2. Due to the fact that these results do not differ from the value of 0.0223 ± 0.0062 obtained earlier by an independent method based on estimation of zygotes' survival, a generalized estimate of the mean number of DLM in the genome for three experiments equal to 0.0204 ± 0.0050 is presented.

3. It is demonstrated that the law according to which the sex ratio of the surviving offspring of irradiated males changes as the number of DLM or the irradiation dose increases is described by an exponential function of $y = ae^{-kx}$ type where a and k are positive constants.

4. It is shown that the contribution of the natural radiation background to spontaneous mutagenesis is too negligible for even multiple changes of the background being able to influence noticeably the mutability in nature; the idea that the time course of mutability in populations is determined by biotic factors, and most probably by the time course of the species abundance, is thereby confirmed.

5. Dependencies of zygotes' mortality from DLM in *D. melanogaster* population on the frequency of occurrence of recessive lethal and visible mutations in X chromosome and chromosome 2 are deduced, and it is demonstrated that the observed increases of mutability in populations of this fly can increase the zygotes' mortality to more than 20% by means of DLM. Due to this, DLM may be a very efficient factor limiting the species' population density.

6. The importance of a comparative study of the number of DLM, and especially their fraction in the spontaneous mutagenesis in species with karyotypes sharply differing in the number and sizes of chromosome arms, for verification and specification of our view on the role of mutations in the species' abundance control, is discussed.

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Ivanov, Yu.N. Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk, 630090, Russia. FAX: (3832) 35 65 58. E-mail: ivanov@bionet.nsk.ru. Estimation of the number of genic dominant lethal mutations in the genome of the fruit fly *Drosophila melanogaster* using ethylmethane sulphonate.

A dominant lethal mutation (DLM) is any newly arisen mutation resulting in an immediate death of zygote in the very first generation. The studies carried out by Muller, Pontecorvo, Haldane and Lea, Demerec and Kaufmann, Catcheside and Lea, and especially McClintock's cytological data have shown that the mortality from DLM affects usually early stages of development (in *Drosophila* – the egg, larva, and rarely pupa) and that a considerable part of DLM represent breakages of chromosome arms with loss of their terminal acentric fragments, i.e. terminal deletions, in the course of subsequent divisions; therein, incorrect healing of the chromosome also happens when its sister chromatids join with their broken ends, due to which the cell division results in formation of a chromosomal bridge which is then broken at a random point, so that the daughter cells obtain unbalanced gene assortments (Hadorn, 1961). DLM have been studied mainly in induced mutagenesis where they are abundant, while the spontaneous mutagenesis remains so far not studied both with respect to the number of DLM and with respect to their nature. Difficulties arise due to the fact that the zygotes' mortality from DLM is directly indistinguishable from accidental death caused by other genetic agents and adverse environmental factors. This requires a rather long special experience. The interest for DLM seems to have abated also due to the fact that they are obviously deprived of any biogenetic meaning. The chromosomal mechanism of DLM has fascinated the audience so much that even doubts have been expressed as to whether single gene mutations could be dominant lethal factors. What fraction of all DLM is made up by the point, or genic DLM, remains yet a problem. In the present work a method for solving this problem on the fruit fly *D. melanogaster* using ethylmethane sulphonate (EMS) is proposed. This supermutagen is remarkable for the fact that, being nontoxic, it induces genic mutations without influencing the frequency of chromosome aberrations. It is just on these properties that the performance of the experiment described here is based. Besides, we also used the result of measuring the mean number of spontaneous DLM in the genome of *D. melanogaster* obtained by us earlier (Ivanov, 1998).

The experiment was as follows. Males of Canton-S population were divided into two groups: 1) untreated and 2) EMS-treated. The treatment was carried out as follows. A pinch of sucrose and 0.05 ml of an almost 100% EMS

solution were added to 20 ml of distilled water, stirred for better dissolvement of EMS in water, and several pieces of filter paper were soaked in the obtained solution in a hermetically closed bowl for 24 hrs. The EMS-solution-soaked pieces of filter paper were placed into several flasks, three in each; into each of the same flasks 100 ether-anesthetized males were placed in paper bags, kept there at 25°C for 24 hrs in a thermostate, whereupon crosses began to be carried out. Males of each group were crossed simultaneously, i.e. in the same flasks, with wild type (+) females from the Canton-S population and with M5 (Basc) females. In the progeny of (+) females, the survival of zygotes at the stages from egg to imago was studied, and so was the ratio of number of females to that of males among the surviving flies. The cultures in dismountable flasks where the zygote survival and the sex ratio were studied were kept under strictly equal conditions at 27°C in order that the accidental death rates were the same. In the progeny of M5 females, the incidence of recessive lethals (RLM) and visible mutations (VM) in the X chromosome of males with which they mated was measured by the well known technique. The general scheme of the work is shown in the Figure. Details of the experiment are described in our previous work, and the difference from it consists only in the fact that EMS, and not irradiation was used here as mutagen (Ivanov, 1998).

Let Q be the proportion of zygotes that survived at stages from egg to imago, r be the ratio of the number of females to that of males among the survived flies, u – incidence of RLM and VM in the X chromosome of male parents, A – the mean number of DLM in the whole genome (autosomes + X chromosome), A_1 – the mean number of genic DLM in the whole genome, and a – the mean number of RLM and VM arising in the X chromosome without EMS treatment of male parents; $\tilde{Q}$, $\tilde{r}$, $\tilde{u}$, $\tilde{A}$, $\tilde{A}_1$, and $\tilde{a}$ – the same quantities when the male parents are treated with EMS, respectively; R – the proportion of zygotes dying from accidental causes, R_f and R_m – accidental mortality of females and males, respectively; s – proportion of the genes of X chromosome in the genome.

Let us deduce some relations from which we will calculate the quantities we need, such as the mean number $\tilde{A}$ of induced DLM in the whole genome under the influence of EMS, the mean number A_1 of spontaneous genic DLM in the whole genome, etc.

