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The Nature of Mildly Deleterious *Mutations* Eliminated upon Sexual Reproduction in Meiosis

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Abstract Mildly deleterious *mutations* (MDMs) with incomplete dominance, which decrease the viability of the progeny, apparently play a significant role in the evolution of sexual reproduction. In particular, they are thought to be eliminated in meiosis. The nature of MDM remains unclear. By studying the accumulated MDMs in *Drosophila* strains carrying various meiotic *mutations* (*c(3)G*^{17}*, mei-P22, mei-W68, mei-41, mei-218*), we found that impair the formation of DNA breaks is more effective in accumulation of MDMs. The relationship between survival and generation number upon MDMs accumulation suggests that MDMs interaction corresponds to their synergistic epistasis. The viability in progeny after meiosis in heterozygotes with chromosome with accumulated MDMs and normal chromosome, and in heterozygotes with independently accumulated MDMs chromosomes was shown to be restored. Our results support the hypothesis that MDMs have epigenetic nature. It is proposed that: during the life cycle "mutant" variants of the formation of structural and functional loop domains appear in the chromosomes; these variants are normally corrected in meiosis; an abnormal loop alters the activity of many genes (~17), increasing (+) or decreasing (–) it. The hybrids with chromosomes carrying independently accumulated MDMs partially restore viability due to complementary interaction of + and – genes.

Keywords Sex Evolution, Mildly (Slightly) Deleterious *Mutations*, Meiosis, Chromosome Loop Domains, Epigenetic *Mutations*, *Drosophila Melanogaster*

1. Introduction

Diploidy, multicellular organization, the increase in the genome size and the structural complexity of organisms are associated with the development of sexual reproduction. The evolutionary advantage of sexual reproduction (which is in fact less effective and energetically less beneficial) over asexual one is not quite understood[1],[2]. More than 20 hypotheses have been advanced to explain this phenomenon[3]. In the evolution of sex, mildly deleterious *mutations* (MDMs), the effects of which are intermediate between neutral and deleterious, are thought to play a significant role. MDMs in homozygous state result in mortality of only part of the progeny. Moreover, they are semidominant and manifest in heterozygotes and these properties of MDMs provide their inheritance and maintenance in populations[1-5]. A high rate of appearance of new MDMs makes evolution with accumulation of advantageous *mutations* impossible[1]. Herman Muller was the first to theoretically demonstrate (later it was documented experimentally) that in asexual organisms, the gene pool is slowly but consistently degraded owing to the accumulation of MDMs ("Muller's ratchet")[4],[5]. The MDM number in such organisms increases with decreasing population size and increasing complexity of the genome (increasing number of genes). According to Muller, asexual populations, in spite of the mutation pressure, exist because of the simplicity of their organization (small genome size), extremely large population sizes, and strong stabilizing selection, rapidly eliminating MDM carriers, which are replaced by mutation-free clones. In this connection, an alternative explanation is that MDMs are eliminated in sexual reproduction[6],[7]. The mechanism of the elimination and its association with meiosis are unclear. Hypotheses highlighting the role of recombination (generation of MDM-free recombinant forms) in effective elimination of MDMs and, consequently, in reducing the mutational load of sexual populations[3],[5], hold only on unlikely condition of constant environmental changes, when in each generation new genotypes with high fitness would be required[1]. Indeed, the axiom on the evolutionary role of recombination has a serious defect, which lies in recombination itself. The high recombination rate, which is actually observed in nature, would destroy a beneficial gene combination not later than in the following generations. A simple reshuffling of not eliminated MDMs would have no effect on the progeny viability at the population level, which is the same proportion of the progeny as before recombination.

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In papers devoted to the study of MDMs, less discussed issue is their nature. Is MDM the "classical" *mutations* with changes in the nucleotide sequences of structural genes, or there are changes in the regulation of their activity? It is unknown. Incomplete dominance is used for classification of regulatory *mutations* and is associated with changes in the activity of structural genes[8]. Based on this MDM property, Mukai[9] suggested that MDMs are located in noncoding regions. Another suggestion connects reduce the viability of offspring with numerous insertions of mobile elements[10], which affect gene activity. Finally, it was concluded about the absence of MDMs upon mutagenic exposure[11].

The method of MDM accumulation in *Drosophila*, consisting in disturbance of main meiotic processes in an individual chromosome pair, confirms the role of meiosis in their elimination. A circuit method of MDM registration and accumulation in Drosophila was first proposed by Muller in 1928[12]. This method involves suppression of meiotic pairing and recombination in the examined chromosomes in many generations. With this aim, only heterozygotes with the corresponding chromosomes carrying multiple inversions and transpositions (balancers and crossover suppressors) were employed for reproduction. Chromosomes with multiple rearrangements are usually lethal in homozygous state. The main sign of the MDM manifestation and accumulation in *Drosophila* is mortality of organism from embryo to eclosion of imago from pupae[13]. In adult flies, MDMs affect individual adaptation to the environment. In such experiments, a reduction in viability and partial mortality of the progeny increases in 20[14], 40[15], 250[16] generations. These MDMs appear practically in each individual in the progeny at a rate an order of magnitude higher than that of recessive lethals and manifest in heterozygotes with the coefficient of dominance 0.2-0.5[15],[17].

Our approach was to study the progeny viability in the strains of *Drosophila* carrying recessive meiotic *mutations* (mei-*mutations*) maintained using balancer chromosomes or transmitted from father to son. In this case, selection for reproduction of only heterozygotes with balancers is not required for MDM accumulation. Mei-*mutations* in homozygote do not affect viability of their carriers. They only disturb meiosis, thus affecting fertility (due to the formation of abnormal gametes) and promote MDM preservation and accumulation in the progeny of small laboratory populations. The absence of recombination in male meiosis upon only paternal X-chromosome inheritance also promotes MDM accumulation.

The purpose of this paper is to clarify the nature and mechanism of MDM elimination at meiosis.

2. Materials and Methods

2.1. Fly strains

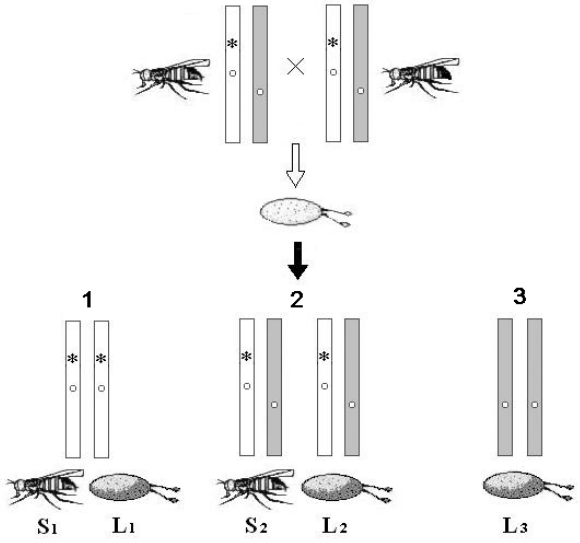
Flies were reared at 25°C on standard medium. We used the following strains of *Drosophila melanogaster* (the abbreviated designation is given):

