A Theory for the Frequency Distribution of Cluster Mutants^{1),2)}

Sohei KONDO

National Institute of Genetics, Misima, Japan

Received October 12, 1960

Introduction

Nagai and Locher (1938) and Nishina and Moriwaki (1939) concluded from their results that neutrons differ from X rays in the yield of recessive lethal mutations induced in *Drosophila* sperm in that there is a clusterwise tendency of sperm in a given irradiated male to simultaneous mutation. Fano (1944) and Lea (1955), however, showed that the grouping effects observed by those authors can be satisfactorily explained by a Poisson distribution of lethal mutants based on non-grouping effect. That is, the distribution of the number of mutants is no other than a random one. Fano and Lea's conclusion, however, does not hold for mutations recovered from irradiation of immature germ cells which have to undergo multiplication and produce many gametes per each immature germ cell (see, e.g., Singleton 1954). The reason is that if mutation occurs in any one of these cells, it eventually develops into a group of mutated gametes. In general, mutants will show up in clusters in a particular individual if mutations are induced in it at an early gametogenic stage; the resulting probability distribution of the number of mutants recovered per individual will deviate from a simple Poisson distribution. The larger the mean multiplication factor of the irradiated germ cells, the bigger will be the deviation from a Poisson distribution.

Based on such consideration, a theory for cluster mutation can be developed by taking account of a large multiplication factor. For simplicity, this paper deals with the case in which only two types of germ cells are present with different but constant multiplication factors and are acutely treated with a mutagenic agent. Generalization covering many types of cells is simple.

Comparison of the theory with experimental results will be shown for a case of irradiation of silkworm gonad so as to illustrate how to apply the theory to experiments and how good the agreement is. Further applications to experiments and further studies of a case of a more general nature will be given elsewhere.

Theory

1. *Assumptions for Theoretical Derivation of Frequency Distribution of Cluster Mutants*

1) Contribution from the National Institute of Genetics, Japan, No. 336.

2) This research has been partly supported by a grant from The Rockefeller Foundation.

It is apparent that the number of mutants recovered per individual depends on the number of mutants initially induced per individual and also on a complicated series of gametogenic processes which the treated germ cells have to undergo during the long period between the initial induction of mutations and the final recovery of observable mutations.

The following are the assumptions for the processes involved for deriving the probability distribution function of the number of mutants:

(i) *Two types of cells*: Each individual of a strain under consideration contains a cell population of two types 1 and 2 of diploid cells with cell numbers n_1 and n_2 , respectively.

(ii) *Poisson distribution of the initial mutative events*: Consider a mutation of gene A at an arbitrary locus a to a specific allele a induced by acute treatment of each individual with a mutagenic agent. We take p_1 and p_2 as the *initial rates** of mutations of this type induced in the germ cells of types 1 and 2, respectively. The induction of the initial mutations is a random process with very small values of p_1 and p_2 . That is the actual number k_i of mutated loci in n_i cells of an individual is distributed in a Poisson distribution around the mean λ_i equal to $2p_i n_i^{**}$ where $i=1, 2$.

(iii) *Cell multiplication without fluctuation*: After treatment with a mutagenic agent, any of loci a 's in n_i cells undergoes multiplication by the factor of μ_i where $i=1, 2$. Fluctuation in μ_i is negligible. Hence, the maximum number of gametes or of chromosomes with locus a at a certain gametogenic stage, amounts to $N=2\mu_1 n_1+2\mu_2 n_2$. Considering the case of germinal selection, we assume that the mutated locus a with allele a has a different multiplication factor $s_i \mu_i$. We assume, however, that the maximum number N is not affected because of the number of mutants being very small compared with that of non-mutants.

(iv) *Random sampling of gametes*: r gametes randomly sampled from the total N gametes are utilized for fertilization. In females, the random sampling process will take place when oögonia differentiate into ova.

