

# FREQUENCY OF SPONTANEOUS MUTATIONS IN CERTAIN STOCKS OF *DROSOPHILA MELANOGASTER*

M. DEMEREC

*Carnegie Institution of Washington, Cold Spring Harbor, New York*

Received April 24, 1937

**D**URING the investigations dealing with various problems in which the frequency of X chromosome lethals was measured, an unusually high frequency of spontaneous lethals was noticed in the inbred Florida stock. This marked the beginning of an attempt to ascertain the rate of spontaneous mutability in various wild type stocks. The results of these studies will be reported in the present paper. A preliminary account was presented at the 1936 summer meeting of the Genetics Society of America (DEMEREC 1937a). At the same meeting PLOUGH and HOLTHAUSEN (1937) reported a high frequency of visible mutants in crosses where the Florida stock was used.

## METHODS

The major part of the experiment deals with the determination of the rate of occurrence of spontaneous X chromosome lethals. Throughout this work a uniform procedure was used. Lethals were detected by the CIB method according to the following scheme:

- (1) CIB/*ec ct v g* ♀ by + ♂
- (2) F<sub>1</sub> CIB/+ ♀ by *ec ct v g* ♂
- (3) Females from a culture without males were bred as follows:  
+ (*l*)/*ec ct v g* by *y Hw*, In dl-49.

Flies for (1) were raised in  $\frac{1}{4}$  pint milk bottles, for (2) in 25×95 mm vials and (3) in  $\frac{1}{4}$  pint milk bottles. In all cases corn-meal, molasses, and agar food was used, brewers' yeast having been added to make it richer. The food contained about 0.10 percent of Moldex-A.

## RESULTS

Results of experimental tests of fifteen wild type stocks are summarized in table 1. These stocks, obtained from various laboratories, came from places so widely separated geographically that they could be only distantly related. The data show that three of them, Florida-inbred; Wooster, O.; and Formosa, Japan, have a significantly higher mutability rate than the other twelve stocks tested. Comparing the high mutability stocks with the low mutability Oregon-R, it was found that the Formosa stock has a mutability rate about five times as high as that of the Oregon-R, while Wooster showed a mutability rate about ten times and Florida almost twenty times as high as that of the Oregon stock.

TABLE I

*Frequency of spontaneous lethals in X chromosomes of various wild-type stocks.*

STOCK	RECEIVED FROM	NUMBER OF		PERCENT LETHALS
		X CHRO- MOSOMES	LETHALS	
Florida-inbred	Columbia University, 1928	2108	23	1.09 ± 0.15
Wooster, O.	Spencer, June 1936	1266	8	0.63 ± 0.15
Formosa, Japan	Zool. Inst. Kyoto, Aug. 1936	2054	8	0.39 ± 0.09
Oregon-R	Cal. Inst. Tech. (?)	3049	2	0.066 ± 0.03
Swedish-b	Cal. Inst. Tech., April 1936	1627	3	0.18 ± 0.07
California-c	Cal. Inst. Tech., June 1936	708	2	0.28 ± 0.13
Huntsville, Tex.	Univ. of Texas, June 1936	938	—	—
Urbana, Ill.	Univ. of Illinois, July 1936	1016	1	—
Canton, O.	Amherst Col., June 1936	922	—	—
Amherst, Mass.	Amherst Col., June 1936	572	1	—
Woodbury, N. J.	New York Univ., Nov. 1936	1159	1	—
Tuscaloosa, Ala.	B. Kaufmann, Aug. 1936	545	1	—
Lausanne	V. Jollos, July 1936	955	2	0.21 ± 0.09
Seto, Japan	Zool. Inst. Kyoto, Aug. 1936	1236	—	—
Kyoto, Japan	Tokyo Imp. Univ. Kyoto, Sept. 1936	875	1	—
		13602	14	0.10 ± 0.02

*Analysis of the Florida stock*

In order to determine whether the basis for the high mutability rate in Florida stock is genic, the males from the backcross, in which the female parent was *ClB/g pl* and the male parent + Florida, were tested for mutability. Among 1338 X chromosomes tested, four (0.30 ± 0.10 percent) carried a lethal. This test revealed a mutability rate lower than that of the original Florida stock but higher than the average mutability rate of wild stocks. Since all of the males tested had the Florida X chromosome, this experiment showed that if the factor responsible for high mutability is genic, it is not located in the X chromosome.

