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Effect of caffeine on the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system of *Drosophila melanogaster*

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Abstract

The possible effect of caffeine on the *c3G* female-*ywmf-2* male system which is proposed to be responsible for an extraordinarily high yield of production of spontaneous *Minute* mutations (Miyamoto, 1992) was studied. Prior to mating, the *c3G* females were fed on caffeine in honey solution at their age of 0-8 h for three days. These females were mated and allowed to lay their eggs for 12 days by transferring them every day to fresh food(summarized as six two -day broods, broods A-F). The yield of spontaneous *Minute* mutations was determined and compared between the caffeine and non-caffeine treated series of experiments. The results show that the yield of spontaneous *Minute* mutations recorded in broods A and D of caffeine series is significantly lower than that in the non-caffeine series. The significant difference between these two series is also observed in the total yield of these mutations of the six broods. These findings clearly indicate that the yield of *Minute* mutations is reduced by the caffeine pretreatment of *c3G* females. This possibly suggests that the *c3G* female-*ywmf-2* male system may have a caffeine sensitive nature.

INTRODUCTION

Mutagenesis research in prokaryotic organisms has provided the evidence that there exists a close relationship between DNA repair, genetic recombination and mutagenesis (for a 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, 1995 ; Smith, 1976 ; Watson, 1969, 1972).

In an attempt to elucidate further 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 undertaken. During the course of mutagenesis research in female germ cells, an extraordinarily high yield of spontaneous

Minute mutations was recorded incidentally in the F_1 progeny of *c3G* strain female×*y w m f*; *dp* (*ywmf*) strain male crosses. This finding was confirmed in the next study where 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×*ywmf* male crosses. No such a high yield of spontaneous *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 determined using the *c3G* strain females and *y w m f*/$sc^8(y^+)$ Y B^s; *dp* (*ywmf-2*) strain males (Miyamoto, 1990). This *ywmf-2* strain was newly established by introducing the doubly marked Y chromosome into the *ywmf* strain. Expectedly, a strikingly high frequency of spontaneous *Minute* mutations was detected only in the progeny of *c3G* female×*ywmf-2* male crosses. The above findings were interpreted to mean that a combination of *c3G* female and *ywmf* or *ywmf-2* males, recombination-defective strain female and recombination-proficient strain male, permit the operation of a system which causes a high production of spontaneous *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 named *c3G* female-*ywmf-2* male system (Miyamoto, 1992).

To clarify the possible mechanisms of an extraordinarily high production of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system, possible factor or factors which modulates the function of the *c3G* female-*ywmf-2* male system was searched. The information obtained in such a study will be useful also for elucidating the nature of the function of that system. High temperature of 29℃ was used as the first candidate for such a factor (Miyamoto, 1995). The results show that the yield of spontaneous *Minute* mutations recorded in the *c3G* female-*ywmf-2* male system at 29℃ is about four times more than that at 22℃. This finding clearly indicates that the yield of these mutations is enhanced at the high temperature of 29℃. This possibly suggests that the *c3G* female-*ywmf-2* male system may have a temperature dependent nature.

In the present study caffeine was used as the second candidate for the above factor. It is well known that caffeine has a potentiating effect on damage induced by X-rays or chemical

mutagens. Its potentiating effect is related to its ability to cancel the G2 arrest (Painter and Young, 1980 ; Kihlman et al., 1982 ; Lau and Pardee, 1982 ; Lucke-Huhle et al., 1983, Rowley et al., 1984) and to inhibit DNA-repair (Gonzalez-Fernandez et al., 1985). In addition, the protective action of caffeine on the radiation-induced chromosomal damage and on the clastogenic and carcinogenic chemicals is reported in recent years (Kesavan and Natarajan, 1985 ; Wattenberg, 1985 ; Ito et al., 1989 ; Abraham, 1991). More recently, Stoilov et al. (1994) has reported that caffeine treatment causes either potentiation or protection against induction of chromosomal aberration by radiation. When the caffeine is given to the *c3G* females as pretreatment, the caffeine molecules would be present in the body fluids of these females and in the mature oocyte formed by them until the caffeine molecules are disintegrated. During the operation of the *c3G* female-*ywmf-2* male system the caffeine molecules would in turn react with the spontaneously produced premutational damage which is transient in its nature and may result in an increase or decrease in the yield of *Minute* mutations. Thus, it is quite interesting to know whether or not the frequency of spontaneous *Minute* mutations produced in this *c3G* female-*ywmf-2* male system is modulated by the caffeine treatment. To evaluate the extent of modulation of the *c3G* female-*ywmf-2* male system by caffeine, the frequency pattern of spontaneous *Minute* mutations in the caffeine series was compared with that in the non-caffeine series.

