PARAMETERS OF MITOTIC RECOMBINATION IN MINUTE MUTANTS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

A sample of 16 Minutes, representing 12 loci distributed over all the chromosome arms and including 3 pairs of alleles and 4 deficiencies, has been studied with respect to several developmental and recombinational parameters. Cell marker mutants located in most of the chromosome arms were used to assess (1) spontaneous and X-ray-induced mitotic recombination frequencies of each Minute, and (2) clone sizes of the different cell marker clones. These parameters were analyzed both in the wing disc and in the abdominal histoblasts. —— Whereas spontaneous frequencies are not affected by the presence of the Minutes studied, the different Minutes characteristically increase the frequency of recombination clones arising after X-irradiation. The recombinant clones which are $M+/M+$ are significantly larger than clones in the same fly which retain the $M+/M$ condition. This is particularly striking in clones in the wing disc, slightly so in clones in the tergites. The occurrence of mitotic recombination in the fourth chromosome is reported for the first time. —— Chaeta length and developmental delay correlates with the recombinational parameters in different ways. Possible causal interrelationships of the different traits of the Minute syndrome are discussed.

THE Minute mutations of *Drosophila melanogaster* were first described by BRIDGES (BRIDGES and MORGAN 1923) and characterized phenogenetically as a group by SCHULTZ (1929). Minutes are recessive lethal mutations, located throughout the genome, with a series of common dominant characteristics, such as small chaetae, plexated wings, abnormal facets in the eye, developmental delay, etc. Minutes also present peculiar genetic properties. STERN (1936) and KAPLAN (1953) showed that Minutes led to an increase of spontaneous mitotic recombination in their own chromosome arm. Recently MIKLOS (1972) ascribed a new characteristic to them, that of inducing nondisjunction of the *X* chromosome.

STERN and TOKUNAGA (1971) showed in genetic mosaics that both chaeta differentiation and cell lethality of some Minutes are cell autonomous in their expression. MORATA and RIPOLL (1975) demonstrated the autonomous condition of the mitotic division rate for three Minute mutants. In the present paper we will analyze and correlate mitotic recombination parameters with other developmental parameters of Minutes with the aims of (1) understanding the common basis of the Minute phenotype and of (2) exploring the usefulness of different

Minutes in clonal analysis. Minutes located in all chromosome arms, deficiencies for some Minute loci and several groups of alleles were chosen for the present study.

MATERIALS AND METHODS

Stocks employed

a) Minute stocks: *M(1)o/FM6*; *M(1)o^{sp}/FM6*; *T(1,2)Bld/Clb* (the $2^{D}X^{P}$ aneuploid segregant from *T(1,2)Bld* is *M(1)Bld*); *M(2)l^{2}/SM1*; *M(2)S7/SM5*; *M(2)H^{s5}/SM5*; *Df(2R)M-S2^{10}/SM1*; *Df(2R)M-c^{33a}/In(2LR)bw^{v32g}*; *M(3)i^{55}/TM1*; *M(3)h^{y}/In(3L)P*; *M(3)h^{S37}/In (3L)P*; *M(3)w/In(3R)C*; *M(3)w^{124}/In(3R)C*; *M(3)be^{36e}/In(3R)C*; *M(4)57g/ci^{D}*; *DfM(4) 63a/ci^{D}*.

b) Two stocks carrying several cell marker mutants: (i) multiple marker stock I: *al dp pwn/SM5*; *mwh bld/TM2*; (ii) multiple marker stock II: *y; mwh Sb/T(2,3)ap^{Xa}, mwh*. Both stocks share a common cell marker, *mwh*, in order to detect possible variations in frequency due to differences in the genomes (WEAVER 1960).

c) A *sv^{de}/ey^{D}* stock to study recombination in the 4th chromosome.

d) Vallecas wild-type strain. For description of all mutants except *pwn* and *bld* (GARCIA-BELLIDO and DAPENA 1974) see LINDSLEY and GRELL (1968). The mitotic map (see GARCIA-BELLIDO and DAPENA 1974) for the cell markers used and the approximate locations of the Minutes are shown in Figure 1. The scorability of the cell marker mutants used is very good, as described elsewhere (GARCIA-BELLIDO 1972; GARCIA-BELLIDO and DAPENA 1974).

