INDUCTION OF MUTATIONS BY HIGH TEMPERATURE IN DROSOPHILA

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INTRODUCTION

Exposure of the germ cells of living organisms to X-rays or to radium has so far proved to be the only reliable method for artificially inducing mutations, as shown by MULLER and others. In nature, organisms or groups of organisms carrying "favorable" mutations are supposed to be chosen for survival by the sorting action of natural selection, and MULLER and MOTT-SMITH (1930) have concluded that natural radiation is inadequate as a cause of spontaneous or natural mutations. The search continues therefore for some agent in the natural environment of living things which produces mutations under controlled conditions, and is of sufficiently general occurrence to account for mutations in nature.

That this factor may be temperature change is of course an old idea. Temperature changes are ubiquitous and palaeontologists have often suggested that periods of rapid evolutionary change coincided with, or followed, periods of marked climatic fluctuation. Most of the observations of the pre-Mendelian period fail to satisfy the genetic criteria, and until recently, controlled genetic tests have proved negative. More than fifteen years ago the senior author looked for mutations in Drosophila strains which had been exposed to high and low temperatures with negative results (PLOUGH 1917). MULLER and ALTENBERG (1919) and later MULLER (1928) reported a slight but significant increase in the number of lethals from Drosophila cultures bred at 27°C. But the work of GOLDSCHMIDT (1929) was the first which seemed to give clear-cut evidence in support of this view. He reported that a large number of mutations of all classes appeared among the offspring of flies which had been exposed as larvae to sub-lethal temperature (35°–37°) for periods of about 24 hours. This work was confirmed and extended in a series of reports by JOLLOS (1930–1933). The latter has carried out a long series of experiments not yet completely summarized and found a marked increase in the number of mutations as well as of non-inherited variations in successive generations following heat, as compared with untreated controls. Some of the non-inherited variations reappeared among the offspring of females showing them, and many of the mutations found resembled in their expression certain of these non-inherited variations. In certain cases, too, subsequent heating of flies bearing mutations already induced gave rise to more extreme alleles in

successive steps. JOLLOS concludes that brief and repeated exposures to sub-lethal temperature induce simultaneously in this order of frequency: certain particular somatic or cytoplasmic modifications, Dauermodifikationen of the same type, initial mutations of the same manifestation, and finally more and more extreme alleles of the same genes. He states his view thus: (Naturwissenschaften **21**: p. 456) "Da nun die gleichen veränderten Umweltfaktoren bei einem Teil der Individuen Modifikationen und Dauermodifikationen . . . bedingen, bei anderen dagegen ganz analoge, aber auf *Gen*veränderungen beruhende Umstimmungen hervorrufen, so ist anzunehmen, *dass die von den Genen in das Plasma entsandten wirkenden Stoffe und die Gene selbst ihrer Konstitutionen nach wesensgleich oder doch sehr ähnlich sind.* Es ist dabei unschwer einzusehen, dass die im Plasma liegenden Gen*produkte* leichter verändert werden können als die in den Chromosomen liegenden, den Umwelteinflüssen offenbar schwerer zugänglichen Gene selbst."

JUST (1932) has come forward with another interpretation developed on speculative grounds from JOLLOS's evidence and from a consideration of the effects of external agents on the eggs of various animals. He believes that the environment affects primarily the cytoplasm, and secondarily the nucleus. On this view the modifications are the primary results of temperature change, and the altered cytoplasm reacts on the chromatin to produce occasional mutations. JUST contends that the burden of proof rests on those who hold that cytoplasmic modification is a consequence of nuclear change.

There has been a good deal of interest aroused in these observations and in the conclusions, because of their bearing on the theory of evolution. JOLLOS believes he has proved that the high temperature brings about evolution in certain specific directions, in a step-by-step, or orthogenetic series, as has been claimed by some palaeontologists from a study of certain lines of fossils. While there may be reasonable doubt if the evidence as brought forward by JOLLOS justifies the conclusions drawn, the facts constitute an important contribution to genetics, of special interest since they have not been brought to light by the many previous breeding experiments with Drosophila at high temperatures.

Soon after GOLDSCHMIDT'S report was published, a number of Drosophilists, both in America and in Europe, attempted to secure confirmatory evidence. Of these ROKITZKY (1930) appeared to give some confirmation while FERRY, SCHAPIRO and SIDOROFF (1930) reported negative results. The somewhat more extensive series of tests exhibited at the Sixth International Congress of Genetics by STURTEVANT, by DEMEREC, and by REDFIELD and SCHULTZ, were negative. At this Congress, also, MULLER (1932) summarized the work of MACKENSEN in his laboratory in which the Gold-

schmidt method was applied to *ClB* stocks. These tests were on a large scale, and showed a rise in lethal mutation frequency of a slightly greater order than MULLER's earlier data would have led one to expect. Finally PLOUGH and IVES (1932) at the same Congress made a preliminary report of an extensive series of tests, and this was somewhat amplified in reports to the Genetics Society at Atlantic City and at Cambridge (PLOUGH 1932 and 1933). (See also GROSSMAN and SMITH 1933.) Our results, which are here reported in detail, show excellent agreement with those of MULLER and confirm the observations of GOLDSCHMIDT and JOLLOS as to the increase in rate of mutation. In addition they indicate that certain genes are induced to mutate more frequently than others, although we find no evidence of step-by-step mutation in allelic series. It is of interest that our data show many similarities to those of PROMPTOV (1932) for the effect of ultra-violet rays suggesting perhaps that his results may have been due to high temperature. In general also our results are in agreement with those reported by RANDOLPH (1931) who secured a certain number of chromosome mutations in maize by high temperature treatment.

EXPERIMENTAL

Since our purpose was to check the results of GOLDSCHMIDT and JOLLOS, we followed their method as exactly as possible (see JOLLOS 1930). Ordinarily three pairs of flies of the particular mating to be used were placed in one culture bottle for three or four days, and then transferred to a second bottle for one day only. A number of successive transfers of one day each were made, and these one-day cultures were placed in the incubator at 36.5°C on the fifth or sixth day after the parents had been introduced. They were left at this temperature in most cases for 24 hours and then replaced at 24° along with the first series which were the controls. Thus the genetic constitution of controls and heated lines was exactly alike.