MATERIALS AND METHODS

The strains of *Drosophila melanogaster* used were the recombination-defective *ru st 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 nature of *ywmf-2* strain is described elsewhere (see Miyamoto, 1986, 1990). These two stocks were cultured on a rice bran, molasses, dry yeast, and agar medium (normal fly food) at the temperature of 25℃.

In the caffeine treatment series of experiments, the *c3G* females at the age of 0-8 h were fed on 0.2% caffeine in 0.5% honey solution for three days and were mated with the *ywmf-2* males in mass with a ratio of 1 female to 2 males. The parent flies were transferred daily to fresh normal fly food 11 times and discarded on the 13th day (these 12 egg-laying periods are summarized for 6 two-day broods, broods A-F). Fresh males were supplied on the 7th day to

promote the egg-laying of the *c3G* females.

The control series of experiments was done by feeding the *c3G* females on normal fly food and using the same mating and egg-laying procedure as that used in the caffeine series. In addition, another control series was done by feeding such females on honey solution.

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 on these mutations were not included in the present analysis.

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 F_1 progeny of the above matings. They were classified as XXY and XO exceptions, respectively. The incidence of these exceptions was used as a biological indicator of the meiotic defects occurred in the *c3G* females.

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

RESULTS AND DISCUSSION

The present study was designed to examine whether or not the function of the *c3G* female-*ywmf-2* male system proposed by Miyamoto (1992) which is responsible for the high yield of spontaneous *Minute* mutations is affected by the caffeine pretreatment of the *c3G* females. If the function of the *c3G* female-*ywmf-2* male system is somewhat modulated by the caffeine pretreatment of these females, this modulation would be reflected as an increase or decrease in the yield of *Minute* mutations recovered. Three series of experiments were planned and done in the present study. The first series of experiments was done as a control, where the *c3G* females were fed on normal fly food. In the second series these females were fed on honey. The third series was done as main experiments, where such females were fed on caffeine in honey. In each series of experiments the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system was evaluated.

Table 1 summarize the results on the yield of *Minute* mutations recorded in the three series of

experiments. In this table, the 12 egg-laying periods were given as 6 successive two-day broods (broods A-F). As shown in this table, the mutations were detected with a considerably high frequency as compared with that previously reported for spontaneous *Minute* mutation (Glass, 1955 ; Huang, 1977) throughout the 6 broods of the three series of experiments.

Table 1 Effect of caffeine on the induction of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system. The *c3G* females were fed on caffeine for three days when they were 0-8-hour-old

Expt. series	Frequency (%) Two-day broods							
	Brood A	Brood B	Brood C	Brood D	Brood E	Brood F	Total	Ratio
Control[a]	1.771 (70/3953)	0.600 (51/8499)	0.665 (43/6463)	1.044 (38/3641)	1.066 (31/2907)	0.548 (12/2188)	0.886 (245/27651)	1.00
Honey[b]	1.213* (69/5688)	0.630 (61/9676)	0.601 (47/7825)	0.821 (43/5238)	0.588 (18/3063)	0.740 (12/1622)	0.755 (250/33112)	0.85
Honey+ caffeine[c]	0.964** (47/4876)	0.574 (73/12710)	0.546 (56/10265)	0.574* (43/7497)	0.930 (49/5267)	0.791 (20/2527)	0.668** (288/43142)	0.75

*, **Significant at the 5 and 1% level from the control, respectively.
[a] Data were pooled from 2 replicate experiments.
[b] Data were pooled from 3 replicate experiments.
[c] Data were pooled from 4 replicate experiments.
The numbers in parentheses indicate mutants/total progeny.

It is noted that when intra-series comparisons are made, the highest frequency of *Minute* mutations is recorded in the first brood, brood A. This was found throughout the three series of experiments, in spite of the difference in the pretreatment of the *c3G* females. The brood where the second highest frequency was observed, however, is not necessarily coincident among the three series, broods D and E in the control series, brood D in the honey series, and with some delay in the caffeine series, in brood E. At present no satisfactory explanation can be offered for such a delay in the caffeine series. As stated in the section of "MATERIALS AND METHODS", fresh males were supplied in broods A and D. In these two broods the highest

and the second highest frequency of *Minute* mutations are recorded. It may be said that the yield of spontaneous *Minute* mutations produced in the *c3G* female-*ywmf-2* male system seems to be enhanced by mating the *c3G* females with fresh *ywmf-2* males.