(1) st[1] c(3)G^{17} [1] ca[1]/ TM2 ri Ubx[130] e[s] ca[1] ***(c(3)G^{17}/TM2)***; *(2) st[1] c(3)G^{17} [1] ca[1]/TM3 y^{+}ri[1] p[p] sep bx[34e] e[s] Sb[sbd-1] Ser[1]* ***(c(3)G^{17}/TM3);*** *(3) sp[2];st[1]* ***c(3)G^{17}*** *[1] ca[1]/ TM1 Me[1] kni[ri-1] Sb[sbd-1]* ***(c(3)G^{17}/TM1);*** *(4) ru[1] h[1] th[1] st[1] cu[1] sr[1] e[s] ca[1]/ TM6 Hu[1] e[1] Tb[1] ca[1]]* ***(rucuca/TM6 Tb)***; ***(5)*** *h[1] th[1] st[1] cu[1] sr[1] e[s] Pr[1] ca[1]/ TM6B Bri[1] Tb[1]* ***(TM6B Bri Tb); (6)*** *ru[1] h[1] th[1] st[1] cu[1] sr[1] e[s] ca[1]; (7) y[1]; al[1] dp[1] b[1] pr[1] cn[1] mei-W68[L1]/In(2LR) SM1 al[2] Cy cn[2] sp[2] (Cy* suppressed) ***(mei-W68/SM1);*** *(8) y[1] w[1]/Dp(1;Y) y[+]; mei-P22[P22]; sv[spa-pol]* ***(mei-P22/);*** *(9) Dp(1;1) sc[V1] y[1] mei-41[1] car[1] y[+]/C(1)DX y[1] f[1] bb[-]/Y* (not *y[+]*) ***(mei-41/);*** *(10) Dp(1;1} sc[VI] y[1] mei-218[1] car[1] y[+]/C(1)DX y[1] f[1] bb[-]/Y* ***(mei-218/);*** *(11) l[21pn]/FM4 y[31d] sc[8] dm[1] B[1]* ***(/FM4);*** *(12) Df(2L)A267, b cn bw/In(2LR)O Cy dp pr cn* ***(/CyO);*** *(13) wild type (Oregon R)* ***(+/+).***

The information on the genome, *mutations*, and balancers of *D. melanogaster* is presented in the manual by Lindsley and Grell[18] and at http://flybase.bio.indiana.edu.

2.2. The Method of Examining the Viability of the Heterozygous Parent'S Progeny

The crossing of heterozygous parents excludes the effects of recessive mei-*mutations* in the progeny (Fig. 1).



Survival (S, fly) or mortality (L=1-S, dark egg) was studied in homozygous (1) and heterozygous (2) progeny after crossing heterozygous parents - "chromosome with mei-mutation" (star)/"balanser chromosome" (gray). The centromere is marked by circle. The balanser chromosomes are lethal in homozygous state (3). The number of laid eggs is indicated by a light egg. See text for details.

Figure 1. Scheme of studying the effect of MDMs accumulation on the viability of the progeny

To estimate viability, each virgin female was mated with two males in a vial with standard nutrient medium. In total, 30 to 50 females were used in every cross. After 24 h, all flies were transferred to a bottle with the medium. The laid eggs

were counted each 3-4 h. Typically, the bottles were replaced three to four times. The number of laid eggs in the experiments varied approximately from 400 to 2000. Then, the numbers of pupae and hatched flies of different phenotypes were recorded. The viability (S) was measured as the proportion of hatched flies with a particular genotype (and, accordingly, phenotype) in the number of laid eggs with the same genotype. The number of eggs with the given genotype was determined as the proportion in the total number of laid eggs, using the ratio of genotype classes in the progeny of the cross. The progeny mortality (L) was estimated as the proportion of eggs that did not develop to the adult stage in the total number of eggs laid after the cross (L=1-S). Crosses are listed in the first column of Tables 1 and 3.

2.3. Counting the Number of Generations, Accumulating MDMs

The number of generations (N), during which there were violations of the pairing and recombination of chromosomes during meiosis under the influence of different *mutations*, was calculated. Strains with *mutations* were maintained with crossover suppressors from the time of their registration or production to the time of the experiments. The strains carrying mei-c(3)G^{17}, mei-W68, mei-P22, mei-41 and mei-218 are maintained since 1917[19],1972[20], 1992[21], 1972[22] respectively. Taking into account the duration of the Drosophila life cycle and the practice of maintaining strains in laboratory (18 days), consequently, the approximate number of generations elapsed to the time of analysis were different for different strains: 1750 for mei-c(3)G^{17}, 710 for mei-W68, 300 for mei-P22, 730 for mei-41 and mei-218.

2.4. Viability of Progeny in Strains and Their Hybrids Carrying Mei-Mutation C3)G^{17}

Progeny viability was examined in three laboratory strains of D. melanogaster (1-3), carrying meiotic mutation c(3)G^{17}. Laboratory strain 2 (c(3)G^{17}/TM3) is maintained since 1985; it was derived from strain 1 (c(3)G^{17}/TM2) by substitution of the balancer. Strain 2 was supplied by I.D. Alexandrov (United Institute of Nuclear Research, Dubna, Russia). Strain 3 (c(3)G^{17}/TM1) was provided by the Bloomington Drosophila Stock Center, previously, it was kept in the Caltech Stock Center, approximately up to 1970 (http//www.flybase.edu). In addition, we examined viability of the hybrid progeny, homozygous for the mei-mutation and produced by crossing flies of different strains (1 × 2, 1 × 3 and 2 × 3). To facilitate phenotypic marking of the progeny, we transferred the studied chromosome (carrying mutation c(3)G^{17}) to heterozygote with a new balancer chromosomes; the flies were taken in the experiment during three generations. In some cases the reproduction was conducted for about 20 and 50 generations. We used strains 4 and 5 as a source of new balancer chromosomes and strain 6 for generating crossover chromosomes (females st c(3)G^{17} ca/ru h th st cu sr e ca) containing different regions with accumulated MDMs (th st cu c(3)G^{17} (?) ca, ru h st c(3)G^{17} (?) sr e ca). Strain 13 was used as control. Strains 4–6, 13 were also provided by the Bloomington Stock Center.

2.5. Comparative Analysis of Viability in Strains Carrying mei-*mutations* c(3)G^{17}, mei-P22, mei-W68, mei-41, mei-21

Progeny viability was examined in D. melanogaster strains 7-10, homozygous at various mei-*mutations* (mei-W68, mei-P22, mei-41, mei-218). Strains 7 and 9 were provided by A.T.C. Carpenter. In the strain 8 with mutation mei-P22, the balancer chromosome was at some point lost, and this mutation is currently maintained in homozygote (*http://flybase.org*).

Strains 8 and 10 were supplied by the Bloomington Stock Center. Strains 4 and 11 were used as a source of new balancer chromosomes.