The next step in these tests was to substitute autosomes of a low mutable stock for the Florida autosomes and thus to determine whether any of the Florida autosomes carry a factor responsible for high mutability. Swedish-b stock was selected for this purpose and stocks with different combinations of Florida and Swedish-b chromosomes were built up. Mutability tests for these stocks are summarized in table 2. It is evident from this summary that only combinations homozygous for the second Florida chromosome had the high mutability. This indicates strongly that our Florida stock carried in the second chromosome a recessive factor which is responsible for the high rate of mutability in that stock.

TABLE 2

*Mutability in stocks having only certain Florida chromosomes. (F=Florida; S=Swedish-b.)*

CONSTITUTION	NUMBER OF			PERCENT OF LETHALS
	MALES USED	CHROMOSOMES TESTED	LETHALS	
1F 2S 3S	10	1162	—	—
1S 2F 3S	21	1707	17	1.00
1S 2S 3F	10	804	—	—
1F 2F 3S	10	1215	11	0.91
1S 2F 3F	8	790	9	1.14
1F 2S 3F	8	800	—	—

*Action of the Florida factor*

Detailed analysis of the data reveals interesting information regarding the action of the Florida factor. Table 3 gives a summary of the data obtained in tests of stocks containing Florida and Swedish-b chromosomes. This table contains data obtained from the tests of sperm from individual males and also shows the approximate position of the lethals in the chromosome. It is evident that lethals were found in the sperm of certain males only and also that the lethals carried by the sperm taken from any one male tend to be located in the same region of the chromosome. This grouping of lethals indicates that those of the same group are genetically identical. It can be safely assumed that the genetic change producing a lethal effect occurred sometime during the ontogeny of the male and thus was transmitted by a group of sperm which evolved from the affected cell. The supposition that all lethals of a group are identical is also supported by the fact that in male number 5 of the constitution 1F 2F 3S (table 3) all six changes which were classified as lethal by standard tests in vials proved to be semilethals when flies were raised under better conditions for linkage tests. Similarly a group of nine changes obtained from male 8 of 1S 2F 3S constitution proved to be semilethal and in addition every member of that group showed a similar morphological characteristic expressed as a groove on the abdomen.

From the data giving the number of lethals in a group and the number of sperm tested from each male parent, it is possible to estimate the time in ontogeny when the change, which was the progenitor of the group, occurred. This estimate is reached by dividing the number of sperm tested by the number of changes in the group. In table 4 calculations are given for the time of origin of seventeen independent changes. From this table it is evident that changes are not limited to any one stage of the germ cell development. Changes which occurred when the germ cells were in as early

TABLE 3

*Lethals appearing in the sperm of individual males.*

MALE CONSTITUTION	NUMBER	NUMBER OF		POSITION OF LETHALS			
		SPERM TESTED	LETHALS	<i>ec</i>	<i>ct</i>	<i>v</i>	<i>g</i>
1F 2F 3S	1	171	—				
	2	153	1	1			
	3	53	4		1	3	
	4	103	—				
	5	189	6				6*
	6	111	—				
	7	99	—				
	8	96	—				
	9	134	—				
	10	108	—				
1S 2F 3F	1	29	—				
	2	98	6	6			
	3	42	—				
	4	152	—				
	5	127	—				
	6	86	—				
	7	101	3	1		2	
	8	255	—				
1S 2F 3S	1	55	—				
	2	40	1				
	3	36	—				
	4	49	2	2			
	5	75	—				
	6	29	5		3	2	
	7	51	—				
	8	75	9				9**
	9	51	—				
	10	62	—				
	11	72	—				

\* All semilethal.

\*\* All semilethal and showing similar morphological characteristic (groove on the abdomen).

as the 8 to 10 cell stage were observed. This is an early embryonic stage corresponding to about the ninth nuclear division, when germ cell nuclei have just become separated from the nuclei which are to form the soma. Several changes were also observed which probably occurred in spermatogonia at a stage of development when many spermatocytes had been differentiated.

