AN ANALYSIS OF DATA ON X-RAY-INDUCED VISIBLE GENE MUTATIONS IN DROSOPHILA MELANOGASTER[1]

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Received August 1, 1941

INTRODUCTION

IT IS well known that the chromatin material of germ cells is subject to spontaneous alterations. These changes may involve large sections of the chromosomes, or they may be localized about a particular gene. Since these alterations are subject to variations, both random and non-random in origin, the methodology by which this variation is analyzed becomes of major importance to any interpretation to be attached to such data.

Previous irradiation experiments have shown that changes in the chromatin can be produced artificially and at a higher frequency than exists naturally. Differing sources and dosages of radiant energy have been used, and differing types of gene changes have been studied. All those experiments likewise involve, as an integral part of their interpretation, the random and the non-random variation mentioned above, and its proper analysis.

If we have a population of Drosophila in which a certain fraction, p, has mutated from the normal red eye-color to a vermilion eye-color, we say that the probability of observing a mutant fly, on one random observation of this whole population, is p. If the proportion of mutants stays fixed during the period of our interest in the population, it is well known that the probability of observing exactly r mutants in a sample of size n is given by the binomial probability function

$$\frac{n!}{(n-r)!\,r!}\,p^r(1-p)^{n-r}. \tag{1}$$

The population is determined by p, which, however, is usually unknown. Physical or biological considerations supply certain hypotheses which are then used in the estimation of p. We then have a means of determining what we would expect to obtain during random sampling from the given population. If expectation and observation fail to agree, doubt is cast upon the adequacy of the hypotheses adopted.

For the purpose of measuring the agreement between observation and expectation, it has been thought advantageous to approximate the discontinuous function, (1), by a continuous function. The Normal distribu-

[1] Journal Paper No. J-914 of the IOWA AGRICULTURAL EXPERIMENT STATION, Ames, Iowa Project No. 573.

tion has been used for that purpose, but has not been shown to be a good approximation if the expected numbers, np, are small. The probabilities associated with visible gene mutations are nearly always so small that n would need to be much larger than is experimentally possible before the Normal approximation would be—in the light of present knowledge—a safe one to use in the analysis of such irradiation data. In spite of this situation, the literature on gene mutations shows that the Normal approximation has been used in the classical large sample manner to test the variability observed in irradiation data. It seems that the error involved in such analyses should be investigated or else a different method of analysis sought.

This paper has the dual purpose of presenting some new data on X-ray-induced, visible gene mutations and of showing how a satisfactory analysis of such data can be made by means of the χ^2 distribution.

HISTORICAL BACKGROUND

Due to the difficulties attending the collection of data on visible gene mutations, there is not an abundance of such data in the literature. Such data as are found are characterized by large numbers of observations and small numbers of recorded mutations. If the data are analyzed at all, the analysis is accomplished through the Normal approximation described above and upon the basis of large sample theory. These circumstances leave in doubt the conclusions drawn from the data.

The χ^2 test is especially designed for enumeration data. Its purpose is to measure the agreement between the numbers actually observed and the corresponding numbers which would be expected mathematically upon the basis of certain hypotheses. Thus this test seems particularly adapted to the general nature of irradiation research on visible gene mutations. The χ^2 test has two other advantages: (1) it has a form especially adapted to convenient calculation of $2 \times n$ classifications, and (2) its additive property enables one to accumulate evidence on rare events statistically.

However, one big disadvantage has been thought to attach to the use of χ^2 when small expected numbers are involved. Since an exact test is not practical on most data, it has been approximated by means of a Type III Pearsonian curve. This distribution is the one found in the tables. It was thought, until quite recently, that this approximation was so poor, when small numbers such as those which exist in irradiation data are used, that it could not validly be employed.

The closeness of the Type III approximation, in so far as it affects a test of significance, has been investigated by HOEL (1938), NEYMAN and PEARSON (1931), SUKHATME (1938), COCHRAN (1936), and FRYER (1940). These investigators have shown that the tabular χ^2 distribution is satis-

factory for a test of significance even when the expected numbers are as small as 0.5. This closeness of agreement between the exact and the tabular distributions of χ^2 has been demonstrated only empirically; but the validity of that demonstration has no relation to the assumptions accompanying the derivation of the tabular distribution. However the tabular values may have been obtained, they seem to give the exact distribution of χ^2 very satisfactorily. These considerations plus the additive property of χ^2, which makes it possible to collect information on rare events, make it appear that the χ^2 test fits the purposes of irradiation research quite well.

