by introducing the doubly marked Y chromosome into the *ywmf* strain. Expectedly, a strikingly high frequency of *Minute* mutations was detected in the progeny of *c3G* female x *ywmf-2* male crosses. No such a high yield of *Minute* mutations was recorded in the other crosses tested (Miyamoto, 1990). The above findings were interpreted to mean that a combination of *c3G* female and *ywmf* or *ywmf-2* males permit the operation of a system which causes a high production of *Minute* mutations, namely, a system which tends to promote the production of events leading to these mutations. Some kind of premutational damage leading to *Minute* mutations might be produced in such a cross (Miyamoto, 1986, 1990). This system was proposed to be called *c3G* female-*ywmf-2* male system (Miyamoto, 1992). It will be interesting to search for a factor or factors which modifies the function of the *c3G* female-*ywmf-2* male system. The information obtained in such a study will be useful not only for clearfying the nature of possible mechanisms of this high production of *Minute* mutations in this *c3G* female-*ywmf-2* male system, but also for elucidating the nature of the function of that system. In the present study high temperature of 29°C was used as a candidate for such a factor. To evaluate to what extent the function of such a system can be

modified by the high temperature, a comparison was made between the yield of spontaneous *Minute* mutations recorded at 29°C and that at 22°C.

Materials and methods

Two strains of Drosophila melanogaster were used, the recombination-defective *rust c3G* e^s (*c3G*) and *y w m f*/sc^8 (y^+) Y B^s; *dp* (*ywmf-2*) strains (for a description of these markers, see Lindsley and Zim, 1992). The *ywmf-2* strain was made in 1986. This strain retains essentially the same nature as the original *ywmf* strain (Miyamoto, 1986) with regard to the induction of *Minute* mutations when its male is mated with the *c3G* strain female (Miyamoto, 1990). These stocks were cultured on a rice bran, crude sugar, dry yeast, and agar medium at the temperature of 22°C.

In the high temperature series of experiments, the *c3G* females at the age of 3-4 days were mated with the *ywmf-2* males in mass with a ratio of 1 female to 2 males. Immediately after the matings, the parent flies were shifted up to the 29°C. They were transferred daily to fresh food six times and discarded on the 8th day (broods I-VII). Since the number of progeny counted in the brood VII of the 29°C series of the preliminary experiment was small as compared to that in the preceding six broods, the further brooding over the brood VII was not done in the present experiments.

The control series of experiments were done at 22°C using the same mating procedure as that used in the high temperature series.

All the F_1 flies were examined for complete and fractional mutations at the *Minute* locus. The short, thin-bristle, *Minute* phenotype was used for scoring this mutation (Huang and Baker, 1976). Since the yield of fractional *Minute* mutations recorded was not large enough to be analyzed, the data pertaining to these mutations were excluded and will not be considered in this report.

In conjunction with the scoring of the *Minute* mutations, the exceptional females with B^s phenotype and such males with *yellow* (*y*), *white* (*w*), *miniature* (*m*) and *forked* (*f*) phenotype were also detected in the above matings. They were classified as XXY and XO exceptions, respectively.

The statistical tests of the data obtained in the present study were made using Chi-square tests (2 x 2 contingency table) and Kastenbaum and Bowman's tables (Kastenbaum and Bowman, 1970).

Results and discussion

The present study was designed to elucidate whether or not the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system proposed by Miyamoto (1992) is affected by the high temperature of 29°C. Prior to conducting the main experiments, the yield of spontaneous *Minute* mutations in this system at the temperature of 22°C was evaluated (the first series of experiments). This series of experiments was planned and done as a control.

Table 1 Frequencies of *Minute* mutations, B^s (XXY) and *ywmf* (XO) exceptions detected in the *c3G* female-*ywmf-2* male system of *D. melanogaster* at 22°C

Effect studied	Frequency (%)							
	Daily broods							
	I	II	III	IV	V	VI	VII	Total
Minute mutations in total progeny	0.377 (3/795)	0.623 (12/1926)	0.246 (8/3249)	0.518 (17/3279)	0.468 (13/2777)	0.467 (12/2572)	0.482 (11/2280)	0.450 (76/16878)
B^s (XXY) exceptions in female progeny	12.74 (53/416)	16.16 (159/984)	13.20 (216/1637)	15.60 (260/1667)	14.31 (225/1572)	13.96 (185/1325)	12.78 (147/1150)	14.23 (1245/8751)
ywmf (XO) exceptions in male progeny	17.94 (68/379)	17.62 (166/942)	16.13 (260/1612)	15.70 (253/1612)	15.76 (244/1548)	15.16 (189/1247)	17.35 (196/1130)	16.25 (1376/8470)

Data were pooled from 4 replicate experiments. The numbers in parentheses indicate mutants or exceptions/female, male, or total progeny.

