

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 spontaneous dominant lethal mutations in the genome of the fruit fly *Drosophila melanogaster* by the sex ratio.

The most noticeable consequences of induced mutagenesis in males of *D. melanogaster* are 1) an increased death rate of zygotes in their progeny and 2) a shift of the sex ratio among surviving flies in the direction of prevalence of males (Hadorn, 1961). The two phenomena may serve for estimation of the number of dominant lethal mutations (DLM) in the genome. The average number of spontaneous DLM in the whole genome (autosomes + X-chromosome) calculated by us

by zygotes' survival is 0.0223 ± 0.0062 (Ivanov, 1998). However, the reliability of any measurement is determined by its replicability. That is why, apart from indicating the error of the measured quantity, which is although an indispensable yet a conditional formality, one has to try to check whether the given result can be obtained by another independent method. In the given work a method of quantitative estimation of DLM in *D. melanogaster* by sex ratio is described, and results of its application obtained in two similar experiments are presented, which has served both for checking and specifying the mean number of DLM in the genome calculated earlier by zygotes' survival. The importance of DLM studies for understanding of the time course and role of mutagenesis in nature has also been demonstrated.

Let us define the sex ratio r as a ratio of the number of females to the number of males among the surviving flies. When the males are treated with a mutagen (irradiation or chemical agents), the X spermia that give origin to females and contribute to the zygote more genetic material than the Y spermia (giving rise to males), do contribute at the same time more lethal injuries, which accounts for the elevated death rate of females. Y spermia contain a heterochromatin Y chromosome whose even complete loss does not affect the zygotes' viability; that is why injuries of the Y chromosome do not bring lethal factors into the zygote, and males have a lower mortality from DLM than females do. This is valid both for induced and for spontaneous mutagenesis, because in both cases a males zygote obtains from the father on the average only $(1 - s)$ th part of the injuries obtained from the father by a female, if s designates the fraction of X chromosome genes in the whole genome. That is why the progressive decrease of the sex ratio accompanying the increase of DLM frequency or mutagen dose seems surprising at the first glance and requires a quantitative analysis for clear understanding. As it often happens, considering the extreme cases makes things clearer. A qualitative explanation of the phenomenon consists in the fact that at extremely high mutagen doses the females may practically disappear, while the males still remain, and then the r will really decrease to zero. Consequently as the number of DLM increases, the sex ratio r must drop from approximately 1 (in spontaneous mutagenesis) to zero, which is really the case (Hadorn, 1961). A quantitative analysis of the phenomenon is made herein below. The law according to which r decreases will be established as a by-product of the present work, i.e. analytical dependencies of the sex ratio r on the number \bar{A} of DLM in the genome and on the dose D of irradiation of the male parents - $r(\bar{A})$ and $r(D)$ - will be obtained.

Let n_f be the number of females, and n_m the number of males among surviving flies, A - the average number of DLM in the whole genome (autosomes + X chromosome), a - the mean number of recessive lethal (RLM) and visible (VM) mutations arising in the X chromosome, and u - the incidence of RLM and VM in the X chromosome due to spontaneous mutagenesis. Let the symbols with a tilde be respective values when the male parents are irradiated, as, e.g., r and \tilde{r} are sex ratios without and with irradiation of male parents, respectively. Let N_f be the initial number of females in the culture, i.e. their number at the egg stage at the moment of fecundation; R_f be the accidental mortality of females, i.e. their fraction dead from any other factors except DLM; N_m and R_m be the initial number of males in the culture and their accidental mortality, respectively; $r_0 = N_f / N_m$ be the initial sex ratio; $s = 0.19$ be the fraction of genes of the X chromosome in the whole genome (Ivanov, 1998); and e be the base of natural logarithms.

Let us deduce some relations from which, by means of substituting empirical values, we shall find the average number of DLM in the whole genome.

Let us find the sex ratio $r = n_f / n_m$ among the surviving flies in the culture originating from non-irradiated male parents. The fraction of survivors among the daughters of non-irradiated male parents is equal to the product of the probability of a female not dying from spontaneous DLM by the probability of her not dying from accidental causes. The former probability is e^{-2A} where $2A$ is the mean number of spontaneous DLM in the female's zygote. The latter probability is $1 - R_f$. Hence the fraction of surviving daughters is equal to $e^{-2A}(1 - R_f)$, and their number among imagines in the culture originating from non-irradiated male parents is $n_f = N_f e^{-2A}(1 - R_f)$.