THE EXPERIMENT

Materials and methods

The experiments involved three wave lengths of radiation, two dosages with each wave length, and 19 specific gene loci.

The following stocks of females of *Drosophila melanogaster* were maintained:

(1) $\dfrac{sc\ ec\ cv\ v\ f\ car}{sc \qquad v\ ClB} \dfrac{+}{+} \dfrac{+}{+} \dfrac{+}{+}$;

(2) $\dfrac{+}{+} \dfrac{al\ dp\ pr\ c\ px\ sp}{al\ \ Cy \quad L_4\ \ sp} \dfrac{ru\ h\ st\ sr\ e^s\ ca}{Me'\ \ Sbc\ \ e^s} \dfrac{+}{+}$;

(3) $\dfrac{+}{+} \dfrac{+}{+} \dfrac{ru\ h\ st\ sr\ e^s\ ca}{ru\ h\ st\ sr\ e^s\ ca} \dfrac{+}{+}$;

(4) $\dfrac{+}{+} \dfrac{+}{+} \dfrac{ru\ h\ st\ sr\ e^s\ ca}{Me'\ \ Sbc\ \ e^s} \dfrac{+}{+}$; and,

(5) $\dfrac{+}{+} \dfrac{al\ dp\ pr\ c\ px\ sp}{al\ \ Cy \quad L_4\ \ sp} \dfrac{+}{+} \dfrac{+}{+}$.

Normal males from a line which had been inbred for several generations were X-rayed at 24 hours, or less, of age. These Drosophila were irradiated with a gas-type X-ray tube of WYCKOFF and LAGSDIN'S (1930) general design, a description of which has been presented by PINNEY (1939). The targets were the pure metals: silver, copper, and chromium. The particular X-rays were filtered heavily by a window of palladium for the silver target, nickel for the copper target, and aluminum for the chromium target. The thickness of this window was adjusted to absorb fifty percent of the radiation.

The peak kilovoltage across the tube was different for the different metals—56.0 for Ag, 39.6 for Cu, and 34.3 for Cr—as measured by a 10 cm sphere gap. The current through the tube during the irradiations was held constantly at 12.5 milliamperes. The beam intensity at the time of irradiation was measured either by a small ionization chamber or by a

Victoreen dosimeter. The ionization chamber was constructed in the IOWA STATE COLLEGE machine shop after a design by L. E. PINNEY based upon the general plan of TAYLOR and SINGER (1930). The dosimeter was checked frequently with the ionization chamber both for accuracy and for the absorption of the particular rays in the capsule housing the test chamber.

The ionization readings for the different metals were corrected for a temperature of 20°C, a barometer reading of 74 cm, and the loss of radiation due to the amount absorbed in the 5.5 cm layer of air between the surface of irradiation and the volume giving the ionizing current.

The linear absorption coefficient for the male Drosophila was determined for each wave length used. The sperm-storage organs are found throughout the abdomen, but for the purposes of X-ray dosage, average approximately the mid-position. The energy incident upon the sperm is also corrected for this loss of X-ray as they pass into the body of the fly. These corrected readings are those presented in the tables.

It is essentially true that for a constant thickness of biological material the fraction of the incident dosage absorbed by the material is a constant for a given wave length of irradiation. Hence $\log I/I_0 = -\mu x$, where I_0 is the incident dosage. The ratio: I/I_0, was obtained experimentally as follows: a fly was placed directly over the window of the ionization chamber and then irradiated. The dosage incident upon the fly and that passing through the fly were both measured. When the latter had been expressed as a fraction of the former, and the process repeated on several flies, the average was used as the estimate of I/I_0. This gave $I/I_0 = 0.53$, 0.894, and 0.246 for Cu, Ag, and Cr, respectively.

These estimates were then used to plot $\log I/I_0$ against an arbitrary depth scale. From these graphs, it was estimated that 94.4, 72.5, and 49.5 percent of the dosage incident upon the fly was actually incident upon the sperm in the mid-position for Ag, Cu, and Cr irradiation, respectively.