In the majority of lines subsequent generations were secured from pairs derived from the heated (or control) series, but in certain experiments only heated males or heated females were used. Later generations were ordinarily carried at 24°, but in a certain number of cases subsequent generations were exposed to heat as in the first generation. In all cases the generations actually heated as larvae (and the corresponding controls) are designated as generation 1.

A chart of the different experiments showing the stocks used, the treatment, and the number of flies examined is shown in table 1. With a few minor exceptions in experiment 10 all the work on any one experiment was done by a single observer, so that the heated and control lines were given similar attention. Every fly recorded was examined for visible variation and, if any irregularity was observable, was mated. Of course only a small number of the normal flies were continued further.

TABLE 1

List of experiments. Totals for generations 1–5 only.

NO. OF EXPERIMENT AND INVESTIGATOR	STOCK AND MATING	NO. GENERATIONS FOLLOWED	TREATMENT 5 DAY LARVAE EXPOSED AT 36.5°C FOR 24 HRS.	NO. CULTURES IN HEATED LINES	NO. FLIES OBSERVED IN HEATED LINES	NO. CONTROL CULTURES	NO. CONTROL FLIES	NO. MUTATIONS FOUND HEATED	NO. MUTATIONS FOUND CONTROL
1. PLOUGH 1930	Various stocks	1–3	♂ and ♀	49	±6615	22	±4540	2	0
2. PLOUGH 1930	XX yellow—*b* ♀ ×sup. *b*—*b* ♂	1–3	♂ and ♀	46	3085	15	1870	0	0
3. PLOUGH 1931	II ple ♂ (*t[d]b pr c px sp*) ×normal ♀ (backcross)	1–2	♀	17	1307	11	1653	0	0
4. SWIGERT 1931	*ec ct g* ♂ ×normal Florida ♀	1–3	♂ and ♀	52 (3rd gen. missing)	14033	11	1386	2	0
5. PLOUGH 1931	Amherst normal	1–2	♂ and ♀	42	±4220	11	±3135	1	1
6. MCGOUN 1931	*b vg* ♀ ×*Bl* ♂	1–2	♂ and ♀	(500) tests of crossing over only		(30)		—	—
7. PLOUGH 1931–32	*sc cv vf* ♂ ×normal ♀	1–5	♂ and ♀	74	11586	43	7279	3	0
8. IVES 1931–32	*ec ct g* ♂ (or $w^e m$ ♂) ×So. Amherst normal ♀	1–9	♂ and ♀	202	31499	67	12770	6	0
9. PLOUGH 1932–33	II ple ♂ (*t[d]b pr c px sp*) ×So. Amherst ♀ (backcross)	1–6	♀ gen. 1–3, ♂ and ♀ gen. 4–6	86	11657	24	4564	1	0
10. IVES 1933	*ClB* ♀ ×So. Amherst normal ♂	1–10	(1) ♂	522	75449	226	36024	20	2
	So. Amherst ♀ ×*ec ct g* ♂		(2) ♀	98	15755			4	
	So. Amherst ♀ ×♂		(3) ♂ and ♀	70	11220			5	
Totals				1258	186426	430	73221	44	3

GENERAL SURVEY OF THE RESULTS

While certain of the experiments were continued for as many as 10 generations, adequate counts were available for not more than 5. It has seemed best therefore to summarize the results for generations 1–5 only, although, as will appear later, several mutations appeared in heated lines after the fifth generation. The summaries are given in table 2. The percentage of total mutations of all types is given per culture and per total number of flies examined. The latter is the more significant figure in general since, as the table shows, fewer flies hatched per culture from heated than from control lines. The general conclusion to be drawn from table 2 is that the heat treatment caused nearly a sixfold increase in number of mutations of all classes as compared with the controls. The difference is unquestionably statistically significant since the chance that the two are identical is of the order of 250,000 to 1.

Table 2 also makes it possible to estimate the relative frequency of the different sorts of mutations in the heated lines. It is obvious that there were only as many tests for X-linked lethals (col. a) as there were females whose offspring were classified into males and females. This number is slightly less than the total number of cultures. For X-linked visible recessives (b) the number of tests is the total number of male flies counted. For autosomal recessives (c) the number is that of cultures in the last three generations. For visible dominants (d) every fly counted is a separate test for such a mutation, and for chromosome aberrations (e) the number is the number of cultures in which crossing over percentages for at least one chromosome were recorded. This very rough calculation indicates that in the heated lines, if the frequency of dominants is taken as 1, X-linked recessives are also 1, chromosome aberrations are 42, autosomal recessives are 146, and X-linked lethals are about 157. There are about three times as many genes which may give autosomal recessives as X-linked lethals, so that we have here clear evidence that the latter are by far the most frequently occurring class of mutations. In addition also we have a clear demonstration of the fact that dominants are the least frequent mutations in spite of the large number recorded in these experiments. There is no reason to believe that the relative frequency of the different kinds of mutations is any different in these heated lines than in spontaneous mutation generally.

THE MUTATIONS FOUND

All variations revealed by careful examination of either the heated or control cultures were mated in pairs and their offspring continued for at least one further generation. If the variation reappeared it was tentatively classified as a mutation and a stock isolated. By the usual methods the

TABLE 2

Mutations induced by exposure of larvae to high temperature. Summary of all experiments—generations 1–5 only.

TOTAL NO. FLIES	TOTAL NO. CULTURES	AVERAGE NO. FLIES PER CULTURE	MUTATIONS FOUND (a) X-LINKED LETHALS	(b) X-LINKED VISIBLE RECESSIVES	(c) AUTOSOMAL VISIBLE RECESSIVES	(d) VISIBLE DOMINANTS	(e) CHROMOSOME ABERRATIONS	TOTAL NO. MUTATIONS / NO CULTURES	TOTAL NO. MUTATIONS / NO. FLIES
Heated lines—larvae exposed to 36.5°C for 24 hrs. on 5th–6th day.									
186426	1258	150	13	7	9	13	2	$\frac{44}{1258}=0.03498\pm.00349$	$\frac{44}{186426}=0.000236\pm.000024$
Approximate no. tests			1185	93000	880	186000	681		
Approximate rate of mutation per fly			0.01097	0.00007	0.01023	0.00007	0.00294		
Relative frequency			(157)	(1)	(146)	(1)	(42)		
Control lines—kept at 24°C									
73221	430	170	1	1	0	1	0	$\frac{3}{430}=0.00698\pm.00271$	$\frac{3}{73221}=0.000041\pm.000016$
Difference								$0.028\pm.004$	$0.000195\pm.000028$
Difference ÷ Probable Error of difference								6.3	6.7
Percent mutations Heated ÷ Control								5.0	5.8

gene was then located in its proper linkage group and at its approximate locus. In the case of a known locus its identity or allelism was established

TABLE 3

List of mutations by generations. Control mutations are underlined. Roman numerals indicate linkage groups. Arabic numerals show experiment.

