A STUDY OF A CASE OF HIGH MUTATION RATE IN DROSOPHILA MELANOGASTER

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INTRODUCTION

FROM the standpoint of evolutionary theory, as well as for the light shed on the nature of the gene, it is important to know how "spontaneous" mutation is distributed in space and time. The first adequate studies of the rate of occurrence of mutation (MULLER and ALTENBERG 1919; MULLER 1928), utilizing sex-linked lethal mutations in *Drosophila melanogaster*, served to underline this question, for whereas in the initial work, involving two different strains of flies, rates of 1.69 ± 0.46 and 0.98 ± 0.22 percent were encountered, in subsequent experiments a more normal rate of 0.19 ± 0.05 percent was observed. For some time these data remained an isolated instance of a high spontaneous mutation rate. Since 1937, however, seven additional examples of this phenomenon have been described (DEMEREC 1937; PLOUGH and HOLTHAUSEN 1937; GOLDSCHMIDT 1937, 1939; VALADARES 1937; TINIAKOV 1939; PLOUGH 1941), although one of these (GOLDSCHMIDT 1937) perhaps belongs in a separate category. In addition, SPENCER (1935) has described a cyclical variation in the frequency of mutation, involving all the strains in his laboratory. These observations have established the existence of periods of high mutational activity in Drosophila strains, although, with the exception of the case reported by DEMEREC (1937), where the phenomenon apparently depended on some recessive influence of the second chromosome, the exact causes of this mutational variability have remained obscure.

It is the purpose of the present paper to describe and analyze the cause of another case of high mutation frequency. The phenomenon was first detected in February, 1941, in two closely related strains. The first of these was a long-inbred Oregon wild type line, secured from DR. CURT STERN in 1938. During the 60 generations it had been in the author's laboratory it had been inbred in small mass cultures. Throughout this time the line had been under rather close surveillance, for it was used as a genetic background into which various bristle factors were being intro-

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duced, and crosses involving the line were rather constantly in progress. During all this time no mutants had been observed. The second line was a heterozygous *y Hw* strain which by repeated backcrosses continuing up until the discovery of the phenomenon had been rendered genetically very similar to the Oregon line. With the exception of the *y Hw* factors, the two strains were so isogenic that they may be considered as one.

THE HIGH FREQUENCY OF OCCURRENCE OF VISIBLE MUTATIONS

Between February and June, 1941, as many flies from the two lines were examined as time would permit. Whenever an off-type was found it was as a rule mated within the strain, and thus sublines of the original strain were established. The individuals comprising various sublines were likewise carefully scrutinized, and aberrant individuals bred. In this way a total of 72,851 flies was examined. The off-types found fall into three groups: (a) mutants, of which at least 47 were detected, with three more in the probable class; (b) off-types which were sterile and hence unanalyzable, but which nevertheless had the appearance of mutants—17 in number; and (c) numerous aberrant types whose unusual characteristics did not reappear in subsequent generations and which were therefore somatic mosaics or developmental accidents.

Space does not permit a description of the 47 known mutations, but a complete summary is on file with GENETICS. A summary also appears in the sixteenth issue of Drosophila Information Service. The mutations are on the whole a cross section of the types encountered in Drosophila work, with a few possible exceptions to be noted later. Thus, the sex-linked factors consisted of alleles of yellow (12), Notch (2), singed (2), bobbed (2), cut (1), carmine (1), and lozenge (1), as well as six other mutations that could not readily be homologized with any known factors. These latter six were respectively characterized by (1) a carmine-like eye color, (2) supernumerary scutellar bristles, (3) dark eyes, small body, and female sterility, (4) tiny bristles, (5) rough, oval eyes, weak wings, disarranged bristles, rarely body protuberances, and female sterility, and (6) rough eyes, missing, doubled, and disarranged hairs and bristles, and occasionally incised or blistered wings. The autosomal mutations are more difficult to homologize, but thus far have been shown to include alleles of net (1), Delta (1), and polymorph (1), and in addition factors characterized by extra bristles (3), small bristles (2), erect bristles (1), malformed wings (5) abnormal wing carriage (1), abdominal and genital imperfections (2), dark eye color (1), dark body color (1), and rough eyes (1).

A number of the mutations were characterized by small bristles (bobbed, a second sex-linked recessive, an autosomal dominant Minute, a second chromosomal recessive). These mutations became widely disseminated

throughout the strains. Toward the end of the study aberrant types with small bristles appeared with great frequency. It was obviously not feasible to test and attempt to differentiate between all these small bristle types, nor to determine which of them represented outcroppings of already isolated mutations and which new mutations. The estimate of the frequency of mutations characterized by small bristles is therefore probably low, particularly with reference to the frequency of "Minute" mutations. It should be emphasized that in this and subsequent work, the dominant autosomal Minutes were carefully distinguished from the Minute type due to the loss of the fourth chromosome through non-disjunction.

The possibility of contamination, which must always be considered, may be rigorously excluded in the great bulk of this work. The number of stocks kept in the laboratory at DARTMOUTH, where the foregoing portion of the work was carried out, was relatively small (*cf.* Drosophila Information Service #14), and few of the mutations found were duplicates of factors already present in the laboratory. Another consideration which rules out contamination is this: many of the mutations detected in the later stages of the analysis (see below) occurred in strains homozygous for one or more genetic markers. Contamination should have resulted in the disappearance or temporary suppression of these markers; this was observed in only two cases among all the thousands of cultures examined.

