THE EFFECT OF SEX ON THE SPONTANEOUS MUTATION RATE IN *DROSOPHILA MELANOGASTER*

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Introduction

In experiments designed to test the influence of carcinogenic substances on the mutation rate in *Drosophila melanogaster* (1940), pronounced differences were observed between the rates with which sex-linked lethals arose spontaneously in male and female germ cells. The flies used for testing the mutation rate were F_1's from crosses between females of a $sc^8 w^a bb$ stock and males of a $scv\delta 49\, od\, ca$ stock. When only the lethals arising in the $sc^8 w^a bb$ chromosome are considered the figures are: no lethal in 846 chromosomes derived from females, and twenty-seven lethals (from twenty-four different individuals) in 3771 chromosomes derived from males. These results had been guarded against any error due to the occurrence of non-disjunction by the scheme of crossing used. Results pointing in the same direction had been reported by Muller & Altenburg as far back as 1919, but as the differences then observed were not statistically significant, and as significant data were difficult to obtain with the low natural mutation rate soon afterwards found in the stocks used, these indications were not followed up. The present differences, however, were so striking that further investigations into this problem of fundamental importance appeared promising.

Experiment I

Following the above preliminary results, the first test was carried out with the $sc^8 w^a bb$ stock which had been used as one of the parental stocks in the experiments on carcinogenic substances. Males and females to be tested for the occurrence of sex-linked lethals in their germ cells were taken from the same stock bottles. The males were tested by the usual *ClB* method, the females by means of the following scheme of crosses:

P_1 ♀ $\frac{sc^8 w^a bb}{sc^8 w^a bb}$ × ♂ $scv\delta 49\, od\, ca$ (pair-matings, 23 pairs);

F_1 ♀ $\frac{sc^8 w^a bb}{scv\delta 49\, od\, ca}$ × brother $sc^8 w^a bb$ (pair-matings, 30–60 pairs from each P_1 ♀).

In the absence of a sex-linked lethal the F_2 males consist of two types which are readily distinguished through the glass wall of the culture vial. If a lethal arises in the germ track of a P_1 female, one of her daughters fails to produce w^a sons, the production of cross-overs by the F_1 being prevented by the presence of different inversions in the two X-chromosomes. By mating the P_1 in pairs any lethal already present in a P_1 female could be detected by the low sex ratio of her offspring, and such females were excluded from the test. Likewise excluded were P_1 females which produced non-disjunctional *sc v 8 49 od ca* sons, because the appearance by secondary non-disjunction of w^a F_2 males might mask the presence of a lethal on the w^a chromosome. There still remain the possibilities of primary non-disjunction in the F_1, which in the presence of several inversions cannot be neglected, and of an extra Y introduced from the P_1 male causing secondary non-disjunction in the F_1. The precaution against these sources of error in experiment I was not to classify any F_2 progeny as lethal-free unless at least three w^a males were found on superficial inspection through the glass of the vial, and in doubtful cases to rear an F_3. This seems sufficient to exclude cases of primary non-disjunction, but some cases of secondary non-disjunction due to the presence of an extra Y in the P_1 male may have remained undetected and create a source of error which is not altogether negligible.

The results were as follows: no lethal in 843 chromosomes derived from females; five lethals and one semilethal (one male among more than fifty females) in 538 chromosomes derived from males.

Experiments II and III

In order to eliminate differences of genotype—apart from those necessarily existing between the sexes—the following tests were carried out with males and females from Florida wild-type stocks made isogenic by Singh through a sequence of crosses described in his thesis (1940). Two of these stocks were used: "Florida 4" and "Florida 5" (*Fo* 4 and *Fo* 5). As in each of these stocks by far the greater part of the major chromosomes of every individual is derived from one and the same ancestral haploid set, these flies constitute a nearly homogeneous material in respect of genotype—barring, of course, new mutations which may have arisen between the time the stocks were completed and the beginning of our experiments. At the same time, environmental differences between the flies under test were reduced to a minimum by rearing them under controlled and as nearly as possible identical conditions of food, temperature, and moisture, by taking P_1 males and females from the same

bottles in approximately the same numbers, and by randomizing the unavoidable individual differences between P_1 individuals through the use of a fairly high number of P_1 couples in each series. Males were again tested by the *ClB* method, females were tested by the following crosses:

"P_0"[1] ♀ $\frac{Fo}{Fo}$ × ♂ *Fo* (controlled and identical conditions);

P_1 ♀ $\frac{Fo}{Fo}$ × ♂ $sc^{S1}Lw^a sc^4R$ (23 pairs in experiment II, 37 pairs in experiment III);

F_1 ♀ $\frac{Fo}{sc^{S1}w^a sc^4}$ × brother *Fo*.