The frequency Q of zygotes' survival without EMS treatment of male parents is equal to the product of the probability of no spontaneous DLM getting into the zygote by the probability of the zygote not dying from accidental

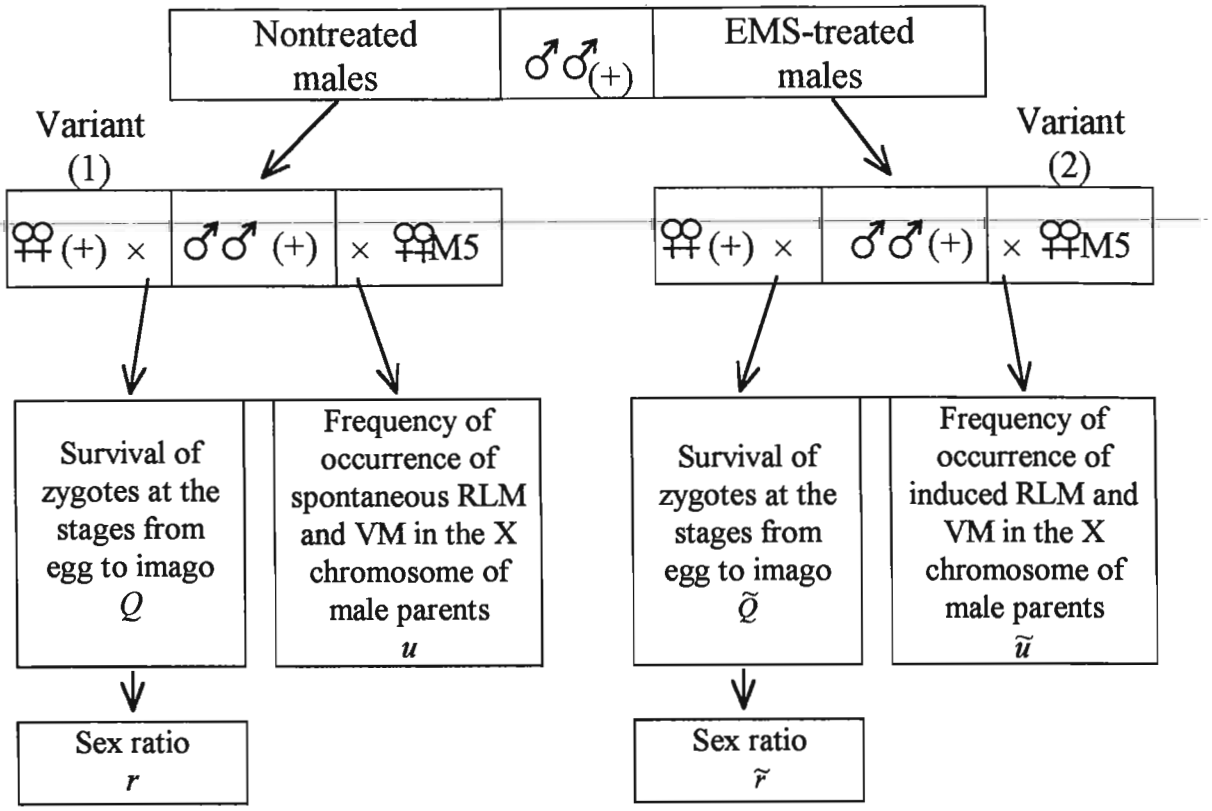

Figure 1. A scheme of experiment on estimation of the number of spontaneous genic ELM in the genome *of D. melanogaster.*

factors. If the mean number of DLM getting into the zygote is designated as X, then the former probability is e^{-X}. The number of whole genomes in the female's zygote is 2, and that in the male's zygote is 2 - s, because the male has, instead of another X chromosome, a genetically empty Y chromosome. Then an average, intersexual zygote, the sex ratio being 1:1, contains $(2-s/2)$ whole genomes, and the mean number of DLM in it is $X = A(2-s/2)$, whence the former probability is $e^{-X} = e^{-A(2-s/2)}$. The latter probability is $1 - R$. Hence

$$Q = e^{-A(2-s/2)}(1-R). \quad (1)$$

The frequency $\tilde{Q}$ of zygotes' survival when male parents are treated with EMS is found in a similar way. One of the genomes of the zygote now is treated with the mutagen, therefore the mean number of DLM in the zygote is $\frac{\left(A+\tilde{A}\right)+\left[A+\tilde{A}(1-s)\right]}{2} = A+\tilde{A}(1-s/2)$, where the first item of the numerator $(A+\tilde{A})$ is the mean number of DLM in the female zygote, and the second one $\left[A+\tilde{A}(1-s)\right]$ is the mean number of DLM in the male zygote. The probability of there being no DLM in an average zygote is $e^{-\left[A+\tilde{A}(1-s/2)\right]}$, and the sought expression is

$$\tilde{Q} = e^{-\left[A+\tilde{A}(1-s/2)\right]}(1-R). \quad (2)$$

Let us find the expression for the sex ratio $r = n_f : n_m$ in culture among the survived imagines without treatment of male parents with the mutagen. The number of female imagines is $n_f = N_f e^{-2A}\left(1-R_f\right)$, where N_f is the initial number of females, i.e. their number at the egg stage, and the product $e^{-2A}\left(1-R_f\right)$ is the fraction of the surviving females. The number of male imagines is $n_m = N_m e^{-A(2-s)}\left(1-R_m\right)$, where N_m is the initial number of males and $e^{-A(2-s)}\left(1-R_m\right)$ is the fraction of the surviving males. Hence

$$r = \frac{N_f e^{-2A}\left(1-R_f\right)}{N_m e^{-A(2-s)}\left(1-R_m\right)} = r_o \frac{1-R_f}{1-R_m} e^{-As},$$

where $r_0 = N_f : N_m$ is the initial sex ratio. In the same way the expression for the sex ratio $\tilde{r} = \tilde{n}_f : \tilde{n}_m$ in culture among imagines is found when the male parents are treated with EMS. Then the number of female imagines is $\tilde{n}_f = N_f e^{-\left(A+\tilde{A}\right)}\left(1-R_f\right)$, and the number of male imagines is $\tilde{n}_m = N_m e^{-\left[A+\tilde{A}(1-s)\right]}\left(1-R_m\right)$. Hence

$$\tilde{r} = \frac{N_f\left(1-R_f\right)}{N_m\left(1-R_m\right)} e^{-\tilde{A}s} = r_o \frac{1-R_f}{1-R_m} e^{-\tilde{A}s}.$$