THE NORMAL FREQUENCY OF DETECTION OF VISIBLE MUTATIONS

For all the effort that has been expended on Drosophila, quantitative information on the normal frequency of detection of visible mutants under the conditions obtaining in such an experiment as this is surprisingly meager. Data on this point are summarized in table 1. In the hands of the average investigator a rate of about five "fair" to "good" mutants per 100,000 flies examined has been normal. After due allowance is made for the fact that some of the mutants found in the present study were "poor" ones, the mutation rate in the Oregon line (64.5 per 100,000) would on the basis of these figures appear to be at least five to ten times greater than normal, and this was the first estimate of the magnitude of the increase which the author made (NEEL 1942).

However, the existing estimates of the frequency of appearance of new mutants in *Drosophila melanogaster* are probably quite inadequate. The reasons behind this statement are chiefly two:

(1) In *ClB* experiments on induced mutations, the ratio of sex-linked lethals to visible sex-linked dominant and recessive, and autosomal dominant, mutations varies between 10 to 1 and 5 to 1, depending on the investigator. Obviously in such a case the lower ratio more nearly approximates the "true" ratio. In the later stages of the present work (see below)

TABLE 1

A compilation of the frequency of detection of visible mutants in Drosophila melanogaster.

INVESTIGATOR	ORIGIN OF FLIES EXAMINED	NUMBER FLIES	NUMBER OF MUTATIONS	RATE PER 100,000
MORGAN (1914)	?	31,168	0	0
DUNCAN (1915)	F_2 of a cross of two unrelated, inbred lines	16,637	2	12.0
GROSSMAN & SMITH (1933)	Inbred wild type stock	45,000	0	0
JOLLOS (1934)	Inbred wild type stock	ca 700,000	22	3.1
PLOUGH & IVES (1935)	Various	73,221	3	4.1
MOORE (1934)	Males from XX matings	13,673	1	7.3
	Male offspring of normal females	12,633	0	0
BUCHMANN & TIMOFEEFF-RESSOVSKY (1935)	Males from XX matings	58,453	5	8.6

a total of 7565 *ClB* F_2 vials was examined, and 25 lethals and seven visibles found. The ratio of lethals to visibles, 3.6 to 1, is sufficiently lower than the ratios encountered in irradiation work to be pertinent to the question of whether there is a difference between spontaneous and induced mutation in this respect. Be that as it may, if approximately two per 1000 is the normal rate of occurrence of sex-linked lethals, then in 100,000 *ClB* F_2 vials there should be some 200 lethals, and, on the basis of a 5 to 1 ratio of lethals to visibles, at least 40 visible mutations of the types indicated above. The *ClB* method is undoubtedly an unusually favorable means of detecting certain kinds of visible mutations. Detailed calculations, involving matters of viability, sterility, penetrance, and observational difficulties, suggest that the system of moderate inbreeding used in the work described in the previous section reduces the relative chance of detecting the average mutant to about 0.5 or so on the probability realized when the *ClB* method is employed. The *ClB* data therefore suggest that about 20 mutants due to autosomal and sex-linked dominant and sex-linked recessive factors should appear in 100,000 flies. Since autosomal recessives constitute about a third of the Drosophila mutations, with inbreeding, such as was practiced in the above described work, the expected rate of recovery of all kinds of mutations should be at least 25 per 100,000 flies. Stricter inbreeding might well result in an even higher rate of appearance of new mutations. About two thirds of these should be of the "fair" to "good" types included in table 1.

(2) Between February, 1941, and January, 1942, the period covered by this investigation, the author kept in stock an average of 20 different strains unrelated to the Oregon line and to each other. These strains were

transferred to fresh culture bottles about every 20 days, at which time some 50 flies of each strain were cursorily examined for the occurrence of contamination or mutation. Over the entire period an estimated 18,000 flies were examined. Five mutations were found, as follows:

(a) misshapen^{41d3}. 3-?. Eye variably small and deformed, overlapping with wild type.
(b) cut-out^{42a14}. Autosomal recessive. Wing margins incised. Found in same stock as (a).
(c) peach^{41d22}. 2-?. Eye color varies from orange in newly emerged flies dark, translucent prunish color in aged flies.
(d) scute^{41i}. 1–0.0. Scutellar bristles descreased in number, head bristles increased.
(e) hairy^{41k}. 3–26.5. Typical hairy-1 allele, with hairs on scutellum and along wing veins.

The occurrence of five mutations in 18,000 hastily examined flies represents a rate of 28 per 100,000, and constitutes excellent agreement with the above calculated normal rate.

The rate of visible mutation in the Oregon line was therefore two to three times greater than normal, although, as will be discussed later, this represents a minimum estimate of the increase which the factor responsible can engender.

THE FREQUENCY OF OCCURRENCE OF SEX-LINKED LETHAL MUTATIONS IN SOME STRAINS

The rate of occurrence of sex-linked lethals was investigated by the *ClB* method. In each *ClB* F_2 vial (except, of course, those indicating the presence of a lethal) an average of five males was inspected, to provide a check on the occurrence of visible mutations in the P_1 males (if all or half of the F_2 males were affected) or in the F_1 of the *ClB* cross (if only a single male was affected). Autosomal recessive mutations would not be detected by this method. The results are summarized in table 2. The first flies tested (Or F_8 1) were from the original Oregon line, seven generations after the

TABLE 2

Rate of mutation in three sublines of the frequently mutating strain.