The average effective wave length of the X-rays for the different metals, under the conditions of this investigation, was determined by absorption experiments through successive sheets of aluminum, 0.1308 mm and 0.0072 mm in thickness. These tests showed the average effective wave lengths of irradiation to be as follows: Ag, 0.7Å, Cu, 1.5Å, and Cr, 2.1 to 2.2Å.

Male Drosophila which had been X-rayed under the above conditions were then mated to virgin females from one of the stocks described. Whenever a mutation was believed found, that fly was mated back to an appropriate stock. Due to the high percentage of sterility found with the high dosages used, those progeny tests frequently produce no offspring. Those mutations which were verified by progeny tests appear under the heading "v"; the rest are classified as "c" if the identification was considered fairly certain.

Under fortunate circumstances, a progeny test also furnishes a test for a deficiency. Since at these dosages, many of our mutations are not classifiable as "deficient" or "non-deficient," that distinction has not been made in the data to be presented below.

Results

The data obtained in the above-described experiment are presented in the tables below. In tables 2, 3, and 4, only those sets of data which are

TABLE 1

Summary of data on visible gene mutations by wave length, dosage, and chromosome.

TARGET	DOSAGE (r units)	I LOCI EXAMINED	I v	I (v+c)	II LOCI EXAMINED	II v	II (v+c)	III LOCI EXAMINED	III v	III (v+c)
Cu	3,625	52,101	16	32	18,850	4	8	13,917	3	7
Cu	7,250	12,455	12	23	3,432	0	0	5,451	2	5
Cr	2,475	7,020	0	1	8,331	2	7	7,478	1	4
Cr	4,950	6,397	2	8	7,576	1	5	6,760	3	4
Ag	3,776	—	—	—	4,412	0	3	4,389	0	5
Ag	4,720	2,955	2	4	3,416	0	2	4,005	1	5
		80,928	32	68	46,017	7	25	41,970	10	30

TABLE 2

Summary of specific mutations on Chromosome I.

LOCUS	Cu_{3625} LOCI EXAMINED	Cu_{3625} v	Cu_{3625} (v+c)	Cu_{7250} LOCI EXAMINED	Cu_{7250} v	Cu_{7250} (v+c)	Cr_{4950} LOCI EXAMINED	Cr_{4950} (v+c)
sc	7,443	3	4	1,765	2	2	667	0
ec	7,443	3	8	1,765	2	5	667	2
cv	7,443	1	2	1,765	0	0	667	0
ct	7,443	4	8	1,765	5	9	667	5
v	7,443	3	6	1,765	1	2	667	1
f	7,443	1	2	1,765	1	2	667	0
car	7,443	1	2	1,765	1	3	667	0
	52,101	16	32	12,455	12	23	4,669	8

sufficiently complete to be useful are included. As with most irradiation data on visible gene mutations, the scarcity of these data limits the definiteness of any conclusions to be drawn from this one set of data. Fortunately, the χ^2 test enables one to add this evidence to that obtained in other experiments testing the same hypotheses.

TABLE 3

Summary of specific mutations on Chromosome II.

LOCUS	Cr_{2475} LOCI EXAMINED	(v+c)	Cr_{4950} LOCI EXAMINED	(v+c)	Cu_{3625} LOCI EXAMINED	v	(v+c)
al	1,843	2	1,638	0	3,483	1	1
dp	929	1	860	1	2,971	1	4
pr	929	2	860	1	2,971	0	0
c	929	2	860	2	2,971	0	0
px	929	0	860	1	2,971	2	3
sp	1.843	1	1,638	0	3,483	0	0
	7,402	8	6,716	5	18,850	4	8

TABLE 4

Summary of specific mutations on Chromosome III.

LOCUS	Cu_{3625} LOCI EXAMINED	v	(v+c)	Cu_{7250} LOCI EXAMINED	(v+c)	Cr_{4950} LOCI EXAMINED	v	(v+c)
ru	2,222	0	1	884	2	787	0	0
h	2,222	0	0	884	1	787	0	0
st	2,222	1	2	884	0	787	1	2
sr	2,222	1	1	884	0	787	0	0
e^s	2,807	1	2	1,031	0	1,638	2	2
ca	2,222	0	1	884	2	787	0	0
	13,917	3	7	5,451	5	5,573	3	4

ANALYSIS OF THE DATA

The above data furnish some evidence regarding questions of interest in irradiation research. Some of these questions are: (1) Are there differential rates of mutation at the gene loci considered? (2) Is the mutation rate of a particular gene directly proportional to the dosage of radiation applied? (3) After due allowance is made for differences in absorption, does wave length affect mutation rate? (4) In so far as the sets of genes observed are representative of their respective chromosomes, what evidence is there of different rates of mutation among the first three chromosomes of *Drosophila melanogaster?*

To obtain evidence on these questions, we shall derive from them certain hypotheses, which then become the "base-lines" from which variation in the data is measured. If the deviations from those "base-lines" are too great to be reasonably assigned to random sampling variation, we shall

conclude that the hypotheses used probably are not taking into account all the sources of non-random variation.