Except for both *M(1)o* alleles, the Minute chromosome was always carried by the parental males. Heterozygous flies for the different Minutes and all the marker chromosomes were selected in the offspring of crosses of Minute-carrying and marker-carrying stocks. Data of male and female offspring were pooled. These crosses were made from two replicated samples of offspring from the same parents: one was irradiated and the other not. As a rule the data of three separate irradiations were pooled. Data of experiments utilizing Minute flies were compared with those

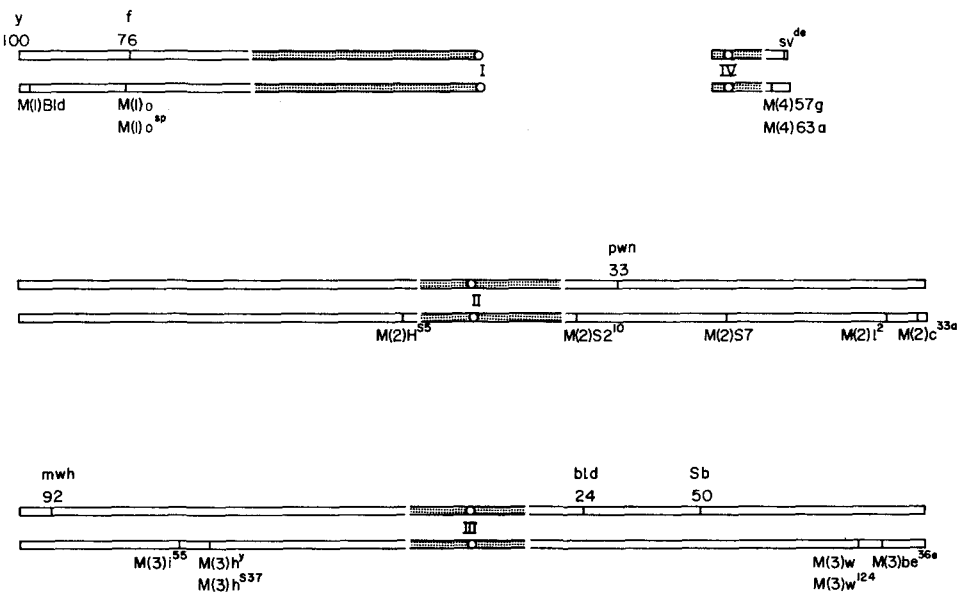

FIGURE 1.—Mitotic map for the cell markers used (GARCIA-BELLIDO and DAPENA 1974) and relative locations of the Minutes.

of wild-type flies. The latter experiments were also done in two kinds of replicas: irradiated and non irradiated.

Larvae of all ages between 48 and 72 hrs., starting from egg deposition, were irradiated with X-rays (Philips MG 151 Be, 150 r/min., 100 kV, 15 mA and 2mm. Al filiter) with a total dose of 500 r. The flies were mounted in Euparal for microscopic examination. In order to estimate the developmental delay the non-irradiated replicas of the Minute experiments mentioned above were used. Delay was evaluated as the time lapse between the moments when 50% of the Minute and 50% of the non-Minute adults emerged, after a 24-hour egg laying period. All experiments were carried out at 25 ± 1°. Length of chaetae was measured in the 15 first-hatching males from crosses between the different Minute males and wild-type females. Minute females were employed when Minutes located in the *X* chromosome were considered. The length of presutural, posterior dorsocentral and anterior scutellar chaetae was measured with a scale ocular.