The initial sex ratio r_0 and respective mortalities are assumed to be constant in all cultures, therefore the final expressions for r and $\tilde{r}$ through r_0 are applicable to sets of all the cultures from intact and EMS-treated males, respectively. Dividing r by $\tilde{r}$, we obtain an expression free from unknown r_0, R_f, and R_m:

$$\frac{r}{\tilde{r}} = e^{s\left(\tilde{A}-A\right)}. \quad (3)$$

The frequency u of the incidence of RLM and VM in the X chromosome of male parents is measured as the probability of the incidence of at least one such mutation in the X chromosome and is equal to the difference between unit and the probability of arising no such mutation. The latter probability is found from the Poisson distribution with a parameter a and is equal to e^{-a}, whence

$$u = 1 - e^{-a}. \quad (4)$$

Analogously, the incidence of the mutations in the X chromosome of EMS-treated male parents is

$$\tilde{u} = 1 - e^{-\tilde{a}}. \quad (5)$$

The assumption that the number of genic DLM in the genome is proportional to that of RLM and VM arising in the X chromosome will be written as a proportion

$$\frac{A_1}{\tilde{A}_1} = \frac{a}{\tilde{a}}. \quad (6)$$

From equation (1) and (2), by dividing one by another, we find that the mean number of DLM in the whole genome with EMS treatment is

$$\tilde{A} = A + \frac{1}{1-s/2} \ln \frac{Q}{\tilde{Q}}. \quad (7)$$

Here and herein below, $A = 0.0223 \pm 0.0062$ is the mean number of spontaneous DLM in the whole genome found by us in a special experiment (Ivanov, 1998).

Another independent estimate of $\tilde{A}$ is obtained from the data on the sex ratio, and namely from the equation (3):

$$\tilde{A} = A + \frac{1}{s}\ln\frac{r}{\tilde{r}}. \qquad (8)$$

For calculation of the mean number A_1 of spontaneous genic DLM in the genome let us transform expressions (1) and (2). Let us designate the mean number of chromosomal DLM in the whole genome as A_2, so that $A = A_1 + A_2$, and assume that $A_2 = \tilde{A}_2$, i.e. that the effect of EMS does not change the number of chromosomal DLM. Hence $\tilde{A} = \tilde{A}_1 + A_2$. It is obvious that if the genic DLM coincide with chromosomal ones, they are not recorded as genic, i.e. the sets of genic and chromosomal DLM do not intersect. Then equations (1) and (2) assume the form of

$$Q = e^{-(2-s/2)(A_1-A_2)}(1-R) \text{ and } \tilde{Q} = e^{-\left[A_1+(1-s/2)\tilde{A}_1+(2-s/2)A_2\right]}(1-R).$$

Supplementing the new expressions for Q and $\tilde{Q}$ with equations (4) - (6), and solving this system, we will obtain expressions for A_1 and $\tilde{A}_1$ through empiric values:

$$A_1 = \frac{\ln\frac{\tilde{Q}}{Q}}{(1-s/2)\left[1-\frac{\ln(1-\tilde{u})}{\ln(1-u)}\right]};\quad \tilde{A}_1 = \frac{\ln\frac{Q}{\tilde{Q}}}{(1-s/2)\left[1-\frac{\ln(1-u)}{\ln(1-\tilde{u})}\right]}. \qquad (9)$$

Since $\tilde{A} - A = \left(\tilde{A}_1 + A_2\right) - \left(A_1 + A_2\right) = \tilde{A}_1 + A_1$, equation (3) may be written as

$$\frac{r}{\tilde{r}}e^{s\left(\tilde{A}_1 - A_1\right)}.$$

Supplementing this with equations (4) – (6) and solving this system, we will obtain other, independent expressions for A_1 and A_1 deduced from the sex ratio:

$$A_1 = \frac{\ln\frac{\tilde{r}}{r}}{s\left[1-\frac{\ln(1-\tilde{u})}{\ln(1-u)}\right]};\quad \tilde{A}_1 = \frac{\ln\frac{r}{\tilde{r}}}{s\left[1-\frac{\ln(1-u)}{\ln(1-\tilde{u})}\right]}. \qquad (10)$$

Assuming that s = 0.19 and using the experimental data, in particular the earlier known value of A = 0.0223, we can now make calculations for all the deduced formulae (7) – (10). The error of any calculated quantity can be found from the dispersion of respective function of several independent variables, which can be easily calculated from the arguments' dispersions.

The results of the experiment are presented in Table 1. In section (a) experimentally measured and , in (b) calculated values and their standard deviations (errors) are given. Noteworthy is the difference between estimates of the number of DLM in the genome found by zygotes' survival from formulae (7) and (9), and by sex ratio from formulae (8) and (10). Tables 2 and 3 show decomposition of dispersion of the mean number A_1 of genic DLM in the genome into contributions corresponding to dispersions of single independent variables from which the A_1 value is calculated. From the Tables one can see that the estimate of dispersion of the A_1 value calculated by the sex ratio ($s^2_{A_1} = 1093.3\cdot10^{-8}$) exceeds by 2 orders the estimate of dispersion of this quantity calculated by zygotes' survival ($s^2_{A_1} = 973.8\cdot10^{-10}$) and that this is accounted for by the strong variability of the sex ratio. That is why the estimates obtained from the zygotes' survival are more efficient, and it is just these that were taken as the basis, since they are closer to real ones. The estimates obtained from the sex ratio are of some interest only because this method is checked here: in spite of its apparent simplicity, it requires rather large samples. In order to diminish the error of the number A_1 of genic DLM in the genome, we measured the spontaneous mutability in the X chromosome with a higher accuracy than it was possible by one measurement in the experiment with EMS carried out in 1977. Due to uniformity of data, we merged the results of 16 measurements of mutability in the X chromosome of males from the Canton-S population for the period of June 1973 to October 1981, increasing the size of the gamete sample to 17243 from 1637 in 1977.