2.6. Mortality of Hybrid Progeny with Chromosomes From Strains Carrying Different Mei-*Mutations* with Accumulated MDMs

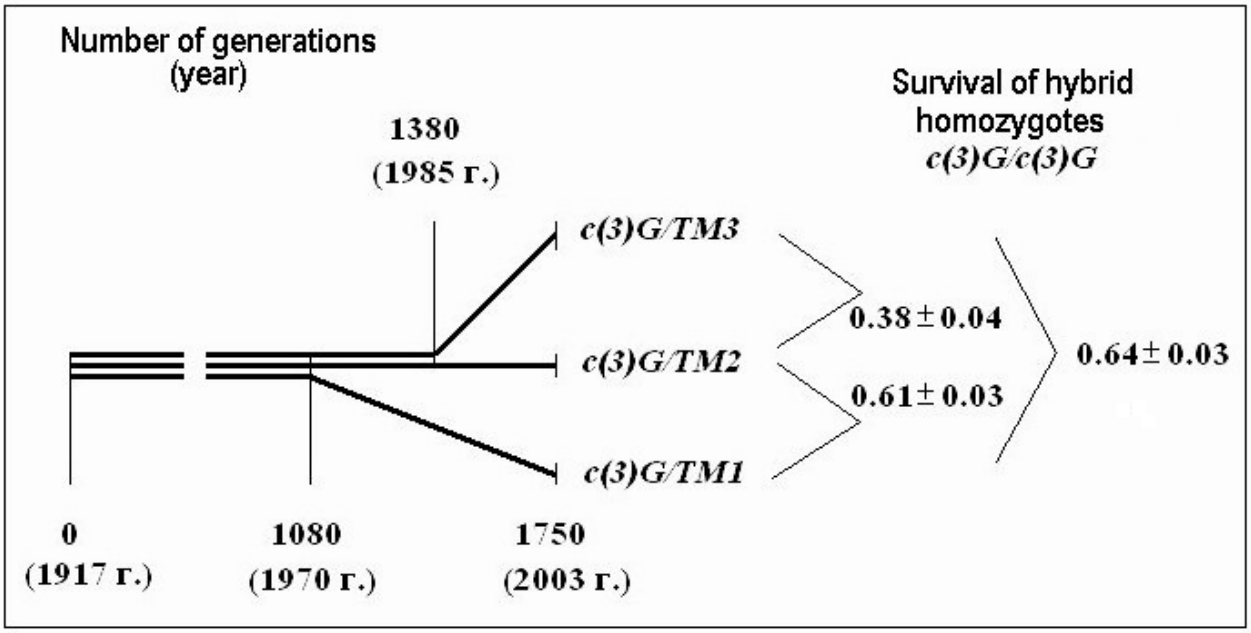
Strains 7, 8, 2 -, containing the mei-*mutations* W68, P22 and c(3)G^{17} respectively were used. Strains 4 and 12 were used as a source of new balancer chromosomes. We examined the mortality of the following hybrid progeny: (a) c(3)G^{17}/ mei-P22; (b) mei-W68/+, c(3)G^{17}/+ and (c) mei-W68/+, mei-P22/+. In addition, mortality of the progeny was studied in heterozygotes c(3)G^{17}/rucuca, c(3)G^{17}/TM6Tb, mei-P22/TM6Tb and mei-W68/SM1.

3. Results and Discussion

3.1. Viability of the Progeny in Strains and Their Hybrids Carrying Mei-mutation c(3)G^{17}

In this section of paper we refer to some historical research data. Since the average value of life cycle duration estimated previously[13],[23] was somewhat lower, here it was standardized for comparing different strains.

The first meiotic mutation, c(3)G^{17}, was found in a natural population in 1917 and has been since then maintained in laboratory strains. Mei-mutation c(3)G^{17} in autosome 3 (3-57.4; 89A5) disturbs the formation of synaptonemal complex (SC), suppresses recombination in homozygous females[24],[25], and, which is worth mentioning, enhances recombination in heterozygotes with the normal chromosome[26]. Note also the exclusive maintenance of autosome 3 in all strains in heterozygote at balancers and the absence of chromosome pairing and crossing over in homozygotes due to the mei-mutation.



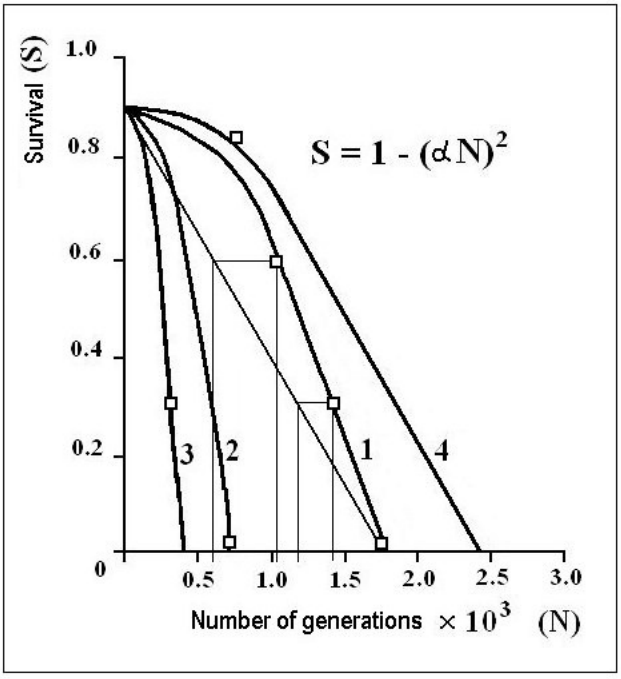
The start of maintenance is marked by thin vertical lines. Survival of hybrid homozygotes (chromosomes are from different mei-mutation strains) is shown to the right.

Figure 2. History of strains carrying mei-mutation *c(3)G*^{17}

All three c(3)G^{17} - strains (1-3) practically totally lacked homozygous progeny (1 – 0.0001 ± 0.0001, 2 – 0.00005 ± 0.00004, 3 – 0) which was represented only by balancer heterozygotes (1 -0.66 ± 0.04, 2 – 0.68 ± 0.04, 3 – 0.70 ± 0.03)[13],[23]. Thus, a labour-consuming study of adaptation of the flies with accumulated MDMs to the environment in this case was excluded. We first noted a very small number of homozygotes in a strain carrying the c(3)G^{17} mutation in 1997, when a few homozygotes were recorded in the progeny[27]. The character of the dependence of the progeny viability on the generation number upon meiosis suppression can be "restored" from the results of viability of hybrids between the initial strain and a dated derivate, typically maintained with another balancer. This is caused by independent random appearance and rapid fixation of MDMs in small populations, which are represented by laboratory strains. MDMs accumulated in the initial strain are shared or homologous (Fig. 2).

In view of the history of the strains, the viability estimates for interstrain hybrids (Fig. 2) are in agreement with the assumption of the quadratic relationship between survival S (L = 1 - S, mortality of progeny) and generation number N upon MDM accumulation (Fig. 3).

This relationship is described empirically by the equation $S = 1 - (\alpha \cdot N)^2$, where α is the reduction in viability resulting from the MDM appearance in one generation. $\alpha = \sqrt{(1-S)}/N$. Considering the lethality of the progeny in wild-type strain +/+ (0.09 ± 0.02), $\alpha = \sqrt{(1-0.09)}/1750 = 5.4 \cdot 10^{-4}$. The factual data correspond to the splitting of the strains from the initial stock in 1970 and 1985, i.e., after their maintenance together for 1010 and 1430 generations, respectively. The above relationship suggests that MDMs interaction corresponds to their synergistic epistasis.



α - is the decrease in viability per generation. The experimental data are marked with squares. See text for further details.