STRAIN	NUMBER OF X CHROMOSOMES TESTED	NUMBER OF MALE P_1	SEX-LINKED LETHAL MUTATIONS	FREQUENCY OF LETHALS (%)	NUMBER OF MALE P_1 GIVING LETHALS	NUMBER OF F^2 EXAMINED (ESTIMATED)	VISIBLE P_1 MUTATIONS	VISIBLE F_1 MUTATIONS
Or F_8 1	985	46	1	0.10±0.10	1	4920	0	0
y Hw F_1 2	1021	37	8	0.78±0.28	6	5065	1	1
y Hw F_8 6	691	29	7	1.01±0.38	6	3420	1	1?

discovery of the phenomenon. The rate of occurrence of lethals appeared to be entirely normal. Two additional strains were then examined. These were selected because, as sublines, each had yielded several mutants, and, more to the point, in each a mutant had been detected quite recently. The first of these (*y Hw* F_1 2) yielded eight lethals and one visible—a new allele of prune (1–0.8)—in 1021 tested X's. Three of these lethals were derived from the same male; two of them were probably alleles, since under favorable conditions both of them behaved as semi-lethals, characterized by small, dwarf flies, but the other always behaved as a complete lethal and so probably represented a distinct mutation. Ten of the 37 males who contributed to the tested X chromosomes proved to be heterozygous for an ebony allele which had not been encountered up until this time. In one of the F_2 vials a single yellow-2 type male was found, product of a mutation in an F_1 female. In the second strain (*y Hw* F_3 6) seven lethals and one visible—characterized by a rough eye and located at 1–0.5—were found in 691 tested X's. Two of these lethals, occurring in the same male, are possibly alleles. One of the lethals, located at 1–37.2, acted under favorable conditions as a semi-lethal, resulting in flies with obliquely truncated wings and disturbed venation. The questionable F_1 visible mutation indicated in the table was a vermilion-1 (1–33.0). This factor is known to be carried within the *ClB* inversion, and it is possible that the so-called mutation was really the result of a rare double cross-over within the inversion. However, the P_1 males carried *cm* (1–18.9), and this was still present in the mutant, and t^2, also carried in the *ClB* inversion, was not associated with the vermilion.

If each lethal encountered in the latter two strains be treated as the product of a separate event, then the combined rate of lethal mutation in these two strains exceeds the rate of the Or F_8 1 strain by a significant amount. If some of the lethals are products of the same mutation, then the difference, so far as lethals alone are concerned, is less significant. However, when the occurrence of visibles in the latter two strains is also taken into account, it seems rather certain that these two strains were throwing significantly more mutations than the first strain tested. The behavior of this first strain can most reasonably be explained in terms of its loss of high mutative properties.

ANALYSIS OF THE CAUSE OF THE INCREASED MUTATION RATE

Experiment 1

A number of experiments were performed, designed to shed light on the factors responsible for the increased rate of mutation. In the first of these, an attempt was made to isolate, from the *y Hw* F_3 6 line, males producing visible mutations in relative large numbers and to establish balanced

stocks of the chromosomes of such flies. Three generations after the experiments leading to the above-described results in this strain had been started, *y Hw* F_3 6 males were mated individually with several *y S*/In(2L)*Cy*, *Cy*; *H*/In(3R)C, *Sb* females and then, three days later, remated with ca. 15 *y v f; bw; e wo ro; ey* females. Of 16 such attempted double matings, 13 were successful, although the fertility of the second mating was in most cases poor. The male offspring of the second cross were examined for mutant individuals. The results are summarized in table 3. Among a total of

TABLE 3

Summary of experiment 1. Explanation in text.

FLY NUMBER	NUMBER OF MALE OFFSPRING BY XX FEMALES	FERTILE MUTANTS	STERILE OFF-TYPES	MOSAICS OR DEVELOPMENTAL ACCIDENTS
1	401	0	0	0
3	202	0	0	0
4	536	1	1	4
5	1105	0	1	1
7	634	1?	0	2
8	982	0	0	2
9	297	0	0	0
10	219	0	2	1
11	382	0	0	1
12	357	0	0	0
13	408	0	0	1
14	349	0	0	0
15	284	0	0	1
13	6,156	1+1?	4	13

6156 males one certain mutation—an autosomal dominant characterized by incompletely expanded wings—and one probable mutation—resulting in a small-eyed condition—were detected. The analysis of this latter was complicated by the presence in the strain of the *ey* factor and the early loss of the strain, but the mutation was probably an autosomal dominant. The *y Hw* F_3 6 males all carried *cm* (1–18.9), which had arisen in the strain as the result of mutation, and the continued presence of which in this and subsequent experiments served as a check on contamination.

Among the 536 male offspring of male 4 there appeared, in addition to the above-mentioned certain mutant, one sterile male characterized by curled wings, rough eyes, and sparsely distributed thoracic hairs, who was in all probability the result of genetic change, and one mosaic male whose left eye was very rough, left thoracic hairs sparse and disarranged, left thoracic bristles slightly contorted, and left wing small, but whose off-type characteristics did not reappear in subsequent generations. In addition, there were three other less striking mosaics among his offspring.