When the yield of *Minute* mutations recorded in brood A is compared among the three series of experiments (inter-series comparison), it is found that the frequency observed in the control series is the highest and that observed in the caffeine series is the lowest. The difference in the yield of these mutations between the control and caffeine series is significant. Almost the same tendency as that found in brood A is also observed in brood D. Total frequency also shows a clear difference between the control and caffeine series. This may be a reflection of the large difference observed in broods A and D. These findings clearly indicate a caffeine effect.

As compared to other series of experiments, the number of progeny per treated female per egg-laying period of the caffeine series is remarkably reduced especially in the first egg-laying period, but not in the subsequent ones. About one-fifth of that of the non-caffeine series is estimated (Data not shown). This is quite probably due to the effect of caffeine. Since this kind of caffeine effect does not persist over the second egg-laying period, caffeine may be metabolized relatively rapidly in the *c3G* female. If so, the possible modulation by caffeine pretreatment of the *c3G* females will probably appear in the first or the second egg-laying periods, namely, in the first brood (brood A). As expected, a clear modification in the yield of *Minute* mutations is found in brood A. The frequency recorded of these mutations in the caffeine series is significantly lower than that in the control one. The finding that such a modification is also observed in brood D is, however, rather unexpected in view of the metabolic nature of the caffeine. At present no satisfactory explanation can be provided for such an unexpected outcome. Anyway, these findings may indicate that the yield of spontaneous *Minute* mutations in the *c3G* female-*ywmf-2* male system is lowered by the caffeine pretreatment of the *c3G* females, suggesting that the function of the *c3G* female-*ywmf-2* male system is affected sensitively when the females are fed on caffeine prior to their matings.

It may worth pointing out that in brood A the yield of *Minute* mutations recorded in the honey series is also significantly lower than that in control. The honey has some kind of deficiency in the nutritious content as compared to the normal fly food. This finding may indicate that the yield of *Minute* mutations is lowered by some kind of deficiency in the nutrition which *c3G*

female has taken prior to mating. In this context, the following observation is also interesting. In broods B and C where the *c3G* female have a chance to take enough the normal fly food prior to their egg-laying, there seems to be no essential difference in the yield of *Minute* mutations recovered between the three different pretreatment series of experiments. This may suggest that the *c3G* female-*ywmf-2* male system is also affected to some extent by the nutritious content of food the *c3G* female took.

The above reduction by the caffeine treatment was not observed in the yield of the B^s (XXY) and *ywmf* (XO) exceptions. These two types of exceptions represent the two types of X-chromosomal aneuploidy. They may be used as an indicator of a defect in the meiotic process. The results for the B^s and *ywmf* exceptions are presented in Tables 2 and 3, respectively.

Table 2 Effect of caffeine on the induction of B^s female exceptions in the *c3G* female-*ywmf-2* male system. The *c3G* female were fed on caffeine for three days when they were 0-8-hour-old

Expt. series	Frequency (%)							
	Two-day broods							
	Brood A	Brood B	Brood C	Brood D	Brood E	Brood F	Total	Ratio
Control[a]	15.78 (324/2053)	14.97 (655/4376)	14.10 (467/3312)	15.39 (295/1917)	14.88 (228/1532)	15.08 (165/1094)	14.94 (2134/14284)	1.00
Honey[b]	16.35 (480/2936)	14.95 (748/5004)	15.34 (609/3970)	15.08 (408/2706)	13.70 (216/1577)	14.37 (121/842)	15.16 (2582/17035)	1.02
Honey+ caffeine[c]	16.65 (413/2481)	13.98 (912/6523)	14.62 (764/5224)	13.79 (535/3880)	15.22 (413/2713)	12.38 (156/1260)	14.46 (3193/22081)	0.97

[a] Data were pooled from 2 replicate experiments.
[b] Data were pooled from 3 replicate experiments.
[c] Data were pooled from 4 replicate experiments.
The numbers in parentheses indicate exceptions/female progeny.

As shown in the Table 2, a very high and almost constant frequency of B^s exceptions is detected throughout the six broods of the three series of experiments. No reduction is observed in the frequency of these exceptions in the caffeine series as compared to the non-caffeine

series. This finding indicates that the yield of B^s exceptions in the *c3G* female-*ywmf-2* male system is not affected by the caffeine treatment of the *c3G* females.