2. Fundamental Equation for Probability Distribution of the Number of Mutants per Individual

Using the assumptions mentioned in the preceding section we can readily write the frequency distribution function $f(m)$ of m mutants per individual as follows:

$$f(m) = \sum_{\substack{k_1, k_2=0 \\ s_1 \mu_1 k_1 + s_2 \mu_2 k_2 \geq m}}^{\infty} \frac{\lambda_1^{k_1} e^{-\lambda_1}}{k_1!} \frac{\lambda_2^{k_2} e^{-\lambda_2}}{k_2!} \frac{\binom{s_1 \mu_1 k_1 + s_2 \mu_2 k_2}{m} \binom{N - s_1 \mu_1 k_1 - s_2 \mu_2 k_2}{r-m}}{\binom{N}{r}} \quad (2-1)$$

The $1-s_1$ and $1-s_2$ are the germinal selection coefficients against mutant cells and $s_1 \mu_1$ and $s_2 \mu_2$ will be called the effective multiplication factors of the mutant cells. Recalling assumptions (ii) and (iii), from Eq. (2-1) we easily find the observed

* *Experimental* mutation rate is observable only after fertilization and hence, in general, differs from the *initial* mutation rate owing to germinal selection and other factors involved in the course of gametogenesis and fertilization.

** Factor 2 is due to diploidy of germ cells.

mutation rate per locus as follows :

$$\dot{p}_{obs} = \frac{1}{r} \sum_{m=0}^{\infty} mf(m) = \frac{s_1\mu_1 n_1 \dot{p}_1 + s_2\mu_2 n_2 \dot{p}_2}{\mu_1 n_1 + \mu_2 n_2} . \quad (2-2)$$

This means that the observed mutation rate is equal to the mean average of the effective initial mutation rates $s_1\dot{p}_1$ and $s_2\dot{p}_2$ multiplied by weighing factors proportional to the fractions of viable gametes originated from cells of types 1 and 2, respectively.

The observable average number of mutants per individual calculated from Eq. (2-2) is given by

$$A = \sum_{m=0}^{\infty} mf(m) = \frac{r}{N} (s_1\mu_1\lambda_1 + s_2\mu_2\lambda_2) = a_1\lambda_1 + a_2\lambda_2 , \quad (2-3)$$

where

$$\lambda_i = 2 \dot{p}_i n_i , \quad (i = 1, 2) \quad (2-4)$$

$$a_i = \frac{r}{N} s_i \mu_i = \frac{r s_i \mu_i}{2\mu_1 n_1 + 2\mu_2 n_2} . \quad (i = 1, 2) \quad (2-5)$$

3. *Explicite Forms of Probability Distribution of the Number of Mutants*

To reduce Eq. (2-1) to simpler forms numerically calculable, we need further assumptions.

3-1. *Case of all gametes utilized for fertilization: $r/N=1$.*

Ep. (2-1) reduces to

$$f(m) = \sum_{k_1, k_2=0}^{\infty} \frac{\lambda_1^{k_1} e^{-\lambda_1}}{k_1!} \frac{\lambda_2^{k_2} e^{-\lambda_2}}{k_2!} \binom{s_1\mu_1 k_1 + s_2\mu_2 k_2}{m} \binom{N - s_1\mu_1 k_1 - s_2\mu_2 k_2}{N-m} . \quad (3-1)$$

$s_1\mu_1 k_1 + s_2\mu_2 k_2 \geq m$

For the theoretical treatment of $f(m)$ in general, it is more convenient to use the generation $G(z)$ (see, e.g., Lea and Coulson 1949, Armitage 1952). The above function for $f(m)$ can be given as the coefficient of z^m in the expansion of the following generating function :

$$G(z) = \exp (\lambda_1 z^{s_1\mu_1} + \lambda_2 z^{s_2\mu_2} - \lambda_1 - \lambda_2) . \quad (3-2)$$

Now, if cells of type 1 alone are present, the above function reduces to

$$G(z) = e^{-\lambda_1} \sum_{j=0}^{\infty} \lambda_1^j z^{js_1\mu_1} / j! . \quad (3-3)$$

That is :

$$f(m) = \frac{\lambda_1^j e^{-\lambda_1}}{j!} . \quad (m = js_1\mu_1; j = 0, 1, 2, \dots) \quad (3-4)$$

This means that the number of mutants is distributed in a Poisson distribution around the mean λ_1 but is a discrete variate by the interval of $s_1\mu_1$. If $s_1\mu_1$ is 2, only an even number of mutants show up in each individual.