The χ^2 test is used to distinguish between the random and non-random variation described above.

The first question stated above leads to this null-hypothesis: for a given wave length, the probability of observing a mutation on one observation of a given gene locus is the same for all the loci considered in that set. The expected numbers under that hypothesis are compared with the corresponding observed numbers in table 5. The calculation of the expected numbers is illustrated from table 3 and for Cr_{2475}.

Let p be the estimated probability of observing a mutation at a particular locus on a single observation. Then we would expect (mathematically) 1843 (p) mutations at "*al*" and "*sp*" and 929 (p) mutations at each of the other loci listed. Hence, keeping the total number of mutations expected the same as the number observed, we must have $2(1843p)+4(929p)=8$. This requires that $p=.001081$; or, the estimated rate of mutation for each locus is 1081 per million observations. One then obtains

$$\bar{x}=1843(.001081)=2.0$$

as the expected number of mutations at the locus "*al.*"

As pointed out by FRYER (1940), we can use

$$\chi^2=\sum_1^N \frac{(x_i-\bar{x}_i)^2}{\bar{x}_i}$$

and disregard the numbers of non-mutants, since the deviations squared are very small compared to the expected numbers of non-mutants.

The tests described above were made on the data of tables 2, 3, and 4. The results for each test separately are given in table 5. χ^2 offers a means

TABLE 5

Tests of the hypothesis that basic rates of mutations of genes within sets are the same.

METAL AND DOSAGE		CHROMOSOME I χ^2	I d. f.	I P	II χ^2	II d. f.	II P	III χ^2	III d. f.	III P
Cu_{3625}	v	4.10	6	.66	5.29	5	.40	2.82	5	.77
	v+c	9.94	6	.12	12.52	5	.035	2.14	5	.83
Cu_{7250}	v	9.08	6	.18	—	—	—	—	—	—
	v+c	15.58	6	.023	—	—	—	6.25	5	.28
Cr_{2475}	v+c	—	—	—	3.50	5	.63	—	—	—
Cr_{4950}	v	—	—	—	—	—	—	3.82	5	.57
	v+c	18.30	6	.007	5.94	5	.31	6.51	5	.27

of combining the results of separate tests if that is desired. A pooling of the data into one test occasionally yields misleading results. For example, the pooled χ^2 for the first chromosome has six degrees of freedom and is far beyond the 0.1 percent level of significance; but during the copper irradiation—where we obtained our best set of data—the χ^2 is well above the five percent level of significance. Also, on the second chromosome, the pooled χ^2 is definitely non-significant. This hides the fact that χ^2 was significant for the results of the copper irradiation.

One sees from table 5 that but three of the fourteen values of χ^2 are significant and but one is highly so. All three of the significant values came on the (v+c) data. It is found from the amounts contributed to χ^2 by the various genes that the "*ct*" locus is causing the large χ^2 in two of the three significant cases. For example, for Cr_{4950} on I, the expected number of mutations at each locus was 1.14. Actually five mutations were observed at "*ct*." Then $(5-1.14)^2/1.14=13.07$ of the 18.30 found for the χ^2 in this case was produced by the deviation of the observed number of "*ct*" mutations from the number of such mutations expected if all of the sex-linked genes considered have the same mutation rate.

In the remaining case, "*dp*" and "*px*"—both more subject to misclassification than most of the genes observed—are contributing most of the deviations from expectation. Assuming that neither wave length nor dosage of irradiation interact with mutation rate, it is proper to use a combined χ^2 test on the data of table 5. Adding the values of χ^2 for "v" mutations, if possible, one obtains: $\chi^2=59.10$, d. f.$=48$, P$=.05$. If this same procedure is used on "v" mutations only, $\chi^2=25.11$, d. f.$=27$, P$=.57$. Hence one concludes that, in general, the genes in a chromosome group have about the same mutation rate. However, there is evidence in our data to indicate that the rates of change at the "*ct*" locus might be greater than the rates of mutation of the other genes in the corresponding chromosome set.