Similarly, the fraction of survivors among the sons of non-irradiated male parents is equal to the product of the same probabilities for a male, the former of which is now equal to $e^{-(2-s)A}$, and the latter $1 - R_m$. Hence the number of surviving males among imagines in the culture without irradiation is $n_m = N_m e^{-(2-s)A}(1 - R_m)$.

The sought sex ratio among the surviving flies in the culture without irradiation is

$$r = \frac{n_f}{n_m} = \frac{N_f(1-R_f)}{N_m(1-R_m)} e^{-sA} = r_0 \frac{1-R_f}{1-R_m} e^{-sA}. \quad (1)$$

The sex ratio $\tilde{r} = \tilde{n}_f : \tilde{n}_m$ among surviving imagines in the culture originating from irradiated male parents is found in a similar way. Now the number of surviving daughters is $\tilde{n}_f = N_f e^{-(A+\tilde{A})} (1-R_f)$, since the probability of a female not dying from DLM is already calculated from their mean number in the female zygote, which is $A + \tilde{A}$. The number of surviving sons in the culture is now $\tilde{n}_m = N_m e^{-[A+(1-s)\tilde{A}]} (1-R_m)$, where the probability of a male not dying from DLM is calculated from their mean number in the male zygote which is $A + (1-s)\tilde{A}$.

The sought sex ratio among the surviving flies in the culture from irradiated male parents is

$$\tilde{r} = \frac{\tilde{n}_f}{\tilde{n}_m} = \frac{N_f(1-R_f)}{N_m(1-R_m)} e^{-s\tilde{A}} = r_0 \frac{1-R_f}{1-R_m} e^{-s\tilde{A}}. \quad (2)$$

Assuming the initial sex ratio r_0 and the sexes' mortality R_f and R_m to be equal in all the cultures, for which appropriate conditions have to be observed, one may exclude these unknown quantities from the equations. By means of term-by-term divisions of equations (1) and (2), we will obtain an equation with only two unknown quantities A and \tilde{A} :

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

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

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

In quite the same way the incidence \tilde{u} of RLM and VM in the X chromosome of irradiated male parents is found:

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

The number of DLM in the genome is proportional to that of RLM and VM arising in the X chromosome. Therefore we have a proportion

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

Equations (3) - (6) form a system with unknown quantities A , \tilde{A} , a , and \tilde{a} whose solution will give us expressions for A and \tilde{A} through $s = 0.19$ and empirically found r , \tilde{r} , u , and \tilde{u} :

$$A = \frac{\ln \frac{\tilde{r}}{r}}{s \left[1 - \frac{\ln(1-\tilde{u})}{\ln(1-u)} \right]}; \quad \tilde{A} = \frac{\ln \frac{r}{\tilde{r}}}{s \left[1 - \frac{\ln(1-u)}{\ln(1-\tilde{u})} \right]}. \quad (7)$$

Due to independence of the empirical quantities each of which is measured in a separate experiment, the error of each of the calculated quantities A and \tilde{A} is found from the dispersion of respective function of independent variables which (dispersion, not the function) can be easily calculated by arguments' dispersions.

The conditions for which the calculations have been done and the final expressions of (7) determine completely the organization of the experiment in which the mean number of DLM in the genome is found from the sex ratio. Two experiments were made: at γ -irradiation doses of 1500 and 2500 r. Each experiment was made as follows. Two groups of males from the Canton-S population were taken: 1) non-irradiated and 2) irradiated with a preset dose of γ -rays. Both were crossed with wild type (+) females from the Canton-S population and simultaneously, in the same tubes, with M5 (Basc) females. In the progeny of (+) females the sex ratio was estimated among the surviving flies: r in variant (1), without irradiation, and \tilde{r} in variant (2), with irradiation. In crosses with M5 females estimated was the incidence of RLM and VM in the X chromosome of spermia: u in variant (1) and \tilde{u} in variant (2). The general scheme of the experiment is shown diagrammatically in Figure 1.