3-2. *Case in which a small proportion of a large number of germ cells are utilized for fertilization in each individual.*

We assume the following conditions:

$$N - r \gg s_1\mu_1k_1 + s_2\mu_2k_2 - m, \quad (3-5)$$

and

$$r \gg m. \quad (3-6)$$

This is the case of silkworm and also approximately the cases of mouse and *Drosophila* (Glückman 1947). For the present case Eq. (2-1) can be rewritten in the approximate but simple form:

$$f(m) \cong \sum_{\substack{k_1, k_2=0 \\ s_1\mu_1k_1 + s_2\mu_2k_2 \geq m}} \frac{\lambda_1^{k_1} e^{-\lambda_1}}{k_1!} \frac{\lambda_2^{k_2} e^{-\lambda_2}}{k_2!} \binom{s_1\mu_1k_1 + s_2\mu_2k_2}{m} \left(\frac{r}{N}\right)^m \left(1 - \frac{r}{N}\right)^{s_1\mu_1k_1 + s_2\mu_2k_2 - m} \quad (3-7)$$

The generating function $G(z)$ for this distribution function can be given by substituting $(rz/N + (1-r/N))$ for z in Eq. (3-2):

$$G(z) = e^{-\lambda_1 - \lambda_2} \exp \left[\lambda_1 \left(\frac{r}{N} z + \left(1 - \frac{r}{N}\right) \right)^{s_1\mu_1} + \lambda_2 \left(\frac{r}{N} z + \left(1 - \frac{r}{N}\right) \right)^{s_2\mu_2} \right]. \quad (3-8)$$

If we may further assume the following condition

$$s_1\mu_1, s_2\mu_2 \gg 1, \quad (3-9)$$

using the assumption that r/N is very small, the $\left(\frac{r}{N}z + \left(1 - \frac{r}{N}\right)\right)$ in Eq. (3-8) can be replaced by $\exp((z-1)r/N)$ (see e.g., Armitage 1952), obtaining a more simplified form

$$G(z) = e^{-\lambda_1 - \lambda_2} \exp [\beta_1 e^{a_1 z} + \beta_2 e^{a_2 z}], \quad (3-10)$$

where

$$a_i = s_i\mu_i r/N; \quad \beta_i = \lambda_i e^{-a_i}. \quad (i = 1, 2) \quad (3-11)$$

Hence we obtain

$$f(m) = e^{-\lambda_1 - \lambda_2} \frac{d^m}{m! dz^m} \left[\exp(\beta_1 e^{a_1 z} + \beta_2 e^{a_2 z}) \right] \Big|_{z=0}. \quad (3-12)$$

3-2a. If the effective multiplication factors of mutant germ cells are unity, putting $s_1\mu_1 = s_2\mu_2 = 1$ in Eq. (3-8) we have

$$G(z) = e^{-(\lambda_1 + \lambda_2)r/N} e^{(\lambda_1 + \lambda_2)r z/N}. \quad (3-13)$$

This means that the number of mutants is distributed in a Poisson distribution around the mean value $r(\lambda_1 + \lambda_2)/N$.

3-2b. If $\lambda_1, \lambda_2 \gg 1$ and both a_1 and a_2 are close to zero, Eq. (3-10) reduces to (see also Section 4-1)

$$G(z) = e^{-(a_1\lambda_1 + a_2\lambda_2)} e^{(a_1\lambda_1 + a_2\lambda_2)z}, \quad (3-14)$$

where $a_1\lambda_1 + a_2\lambda_2$ is equal to the average number of observable mutants per individual (see Eq. (2-3)). This means that the number of observed mutants shows a simple Poisson distribution around the mean number of observed mutants per individual. Except for the above two cases, there would be more or less deviation from a simple Poisson distribution.