From the data of table 4 the approximate frequency of changes at different cell generations can be estimated if the data are arranged so as

TABLE 4

*Data indicating the stage in the male germ cell development at which changes occurred.*

LETHALS FROM THE MALE	NUMBER OF LETHALS IN THE GROUP	NUMBER OF SPERM TESTED	CHANGE OCCURRED WHEN GERM CELLS NUMBERED ABOUT
1F 2F 3S —2	1	153	153 cells
—3	1	53	53 "
—3	3	53	18 "
—5	6	189	33 "
1F 2F 3F —2	6	98	16 "
—7	1	101	101 "
—7	2	101	50 "
1S 2F 3S —2	1	40	40 "
—4	2	49	25 "
—6	3	29	10 "
—6	2	29	15 "
—7	9	75	8 "
291-87— 1	2	297	147 "
— 3	1	95	95 "
291-93—15	2	58	29 "
291-94—10	2	64	32 "
—12	2	61	30 "

to show the frequency of changes per number of cells, divided into classes following a geometric progression. Such a classification is given in table 5.

TABLE 5

*Frequency of changes occurring at different cell generations of germ cell development.*

GERM CELL NUMBER	8	9-16	17-32	33-64	65-124	125-
Frequency of changes	1	3	5	4	2	2
Corrected frequency	1	1.5	1.25	0.5	0.125	0.0625

To obtain the actual frequency of change, the data for each successive class were divided by 1, 2, 4, 8, 16, and 32 respectively in order to correct for the increase in the chance of a change occurring in each succeeding class due to the increased number of cells. When this is done "corrected frequency" values (table 5) are obtained showing the approximate frequency of changes as observed during the six cell generations of the development of the male germ cells. Considering the small number of observations, the values for the first three cell-generations are similar. Values for the last three generations are lower than the values for the first three. This, however, should be expected since such late changes can be detected only if a large number of sperm from individual males is tested. This has not always been done in our experiment.

This evidence favors the assumption that the Florida mutability factor stimulates gene changes throughout the whole period of germ cell development. It suggests also that these changes occur with approximately equal frequency at different stages of that development.

*Visible gene changes*

Special experiments have not been performed to determine the action of the mutability factor on the frequency of visible gene changes. However, linkage tests were made with all lethals found in this experiment and in doing that, the material was inbred for two generations after the original cross between CIB/*ec ct v g* and +Florida was made. In this second generation the mutability factor became homozygous in a certain proportion of individuals and, if effective, should have produced a higher frequency of visible mutations among F<sub>3</sub> offspring which were used in linkage counts.

Among 15,000 individuals examined in linkage tests the following visible changes were found:

yellow	24 times	black	2 times
forked	3 times	blistered	1 time
lozenge	2 times	dwarfish	1 time
vermilion	2 times	curled wings	1 time

This indicates that the Florida mutability factor increases the rate of visible mutations as well as the rate of lethal changes.

A large proportion of yellow mutations among visible changes raises the question as to whether the mutability factor has a specific action or whether it is likely to increase the changes in certain genes to a greater extent than in other genes. Results with lethals show that these are scattered throughout the whole length of the chromosome indicating that a large number of genes must be sensitive to the action of the mutability factor. What appears to be a disproportionately high frequency of yellow mutants may be accounted for in several ways. First of all, in the experiments where these mutants were found, echinus, cut, vermilion and garnet characters were involved which, to a large extent, prevented the detection of new mutant forms expressed as rough eyes, cut wings and certain eye colors. Furthermore, it is probable (PATTERSON 1932, DEMEREC 1933) that the majority of changes in any one locus are lethal changes, presumably deficiencies. If deficiencies for a certain locus are not lethal but if they show up as visible characters, then it might be expected that visible changes in these loci will be more frequent than visible changes in loci where deficiencies are lethal. Yellow seems to be such a "deficient non-lethal" locus. These two reasons, however, may not be sufficient to account for the absence of such distinct mutants as white, miniature, and singed. Therefore, the possibility is not excluded that the mutability factor has a differential effect on various loci.