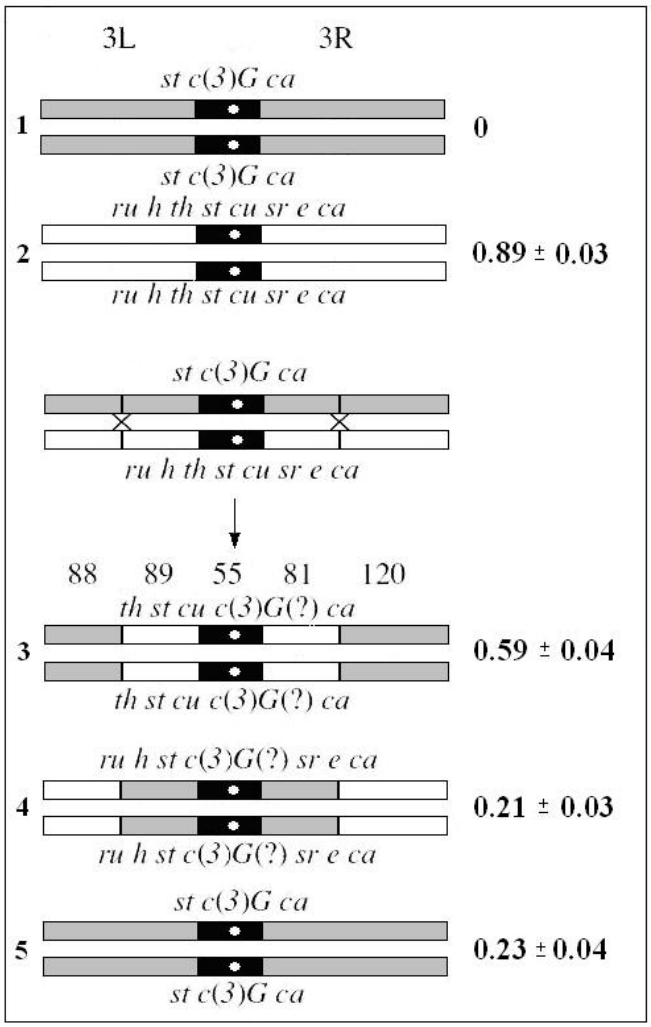
Figure 3. Putative quadratic relationship between the progeny viability *S* and the number of generations *N* that accumulate MDMs in strains carrying mei-*mutations c(3)G*^{17} (1), *W68* (2), *P22* (3), *41* or *218* (4)

Calculations show that with a linear relationship (additive effect, $S = 1 - \alpha N$, $\alpha = 5.1 \cdot 10^{-4}$ and accordingly $N = (1 - S)/\alpha$), the strains would have split from the original stock respectively in 1956 and 1975 (after 550 and 1180 generations), which was not the case (Fig. 3). Our conclusion on the MDM interaction was indirectly supported by the results reported for *Drosophila* by other authors: it was shown that the effect on the progeny viability and coefficient of dominance increase with the number of generations, in which MDM accumulation occurs[16],[28].

To assess the accumulation of MDMs in different region of metacentric autosome 3 and the effect of meiosis on their manifestation, we constructed strains with non-crossover and reciprocally recombinant chromosomes by a double exchange (in different arms) between a chromosome from strain 2 carrying the $c(3)G^{17}$ mutation and a chromosome from strain 6 with normal viability (Fig. 4). Our results show that MDMs located in the middle, pericentromeric part of chromosome 3 are nearly twice as efficient in mortality as those from the distal euchromatic regions. The mortality of homozygous progeny (taking into account the control) per a physical unit of chromosome region length, calculated according to[29] in pericentromeric and distal chromosome regions, respectively, is $(0.89 - 0.21)/ (89 + 55 + 81) = 0.68/225 \approx 0.0030$ and $(0.89 - 0.59)/ (88 + 120) = 0.30/208 \approx 0.0014$ (in Fig. 4, sizes of the chromosome regions are given in arbitrary units above the chromosome schemes). Probably, chromosome recombination as such does not play a significant role in decreasing the pressure of these *mutations*, since after normal meiosis non-crossover chromosomes partially (0.23 ± 0.04) restore viability, which was previously zero ($P > 0.999$). In this case, gene conversion is not excluded. On the other hand, the presence in the control population of MDMs that result is nearly 0.1 mortality in the progeny may be explained by their localization in chromosome regions with less effective pairing and corresponding meiotic processes, namely, in pericentromeric regions.

The substitution of the balancer chromosome in the strain carrying mei-mutation $c(3)G^{17}$ for a new balancer proved to result in a partial (from 0 ± 0.02 to 0.27 ± 0.03) restoration of viability ($P > 0.999$) of $c(3)G^{17}/c(3)G^{17}$ homozygotes during the first 20-30 generations (Fig. 5). Further maintenance of the strain on the same balancer for 20 generations led to decline in viability to the former level (Fig. 5). This phenomenon, albeit long known to drosophila genetics, has not been studied.

Apparently, chromosome recombination as such does not play a significant role in decreasing the MDM pressure, since recombinants of a structurally normal chromosome with multiple inversions in the balancer do not survive because of deletions. In our case, gene conversion (intragenic recombination), also initiated by DNA breaks, successfully occurs[30-32].



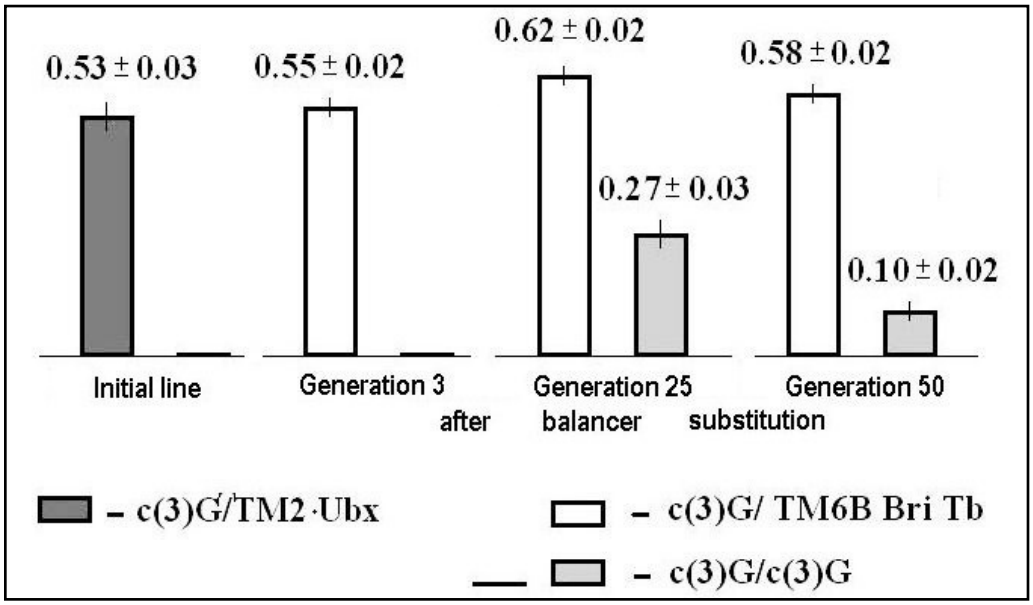
Chromosomes with MDMs are shown in grey; chromosomes with normal viability of the progeny, in white; the pericentromeric heterochromatin, in black. The centromere is marked by circle. The exchange points are marked with a cross. Physical sizes of chromosome regions are shown in arbitrary units above the exchanged chromosomes[Chubykin, Omelyanchuk, 1989]. Viability before (1, 2), after chromosome crossingover (3, 4) and probably after gene conversion in nonexchanged chromosomes (5) shown at right.

Figure 4. Viability of homozygous progeny before and after mei-recombination in heterozygotes *st* $c(3)G^{17}$ *ca/ru h th st cu sr e ca*

3.2. Comparative Analysis of Viability of the Progeny in Strains Carrying Mei-*mutations* $c(3)G^{17}$, *mei-P22, mei-W68, mei-41, mei-218*

To evaluate the role of other meiotic events that promote MDM accumulation, we examined the accumulation of MDMs in strains having other mei-*mutations*: *mei-W68* (2-94; 56D9) and *mei-P22* (3-21.5; 65E9). Both these *mutations* disturb the formation of double-strand DNA breaks without changing SC[21],[33]. In addition, we studied strains carrying mei-*mutations* in the X chromosome (*mei-218* (1-56.2, 15D6) and *mei-41* (1-54.2; 14C3)), disrupting repair

of DNA breaks appearing during meiosis[20]. We note that *mutations* at the time of their generation and description did not affect the viability of their carriers in either homozygote or heterozygote. The effect of mei-*mutations* on MDM accumulation in a laboratory strain is possible only in homozygotes for recessive mei-*mutations*. This condition was met in laboratory strains carrying *mutations* $c(3)G^{17}$, *mei-W68* and *mei-P22*.