Table 1. Estimation of the number of genic DLM in the whole genome (autosomes + X chromosome) of *Drosophila melanogaster* by the effect of EMS.

a) Experimental data

Experiment conditions	Mutability in X chromosome of male parents (%) u	Gamete sample size	Mean value of the fraction of survived zygotes in cultures Q	Number of cultures for estimation of survival	Mean value of the ratio of the number of females to that of males in cultures r	Number of cultures for estimation of sex ratio
With EMS treatment of male parents	34.971	346	0.7355 ± 0.0255	26	0.9852 ± 0.0678	26
Without treatment	0.203	17243	0.9414 ± 0.0075	24	1.1242 ± 0.1239	26

b) Calculated values

Experiment conditions	Mean number of RLM and VM in the X chromosome a	Estimation of the mean number of A of all DLM in the genome		Estimation of the mean number A_1 of genic DLM in the genome	
		by zygotes' survival	by sex ratio	by zygotes' survival	by sex ratio
With EMS treatment of male parents	0.43034	0.2949 ± 0.0398	0.717 ± 0.684	0.274 ± 0.040	0.698 ± 0.687
Without treatment	0.00203	—	—	0.00129 ± 0.00031	0.00330 ± 0.00331

Table 2. Contribution of dispersions of $\tilde{Q}$, Q, $\tilde{u}$, and u to dispersion $s^2_{A_1}$ of the mean number of genic DLM in the genome.

Contribution to $s^2_{A_1}$	$s^2_{\tilde{Q}}$	s^2_{Q}	$s^2_{\tilde{u}}$	s^2_{u}	$s^2_{A_1}$
Absolute x 10^{10}	331.1	17.6	142.2	482.9	973.8
%	34	2	15	49	100

Table 3. Contribution of dispersions of $\tilde{r}$, r, $\tilde{u}$, and u to dispersion $s^2_{A_1}$ of the mean number of genic DLM in the genome.

Contribution to $s^2_{A_1}$	$s^2_{\tilde{r}}$	s^2_{r}	$s^2_{\tilde{u}}$	s^2_{u}	$s^2_{A_1}$
Absolute x 10^{10}	295.0	757.8	9.2	31.3	1093.3
%	27	69	1	3	100

Table 4. Relative numbers of genic and chromosomal DLM in *D. melanogaster* in spontaneous mutagenesis and under the influence of EMS in the given experiment.

Experiment conditions	Mean number of DLM in the genome	Genic A_1	Chromosomal A_2	Total DLM A
Without EMS treatment	Absolute	0.0013	0.0210	0.0223
	%	6	94	100
With EMS treatment of males	Absolute	0.2740	0.0209	0.2949
	%	93	7	100

Data on the relative numbers of genic and chromosomal DLM in spontaneous mutagenesis and after EMS treatment of flies in our experiment are presented in Table 4. The mean number of chromosomal DLM in the genome was obtained as the difference $A_2 = A - A_1$ (or $\tilde{A}_2 = \tilde{A} - \tilde{A}_1$). Genic DLM made up about 6% of all spontaneous DLM, whereas with EMS treatment the proportion of genic DLM increased to 93% of the total number of DLM.

The hypothesis that EMS does not affect the chromosome aberrations, leaving their incidence at the spontaneous level, seems too strong. Let us consider the question of how the result of our calculations would change if the EMS effect

in our experiment increased the number of chromosomal DLM by k times, so that the number of induced chromosomal DLM would be equal to $\tilde{A}_2 = kA_2$, where $k \geq 1$. Let us calculate the A_1 value for this general case. We have equations $A_1+A_2 = A$ and $\tilde{A}_1 + kA_2 = \tilde{A}$. From the data of our experiment (Table 1) we have: $\tilde{A}_1 : A_1 = a : \tilde{a} = 211.8$, whence $\tilde{A}_1 = 211.8A_1$; $\tilde{A} = 0.2949$, and $A = 0.0223$. Substituting these numbers in our equations, we obtain a system of equations with unknown A_1 and A_2:

$$A_1 + A_2 = 0.0223;$$
$$211.8A_1 + kA_2 = 0.2949,$$

solving which we find:

$$A_1 = \frac{0.0223k - 0.2949}{k - 211.8}.$$

Considering A_1 as a function of k, we see that A_1 (1) = 0.00129 and that the derivative of this function

$$\frac{dA_1}{dk} = -\frac{4.428}{(k-211.8)^2} < 0$$

is negative at all values of k, i.e. A_1 (k) is a decreasing function of k. Hence it follows that at k = 1 we found the highest value for A_1 , and in this case the true A_1 value must be lower, while that for A_2 , on the contrary, higher than in our calculation.

However, the value of A_1 = 0.00129 evokes little doubt, since it finds its confirmation from population genetics, and if so, EMS retains its reputation as a mutagen which brings about only genic mutations without any disruption of the chromosome thread. In studies of *D. melanogaster* populations, at least at moderate latitudes, there exists a problem of elimination of lethal chromosomes 2 connected with the fact that the usual frequency of spontaneous incidence of RLM in chromosome 2 supplies the population with by an order more lethals than they are eliminated in compounds l_i / l_j due to identity, or allelism, when the lethal is homozygotized (Dubinin, 1966). The fraction of lethal chromosomes 2 is maintained relatively constant in the population, and therefore it is inevitable to hypothesize that autosomal RLM possess a certain level of dominance and are eliminated mainly due to penetrance in heterozygotes. Thereby the nature of genic DLM is confirmed: these are usual newly arisen RLM with a certain penetrance in heterozygotes. According to the experiment conditions they cannot be preexistent, because in this case the mortality caused by them would be equal in variants (1) and (2), and would be excluded as accidental.

The body of mathematics of the selection theory of Fisher, Haldane, and Wright permits estimating, with a certain degree of inevitable idealization, the mortality from genic DLM in a *D. melanogaster* population, and determining therefrom their number in the genome. Let the population be panmictic, i.e. zygotes be formed in it according to the Hardy – Weinberg rule; then the frequency of lethal chromosomes 2 in it will be q = 0.16; the incidence of spontaneous RLM in chromosome 2 will be u = 0.008; and the probability of allelism (loci identity) of lethals in compounds will be I = 0.004. The coefficient of selection against heterozygotes with respect to lethal chromosome 2 will be expressed through these quantities by an equation

$$s = 1 - \frac{q - u - p + \sqrt{(1-u)^2 - 4p(q-u)I}}{2q(1-I)},$$

where $p = 1 - q$. After substitution of numbers we obtain s = 0.0494. Let us now estimate the mortality from lethal chromosomes 2 on a set of zygotes not affected by chromosomal DLM. The total mortality of the zygotes from lethals in chromosome 2 is

$$S(l) = 2pqs + q^2\left[1 - (1-s)^2(1-I)\right].$$

It can be rather simply divided into 2 items: 1) mortality from preexistent lethals both in homozygotes and heterozygotes:

$$S(l_o) = \frac{q-u}{1-u}\left\{2ps + \frac{q-u}{1-u}I + q(1-I)\left[1-(1-s)^2\right]\right\},$$

and 2) mortality from newly arisen RLM acting mainly due to the dominant effect in heterozygotes and rarely due to allelism with preexistent lethals in compounds:

$$S(l_n) = \frac{pu}{1-u}\left\{2ps + \left(q + \frac{q-u}{1-u}\right)I + q(1-I)\left[1-(1-s)^2\right]\right\}.$$