4. Frequency Distribution of Cluster Mutants

We can easily find the following relations :

$$-\frac{d'}{dz'} e^{\beta eaz} \Big|_{z=0} = \beta e^{\beta} a' g_t(\beta) , \quad (4-1)$$

where

$$\left. \begin{aligned} g_t(\beta) &= \left[\left(\beta \frac{d}{d\beta} \right)' e^{\beta} \right] / (\beta e^{\beta}) : \\ g_0(\beta) &= 1/\beta, \quad g_1(\beta) = 1, \quad g_2(\beta) = 1 + \beta, \quad g_3(\beta) = 1 + 3\beta + \beta^2, \\ g_4(\beta) &= 1 + 7\beta + 6\beta^2 + \beta^3, \quad g_5(\beta) = 1 + 15\beta + 25\beta^2 + 10\beta^3 + \beta^4, \\ g_6(\beta) &= 1 + 31\beta + 90\beta^2 + 65\beta^3 + 15\beta^4 + \beta^5, \dots\dots\dots, \\ g_t(\beta) &= 1 + (2^t - 1) \beta + \dots\dots\dots, \dots\dots\dots \end{aligned} \right\} \quad (4-2)$$

4-1. For simplicity, we first consider the case of only type 1 cells being present. Then from Eq's (3-12), (4-1) and (4-2) we find

$$f(m) = e^{-\lambda_1 + \beta_1} \lambda_1 \frac{a_1^m e^{-a_1}}{m!} g_m(\beta_1) . \quad (4-3)$$

The deviation from a Poisson distribution should be noted. Though the relative frequency of m mutants for $m \geq 1$ is distributed in a Poisson-like distribution $a_1^m e^{-a_1} g_m(\beta_1) / m!$ it is distributed not around the average number $a_1 \lambda_1$ of mutants but around a_1 and the $m!$, deviation factor $g_m(\beta_1)$ becomes appreciably larger than unity with increasing m if β_1 is appreciably larger than zero as seen from Eq. (4-2). However, if $\beta_1 \rightarrow \infty$, $\beta_1 g_m(\beta_1)$ tends to β_1^m and hence $f(m)$ given by Eq. (4-3) approaches $e^{-a_1 \lambda_1 (a_1 \lambda_1)^m} / m!$ as $a_1 \rightarrow 0$. This is the case considered in Section 3-2b).

If the following conditions hold

$$e^{-\lambda_1} \ll 1 , \quad (4-4)$$

$$\lambda_1 \ll 1 , \quad (4-5)$$

then, Eq. (4-3) can be approximated by

$$f(m) \cong \begin{cases} e^{-\lambda_1} ; & m = 0 \\ \lambda_1 e^{-\lambda_1} \frac{a_1^m}{m!} e^{-a_1} ; & m \geq 1 . \end{cases} \quad (4-6)$$

The order of the approximation of Eq. (4-6) may be estimated by

$$\Delta_f = 1 - \sum_{m=0}^{\infty} f(m) = \frac{\lambda_1^2}{2} \left(1 + \frac{2}{3} \lambda_1 + \dots\dots \right) , \quad (4-7)$$

and

$$\Delta_\lambda = \left[a_1 \lambda_1 - \sum_{m=0}^{\infty} m f(m) \right] / (a_1 \lambda_1) = \lambda_1 \left(1 - \frac{\lambda_1}{2} + \dots \right) . \quad (4-8)$$

That is, $100\lambda_1^2$ and $100\lambda_1$ are the order of the overall errors in % involved in Eq. (4-6) for the probability distribution and for the average number of mutants per in-

dividual, respectively. Eq. (4-6) means that the number of mutants is distributed in a Poisson distribution with mean value a_1 if a mutation had occurred, because the average probability of the occurrence of an initial mutative event in an individual is equal to $\lambda_1 e^{-\lambda_1}$. In Eq. (4-6) the probability of the simultaneous occurrence of more than one initial mutant per individual has been neglected.

4-2. Now we shall consider the case of cells of type 2 being present together with the cells of type 1 mentioned above. Expanding Eq. (3-10) with respect to β_1 we have

$$G(z) \cong e^{-\lambda_1 - \lambda_2} (1 + \beta_1 e^{a_1 z}) \exp(\beta_2 e^{a_2 z}), \quad (4-9)$$

where we have neglected terms of the order of β_2 because of Eq's (4-4), (4-5) and (3-11).