On the assumption that male 4 might carry mutability-stimulating factors, an attempt was made to establish balanced stocks of his chromosomes. Accordingly, $+^m$; *S* or In(2L)*Cy*, *Cy*/$+^m$; *H* or In(3R)C, *Sb*/$+^m$ male offspring from the first cross of male 4 with *y*; *S*/In(2L)*Cy*, *Cy*; *H*/In(3R)C, *Sb* females ($+^m$ indicates the appropriate chromosome derived from the *y Hw* F_3 6 strain) were mated individually in a manner designed to balance the X chromosome against an attached-X chromosome homozygous for *y*, the second against a rearranged second chromosome with the dominant *Cy* factor in one limb and L^4 in the other (In(2L)*Cy*, *Cy*, In(2R)*Cy*, $L^4 sp^2$), and the third against a complexly rearranged third chromosome marked by the dominant Mé factor (T(2; 3)*Mé*). It had originally been planned to establish six or seven strains combining these balancer chromosomes with homologues derived from male 4. At a late point in the experiment it was discovered that female flies carrying all three balancers were too highly inviable and sterile to be useful. While a number of strains containing balanced first and second chromosomes derived from male 4 were secured, none completely balanced for the third was obtained. However, a number of strains with a balanced first and second, and originally heterozygous for an unbalanced third, were established, and it was also possible to establish some strains with a completely balanced first and second, and with the right end of the third balanced against In(3R)C, *Sb*, while the left end of the same chromosome remained unbalanced. No attempt was made to follow the fourth chromosome. Table 4 shows the types of balanced strains

TABLE 4

The rate of apperance of mutants in balanced stocks of the chromosomes of male 4 of experiment 1. Numbers in parentheses indicate the number of different balanced lines of the composition indicated that were followed. The question mark after certain chromosomes, or parts of chromosomes, indicates that these chromosomes may or may not have been represented in the strains.

CHROMOSOMES PRESENT IN THE STOCK	NUMBER OF FLIES EXAMINED	FERTILE MUTANTS
I, II (11)	5011	0
I, II, III? (4)	2025	1
I, II, IIIR, IIIL? (5)	2441	1
	9477	2

established, the number of flies from each examined, and the number of mutations found. The two mutations were a singed allele (1–21.0) and an autosomal recessive factor characterized by extra venation. Both of these were detected in strains in which the third chromosome of the *y Hw* F_3 6 line was either possibly or certainly represented. It is obvious that the behavior of these strains does not bear out the performance of the founder

male. The possibility that this was due to failure to control the third chromosome adequately remains open (see below).

Among the relatively few offspring of male 11 there was one off-type, a male with an abnormally oval right eye. This male, of the constitution *cm; +^{m}/bw; +^{m}/e wo ro; +^{m}/ey*, was mated with several *y v f; bw; e wo ro; ey* females. The oval right eye did not reappear in the next generation. However, among the 74 male offspring of the cross there was one lozenge-eyed male, subsequently found to be the result of mutation at the lozenge locus (1–27.7). It seemed possible that male 11 had actually carried a mutability stimulating factor and that its effects had not been detected because of the small number of offspring from this male. Accordingly, a balanced stock of these chromosomes was established by selecting *cm; +^{m}/bw; +^{m}/e wo ro; +^{m}/ey* males from the bottle which had yielded the lozenge male, crossing these with *y v f; bw; e wo ro; ey* females, and repeating the cross each generation. In order to test the frequency of mutation in these males, they were crossed with attached-X *y v f car* females, and the male offspring examined for mutants. A total of 10,084 flies was inspected. Only one mutant was detected among these—a Minute due to a dominant factor in the third chromosome—plus 11 sterile types and 22 types which bred without the reappearance of the aberrant characteristic. One of these latter was a right-left singed or forked bristle mosaic. This mutation rate did not seem to be sufficiently high to warrant further work with the strain.

Experiment 2

The second attempt to get at the factors responsible for the increased mutation rate was designed to utilize the rate of occurrence of lethal rather than visible mutation as an index of the properties of a male. Fourteen *y Hw* F_3 6 males were crossed singly with *y; S*/In(2L)*Cy, Cy; H*/In(3R)C, *Sb* females. Three days later the males were crossed with six to eight *ClB/y Hw*, In(1) dl–49, m^2 g^4 females. As many X chromosomes as could be obtained from each male were tested for lethals. Ten of the 14 double matings were successful. In each of the *ClB* F_2 vials an average of 10 males was examined for the occurrence of new mutants.

The results of the experiment are summarized in table 5. A number of interesting facts are obvious. First of all, the rate of lethal mutation in these ten males—at most 0.19 ± 0.08 percent—appears to be entirely normal and significantly less than what it had been in this same strain two months earlier. A χ^2 test, with Yates's correction for continuity, yields a P of less than 0.01. Secondly, the rate of visible mutation in the parental flies appears to remain high, since four visibles were found in 3103 sets of chromosomes. Two of these were sex-linked recessives—another yellow-2 type, for which male 5 was a germinal mosaic, and a tiny bristle factor

TABLE 5

Summary of experiment 2. Explanation in text.