The latter is just the mortality from genic DLM in chromosome 2. Substituting our numbers, we obtain:
$S(l) = 15.84 \bullet 10^{-3}$ (100%); $S(l_o) = 15.16 \bullet 10^{-3}$ (96%), and $S(l_n) = 0.68 \bullet 10^{-3}$ (4%).

A strict extrapolation of zygotes' mortality from genic DLM of chromosome 2 to the whole genome is made difficult by the fact that the pair of sex chromosomes has its peculiarities which we have to ignore for want of necessary data; that is why our results become rather rough. Let us assume sex chromosomes being similar to autosomes and

assume the proportion of genes of chromosome 2 in the whole genome as equal to 0.36. This fraction is obtained as the fraction of euchromatin of chromosome 2 in the euchromatin of the whole genome according to the data borrowed from compendia made by Zakharov (1979), Korochkina (1977), and Zhimulev (1993). Then the zygotes' mortality from genic DLM arising in the whole genome is equal to 0.00068:0.36 = 0.00189. The zygotes' mortality from total DLM amounts to 0.0415 (Ivanov, 1998), therefore the fraction of genic DLM among them is 0.00189 : 0.0415 = 0.0455, whence the average number of genic DLM in the whole genome is approximately equal to A_1 = 0.0455 x 0.0223 = 0.00101. Our independent estimates of the average number of genic DLM in the whole genome 1) 0.0013 and 2) 0.0010 are rather close to each other and confirm each other.

Extrapolation of data on the number of DLM from *D. melanogaster* to species of other taxa will undoubtedly come across limitations due to the difference in their karyotypes with respect to the number of chromosome arms. As the number of arms increases, their affection by DLM decreases, since the genetic content of arms thereby diminishes, due to which their breakages accompanied by formation of terminal deletions become less dangerous. In larger chromosomes, the harmful effect of even very small terminal deletion may be enhanced to lethality due to growing together of sister chromatids and to the chromosome bridge which is in this case formed in cell division, is broken in a random site and forms unbalanced gene assortments in daughter cells. The chromosomes broken at bridges may again have a defective healing, i.e. growing together of sister chromatids, which results in a new bridge at the next cell division, etc. When chromosomes are small, the bridges will not be as harmful, which is especially well seen on the chromosomes that can be lost completely without any lethal consequences. If one ignores such an influence of chromosome bridges and makes some other simplifying assumptions, then, at such idealization, the dependence of the mean number A_2 of chromosomal DLM in the genome on the number f of chromosome arms is a function $A_2 = k(1-\alpha f)$, where k and α are positive constants, α being the mean size of the maximal non-lethal terminal arm deletion in the genome expressed as a genome fraction, so that $1 - \alpha f$ is the genome fraction vulnerably to chromosomal DLM, i.e. its part whose breakages result in deletions of lethal size. As f increases due to karyotype fragmentation into progressively smaller chromosomes on the condition that the largest chromosome arms in the genome do not remain fixed, but decrease infinitely in size, the number A_2 of chromosomal DLM decreases and becomes equal to zero as soon as the relation $\alpha f \geq 1$ is attained. Species with large numbers of chromosomes in which even the largest chromosome arms are very small and are close by their fraction in the genome to the α value may be free from chromosomal DLM and have a low DLM level in general, because the number of genic DLM, apparently similar in various species, is rather negligible. On the contrary, species of the genus *Drosophila* and those karyotypically similar to them, which have few chromosomes and whose chromosome arms are large as compared to α, must have many chromosomal DLM and therefore DLM in general. As contrary to the few-chromosome karyotype of *Drosophila*, one may refer to that of grayling *Thymallus thymallus* which contains 100 – 106 , and on the average 102 chromosomes and 170 chromosome arms (Severin, 1979). DLM in grayling do not seem to play any essential role, since the genetic content of each of so many chromosome arms is negligible, their breakages are not dangerous and are not DLM, which is probably confirmed by the variation of the number of chromosomes in the karyotype. The mutagenesis as regulator in the ecosystem must be especially efficient when it limits the population number of few-chromosome species like *Drosophila*, and it is probably not without reason that the first factual data on the regulatory action of mutations on the population density were obtained just on *D. melanogaster* (Ivanov and Ivannikov, 1997).

To summarize, the use of EMS for induction of mutations throws some light on the nature of DLM. It is believed that EMS causes mainly genic mutations and much less often chromosome breaks. If all the spontaneous mutations were genic, then EMS treatment would increase their number as efficiently as that of genic mutation in the X chromosome. However, the effect of EMS in the experiment was accompanied by an extraordinary increase in the number of RLM and VM in the X chromosome and by a comparatively small increase in the number of DLM – by 212 and 13.3 times, respectively. Therefore the overwhelming majority of spontaneous DLM represent chromosome aberrations, i.e., as it has been established cytologically (Hadorn, 1961), losses of chromosome fragments during the division of the developing zygote and chromosome bridges. Calculation of the mean number of genic DLM in the whole genome (autosomes + X chromosome), on assumption that EMS does not bring about chromosome breaks, gives a value of $A_1 = 0.00129 \pm 0.00031$, which is about 6% of the whole number of spontaneous DLM in the genome measured by us earlier as $A = 0.0223 \pm 0.0062$, whereas the chromosomal DLM make up the remaining 94% of this quantity. Data on *D. melanogaster* population genetics show that a considerable part of autosomal RLM are eliminated in heterozygotes. Such elimination, if it concerns newly arisen mutations, is a phenomenon recorded as DLM. Zygotes' mortality from newly arisen RLM, i.e. from genic DLM, can be measured, whence another, independent estimate of the mean number of genic DLM in the whole genome $A_1 \approx 0.0010$ rather close to the former, is obtained. It has been demonstrated that species must differ in the number of DLM in their genomes, and an idealized dependence of the number of chromosomal DLM in the genome on the number of chromosome arms in the species' karyotype has been considered.