Now we assume the following condition

$$a_2 < 1. \quad (4-10)$$

Then, combining with Eq. (4-4), we find that we can neglect the terms of the order of $(a_2/a_1)^2$ in Eq. (4-9), obtaining

$$G(z) \cong e^{-\lambda_1 - \lambda_2} [\exp(\beta_2 e^{a_2 z}) + \beta_1 e^{\beta_2} e^{(a_1 + \beta_2 a_2)z}] \quad (4-11)$$

In a way similar to Eq's (4-7) and (4-8), we may estimate the order of approximation of Eq. (4-11) by

$$A_F = 1 - \sum_{m=0}^{\infty} f(m) = 1 - G(1) = \frac{1}{2} [\lambda_1^2 + a_2^2 \lambda_1 \lambda_2 + \dots] \quad (4-12)$$

To the order of the approximation given by Eq. (4-12) we may modify Eq. (4-11) into a convenient form so that Eq. (4-12) vanishes:

$$G(z) \cong (1 - \lambda_1) e^{-\lambda_2} \exp(\beta_2 e^{a_2 z}) + \lambda_1 e^{-\lambda_1 - a_2 \lambda_2} e^{(a_1 + a_2 \lambda_2)z} \quad (4-13)$$

Recalling Eq. (4-3), from the above equation we obtain the desired expression for $f(m)$:

$$f(m) = c(m) + h(m), \quad (4-14)$$

where

$$c(m) = \lambda_1 e^{-a_1 - a_2 \lambda_2} (a_1 + a_2 \lambda_2)^m / m!, \quad (4-15)$$

$$h(m) = (1 - \lambda_1) e^{-\lambda_2 + \beta_2 \lambda_2} g_m(\beta_2) e^{-a_2 a_2^m} / m! \quad (4-16)$$

$c(m)$, the cluster distribution function, is the contribution of the initial mutations which occurred in type 1 of cells alone or in both types 1 and 2, to the yield of mutants of large clusters having the average size $(a_1 + a_2 \lambda_2)$, and $h(m)$, the non-cluster distribution function, represents the contribution of the occurrence of mutations only in type 2 of cells which results in yielding only a small number of mutants per individual.

Application of the Theoretical Results to Silkworm Gonias

5-1. Probability Distribution of the Number of Mutants per Insect

Two examples of the observed probability distribution $f(m)$ of m mutants are shown

in Table I. The experimental values of $f(m)$ listed in this table were calculated from the experimental data recently obtained by Tazima (1960). Silkworm of a wild type at the early gonial stage were exposed to 1,000 r or X or ^{137}Cs γ rays and the number of pe mutants, which are recognizable by the color of fertilized eggs, were counted after crossing the treated insects with testers homozygous for the recessive gene pe . The results of two series, EX-594 and EX-601, of Tazima's experiments were pooled together neglecting the small difference in the distribution $f(m)$ between the irradiated male and female silkworm. Correction for eventual contamination by spontaneous mutation to the observed frequency distribution of the number of mutants recovered in the irradiated silkworm was made by subtracting the observed frequency distribution of the number of mutants in the control silkworm with the use of successive approximation. Though the present theory is applicable only to the case of acute irradiation, the results obtained by chronic irradiation of silkworm are also shown for comparison.

Table I. Comparison of theoretical and experimental frequency distribution of the number of pe mutants of silkworm induced by irradiation at gonial stage.

Number of mutants per insect, m	Acute irradiation			Chronic irradiation		
	Observed ¹⁾	Calculated		Observed ¹⁾	Calculated	
		present theory	Poisson ²⁾ distrib.		present theory	Poisson ²⁾ distrib.
0	.922	.922	.914	.865	.865	.854
1	.0615	.0622	.0801	.1076	.1077	.127
2	.0115	.0105	.00383	.0155	.0169	.0100
3	.0013	.00174	.000650	.0046	.00303	.00144
4	.0015	.00033	.00069	.0026	.00158	.00136
5	.00079	.00070	.00070	.0014	.00141	.00141
6	.00055	.00057	.00057	—	.00124	.00124
7	—	.00041	.00041	.0012	.00095	.00095
8	—	.00025	.00025	—	.00064	.00064
9	—	.00014	.00014	—	.00038	.00038
10	—	.000071	.000071	.00059	.00020	.00020
11	—	.000032	.000032	.00059	.00010	.00010

1) These figures were calculated from Tazima's (1960) experimental data by subtracting the contribution of spontaneous mutations. Mosaics for pe were included as pe mutants in this calculation. The total numbers of observed eggs were 851,192, 734,600 and 669,640 for "control", "acute irradiation" and "chronic irradiation", respectively.

