EFFECT OF TEMPERATURE ON THE RATE OF CHANGE OF THE UNSTABLE MINIATURE-3 GAMMA GENE OF DROSOPHILA VIRILIS

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It has been shown by Muller[1] and later by Goldschmidt[2] and by Jollos[3] that high temperature increases the mutation rate of genes of *Drosophila melanogaster*. It was of interest, therefore, to ascertain what effect the temperature has on the rate of change of the unstable miniature gene.

Material.—Miniature-3 gamma was selected as the most suitable material for measuring the temperature effect since this gene is unstable in somatic cells only and in addition to that changes with a relatively low rate. Changes from the miniature gene to the wild-type gene manifest themselves as wild-type regions in miniature wings. The frequency of the change is such that between five to ten per cent of flies may be expected to have mosaic wings. This frequency is low enough so that two wild-type spots rarely occur on the same fly and the chances of two spots overlaping are negligible.

The line used in experiments was highly inbred. Its history is as follows: about 50 generations of mass matings, two generations of pair matings, ten generations of mass matings, one generation of pair mating, two generations of mass matings, one generation of pair mating and finally two generations of mass matings. Since the gamma gene is capable of changing into an alpha or a beta gene, both of which differ from gamma in their stability, the line has been tested whenever it was propagated by pair matings and only flies having gamma genes were used to continue the line.

Methods.—Since the gamma line was inbred for about 68 generations it could be expected that the flies were genetically homogeneous. To eliminate the effect of mutations, which might possibly have occurred in the line, offspring from mass matings were used in the experiments. Four sets of 25 pairs of flies, all taken from the same culture bottle, were used as parents. These sets were numbered 1, 2, 3 and 4, respectively. Parent flies were allowed to lay eggs for 24 hours and were then transferred into fresh culture bottles. This process was continued every 24 hours until 33 transfers were made giving a total of 132 culture bottles. A part of these was then kept at 20 ± 0.2 degrees centigrade, another part was kept at 25 ± 1 degrees centigrade and the remaining part at 30 ± 0.2 degrees centigrade. Thus the eggs from the same set of parents

were allowed to hatch and develop into flies at three different temperatures.

Wings of the newly hatched flies were examined under 40.8× magnification for the wild-type regions. By using weak light reflected from the sub-stage mirror through the wing it has been possible to detect minute wild-type spots.

As already mentioned only in a very few instances more than one wild-type area has been found on one fly. In these cases each wild-type area was considered as a separate mosaic.

Results.—In table 1 summaries of results are given. It is evident from

TABLE 1

NUMBER OF MOSAIC FLIES IN MINIATURE-3 GAMMA CULTURES REARED AT DIFFERENT TEMPERATURES

Set	Number of cultures	Mosaic		Miniature		Total		Per cent of mosaics		
		♀	♂	♀	♂	♀	♂	♀	♂	Total
		Temperature 30 ± 0.2 Degrees Centigrade								
1	11	45	43	410	409	455	452	9.89	9.51	9.70
2	14	53	51	607	612	660	663	8.03	7.69	7.86
3	15	70	72	697	733	767	805	9.13	8.94	9.03
4	11	41	39	353	335	394	374	10.41	10.43	10.42
	51	209	205	2067	2089	2276	2294	9.18 ± 0.45	8.94 ± 0.44	9.06 ± 0.35
		Temperature 25 ± ½ Degrees Centigrade								
1	4	34	41	280	252	314	293	10.83	13.99	12.36
2	4	37	26	273	245	310	271	11.94	9.59	10.84
3	4	25	35	268	256	293	291	8.53	12.03	10.27
4	4	25	21	209	196	234	217	10.68	9.68	10.20
	16	121	123	1030	949	1151	1072	10.51 ± 0.56	11.47 ± 0.93	10.98 ± 0.47
		Temperature 20 ± 0.2 Degrees Centigrade								
1	7	41	41	376	371	417	412	9.83	9.95	9.89
2	7	49	55	353	358	402	413	12.19	13.32	12.76
3	7	59	73	491	468	550	541	10.73	13.49	12.10
4	7	46	47	437	449	483	496	9.52	9.48	9.50
	28	195	216	1657	1646	1852	1862	10.53 ± 0.51	11.60 ± 0.54	11.07 ± 0.41