Table 1 presents the results of the first series of experiments. As shown in this table, *Minute* mutations were detected with a considerably high as compared with previously reported spontaneous *Minute* mutation frequency (Glass, 1955; Huang, 1977) and almost constant frequency throughout the seven broods. As a total frequency 0.450% is recorded. Unexpectedly, the value of 0.450% is about 60% of that of 0.752% which has already been recorded for these mutations at 25°C (see Table 4). In addition, this difference between these two different temperature series is significant at the 0.1% level in Chi-square tests. This finding clearly indicates that the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system is lowered significantly by lowering the rearing temperature by only three degrees, suggesting that the function of the *c3G* female-*ywmf-2* male system is affected sensitively when the rearing temperature is lowered.

Table 1 also shows the results for the B^s (XXY) and *ywmf* (XO) exceptions. These two types of exceptions represent the two types of X-chromosomal aneuploidy, X-chromosome gain (XXY) and X-chromosome loss (XO). The XXY exceptions

may be used as a more reliable indicator of a defect in the meiotic process, for the incidence of XXY exceptions directly reflects the incidence of X-chromosome non-disjunction. As expected, a very high and constant frequency of B^s exceptions is detected throughout the seven broods. A very high and constant frequency is also recorded for *ywmf* exceptions. In addition, the total frequencies recorded for these two types of exceptions at 22°C are almost equivalent to those at 25°C (see Table 4). This finding indicates that the yield of XXY and XO exceptions in the *c3G* female-*ywmf-2* male system is not affected by lowering the rearing temperature.

In the second (main) series of experiments, immediately after the *c3G* females were mated with the *ywmf-2* males, they were shifted up to 29°C. If the function of the *c3G* female-*ywmf-2* male system is somewhat modified by the high temperature of 29°C, this modification would be reflected as an increase or decrease in the yield of *Minute* mutations recovered.

Table 2 Frequencies of *Minute* mutations, B^s (XXY) and *ywmf* (XO) exceptions detected in the *c3G* female-*ywmf-2* male system of *D. melanogaster* at 29°C

Effect studied	Frequency (%) Daily broods I	II	III	IV	V	VI	VII	Total
Minute mutations in total progeny	2.725 (40/1468)	1.866 (58/3109)	1.094 (30/2742)	1.431 (31/2167)	1.482 (24/1619)	1.566 (16/1022)	0.637 (3/471)	1.603 (202/12598)
B^s (XXY) exceptions in female progeny	14.73 (114/774)	15.60 (249/1596)	15.15 (222/1465)	15.75 (180/1143)	12.63 (108/855)	10.84 (57/526)	11.35 (27/238)	14.51 (957/6597)
ywmf (XO) exceptions in male progeny	20.46 (142/694)	18.37 (278/1513)	19.34 (247/1277)	16.90 (173/1024)	13.61 (104/764)	19.96 (93/466)	13.48 (31/230)	17.90 (1068/5968)

Data were pooled from 6 replicate experiments. The numbers in parentheses indicate mutants or exceptions/female, male, or total progeny.

The results of this series of experiments are shown in Table 2. As can be seen in this table, an extraordinarily high frequency of *Minute* mutations is recorded in brood I, very high frequency in broods II-VI and a considerably high frequency in brood VII. As a total frequency 1.603% is recorded in these mutations. This total frequency is significantly higher not only than that of 0.450% at 22°C but also than that of 0.752% at 25°C (see Table 4). This finding clearly indicates that the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system is enhanced greatly by elevating the rearing temperature. This may be interpreted to mean that the function of the *c3G* female-*ywmf-2* male system is affected greatly when the

rearing temperature is raised.

Clearly from Table 2, an almost the same tendency as that observed at 22°C is found in the frequency patterns for the B^s and *ywmf* exceptions over the seven broods. The total frequencies for these two types of exceptions recorded at 29°C are almost equivalent to those at 22°and 25°C (see Table 4). This finding indicates that the yield of XXY and XO exceptions in the *c3G* female-*ywmf-2* male system is not affected by elevating the rearing temperature. This is in highly contrast with the observation for the *Minute* mutations in this system.

Table 3 Comparison of the frequencies of the *Minute* mutations, B^s (XXY) and *ywmf* (XO) exceptions recorded between two different temperature series

