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:

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

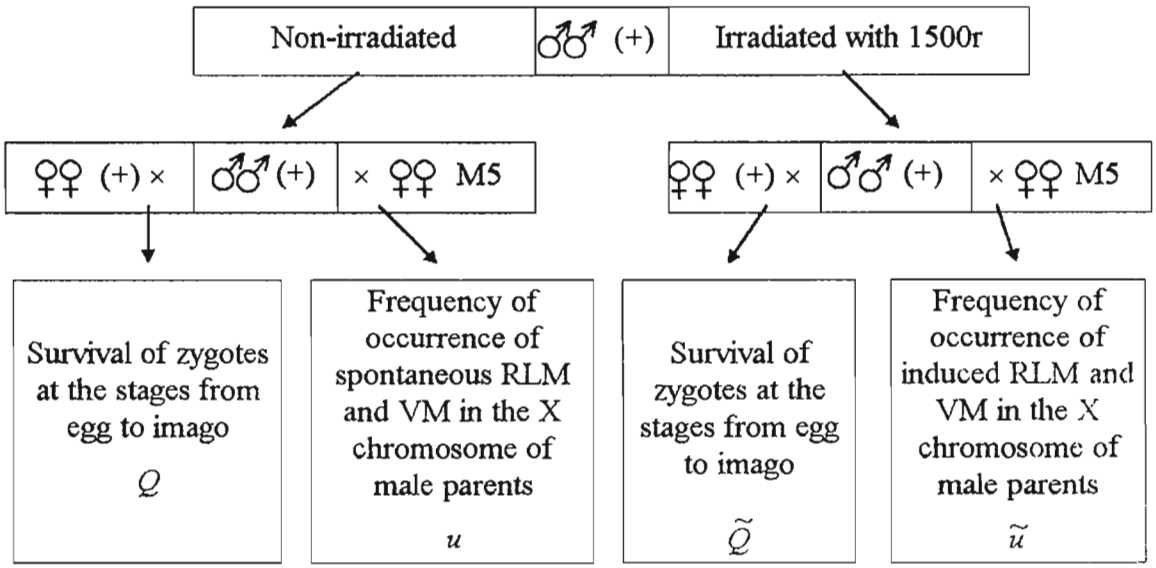

Figure 1. Scheme of the experiment on quantitative estimation of spontaneous DLM in the genome of *D. melanogaster.*

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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