The data in table 1 furnish evidence regarding the proportionality of mutation rate to dosage. If one makes the comparisons: Cu_{3625} with Cu_{7250} on the first and third chromosomes, and Cr_{2475} with Cr_{4950} on the second and third chromosomes by assuming that mutation rate is proportional to dosage applied, one obtains these results in that order, using "*v*" mutations:

$\chi^2=1.37$, d. f.$=1$, P$=.25$,
$\chi^2=0.032$, d. f.$=1$, P$=.85$,
$\chi^2=1.16$, d. f.$=1$, P$=.29$, and
$\chi^2=0.176$, d. f.$=1$, P$=.68$.

Since none of the χ^2 values above is significant, it is concluded that the hypothesis of proportionality of mutation rate to dosage is in accord with the data in table 1.

In studying the effect of wave length on mutation rate by means of χ^2, we shall assume that wave length—for a given dosage and gene locus—has no effect on mutation rate. That is the hypothesis to be tested against the observed data. Since previous analyses cast no doubt on the hypothesis that mutation rate and dosage of radiation are proportional, we have assumed that to be true in the analyses below. Using the appropriate parts of table 1, we have derived the contents of tables 6 and 7.

TABLE 6

Comparison of wave-lengths using (v) frequencies and sex-linked mutations.

WAVE LENGTH	(1) DOSAGE (r units)	(2) LOCI EXAMINED	(1)×(2) TOTAL	MUTATIONS OBS.	MUTATIONS EXP.
0.7Å (Ag)	4,720	2,955	.04295	2	1.4
1.5Å (Cu)	3,625	52,101	.5815	16	18.6
	7,250	12,455	.2780	12	8.9
2.1 to 2.2Å (Cr)	4,950	6,397	.09755	2	3.1
Total (1)×(2)=	324,777,625		1.00000	32	32.0
	χ^2=2.09, d. f.=3, P=.56.				

The results in table 6 indicate that the hypotheses adopted account for the variation among the data quite well.

TABLE 7

Comparison of wave lengths using (v) frequencies and third chromosome mutations.

WAVE LENGTH	(1) DOSAGE (r units)	(2) LOCI EXAMINED	(1)×(2) TOTAL	MUTATIONS OBS.	MUTATIONS EXP.
0.7Å (Ag)	4,720	4,005	.1175	1	1.2
1.5Å (Cu)	3,625	13,917	.3137	3	3.1
	7,250	5,451	.2457	2	2.5
2.1 to 2.2Å (Cr)	2,475	7,478	.1151	1	1.1
	4,950	6,760	.2080	3	2.1
Total (1)×(2)=	160,842,525		1.0000	10	10.0
	χ^2=0.531, d. f.=4, P=.96.				

While the agreement between observation and expectation is abnormally good for these data, it is not excessively so. In consideration of previous analyses, this result seems to confirm that in table 6.

A comparison of the rates of mutation on the three chromosomes studied can be made if one makes two assumptions in addition to the null hypothesis that the three chromosomes have the same basic rate of mutation.

These assumptions are: (1) that the sets of genes we have observed are equally representative of their respective chromosomes, and (2) that differences among the stocks of flies used do not spoil the comparisons being made.

In view of the results of previous analyses, we may use all the data in table 1 in an analysis similar to that used in tables 6 and 7. This is done in table 8.

TABLE 8

Comparison of mutation rates on the first three chromosomes of Drosophila melanogaster.

CHROMOSOME	"UNITS"	EXPECTED PROPORTION OF MUTATIONS	MUTATIONS	
			OBS.	EXP.
I	342,152,125	.4862	32	23.8
II	184,116,907	.2616	7	12.8
III	177,415,389	.2521	10	12.4
	703,684,421	1.0000	49	49.0

$\chi^2 = 5.918$, d. f. = 2, P = .056.

Although the probability, P, in table 8 is not quite down to the conventional five percent level, one must conclude that the variation from expectation probably is not just random. We cannot say whether the nonrandom element is produced by stock differences, or by different mutation rates on the chromosomes.