Intact and irradiated males were taken in a number of 300 each; 4 - 6 males, 2 (+) females and 2 - 5 M5 females were placed in each tube containing currant medium in which crossing was performed. They were kept together for 24 hours whereafter (+) and M5 females were transferred to tubes with enriched medium and placed separately, two (+) females or three M5 females in each tube. In order to increase the number of cultures in which the sex ratio was counted, the tubes with currant medium where the flies had mated also served for counting, since the females had already laid

Table 1. Estimation of the number of DLM in the whole genome (autosomes + X chromosome) by sex ratio at a γ -irradiation dose of 1500 r (experiment 1)

| Experiment conditions | Experimental data | | | | Calculated values | | | |
|----------------------------------|-------------------|-----------------|--|--------------------|--|--------------------|---|--------------------------------------|
| | Number of females | Number of males | Mean value of $r = n_f / n_m$ in culture | Number of cultures | Mutability in X chromosome of male parents (%) u | Gamete sample size | Mean number of RLM and VM in X chromosome a | Mean number of DLM in the genome A |
| With irradiation of male parents | 8204 | 8343 | 1.0169 \pm 0.0222 | 120 | 3.553 \pm 0.672 | 760 | 0.03617 | 0.1723 \pm 0.1640 |
| Without irradiation | 10392 | 10390 | 1.0478 \pm 0.0191 | 119 | 0.309 \pm 0.073 | 5828 | 0.00309 | 0.0147 \pm 0.0148 |

Table 2. Estimation of number of DLM in the whole genome (autosomes + X chromosome) by sex ratio at a γ -irradiation dose of 2500 r (experiment 2)

| Experiment conditions | Experimental data | | | | Calculated values | | | |
|----------------------------------|-------------------|-----------------|--|--------------------|--|--------------------|---|--------------------------------------|
| | Number of females | Number of males | Mean value of $r = n_f / n_m$ in culture | Number of cultures | Mutability in X chromosome of male parents (%) u | Gamete sample size | Mean number of RLM and VM in X chromosome a | Mean number of DLM in the genome A |
| With irradiation of male parents | 4845 | 5104 | 0.9957 \pm 0.0231 | 122 | 7.023 \pm 1.480 | 299 | 0.07282 | 0.3759 \pm 0.1790 |
| Without irradiation | 7199 | 7119 | 1.0658 \pm 0.0241 | 122 | 0.345 \pm 0.089 | 4353 | 0.00345 | 0.0178 \pm 0.0106 |

Table 3. Contribution of dispersions of \bar{r} , r , \bar{u} , and u to the dispersion s_A^2 of the mean number of DLM in the genome in 2 experiments

| Year and γ -ray dose | Contribution to s_A^2 | s_A^2 | | | | | $s_A^2 \times 10^6$ |
|-----------------------------|-------------------------|-----------------|---------|-----------------|---------|-------|---------------------|
| | | $s_{\bar{r}}^2$ | s_r^2 | $s_{\bar{u}}^2$ | s_u^2 | | |
| 1979 1500 r | Absolute $\times 10^6$ | 115.7 | 80.7 | 9.7 | 14.4 | 220.5 | |
| | % | 53 | 37 | 4 | 6 | 100 | |
| 1982 2500 r | Absolute $\times 10^6$ | 36.9 | 35.0 | 16.7 | 23.3 | 111.9 | |
| | % | 33 | 31 | 15 | 21 | 100 | |

Table 4. Results of estimation of the number of spontaneous DLM in the genome of *D. melanogaster* in 3 experiments

| Estimation method | Mean number of DLM in the genome A | Dispersion s_A^2 |
|---------------------------|--------------------------------------|------------------------|
| By zygotes' survival | 0.0223 \pm 0.0062 | 38.1 $\times 10^{-6}$ |
| By sex ratio, dose 1500 r | 0.0147 \pm 0.0148 | 220.5 $\times 10^{-6}$ |
| By sex ratio, dose 2500 r | 0.0178 \pm 0.0106 | 111.9 $\times 10^{-6}$ |
| Average | 0.0204 \pm 0.0050 | 25.2 $\times 10^{-6}$ |