A lethal in the *Fo* chromosome becomes apparent by the absence of wild-type males in F_2. As before, precaution was taken against lethals present from the start, and against secondary non-disjunction due to a *Y*-chromosome introduced from a P_1 female. Moreover, in each batch of F_1 females derived from a P_1 pair, a number of females were mated as virgins to yw^aB males. If a *Y*-chromosome had been handed on from the father, some of these females would be expected to produce sons of paternal type, and in this case the whole batch was discarded. By accepting as lethal-free only those F_2 progenies in which at least three wild-type males were observed through the glass of the vial and by subjecting the doubtful cultures to further breeding tests, precaution was taken against occurrences both of primary non-disjunction in cells of the F_1 ♀ and of double crossing-over between the two *X*-chromosomes of the F_1 female. The results were as follows:

Experiment II. No lethal in 815 chromosomes derived from *Fo* 5 females. Nine lethals (from six different males) in 841 chromosomes derived from *Fo* 5 males.

Experiment III. No lethal in 796 chromosomes derived from *Fo* 4 females. One lethal and one semilethal (three males among more than seventy females) in 790 chromosomes derived from *Fo* 4 males.

EXPERIMENT IV

The data of experiment III, though not disproving the earlier results, yet do not confirm them. It was therefore deemed desirable to test the question again on a larger scale. One more experiment was carried out, using *Fo* 5. The technique was the same as before except for four alterations: (1) Special care was taken to test germ cells of young individuals

[1] "P_0" is used to designate the generation preceding that of the flies ("P_1") whose mutation frequency was tested.

only, by mating the P_1 flies a few days after collection, keeping them on syrup food between collection and mating, and removing them from the vials after 3–4 days. (2) $sc^{S1}Lw^aIn\text{-}Ssc^8R$ males that had been made up for such purposes by Muller were used for the P_1 instead of the rather inviable $sc^{S1}w^asc^4$ males. The presence of inversion S in the middle of the X-chromosome (Muller, 1935) renders the suppression of cross-overs complete. (3) In the later part of the experiments, the F_1 females were mated to $y^2sc^8w^aB$ males. Though the females were not virgins, a sufficient number of B daughters were usually produced to allow an easy decision whether the absence of w^a males in certain F_2 progenies was due to a lethal in the $sc^8w^aIn\text{-}Ssc^{S1}$ chromosome or to the mother (F_1) having been a homozygous wild-type ♀ derived by primary non-disjunction in the P_1 female or by her non-virginity; thus simultaneous observation of lethals in both chromosomes could be carried out with only a little more labour. (4) For detecting an extra Y in the P_1 males each male was tested by mating it to a virgin female carrying $bw^{a\text{-}4}\ Bl\,L^3$. The presence of an extra Y is easily discovered in the offspring by the appearance of a number of non-Bl non-L^3 flies in which the mottling of the eye has been suppressed. All daughters of P_1 males with extra Y's were excluded from the test.

The results of experiment IV were as follows: three lethals (two of them from the same female) and one semilethal (two wild-type males) in 2744 chromosomes derived from *Fo* 5 females; fifteen lethals and one semilethal (five males among over fifty females) in 2691 chromosomes derived from *Fo* males. In addition, twelve lethals (from eight different P_1 males) were found among the 2744 $sc^{S1}LIn\text{-}Sw^asc^8R$ paternally derived chromosomes in the series in which the maternally derived *Fo* chromosomes were being tested. This latter finding may be taken as to some degree confirmatory of the relatively high mutability of the X-chromosome in the male, although of course the flies supplying this w^a-containing chromosome were genetically different from those of *Fo* 5.

When the data, as tabulated in Table 1, are pooled according to the method developed by Muller (1940)—disregarding the semilethals, and in the male series counting as separate only mutations which arose in different males—the difference in the percentage of sex-linked lethals turns out to be 0·48 % with a standard error of 0·11 %. As the difference is 4·4 times its standard error the result is statistically well secured. Analysis of the data gained in this experiment showed that the apparent discrepancy of the results gained in experiment III from the rest was in all probability only a result of "accidental" circumstances: in experi-

ment IV, also, there occurred one run of over 600 F_2 families without a single lethal.

Table 1. *Summary of experiments I–IV*

		Chromosome derived from female				Chromosome derived from male			
No. of experiment	Chromosome tested	No. of fertile F_1 cultures	No. of lethals (in brackets: semi-lethals)	From how many different ♀♀	Percentage of lethals	No. of fertile F_1 cultures	No. of lethals (in brackets: semilethals)	From how many different ♂♂	Percentage of lethals
I	sc^8w^ab	843	0	—	0	538	5 (+1)	6	0·93
II	*Fo* 5	815	0	—	0	841	9	6	1·07
III	*Fo* 4	796	0	—	0	790	1 (+1)	2	0·13
IV	*Fo* 5	2744	3 (+1)	3	0·15	2691	15 (+1)	16	0·56

DISCUSSION

The data presented above appear to establish a difference in the rate at which sex-linked lethals arise spontaneously in the sexes, the males having the higher mutation rate. From what we know about the different types of mutation, there is no reason to suspect that this sex difference should not extend to viable and autosomal gene mutations as well. As to its causes, only assumptions can be put forward as yet. If subsequently it should become possible to decide between them experimentally, this might bring us one step nearer the truth about the origin of natural mutations.

