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THE IMPORTANCE OF MUTATION RATE GENES IN EVOLUTION

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INTRODUCTION

One of the problems of longest interest in genetics and evolution is the spontaneous appearance of gene changes, mutations. It is only within the past thirteen years that it has been clearly shown that the rate of spontaneous mutation depends in part at least upon the genotype of the individual and that certain individual genes are themselves capable of causing marked changes in this rate. (See particularly Demerec, 1927 a, b; Neel, 1942; Mampell, 1943; Ives, 1945.)

The mechanism by which mutagenic agents work—whether they be physical, chemical or genetic agents—is still essentially unknown. It is important, therefore, to report studies on any level showing the kinds and quantities of mutations produced by a given agent under specified conditions. It is particularly interesting to report such studies on a genetic agent derived directly from a natural population. Presumably this type of agent is a major source, possibly *the* major contributor, of new mutations in wild populations. Students of genetics generally recognize that such an agent may play a role of exceptional importance in organic evolution.

The origin of the mutator under consideration and some preliminary studies on it have been presented in Ives (1945). Other studies have appeared in abstract form in Ives (1943, 1947, 1949) and in Ives and Andrews (1946). The major aim of these studies and the present report is to show something of what the gene in question does genetically and to discuss the evolutionary significance of these observations. In two of the earlier studies on mutators the genes were lost within a year or two after their first discovery, for reasons not clearly demonstrated. It seemed more valuable, therefore, to attack the mutagenic properties of the present mutator at once rather than to determine the exact chromosome locus involved. As it turns out, since the gene's mutagenic properties are so complex and since only by its ability to raise the mutation rate is the gene phenotypically recognizable, it will be a task of considerable difficulty to determine even roughly its locus. However, since the gene is now successfully balanced with marked chromosomes, the problem of its exact locus is of minor importance.

This mutator gene has been named *high* (hi). The studies completed so far with *hi* have been particularly designed to show what *hi* could contribute genetically to a given wild population of *Drosophila melanogaster,* as regards gene mutations and chromosome rearrangements, under different controlled genetic and environmental conditions. Specifically, they have measured the rate and variety of second chromosome lethal mutations, of X-chromosome lethal and easily noticeable visible mutations, and of X-chromosome inversions when these are associated with sex-linked lethal or visible mutations. In some of these experiments *hi* was homozygous; in others, heterozygous. In some cases the mutations arose in sperm deposited by young males; in others, in sperm deposited by males aged up to four weeks.

Taken together these studies show the following facts. First, *hi* affects the mutation rate of many genes, but not all of them with equal frequency. It increases the general mutation rate up to ten times the normal rate. Second, whether it acts as a dominant or a recessive appears to

depend on the genotype within which it is acting. Third, in some lines its effect on the mutation rate during spermatogenesis falls off rapidly to, or close to, the normal mutation rate as the males grow old; while in other lines there is no such decrease. Finally, it produces chromosomal rearrangements with measurable frequency.

The Origin of the Mutator

In the summer of 1943 ten lines of wild flies were tested for their second chromosome lethal mutation rate by the use of *Bl/Cy sp*^{2} marker stock. Each of these lines was homozygous for a second chromosome collected in Florida in April and contained their other chromosomes in non-regulated proportions from the Florida wild population and the marker stock. The history of these lines, the method of genetic analysis and a summary of the results obtained were included in Ives (1945).

The detailed data from these tests are presented in table 1. The line number refers to the number of the Florida wild male from which the second chromosome of the line was derived. Chi-square tests of the variance of these samples in various combinations shows the following results, expressed in terms of p, the probability of getting as bad a fit, or worse, to a perfect distribution as a result of random sampling. Considering all ten lines together, p is less than .0001, an extremely unlikely random result. When the highest line, 30, is omitted and the remaining nine lines are tried together, p increases to .14, which is not an unreasonable random result. However, the total chi-square in this case is 12.36 and 9.17 of that total is contributed by line 73. This indicates that line 73 does not fit in well with the other eight lines. When the other eight lines are tried together, p rises to .91, an exceptionally close fit to an even distribution. These eight lines are interpreted as representing one mutation rate of .87 per cent. When lines 30 and 73 are tried together, p is .06, not a good fit. It was also found that line 73 was homozygous for the second chromosome inversion, In(2R)NS, while line 30 carried the normal gene sequence. It is probable, therefore, that 30 and 73 do not carry the same mutator. In any case there can be no doubt that line 30 is clearly a high mutation rate line. All subsequent work has been done with this line, the bearer of *hi*.

Table 1. *Chromosome 2 mutation rate in 10 Florida lines*

Line No.	Tests	Lethals	%
21	247	1	0.40
36	371	4	1.08
41	53	0	0.00
43	215	2	0.93
45	87	0	0.00
54	98	1	1.02
68	255	3	1.18
70	161	2	1.24
Total	1,487	13	0.87
73	235	8	3.40
30	349	25	7.16
All	2,071	46	2.22

Evidence that *high* is in the Second Chromosome

The data already presented suggest strongly that *hi* is a second-chromosome gene, since the second chromosome is the only one by which the ten lines differed in regulated proportions and since there was no significant variation in distribution of the lethals in the families of tests comprising the data of line 30 in table 1. Additional evidence of the same kind came out of testing the effects of *hi* on the sex-linked mutation rate. In the first experiment 7 lethals appeared in 657 tests (Ives, 1945, table 8, item 5), which is as much above the normal rate as the second chromosome rate noted above. A year later, however, only 5 lethals appeared in 1,724 tests of the line 30 stock. The value of p in the comparison of these two samples of data is only .017, a very poor fit, indicating a strong probability that

something had happened to the line 30 stock, possibly a contamination from another wild-phenotype stock in the laboratory.