Balancer TM2 was substituted with balancer TM6B.

Figure 5. Effect of balancer replacement on the viability of heterozygotes and homozygotes for mei-mutation $c(3)G^{17}$ depending on the number of generations

Table 1. The survival rate of progeny in the strains carrying *mutations* mei-W68 and mei-P22 in the autosomes 2 and 3, respectively, and *mutations* mei-218 and mei-41 in the X-chromosome

Cross	Number of laid eggs	Genotype and expected number of eggs	Number of flies emerged	Survival rate (S)
1 +/+ × +/+	696	+/+ 696	633	0.91 ± 0.02
2 *mei-W68/SMl* × *mei-W68/SMl*	1112	*mei-W68/mei-W68* 278	3	0.01 ± 0.05
		mei-W68/SMl 556	275	0.50 ± 0.02
		SMl/SMl 278	0	0
3 *meiP22/TM6Tb* × *mei-P22/TM6Tb*	1312	*mei-P22/mei-P22* 328	128	0.39 ± 0.03
		mei-P22/TM6Tb 656	341	0.52 ± 0.02
		TM6Tb/TM6Tb 328	0	0
4 *mei-218/FM4* × *mei-218/Y*	1987	*mei-218/mei-218* 467	390	0.83 ± 0.02
		mei-218/FM4 467	438	0.94 ± 0.01
		mei-218/Y 467	393	0.84 ± 0.03
		FM4/Y 467	25	0.05 ± 0.03
5 *mei-41/FM4* × *mei-41/Y*	1053	*mei-41/mei-41* 263	219	0.83 ± 0.02
		mei-41/FM4 263	234	0.89 ± 0.02
		mei-41/Y 263	221	0.84 ± 0.02
		FM4/Y 263	15	0.06 ± 0.01

Table 2. Characteristics of strains carrying different mei-*mutations*

Strains with mei-*mutations* in homozygote (year of obtaining)	Number of generations *N* (proportion of perished progeny *L*) at the time of study	Viability reduction per generation (α) with linear dependence of progeny mortality on time ($L = \alpha N$)	Viability reduction per generation (α) with quadratic dependence of progeny mortality on time ($L = (\alpha N)^2$)	Putative number of generation with total mortality of progeny carrying MDMs	
				without their interaction	with their interaction
1 c(3)G^{17} (1917)	1750 (0.91 ± 0.02)	0.00051	0.00054	1750	1750
2 *mei-W68* (1972)	710 (0.90 ± 0.03)	0.00127	0.00134	710	710
3 *mei-P22* (1992)	300 (0.52 ± 0.02)	0.0017	0.0024	530	400
4 *mei-41* or *mei-218* (1972)	730 (0.08 ± 0.02)	0.0001	0.00039	9000	2430

Note: The data were summarized and corrected for the control (+/+).

The results of estimating progeny viability in crosses of *mei-W68* and *mei-P22* heterozygotes are presented in Fig. 3 and Table 1. The viability of the progeny homozygous for mei-*mutations* - is 0.01 ± 0.05 (*mei-W68/mei-W68)* and – is 0.39 ± 0.03 (*mei-P22/mei-P22)*. Taking into account the presence of MDM in wild-type strain Oregon R (0.91 ± 0.02 progeny survives), the progeny mortality in mei-mutation homozygotes in one generation varied among the strains. In the case of MDM interaction, i.e., according to the quadratic dependence of progeny mortality on time, the viability reduction per generation (α) was as follows: 0.00054 in strain *c(3)*G^{17}, 0.00134 in strain *mei-W68* and 0.0024 in strain *mei-P22* (Fig. 3, Table 2).

The highest efficiency in the MDM accumulation was observed in the strain carrying the *mei-P22* mutation. For example, the efficiency of viability decline per generation in this strain was more than four times higher than in the *c(3)*G^{17} strain (0.0024/0/00054 ≈ 4.44). This is explained by partial maintenance of this mutation in homozygote. In that case, MDMs can accumulate in the whole genome. On the other hand, if a mutation is maintained with a balancer, normal meiotic processes take place, in heterozygous individuals in all chromosomes except the one carrying the mei-mutation and paired with the balancer. It is exactly in this chromosome the MDMs are accumulated. Strictly speaking, we can compare only two strains with the identical conditions of maintaining mei-*mutations* in autosomes on a balancer, *c(3)*G^{17} и *mei-W68*.

Based on this comparison, we can conclude that changes in the topological heterochromatin structure caused by double-strand DNA breaks, controlled by gene MEI-W68, in comparison to disruption of SC formation, controlled by gene MEI- c(3)G^{17}, play a significant role in controlling the MDM rate and accumulation - the efficiency of their accumulation was more than two times higher ($\alpha_{c(3)G^{17}}/\alpha_{W68}$ = 0.00134/0.00054 ≈ 2.5, Table 2). It is not clear whether such DNA breaks occur in homozygous *c(3)*G^{17} mutants. Apparently, the answer to this question is no, because the SC formation in these mutants is disturbed. However, we found homologous chromosome pairing in half of their oocytes[27], which does not exclude this possibility.

X-chromosomes carrying *mutations mei-218* and *mei-41* showed exclusively paternal inheritance (*Drosophila* males SC and chromosome recombination are undefined). Females carried one chromosome with two sets of the X-chromosome genetic material, i.e., acrocentric compound *C(1)DX*. Thus, in this case, as in the *c(3)*G^{17} strain, we recorded the effect of the complete absence of the SC and recombination rather than the *mutations*. The viability of the progeny with accumulated MDMs in strains carrying *mutations mei-41* and *mei-218* in X-chromosomes is presented in Fig. 3 and Table 1. In both strains, the same proportion of females (0.83 ± 0.02) and males (0.84 ± 0.03) survived (P<<0.95). Taking into account the progeny viability in the wild-type strain (0.91 ± 0.02), the proportion of viability reduction in homozygous females *mei-41/mei-41* and *mei-218/mei-218* and hemizygous males *mei-41/Y* and *mei-218/Y* per generation was identical in both strains. Corrected for the gene interaction, this parameter (α) constituted 0.00039 (Table 2). The difference of this estimate from that in the *c(3)*G^{17} strain (nearly 1.4-fold, 0.00054/0.00039) is apparently explained by the difference in physical size between the X chromosome and autosome 3 or by specific features of sex chromosome functioning in *Drosophila*.