ANALYSES OF ALLIED DATA

The literature contains a number of sets of data which are closely related to this study. A few of these sets have been analyzed in order to illustrate the kind of information they can yield. The data selected for analysis pertain to these subjects: (1) continuous versus interrupted administration of the dosage of radiation; (2) the effectiveness of heat in producing sex-linked lethals in Drosophila; (3) the effectiveness of heat in producing visible gene mutations; (4) differential mutation rates among different species of Drosophila; (5) rates of reverse mutation; that is, from recessive to normal, and (6) mutation rates of the alleles in "white" series of *Drosophila melanogaster*.

The data for the first illustration are from PATTERSON (1931) and are presented in table 9.

The only one of the above experiments to yield a significant χ^2 was the second. The author expressed the feeling that since it was the smallest scaled and for the shortest total time, it was the least reliable. Accepting that point of view and noting the total χ^2, the general conclusion to be

TABLE 9

Continuous versus interrupted administration of the radiation as it affects rate of lethal mutation in Drosophila.

TIME INTERVAL OF INTERRUPTION	TOTAL TIME	DOSAGE (r units)	NUMBER OF FLIES	NUMBER OF LETHALS OBS.	NUMBER OF LETHALS EXP.	NUMBER OF NON-LETHALS OBS.	NUMBER OF NON-LETHALS EXP.
0 hrs.	16 mins.	1,654	971	49	60	922	911
12 hrs.	16 mins.	1,654	993	62	61	931	932
6 hrs.	16 mins.	1,654	981	71	61	910	920
		$\chi_1^2=3.92$, d. f.$=2$, P$=.15$					
0 hrs.	8 mins.	2,558	518	39	50	479	468
12 hrs.	8 mins.	2,558	345	45	34	300	311
		$\chi_2^2=6.63$, d. f.$=1$, P$=.01$					
0 hrs.	10 mins.	1,234	863	28	31	835	832
24 hrs.	10 mins.	1,220	876	31	32	845	844
12 hrs.	10 mins.	1,221	936	40	34	896	902
8 hrs.	10 mins.	1,219	856	34	31	822	825
1 hrs.	10 mins.	1,220	1,014	32	36	982	978
30 mins.	10 mins.	1,234	962	33	35	929	927
		$\chi_3^2=2.31$, d. f.$=5$, P$=.80$					
0	12 hrs.	radium	544	58	57.9	486	486.1
12	12 hrs.	radium	452	48	48.1	404	403.9
		$\chi_4^2=.000425$, d. f.$=1$, P$=.98$					
		$\sum_1^3 \chi_i^2=12.86$, d. f.$=8$, P$=.12$					

TABLE 10

*Summary of an investigation of the production of sex-linked mutations by temperature shocks.**

TYPE OF INVESTIGATION	TREATMENT	GAMETES EXAMINED	SEX-LINKED LETHALS OBS.	SEX-LINKED LETHALS EXP.
Lethals (using *ClB* Method)	Controls	6,495	10	15.7
	♂, 35°–38°C	4,635	19	11.2
	♀, 35°–38°C	7,052	15	17.1
		18,182	44	44.0
	$\chi^2=7.76$, d. f.$=2$, P$=.02$			
			VISIBLE MUTATIONS	
Visible mutations (with attached-X stocks)	Controls	84,015	8	12.8
	♂, 35°–38°C	88,198	17	13.4
	♀, 35°–38°C	64,300	11	9.8
		236,513	36	36.0
	$\chi^2=2.91$, d. f.$=2$, P$=.24$			

* See BUCHMANN and TIMOFEËFF-RESSOVSKY 1936.

drawn from the above data would be that interruption of the process of irradiation probably does not affect the production of lethal mutations.

On the subject of heat-induced mutations, BUCHMANN and TIMOFEËFF-RESSOVSKY (1936) have reported an investigation during which they produced sex-linked lethals in one stock of Drosophila and visible gene mutations in an attached-X stock of Drosophila. They used both X-rays and heat, but only the data from the latter treatments are given here.