Of the 24 yellows found, 10 have both yellow setae and yellow body and resemble yellow-1; and 14 have gray setae and yellow body and resemble yellow-2. Both lozenge changes found are sterile in homozygous females. One forked change has slightly forked bristles, and the other has both hairs and bristles strongly forked.

*Mutability factor and ontogeny*

Evidence was presented earlier indicating that the Florida mutability factor stimulates the occurrence of lethal changes during the whole period of the development of male germ cells. The majority of the visible changes occurred in females, and a large proportion of them showed up with several mutant males. This means that in females, as well as in males, changes occur during the development of the germ cells.

It is known that in *Drosophila* the fertilized nucleus undergoes about eight simultaneous divisions before a blastoderm is formed. After eight such divisions when there are 256 nuclei in the egg, a few of them, about 5-15, migrate toward the posterior end of the egg to form the rudiments of the germ cells. All changes occurring prior to the separation of the germ cells should, as a rule, produce mosaics affecting both germ cells and soma. Changes occurring after the separation of the germ cells produce somatic mosaics if they occur in somatic cells, and germinal mosaics if they occur in the germ cells.

Among a relatively small number of flies examined, 24 germinal changes to yellow were found. Somatic changes to yellow show up on all setae and if the flies are examined carefully such a change can be detected on a single seta. Since a fly has about 1500 setae, it is evident that it should not require many flies to detect somatic changes if they occur with the same frequency as observed for germinal changes. More than 150 males were carefully examined for yellow setae. Only one yellow-mosaic case was found. Unfortunately, it was lost before it could be rechecked. This indicated that yellow changes in somatic cells occur with much lower frequency than in germinal tissues. This in turn suggests that the action of the Florida mutability factor may be limited to germ cells only. If that is true, then the factor is inactive during the first eight nuclear divisions, its activity being limited to the nuclei developing into germinal tissues.

*Normal mutability rate in the Florida stock*

About one year after these experiments with the Florida stock were completed another set of experiments was started to test the effect of temperature on the mutability factor, and to accumulate more data on the frequency of changes at different stages of ontogeny. To my great surprise it was found that the Florida line used in these experiments had a very low mutability rate. Seventy-two males were tested individually,

but lethals were found among the sperm of only five of them. In the case of four males, two lethals were obtained from each and in one case one lethal was found. Altogether 6743 sperms were tested and since 5 lethals were found, the rate of change was 0.074 percent.

The original Florida line apparently was heterozygous for the mutability factor and during the year which passed between the two sets of experiments, that factor was almost entirely eliminated from the stock. The Florida stock without that factor, therefore, has a very low rate of spontaneous mutability.

#### DISCUSSION

It is known that variations in the spontaneous mutability rate do occur. A number of so-called "unstable genes" have been investigated (DEMEREC 1935), all of which have a relatively high rate of change. If changes responsible for the appearance of mutations are chemical changes, as seems probable, then unstable genes are in such a chemical state that even ordinary environmental conditions are close to the threshold where changes occur. Since only slight variation in the environment is sufficient to bring conditions to this threshold point, it is to be expected that the rate of change in unstable genes would be readily affected by various factors. Such behavior has been described for unstable miniature of *Drosophila virilis* (DEMEREC 1929a, 1930) where five genetic factors are known to stimulate the rate of mutations in that unstable gene.

A similar interpretation can explain the action of the Florida mutability factor. It can be assumed that this mutability factor influences the gene environment to such an extent that changes in a large number of genes are likely to occur. Variations in the environment as it exists in the different cells are sufficient to cause changes in genes.

Specificity of the gene behavior observed on many other occasions is again evident in the present case. The experimental evidence now available indicates that the changes in genes occur only during the development of the germ cells. This means that in these cells only the mutability factor is able to exert a sufficiently strong influence on the gene environment to bring about a condition which causes a mutation to occur. This situation is similar to that found in the unstable miniature-gamma of *virilis* (DEMEREC 1929b), this gene being unstable in somatic cells only.

In the material described here, the spontaneous frequency of the X chromosome lethals was 1.09 percent. Since the stock used in experiments was not homozygous for the mutability factor, it is evident that the rate of mutations should be higher in the homozygous material. The observed frequency of lethals, therefore, can be taken as the lowest value for the effect of the Florida mutability factor. This value is equivalent to



the one obtained from the X-ray treatment of sperm by about 300 r-units. Since both the mutability factor and the X-ray treatment produced a similar effect, the question may be raised whether or not both results could be due to the action of a similar mechanism.