3.3. Mortality of the Hybrid Progeny With Chromosomes from the Strains Carrying different Mei-*Mutations* with Accumulated MDMs

The MDM effect on viability of hybrids containing heterozygous (from different strains) chromosomes with MDMs in autosome 3 (*cis* position) with *c(3)*G^{17}/ *mei-P22* was examined (Fig. 6a). We also examined hybrids with combination of autosomes 2 and 3 with MDMs from strains with meiotic *mutations c(3)*G^{17}, *mei-P22* and *mei-W68* in

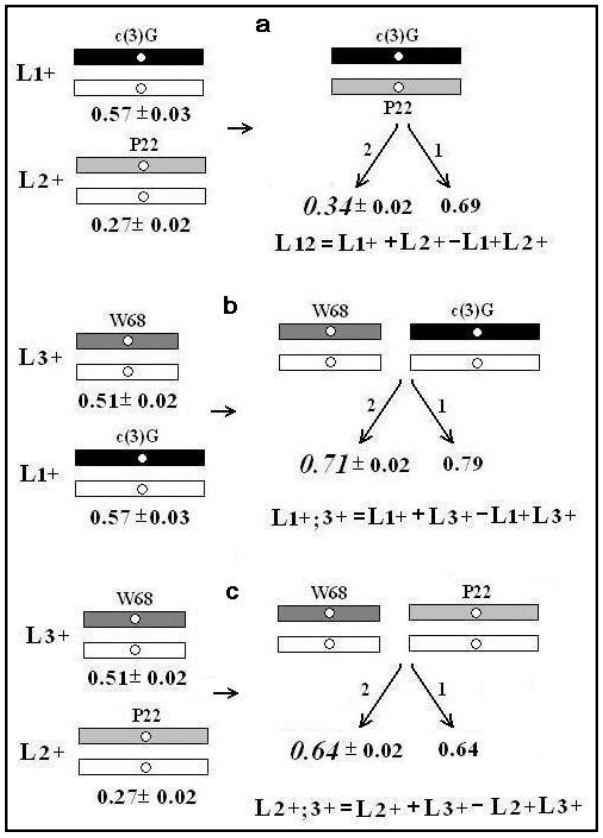
heterozygote with a chromosome that conferred normal viability (*trans* position), *mei-W68/+, c(3)G^{17}/+* (Fig. 6b) and *mei-P22/+, c(3)G^{17}/+* (Fig. 6c). The results produced in different crosses are presented and analyzed in Fig. 6 and Table 3.

The mortality of the progeny of the wild-type strain +/+ (0.09 ± 0.03) and homozygotes for multiple phenotypic markers *rucuca/rucuca* (0.11 ± 0.02) showed practically no difference (P<<0.95). The high mortality of progeny *rucuca/TM6Tb* (0.42 ± 0.03 and 0.47 ± 0.03 in different crosses) suggests that the balancer chromosomes, in addition to lethal *mutations*, carry MDMs]. However, the equal mortality of the heterozygous progeny with *c(3)G^{17}/rucuca* and *c(3)G^{17}/TM6Tb* (0.57 ± 0.03 and 0.57 ± 0.03 respectively, Table 3) cast doubt on this assumption. Nevertheless, these results indicate that there is no difference between the mortality of heterozygous progeny with chromosomes carrying accumulated MDMs in balancers and with structurally normal chromosomes, which was established earlier in classical studies[14],[15].

Table 3. The mortality rate of progeny with different genotypes, including hybrids

Cross	Number of laid eggs	Genotype and expected number of eggs	Number of flies unemerged	Mortality rate (L)
1 +/+ × +/+	696	+/+ 696	63	0.09 ± 0.02
2 *rucuca/TM6Tb* × *rucuca/TM6Tb*	441	*rucuca/rucuca* 110	12	0.11 ± 0.03
		rucuca/TM6Tb 220	93	0.42 ± 0.03
		TM6Tb/TM6Tb 110	110	1.0
3 *c(3)G^{17}/TM6Tb* × *rucuca/TM6Tb*	1107	*c(3)G^{17}/rucuca* 277	161	0.57 ± 0.03
		c(3)G^{17}/TM6Tb 277	157	0.57 ± 0.03
		rucuca/TM6Tb 277	131	0.47 ± 0.03
		TM6Tb/TM6Tb 277	277	1.0
4 *mei-P22/TM6Tb* × *mei-P22/TM6Tb*	866	*mei-P22/mei-P22* 214	129	0.60 ± 0.02
		mei-P22/TM6Tb 428	115	0.27 ± 0.02
		TM6Tb/TM6Tb 214	214	1.0
5 *mei-W68/SM1* × *mei-W68/SM1*	1112	*mei-W68/mei-W68* 278	275	0.99 ± 0.05
		mei-W68/SM1 556	281	0.51 ± 0.02
		SM1/SM1 278	278	1.0
6 *c(3)G^{17}/TM3* × *mei-P22/mei-P22*	1796	*c(3)G^{17}/mei-P22* 998	339	0.34 ± 0.02
		mei-P22/TM3 998	320	0.32 ± 0.02
7 *mei-W68/CyO* × *c(3)G^{17}/TM3*	1765	*mei-W68/+; c(3)G^{17}/+* 441	299	0.71 ± 0.02
		mei-W68/+;TM3/+ 441	207	0.47 ± 0.02
		CyO/+; c(3)G^{17}/+ 441	191	0.43 ± 0.02
		CyO/+;TM3/+ 441	361	0.82 ± 0.02
8 *mei-W68/CyO* × *mei-P22/mei-P22*	983	*mei-W68/+; mei-P22/+* 492	307	0.64 ± 0.02
		CyO/+;P22/+ 492	289	0.59 ± 0.02

Figure 6 schematically presents the hybrids and the progeny mortality in case of changed gene activity in all chromosomes with independently accumulated MDMs, expected if they are not allelic (arrows 1) and the experimental data (arrows 2).



Chromosomes with accumulated MDMs from strain c(3)G^{17} are shown in black; from strain P22, in light grey; and from strain W68, in dark grey. The centromere is marked by circle. Chromosomes without MDMs are not coloured. Heterozygotes are presented in the left column, the hybrids of the cross, in the right column. The numerals show the mortality rate L. Arrow 1, the expected results in the case of non-allelic independently arisen MDMs, calculated using the formulae given below. Arrow 2, the observed data, indicating partial complementarity of independently arisen MDMs in homologous chromosomes. The mortality rate L subscript shows chromosome in the zygotes containing different mei-*mutations*: 1, c(3)G^{17}; 2, P22; 3, W68. Chromosomes lacking mei-*mutations* are marked by +.

Figure 6. Mortality rate of heterozygotes and hybrid progeny carrying homologous (a) and nonhomologous (b, c) chromosomes with independently accumulated MDMs in different mei-mutant strains

The mortality rates of the hybrid progeny c(3)G^{17}/ mei-P22 (a), mei-W68/+, c(3)G^{17}/+ (b) and mei-W68/+, mei-P22/+ (c) are presented in Fig. 6 (marked by arrow 2) and in Table 3. These experimental results were unexpected. Collectively they indicate altered activity of genes in all chromosomes with independently accumulated MDMs. The surprising result was a significant decrease in mortality of two hybrids relative to the expected value, which was observed at a different extent (Fig. 6, arrow 1). The survival frequency of the progeny with genotype (a) c(3)G^{17}/ mei-P22 was higher then expected by about twice (0.69/0.34 = 2.03), with genotype (b) mei- W68/+, c(3)G^{17}/+, by 1.11 (0.79/0.71), while the progeny with genotype (c) mei-W68/+, mei-P22/+ show the expected viability (0.64/0.64). Upon independent MDM accumulation, these results are unlikely, if we assume that gene mutate. The MDM frequency P in the chromosome is calculated as

$$P = \frac{N \cdot q \cdot k}{G} \cdot U \qquad (1).$$

Where N is the number of generations accumulating MDM, q is the proportion of the genes in the chromosome in the total gene number G = 13767, k is the coefficient of relative MDM accumulation efficiency, and U is the number of MDMs generated per generation. The frequency of coincidence (complementation) of MDMs in hybrids containing heterozygous (from different strains) chromosomes with independently accumulated MDMs in autosome 3 (c(3)G^{17}/mei-P22) is equal to the product P(1) for Drosophila strains with different meiotic *mutations* $P_{c(3)G^{17}} \cdot P_{P22}$. The values of these parameters are presented at http://flybase.bio.indiana.edu and in[29]. According to these data, the proportion of genes in autosome 3 is ≈ 0.38. Parameter k was estimated as the ratio of the rates of progeny mortality per generation (α) in strains carrying *mutations* mei-P22 и c(3)G^{17} (0.0024/0.00054 ≈ 4,4).