There remained from the earlier work with line 30 a number of balanced *lethal/Curly* (*le/Cy*) stocks. In each of these lines the lethal chromosome was a descendant of the original line 30 and should also carry *hi*. A cross of two different *le/Cy* lines should then give non-*Cy* flies which were homozygous for *hi*. The second chromosome constitution of these flies should be

$$\frac{le_a\ hi\ +}{+\ hi\ le_b},$$

with subscripts *a* and *b* indicating that the lethals are different loci.

Several such crosses have been made in the course of subsequent experiments. The results indicated the presence of *hi* in the *le* chromosome each time. In a number of instances *le hi/Cy* stocks have grown progressively weaker in viability and fecundity and had to be crossed to other *Cy* stocks for strengthening. In each case the recovered and rejuvenated *le hi/Cy* lines proved to be carriers still of *hi*, as evidenced by a high sex-linked mutation rate. All of the remaining data to be presented in this report have come from such crosses of recovered lines.

In the light of the above evidence there can be little doubt that *hi* is a second chromosome gene. It probably originated in the wild population of Winter Park, Florida, and was transferred from there to the balanced stocks now carrying it. It would require a most extraordinary set of coincidences for it to be in any of the other three chromosomes and still have come through in either balanced or homozygous condition in these stocks.

Methods of Genetic Analysis of the X-Chromosome

Early in the experiments with sex-linked mutation rates it was found that the use of the standard *ClB* technique was impractical because of the presence of a very high proportion of XXY females in our several *ClB* stocks. (Later analysis showed that nearly 50 per cent of the females were of this constitution.) A lucky crossover in our stock of *y ct ras f/ClB* made possible the complete control of this situation. The crossover female was *y ct ras f/ct ras ClB*, hence *ct ras B* in appearance. Later this new chromosome was reduced to *ct ClB* by another crossover. Since this form proved to be more viable and fecund, it was chosen for experimental use.

The essential feature of the new *ClB* stock is the sharing of a common marker gene, *cut* wings, by the two marked chromosomes. Using Oregon-R standard wild stock as a source of normal X-chromosomes, two complementary stocks were established, normal males × *y ct ras f/ct ClB* and *y ct ras f* males × normal/*ct ClB* females. Regular males and *ClB* females are used to continue the stocks. Non-disjunction females—in appearance, *ct B* in the F_1 of the first cross and *B* in the second—can be eliminated at once. They can be similarly eliminated when experimental males are mated to the double-marker females. One could, of course, substitute a third marked chromosome, not carrying *ct*, for the normal one in these stocks and insure himself against the undetected accidental use of non-virgin females in his experimental crosses. In the present case it has proven more advantageous to outcross the marker stock to Oregon-R from time to time to improve the fecundity of the marker stock.

The following method of matings has been used in these tests. Males of the desired constitution with respect to *hi* or its normal allele were mated individually to *y ct ras f/ct ClB* females. The F_1 *ClB* (not *ct*) females, carrying the testing X-chromosomes, were mated to their *y ct ras f* brothers. It was found that if these flies were mated at once in cultures containing a single female and two males each, an annoying amount of sterility, often more than 20 per cent, developed in these test vials. However, if groups of

30 to 35 females were mated with equal numbers of males for two or three days and then put into cultures of one pair each, the sterility was generally less than five per cent. These matings were done in shell vials.

The offspring of the *ClB* test matings were examined under a binocular dissecting microscope without anesthesia. In cases where there were no males, or where the males were obviously abnormal, non-*ClB* females were mated to *y ct ras f* stock males and the locus of the lethal or visible mutation was determined. In a few cases an apparent lethal by *ClB* test proved in larger crossover counts to be either semi-lethal or normal. In such cases, where the chromosome gave a viability of more than five per cent, it was reclassed as non-lethal.

Visible mutants and lethals were grouped together for mutation rate determination. There were approximately eight times as many lethals as visibles, even when including among the visibles some strong semi-lethals which happened to have phenotypic effects. In the tables of data all of these are grouped under lethals.

When it was desired to make more tests of an experimental male than his *ClB* offspring made possible, the sibling non-*ClB* females were also tested in similar manner. Offspring from these tests were normally wild type, *y ct ras f*, or any of the possible crossover types. After some practice I was able to class these tests, too, for lethals and visibles without anesthetizing the flies. The trick seems to be to "photograph" a moving male fly in one's eye and then examine the photograph for the presence or absence of the marker genes. Only *forked* of the four genes used presents difficulty. The most likely loss of lethals through classification error is that group lying several units to the right of *f*. Actually, the proportion of such lethals found in normal/*y ct ras f* tests was as great as in the *ClB* tests. In all doubtful cases the flies were anesthetized for final check. While the classing of normal/*y ct ras f* tests was much slower than the *ClB* tests it was possible to class them much more rapidly without anesthesia. Their use made it possible to secure significant data in many cases that would otherwise have been lost, particularly cases of aged males with greatly reduced fecundity.

Effects of Increasing Age of the Male on the Mutation Rate

In the fall of 1944 experiments were begun to determine the mutation rate manifested in sperm deposited by homozygous *hi* males during the course of their first four weeks of life. Since none of males used in preliminary tests deposited significant numbers of active sperm in the fifth week, these tests cover essentially the lifetime production of *hi* males.

In these experiments the males were raised at 25° C. and mated individually within 24 hours after emergence to two or three marker females. Each male was transferred to a new set of females twice weekly without anesthesia. (The normal male had much better phototropism as well as greater flying ability than the *ct ClB* females.) At the time of his transfer the females were shaken to a new vial to increase their productivity.

Four different *le hi/Cy* stocks were used to produce *hi/hi* males for these experiments. The rest of the 25 stocks did not give sufficiently viable flies in such crosses to be useful. The stocks used were numbers 1, 5, 14 and 29. The crosses proving useful for these tests were 1 × 5, 5 × 14, 5 × 29 and 14 × 29. The most satisfactory of these over several years of time were males of 5/14 and 14/29 constitution. Not all of the combinations were tested at one time, but each one was tested in at least two groups of males.