In order to evaluate the data, a test of statistical significance was applied to detect differences not due to sampling between the Minutes and the wild-type controls. The distribution of flies into classes according to the number of abdominal clones present follows a Poisson distribution (GARCIA-BELLIDO and MERRIAM 1971b). The value of the following coefficient was calculated: $(f_c - f_M)/\sigma_D$, f_c being the clone frequency in wild-type control flies, f_M the clone frequency in the Minute flies tested, and σ_D the standard deviation between these two frequencies. The numbers were considered significantly different when this value was higher than that of "*t*" for $n_c + n_M - 2$ degrees of freedom (n_c being the number of wild-type control flies and n_M the number of Minute flies), and 0.05 or 0.10 as limits of significance.

RESULTS

I. *Mitotic Recombination in the Abdominal Histoblasts*

The clonal analysis of GARCIA-BELLIDO and MERRIAM (1971b), and GUERRA, POSTLETHWAIT and SCHNEIDERMAN (1973) shows that the imaginal cells (histoblasts) of the tergites remain quiescent throughout larval development. Mitotic recombination frequencies were evaluated in flies irradiated in the larval period 48–72 hrs. after egg deposition. Tergites II to VI have been scored and only spots embracing two or more chaetae were counted (see GARCIA-BELLIDO 1972).

The data of spontaneous mitotic recombination in Minute flies are shown in Table 1. Due to the yellow condition of *M(1)Bld* flies, f^{36a}; e^{11} females were crossed to *T(1,2)Bld* males and the frequency for f^{36a} was calculated. Spontaneous frequencies of Minutes relative to wild-type controls are not significantly different except in three cases. Two of the three cases involve *mwh* clones in multiple marker stock II, but in both cases the frequency of *mwh* clones utilizing stock I was no different in the Minute and in the wild-type flies. In general, frequencies of *mwh* in crosses involving stock II are slightly higher than those involving stock I, which suggests a variability due to genome differences (WEAVER 1960). The third exception corresponds to the frequency of f^{36a} in *M(1)Bld* flies. It could be interpreted in the same way, but another possible explanation will be considered in the DISCUSSION.

The frequencies of X-ray-induced mitotic recombination in Minute flies are presented in Table 2. Some Minutes (*M(1)Bld*, *M(1)*o^{sp}, *M(2)*l^{s}, *M(2)S7*, *M(3)*i^{55}, *M(3)*h^{y}, *M(3)*h^{S37}, *M(4)57g*, *Df(4)*M^{63a}) show a significant increase for markers located in all or most of the chromosome arms tested (*1, 2R, 3L, 3R*). Another group of them (*M(2)*H^{S5}, *Df(2R)M-S2*^{10}, *Df(2R)M-*c^{33a}, *M(3)*be^{36e})

TABLE 1

Frequencies (per abdomen) of spontaneous clones in tergites II-VI

Minute	n	*pwn* 2R	*bld* 3R	*mwh* 3L	n	*mwh* 3L	*y* 1	*Sb+* 3R
WT-Controls	200	0.03	0.02	0.06	200	0.09	0.30	0.13
M(1)Bld	70	0.03	0.01	0.10	50	0.14	a	0.18
M(1)o	90	0.02	0.02	0.12	80	0.10	0.31	0.13
M(1)o^{sp}	70	0.04	0.03	0.15	60	0.06	0.28	0.09
M(2L)H^{s5}	126	0.03	0.01	0.04	160	0.06	0.15	0.09
M(2R)l^{2}	157	0.04	0.04	0.04	192	0.10	0.40	0.18
M(R)S7	84	0.03	0.01	0.04	67	0.10	0.32	0.17
M(2R)S2^{10} (Df)	100	0.00	0.03	0.04	73	0.08	0.31	0.11
M(2R)c^{s3a} (Df)	87	0.02	0.04	0.10	41	0.08	0.33	0.11
M(3L)i^{55}	140	0.03	0.01	0.05	90	0.31**	0.36	0.12
M(3L)h^{y}	111	0.03	0.01	0.07	85	0.18	0.41	0.18
M(3L)h^{S37}	100	0.05	0.01	0.06	70	0.11	0.20	0.13
M(3R)w	80	0.03	0.02	0.04	70	0.08	0.18	b
M(3R)w^{124}	141	0.02	0.01	0.03	75	0.05	0.23	b
M(3R)be^{s6e}	80	0.03	0.01	0.03	70	0.08	0.20	b
M(4)57g	107	0.02	0.01	0.07	87	0.13	0.24	0.14
M(4)63a (Df)	120	0.04	0.02	0.03	65	0.27**	0.33	0.17