FLY NUMBER	NUMBER OF FERTILE *ClB* VIALS	SEX-LINKED LETHAL MUTATIONS	VISIBLE P_1 MUTATIONS	NUMBER OF F_2 FLIES EXAMINED (ESTIMATED)	VISIBLE F_1 MUTATIONS	STERILE OFF-TYPES IN F_2	MOSAICS OR DEVELOPMENTAL ACCIDENTS IN F_2
3	423	0	1	4230	0	0	2
4	112	1?	0	1110	0	0	0
5	183	1	1	1820	0	1	3
6	225	0	1	2250	0	4	1
9	137	0	0	1370	0	0	0
10	558	3	0	5550	0	3	3
11	212	0	0	2120	0	2	0
12	491	0	0	4910	2	3	4
13	419	0	1	4190	3+1?	1	3
14	343	1	0	3420	0	0	1
10	3,103	5+1?	4	30,970	5+1?	14	17

(1–19.5) found in male 3—and the other two were "poor" autosomal dominants—one characterized by extra anterior scutellar bristles, and the other by slightly small, wavy-surfaced wings, which were often carried at an angle with the body. The dominant autosomal mutations may have occurred in either the male or female parents. Thirdly, among the approximately 30,970 F_2 males examined, six mutants were encountered as single individuals in different vials. All of these were found in the progeny of two males. Male 12 yielded two autosomal dominant Minute mutations, one of which was localized to the third chromosome, while male 13 yielded two autosomal dominant Minutes, one second chromsome semi-dominant characterized by curly wings, and a vermilion allele (1–33.0). As previously, the possibility exists that the vermilion appeared as the result of a double cross-over involving the *ClB* inversion. The continued presence of *cm* and the non-appearance of t^2 in the strain again provide some evidence against such an origin. The problem of determining whether or not the observed distribution of mutation in the offspring of these ten males is random is a thorny issue. However, calculations based upon any one of several different methods suggest that the "clumping" of visible mutations in the descendants of males 12 and 13 is significant. Fourthly, the occurrence of visible mutations in the F_2 of certain males would suggest that if the factors involved were genetic, they were dominant.

The chromosomes of males 12 and 13 were saved for study of their properties, using a modification of the method described under experiment 1, which made it possible to secure some lines with a completely balanced

third chromosome. The attached-X chromosome used in this experiment was *y v f*. Table 6 shows the composition of the balanced strains estab-

TABLE 6

The rate of appearance of mutants in balanced stocks of the chromosomes of males 12 and 13 of experiment 2. Numbers in parentheses indicate the number of different lines of the composition indicated that were followed. The question mark after certain chromosomes indicates that these chromosomes may or may not have been present in the strain.

CHROMOSOMES PRESENT IN THE STOCK	NUMBER OF FLIES EXAMINED	FERTILE MUTANTS
I, II (12)	13,001	2
I?, II, III (4)	4,619	0
I?, II?, III (2)	1,441	4
I?, III (8)	10,203	7
	29,264	13

lished, the number of flies examined, and the mutations found. Among a total of 29,264 flies there were 13 different mutants. These include a hairy-2 allele (3–26.5), two forked alleles (1–56.7), a singed allele (1–21.0), a yellow-1 and two yellow-2 types (1–0.0), a third chromosome recessive characterized by plexate wings, a sex-linked rough eye, and four mutants due to as yet unlocalized factors, characterized respectively by plexate wings; short, blunt bristles; Minute-like bristles; and abnormal abdomen. Only two of the mutants were detected among the 13,001 offspring of flies with balanced first and second chromosomes, while among 16,263 offspring of flies with a balanced third, which might also have a first or second from that line, there were 11 mutants. The probability that these two series have the same mutation rates, as tested by χ^2 with Yates's correction for continuity, is less than 0.01. These results indicate a role of the third chromosome or, less probably, of a combination of the first and third, in the increased mutation rate. Since, moreover, that chromosome was carried in the heterozygous state in the bulk of the flies and since the strain was perpetuated by the heterozygous flies, the influence was apparently dominant.

It should be emphasized that the presence of the various genetic markers —*y*, *v*, and *f* in the attached-X females, and *Cy*, L^4, and *Mé* in the balancing chromosomes of both males and females, together with some other markers introduced to follow certain chromosomes—tended to interfere with the detection of mutants, not only because they rendered the detection of mutants more difficult, but also because they lowered the viability and fertility of the flies to such an extent that the occurrence of further mutations might make the fly inviable or sterile.

Experiments 3 and 4

Two further experiments were undertaken to get additional information on the rate of lethal mutation. At this time there were on hand some 50 sublines of the high mutation strain. Each generation, when these lines were transferred to fresh bottles, they were given a quick inspection, and a record was kept of any mutant individuals encountered. In this way two lines were isolated which seemed to be particularly "active." The first line (*y Hw* F_3 2) had the following history: On March 9, 1941, a female with a notched right wing was found during a count of the third generation of the high mutability strain. She bred as if heterozygous for a factor designated as Notch 264–130 (1–3.0). On May 4 the subline established from her yielded two flies with slightly plexate wings, heterozygous for a new semi-dominant factor, net^{41e4} (2–0.3). On July 31, during the course of a cross to localize this factor, a dumpy allele (2–13.0) was found, but it was not clear whether this had been present in the *y Hw* F_3 2 strain or had been introduced by the second strain involved. Likewise, on August 14, in the F_2 of an outcross made for the purpose of studying the salivary gland cell chromosomes of the net flies, one of the two strains involved was found heterozygous for a recessive autosomal factor characterized by rough eyes. On September 30 two new mutants were discovered in the same bottle. One of these, characterized by very small bristles, a tarsus-like antenna, and poor fertility, was found to be due to a recessive spineless-aristopedia allele (3–58.5). The other was the resultant of a new cut allele (1–20.0). Exclusive of the original Notch, there were thus three to five mutations in about 2,000 flies.