The experiment was carried on during four two to three months periods in three different years. The results of the different series were consistently alike. They have been pooled for consideration in this report.

The data from analysis of 1/5, 5/29 and 14/29 males indicated that *hi* had similar effects in each set of males. Their pooled totals are presented in table 2A. This table shows the number of completed tests on spermatozoa deposited in each of four consecutive weeks by 27 individually mated males all of whom are represented in each week's tests. These 27 males represented 10 of 1/5, 11 of 5/29 and 6 of 14/29.

A distinction is made between new and duplicate lethals. Whenever a crossover test of two or more lethals from the sperm of one male indicated that they were in apparently the same locus they were considered to represent but a single mutation. They were entered in the column of new lethals as one mutation in the week in which the first one was recovered. The one or more duplicates were entered in the duplicate column in the appropriate week or weeks. Thus the total number of lethal chromosomes recovered is the sum of the new and duplicate columns in each week. The total per cent of recovered lethals is calculated from this combined total. Most of the information concerning activity of *hi*, however, is found in the third column of table 2, per cent of new lethals recovered, since this is directly related to the number of loci which had mutated.

TABLE 2. *Sex-linked mutation rate in sperm deposited during successive weeks after emergence*

A. 27 Males of Combinations 1/5, 5/29 and 14/29

Week	Tests	New le's	%	Dup. le's	Total %
1	1,783	23	1.29	5	1.57
2	1,697	10	0.59	6	0.94
3	1,977	6	0.30	1	0.35
4	1,555	4	0.26	2	0.39
Total	7,012	43	0.61	14	0.81

B. 9 Males of Combination 5/14

Week	Tests	New le's	%	Dup. le's	Total %
1	631	4	0.63	0	0.63
2	642	10	1.56	2	1.87
3	884	11	1.24	2	1.47
4	886	12	1.35	9	2.37
Total	3,043	37	1.22	13	1.64

C. Male No. 32, Combination 5/14

Week	Tests	le Chrom's	%	Corrected B %
1	82	3	3.7	.70
2	122	10	8.2	1.31
3	48	4	8.3	1.18
4	37	2	5.4	1.30
Total	289	19	6.6	1.14

The data of table 2A show that in the combinations represented here the mutation rate falls off rapidly week by week, from a high of 1.29 per cent in the first week to a low of 0.26 per cent in the fourth week, the rate in the third week being essentially the same as that in the fourth. The number of duplicate lethals recovered also decreased sharply in the third and fourth weeks.

Since the sperm of a given male were by no means exhaustively tested the presence of even a few duplicate mutations is enough to indicate that *hi* produced its mutations principally in the spermatagonial cells or their progenitors in the males represented in table 2A. The duplicate lethals represent several loci in different males, apparently at random.

The combination of 5/14 produced quite different results. Data from nine males of this type are shown in table 2B. In this case the homozygous *hi* continued its effect throughout the normal period of spermatogenesis at a rate (note the total rate for the four weeks together) very close to that evident in the first week sperm of males represented in table 2A. If anything, the rate was higher in the last three weeks than in the first week; and the highest proportion of duplicate lethals appeared in the fourth week.

One male, number 32, of combination 5/14, was quite exceptional to the other 36 males tested. The data from this male are presented in table 2C. He produced an exceptionally high frequency of lethal

chromosomes, all of the lethals being in apparently the same locus. From the total of 6.6 per cent lethals it seems probable that this mutation took place early enough so that roughly one-eighth of the spermatagonia of one testis were derived from the mutant cell. It did not seem right to include these duplicates in those of table 2B; but the tests of this male should be added to those of that table for rate of new lethals, these lethals representing one more new mutation recovered in week 1. The last column of table 2C shows the percentage of new lethals in the combined data of tables 2B and 2C. It can be seen that the percentage of new mutations in the total of four weeks, 3,332 tests, is reduced to 1.14, still very close to the 1.29 per cent observed in the first week in the males of table 2A.

No large scale attempt was made to check the mutation rate in non-*hi* males under conditions similar to those obtaining in the experiments recorded above. However, the following stocks were constructed and used in a small scale control test. Line 43 of the stocks recorded in table 1 was chosen as a representative low or normal mutation rate stock. At the beginning this stock was homozygous for its second chromosome and contained not more than four different third chromosomes. Its two lethal sublines, 47 and 60, each *le/Cy,* were crossed in the same way that the *hi* line lethals were crossed. The resultant 47/60 males, homozygous non-*hi,* were then tested in the same manner as the above males for X-chromosome mutation rate, 8 males over a four weeks' period. The tests totaled 928, fairly evenly divided among the four weeks. Two lethals were found, one in sperm of the first week and the other in the fourth week, showing only that the lethal mutation rate is comparatively low in this combination.

Professor H. J. Muller was carrying on experiments with similar purpose at that time in this laboratory (Muller, 1946 and unpublished). It was intended that his results be considered in part a control for the present experiments. However, his methods were somewhat different and he tested X-chromosomes which were not normal, phenotypically and with respect to viability, so that it is not proper to compare the actual mutation rates obtained in the two sets of experiments. His rate in the first week of sperm production was higher than that found in the 47/60 data to be presented later in this report, possibly due to additive effects of new semi-lethal mutations with the lowered viability already present in his test chromosomes.

In Muller's tests of a low mutation rate stock there was a distinct drop in mutation rate in sperm deposited after the first week of activity, a drop which is similar in principle at least to that which appears in table 2A of this report. Zuitin (1941) has reported a similar mutation rate decline with increasing age in his studies on wild-type males derived from each of two natural *Drosophila* populations in Russia. Therefore, the data of table 2B and 2C together are more abnormal than those of table 2A. The males of 5/14 combination are less like normal males than are males of the other crosses.