Cell marker mutants carried for the following stocks:
(i) Multiple marker stock I: *al dp pwn/SM5; mwh bld/TM2*
(ii) Multiple marker stock II: *y; mwh Sb/T(2,3)ap^{Xa}, mwh.*
n = Number of abdomens scored.
** Significantly different ($P < 0.05$) from WT-Controls.
a: Frequency of *f^{s6a}* in WT-Control flies is 0.31. Frequency of *f^{s6a}* in *M(1)Bld* flies is 0.69*.
b: *Sb/Sb* clones indistinguishable from *Sb/Sb+* clones.

TABLE 2

X-ray-induced (500r) clone frequencies in the tergites II–VI

Minute	n	*pwn* 2R	*bld* 3R	*mwh* 3L	n	*mwh* 3L	*y* 1	*Sb+* 3R
WT-Controls	123	0.28	0.14	0.40	70	0.46	1.20	0.40
M(1)Bld	60	0.41	0.16	0.80**	40	1.17**	a	0.67*
M(1)o	155	0.41*	0.16	0.53	52	0.65	1.43	0.63*
M(1)o^{sp}	81	0.41	0.23	0.58*	40	0.60	1.85**	0.80**
M(2L)H^{s5}	74	0.28	0.10	0.30	40	0.46	1.36	0.54
M(2R)l^{2}	107	0.70**	0.23	0.60**	45	0.81**	2.30**	1.00**
M(2R)S7	53	0.77**	0.30*	0.50	55	0.40	1.70**	0.72**
M(2R)S2^{10} (Df)	50	0.06	0.20	0.35	45	0.40	1.18	0.39
M(2R)c^{s3a} (Df)	50	0.38	0.17	0.33	40	0.40	1.93**	0.48
M(3L)i^{55}	179	0.43**	0.18	0.52	42	1.31**	1.00	0.80**
M(3L)h^{y}	118	0.45**	0.20	0.70**	54	1.09**	1.60*	0.80**
M(3L)h^{S37}	148	0.42*	0.26*	0.44	40	0.66	1.45	0.60
M(3R)w	90	0.33	0.18	0.20**	50	0.27*	1.50	b
M(3R)w^{124}	100	0.47**	0.12	0.14**	60	0.12**	1.08	b
M(3R)be^{s6e}	108	0.45**	0.20	0.30	50	0.45	1.19	b
M(4)57g	73	0.43*	0.13	0.50	40	0.60	1.60*	0.70**
M(4)63a (Df)	100	0.47**	0.20	0.40	50	0.50	1.51	0.71**

* Significantly different ($P < 0.10$) from WT-Controls.
a: Frequency of *f^{s6a}* in WT-Control flies is 1.04. Frequency of *f^{s6a}* in *M(1)Bld* flies is 3.00.**
Remaining symbols are as in Table 1.

show no increase. Both alleles of *M(3)w* show an apparent *decrease* in the marker located in the *3L* chromosome arm. The low frequency for *pwn* in *Df (2R)M-S2*^{10} flies is probably due to the reduction in length of the Minute-carrying chromosome arm proximal to the locus of the marker.