NO. GENERATION	X-LETHALS	VISIBLE RECESSIVES	VISIBLE DOMINANTS
1.			(5) vg^{No_2} II
			(8b) Stubby (Bristle allele?) II
2.		(4) cut ^{n}I(2♂1♀)	(1) Stubby (Bristle allele)? II
			(4) vg^{No_1} II
			(5) Bar rev. to normal I
			(7) Star allele II
			(10_1) Minute allele III
			(10_1) Trident III
			(10_2) Minute allele III
3.	(10) 1–(58 locus)	(10) w-allele like blood I	(1) Star allele II
			(7) Trident (weak sooty?) III?
	(8b) 1–0+ ?	(7) garnet I♂	(10_1) vg^{No_3} II
	(10_1) 4–(32 locus)	(10_1) lozenge I♂	
	(10_1) 1–(28 locus)	(10_1) rough-eye III	
	(10_1) 1–(48 locus)	(10_1) missing d. c. bristles with basal ring. D? ?	
	(10_1) 1–(54 locus)		
	(10_3) 1–(28 locus)	(10_1) sunken thorax ?	
	(10_3) 2–?	(10_2) vg^{No_5}–D? II	
4.	(10_1) 1–(52 locus)	(8b) cut^{n} I♂	(10_1) vg^{No_4} II
	(10_1) 1–?	(10_2) brown II	
		(10_2) black body? II (or III?)	
		(10_3) brown II	
5.	(10_1) CO. inhibitor of *ec ct g.*	(8a) Inversion (C III r?)	(8a) Glued III
		(8b) dark-eye 1 (safranin allele?) II	
		(9) dorso-central bristle missing (*sc?*) I	
		(10_1) rough I (facet?)	
		(10_1) balloon-wing? II	
		(10_3) garnet I	
6–10.		(8b) lethal II	(10_1) Lobe II
		(8b) white I	
		(10_1) rough I ?	
		(10_1) garnet I	
Totals			
Heated	14	21	14 49
Control	*1*	*1*	*1* *3*

when the necessary stocks were available. Nearly all the sex-linked lethals were isolated from the final *ClB* experiment (No. 10) and they were located by crossover tests with *ec-ct-g* chromosomes. The complete list of mutations is given in table 3. This includes five which appeared in heated lines in generations 6–10. No doubtful cases of irregularly appearing mutants are included and wherever doubt exists as to locus, that fact is indicated by a ?. Stocks of most of the visible mutations are still available.

In the course of the experiments, mutations came to light in both the experimental and the corresponding control lines, or in one or the other and in the stocks from which they came. In either case the mutation was removed from the list of mutations produced in the course of the experiments since it was probably present in the original stocks. In all, 12 different mutations—several of which occurred more than once—fell into this group and they are listed in table 4. It is obvious that inclusion of all of these in either the heat line totals or in the control would have completely changed the picture, and it seems reasonable to inquire whether any such series was set aside in the very large list of mutations from heated lines given by GOLDSCHMIDT and JOLLOS. The fact that they reported no mutations whatever from the control lines suggests that this was not done. In the work of MULLER (1928) special methods were used to secure mutation-free stocks. While that was not done in the present work, it is believed that adequate examination of control and stock cultures has largely eliminated this hazard of the method.

TABLE 4

List of mutations isolated from both heated and corresponding control lines.

MUTATION	STOCK
1. kidney or bulge-eye	—Florida wild
2. rough-eye	—black
3. spread-wing	—suppressor black
4. blistered-wing	—*ec ct g*
5. translucent-eye	—*ec ct g*×So. Amherst wild
6. plexus	—*b pr c*
7. slight plexus	—*sc cv v f*
8. trident	—So. Amherst wild, *ClB* and others
9. dark-eye	—*ClB*×So. Amherst wild
10. dorso-central bristles missing (no basal ring)	—*ClB*×So. Amherst wild
11. abdominal sclerites broken (abnormal abodmen)	—*ClB*×So. Amherst wild
12. stubby bristles	—So. Amherst wild

It will occur to anyone familiar with Drosophila work that the method here outlined is not likely to have picked out all the mutations which occurred. We are certain that this is true, since a number of slight eye color

differences as well as other minor variations were either passed by, or discarded after being bred for a generation or two. In addition the personal factor of the observer is important in the number of variations or mutations found. MULLER and many other Drosophilists have therefore given up the attempt to secure accurate data on the relative numbers of all classes of mutations in work on mutation rate, and confine themselves only to listing lethal mutations. It should be emphasized, however, that we set ourselves the problem of testing the Goldschmidt-Jollos technique, and this required listing visible as well as lethal mutations. Since adequate controls were run, and the same observer carried the whole of each set of experiments, we believe that our results give a valid estimate of the effect of temperature on mutation. They certainly show a minimum rather than a maximum effect.

A word may be said of the only other possible source of inaccuracy in the number of mutations recorded, namely contamination of the cultures from flies outside the experiment. This possibility can never be entirely eliminated from a series of tests of this kind, but there is reason to believe that it is unimportant in the final result. Obviously it is as likely to occur in controls as in the heated lines, and the difference between the final percentages should still be accurate. In addition more than half of the visible mutations—and it does not apply to lethals—were new to our laboratory; therefore these could not have been contaminations. Finally most of the mutations appeared first in single flies. If contamination had occurred it is likely that there would have been a considerable number of examples. Since most of the stocks carried in the laboratory are multiple stocks, cases of contamination would involve therefore several known gene series. As a matter of fact we recorded no case of simultaneous mutations in more than one gene. Seven cases of contamination were recorded in the course of the experiments—involving in all over 400,000 flies—and every one showed the presence of an echinus-cut-garnet chromosome. They all occurred in line (3) of experiment 10 in which a set of 100 bottles were used with cardboard milk bottle stoppers. These stoppers were immediately discontinued and cotton plugs used in all other cases.