In their qualitative gene content, males and females of an isogenic stock differ only by the presence of the *Y*-chromosome in the former. It does not seem very likely that the *Y*-chromosome should influence the occurrence of mutations. Females carrying a *Y*-chromosome or a portion of it might be used to test this possibility.

When the mutations for which we test are sex-linked lethals, the possible occurrence of germinal selection in the male but not in the female has to be taken into account. Its effect would be to reduce the number of observable lethals in the male. If, therefore, it had occurred in the present experiments to any considerable degree, the observed difference between males and females would assume even more significance.

A possible difference between the sexes which might be considered as underlying the observed difference in mutation rate is one in respect of the number of cell divisions intervening between the fertilized egg that is to develop into the P_1 and that of the next generation (F_1), in which the mutant gene is found to have been present. If this number were considerably higher in the male, and if mutation occurred exclusively or mainly during the process of reduplication of the genes (a possibility

tentatively suggested by Muller, 1928, and apparently supported by results of Olenov, 1939, and of Singh, 1940), a superiority of the male in respect of mutation rate would be expected. Both assumptions, however, are as yet unproved. Muller's suggestion would find support if it could be shown that correlated with the higher frequency of mutants in the *Drosophila* sperm as compared with the egg was a markedly greater number of mitoses during its life history. Unfortunately, the proof for this is not easy to adduce, though at first sight one would suppose that the larger number of spermatozoa would require a larger number of preceding divisions. To arrive at a rough idea of the number of mitoses between fertilized egg and mature reproductive cell in either sex, the following calculations can be made, taking the female first.

According to Huettner (1923), the polar cells are differentiated from the blastoderm cells at the 256 nuclei stage, i.e. after eight previous divisions. There are five to eleven of them, and they form an average of fifty egg strings (ovarioles) in the mature female (Donald & Lamy, 1937). To obtain fifty initial cells for the fifty egg strings from eight to ten pole cells two to three mitoses are required. The total output in eggs of a *D. melanogaster* female averages about 1000, i.e. about twenty eggs per ovariole. Assuming that twenty oögonia are formed in the end filament as forerunners of the twenty eggs to be produced, and that these twenty oögonia are formed by simple dichotomous division, the numbers of cells after each subsequent division proceeding as the powers of two, four to five oögonial divisions have to be postulated. Almost certainly this figure is too low: if oögenesis followed this system no cells would be left in store at all. One division at least has to be set aside for the purpose of providing a store. Possibly there is considerably more storing. Also, there is no reason to assume that oögonial division always or mostly follows a dichotomous scheme. Certain mitoses may result in two cells, one of which only would go on dividing, the other being kept in store (or possibly becoming non-germinal). The extreme case of this type would be a division scheme in which one apical cell gives off one oögonial cell at a time, all oögonial cells being direct progeny of this apical cell. If, then, the oögonia developed directly into the egg, the first egg to be formed would require one oögonial division, the second two, etc. Twenty divisions would precede the formation of the twentieth egg, and ten divisions would be the average for all eggs formed during the lifetime of the fly. For the first eggs, however, which alone were used in experiment IV, the average would be much lower, perhaps two or three. If we allow each oögonium two more divisions before reaching the oöcyte stage the figure is raised to

four or five, i.e. the same as assumed above for a purely dichotomous mode of oögenesis. Next come four divisions producing the fifteen nurse cells and the egg proper, and finally the two oöcyte divisions. Adding up, we arrive at an estimate of $8+3+5+4+2=22$ mitoses preceding the formation of the mature egg.

In the male-forming egg development up to the formation of the polar cells is the same as in the female-forming egg, i.e. eight initial mitoses have to postulated. The five to eleven polar cells thus formed produce the two testes, which together, according to Kaufmann (oral report from Dr Koller), contain 8000–10,000 completed spermatozoa in the newly hatched male. In order to produce 10,000 spermatozoa from, say, ten initial cells by pure dichotomy, ten divisions (including the two spermatocyte divisions) are required. With an exclusively apical cell scheme of division, one primary cell in each testis would have to give off the 1000–1250 primary spermatocytes necessary to produce 4000–5000 spermatozoa. The number of mitoses preceding the primary spermatocytes would thus range from one for the first to at least 1000 for the last, with an average at 500–625. The spermatocytes then undergo two more divisions. Whereas the apical division scheme allows of a continuous formation of spermatogonia for the subsequent production of spermatocytes, the dichotomous scheme requires some previous storing (say four to five divisions in analogy to the estimate for the female). Adding up, we arrive at a minimum of $8+10+4=22$, and a maximum of $8+500+2=510$ or more mitoses preceding the formation of the sperm in the newly hatched male.