Again, as in the non-irradiated experiments, slightly higher frequencies of *mwh* appear in crosses involving stock II than in those involving stock I. Thus, these differences possibly have no relation with the Minute-carrying chromosomes and only reflect genomic differences.

II. *Mitotic Recombination in the Wing Disc*

The clonal analysis of the growth of the wild-type wing disc (BRYANT 1970); GARCIA-BELLIDO and MERRIAM 1971a) has shown that this disc is already engaged in cell proliferation at 48–72 hrs. after egg deposition. However, GARCIA-BELLIDO, RIPOLL and MORATA (1973) have found that the wing disc in *M(3)i*^{55} flies has not started to grow at this time. If this situation is general in all or most of the studied Minutes, irradiation at this time will affect a mitotically quiescent cell population.

The frequencies of spontaneous mitotic recombination of the different Minutes are not shown. Their clones are of different sizes (indicating their initiation at different developmental stages) and appear in extremely low frequencies, making a statistical evaluation impossible. The results of X-ray-induced mitotic recombination are shown in Table 3. Although the number of clones in the wing disc at this age of irradiation is lower than in the abdomen, resulting in poorer statistical security, it may be concluded that probably the same Minutes increase the clone frequencies in both wing and tergites. General frequencies in both systems are significantly correlated ($r = 0.44$, see Table 4).

III. *Clone Size*

Crossovers between the centromere and the locus of the Minute result in M^+ clones; crossovers distal to the Minute locus or in other chromosome arms will give rise to clones that retain the Minute genotype. MORATA and RIPOLL (1975) showed for three Minutes that M^+ clones are larger than M clones initiated at the same stage of development. We have studied the average size (number of cells) of wing disc clones of several Minutes irradiated at 48–72 hrs.

As seen in Table 3, the Minute$^+$ clones are always larger than the Minute ones and the latter (M clones in an M background) are not significantly different in size from the WT-controls (M^+ clones in an M^+ background).

The actual increase in size of the M^+ clones in the wing disc is in some instances probably larger than reported in the table. This is so because in those cases in which the Minute locus is proximal to the locus of the cell marker (see Figure 1), these figures include M^+ and M marked clones. We also corroborated the finding of MORATA and RIPOLL (1975) that the average size of the M^+ clones in the tergites of all the Minutes studied is only slightly larger than in wild-type controls.

TABLE 3

Frequencies and sizes of wing clones resulting from X-ray-induced mitotic recombination

Minutes	*n*	*2R pwn* Freq.	Size	*3R bld* Freq.	Size	*3L mwh* Freq.	Size	*n*	*3L mwh* Freq.	Size	*1 y* Freq.	Size	*3R Sb+* Freq.	Size
WT-Controls	240	0.02	12	0.004	4	0.05	12	137	0.08	10	0.03	8	0.02	9
M(1)Bld	115	0.02	**10**	0.008	6	0.03	9	80	0.07	9	a		0.02	**10**
M(1)o	303	0.05*	**10**	0.007	3	0.08	9	101	0.10	9	0.30**	**15**	0.07*	**11**
$M(1)o^{sp}$	157	0.05	9	0.06	5	0.10*	9	80	0.10	10	0.17**	**15**	0.02	7
$M(2L)H^{s5}$	140	0.02	**11**	0.00	—	0.06	9	80	0.08	10	0.04	7	0.03	7
$M(2R)l^{2}$	211	0.06**	**20**	0.00	—	0.04	12	87	0.05	9	0.03	7	0.02	10
M(2R)S7	106	0.08**	**28**	0.00	—	0.05	9	110	0.07	9	0.05	7	0.03	9
$M(2R)S2^{10}$ (Df)	100	0.00	—	0.00	—	0.05	9	89	0.07	9	0.04	9	0.02	8
$M(2R)c^{33a}$ (Df)	99	0.05	**55**	0.00	—	0.05	9	80	0.04	11	0.06	6	0.02	7
$M(3L)i^{55}$	356	0.03	12	0.006	3	0.10*	**32**	82	0.15	**43**	0.07	8	0.05	10
$M(3L)h^{y}$	234	0.03	9	0.008	3	0.15**	**26**	106	0.26**	**34**	0.10**	10	0.01	10
$M(3L)h^{S37}$	294	0.02	12	0.003	4	0.15**	**59**	179	0.20**	**42**	0.07*	8	0.03	10
M(3R)w	180	0.02	11	0.005	**7**	0.03	9	100	0.09	10	0.05	8	b	
$M(3R)w^{124}$	193	0.04	9	0.005	**6**	0.04	10	117	0.03	9	0.02	9	b	
$M(3R)be^{36e}$	114	0.05	10	0.009	**6**	0.07	9	100	0.11	9	0.04	9	b	
M(4)57g	144	0.03	**12**	0.00	5	0.05	12	130	0.08	10	0.08*	7	0.00	
M(4)63a (Df)	198	0.02	11	0.005	4	0.02	**11**	97	0.04	10	0.04	6	0.02	8