2) The figures were calculated from the distribution function obtained by substituting a Poisson distribution function for function $h(m)$ used in the present theory (see Section 5).

In order to apply Eq. (4-14) to experimental results, we have to determine four parameters a_1 , a_2 , λ_1 , and λ_2 . So we have to establish at least four equations. For example, from Eq.'s (4-14), (4-15) and (4-15) we have

$$F_c = \sum_{m=1}^{\infty} c(m) = \lambda_1(1 - e^{-a_1 - \lambda_2}) \quad \left(\begin{array}{l} \text{Total frequency of} \\ \text{cluster mutation} \end{array} \right) \quad (5-1)$$

$$A_c = \sum_{m=0}^{\infty} mc(m) = \lambda_1 a_1 + \lambda_2 a_2 \lambda_1 \quad \left(\begin{array}{l} \text{Average number of mutants per in-} \\ \text{dividual for cluster mutation} \end{array} \right) \quad (5-2)$$

$$F_h = \sum_{m=1}^{\infty} h(m) = (1 - \lambda_1) (1 - e^{-\lambda_2 + 2p}) \quad \left(\begin{array}{l} \text{Total frequency of non-} \\ \text{cluster mutation} \end{array} \right) \quad (5-3)$$

$$A_h = \sum_{m=0}^{\infty} mh(m) = (1 - \lambda_1) \lambda_2 a_2 \quad \left(\begin{array}{l} \text{Average number of mutants per in-} \\ \text{dividual for non-cluster mutation} \end{array} \right) \quad (5-4)$$

We can make many other sets of equations. Furthermore, strictly speaking, the four parameters would better be determined by the least-squares method but this method is too cumbersome in the present case.

Strictly speaking both $c(m)$ and $h(m)$ contribute to the yield of m mutants for any value of m . However, we can draw a borderline to separate the whole group of observed insects into the non-cluster group, approximately corresponding to the contribution of $h(m)$ only, and the cluster group approximately corresponding to that of $c(m)$ only. In Table I we have assumed that the borderline passes through $m=3$, two thirds of $f(3)$ being classified into the non-cluster group and the rest into the cluster group. The determination of the location of the borderline can be improved by the successive approximation method so that the calculated $f(m)$ becomes self-consistent with the assumed borderline.

Using the experimental values of F_c , A_c , F_h and A_h calculated from Table I with use of the above mentioned borderline (see Table II), we can easily solve Eq's (5-1) to (5-4) for the four parameters. The obtained numerical values for them are also listed in Table II. The probability distributions of the number of mutants calculated from Eq's (4-14) to (4-16) by using these parameters are given in Table I in the columns next to "observed" for both cases of acute and chronic irradiation, respectively. The agreement between the calculated and the observed distribution is excellent for acute irradiation but is somewhat less satisfactory for chronic irradiation. The small disagreement in the latter case will be attributable to the change of the stage of germ cells during the irradiation period of about a week. To apply the present theory to chronic irradiation, a modification is necessary taking account of the cell development in a way similar to the methods adopted by Lea and Coulson (1949) and Armitage (1952) for the mutation of bacterial populations.

Table II. The characteristic parameters for frequency distribution function of the number of pe mutants of silkworm calculated from experimental results and the relevant experimental data.

		Acute irradiation	Chronic irradiation
Experimental data used	F_c	.00388	.00798
	A_c	.0195	.0430
	F_h	.0739	.126
	A_h	.0872	.148
Characteristic parameters	a_1	4.92	5.21
	λ_1	0.00391	0.00802
	a_2	0.260	0.180
	γ_1	0.336	0.828
	$a_1 + \lambda_2 a_2$	5.00	5.36

For comparison in Table I is also shown the frequency distribution calculated from the function obtained by substituting the Poisson distribution function $(a_2\lambda_2)^m e^{-\lambda_2}/m!$ for $e^{-\lambda_2+\beta_2\lambda_2} a_2^m e^{-a_2} g_m(\beta_2)/m!$ in $h(m)$ in Eq. (4-16). It should be noted that when compared with the Poisson distribution the observed frequency is considerably less than the calculated one for one mutant while it is larger for 0,2,3,... mutants. This means that the high frequency of one mutant is smoothed because of the large multiplication factor, lessening the high frequency for one mutant and raising the frequencies of 0,2,3,... mutants.