that table that of the flies reared at 30 degrees centigrade 9.06 ± 0.35 per cent were mosaics, of the flies reared at 25 degrees centigrade 10.98 ± 0.47 per cent were mosaics and of those reared at 20 degrees 11.07 ± 0.41 per cent were mosaics. The difference in the percentage of mosaics is 1.92 ± 0.58 between 30 and 25 degrees, 2.01 ± 0.54 between 30 and 20 degrees and 0.09 ± 0.62 between 25 and 20 degrees. The value for the difference divided by its probable error is 3.3 in the first case, 3.7 in the

second case and 0.15 in the third case. In the first two cases the difference is significant and in the third case it is not.

Discussion.—Temperature Effect.—There is very little doubt that among the flies reared at 30 degrees centigrade significantly fewer mosaics were observed than among the flies reared at either 25 or 20 degrees. The question arises, however, whether that difference is due to temperature or whether it is a result of some other cause.

The flies reared at 30 degrees are appreciably smaller than the flies reared at either 25 or 20 degrees. The size of the wings of these flies is about half of the size of the wings of the flies raised at 20 degrees. In addition to that the wings of the 30 degree flies have a tendency to wrinkle which tendency is accentuated in cultures having even a slight excess of moisture. It is very probable, therefore, that small and wrinkled wings are responsible for the smaller number of observed mosaics in the 30 degree cultures, since the minute wild-type spots are easily missed on such wings. If the smaller number of mosaics were due to temperature it could be expected that the flies reared at 25 degrees would have the number of mosaics intermediate between the flies raised at the 20 and 30 centigrades, which, however, has not been observed. The explanation appears feasible, therefore, that the lower number of mosaics observed in the 30 degree cultures is due to an observational error rather than to the effect of temperature.

However, before reaching the conclusion that ten degrees difference in temperature does not affect the frequency of change of the miniature-3 gamma gene it is necessary to consider another factor in which the cultures raised at different temperatures differed. That factor is the time which it takes for a fly to develop from an egg to an imago. At 30 degrees centigrade this period is 11 days and at 20 degrees it is 19 days. It is evident that if the rate of the change in the gene were a function of time then the possible increase due to high temperature might have been obscured in our experiment. Another experiment was performed, therefore, to clear up this point. Flies were raised at 30 degrees but the period of the development was prolonged by crowding which was accomplished by using small culture bottles. In this experiment, also, 25 pairs of flies were allowed to lay their eggs for a period of 24 hours and then they were transferred into another set of bottles. The flies began to hatch on the thirteenth day and continued hatching for fourteen days. The flies in the previous experiment began to hatch on the eleventh day and all hatched within four days. Of the 175 flies which hatched after 13 to 17 days from the time the eggs were laid, 17 or 9.71 per cent were mosaics and of the 82 flies which hatched from the same two cultures after 18 to 24 days from the time of the egg laying, 7 or 8.54 per cent were mosaics. It is evident, therefore, that the change in the gene is not a function of

time and that the inequality in the duration of the development from an egg to an imago has not affected the results of the temperature experiment.