Dominant Effect of *hi*

Experiments were also planned to test the dominant effect of *hi.* In order to simplify the procedure, tests were made of normal/*hi* which were non-*Cy*. This also made it possible to test the effects of heterozygous chromosomes of recent origin from natural populations.

Control tests of normal (+/+) consisted of earlier data from males of the original Florida 43 stock and from concurrent tests involving males of lethal sublines 47/60. Control tests of *hi/hi* consisted of data gathered from crosses of subline 29 to other subline *le-Cy* stocks derived from the original line 30, principally to subline 14. The *hi*/+ data came from crosses of 29 to 47, 60, 68 and 72. The last two sublines are *le/Cy* stocks isolated from the Florida 43 stock after it had been bred in bottle cultures

for some 70 generations of about 100 pair matings. For one series of tests, lines 29 and 60 were made homozygous for their third chromosomes by means of a *Cy; DcxF* stock, these homozygous lines being noted as 29–1 and 60–4, respectively. Lines 68 and 72 were also homozygous for their third chromosomes. Extensive tests of *hi/hi* were run at approximately six-month intervals to make sure that significant changes did not occur in the *hi* chromosome of line 29.

Because of the effects of ageing on the mutation rate, only sperm deposited in the first ten days were used. Generally, sperm deposited in the first week were enough for the number of tests desired. However, the development of these males was slower in all series than in the previous tests in spite of no obvious change in technique or conditions. Calculated from the time of egg laying, these males were from two to four days older at emergence than were those of earlier experiments. Their sperm in the first ten days may, therefore, have been more nearly equivalent in developmental age to the sperm of the first two weeks in earlier experiments. This may account for the lower mutation rate in *hi/hi* control tests in these experiments on dominant effect of *hi*. Strict care was taken to make sure that *hi*/+ males were not different in age from their control males of both types. All of them were raised under similar environmental conditions.

The results of these tests are presented in table 3. The +/+ data are separated into two groups, those coming from tests of the Florida 43 stock itself and those from males of the 47/60 combination. There is obviously no difference between the two rates. Together they show a rate very close to one lethal per thousand tests. At the other extreme are the data from *hi/hi* which show a rate close to one lethal per hundred tests, ten times as high, approximately.

The *hi/hi* data were tested for consistency by dividing the total into eight groups of tests numbering between 969 and 1,758 tests per group, in chronological order. That the variation in mutation rates between groups was random is indicated by the p value of 0.68 for their distribution around their mean. That there

TABLE 3. *Mutation rates in hi/+ crosses*

Cross	Lines	Tests	Lethals	%	Item No.
Control +/+	Fla. $	4,618	5	.108	1
	47/60	7,100	7	.099	2
	Total	11,718	12	.102	3
Control *hi/hi*	29/others	10,323	100	.969	4
hi/+	29/75	3,337	7	.210	5
	29/68	4,334	10	.231	6
	Total	7,671	17	.222	7
	29/47	9,206	34	.369	8
	Total	16,877	51	.302	9
	29/60	4,234	33	.779	10
	29–1/60–4	5,917	39	.659	11
	Total	10,151	72	.709	12
hi/+	Grand Total	27,028	123	.455	13

was no gradual shift in rate with time is indicated by the terminal rates of 1.03 per cent in Nov. 1944 and 1.00 in May 1948. The extremes were 0.64 and 1.34 in the two smallest groups, in the spring and fall of 1945.

The results from the *hi*/+ crosses, items 5–13 of table 3, show marked differences between crosses. The results within each cross were, however, consistent. In crosses of 29 by 68 and 75 the mutation rate in *hi*/+ was at most twice the +/+ rate. Neither line is strongly above the control statistically, the *p* values being .13 for 75 and .050 for 68. Their combined total, item 7, is significantly different from the control with a *p* value of .038. The mutation rate in 29/47 flies, item 8, is well above the control +/+, the value of *p* being less than .0001. It is not clearly above the rates of items 5, 6 and 7. When the three crosses are tried together the value of *p* is .22. When the comparison is between the totals, items 7 and 8, the value of *p* drops to .080, suggesting that line 47 did produce a slightly higher rate. The difference is not statistically certain, however. The combined total of these three crosses (item 9) is statistically above the control, the value of *p* being .0004. If one considers these three groups of data (items 5, 6, and 8) as essentially similar, it is clear that they represent a mutation rate above that of the control +/+ tests.

In the tests of *hi*/+ in crosses 29 by 60 and 29–1 by 60–4 (items 10 and 11) the mutation rates are well above the control +/+ rate, the values of *p* in each case being far below .0001. A *p* value of .42 indicates that there is no difference between these two rates. A comparison of their total (item 12) with *hi/hi* (item 4) gives a *p* value of .045, a significant though not compelling difference. The other *hi*/+ rates in this table are all statistically far below the *hi/hi* rate.

This table of data shows at least two different *hi*/+ mutation rates, one near the +/+ rate and the other near the *hi/hi* rate. There is a suggestion that the lower of these two may actually represent two different rates. At most, the data show rates in *hi*/+ that are 2, 4 and 7 times the control rate; at least, they show rates that are 3 and 7 times the normal mutation rate. The rate in *hi/hi* is indicated as 10 times the normal rate.

In the data resulting from the tests of *hi/hi* and *hi*/+ there were many instances in which one male produced more than one mutant locus in the sample of X-chromosomes tested in his offspring. The occurrence of families of 0, 1, 2 and more lethal loci per family of tested chromosomes should follow a Poisson series if all the males of any one cross produce mutations at the same rate. If some produce at a rate much higher than others, however, the result will not be a Poisson series. Thus, a test for goodness of fit to a Poisson series should indicate if there is marked variability among the males in each cross represented in table 3.