Table 3 Effect of caffeine on the induction of *ywmf* male exceptions in the *c3G* female-*ywmf-2* male system. The *c3G* females were fed on caffeine for three days when they were 0-8-hour-old

Expt. series	Frequency (%)							
	Two-day broods							
	Brood A	Brood B	Brood C	Brood D	Brood E	Brood F	Total	Ratio
Control[a]	19.47 (370/1900)	18.58 (766/4123)	16.38 (516/3151)	17.11 (295/1724)	20.58 (283/1375)	17.18 (188/1094)	18.09 (2418/13367)	1.00
Honey[b]	20.57 (566/2752)	17.69 (827/4674)	17.54 (676/3855)	17.58 (445/2532)	17.09 (254/1486)	16.67 (130/780)	18.02 (2898/16079)	1.00
Honey+ caffeine[c]	18.87 (452/2395)	16.86 (1043/6187)	18.83 (949/5041)	17.45 (631/3617)	17.14 (431/2514)	17.34 (218/1257)	17.72 (3724/21011)	0.98

[a] Data were pooled from 2 replicate experiments.
[b] Data were pooled from 3 replicate experiments.
[c] Data were pooled from 4 replicate experiments.
The numbers in parentheses indicate exceptions/male progeny.

Clearly from Table 3, an almost the same tendency as that observed with the B^s exceptions is found in the frequency patterns for the *ywmf* exceptions over the six broods of the three series of experiments. This finding indicates that the frequency of *ywmf* exceptions produced in the *c3G* female-*ywmf-2* male system is not affected by the caffeine treatment.

The above findings clearly indicate that the yield of XXY and XO exceptions produced in the *c3G* female-*ywmf-2* male system is not affected by feeding the *c3G* females on caffeine. This is in highly contrast with the observation for the *Minute* mutations in this system. It may be said that the process or processes involved in the formation of XXY and XO exceptions in the *c3G* female-*ywmf-2* male system are not affected by the caffeine pretreatment of the *c3G* females. The incidence of XXY and XO exceptions directly or indirectly reflects the incidence of X-chromosome non-disjunction, which is due to some kind of defect in the meiotic process of the *c3G* female. Therefore, it may also be said that this kind of defect of which formation is

closely relates to the meiotic process of the *c3G* female is not affected by the caffeine treatment.

Probably, the *c3G* female-*ywmf-2* male system (Miyamoto, 1992) may operate with some kind of unstableness. This unstableness may be closely related with producing the events leading to an extraordinarily high yield of spontaneous *Minute* mutations. A factor or factors which modulates this unstableness may affect the function of this system, thus resulting in an increase or decrease in the yield of *Minute* mutations produced by this system. The result of an earlier study (Miyamoto, 1992) revealed that the yield of *Minute* mutations recorded in the *c3G* female-*ywmf-2* male system was enhanced greatly when the various kinds of lesions induced by X-rays in the various stages of spermatogenesis of the *ywmf-2* males were introduced into the above system as such a factor. It also revealed that the magnitude of enhancement was highly dependent on the stages of spermatogenesis 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 by the *ywmf-2* males, resulting in the enhanced production of the events leading to *Minute* mutations.

Recent results (Miyamoto, 1995) 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℃ as compared to that at 22℃, 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℃ is compared with that at 25℃, the former is significantly lower than the latter. This finding may suggest that even at 25℃ the yield of spontaneous *Minute* mutations produced in such a system is enhanced.

In contrasts, the present results have revealed that the yield of spontaneous *Minute* mutations recorded in the caffeine series is lower than that in the non-caffeine series in the two broods (broods A and D), where the fresh *ywmf-2* males were supplied and the first and the second highest frequency is recorded in the yield of *Minute* mutations in the control series. In the remaining four broods, there is no essential difference in the yield of these mutations among these broods. These findings clearly indicate that the *c3G* female-*ywmf-2* male system is modulated by caffeine pretreatment of the *c3G* females and that this modulation seems to be

evident in the condition under which the *c3G* females are provided with the fresh *ywmf-2* males. In view of the above reasoning, it may be concluded that the *c3G* female-*ywmf-2* male system has unstable nature, which seems to be closely connected with the production of premutational damage leading to the spontaneous *Minute* mutations. It may also be said that the unstableness of the *c3G* female-*ywmf-2* male system can be made more unstable by an additional load such as X-ray-induced lesions introduced into this system, by the high temperature of 29℃ under which this system functions, or by mating the *c3G* females with fresh *ywmf-2* males. Such an unstable state, however, seems somewhat stabilized by the low temperature of 22℃ and caffeine pretreatment of the *c3G* females. In another word, the *c3G* female-*ywmf-2* male system may have load, temperature, caffeine and mating dependent nature. The detailed nature of the *c3G* female-*ywmf-2* male system, however, still remains to be elucidated.

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