Frequencies per wing disc. Sizes of $M+$ clones are printed in heavy type.
Sizes: Average number of cells ($\times 10^2$).
n: Number of wing discs scored.
a: Frequency of f^{36a} in WT-Control flies is 0.02 (size of about 8×10^2 cells). Frequency of f^{36a} in *M(1)Bld* flies is 0.07* (size of about 60×10^2 cells).
Remaining symbols are as in Table 1 and Table 2.

TABLE 4

Some parameters of the studied Minutes

	α_g tergites frequency of clones	α_x wing frequency of clones	Ω size of M+ clones	γ bristle length	D developmental delay (hrs)
M(1)Bld	0.95	1.07	5.92	0.58	76
M(1)o	0.35	2.43	0.83	0.67	35
M(1)o^{sp}	0.57	1.30	0.87	0.71	34
M(2L)H^{85}	—0.04	0.20	—	0.70	30
M(2R)l^{2}	0.97	0.28	1.14	0.71	19
M(2R)S7	0.70	0.80	2.31	0.75	24
M(2R)S2^{10} (Df)	0.03	0.05	—	0.88	13
M(2R)c^{33a} (Df)	0.18	0.40	5.14	0.68	49
M(3L)i^{55}	0.62	0.95	3.60	0.72	45
M(3L)h^{y}	0.75	1.22	2.71	0.72	48
M(3L)h^{S37}	0.43	0.84	4.91	0.72	57
M(3R)w	—0.04	0.12	0.24	0.64	36
M(3R)w^{124}	—0.19	0.02	0.20	0.65	41
M(3R)be^{36e}	0.15	0.63	0.22	0.69	31
M(4)57g	0.37	0.54	1.39	0.76	33
M(4)63a (Df)	0.37	—0.10	—	0.80	49

The different parameters are described in text.

IV. *Recombination in Chromsome IV*

In this analysis we include the data of mitotic recombination in the fourth chromosome, using *sv^{de}* as a cell marker. This cell marker has a slightly dominant effect in heterozygous flies that prevents (GARCIA-BELLIDO, personal communication) studying recombination frequencies in the tergites. The property of Minute^{+} recombinant cells of overgrowing their surrounding cells was used as a tool for detecting *sv^{de}* mitotic recombination clones in the wing disc. *sv^{de}* clones appeared following irradiation of *M(4)57g/sv^{de}* and *ci^{D}/sv^{de}* larvae. The frequency of *M^{+} sv^{de}* spots in irradiated Minute larvae was 0.07 (based on 185 flies), with an average clone size of 22×10^2 cells. In irradiated *ci/sv^{de}* larvae the frequency was 0.03 (based on 110 flies) with an average clone size of 11×10^2 cells. This is the first known case of mitotic recombination in chromosome IV.