The second line (Or F_7 2) was derived from two male and three female flies with a medially-placed dark patch on the thoracic dorsum, found in the seventh generation of the count. The character was due to an autosomal recessive. On July 17 two males with small, rough eyes were found; they were due to a new sex-linked recessive factor which was also characterized by the complete sterility of the homozygous females. August 8 the strain yielded a yellow-2 type male, and on September 11 a male with garnet-like eyes, who was sterile. Finally, on September 30 a male with tiny bristles was discovered, the result of an autosomal recessive mutation. Exclusive of the original character, there were thus three to four mutants in about 1200 flies.

The results of *ClB* tests of these two strains are summarized in table 7. There were no lethals nor visibles in a total of 849 X chromosomes examined from the *y Hw* F_3 2 strain; among ca. 8490 F_2 males two F_1 mutants were found—a Minute due to a dominant factor in the second chromosome and a spread wings due to a sex-linked recessive. However, two of the P_1 males were heterozygous for a new recessive factor in the second chromo-

TABLE 7

Summary of experiments 3 and 4.

STRAIN	NUMBER OF X CHROMOSOMES TESTED	NUMBER OF MALE P_1	SEX-LINKED LETHAL MUTATIONS	FREQUENCY OF LETHALS (%)	NUMBER OF MALE P_1 GIVING LETHALS	NUMBER OF F^2 EXAMINED (ESTIMATED)	VISIBLE P_1 MUTATIONS	VISIBLE F_1 MUTATIONS
y Hw F_3 2	849	28	0	0.0	0	8,490	0	2
Or F_7 2	916	31	3	0.33±0.19	3	9,130	1	2+1?

some, resulting in contorted, "hooked" bristles, and a third male was heterozygous for a new third chromosomal recessive, resulting in wings with very scalloped edges. Since the *y Hw* F_3 2 strain was carried by small mass matings, resulting in rapid elimination or homozygosity of new factors, these two mutations had probably arisen at a relatively recent date. In the light of experiments 1 and 2 the results with this strain can most reasonably be explained in terms of the recent loss from the strain of the dominant factor thought to be responsible for the phenomenon.

The Or F_7 2 line yielded three lethals and one visible, a sex-linked recessive characterized by weak, uneven-surfaced wings and rotated genitalia, in a total of 916 X chromosomes. Among the approximately 9130 F_2 males inspected were three single mutants, a Minute due to an unlocalized autosomal dominant, a vermilion-2 type eye, and a vermilion-1 type eye (both 1–33.0). The latter might well have arisen by double crossing over involving the *ClB* inversion, since there was no check on such an event in this experiment. The same possibility does not hold for the vermilion-2, since it is phenotypically distinct from the vermilion carried within the inversion. This rate of mutation, although possibly high, was not thought sufficiently greater than normal to warrant further investigation.

THE NON-RANDOM DISTRIBUTION OF MUTANTS IN THE PROGENY OF CERTAIN FLIES

In previous sections, several cases of a possible non-random distribution of mutations in the offspring of certain flies have been described. A more significant example than any of these is the following: In the F_2 of a cross of a male with rough eyes and missing bristles, found in the second generation of the study, to normal females, the ratio indicative of a sex-linked mutation was secured. In establishing a pure stock of this, mutant males were crossed with their phenotypically normal sisters. Among the 384 offspring of this cross there appeared, in addition to the expected types, two new phenotypes, both represented by a single fly. The first was a male characterized by small, blistered wings and contorted, gnarled bristles, and was subsequently shown to be the result of a recessive mutation in the third

chromosome. The second was a female characterized by a deep, dull red eye. Crossed with her normal-eyed brothers, she produced 149 normal-eyed offspring and 32 with the aberrant eye color, as well as one female with an extreme type of plexus wings. The latter was sterile. Subsequent analysis showed that the eye color was due to a third chromosomal recessive, which later cropped out in several other lines. Males and females showing the new eye color, bred together to establish a homozygous stock, yielded in the next generation ca. 150 flies showing the trait in question, and in addition a yellow-1 mutant and a lozenge mutant (1–27.0).

Several more less striking cases of this nature could be added. But while the data are suggestive of a non-random distribution, they are by no means critical. In an extensive series of observations like the present a clumping of mutations is occasionally to be expected on the basis of chance alone. However, such a clumping is also exactly what would be expected if the factor responsible for the increased mutation rate was a dominant carried by a relatively few flies. No outbursts of mutation similar to the cases briefly described by GOLDSCHMIDT (1937), involving not only the simultaneous appearance in a strain of as many as four mutations but also the simultaneous disappearance of recessive markers, were observed.

THE LOCALIZATION OF THE MUTATIONS

One question which arises in the course of an investigation such as this concerns the distribution of the mutations. Are they scattered at random throughout the chromosomes, or is there a specific effect on certain regions? This question is most conveniently discussed in connection with the 42 new sex-linked factors which were analyzed to a point where they could be assigned a definite locus. These factors were as follows (the first figure in parentheses gives the number of times the mutation occurred; the second, the location): yellow (18) (0.0), multiple (1) (0.0), rough (1) (0.5), prune (1) (0.8), Notch (2) (3.0), carmine (1) (18.9), tiny bristle (1) (19.5), cut (2) (20.0), singed (3) (21.0), scruff (1) (22.0), lozenge (2) (27.0), vermilion (4) (33.0), oblique (1) (37.2), forked (2) (56.7), and bobbed (2) (66.0).