Most of the males tested contributed between 50 and 100 tests to the data. Those few which contributed more than 100 tests were divided into two equal families for the sake of this analysis. Those contributing less than 50 tests were added together consecutively as they appeared in the records to form families of between 50 and 100 tests per group, each group being considered one family of data. When these family limits were strictly followed, it was found that in every cross the number of families with 0, 1, 2 or more lethals was statistically not different from a Poisson series. The fact that most of the values of *p* lay between .3 and .1 is probably due to the size spread of the families. Actually, family size should be the same, not variable between such limits as used here, when employing a Poisson series test. Otherwise, as in the present instance, one underestimates the value of *p*. Accordingly, the *p* values found in the present case may be considered very satisfactory in indicating a Poisson distribution of lethals. This, in turn, indicates that the males within each cross produced mutations at similar rates.

THE EFFECTS OF *hi* ON DIFFERENT LOCI

It is important to know if the effects of *hi* are upon a few genes specifically or if it causes mutations in many different genes. The first experiments shedding light on this question tested the effect of *hi* on the production of second chromosome lethals. It is a simple matter to determine the relationship of such lethals to each other by crosstesting. This was done for a total of 27 lethals which occurred in these tests. The crosstests showed that there had been mutations in 25 loci, two of which had mutated twice each. Data on recurrent lethal mutation rates in normal mutation rate stocks (Ives, 1945) indicated a probability of obtaining in a sample of this size one duplicate lethal due to a second mutation of a locus. It was, therefore, concluded that the effect of *hi* was random in this sample of mutated genes.

Since that first experiment, however, data have come out of the experiments with sex-linked mutations which are not entirely in line with such a simple picture. These data pertain to the occasional visible mutations which occurred in these experiments. Because of the author's own experiences in finding a large proportion of *yellow* alleles among the mutants appearing spontaneously, particularly in the hundreds of *le/Cy* stocks worked with in the past dozen years, it was expected that a large proportion of visibles found in these experiments would be *yellow* alleles. Both Demerec and Neel have reported a high proportion of *yellow* alleles in their studies. As the number of visible mutations accumulated, however, it became obvious that *yellow* alleles were actually rare among the visibles produced by *hi*. Finally, in order to get more accurate data for comparison, records were kept on all sex-linked mutations occurring in about 140 *le/Cy* stocks during the period September, 1948, through June, 1949. As in the *hi* tests, only easily seen visibles were recorded and tested. These included genes affecting eye color, body color, wing size and shape, and bristle size and shape. The stocks contained lethals isolated from the local wild population in the summer of 1948.

The flies in whose germ cells the mutations occurred in the *hi* and *le/Cy* series differed from each other chiefly in their second chromosomes. In the *hi* tests these were both of Florida origin, carried a lethal, and either one or both of them carried *hi*. In the *le/Cy* stocks one second chromosome was *Cy*, with its lethal effect and two inversions; the other was a lethal-bearing chromosome with unknown other factors present, only a small proportion of these chromosomes being closely related to each other. The third and fourth chromosomes were of inbred Florida stock origin in the *hi* series; while in the *le/Cy* stocks, they were on the average three-quarters of this type and one-quarter of local wild population origin. The X-chromosomes, in which the mutations actually took place, were the same in both series and were of inbred Florida stock origin. Inasmuch as *Cy* was not a factor in producing *yellow* alleles in the experiments of Demerec and Neel, the major contrast in these two series is between the effects of *hi* on the one hand and of a random sample of second chromosome wild-population dominant genes on the other. These latter represent an estimate of what could be expected to happen in the highly heterozygous wild population from which they came and are accordingly called controls. An estimated 70,000 males were examined in these control tests.

The data from these two series of tests are summarized in table 4. The most striking difference between the two series is the presence of many *yellow* alleles in the control group, each one of which occurred in a different *le/Cy* stock. Only one of 42 visible mutations in the *hi* tests was a *yellow* allele; 19 of 37 mutations noted in the *le/Cy* stocks were *yellow* alleles. The sterile mutants in the control group are included because of the

strong bilateral symmetry of the phenotypes. Most of these six cases also occurred in more than one male in their respective stocks. Some of the *hi* visibles were known to be male-sterile; but not all of them were tested for this characteristic, since the necessary data on locus and allelism could be obtained from heterozygous females. With the exception of *yellow,* no locus was found certainly more than twice in the controls. One of the steriles may have been a third occurrence of *forked.* It looked more like *singed,* but there is no absolute difference between these two phenotypes. Of the three mutants occurring twice in the controls, two —*lz* and *w*—occurred once in the *hi* series. Found once in each series was *wy.*

The data on *folded* (*fo*) wing are of special interest. None was found in the controls. More than that, I have tested seemingly dozens of *folded*-like flies in the past 18 years without once finding an allele of folded—until these in the *hi* series appeared. It does not seem to be a locus with a mutation rate comparable to that of *y, w, pn, sn, lz, ras, m, v, wy, g* or *f,* each of which has appeared several times in this laboratory. Yet, here *fo* appeared four times (in unrelated sperm) in tests of *hi*/+.

The general conclusion from this table of data is that certainly the frequency of mutations at the *yellow* locus, and very probably that at the *folded* locus, are different in the two series of tests. The mutation rate of the *y* locus seems to be either not affected at all, or else actually decelerated, by *hi;* while that of the *fo* locus seems to be accelerated more than the others.

A comparison of the data on sex-linked visibles and second chromosome lethals in tests of *hi* shows that in both instances many different genes were affected. No such case as *y* could be expected to appear in the second chromosome lethal tests; and the data on *fo* stand out because of the complete lack of *fo* alleles in previous mutation work, not because of any statistical difference (which is nil) between its rate of mutation in this experiment and the mutation rate of the two recurrent second chromosome lethals. Thus it can be seen that the data on sex-linked visibles are an extension of the findings with second chromosome lethals. The added information of specific effect on the *y* and *fo* loci results from advantages associated with the use of data on sex-linked visible mutations.