VISIBLE MUTATIONS

The visible mutations listed in table 3 are classified according to their linkage groups in table 5. Most of the mutants are at known loci, 5 only appear to be new. Mention may be made of a very sharp chromosome III dominant, discovered by IVES in experiment 8 and called Glued-eye. It shows a reduced number of facets, somewhat as in the second chromosome Glass, with a clear area about the eye as in MULLER'S X-ray-induced spectacled. The surface of the eye is smooth and shiny as though covered with

dried glue. It is located so close to Dichaete that no crossing over has occurred between the two genes in 5000 test flies. A drawing of the Glued-eye is shown in figure 1a. One of the Star alleles is shown in figure 1c, and a normal eye for comparison.

The most frequent visible is a dominant vestigial allele called vg^{No}. It was first discovered by SWIGERT in experiment 4. It first appeared in several flies with a clear notch in each wing as shown in figure 2b. In homozygous condition the wings are reduced to mere stubs and the balancers are absent. It was therefore called "wingless." This was kindly

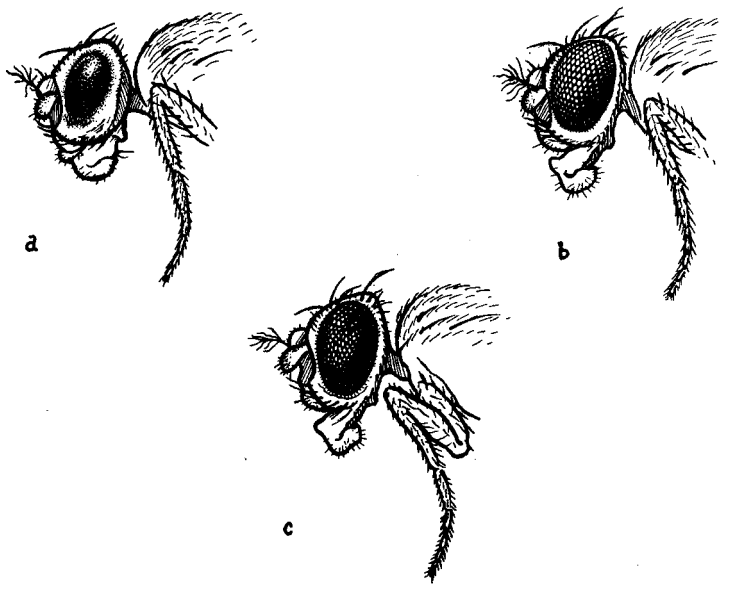

FIGURE 1.—Heat-induced mutations. a—Glued-eye. b—normal-eye. c—Star-eye.

identified for us as a vestigial allele by Dr. OTTO L. MOHR of Oslo, Norway, who describes it as follows (personal communication): "It is a new dominant allelomorph, but non-lethal in homozygous condition. In compounds with the weak allelomorph nicked $\frac{vg^{No}}{vg^{ni}}$ we get large marginal incisions, spade-like wings, and with notched $\frac{vg^{No}}{vg^{no}}$, wings strap-like, divergent 45°, slightly erect scutellars, bulb of balancers rudimentary—in all like a weak vestigial (changed by temperature). vg^{No} comes closest to MORGAN'S vg^{NW} (no wings). It differs from it in the stronger tendency to marginal notches and better viability when homozygous." $\frac{vg^{No}}{vg^{No}}$ females (figure 2c)

are sterile, but males are fertile. $\frac{vg^{No}}{vg}$ flies show extreme vestigial wings.

The second *vg* allele occurred about six months later in one fly of the first generation of the control series of experiment 5. In heterozygous manifestation it is more extreme than the former, the wings being more strap-like (figure 2c). With some initial selection it has become a fairly constant strap-like stock in heterozygous condition.

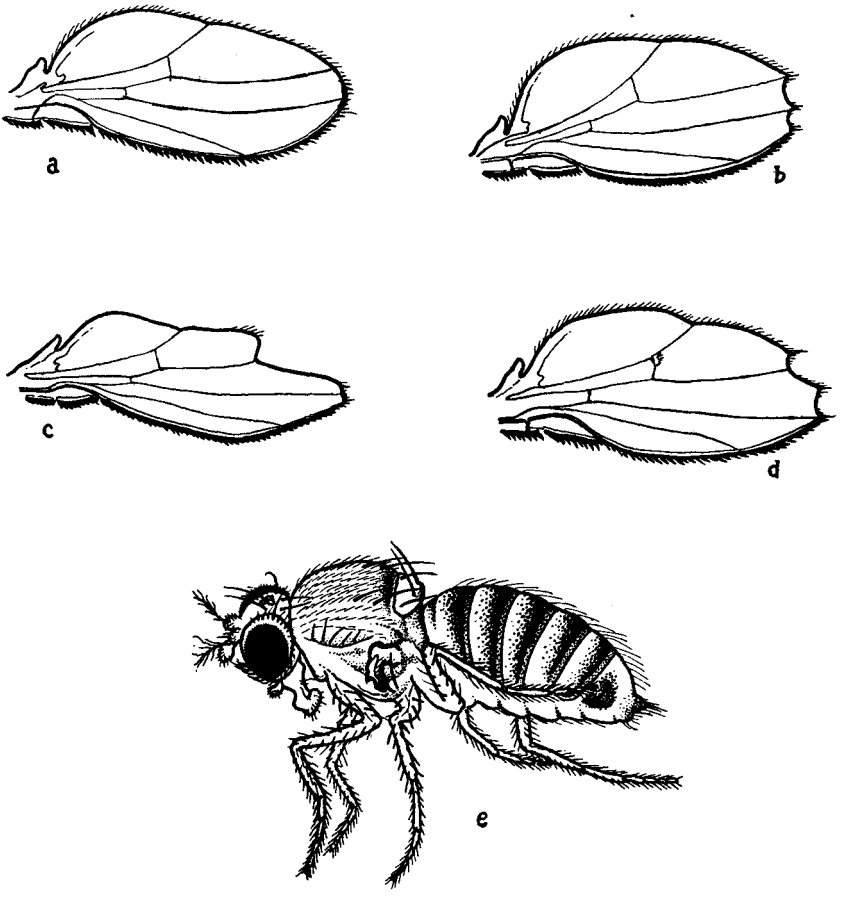

FIGURE 2.—Heat-induced mutations. a–normal wing. b–e, various vestigial alleles.

b $-\frac{vg^{No}1}{+}$ c $-\frac{vg^{No}2}{+}$ d $-\frac{vg^{No}3}{+}$ e $-\frac{vg^{No}}{vg^{No}}$

No less than three additional independent appearances of *vg* alleles were found in subsequent experiments, all in heated lines. One of these (figure 2d) is similar to the above strap-like allele, while a second is identical with the allele first found. The third is much less extreme, producing only an occasional slight notch when heterozygous, except in a selected stock. In homozygous manifestation it is wingless like the others.