$$\left(\frac{1750 \cdot 0.38}{13767} \cdot U\right) \times \left(\frac{300 \cdot 0.38 \cdot 4.4}{13767} \cdot U\right) = 0.0018U^2 \qquad (2)$$

If we take the maximum U value, earlier theoretically estimated in Drosophila for recombination suppression[11] (one gene mutates per zygote), then the probability of complementation is 0.0018.

The chromosomal cis-trans test of independently arisen MDMs showed functional "complementarity" of ~ 0.5 *mutations* manifested as restoration of the normal phenotype in heterozygotes. What is the minimum number of genes that should simultaneously mutate in this case? Using equation (2), we obtain $0.0018\,U^2 = 0.5$ or $U \approx 17$. This suggests that a gene cluster simultaneously mutates in the chromosomes. The term complementarity is taken in quotation marks because, apparently, the reduced viability is caused not by gene *mutations*, but by alteration of structural chromosomal segments that are far larger than genes.

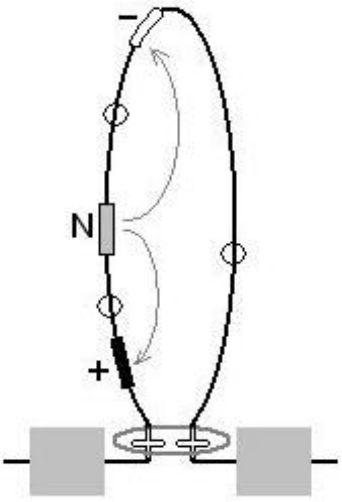
3.4. The Nature of MDM

The nature of MDMs is still unclear. The MDM expression is similar to the position-effect variegation - all progeny carries MDMs but only part of the progeny perishes. This similarity in mutation expression also suggests that MDM impair the gene activity regulation. We have shown that normal meiosis in heterozygote of chromosomes with accumulated MDMs and structurally normal, MDM-free chromosomes restores viability both in recombinant and in non-crossover chromosomes with accumulated MDMs (Fig. 4). Chromosomal recombination as such apparently does not significantly reduce MDM pressure, since we cannot exclude the involvement of gene conversion (intragenic recombination), which is also initiated by DNA breaks [30-32]. This is evidenced by the data on partial viability restoration during 20 generations by changing the balancer

(Fig. 5). The MDM elimination in meiosis is problematic to explain on the basis of gene nature of MDMs, changing DNA sequence, since the probability of their reverse mutation is very low. All facts listed above suggest that MDMs in some manner change gene expression rather than changing DNA.

In contrast to the effect of mutation $c(3)G^{17}$, the mei-*mutations* examined in this study (mei-W68, mei-P22) do not disturb homologous chromosome pairing and the SC formation. These *mutations* affect the initiation of recombination[21],[33] by means of impairing the generation of DNA breaks, produced by topoisomerases after the SC formation in Drosophila oocytes. It is known that DNA breaks, releasing structural tension in the packaged chromatin, promote its reorganization and accessibility in structural modification[34] (for instance, in the processes of inter- and intragenic recombination and DNA repair). Thus, the results of the present study do not contradict our suggestion of the epigenetic nature of MDMs, disturbing the formation and inheritance of specific functional structures of the genome[35]. These are probably structural-functional chromosome domains arranged in chromatin loops and containing gene clusters comparable with their putative number (≈ 17). The number of the loops is nearly by one order of magnitude lower than that of the genes (approximately 13767/17 ≈ 810, where the numerator is the total number of genes and the denominator, the calculated average number of genes per loop). According to the DNA content in female chromosomes[29], the numbers of loops in the X chromosome and autosomes 2, 3, and 4 are respectively 187, 286, 309, and 28.

The chromatin loops are functional and structural chromosome units responsible for gene expression, replication and recombination in eukaryotes[36]. The loops are of different size. In the telomeric and pericentromeric chromosome regions, where the rates of recombination and MDM accumulation are highest (Fig.4), the loops are most abundant, but smaller than in other chromosome parts[37]. The repeated DNA sequences at the loop base are highly variable and associated with the chromosome axial element or nuclear matrix (S/MARs). Similar sequences were detected in loops beyond the axial element, where they may act as potential triggers for the formation of new domains [38],[39]. Apparently, their secondary structure (hairpins made of inverted repeated sequences) is essential for their functioning (anchoring on the axis and forming the loops) (Fig. 7)[40]. The loops size range from 3 to 200 kb; the size of interloop stretches also vary, but in the narrower range (from 3 to 30 kb)[41]. FISH visualization of DNA probes on preparation of nuclei extracted by 2M NaCl (nuclear halos) showed that active genes are localized in the loop bases harboring the complexes for replication, transcription, DNA repair and recombination, where various topological problems of the chromatin are resolved[42]. Most results obtained using different methods of DNA loop mapping, indicate their nonrandom standard organization [43]. The issue on association of the loop structure with epigenetic regulation of gene expression, including genetic imprinting and gene-position variegation, with meiotic chromatin remodeling has long been discussed[44],[45]. The loop domains linearly and spatially border a large group of genes, ensuring interaction enhancers and promoters within these limits, i.e., act also as insulators[46-48]. The major properties of loop domains are shown in (Fig. 7).