With three possible exceptions they appear to be distributed at random. These exceptions are (1) a high frequency of yellow mutations, also noted by DEMEREC (1937) and TINIAKOV (1939) in their work, (2) the absence of detected mutation at the white locus, although this is thought to be one of the most frequently mutating loci of Drosophila, and (3) the apparent grouping of mutations at about map position 20. Four of the yellow mutations were of the yellow-1 type, and 14 the yellow-2 variety. Except for the yellows, the concentration of mutations at the genetic tip of the X is consistent with the known normal distribution of Drosophola mutations.

DISCUSSION

The methods used in this study to detect visible mutations are undoubtedly of different relative efficiencies. There can be little doubt that the *ClB* method is the most effective when applied to certain types of visible P_1 mutations, that inbreeding of a strain is next in effectiveness, and that the attached-X method and the examination of the F_2 of a *ClB* cross for single mutants appearing as a result of mutation in the F_1 are relatively inefficient procedures. If, notwithstanding, we bring together all the results obtained by any method, we find that a total of 80–84 mutants were detected anong 189,827 flies, exclusive of the controls and exclusive of mutants picked up when exact quantitative records were not being kept. This corresponds to a rate of 43.2 per 100,000 flies and is 1.73 times the normal estimated rate of 25 per 100,000 flies. The total number of lethal mutations detected in the 7565 X chromosomes analyzed was 24–25 which represents a rate of 0.32 ± 0.06 percent. This is 1.8 times the normal rate of 0.18 ± 0.03 as summarized by SCHULTZ (1936). These represent the minimum possible estimates of the increase, since they are undoubtedly based in part on flies in which the factor responsible for the increase was not represented. If we exclude the results with Or F_8 1 (table 2), *y Hw* F_8 2 (table 7), and the balanced b11 line, on the grounds that the rate in these cases appears entirely normal or even, owing to the methods of detection, subnormal, the rate of visible mutation is 47.5 per 100,000 flies, and the rate of lethals, 0.43 ± 0.09 percent. This corresponds to an approximate twofold increase in the frequency of both lethals and visibles. This is still a minimum estimate, since an unknown fraction of the flies within these groups carried the factor responsible for the increase. Thus, in experiments 1 and 2 (tables 3 and 5), out of a total of 23 males tested from a strain showing an appreciably high mutation rate, not more than four or five showed evidence of throwing mutations in unusually high numbers.

The behavior of strains established from three of these flies, and carrying various combinations of their chromosomes, as summarized in tables 4 and 6, indicates that a dominant influence associated with the third chromosome of these males, or perhaps a combination of the first and third, is at the basis of the phenomenon. The combined data of the two tables show that in 18,012 flies whose parents carried a first and second chromosome derived from some one of the three "mutating" males, only two mutations were found, while in 20,729 offspring of parents which either certainly or possibly carried a third from these males, as well as a first and/or second, there were 13 mutations. The difference between these two series, as measured by χ^2, with Yates's correction for continuity, is highly significant. The difficulty of detection of mutations in these balanced strains

is such as to suggest that the rate observed in the second group (62.8 per 100,000) is actually a considerable underestimate of the true rate. Moreover, since the factor is behaving as a dominant and since only a relatively few males appear to carry it, the founder males were probably heterozygous, and in that case only half of the stocks balanced for the third chromosome might be expected to carry the factor. A wild type strain heterozygous for the factor should therefore mutate at approximately four to five times the normal rate.

In the analysis of the results obtained with the balanced lines, the assumption is implicit that the various chromosomes introduced as balancers, or carrying marker genes, themselves had no effect on the mutation rate. There is no reason to doubt the validity of this assumption.

The above interpretation encounters one signal difficulty. How can the significant decrease in the frequency of lethal mutation in the *y Hw* F_3 6 line, from 1.01 ± 0.28 percent to 0.19 ± 0.08 percent in three generations, be explained? If the increase was due to a dominant factor which was certainly not homozygous in the strain and which may be unstable, chance alone might be sufficient to account for the difference between the results of the two tests. Even so, if males 12 and 13 carried a factor increasing the mutation rate by a factor of four, then among the 910 X chromosomes from these two males tested for lethals, there should have been six, instead of none. This point remains the chief complication in the present interpretation of the results.

The estimate of the normal frequency of detection of visible mutation adopted in this work is so much higher than the generally published data that it is certain to elicit doubt. Only further independent work by competent investigators can settle this question. If the lower estimates of the frequency of visibles be accepted, then in the case here analyzed we are forced to assume a considerable increase in the frequency of visibles without a corresponding increase in the frequency of lethals.