V. *Developmental Parameters*

The method used to measure the developmental delay gives an estimation of the total delay, but nothing indicates about its distribution throughout development. However, previous data (MORATA and RIPOLL, 1975) and our personal experience in some instances indicate that the development delay takes place in the egg and larval phases of development. Usually the delay was measured in crosses involving stock I. The figures shown in Table 4 (*D* column) are the average of the data obtained in both types of crosses.

It is interesting to point out that the developmental delay shows a maternal influence in some Minute flies; i.e., the delay is increased when the parental females are Minute. Thus, *M(3)i*^{55} shows about 45 ± 6 hrs. of delay when Minute parental males were employed and 68 ± 9 hrs. of delay when Minute parental females were employed. Another Minute tested, *M(2)l*^{2}, shows no maternal effect.

Another developmental feature of Minute flies is to differentiate chaetae shorter than wild-type. We measured the length of three macrochaetae: anterior scutellar, posterior dorsocentral and presutural in different Minutes. Figures of the first 15 males (females in Minutes *M(1)o*, *M(1)o*^{sp} and *M(1)Bld*) and 15 non-Minute flies of the same sib were compared.

VI. *Correlation Analysis of Some Phenotypic Parameters*

An analysis of the correlations between the different traits of the Minute phenotype is interesting in order to understand the causal basis of the Minute phenotypic pleiotropy. The absolute values of the measured parameters have been transformed into the following coefficients:

1) Coefficient α: Considering mitotic recombination frequencies in tergites (Tables 1 and 2) or in the wing (Table 3), the coefficients α were defined as:

$$\alpha = \frac{\text{Clone freq. in Minutes minus Clone freq. in WT–Controls}}{\text{Clone freq. in WT–Controls}}$$

The biological meaning of these coefficients is the relative increase in spot frequency for each marker caused by the Minute genotype. An α_x (irradiated) and an α_s (spontaneous) were considered. In order to give a single coefficient for each Minute and each developmental system, the α_x coefficients for the different markers were averaged (Table 4). The α_s values are all close to 0 as discussed earlier (see above and Table 1).

2) Coefficient Ω: Considering the clone sizes in the wing (Table 3), this coefficient was defined for each Minute as:

$$\Omega = \frac{\text{Size of } M^+ \text{ clones (own arm) minus size } M \text{ clones (other arms)}}{\text{size of } M \text{ clones (other arms)}}$$

The biological meaning of this coefficient is the relative increase in clone size caused by the autonomous condition of the division rate of M^+ cells in the wing disc. Data are given relative to cell markers in other chromosome arms and not to wild-type controls to avoid variations in the number of wing cells of the different Minutes at the age of irradiation (48–72 hrs.). As discussed earlier, values are underestimated in some Minutes.

3) Coefficient γ: A coefficient γ was calculated for each Minute and each chaeta as:

$$\gamma = \frac{\text{Average length of a given chaeta in Minute flies}}{\text{Average length of the same chaeta in sib Minute}^+ \text{ flies}}$$

TABLE 5

Values of the correlation coefficients for the different parameters

	α_x terg	α_x wing	Ω	γ	D
α_x terg	—	0.44	0.44	—0.10	0.28
α_x *wing*	—	—	0.09	—0.30	0.21
Ω	—	—	—	—0.16	0.80
γ	—	—	—	—	—0.58
D	—	—	—	—	—

For all the Minutes the correlation between the three coefficients was high (anterior scutellar — posterior dorsocentral, $r = 0.71$; anterior scutellar — presutural, $r = 0.85$; posterior dorsocentral — presutural, $r = 0.72$). In order to give a single coefficient γ for each Minute, the three coefficients were averaged in each Minute (Table 4, γ column).

The coefficients so defined were tested for correlations (Table 5).