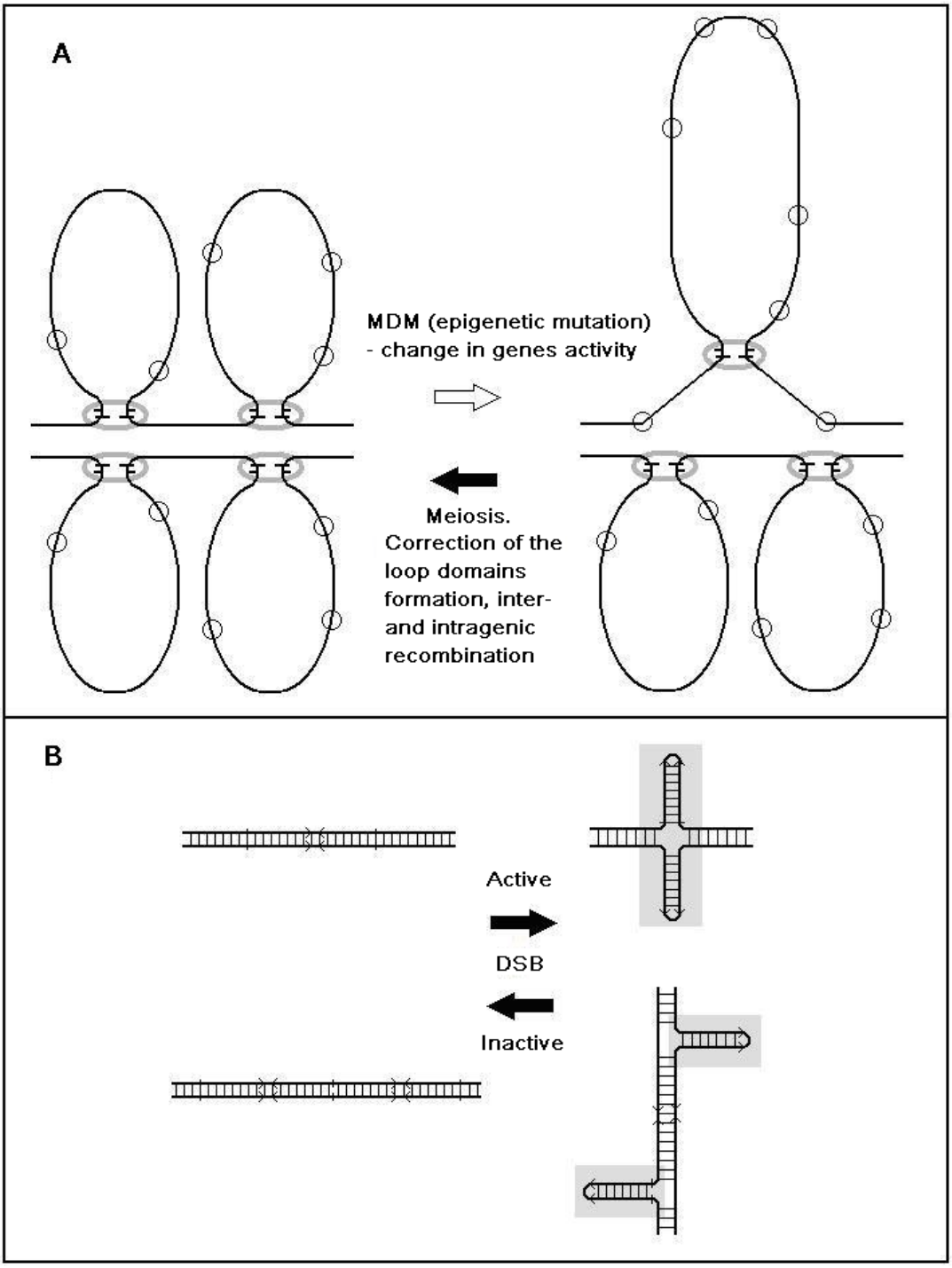


N – normal gene expression, + - high gene expression upon transfer to proximal loop region, - - low gene expression upon transfer to distal loop region.
DNA sequences at the loop base are formed hairpins and are associated with chromosome axial element or nuclear matrix (S/MARs). Analogous sequences (circle) were identified in loops beyond the axial element. Chromatin loops are formed by means of cogesion proteins (gray ellipse). Replication, transcription, repair and recombination complexes (gray square) are at the loop base.

Figure 7. Relationship between gene activity and gene position in the chromatin loops

It is hypothesized that during the life cycle, from zygote to adult, "mutant" variants of the loop domain formation appear in the chromosomes with altered gene localization relative to the loop base and the potential expression of the corresponding genes (Fig. 7). The number of abnormal loops (MDMs) per generation depends on the life-cycle duration[49]. Thus, MDMs disrupt the formation and inheritance of a specific functional state of the genome rather than alter DNA structure[35], i.e., are epigenetic in their nature. The viability restoration in hybrids carrying chromosomes with independently appeared *mutations* suggests that both an increase and a decline in the gene activity are disadvantageous for the development. In hybrid heterozygotes, such opposite in activity mutant homologous genes within symmetrically mutant loops can compensate one another, providing normal development. Since the formation of a "mutant" loop involves on average two standard loops, the number of genes with altered activity can exceed more than 17. Moreover, the viability restoration is possible upon interaction of "mutant" but nonhomologous genes, whose products are involved in the same or connected developmental chains (an analogue of interallelic complementation). This is also evidenced by the results of

the present study (significant viability restoration (P>0.99) in hybrids heterozygous for different chromosomes with MDMs mei-W68/+, c(3)G^{17}/+ (0.71 ± 0.02 in comparison with 0.79) (Fig. 6).



SK is not shown. See text and Fig. 7 for details.

Figure 8. The directional correction of the loop domain formation in meiosis (A) is accompanied by the rearrangement of the secondary structure of S/MAR repeats (B) by means of double-strand DNA breaks (DSB)

Normally, nonstandard loop domains are probably corrected during meiosis (Fig. 8A). Their correction, mediated by stabilizing function of the genome of each species[50] seems to be based on the rearrangement of the secondary structure of S/MARs (palindrome hairpins], also accompanied by DNA breaks (Fig. 8B). Thus, the function of meiosis, in addition to recombination and chromosome segregation, includes directional correction of invariant development of organisms (zygotes), depending on the structural chromatin organization in chromosomes. This correction is very effective: approximately 20% MDMs per meiotic cycle are eliminated in a heterozygote with the normal chromosome[23]. This is much higher than could be compared to the restoration of the original genetic material by means of recombination, to say nothing of reverse *mutations* whose rate is orders of magnitude lower than that of direct *mutations*. The maximum probability of random reverse mutation of loop domains is $(1/690)^2 \approx 0.0000021$. Classical point mutagenesis is practically irreversible: $(1/13767)^2 \approx 0.0000000053$ (without taking into account specific base substitutions in DNA). Since most of genic *mutations* are deleterious, they are either eliminated, in case of being very disadvantageous or lethal, or are inherited but they can never return to their initial state. In the case of MDMs, we deal with the functional restoration of the loop domain structure standard for the species - the code of the species ontogeny. The restoration of the original functional state of the genes in meiosis, which we report, is a characteristic feature of "epi*mutations*".

Ontogeny can be viewed as unfolding a branched chain of gene activities. Each link of this chain relative to the subsequent links represents only a possibility for development, which is determined, apart from quality, by the number and the functional properties of critical products (regulators). Because of this, the MDM manifestation is probabilistic and increases with their accumulation. It may well be that in a genotype with altered activity of the gene cluster, genes of development exhibit altered sensitivity to the gradient and/or the concentration of the regulators is changed. In this case, due to the existence of a sensitivity threshold, defects in the temporal pattern of the gene expression (heterochronization) are inevitable. All this at some stage of development of organisms[51] should lead to changes in adaptation to the environment. Naturally, the influence of MDMs starts at the early developmental stages and continues throughout the development.

4. Conclusions

Thus, the relationship between survival and generation number upon MDMs accumulation suggests that MDMs interaction corresponds to their synergistic epistasis, that impair the formation of DNA breaks is more effective in accumulation of MDMs. The viability in progeny after meiosis in heterozygotes with chromosome with accumulated MDMs and normal chromosome, and in heterozygotes with independently accumulated MDMs chromosomes was shown to be partially restored (20% and 50% respectively). Our results support the hypothesis that MDMs have epigenetic nature. The potential polyvariant character of the chromatin loop formation and the existence of genes with the expression decreasing from the loop base, taken together with our results unambiguously support our conclusion on the nature of MDMs.

The sexual reproduction is important for generating the evolutionary potential of the species and its further evolution, but it's most vital function is conferring stability to the species by means of meiosis. In essence, resolving this issue will provide insight in understanding evolution, which implies increasing the complexity of the structural organization of life upon the presence of nearly equal standard gene set in the majority of higher organisms.

The simple argument for epigenetic nature MDM may be obtained through using the method of comparative DNA loop by mapping a number of genes in wild chromosomes and chromosomes carrying mei-*mutations*.

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