TABLE 4. *Kinds of sex-linked visible mutations*

Class	No. Mut's.	Steriles	y's	Loci > 2×	Loci 2×	Loci once
Control	37	6	19	none	*f, lz, w*	6
hi	42	?	1	*fo*(4)	none	37

THE RATE OF X-CHROMOSOME INVERSIONS

Because lethal and visible mutations in the X-chromosome were tested to determine their locations on the chromosome, it was possible to determine at once if one of these mutations was associated with an inversion. A total of 351 mutant chromosomes so tested revealed 17 cases in which crossingover was so reduced in repeated tests in one or more regions that an inversion was indicated. In addition, two other probable inversion chromosomes were lost before retests could be made. Of the 17 inversions that were certain, 16 were associated with lethals and one was associated with a visible mutation of good viability. The associated lethals and visible were not tested further but are presumably position effects of the inversions. The ratio of non-inversion to inversion chromosomes in these 351 tests is roughly 19 to 1.

Crossover data are not sufficient to indicate more than the approximate location of an inversion in the chromosome. This requires the study of salivary gland chromosome preparations. *Dr. Taylor Hinton* of this laboratory has begun such an analysis of those of the above inversions, 12 in number, which have been successfully continued in stock cultures.

Preliminary analysis indicates that the distribution of breaks in the chromosome is probably similar to that reported by Kaufmann (1946) in a large sample of breaks induced by X-rays.

Discussion

1. *The effects of ageing*

The data presented in table 2 show that the effects of the mutation rate gene, *hi*, depend upon additional genetic factors. In this instance, crosses of four *le hi/Cy* stocks to each other to produce *hi/hi* flies revealed one combination, the cross of line 5 to line 14, which gave distinctly different results than came from the other three crosses. The design of the experiments was not such as to reveal precisely the genetics of this difference; but the consistency of the results leaves little doubt that the difference was genetic and that the factors responsible were located in the *le hi* second chromosomes. There are several possible genetic means by which the observed results could have been achieved. The simplest hypothesis is that before the extraction of these four sublines from the original stock a recessive mutation occurred and was eventually included in those chromosomes leading to lines 5 and 14, but not in those chromosomes which were eventually isolated in lines 1 and 29. This recessive gene, homozygous in 5/14 flies, then modified the effect of *hi* in the manner indicated by the data.

While the direct genetic cause of the differences observed is obscure, it is possible to analyze the result of this cause and to suggest how it may have been produced. The mutation rate in the three crosses which were alike (table 2A) was characterized by being high in sperm deposited in the first week and dropping off rapidly in sperm deposited in subsequent weeks. This phenomenon was first observed in the X-chromosome lethal mutation rate following X-ray treatment and has been explained in part by germinal selection (Timofeef-Ressovsky, 1937). In the present instance, the number of duplicate lethals also decreased sharply after the second week, as would be expected if germinal selection were the cause of the drop in the observed mutation rate. Apparently, in this case, spermatogonial cells carrying a lethal-bearing X-chromosome generally cease producing spermatocytes (perhaps because of metabolic deficiencies resulting from the lethal gene) before normal spermatogonial cells do. In addition, it appears that *hi* produces most of its effect when the males are young. Possibly it ceases to function after the males have emerged as adults. These conditions would cause a sharp drop in mutation rate such as observed in these crosses.

In the case of 5/14 males, the mutation rate did not drop as the males grew older and the proportion of duplicate lethals clearly increased. The effect of the postulated modifying gene seems to be to extend the active period of *hi* so that it causes the production of new lethal-bearing spermatogonial cells at least as fast as the old ones are inactivated by germinal selection. The active period of *hi* in the spermatogonial tissue apparently continues through the period of spermatocyte formation in the 5/14 males.

Obviously, the modification present in the 5/14 cross is one of evolutionary importance. Males of this type produce twice as many mutations in their lifetime as do males not homozygous for this modified condition. Old males of this type produce four or five times as many mutations as occur in old males of the unmodified type.

An attempt was made to test the postulates involved in this analysis by experiments with *hi/hi* females from the same crosses. Since there is no germinal selection of recessive lethals in females, the mutation rate would not drop in any of the crosses and it should increase in the 5/14 cross. The females produced in these crosses were consistently low in viability and productivity and could not be used for such tests.

The case of male number 32 (table 2C)

is of interest because it represents an instance in which spermatogonial cells carrying a certain lethal gene hemizygously were unaffected by germinal selection. Apparently the normal allele of this lethal gene is not involved in spermatogenesis. Or, possibly the lethal allele is not deleterious in its effect on the spermatogonia during spermatogenesis. There was no other case of this kind in these experiments.

2. *The significance of variable dominant effects*

The data of table 3 show that the mutation rate in *hi*/+ flies also depends upon the genotype in which *hi* is working. The consistency of the results obtained would be expected only if the additional genetic factors were brought in by the + second chromosome in each case. The + chromosome of each of the two lowest rate *hi*/+ crosses was derived from the original + stock many generations after the extraction of the two higher rate + chromosomes. They could have carried a mutant modifying gene not found in the earlier ones. Presumably this is a dominant modifying gene. Since it traces back to a different stock than the modifying gene postulated in the analysis of ageing effects, it need not be an allele of that gene.

The relationship of *hi* and the two postulated modifying genes to each other is not indicated by any of the experiments of this report. One can reasonably predict from these results, however, even without specific knowledge of the genetic nature of these particular modifying agents, that the effectiveness of mutators in a wild population depends very much upon the genetic modifying agents which are present or which arise in the population.

A mutator which can act sometimes as a dominant and sometimes as a recessive should produce a longer and more lasting effect in a natural population than one which is either completely dominant or recessive. The completely dominant gene will be quickly eliminated along with the bad mutations it produces. The completely recessive mutator will produce very little effect when present in only a small portion of the chromosomes of the population, since even in comparatively small natural populations of *Drosophila* the actual amount of inbreeding and homozygosis is low (Dobzhansky, 1941).