Conclusions

1. A genetic method of studying the nature of spontaneous dominant lethal mutations (DLM) and estimating the relative number of their types in the genome of the fruit fly *D. melanogaster* with the help of ethylmethane sulphonate (EMS) is described.
2. DLM are divided into 1) chromosomal and 2) genic. The mean number of genic DLM in the whole genome (autosomes + X chromosome) measured in an experiment with EMS, on assumption that it causes only genic mutations and does not break chromosomes, is 0.00129 ± 0.00031, i.e. about 6% of all the DLM. The rest 94% are chromosomal DLM.
3. In classical works it has been established that chromosomal DLM are chromosome breaks with loss of their acentric fragments and formation of chromosome bridges between daughter cells in the course of subsequent divisions of the developing zygote. The present work evolves the idea that genic DLM are ordinary genic mutations with some penetrance of lethal effect in heterozygote which are recorded as DLM only when they cause zygotes' death immediately at their origin, i.e. in the 1st generation.
4. Estimation of the mean number of genic DLM in the genome obtained from the mortality from newly arising recessive lethals in the population of *D. melanogaster* under usual conditions gives a value of 0.0010 which is rather close to the empirical one.
5. Limitations to extrapolation of the results of measurement of the number of DLM in the genome of *D. melanogaster* to species of other taxa are discussed, and an idealized dependence of the number of chromosomal DLM in the genome on the number of chromosomal arms the species' karyotype is presented.

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Estimation of the number of spontaneous dominant lethal mutations in the genome of *Drosophila melanogaster.*

A dominant lethal mutation (DLM) is any newly arisen mutation that causes death of the zygote immediately, in the 1st generation (Hadorn, 1961). Any, including recessive lethal (RLM) or visible (VM) mutation, due to its negative influence on the viability, may cause immediate death of the zygote, and in this case it is recorded as DLM. However, if it survives at first and causes death afterwards, in subsequent generations, it may not be considered as DLM, although it manifests its lethal effect in heterozygote: the death caused by it will be attributed to chance mortality. It is in this way that DLM are understood, which will be discussed in the present paper.

The difficulty in estimating the number of DLM consists in the fact that it is impossible to separate DLM-induced death of zygotes from occasional death: it can be done only by means of a special organization of the experiment. The present work contains a description of such an experiment and of its result in solution of this problem on the fruit fly *D. melanogaster.*

In order to remove all the obstacles brought in by accidental death, the following method was used. Under equal conditions, in the Canton-S population, the total death rate of zygotes at the stages from the egg to imago was counted 1) without irradiation and 2) with γ-irradiation of male parents, so that one genome of each zygote was irradiated in order to heighten the DLM frequency. For estimation of the degree of its heightening, in the same male parents the frequency of occurrence of mutations in the X chromosome was determined by the M5 (Basc) method 1) without irradiation and 2) with irradiation. The number of DLM in irradiated genome increased just like that of RLM and VM in the X chromosome, while the accidental death rate in variants (1) and (2) remained equal, which permitted excluding it from respective equations.

Let Q be the proportion of zygotes that have survived from the egg to imago stage, u be the frequency of arising of RLM and VM in the X chromosome, A be the mean number of DLM in the whole genome (autosomes + X chromosome), and a be the mean number of RLM and VM arising in the X chromosome without irradiation; $\tilde{Q}$, $\tilde{u}$, $\tilde{A}$, and $\tilde{a}$ be the same quantities under γ-irradiation of male parents; R be the accidental death rate (caused by lethal genetic factors apart from DLM or by adverse environmental factors); s be the proportion of genes of the X chromosome in the whole genome, and e be the base of natural logarithms.

Then we obtain the following relations. The frequency Q of survival of non-irradiated zygotes is equal to the product of the probability of no DLM occurring in the zygote by the probability of the zygote not dying from chance causes. The former probability is equal to e^{-X} where X is the mean number of DLM in the zygote, i.e. a Poisson distribution parameter that the number of DLM in the zygote obeys. The number of whole genomes in a female's zygote is 2, and in a male's zygote 2 - s., since the male contains, instead of the second X chromosome, genetically empty Y chromosome. Then an average, intersexual, zygote, the sex ratio being 1:1, contains $2-s/2$ whole genomes, and the mean number of DLM in it is $X = A(2-s/2)$. Hence, the former probability is $e^{-X} = e^{-A(2-s/2)}$. The latter probability is 1 - R. Therefore

$$Q = e^{-A(2-s/2)}(1-R). \tag{1}$$

In a similar way the expression for the frequency $\tilde{Q}$ of survival of zygotes of γ-irradiated male parents, when one of the zygote's genomes turns out to be irradiated, is found. However, this time the mean number of DLM in an average zygote will be $\frac{\left(A+\tilde{A}\right)+\left[A+\tilde{A}(1-s)\right]}{2} = A+\tilde{A}(1-s/2)$, where the first item in the numerator, $\left(A+\tilde{A}\right)$, is the number of DLM in the female's zygote, and the second one, $\left[A+A(1-s)\right]$, is the number of DLM in the male's zygote. The probability of there being no DLM in an average zygote is $e^{-\left[A+\tilde{A}(1-s/2)\right]}$, and the sought expression will be

$$\tilde{Q} = e^{-\left[A+\tilde{A}(1-s/2)\right]}(1-R). \tag{2}$$

The frequency u of mutation occurrence in the X chromosome is measured as the probability of there being at least one mutation in it and is equal to the difference between unit and the probability of there occurring no such mutation. The latter probability is found from Poisson distribution with parameter a and is equal to e^{-a}, whence

$$u = 1-e^{-a}. \tag{3}$$

Similarly, the frequency of occurrence of mutations in the X chromosome when male parents are irradiated is

$$\tilde{u} = 1-e^{-\tilde{a}} \tag{4}$$

where ã is a parameter of Poisson distribution for the number of mutations arising in the X chromosome under irradiation.