The summary in table 5 shows that in our heated lines there appeared: visible mutations in 24 loci in all chromosomes, lethal mutations in 10

TABLE 5

Mutations classified according to chromosomes. Mutations in control lines are underlined.

DOMINANT	LETHAL	RECESSIVE
	Chromosome I	
Bar reversion	4—locus undetermined	3 garnet
	4—locus 32	2 roughened-eye (facet?)
	2—locus 28	white
	1—locus 48	cut ^{n}
	1—locus 52	scute^{x}
	1—locus 54	
	C.O. modifier of *ec ct g* region	(w^{x}-blood?)
	1—locus 58	
	Chromosome II	
4 vg^{No} (3 different)	Lethal II	2 brown
2 Star allele		*sunken thorax
2 Stubby (Bl. allele)		*dark body? D?
Lobe		dark-eye (safranin allele?)
		balloon
vg^{No}		
	Chromosome III	
2 Minute	Inversion C III r	rough
2 Trident (?)		
*Glued-eye		
	Doubtful	
		*missing d. c. bristles (basal ring present)

Summary

	Heated	*Control*
Total mutations	49	3
Total loci	34	3
Total chromosome aberrations	2	0

* Indicates new locus.

loci (assuming 4 undetermined were different), and 2 chromosome aberrations. In the controls there were 2 visibles and 1 lethal. While the controls gave no duplicates, the heated lines showed many recurrent mutations. Of the 34 loci in the latter group which mutated at least once, 10 gave 2 or more mutations in the same locus, 3 gave 3 or more, and 2 gave 4. From these data it is possible to make a rough calculation of the re-mutation rate using the formula of MULLER (1929):

$$r \text{ (a constant)} = \frac{Nx+1}{Nx} .$$

Assuming that there is an equal chance of mutation in all genes, the ratio of the number of genes mutating once to those mutating twice should be the same as that shown by those mutating twice to those mutating three times, et cetera. The ratios in this case are 0.29, 0.33, 0.66. The first two are the most significant and are not far apart, but they are two or three times higher than the value found by MULLER or by GOWEN (1932). Using the first ratio, the total number of genes indicated in all chromosomes would be only about 100, which is obviously lower than the known number, and more than 100 times lower than the total calculated by GOWEN. There are other factors which should be taken into account to secure an accurate estimate, but the rough calculation is sufficient to establish the fact that our heated lines show a greatly increased tendency to second or third appearances of mutations in the same loci. No such tendency has been reported in the studies of the effects of X-rays. It is of interest also that the loci which have re-mutated most frequently—namely vestigial and garnet —are not those which have previously been listed as giving the highest re-mutation rates as spontaneous mutations. The data indicate quite clearly therefore that in addition to increasing the total number of mutations, the heat treatment also causes certain particular genes to mutate much more frequently than others.

We thus bring added confirmation to the observations of JOLLOS. He also finds that certain particular mutations recur following heat treatment with greater frequency. On the other hand, the vestigial and garnet loci which have shown the largest number of mutations in our experiments do not appear in his lists. In addition he had varying results from one set of experiments to another. He attempts to account for these differences in his more recent papers (JOLLOS 1932 and 1933) by suggesting that a wet or a dry environment may be the external agent which determines which genes shall respond by mutation to the effects of elevated temperature. This of course may be true, but until JOLLOS is able to give much more accurate data on relative amounts of moisture in the different culture bottles, the point can certainly not be considered established. The specific mutations are—in JOLLOS's cultures as in ours—entirely unpredictable. It would be quite possible to make out a good case in favor of different genetic constitution as the determining factor—especially in view of TIMOFEEFF'S (1933) recent demonstration that the normal alleles in different stocks may actually be different genes. In view of the lack of agreement between different experiments in the specific genes affected, the only significant general conclusion is that the treatment with high temperature increases the general mutation rate.

NON-INHERITED SOMATIC VARIATIONS

Since every variation noted was recorded and its offspring bred for at least two generations, accurate data are available on those which turned out to be non-genetic. The flies which were actually heated as larvae (generation 1) showed—as would be expected—a very large number of such variations which in this case were direct developmental modifications produced by the elevated temperature. In many cases almost every fly hatched showed some anomaly, and the nature of these direct effects was the same as were those which appeared in smaller numbers in later generations. Actual tests showed that these anomalies were more numerous if the larvae were heated on the fifth and sixth day after the eggs were laid than at any other time in the pre-imaginal stages. Only one such direct modification ever gave rise to a similar mutation, namely a Stubby bristle recorded in table 3 as having appeared in generation 1 of experiment 8. This must be considered as a case of parallel induction.

Direct effects of the elevated temperature are naturally to be expected, and they have little genetic significance. None of these first generation modifications are included in the tables given below, except in the control

TABLE 6

List of somatic variations, or modifications, isolated from cultures not heated as larvae.