In the present case, *hi* would probably contribute most of its mutations while acting as a dominant gene and would be continued from one generation to the next, without significant loss due to selection, in flies in which it acted as a recessive. Since it was derived from an essentially large population, the probability of its complete elimination by chance (without selection) is at a minimum. It should continue in existence much longer than it would in a small population where chance elimination of genes existing in low frequency is often greater than elimination by selection.

3. *The mutation rate in large vs. small populations*

Because mutators like *hi* have a longer span of existence, on the average, in a large population than in a small one, large populations should have, generally, the higher frequency of active mutators. Accordingly, the mutation rate in large populations should be higher than in small populations, on the average, the difference in rate depending both upon the rate of appearance of new mutators and the effective difference in population size. Since laboratory stocks breed in small sized populations in bottle or vial cultures, such stocks should normally have mutation rates well below those present in natural populations in the case of *D. melanogaster*. The reduction of heterozygosis by chance, together with natural selection against mutators producing high mutation rates, should eliminate such mutator alleles comparatively rapidly in laboratory stocks. Laboratory stocks only recently derived from a natural population should carry more active mutators (and their modifiers) than stocks of long lab-

oratory history and should therefore show higher mutation rates.

Zuitin (1941) has reported just such a difference in spontaneous mutation rate between natural populations and a laboratory stock of long standing. In his case, there was an X-chromosome lethal mutation rate of 0.62 per cent in 7,776 tests of males whose chromosomes were entirely of recent Russian natural population origin, in contrast to a rate of 0.16 per cent in 8,614 tests of males from a standard laboratory stock of American origin. The data of Olenov *et al.* (1939) and of Zuitin and Pavlovetz (1940) show spontaneous mutation rates in both sex-linked and autosomal genes which are generally above those found in normal laboratory stocks when they tested flies whose chromosomes were either wholly or partly of wild population origin. The quoted data of Zuitin above are particularly indicative that the act of hybridizing laboratory and natural population chromosomes is not the major cause of increased mutation rate (by genic interaction). They show that genes causing a higher level of mutation rate are already present in the wild population chromosomes.

None of the many stocks of long standing in our laboratory has ever shown a mutation rate for sex-linked visibles comparable to that observed consistently through the past twelve years in collections of *le/Cy* stocks in the year following their isolation from different wild populations. The data of the present study can also be used to demonstrate this point. The mutation rate in the control +/+ flies of table 3 is representative of that in a stock of long laboratory history. The sample of *le/Cy* stocks under observation through the 1948–1949 period represents stocks recently isolated from the wild population. By using the 8 : 1 ratio of lethals to visibles which was observed in the experiments with *hi,* one can calculate that there was a total mutation rate in these stocks which was not less than four times the control rate. This is a minimum estimate, because many of the semilethal visibles on which the 8 : 1 ratio is based would fail to reach maturity and would not be observed, when occurring in the *le/Cy* stocks. It seems most probable that dominant mutators, brought in principally in the second chromosomes from the wild population, were the major cause of the significant increase in mutation rate in the *le/Cy* stocks.

The total frequency of mutators can hardly be as great in the *le/Cy* stocks as it is in the natural population from which they came, since most of the third chromosomes of these stocks are of inbred laboratory stock origin and would therefore carry alleles causing only low mutation rates. The natural population is known to carry a large amount of genetic variability in all of its major autosomes. The mutation rate in the natural population, therefore, should be higher than that observed in the *le/Cy* stocks.

The above data and considerations do not support the conclusions of Sturtevant (1937), whose theoretical analysis led him to infer that mutation rates determined by the use of highly inbred stocks and the usual mating procedures are probably higher than those which occur in nature. The reverse seems more likely to be true.

4. The significance of differential effects on various loci

The differential mutation rates of *folded* and *yellow* in the present experiments suggest that mutation rate genes can endow a natural population with a potentiality for a kind of directed evolution. A population carrying *hi* in ten per cent or more of its chromosomes would be offered a much larger proportion of alleles of *folded* than would occur in a population lacking *hi.* It would receive fewer alleles of *yellow* than would populations carrying either the Demerec or the Neel mutator. Thus, if alleles of *folded* or *yellow* became of selective value, such populations would diverge from each other much more rapidly than a knowledge of the simple average mutation rate

of all genes combined might lead one to suspect.

The cases of *folded* and *yellow* are used here only as potential examples. They are mutations which are at present selectively bad. There are, however, paleontological instances (Wood, 1947, for example) in which evolutionary rise of species has occurred much more rapidly than normal mutation rates seem to allow, apparently as a result of a series of rapid adaptive changes in individual organs or systems. High mutation rate genes, accelerating the production of mutations causing such specific changes, could be an important contributory means of explaining such paleontological findings. Because of their specific as well as their general effects on mutation rates, then, mutators may be factors of prime importance in evolution.

5. *The significance of the production of inversions*

The data of this report indicate that *hi* produces chromosomal inversions at a rate of approximately one inversion to 19 lethal or marked visible mutations. The inversions occurred in both homozygous and heterozygous *hi* flies. Presumably, inversions occur in each of the four arms of the major autosomes at a rate approximately equal to that found for the X-chromosome. This leads to an estimated minimum rate of inversions amounting to approximately one in 400 germ cells for *hi/hi* and to between one and four per 2,000 germ cells in *hi/+* flies. The actual rates may be well above these estimates since they will include inversions not associated with position effect lethals and visibles. Also, the actual lethal mutation rate of *hi/hi* in the two arms of the second chromosome was seven to eight times that in the X-chromosome, not just twice as high.

Inversions and translocations have occurred so rarely spontaneously in tests of average laboratory stocks that it has seemed possible that those which do occur may result from natural radiation. (See Plough, 1941, and the discussion following that paper.) The much higher inversion rate in the presence of *hi,* without increased radiation, indicates that radiation is not necessary to account for spontaneous inversions any more than it is to account for spontaneous mutations in general.