Table 5. Dependence of the number of DLM in the genome of *D. melanogaster* on the γ -ray dose in 3 experiments

| Experiment and DLM recording method | Dose (r) D | Mean number of DLM in the genome \bar{A} | Dispersion s_A^2 |
|-------------------------------------|-------------------|--|-------------------------|
| All the experiments | 0 | 0.0204 \pm 0.0050 | 25.2 $\times 10^{-6}$ |
| 1976, by zygotes' survival | 1500 | 0.3559 \pm 0.0467 | 2184.5 $\times 10^{-6}$ |
| 1979, by sex ratio | 1500 | 0.1723 \pm 0.1640 | 26886 $\times 10^{-6}$ |
| 1982, by sex ratio | 2500 | 0.3759 \pm 0.1790 | 32037 $\times 10^{-6}$ |

The number of DLM in irradiated genome was counted in all experiments, i.e. for various irradiation doses. The results are presented in Table 5 as a dependence of the mean number \bar{A} of DLM in the complete genome on the dose D of γ -rays in roentgens. According to data of all the three experiments, by the method of least squares with weights, an approximation of this dependence was obtained:

$$\bar{A}(D) = 0.02048 + 0.0002035D. \quad (9)$$

It permits calculating, by the found \bar{A} value, the mortality of zygotes from DLM at the given irradiation dose for all the cases when 1) both sexes, 2) only males, or 3) only females are irradiated. It is quite easy to obtain respective formulae.

Let us come back to analysis of the shift in the sex ratio accompanying the increase of the number of DLM or of the mutagen dose. The law according to which this shift occurs has in fact been found by us herein above. Assuming in equation (2) $r_o \frac{1 - R_f}{1 - R_m} = K$, we have $\bar{r} = Ke^{-s\bar{A}}$, whence, designating \bar{r} as $r(\bar{A})$, we obtain a decreasing exponential relation

$$r(\bar{A}) = Ke^{-s\bar{A}}, \quad (10)$$

which tends asymptotically to zero.

The dependence of the number of DLM on the irradiation dose is a linear function

$$\bar{A} = A + kD, \quad (11)$$

where k is a coefficient of proportionality or increment of the mean number of DLM in the genome per 1 r irradiation. By substituting the expression (11) into equation (10) we obtain:

$$r(D) = Ke^{-s(A+kD)} = Ke^{-sA} \cdot e^{-skD}.$$

Designating here $Ke^{-sA} = \rho$ and $sk = \alpha$, we obtain again a decreasing exponential function

$$r(D) = \rho e^{-\alpha D},$$

where ρ is the sex ratio without irradiation of male parents. One has to note that the sex ratio does not depend on the irradiation of females; therefore, as it is easy to see in deducing equation (2), the dependence of r on \bar{A} or D is irrespective of whether we irradiate only males or also females.

We found dependencies of the sex ratio r on the mean number \bar{A} of DLM in the genome and on the irradiation dose D in a most general form where K , ρ , and α are positive constants whose values in each concrete case should be selected by the least squares method. So, having treated the data of Catcheside and Lea (1945) (Hadorn, 1961) on dependence of r on the X-rays dose by the least squares method, we found it as a function $r(D) = 0.9973e^{-0.00004466D}$. The constant s which for *D. melanogaster* is equal to 0.19 (Ivanov, 1998a), in other species has different values, and then it also requires an experimental estimation. In the absence of sex chromosomes in the genome or when these are indistinguishable from autosomes, which in this context is the same, the fraction of X chromosome in the genome is $s = 0$; therefore the relation (10) degenerates into a constant $r(\bar{A}) = K$, i.e. r ceases to depend on the number \bar{A} of DLM in the genome and cannot serve for its estimation. In this way, the presence of heterochromosomes and large X chromosome in *D. melanogaster* is very favourable to measurement of the number of DLM in the genome of this species, which we used in the present work.