GOLDSCHMIDT (1939) has vigorously espoused the role of chromosomal rearrangements, as leading to further rearrangements which are detected through the mutant phenotypes they produce, in such periods of high mutation rate. Both VALADARES (1937) and GOLDSCHMIDT (1939) report the presence of an inversion within their strains. However, it must be emphasized that inversions are sufficiently widespread in Drosophila stocks that any such claims to be accepted must be accompanied by a rigorous demonstration of a causal relationship between rearrangement and mutation, and such a demonstration is thus far lacking. MISS JEAN LANE, of the Department of Genetics, CARNEGIE INSTITUTION OF WASHINGTON, very kindly examined the salivary gland cell chromosomes of the *y Hw* F_3 6 line shortly after the termination of the experiment summarized

in table 2 and was able to detect no gross aberration. The only possible departure from normal observed was a slightly exaggerated tendency toward asynapsis. Moreover, in none of the linkage experiments localizing the various mutations encountered was any evidence of rearrangement found. DR. E. SUTTON found one of the Notch mutations to be characterized by the loss of the 3C7 band from salivary gland cell chromosomes but was unable to detect any abnormality in the chromosomes of the five other sex-linked mutations which she kindly analyzed.

The factor responsible for the present outburst is obviously different from that found by DEMEREC (1937). There are therefore at least two factors, one associated with the second chromosome and the other with the third, which exert a profound effect upon mutation rate in Drosophila. Such factors as these may without exaggeration be regarded as pacemakers of evolution, since by controlling the mutation rate they indirectly control the maximum rate at which a species may change.

As GOLDSCHMIDT (1937) has pointed out, the usual design of Drosophila work militates strongly against detecting such cases of high mutation rate. Further investigation may show that a very considerable portion of the mutation occurring in nature comes in such spurts, resulting in the formation of localized populations of relatively high genetic heterogeneity. Some evidence of this has already been obtained by SPENCER (1942). Should this be the case, then here is a mechanism which may rank in importance with geographical isolation in the process of species differentiation, and one which, in any event, offers a valuable supplement to isolation.

SUMMARY

A case of high mutation frequency in an inbred Oregon wild type line of *Drosophila melanogaster* is described and analyzed. From data based on approximately 208,000 control and experimental flies it is concluded that:

(a) The normal rate of detection of new mutants is about 25 per 100,000 flies under conditions of moderate inbreeding.
(b) In this particular case there was a two to fourfold increase in the frequency of occurrence of sex-linked lethal and sex-linked and autosomal visible mutation.
(c) A dominant influence of the third chromosome, or, less probably, of a combination of the first and third chromosomes, was responsible for the increase.

LITERATURE CITED

BUCHMANN, W., and N. W. TIMOFEEFF-RESSOVSKY, 1935 Über die Wirkung der Temperatur auf den Mutationsprozess bei *Drosophila melanogaster*. II. Behandlung der Männchen mit Temperaturschocks. Z.i.A.V. **70**: 130–137.

DEMEREC, M., 1937 Frequency of spontaneous mutations in certain stocks of *Drosophila melanogaster*. Genetics **22**: 469–478.

DUNCAN, F. N., 1915 An attempt to produce mutations through hybridization. Amer. Nat. **49**: 575–582.

GOLDSCHMIDT, R., 1937 Spontaneous chromatin rearrangements and the theory of the gene. Proc. Nat. Acad. Sci., Wash. **23**: 621–623.

1939 Mass mutation in the Florida stock of *Drosophila melanogaster*. Amer. Nat. **73**: 547–559.

GROSSMAN, E. F., and T. SMITH, 1933 Genic modifications in Drosophila induced by heat irradiation. Amer. Nat. **67**: 429–436.

JOLLOS, V., 1934 Inherited changes produced by heat treatment in *Drosophila melanogaster*. Genetica **16**: 476–494.

MOORE, W. G., 1934 A comparison of the frequencies of visible mutation produced by X-ray treatment in different developmental stages of Drosophila. Genetics **19**: 209–222.

MORGAN, T. H., 1914 The failure of ether to produce mutations in Drosophila. Amer. Nat. **48**: 705–711.

MULLER, H. J., 1928 The measurement of gene mutation rate in Drosophila, its high variability, and its dependence upon temperature. Genetics **13**: 279–357.

MULLER, H. J., and E. ALTENBURG, 1919 The rate of change of hereditary factors in Drosophila. Proc. Soc. Exp. Biol. N. Y. **17**: 10–14.

NEEL, J. V., 1942 A case of high mutation frequency in *Drosophila melanogaster*. Genetics **27**: 158–159.

PLOUGH, H. H., 1941 Spontaneous mutability in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 127–137.

PLOUGH, H. H., and C. F. HOLTHAUSEN, 1937 A case of high mutation frequency without environmental change. Amer. Nat. **71**: 185–187.

PLOUGH, H. H., and P. T. IVES, 1935 Induction of mutation by high temperature in Drosophila. Genetics **20**: 42–69.

SCHULTZ, J., 1936 Radiation and the study of mutation in animals. Biological Effects of Radiation (B. M. DUGGAR, editor), vol. **2**, 1209–1261.

SPENCER, W. P., 1935 The non-random nature of visible mutations in Drosophila. Amer. Nat. **69**: 223–238.

1942 Are natural mutations in wild Drosophila distributed in a Poisson series? Genetics **27**: 171.

TINIAKOV, G., 1939 Highly mutable stock from a wild population of *Drosophila melanogaster*. C. R. DOKLADY Acad. Sci. U.R.S.S. **22**: 609–612.

VALADARES, M., 1937 Declanchement d'une haute mutabilité chez une lignée pure de *Drosophila melanogaster*. Rev. Agron. Lisbon **25**: 363–383.