Similar trends are also found in the results obtained by Nagai and Locher (1938), Nishina and Moriwaki (1939) and Fano (1944) with sperm of *Drosophila* exposed to fast neutrons. It is interesting to note that non-Cluster distribution function given by Eq. (4-16) fits all those results very well for $a_2 = 0.3$ and $\lambda_1 = 0$. However, the cause of the deviation in the case of the irradiated sperm cannot be attributable to multiplication because mature sperm undergo no more multiplication and so a Poisson distribution should hold as shown in Section 3-2a provided that the initial induction of mutations follows a Poisson distribution. That is, Eq. (4-16) is not applicable to irradiation of sperm and so further studies of this deviation are necessary.

6. Theoretical Estimation of the Number of Gonial Cells

One of the interesting byproducts of the present theory is that we can estimate how many germ cells, when irradiated, could have been present from the values of parameters characteristic for the frequency distribution function, a_1 , a_2 , λ_1 and λ_2 .

To make a rough estimation of n_1 and n_2 with the use of Eq's (2-4) and (2-5), we assume the following simplified conditions:

$$p_1 = p_2 = p, \quad (6-1)$$

$$s_1 = s_2 = 1. \quad (6-2)$$

Then, combining with Eq's (2-4) and (2-5) we have

$$\mu_1/\mu_2 = a_1/a_2, \quad (6-3)$$

and

$$n_1 = rf_1/(2a_1); \quad n_2 = rf_2/(2a_2), \quad (6-4)$$

where

$$f_1 = a_1\lambda_1/(a_1\lambda_1 + a_2\lambda_2); \quad n_2 = a_2\lambda_2/(a_1\lambda_1 + a_2\lambda_2). \quad (6-5)$$

The experimental value of r , which for the present case represents the number of eggs laid per silkworm, is 430 ± 130 . Using this value of r and those of a_1 , a_2 , λ_1 and λ_2 given in Table II, we can easily calculate from the above equations the values of n_1 and n_2 and the relative values of multiplication factors as shown in Table III.

On the other hand, from cytological observation, the total number of primary spermatogenic cells, which are supposed by Sado (1959) to correspond to gonial cells of Type A in mouse (see Oakberg 1959), is about 5×10^3 for non-irradiated silkworm (Sado 1960). So from the value of n_2^0 given in Table III we may consider the cells of type 2 as gonial cells of type A. The final number of cells of type 2, n_2^0 , was calculated

Table III. The calculated numbers n_1 and n_2 and relative values of multiplication factors of cells of types 1 and 2, μ_1 and μ_2 .

			Acute irradiation	Chronic irradiation
The number of cells of	type 1	n_1	7.8	9.0
The number of cells of	type 2	n_2	680	930
The initial number of cells of	type 1	n_1^0	44	41
The final number of cells of	type 2	n_2^0	830	1200
Ratio of multiplication factor of type 1 cells to that of type 2	type 2	μ_1/μ_2	19	29
Ratio of multiplication factor of acutely irradiated cells to that of chronically irradiated ones	type 1	$\frac{\mu_1-Acut.}{\mu_1-Chro.}$	0.94	
	type 2	$\frac{\mu_2-Acut.}{\mu_2-Chro.}$	1.5	

by adding the value of n_1 multiplied by the ratio of multiplication factors, μ_1/μ_2 , to the value of n_2 ; and the initial number of cells of type 1, n_1^0 , by adding the value of n_2 divided by μ_1/μ_2 , to the value of n_1 . From the values of μ_1/μ_2 given in Table III the cells of type 1 must be the stem cells which develop into type A gonial cells after four to five divisions.