DISCUSSION

The 16 Minutes analyzed here are possibly a representative sample of the number of known Minutes in the genome of *Drosophila melanogaster* (LINDSLEY *et al.* 1972). Thus, the following considerations should be expected to be valid for Minutes in general.

STERN (1936) and KAPLAN (1953) found an arm-specific increase in the frequency of spontaneous mitotic recombination. They analyzed the data of marker mutants (only some of which can be considered as cell markers) affecting all kinds of cuticular structures and body regions. Our results, using now-available cell markers, scored on the tergites, do not seem to confirm that conclusion. STERN (1936) and MORATA and RIPOLL (1975) already showed that mitotic recombination is not restricted to the locus of the Minute but occurs proximal and distal to it. It is possible that the arm specificity of mitotic recombination clones described by STERN (1936) and KAPLAN (1953) is an artifact possibly due to a higher scorability of the larger M^+ clones. The lack of arm-specific effect of M^+ clones in tergites possibly reflects the poor overgrowth of M^+ clones in this system (see MORATA and RIPOLL 1975). However the spontaneous frequency of f^{s6a} in *M(1)Bld* flies (see legend of Table 1) could be an exception. Recall that single-bristle clones were not scored. Therefore, the M^+ clones which in wild-type flies embrace a single bristle, might, because of the overgrowth of M^+ cells in an *M(1)Bld* background, encompass two or more chaeta and thus be scored. (*M(1)Bld* has high values in all the parameters measured, and can be considered as a "strong" Minute).

In experiments of X-ray-induced mitotic recombination we could not find arm-specific effects in the tergites either. In the wing disc, though, there were 7 Minutes which showed an arm-specific increase in frequency, relative to wild-type controls, and to other chromosome arms. This could be due to a higher

scorability of the large M^+ clones, which occur only following mitotic recombination in their own chromosome arm. Early-irradiated clones of markers in other chromosome arms are large enough, though, to be unequivocally detected. It is possible that M^+ cells arising by X-ray-induced mitotic recombination in a Minute background have a higher probability of surviving (and of giving rise to a visible clone) than the same recombinational events that do not change the Minute constitution.

Ten out of the 16 Minutes studied significantly increase the frequency of induced mitotic recombination in the tergites relative to wild-type controls. Nine of them also show a similar effect in the wing disc. Differences of degree exist among these Minutes, and it is questionable whether those Minutes not exhibiting this effect are in fact "weak" examples of the same phenomenon. The similar behavior of alleles of the four loci studied points to locus-specific response of Minutes to irradiation. Moreover, the significant decrease of both alleles of *M(3)w* studied suggests qualitative differences in the wild-type function of the different Minutes considered. Yet the similar pleiotropic phenotype of the different Minutes indicates a common phenogenetic failure (SCHULTZ 1929).

An analysis of the different parameters studied has shown that developmental delay, increase in M^+ clone size, chaeta length and α_x (both wing and tergites) are correlated. Thus, it is conceivable that most of these Minute phenotypic traits are causally interrelated and the increase in clone frequency actually represents a higher cell sensitivity to X-ray-induced recombination. The differential sensitivity of Minute and non-Minute flies could be caused by inefficient chromosome repair in the former. This would give the broken chromatids more opportunities for wrong rejoining (MERRIAM and FYFFE 1972; HAENDLE 1974). This explanation is consistent with suggestions that the *M* syndrome is a result of deficient protein metabolism (RITOSSA *et al.* 1965; GOLDIN 1968; FARNSWORTH 1970).

The high frequency of clones induced by mitotic recombination in heterozygous Minute flies, as well as the increase in size of the M^+ clones, are useful in the developmental analysis of imaginal discs. This is especially so in those discs, such as the wing, where the number of cells in early stages of development is low (GARCIA-BELLIDO, RIPOLL and MORATA 1973). The present analysis, carried out on a sample of 16 Minutes, will allow us to choose those Minutes which show the most suitable parameters for a clonal analysis of development.

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