It will be seen that the minimum estimates do not differ for the two sexes. The maximum estimates, on the other hand, differ considerably. It is, however, almost certain that the pure apical scheme is not realized in spermatogenesis. Not only do the results gained by Harris (1929) provide evidence of at least two apical cells in each testis, but also cytological evidence on mitoses in the testes and on the time required is in contradiction to rigid apical proliferation. Most probably, actual spermatogenesis follows a system combining both modes of division. It can be seen that figures to fit any ratio between the two sexes could easily be made up by supposing a suitable intermediate between the two extreme schemes of gametogenesis. However, these remain mere speculations until independent information concerning gametogenesis has been gained.

Harris (1929), arguing from the fact that a mutation produced by X-rays 2–3 weeks previous to mating occurs in one-quarter of the sperm,

comes to the conclusion that "the proliferation of germ cells in the testis probably occurs through a system of one or a very few indefinitely reproducing cells functioning like apical cells". His data are, in fact, reconcilable with any other system of division as long as it is assumed that the sperm used in the late mating goes back to two spermatogonial cells present at the time of raying, and only if the situation as found by Harris after a definite time interval between raying and mating were true in general would it imply the existence of apical cells. Experiments of the same kind as those carried out by Harris, with raying at different ages, and checking on group formation of lethals at intervals of a few days, might, as Muller suggests, help to narrow down the scope of possibilities. Before more evidence on the method of gametogenesis is available, all that can be said in respect of its bearing on the sex difference in mutation rate is that it may conceivably be explained by a corresponding difference in the number of mitoses (including gene reduplications) during gametogenesis.

If we discard this explanation as unfounded, there still remains the possibility that the higher mutation rate in the male is an effect of some other physiological difference between the sexes. It is known for example that the catabolic processes differ between the sexes in many animals, the males having the higher catabolic rate. The hypothesis that metabolic processes should be able to influence mutation rate does not appear too fantastic in view of the influence on mutation rate found to be exercised certainly by temperature (Muller & Altenburg, 1919; Muller, 1928; Plough & Ives, 1932, 1935; Promptov, 1934; Timoféeff-Ressovsky, 1935; Buchmann & Timoféeff-Ressovsky, 1935, 1936; Zuitin, 1937, 1938*a*, *b*), and possibly by certain chemicals (Sacharov, 1932, 1933, 1935, 1936, 1938; Lobashov & Smirnov, 1934; Lobashov, 1935; Magrzhikovskaja, 1936, 1938), and by nutrition (Döring, 1937; Stubbe & Döring, 1938; Olenov, 1939). Higher rates of oxidation might influence the mutation rate through direct chemical effects or indirectly through influencing the nature of the medium in which the nuclei exist. If this explanation of the observed differences in germinal mutation rate were true, one would expect to find a corresponding difference in respect of somatic mutations. A higher rate of somatic mutations in the male could not be explained by a greater number of preceding mitoses; an influence of the *Y*-chromosome might be regarded as responsible, but could easily be tested in *XXY* females. Another test of the explanation by such differences in metabolism would consist in direct studies of the influence of altered metabolic rates on the occurrence of mutations. Studies of this kind are now in progress at this institute.

Incidentally our data also provide new evidence of the considerable amount of fluctuation of unkown origin in mutation rate already commented upon by others (cf. Muller, 1928), and it is notable in our work that this applies even to material very strictly controlled for genetical and environmental uniformity. Observations like this should serve still further to caution investigators working on spontaneous mutation rates or using them for control data. Reliable figures for spontaneous mutation rate can only be expected by using devices for maintaining such uniformity, by randomizing the remaining variations through the use of a fairly large number of parents, by taking precautions against sources of error through non-disjunction, crossing-over and the like and, above all, by working with sufficiently large numbers.

SUMMARY

The spontaneous mutation rate in the two sexes was studied in flies from various stocks, mainly isogenic wild-type, reared under controlled and identical conditions. It was found to be markedly higher in the male, the difference being statistically significant. Fluctuations were considerable, even within the same experiment, and point to the necessity for strictest control of all conditions when gaining data on spontaneous mutations. Possible explanations for the observed results are discussed, but without further evidence along other lines no decision between them appears possible.

ACKNOWLEDGEMENTS

The author wishes to express her gratitude to Dr H. J. Muller for his sustained interest in the work and for many helpful suggestions, also to Prof. F. A. E. Crew and Dr A. W. Greenwood for generously providing working facilities. Grateful acknowledgement is also due to the Scottish Cancer Control Organization for their financial help.

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