In the present treatment we have neglected the difference between the spermatogonia and oögonia. The primary purpose of the present section was to show the applicability of the theory to the estimation of the number of gonial cells. Further detailed treatments of such estimation will be presented elsewhere.

Discussion and Conclusions

It is often assumed that the number of mutants per individual is distributed in a Poisson distribution (e.g., Fano 1944, Lea 1955, Bateman 1959). This is a very good approximation for the distribution of the *initial* mutations induced by radiation in an organism because the induction of mutation is usually an independent random process with a very low probability. However, it has been proved from the theory derived above that the number of observed mutants per individual should deviate from a Poisson distribution if mutations take place in germ cells at early gametogenic stages.

The experimental results of irradiated silkworm obtained by Tazima (1960) show an appreciable deviation from a Poisson distribution including a group of cluster mutants. The comparison of the present theory with Tazima's experiments shows excellent agreement.

Furthermore, the order of the number of irradiated cells per individual estimated from the deviation from a Poisson distribution is in agreement with the observed number, though further improvement of the analysis of experimental data is necessary in order to increase the accuracy of the estimated values. This method will be useful

for the study of gametogenesis, especially the very early stages such as spermatogonia, oögonia, stem cells.

From the above two facts we may conclude that there exists an appreciable deviation from a Poisson distribution for the number of mutants when germ cells are treated with mutagenic agents at their early stages. We can expect such a deviation from a Poisson distribution also for the distribution of the number of spontaneous mutations. So in any experiment on induced mutations where a Poisson distribution of the number of mutants is assumed for analysis, it is important to correct the experimental data by subtracting the contribution by contamination with spontaneous mutations.

As to cluster mutants, it is to be noted that in order to estimate the mutation rate of the treated cells, each mutant of a large cluster should be counted in the same way as in one mutant mutation per individual as seen from Eq. (2-2). This equation also implies that the mutation rate calculated in this way does not depend on the sample size r of germ cells picked up from the total number N of the available germ cells for the recovery of mutations. This consideration can be applied in spikes of plants such as wheat, barley. It is customary to select a few spikes among the total grown from each irradiated seed and observe the F_2 segregation for the estimation of the mutation rate in the irradiated seeds (e.g., Gustafsson 1947, Matsumura and Fujii 1955). From the above conclusion, the number of the selected spikes should make no change in the estimated value of the mutation rate*.

In the present theoretical considerations we have neglected the fluctuations of the multiplication factor, the sample size of gametes, r , and the total number of gametes, N , and considered only the case in which two types of cells are present when treated acutely with a mutagenic agent. Among those restrictions only the constancy of the multiplication factor is difficult to remove because of the difficulty in the estimation of its fluctuation. The rest can be rather easily removed and generalization of the present theory is easy. A small but not inconsiderable disagreement between theory and experiment shown in Table I for chronic irradiation of *slikworm* may be attributable to the progressing stages of the germ cells during the exposure period and will be accounted for by a theory generalized from the present one.

Summary

- i) A general theory for the frequency distribution of the number of mutants per individual has been developed on the basis of proper assumptions.
- ii) The conditions for leading to a Poisson distribution of the number of mutants have been obtained.
- iii) The comparison of the theory with the experiments which disclosed an appreciable deviation from a Poisson distribution shows excellent agreement. The oc-

* If the total number of spikes shows an appreciable fluctuation, a correction for the fluctuation is necessary for estimating the mutation rate. The present conclusion holds also in this case.

currence of mutants in a large cluster is also in good agreement with the calculated frequency.

iv) A method for estimating the number of germ cells present when treated with a mutagenic agent has been proposed. The estimated number of germ cells is in agreement with the observed number of the irradiated silkworm gonad.

v) A connection of cluster mutants and sample size of germ cells (used for recovery of mutations) per individual with the estimated mutation rate has been discussed.

Acknowledgments

It is a pleasure to record the authors' indebtedness to Mr. T. Fujii who introduced him to the subject created theoretically in the present paper. The author wishes to acknowledge with appreciation the kindness given by Dr. Y. Tazima and Mr. T. Sado in permitting the author to use their unpublished experimental results and in making valuable discussion on the biological aspects of the present paper. The author is also indebted to Dr. M. Kimura for the literatures relevant to the theoretical part of the present work

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