HEATED LINES	CONTROL LINES
*rough-eye—50 times (various degrees)	rough—15 times
abnormal abdomen—18	straplike wing—7
straplike wing—15	dark body—3
blistered or balloon-wing—13	abnormal abdomen—2
*minute bristles—12	balloon-wing—2
small or deformed-eye—8	minute bristles—2
*trident—7	spread-wing—2
notch-wing—6	dwarf—2
*forked bristles—5	dark body
small- or miniature-wing—5	short legs
spread- or extended-wing—5	
dwarf or small fly—4	
dark-eye—3	
missing bristles—3	
crumpled wing—3	
dark body—2	
stubby bristles—2	
eye mosaics—2	
bithorax	
glassy-eye	
scarlet-eye	
reduplicated legs	
extra bristles	

* Reappeared in a few flies in later generations.

lines. The somatic variations appearing in later generations are of more interest, and a list of those recorded is given in table 6. While there are more in the heated lines the characters recorded for the controls are much the same. The most frequent in either series is rough-eye, showing varying degrees of disarrangement of the ommatidia. Another frequent variation was some form—usually unsymmetrical—of straplike (or reduced) wings. It is of interest that the most frequent mutations were also varying manifestations of these same characters. It is entirely possible that every mutation ever found in Drosophila could occasionally be paralleled by a similar non-genetic variation. This fact probably has no significance other than that genes act at varying stages in development, and there are only a limited number of possible variations in an adult fly. Naturally the limits of mutation are the limits in the possible variability of the animal. No Dauermodifikationen as described by JOLLOS were found, although in a few cases (marked * in the table) a few similar variations appeared among the offspring. We thus find no specificity in the modifications appearing following heat, nor any significant parallelism with the mutations.

The fact of most interest in regard to the somatic variations in generations after No. 1 is the marked excess in their numbers when the female parent is derived from a heated line. Table 7 summarizes these facts for three experiments in which the data are most adequate. It is shown that when both the male and the female parent, or the female parent alone,

TABLE 7

Summary showing the number of non-inherited variations (somatic variations) isolated and tested from generations not directly heated.

EXPERIMENT NUMBER	TREATMENT	NO. GENERATIONS	NO. FLIES	NO. SOMATICS	PERCENT NO. SOMATICS / NO. FLIES	DIFFERENCE HEATED—CONTROL	DIFFERENCE / P.E. OF DIFFERENCE
VII	♂+♀ heated	2–6	9,898	58	.00585±.00052	.00337±.00065	5.2
IX	♀ heated	2–6	11,657	73	.00626±.00049	.00517±.00059	8.7
X	♂+♀ heated	2	6,702	42	.00627±.00065	.00346±.00069	5.0
X	♀ heated	2	7,407	50	.00675±.00064	.00394±.00068	5.8
Total VII–X	♂+♀ and ♀ only heated		35,664	223	.00625±.00028	.00373±.00034	11.1
X	♂ heated	2–3	45,761	116	.00253±.00005	−.00028±.00023	1.2
						Difference ♀ heated—♂ heated .00372±.00029	12.9
VII	Control	2–6	7,279	18	.00248±.00039		
IX	Control	2–6	4,564	5	.00109±.00033		
X	Control	2–3	24,197	68	.00281±.00023		
Total	Control		36,040	91	.00252±.00018		

were derived from a heated line their offspring show about 2.5 times as many obvious somatic variations as the corresponding controls. Although the increase in rate is less than half as great as that for mutations, the difference is more than 11 times its probable error. The increase is about the same for the total of five generations as for generation 2 alone, indicating that the variability has not decreased materially in the course of five generations. In marked contrast is the result when the male parent comes from a heated line and the female parent from control. Here the number of somatic variations is almost exactly the same as when neither parental line has been heated. The data thus clearly prove that a heat-induced increase in rate of production of non-genetic (in the sense of non-chromosomal) variations is transmitted through female flies but not through males. This is to be interpreted probably as cytoplasmic inheritance. It indicates that the heat induces changes in the cytoplasm manifesting themselves as non-specific modifications, and *these changes* are inherited, at least for several generations.

ARE MUTATIONS INDUCED AT THE TIME OF HEATING?

Since non-inherited (or cytoplasmic) variations continue to appear in generations later than those actually heated, it is of interest to inquire whether the mutations, or gene changes, are produced directly at the time of the temperature change. The data on the mutations are given in table 8. It is convenient here to classify the mutations into the four groups indicated. In all cases given here the heat was applied to generation 1 larvae. Since the germ cells of generation 1 develop into the flies of generation 2, any mutations which came to light could have been—and probably were—produced at the time of heating. X-linked mutations would show in male offspring only, and any autosomal recessives would appear only if the same mutation was produced in both male and female parent. Eight mutations actually did appear in generation 2, of which 7 were dominants and 1 a sex-linked recessive. (We omit from consideration the dominant—Stubby—which came to light in generation 1.) In generation 3, X-linked lethals and autosomal recessive mutations could still have been produced at the time of the heat in generation 1. X-linked visible recessives appearing in males only, and all dominants, clearly must have occurred later than the heat exposure. In the fourth and fifth generations only autosomal recessives could still have been carried without previous appearance from generation 1. All other mutations must have occurred later than the time of heating. It seems certain then—as the table shows—that at least 14 (or 33 percent) of the total number of mutations in heated lines occurred later than the heated generation. It has already been demonstrated that the total number of mutations in heated lines is significantly larger than

TABLE 8

Table of mutations by generations (including experimental lines carried at least 5 generations only).

NO. GENERATION	NO. FLIES	X-LINKED LETHALS (a)	X-LINKED RECESSIVES (b)	AUTOSOMAL RECESSIVES (c)	VISIBLE DOMINANTS (d)	MUTATIONS PRODUCED AT TIME OF EXPOSURE TO HEAT (e)	MUTATIONS OCCURRED LATER THAN TIME OF EXPOSURE TO HEAT (f)
Heated lines							
2	45140	0	1	0	7	(a+b+c+d) = 8 } = 23* = .000216 ± .000030	
3	61124	11	2 (♂ only)	4	3	(a+b) = 15 }	(b+d) = 5
4	34336	2	1	3	1	(c) = 3	(a+b+d) = 4 } = 9** = .000130 ± .000029
5	34780	1	3	3	1	(c) = 3	(a+b+d) = 5 }
Total heated	175380	14	7	10	12	(43) Mutations ÷ No. Flies .000245 ± .000025	Difference ÷ P.E. of difference .000204 / ± .000030 = 6.8
Control lines 1–6	73221	1	1	0	1	(3) .000041 ± .000016	

This table shows that about 33 percent of the mutations in the heated lines must have occurred later than the time of exposure.