Effect studied	Temp. (°C)	Frequency (%)±SE Daily broods I	II	III	IV	V	VI	VII	Total
Minute mutations in total progeny	a. 29	2.725±0.425**	1.866±0.243**	1.094±0.199**	1.431±0.255**	1.482±0.300**	1.566±0.388**	0.637±0.367	1.603±0.112***
	b. 22	0.377±0.217	0.623±0.179	0.246±0.087	0.518±0.125	0.468±0.130	0.467±0.134	0.482±0.145	0.450±0.052
	a/b	7.23	3.00	4.45	2.76	3.17	3.35	1.32	3.56
B^s (XXY) exceptions in female progeny	a. 29	14.73±1.27	15.60±0.91	15.15±0.94	15.75±1.08	12.63±1.14	10.84±1.36	11.35±2.06	14.51±0.43
	b. 22	12.74±1.64	16.16±1.17	13.20±0.84	15.60±0.89	14.31±0.88	13.96±0.95	12.78±0.99	14.23±0.37
	a/b	1.16	0.97	1.15	1.01	0.88	0.78	0.89	1.02
ywmf (XO) exceptions in male progeny	a. 29	20.46±1.53	18.37±1.00	19.34±1.11	16.90±1.17	13.61±1.24	19.96±1.85	13.48±2.25	17.90±0.50
	b. 22	17.94±1.97	17.62±1.24	16.13±0.92	15.70±0.91	15.76±0.93	15.16±1.02	17.35±1.13	16.25±0.40
	a/b	1.14	1.04	1.20	1.08	0.86	1.32	0.78	1.10

, * Significant at the 1 and 0.1% level from the 22°C series, respectively.

To determine more precisely the degree of enhancement of the yield of *Minute* mutations in the *c3G* female-*ywmf-2* male system when the parent flies are kept at the high temperature of 29°C for seven days. The results of the above two series of experiments are compared in Table 3. In this table, the first and second series of experiments are designated as b and a, respectively. In order to quantify to what extent the yield of *Minute* mutations is enhanced by the high temperature, the ratio of a to b is calculated and given in the row of a/b. Values of 1.32-7.23 are obtained for these *Minute* mutations. These figures clearly indicate an enhancement of the yield of *Minute* mutations in the *c3G* female-*ywmf-2* male system throughout the

seven broods, suggesting that some kind of promotion of the premutational events to *Minute* mutations might have occurred at this high temperature. Such figures also show that there exist some differences in the extent of enhancement among the broods. The highest enhancement in brood I, moderate one in broods II-VI, and the lowest one in brood VII. At present no satisfactory explanation can be offered for these differences.

For the B^s and *ywmf* exceptions, ratios of 0.78-1.16 and 0.78-1.32 are calculated, respectively. These values may indicate that the above enhancement might not have occurred for these two types of exceptions. It may be said that the process or processes involved in the formation of B^s and *ywmf* exceptions in the *c3G* female-*ywmf-2* male system are not affected by the high temperature of 29°C.

Table 4 Comparison of the total frequencies of *Minute* mutations, B^s (XXY) and *ywmf* (XO) exceptions recovered among three different temperature series

Effect studied	Frequency (%)±SE		
	22°C	25°C*	29°C
Minute mutations in total progeny	0.450±0.052 (76/16878)	0.752±0.071 (111/14763)	1.603±0.112 (202/12598)
B^s (XXY) exceptions in female progeny	14.23±0.37 (1245/8751)	14.76±0.40 (1136/7698)	14.51±0.43 (957/6597)
ywmf (XO) exceptions in male progeny	16.25±0.40 (1376/8470)	17.06±0.45 (1205/7065)	17.90±0.50 (1068/5968)

*See Miyamoto (1990).
The numbers in parentheses indicate mutants or exceptions/female, male, or total progeny.

Probably, the *c3G* female-*ywmf-2* male system (Miyamoto, 1992) may function with some kind of unstable nature, which is proposed to be responsible for producing the events leading to an extraordinarily high yield of spontaneous *Minute* mutations. If so, the function of this system may be affected by a factor or factors which modifies the unstable nature. The result of an earlier study (Miyamoto, 1992) revealed that the frequency of X-ray-induced *Minute* mutations was enhanced greatly when the various kinds of lesions induced by X-rays were introduced into the above

system as such a factor. This was done by mating the X-irradiated *ywmf-2* males every second day with the *c3G* female six times. It also revealed that the magnitude of enhancement was highly dependent on the stages of spermatogenesis involved, where different amounts and kinds of premutational damages were induced by X-rays. These findings were interpreted to mean that the unstable state of the *c3G* female-*ywmf-2* male system might become more unstable by the nature and quantity of an additional load introduced, resulting in the enhanced production of the events leading to *Minute* mutations.

Present results have revealed that the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system is enhanced greatly when the parent flies are kept at the high temperature of 29°C as compared to that at 22°C, suggesting that further promotion in the production of the premutational events to *Minute* mutations might have occurred at this high temperature. They have also revealed that when the yield of such mutations at 22°C is compared with that at 25°C, the former is significantly lower than the latter (see Table 4). In view of the above reasoning, it may be concluded that the *c3G* female-*ywmf-2* male system have some kind of unstable nature. It may also be said that the unstable state of the *c3G* female-*ywmf-2* male system can be made more unstable by an additional load such as X-ray-induced lesions which are introduced into this system or by the high temperature of 29°C under which this system functions. Such an unstable state, however, seems somewhat stabilized by the low temperature of 22°C. In another word, the *c3G* female-*ywmf-2* male system may have load dependent and temperature dependent nature. The detailed nature of the *c3G* female-*ywmf-2* male system, however, still remains to be elucidated.

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