Another equation is assumption that at our rather low irradiation dose the number of DLM in the genome is proportional to the number of mutations arising in the X chromosome:

$$A : \tilde{A} = a : \tilde{a}. \tag{5}$$

Equations (1) – (5) form a system with unknown A, $\tilde{A}$, a, $\tilde{a}$, and R, by whose solution we find the expression for the mean number of spontaneous DLM in a whole genome:

$$A = \frac{\ln\frac{\tilde{Q}}{Q}}{(1-s/2)\left[1-\frac{\ln(1-\tilde{u})}{\ln(1-u)}\right]}. \tag{6}$$

Due to independence of the quantities $\tilde{Q}$, Q, $\tilde{u}$, and u which are obtained in independent experiments, the error of the A value is found by a simple formula which, for the sake of brevity, may be given in a general form as an estimate of dispersion of a function of several variables:

$$D\left[f(x_1, x_2, \ldots, x_n)\right] = \sum_{i=1}^{n}\left(\frac{\partial f}{\partial x_1}\right)^2 s_i^2, \tag{7}$$

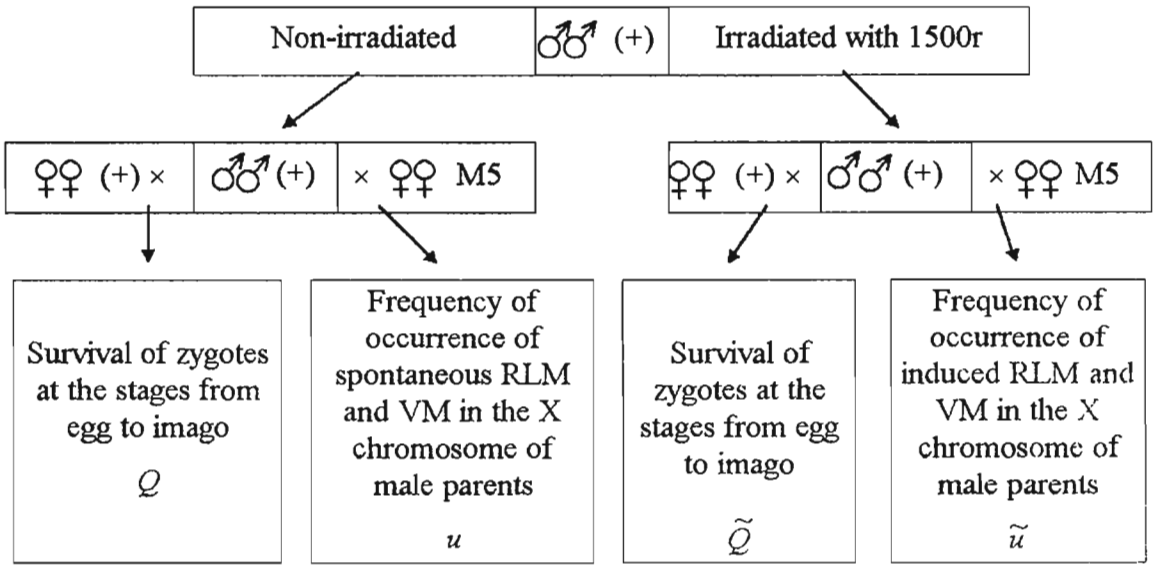

Figure 1. Scheme of the experiment on quantitative estimation of spontaneous DLM in the genome of *D. melanogaster.*

where $\frac{\partial f}{\partial x_i}$ is the partial derivative of the function f with respect to x_i at the point $(\hat{x}_1, \hat{x}_2, ..., \hat{x}_n)$, $\hat{x}_i$ is the empirical value of x_i, and s_i^2 is the estimate of dispersion of the $\hat{x}_i$ value.

In the experiment, two groups of males from the laboratory Canton-S population – 1) non-irradiated and 2) γ-irradiated with a dose of 1500 r - were used. Both of them were crossed with wild type (+) females from the Canton-S population and simultaneously, in the same tubes, with females of the strain M5 (Basc). In the progeny of (+) females, the survival of zygotes from the egg to the imago stage was estimated: Q in variant (1) and $\tilde{Q}$ in variant (2). In crosses with M5 females, the frequency of occurrence of X-linked mutations in spermia was estimated: u in variant (1) and $\tilde{u}$ in variant (2). The general scheme of the experiment is diagrammatically presented in Figure 1.

100 irradiated and 100 non-irradiated males were taken. Every 4 males were placed into a tube with 6 (+) females and 4 M5 females and kept for 1 – 2 days for mating. Every 6 (+) females were placed into dismountable flasks fixed with adhesive tape, in which eggs and imagos were counted. The counting of laid eggs was performed in 7 – 20 hours (till larvae hatching). The survived imagos were counted in 9 days for several days until depupation of all the developed flies. In variants (1) and (2) there were 23 and 25 dismountable flasks, respectively. M5 females were placed in 4s into simple tubes containing medium, and the experiment on estimation of the X chromosome mutability of males with which they were mated was carried out with them in the usual manner. Considered as RLM were both lethals and semilethals. Considered as lethals and semilethals were mutations that reduced the number of (+) males in F_2 to 0 – 5 and 5 – 20% of the expected one, respectively. Assumed as the expected number of (+) males was 1/3 of all F_2 flies with other phenotypes. The tubes in which the initial crosses were carried out and the dismountable flasks were kept at 27°C until the complete development of flies, and the rest of crosses for estimation of X-linked mutability were carried out at room temperature.

The results of the experiment are presented in Table 1. In section (a) the measured, and in (b) the calculated values are given. When survival at the stages of egg to imago was estimated, it turned out that the proportion of survived zygotes Q or $\tilde{Q}$ depended on the number of flies developed in the flask. At large numbers of flies, i.e. at a high population density, the survival was lower, and *vice versa*. Linear approximations of this dependence in our variants are

1) $Q(x) = 0.9664 - 9.988 \cdot 10^{-4} x$ and 2) $\tilde{Q}(x) = 0.7208 - 7.991 \cdot 10^{-4} x$.