Estimation of the number of DLM in the genome acquires a special importance in connection with the fact that mutagenesis plays a regulatory role in the ecosystem: there are data on dependence of mutability in *D. melanogaster* and probably other species on the population density, so that at a higher density it is also higher, and vice versa. A heightened mutability brings about an increased mortality and thereby limits the species' population density, when this is advantageous for the ecosystem (Ivanov and Ivannikov, 1997). Those who do not know the role of DLM in spontaneous mutagenesis usually doubt that the mutagenesis can bring about a high and, what is the most important, an immediate death of zygotes. It seems that mutations must first be accumulated and only after this will they become homozygotized and entail a considerable increase of mortality. However, in fact even at a low level of mutability, when, e.g., in X chromosome RLM and VM arise at a rate of $u = 0.3\%$, DLM bring about death of over 6% of zygotes.

In order to get convinced thereof, one has to know the dependence of zygotes' mortality on DLM in the population on the incidence of RLM and VM in the X chromosome which can be found very simply from decomposition of the process of mutagenesis into the main mutation types (Ivanov, 1991; Ivanov, 1998a). According to our results, the spontaneous mutagenesis in *D. melanogaster* may be decomposed into the following types of mutations and their proportions: DLM make up 68%, RLM 29%, and VM 3% of all the spontaneous mutations arising in the genome and recorded in usual experiments on measurement of mutability. Those mutations whose detection requires special method, e.g. mutations of sterility, inversions, translocation, duplication, etc., are detected by no less than an order more seldom than VM - the rarest of the main mutation types; they may therefore be ignored in this case. The zygotes' mortality from DLM in the population at a primary sex ratio of 1:1 is $S(A) = 1 - e^{-(2-s/2)A}$, or, at $s = 0.19$,

$$S(A) = 1 - e^{-1.905A}. \quad (12)$$

Designating the incidence of RLM and VM in the X chromosome as u_1 , we may write an expression for the incidence of these mutations in the whole genome as $u_1 / 0.19 = 5.26u_1$. From the decomposition of the spontaneous mutagenesis given here, a proportion $A / 5.26u_1 = 68 / 32$ follows, whence $A = 11.2u_1$. Substituting this expression of A through u_1 into formula (12), we obtain the dependence of the mortality caused by DLM on the incidence of RLM and VM in the X chromosome:

$$S(u_1) = 1 - e^{-21.3u_1}.$$

Expanding the exponent into a series and limiting ourselves to its first three terms, we obtain an approximation

$$S(u_1) = 21.3u_1 - 226.8u_1^2, \quad (13)$$

which demonstrates that the zygotes' death from DLM is practically directly proportional to the mutability u_1 in the X chromosome, for u_1^2 is a sufficiently small number.

In a similar way we obtain the expression of the mortality from DLM in the population through the incidence u_2 of RLM and VM in chromosome 2. The fraction of chromosome 2 genes in the whole genome is 0.36 (Ivanov, 1998b). The incidence of RLM and VM in the whole genome is $u^2 / 0.36 = 2.78u_2$. From the proportion $A / 2.78u_2 = 68 / 32$ we obtain $A = 5.90u_2$. Substituting this expression into (12), we obtain a function

$$S(u_2) = 1 - e^{-11.2u_2},$$

whose approximation gives a practically directly proportional relation

$$S(u_2) = 11.2u_2 - 62.7u_2^2. \quad (14)$$

Variations of mutability in the population are larger than those expected from the actual change of abiotic agents of spontaneous mutagenesis. They cannot be accounted for either by the change of cosmic rays flow, or by solar activity, or by chemical factors. If all these factors were responsible for the strong increases of mutability in populations of *D. melanogaster*, the same would simultaneously occur in other species, however, there are no confirmations thereof. According to data borrowed from C. Stern's textbook (1960), the dose that doubles the incidence of mutation in *D. melanogaster* is about 50 r, and a fly receives for the 1 month of its life, due to natural radioactivity background, a dose of no more than 0.01 r, so that the gametes produced by it get on the average 0.005 r. Let us calculate the fraction α of spontaneous mutability u which is referred to as the natural radioactivity background $D_\alpha = 0.005$ r, if the doubling dose is $D_1 = 50$ r. The incidence of mutations from the radiation background D_α is αu , and at the doubling dose D_1 the mutability is increased by a quantity u equal to itself. Due to the fact that the mutability increment is directly proportional to the dose increase, we have $\alpha u / u = D_\alpha / D_1$, whence $\alpha = D_\alpha / D_1$, which at our figures gives $\alpha = 0.0001$. In fact, this fraction is still smaller, since the calculation is made for an acute doubling dose, while the chronic doubling dose is higher than the acute one by about 4 times (Stern, 1960). Besides, the acute DLM-doubling dose calculated by our data from equation (9) is 100 r. It is clear that at such a small contribution of the natural radiation background to the spontaneous mutagenesis, even highly repeated changes of the cosmic rays level and of other natural radiation sources cannot have brought about the observed changes of mutability in nature. Hence a conclusion of biotic nature of the main factors of spontaneous mutability.