It has been noted on several occasions that new spontaneous mutations are discovered in waves, that is, that sometimes a large number of them is found within a relatively short period of time (SPENCER 1935). Presence of a mutability factor in experiments carried on during a mutation wave can readily account for this behavior.

For 29 lethals of independent origin investigated in experiments reported here, genetic tests were made to determine whether they were connected with chromosomal aberrations. In these tests *ec ct v g* stock was used so that reductions in crossing over produced by a chromosomal aberration to the left of *B* should have been detected. Since the region to the right of *B* was not covered in these tests lethals located in that region were not included among 29 lethals considered here. Genetic tests showed that none of the spontaneous lethals was connected with a chromosomal aberration. It is known that lethals induced by X-ray treatment are frequently connected with chromosomal aberrations (inversions and translocations). That does not mean, however, that X-ray lethals should be different from spontaneous lethals. It has been observed that the simultaneous occurrence of a lethal and of a chromosomal aberration increases with the dosage applied and that lethals from low dosage treatments are rarely connected with chromosomal aberrations. This may mean that when the frequency of lethals is low they are independent of chromosomal aberrations and as the frequency increases coincidence of two types of changes increases also (DEMEREK 1937b). According to that interpretation the frequency of spontaneous lethals investigated here has not reached the level at which chromosomal aberrations occur.

The data of table 1 reveal a significant difference in the spontaneous mutability rate between different lines of *D. melanogaster*. Of the fifteen lines tested, three had a significantly higher rate of mutations than the other lines. If the genetic constitution of the line is responsible for the increased mutability, then it appears probable that there are a number of mutability factors acting in a similar manner as the Florida factor. Since such factors increase the variability in the line in which they are present, they may play a significant role in the evolutionary processes of a species.

#### SUMMARY

Frequency of spontaneous X-chromosome lethals was measured by CIB method in fifteen stocks of *D. melanogaster*. In three of them the frequency was significantly higher than in others, the highest being in the Florida stock.

Genetic tests showed that a recessive second chromosome gene (*mu-F*) was responsible for the high frequency of lethals in the Florida stock. It has been found that this gene also increases the rate of visible mutations.

Data show that this mutability gene acts during the development of germ cells, both in males and females. The available evidence indicates that the rate of action is similar during successive cell generations of the developing male germ cells.

It is suggested that the Florida mutability factor affects the gene environment in such a way as to bring it to the threshold condition at which changes in various genes occur.

Twenty-nine spontaneous lethals of independent origin have been tested by genetic methods for chromosomal aberrations. None has been found.

#### LITERATURE CITED

- DEMEREK, M., 1929a Genetic factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. Proc. Nat. Acad. Sci. **15**: 834-838.  
 1929b Changes in the rate of mutability of the mutable miniature gene of *Drosophila virilis*. Proc. Nat. Acad. Sci. **15**: 870-876.  
 1930 A genetic factor affecting germinal mutability of miniature-alpha wing character of *Drosophila virilis*. The Laws of Life, 45-56 pp. Prague.  
 1933 What is a gene? J. Hered. **24**: 368-378.  
 1935 Unstable genes. Bot. Rev. **1**: 233-248.  
 1937a A mutability stimulating factor in the Florida stock of *Drosophila melanogaster*. (Abstract) Genetics **22**: 190.  
 1937b Relationship between various chromosomal changes in *Drosophila melanogaster*. Cytologia (in press).
- PATTERSON, J. T., 1932 Lethal mutations and deficiencies produced in the X chromosome of *Drosophila melanogaster* by X-radiation. Amer. Nat. **66**: 193-206.
- PLOUGH, H. H., and HOLTHAUSEN, C. F., 1937 A case of high mutation frequency without environmental change. (Abstract in Genetics **22**: 203) Amer. Nat. **71**: 185-187.
- SPENCER, W. P., 1935 The non-random nature of visible mutations in *Drosophila*. Amer. Nat. **69**: 223-238.