Table 1. Estimation of the number of DLM in the whole genome (autosomes + X chromosome) by the zygotes' survival.

a) Empirical data

Conditions of experiment	Number of eggs N	Number of imagos n	Survival n/N	Survival under equalized population density Q	Mutability in the X chromosome of male parents (%) u	Gamete sample size
With irradiation of male parent	3198	2038	0.6373	0.6377 ± 0.0205	3.197	1220
Without irradiation	3593	2925	0.8141	0.8625 ± 0.0197	0.203	17243

b) Calculated data

Conditions of experiment	The mean number of RLM and VM in the X chromosome a	The mean number of DLM in the genome A	Accidental death R	Zygotes' death from DLM
With irradiation of male parent	0.03249	0.3559 ± 0.0467	0.1002	0.2913
Without irradiation	0.00203	0.0223 ± 0.0062	0.1002	0.0415

The mean number of imagos in a flask in variants (1) and (2) was 127.2 and 81.5, respectively. Such a difference in population density brought about also a difference in chance mortality R. However, this obstacle is easily removed by reducing the zygotes' survival in the two variants to the same flies' population density in the culture. If, for a higher accuracy, the mean number of flies for both variants is assumed to be $\frac{1}{2}(127.2+81.5) \approx 104$, and the values $Q(104)$ and $\tilde{Q}(104)$ are calculated, they will correspond already to conditions of equal population densities under which the accidental mortality R will also be equal. It is just these values that are presented in Table 1(a) as those correcting the survival value of n/N, where N is the total number of eggs and n is the number of developed flies in all the cultures of the variant.

Now let us estimate the needed fraction s composed of the genes of the X chromosome in the whole genome. The length of the X chromosome amounts to 70.4 map units or, in the cells of salivary glands, 220 μ. The length of the whole genome is 287.7 map units, or in the cells of salivary glands, 1180 μ (Lindsley and Grell, 1968). Hence the X chromosome makes up 70.4 : 287.7 = 24% or 220 :1180 = 19% of the whole genome. "Cytologically chromosome 2 is longer than the X chromosome by 2.5 times. N.I. Shapiro and R.I. Serebrovskaya (1934) in experiments with X-rays demonstrated that the frequency of induced mutations in chromosome 2 was also by 2.5 times higher than in the X chromosome" (Dubinin, 1967). Assuming that large chromosomes 2 and 3 have equal lengths, and therefore taken together exceed by 5 times the X chromosome, we obtain the proportion of the latter in the genome $s = 1/6$. Probably the most correct estimate of fraction of the genes of X chromosome in the genome will be the proportion of its euchromatin in that of the whole genome. According to our calculations based on the data borrowed from the reviews of Korochkina (1977) and Zhimulev (1993), the length of euchromatin of the X chromosome amounts to about 1.0 μ, that of euchromatin of the whole genome about 5.2 μ, whence $s = 0.19$. If a chromosome DNA thread is measured not by the length but by the mass (Kavenoff and Zimm, 1973), which seems to be more accurate, then the X chromosome euchromatin mass amounts to $14.3 \cdot 10^9$ daltons, and the mass of euchromatin of the whole genome does so to $74.95 \cdot 10^9$ Daltons, whence the same estimate, $s = 0.19$, is obtained. The calculated values in Table 1(b) were obtained for $s = 0.19$.

In Table 2, the relative role of the quantities included in formula (6) in the error of the mean number A of spontaneous DLM in the genome is shown. The contribution of this variable to the estimate of the function dispersion is found as the ratio of respective item in the right-hand part of the formula (7) to the total sum. It became clear that the largest contribution to the estimation of dispersion of A was made by the dispersion of the u value, i.e. variance of the spontaneous mutation rate in the X chromosome.

In order to diminish the A error, we measured the u value with a higher accuracy already after the completion of the experiment performed in 1976. The spontaneous mutation rate in the males' X chromosome in our Canton-S population practically had not changed for several years. and this permitted merging the data of its measurements obtained from June 1973 to October 1981 (totally 16 samples containing 17243 gametes). In Table 2, already more

accurate data are presented, but the contribution of s_u^2 to s_A^2 remains nevertheless the largest (42%). In this way, development of dispersion of the function under calculation into its components corresponding to independent variable is a very useful method. It shows the researcher the critical points in the experiment and permits diminishing the error in its replication, increasing the accuracy where this increase gives the highest effect.

The mean number of spontaneous RLM and VM arising in the whole genome, calculated from their mean number in the X chromosome $a = 0.00203$ and its fraction in the genome $s = 0.19$, is equal to 0.0107. Totally, together with DLM, on the average about 0.0107 + 0.0223 = 0.0330 mutations arise, and the proportion of DLM among them is 68%. In this way, the DLM are the most numerous class of mutations in spontaneous mutagenesis whose significance has not so far been understood quite well. It becomes clear that mutations play a regulatory role in the ecosystem, and DLM must have here a decisive importance as a factor of mortality (Ivanov and Ivannikov, 1997).

Table 2. Contribution of dispersions of $\tilde{Q}$, Q, $\tilde{u}$, and u to dispersion s_A^2 of the mean number of DLM in the genome.

Contribution to s_A^2	$s_{\tilde{Q}}^2$	s_Q^2	$s_{\tilde{u}}^2$	s_u^2	s_A^2
Absolute x 10^6	5.4	2.8	13.9	16.0	38.1
IN %	14	7	37	42	100

DLM are the most important factors of embryonic death in induced mutagenesis (Hadorn, 1961), and therefore the frequency of zygote death from spontaneous DLM is undoubtedly of a special interest. As we saw, the average number of spontaneous DLM in an average zygote at an equal frequency of sexes in the population is $A(2-s/2)$, and the fraction of zygotes having no DLM is $e^{-A(2-s/2)}$. Then the fraction of zygotes that died from DLM is $1-e^{-A(2-s/2)}$, which at $A = 0.0223$ and $s = 0.19$ yields a value of 0.0415, i.e. about 4%.

Extrapolating the results obtained on our Canton-S population to other *D. melanogaster* populations, one may conclude that:

1) the mean number of spontaneous dominant lethal mutations (DLM) in the whole genome (autosomes + X chromosome) is (223 ± 62). 10^{-4}, which makes up about 2/3 of all the mutations arising in the genome.
2) the frequency of zygote death from spontaneous DLM is about 4%.

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