Numerous measurements of the frequency of occurrence of RLM show that it varies in the X chromosome from 0.05 to 1.1%, and in chromosome 2 from 0.3 to 1.27% (Dubinin, 1966). In our 45 measurements of the RLM and VM incidence in the X chromosome in natural populations and in Canton-S (1970 - 1993), it varied from 0 to 1.3%. The highest values of mutability exceed its usual level by 3 - 5 times. From the data presented here, by formulae (13) and (14), it is possible to estimate the highest values of zygotes' mortality from DLM in populations, i.e. those obtained from the highest values of mutability $\hat{u}_1 = 1.3\%$ in the X chromosome and $\hat{u}_2 = 1.27\%$ in chromosome 2: $S(\hat{u}_1) = 23.9\%$ and $S(\hat{u}_2) = 13.2\%$.

These not at all small values of mortality from DLM point to a considerable scope of its fluctuations and to these fluctuations being a most important consequence of mutability change in the population. If the mutability plays the role of a regulator of the species number, then its time course must be more or less correlated with that of population number, and therefore the results of measurement of mutability may not be extrapolated from some species to other. The mutability of each species has an independent time course of its own and, according to all data, is determined by biotic factors. Just like the selection, it has nothing to do with the biogenesis, but plays a regulatory role in the ecosystem. The selection as a repressive, and the mutability as a destructive, principles cannot be sources of transmutation (speciation), or else ecosystems could not have existed, but both are regulators of the biotic circulation. The mutation process, evolutionary by its nature, i.e. random, chaotic, and destructive, serves nevertheless the high goal of maintenance of order, constancy, and system in nature: it causes death for the sake of life.

Due to the fact that mutability must depend on the phase at which the population is during the fluctuations of the numbers so that at large numbers the mutability is higher and vice versa, otherwise it would not have been a regulatory factor, specification of the absolute number of DLM in the genome has by itself no great meaning. However, in connection with establishment of the regulatory role of DLM in populations, specification of their relative number in the genome among other main types of spontaneous mutations conserves its importance. It is especially important to study DLM in other species than *D. melanogaster* with different, contrasting, karyotype structures. According to our concept (Ivanov, and Ivannikov, 1997; Ivanov, 1998b), a non-adaptive karyotype structure, and namely concentration of the genome in a small number of large chromosome arms, a large fraction of non-coding DNA regions in the genome, terminal positions of euchromatin in the arms, etc., i.e. everything that increases the probability of disruptions of the chromosome thread or enhances their damaging effect, makes the genome vulnerable to chromosomal DLM and suggests that they play an important role in control of the species abundance. Nevertheless, however verisimilar the theoretical statements could be, they require factual corroboration. That is why a comparison of the relative numbers of DLM in the genome in species having karyotypes with sharply different numbers and sizes of chromosome arms is very interesting from the viewpoint of verification and specification of the ecological role of the mutation process as one of factors limiting the species population numbers.

To summarize, one may make the following conclusions:

1. A method of estimation of the mean number of spontaneous dominant lethal mutations (DLM) in the genome of *D. melanogaster* by the sex ratio is described, and its results obtained in two experiments at γ -irradiation doses of 1500 and 2500 r are presented: they are 0.0147 ± 0.0148 and 0.0178 ± 0.0106 , respectively.

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.

Acknowledgments: The author thanks sincerely V.G. Kolpakov for his help in translation of this paper into English, T.M. Mischenko for preparation of the manuscript for publication, and V.S. Pykhalov for his assistance in statistical treatment of materials.

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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