* Total mutations generations 2+3 column (e) compared with controls shows: Difference ÷ P.E.

$$\text{Difference} = \frac{.000175}{\pm .000034} = 5.2$$

** Total mutations generations 4+5 column (f) compared with controls shows: Difference ÷ P.E.

$$\text{Difference} = \frac{.000089}{\pm .000033} = 2.7$$

that in the controls. The same is true if the generation 2 and 3 mutations produced at the time of the temperature change (column e) are compared with the controls. Comparing now the number of mutations which appeared in generations 4 and 5, but which occurred after the time of the heat treatment (column f) with the control number, we get a difference which is 2.7 times its probable error. This is certainly a minimum value, since the 5-generation 3 mutations of column f are omitted from the calculations, as are several true somatic mutations, such as a mosaic with white in one eye only. There seems to be no doubt therefore that there is a significant excess of mutations which could not have occurred at the time of heating. This shows clearly that mutations—like non-genetic variations—are increased in number for several generations after the actual exposure to high temperature. Indeed our data indicate an increased mutation rate for generations 6–9 (table 3), but the numbers of flies were not completely recorded.

Our final experiment No. 10 only gave sufficiently large numbers of flies and of mutations to give answers to the question as to how this increased mutability in the heated lines is passed on. The data are shown in table 9. It is clear that the percent of mutations per fly examined in

TABLE 9

*Summary of mutations in experiment 10.**

LINE NO.	TREATMENT	NO. GENERATIONS	NO. FLIES	TOTAL NO. MUTATIONS	PERCENT NO. MUTATIONS / NO. FLIES	DIFFERENCE HEATED—CONTROL	DIFFERENCE / P.E. OF DIFFERENCE
3	♂ *heated* × ♀ *heated*	2–5	11220	5	.000446 ± .000134	.000390 ± .000137	2.9
2	♂ control × ♀ *heated*	2–5	15755	4	.000254 ± .000085	.000198 ± .000089	2.2
1	♂ *heated* × ♀ control	2–5	75449	20	.000265 ± .000040	.000109 ± .000048	2.3
Total heated			102424	29	.000283 ± .000035	.000227 ± .000044	5.1
Control		2–5	36024	2	.000055 ± .000026		

* This table indicates that the number of the mutations is about 5 times that of the controls when either ♂ or ♀ parent is heated. When both parents are heated the number of mutations is nearly 9 times as great.

generations 2–5 is the same whether the male or female line (line 1, 2) has been heated. When both parents come from a heated line the percent of mutations is very nearly doubled. None of these figures is beyond ques-

tion a statistically significant difference from the control, since the differences are between 2 and 3 times their probable error values. However, the total number of mutations is significantly larger than the control, and the trends are all consistent. We seem justified in concluding therefore that there is some effect of the elevated temperature which results in increased mutability in later generations, and that this is transmitted equally through males as well as females. This certainly means that the temperature has an effect on certain genes in either male or female germ cells such that either a mutation occurs immediately, or a mutation is more likely to occur during the next few generations, and it is as likely to occur among the offspring of heated males as of heated females.

Thus it appears that both the relative number of non-inherited variations and the relative number of mutations is increased in several generations subsequent to brief treatment with high temperature, but there is an important difference between the two. The tendency to produce more variations is transmitted through females only (that is, cytoplasmic), while the tendency to produce more mutations is carried through both males and females (that is, chromosomal). The original effect of the temperature would appear to be on the cytoplasm in the one case, and on the chromatin in the other, and there is no evidence that this situation is changed in successive generations, until the effects gradually disappear.

It is of interest to review the previous conclusions in the light of our data just recorded. JOLLOS reported that in certain heated lines a small number of non-inherited somatic variations predominated, and the same characters also appeared as mutations. For instance, in one experiment (JOLLOS 1933b) an extended-wing—shown in his beautiful microphotographs—was a frequent modification, and a mutation with extended-wings occurred. In another case a stock gave a number of somatic variations with curled-up wings, and later a Curly wing mutation ("Curloid") appeared. Occasionally JOLLOS found cytoplasmically transmitted cases of particular modifications, which he calls "lasting modifications" (Dauermodifikationen). He believes that the high temperature caused certain changes in the (germ cell?) cytoplasm which manifested themselves as modifications. These same temperature changes also occasionally brought about some effect on the genes which normally influence the same characters as the modifications. The result was a mutation having a manifestation similar to the modification. The idea is thus paraphrased by GOLDSCHMIDT (1933) p. 547: "The genes produce within the protoplasm active stuffs which are of the same constitution as the genes themselves. Both will react in the same way upon (i.e., in response to?) external conditions, but those within the protoplasm easier than those protected within the chromosomes."

JOLLOS has not published sufficiently complete data to allow an independent judgment of the validity of his ideas, but they have been tentatively accepted by GOLDSCHMIDT. Our data, however, show no specific effect of temperature, either on modifications, or on mutations, nor is there a significant correspondence between the two. Indeed our data do not bear out the idea that the protoplasm is more easily affected by the temperature than the genes, if we leave out of consideration the direct effects of generation 1. It is true that if a comparison be made between $\frac{\text{no. of somatics}}{\text{no. of flies}}$ from the heated male-female and heated female lines of table 7, and $\frac{\text{no. of mutations}}{\text{no. of flies}}$ in the heated lines of table 2, the rate of occurrence of somatics (0.00625) appears to be more than two hundred fifty times greater than that of mutations (0.000024). Such a comparison is entirely inaccurate however, since it does not take into account the number of tests involved. All flies are tests for modifications, but the approximate number of tests for mutations is given by the sum of the rates for the different classes of mutations in table 2, column a–e (0.02428). The rate of mutation thus secured is nearly four times the rate of somatic variations, but the difference is not significant because of the small number of mutation tests in certain classes. Taken in conjunction with the fact already noted that heating increases the number of somatics less than half as much as mutations, these figures indicate that mutations in heated lines actually occur *more frequently per gene* than do protoplasmic variations per cell. There is certainly no evidence that both genes and cytoplasm react in the same specific way under external conditions, and no evidence that "the genes produce within the cytoplasm stuffs of the same constitution as the genes themselves."

Finally our data completely disprove the theory urged by JUST (1932) and reviewed on an earlier page. He held that the cytoplasm responded to external changes, and that the cytoplasmic modifications reacted on the chromatin to produce mutations. Our data show that the tendency to produce an increased number of somatic modifications following heat is carried over by females only, while the increased rate of mutation is carried over by both males and females. The female gametes have large amounts of cytoplasm, while male gametes have little or none. There is no difference in the chromatin. Were the mutations always preceded by cytoplasmic modifications, the increased mutation rate should appear among the offspring of females only. Since it appears in the offspring of both sexes, we may conclude that the effect of heat is directly on the chromatin. Thus heat appears to affect both cytoplasm and chromatin independently.