The now large amount of literature on inversions in natural populations of Drosophila is ample to show the importance of these changes in gene arrangement both in maintaining the population (Dobzhansky and Levene, 1948) and in supporting incipient evolutionary changes (Sturtevant and Novitski, 1941; Ives, 1947; Dobzhansky, 1948 and others). It is understood, of course, that generally it is not the inversions themselves but the genes included in them that have such effects.

Any agent capable of producing inversions at an appreciable rate in natural populations becomes at once an important factor in evolution. Mutator genes can, therefore, be considered important factors because of the inversions they produce, as well as for their gene mutations, in natural populations.

6. *The nature of hi and its effects*

At first glance it may appear that *hi* is the same gene which was reported by Demerec (1937). Both genes are in the second chromosome and originated in Florida stocks. There are three considerations, however, which make this relationship seem unlikely: the actual origin of the genes, the mutation rates observed, and the visible mutations found in experiments with the mutators. The Demerec mutator appeared in a Florida stock which had been bred in comparatively small cultures for many years before Demerec worked with it. The present mutator came directly from the wild population in Winter Park, Florida, to the balanced stocks now carrying it. Under these circumstances, it is unlikely that the two mutators are descended from the same mutation. The Demerec mutator showed a

mutation rate (not including duplicates) which was approximately one-third as large as the rate reported here. This could be due to possible heterozygosis of the Demerec mutator in some of his tests and may not be a true difference. Most critical of all is the fact that the Demerec gene produced an abundance of *yellow* mutants while *hi* produces virtually no *yellows*.[1] This is a clear-cut difference. The two genes may be alleles; there is no evidence either way on that point.

Since Neel's (1942) mutation was located on the third chromosome and Mampell's (1943) is in a different species and has different characteristics, it can be stated at once that *hi* is genetically different from each of those genes.

The nature of the genetic effect of *hi* is largely obscure. The time of its effect has already been discussed. Its effect seems to be limited to germinal tissue, since no increases have been noted in the frequency of unilateral somatic mutations in the experiments with *hi*. If *hi* produced this type of anomaly up to ten times as often as it normally occurs, the results could hardly have gone unnoticed, particularly since the author was aware that such events might occur more frequently in stocks and tests of *hi*. It seems very unlikely that *hi* is as effective in somatic as in germinal tissue.

How *hi* produces its effects can, at present, only be conjectured. Generally accepted today is the view that every gene achieves its phenotypical ends by biochemical means, perhaps by the production of a specific enzyme. On this view, *hi* might produce an enzyme which competes with the enzymes involved in the normal reproductive synthesis of genes. When it is successful in this competition, the ultimate result is the synthesis of a mutant instead of a normal gene. The effect of such a common environmental agent as temperature on the mutation rate (Muller, 1928; Plough and Ives, 1935; Plough, 1942 and others) may result from its effects on the activity of mutator alleles or their products, although the earlier studies on *hi* (Ives, 1945) failed to show temperature effects in this particular case. No experiments have been attempted to indicate the nature of an enzymatic activity of *hi*.

7. *General conclusions on the importance of mutation rate genes*

In the foregoing discussion emphasis has been placed upon the probable importance of mutation rate genes as a source of new genetic variability in natural populations. A question which arises naturally from this discussion is whether there are actually any spontaneous mutations. Natural radiation is undoubtedly the cause of some mutations, but it is not generally believed to be normally an important source (Muller and Mott-Smith, 1930). Temperature effects, as pointed out above, may be expressed possibly only through their effects upon mutation rate genes or their products. It is quite conceivable that spontaneous mutations in general result from the changes produced by mutators in the chemical environment in which genes reproduce themselves. On this view, the difference between a low mutation rate stock and a high one is not the absence and presence, respectively, of a high mutation rate gene. It is, instead, the difference in the alleles of mutators which are present in the two stocks. In the case of the low stock, the mutator alleles present are ones whose products cause only a low rate of change in other genes during their reproduction.

[1] It has been suggested that this may reflect a difference in the normal alleles of *yellow* used in the two studies. The normal alleles were not closely related but both have shown themselves to be potentially highly mutable. In the present case the normal allele showed a relative mutation rate (control of table 4) similar to the relative mutation rate observed by Demerec. From this it appears that the two normal alleles were essentially similar in this important characteristic and that the Demerec allele was not a less stable one than the allele used in the present study.

On this view, by far the majority of mutations occurring in a natural population arise from causes *within the cells* of the individual members of the population. Spontaneous mutation is to be thought of not as a "chance" failure of a gene to reproduce itself exactly, but as the result of the influence of the biochemical product of one gene upon the reproductive synthesis of another.

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Summary

Experiments with a high mutation rate gene derived from a natural population of *Drosophila melanogaster* show that this second-chromosome gene produces mutations in many other genes at an average rate of ten times the normal rate when acting homozygously and two to seven times normal when acting heterozygously. Genetic modifiers affect both its dominance and the duration of its activity in the production of sex-linked mutations. Its major effect is in germinal tissue where it produces both lethal and visible mutations at a ratio of approximately 8 : 1. Inversions are also produced. An average of one mutation in twenty was associated with an inversion. The calculated minimum rate of inversion in all chromosomes is one per 400 germ cells in homozygous *high* flies and one to four per 2,000 germ cells in heterozygous flies. The mutation rate of the gene *folded* is increased more than that of other genes, while that of *yellow* is not increased by *high*.

The mutation rate in stocks recently isolated from the local wild population was calculated to be at least four times as high as that in an inbred laboratory stock. Theoretical considerations lead to the interpretation that this reflects the presence of many high mutation rate genes in natural populations.

The evolutionary importance of mutator genes, as exemplified by the present data, is discussed. The thesis is advanced that mutators are the major cause of both gene mutations and inversions in natural populations, and that the majority of "spontaneous" mutations are caused by the biochemical activity of mutation rate genes.

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