The experiment, therefore, indicates that ten degrees centigrade difference in temperature has not affected the rate of change in the unstable miniature-3 gamma gene in a detectable degree. The question now arises how this finding can be brought in accord with observations of the others, namely, that the high temperature increases the mutability of genes. As a possible explanation the following working hypothesis might be offered. Studies made with the unstable rose flower color of Delphinium[4] and with the unstable chlorophyll color of Polystichum[5] indicate that the change in the gene occurs at the time of cell division. It seems probable, therefore, that this change is caused by the failure of the gene to reproduce itself exactly, viz., that the new gene formed next to the old one differs from the old gene. If a gene is considered to be a large organic molecule the changed gene may differ only in the position of groups inside that molecule (isomere). The characteristic of an unstable gene, then, would be that a shift in the molecular organization of the gene frequently occurs during gene reproduction. That process, apparently, is not affected by high temperature. A similar shift in the molecule organization might, however, be produced by environmental agencies such as x-rays and high temperature. As shown in the x-ray work the change in the gene may be produced when cells are not dividing as well as when they are dividing. X-rays, therefore, can accomplish the molecular rearrangement in a molecule already formed. If it is assumed that a similar effect could be produced by high temperature then the explanation of our results becomes evident. In the case of miniature-gamma certain percentage of new genes having gene molecule arranged differently from that of the parent gene is formed during the reproduction of the gene. This process is characteristic of the unstable gene and is not affected by the temperature. Identical rearrangement in gene molecule could probably be produced by high temperature in a gene already formed. The work of Muller and others indicate that the rate of this change for any one particular gene would be so low that it could not have been detected in our experiments.

The Effect of Sex on the Instability of the Gene.—Another point brought out in this experiment is the effect of sex on the instability of miniature-3 gamma gene. Since this gene is sex-linked every female carries approximately twice the number of genes carried by a male. If the rate of instability were the same in both sexes about twice as many mosaic spots should be found in females as in males. The data in table 1 indicate that mosaic spots occurred in both sexes with the same frequency. This means that the rate of instability of the gene is about twice as high in males as it is in females.

It is known that the rate of instability of the gamma gene can readily

be influenced by several genetic factors.[6] The higher rate in the males might be accounted for by the assumption that the male sex stimulates the instability. Experiments are now in progress which are expected to throw more light on this problem.

Summary.—Miniature-3 gamma gene is unstable in somatic cells. The results of experiments in which the flies carrying this gene were reared at 30 ± 0.2, 25 ± 1 and 20 ± 0.2 degrees centigrade, respectively, are interpreted to indicate that these temperature differences have not produced any effect on the instability of the gene.

It has been found that the frequency of gene changes was alike in males and in females in spite of the fact that a female carries approximately twice the number of miniature genes carried by a male.

[1] Muller, H. J., *Genetics*, **13**, 279–357 (1928).
[2] Goldschmidt, R., *Biolog. Zentralblatt*, **49**, 437–448 (1929).
[3] Jollos, V., *Ibid.*, **50**, 541–554 (1930).
[4] Demerec, M., *Jour. Gen.*, **24**, 179–193 (1931).
[5] Andersson-Kottö, I., *Zeitschr. ind. Abst. Vererbungslere*, **56**, 115–201 (1930).
[6] Demerec, M., these PROCEEDINGS, **15**, 834–838 (1929).

AN INTERCHANGE IN MAIZE GIVING LOW STERILITY AND CHAIN CONFIGURATIONS

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The culture of maize which gave semisterile-1 plants (Brink, 1927) contained also two plants with a low percentage of pollen abortion (10–18 per cent based on counts made at that time). This culture had not been x-rayed. Low steriles appeared in the first generation crosses obtained from one of these plants. Pollen counts show them to be about 25 per cent sterile. Thirty-seven plants were counted, giving a total of 58,000 pollen grains, of which 26.3 per cent were practically devoid of starch.

Root tip counts showed that the low sterile plants have 20 chromosomes, demonstrating that this low sterile line differs from the 21 chromosome low steriles arising from irregular (3–1) disjunction of the chromosomes in the rings of semisteriles (Burnham, 1930, McClintock, 1931).

Diakinesis configurations in the microsporocytes of low sterile plants showed 8 bivalents plus a chain of four chromosomes, while their normal sibs had 10 bivalents. In the low sterile plants, a chain of four chromo-