TABLE 11

Comparison of rates of lethal, sex-linked mutation in D. funebris and D. melanogaster (from TIMOFEËFF-RESSOVSKY *1937).*

SPECIES	TREATMENT	NUMBER EXAMINED	LETHALS		NON-LETHALS	
			OBS.	EXP.	OBS.	EXP.
funebris	controls	2,869	2	1.8	2,867	2,867.2
	X-rayed	1,037	84	96.8	953	940.2
melanogaster	controls	1,837	1	1.2	1,836	1,835.8
	X-rayed	731	81	68.2	650	662.8
		$\chi^2=4.57$, d. f. = 2, P = .11				

TABLE 12

Comparison of rates of lethal sex-linked mutation in D. simulans and D. melanogaster (from KOSSIKOV *1935).*

SPECIES	TREATMENT	NUMBER EXAMINED	LETHALS		NON-LETHALS	
			OBS.	EXP.	OBS.	EXP.
simulans	Controls	1,446	12	10.6	1,434	1,435.4
	X-rayed	842	42	38.0	800	804.0
melanogaster	controls	469	2	3.4	467	465.6
	X-rayed	1,019	42	46.0	977	973.0
		$\chi^2=1.57$, d. f. = 2, P = .47				

It appears from table 10 that the variability between control ♂ and ♀ could very well be random as far as the visible mutations are concerned. As regards the sex-linked lethals, it seems logical to conclude that heat treatments probably did increase their rate of mutation somewhat.

The third type of comparison we shall make concerns different species of Drosophila. The rates of mutation of these species are compared in tables 11 and 12, using lethal mutations for the comparison.

It is seen from tables 11 and 12 that the variability exhibited by those sets of data as wholes (which is the way the experiments were planned)

may reasonably be supposed to be random variability rather than being due to species differences in mutation rate. However, it is of interest to note that if in table 11 one omits the controls from consideration, one ob-

TABLE 13

Rates of mutation from visible recessive to normal.

LOCUS	(A) DATA FROM TIMOFEËFF-RESSOVSKY 1937*			(B) DATA FROM JOHNSON AND WINCHESTER 1934 3975 r.		
	NUMBER OF GAMETES	MUTATIONS		NUMBER OF GAMETES	MUTATIONS	
		OBS.	EXP.		OBS.	EXP.
y	21,897	0	1.9	69,923	1	2.9
sc	17,676	3	1.6	101,042	5	4.3
w	29,233	2	2.6	—	—	—
w^e	23,472	2	2.1	—	—	—
w^a	—	—	—	69,302	0	2.9
ec	17,676	0	1.6	57,323	0	2.4
cv	16,460	2	1.4	—	—	—
ct	12,914	0	1.1	57,323	1	2.4
v	29,384	2	2.6	61,119	1	2.6
m	—	—	—	39,923	2	1.7
g	12,914	0	1.1	57,323	4	2.4
f	34,811	8	3.0	130,421	15	5.5
car	—	—	—	69,302	1	2.9
Total	216,437	19	19.0	713,001	30	30.0
	$\chi_1^2=15.80$, d. f.$=9$, P$=.08$, $\chi_2^2=27.24$, d. f.$=9$, P$=.002$					
	$\chi_1^2+\chi_2^2=43.03$, d. f.$=18$, P$<.001$					
ru	12,755	0	0.6			
h	27,155	1	1.2			
th	5,681	0	0.2			
st	27,155	1	1.2			
p^p	21,474	4	1.0			
cu	5,681	0	0.2			
ss	21,474	0	1.0			
sr	5,681	0	0.2			
e^s	27,155	1	1.2			
ca	5,681	0	0.2			
Total	159,892	7	7.0			
	$\chi_3^2=11.50$, d. f.$=9$, P$=.25$					

* TIMOFEËFF-RESSOVSKY's data are based on two or more experiments having different exposures to X-rays. Doses in Mutationsforschung in der Vererbungslehre 3,900 and 5,000 r.

tains a χ^2 which is significant at the five percent level of significance. This is not due merely to the omission of small numbers from the calculations as is shown in table 12 where more than one-third of the χ^2 was contributed

by the control data. The results of this further analysis suggest the possibility that the two species, *funebris* and *melanogaster*, may react differently to the X-rays even though their normal rates of mutation are apparently quite similar. The data on the normal rates of mutation for each of the two species being considered are too scant to allow a more definite conclusion to be drawn.

The next type of data to be considered is from two sources—(1) TIMOFEËFF-RESSOVSKY (1937) and (2) JOHNSON and WINCHESTER (1934)—and is on the subject of reverse mutations. These data include both v and c types of observations.

TABLE 14

Summary of all mutations in different directions within the white series of Drosophila melanogaster, produced by X-ray treatment (dosage approximately 4,800 r).

ORIGINAL GENE	MUTATED TO							TOTAL
	w	w^{bf}	w^{e}	w^{a}	w^{b}	w^{x}	W	
W, wild	25	1	3	1	2	5		48,500
w^{co}, coral	1							6,000
w^{b}, blood	3		1					12,000
w^{c} cherry	1							5,000
w^{a}, apricot	2		1					11,000
w^{e}, eosin	13				1	2	2	39,000
w^{bf}, buff	1							5,500
w^{t}, tinged	1							7,000
w, white		1	1		1			54,000

The conclusions to be drawn from table 13 are essentially those of the corresponding analyses of our own data with the exception that the "*f*" locus now appears to possess the higher rate of mutation in the JOHNSON-WINCHESTER data. It was found that 24.74/43.03 of the combined χ^2 for the sex-linked genes was contributed by the "*f*" locus.

If one uses TIMOFEËFF-RESSOVSKY's data to test the rate of reverse mutation on chromosome I against that rate on chromosome III, he obtains $\chi^2 = 1.93$, d. f. = 1, P = .17. This is essentially the same result as that obtained from our own data for direct mutations.

TIMOFEËFF-RESSOVSKY's study (1933) of the mutation rates of the alleles in the white locus of Drosophila is adapted to the χ^2 test in interpreting the significance of the observed differences.

Two questions will be asked about the data of table 14: (1) Is the rate of change of the different alleles to the "white" gene fundamentally the same for all alleles? To test this question, we take the white mutations in the first column aside from the "white" gene itself and test the eight remaining alleles for homogeneity of mutation rate. The eight observation classes give 134,000 observations with 47 mutations, or an average rate of

0.00035. Seven degrees of freedom are available for deviations from expectation. One finds that the χ^2 is 7.55 and is associated with a probability of .35. There seems to be nothing in the above data to indicate that the different alleles are significantly different in their rates of mutation to "white." (2) Are the frequencies with which the various alleles change to genes other than "white" fundamentally different? To answer that question, we test the homogeneity of the rates of mutation for all nine genes, omitting the data of column one. There are 188,000 observations and 22 mutations, which gives an estimated mutation rate of 0.00012. Eight degrees of freedom are now available, χ^2 is 11.8, and P is .16. It thus appears that no significant difference in rate of mutation to genes other than "white" is indicated by these data.

SUMMARY

The data obtained from irradiation research on visible gene mutations follow binomial distributions which are defined by probabilities less than 0.01. HOEL (1938), SUKHATME (1938), COCHRAN (1936), NEYMAN and PEARSON (1931), and FRYER (1940) have given evidence to show that the tabular χ^2 test is valid and useful for such data even when the expected numbers are as low as 0.5.

New data were obtained on visible mutations at 19 loci on the first three chromosomes of *Drosophila melanogaster*. These data are analyzed by the χ^2 test on the basis of these hypotheses: (1) that the genes within the sets observed have the same basic mutation rate, (2) that the mutation rate at a specific locus is directly proportional to the dosage of radiation applied, (3) that in so far as the sets of genes used are representative of their respective chromosomes, the fundamental rates of mutation on these chromosomes are the same, and (4) that for a particular gene and a fixed dosage, the wave-length of X-ray used does not affect the mutation rate. Our data indicate that the first hypothesis is not adequate to explain the variation observed when the "*ct*" locus and possibly the "*dp*" and "*px*" loci, are among those observed; but the other hypotheses fit the data quite satisfactorily.

Other sets of data were analyzed by χ^2 as illustrations of methodology. These data were on the following subjects: (1) continuous versus interrupted irradiation (from PATTERSON 1931), (2) reverse mutations (from TIMOFEËFF-RESSOVSKY 1933), (3) the production of mutations by heat (from BUCHMANN and TIMOFEËFF-RESSOVSKY 1936), (4) comparisons of mutation rates among the species of Drosophila: *funebris*, *melanogaster*, and *simulans* (from TIMOFEËFF-RESSOVSKY 1937), and (5) comparison of mutation rates at different alleles in the "white" series of *Drosophila melanogaster* (from TIMOFEËFF-RESSOVSKY 1933).

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