THE EFFECT OF SUB-LETHAL TEMPERATURE ON CROSSING OVER

Some years ago PLOUGH showed that exposure of the female parent to temperatures above and below 25° resulted in increased crossing over among the offspring in certain sections of chromosomes II and III (PLOUGH 1917 and 1921). The maximum temperature used in this study was 33°, but GOLDSCHMIDT's work showed that brief exposures to still higher temperatures were possible. It was therefore of interest to discover if such brief exposures also increased crossing over, and if so whether such increased values were transmitted as was the tendency to produce somatic variations.

The first question was answered by the work of R. C. MCGOUN, JR., part of which he kindly allows us to quote, since it is still unpublished. He ran a very extensive series of tests using black-vestigial stock crossed to Bristle. (See experiment 5, table 1.) Part of the results are summarized in section A, table 10. It is conclusively shown that even so short an exposure as 4 hours at 38° gives a marked increase in crossing over for the Bristle-vestigial region of chromosome II. This indicates that extreme temperatures affect crossing over like X-radiation, since the effect of 4 hours exposure shows itself among the offspring for at least 12 days. Obviously here, as with the X-ray effect, a change occurs in the chromosomes long before the actual stage of crossing over itself.

Each of us has run several series of tests to determine if this extreme elevation in the crossing over values is inherited. Selected parts of the ten day brood data are summarized in sections B and C, table 10. Section B shows a significant increase in crossing over for the echinus-cut region of chromosome I, not only in the offspring of heated females, but also in the following generation without further treatment. In the fourth generation this effect has disappeared. In section C similar crossing over data for the black-purple region of chromosome II are given. While the increases shown here are less consistent, nevertheless there are indications of a cumulative increase after repeated heat treatments in successive generations. These with other data of the same kind suggest that the heat-induced increase in crossing over in "sensitive" chromosome regions tends to persist for one or more generations after the exposure to heat, in much the same way as does the tendency to produce modifications and mutations.

SIGNIFICANCE OF THE RESULTS FOR EVOLUTIONARY THEORY

JOLLOS (1931) believes that his results prove not only that an agent in the natural environment—high temperature—induces mutations, but that it has a directive influence. He finds that successive exposures of flies

TABLE 10

Summary showing effects on crossing over of short exposures of larvae to sub-lethal temperatures.

A. Percent of crossing over for Bristle-vestigial region chromosome II. Second generation only—Data of R. C. MCGOUN, Jr.

2-DAY BROODS	CONTROLS 25° C	FEMALE PARENTS AT 25° EXCEPT AS INDICATED		
		32° C FOR 24 HRS. ON 5–6 DAY	36°C FOR 24 HRS. ON 5–6 DAY	38°C FOR 4 HRS. ON 6TH DAY
2	6.5±.4	7.0±.5	8.1±.5	9.6±.3
4	6.1±.4	5.6±.4	6.2±.4	9.0±.4
6	5.6±.3	4.5±.3	6.9±.4	8.9±.4
8	7.0±.3	5.8±.3	9.6±.5	9.3±.4
10	6.7±.3	5.3±.3	10.3±.6	8.8±.5
12	7.7±.3	7.3±.4	10.1±.7	8.2±.5

B. Percent of crossing over for echinus-cut region of chromosome I in successive generations. Data of P. T. IVES

TREATMENT	GENERATIONS		
	2	3	4
Controls 25°C	Total	14.8±.3	
Heated generation 1 only	16.4±.3	16.4±.5	14.7±.5

C. Percent of crossing over for black-purple region of chromosome II in successive generations. Data of H. H. PLOUGH

TREATMENT	GENERATIONS			
	2	3	4	5
Controls 25°C	Total		04.0±.29	
Heated Generation 1	05.3±.4	02.4±.3	02.6±.3	03.3±.3
Heated Generation 1, 3			04.8±.3	05.2±.3
Heated Generation 1, 3, 4				07.7±.4

Note:—In the heated lines values which show significant increases over the controls are underlined.

carrying the mutations first induced show allelic mutations in a step-by-step series to an extreme condition. He points out in a footnote (p. 282) that this does not mean that the series of changes would be limited to those whose genes are known to show a series of multiple alleles, for it may be assumed that all genes may show several alternative conditions. He contends therefore that short exposures to high temperature bring about the appearance of a graded series of characters, not necessarily adaptive, which

are then subjected to the sorting action of natural selection. Those which have survival value will be preserved, and because of their appearance in successive steps, the end result will be a straight line or orthogenetic series. Paleontologists have cited many supposed cases of such orthogenetic evolution, and JOLLOS refers especially to the studies of SEWERTZOFF showing a step-by-step reduction of the limbs in the snake-like lizards.

Interesting as this argument is, it will not stand the test of detailed analysis. With the initial statement that short exposures to sub-lethal temperature induce mutations we agree, since our results clearly confirm those of GOLDSCHMIDT and JOLLOS in this respect, and indeed MULLER (1928) had already published data indicating such a result before GOLDSCHMIDT's publication appeared. The second contention of JOLLOS that high temperature has an influence on the direction of evolution by the particular mutations induced can be true only if *all* natural mutations are induced by temperature changes. Now it is probable that a certain portion of natural or spontaneous mutations are so produced, but hardly all of them. Radiation—X and ultra-violet—and perhaps chemical agents may be expected to induce a certain fraction of natural mutations. We have found 3 mutations in 73,000 control flies kept at constant temperature, and there are dozens of "spontaneous" mutations which have appeared in cultures carried under similar controlled conditions. If a portion of the natural mutations arise from other causes, then all the different kinds of mutant alleles may be expected to appear during a long interval of time. High temperature then merely increases the rate of mutation for all or for certain specific genes. The mathematical result of an increase in mutation rate in a population subject to natural selection has been analyzed independently by HALDANE, FISHER and WRIGHT. Each one has reached the conclusion that there could be no appreciable effects on the direction of evolution.

There are two classes of mutations involved. First there may be mutations which confer a definite advantage on their possessors in natural selection. In his book *The Genetical Theory of Natural Selection* (1930) FISHER concludes (p. 77): "A mutation, even if favorable, will have only a very small chance of establishing itself, if it occurs once only. If its selective advantage is only 1%, it may well have to occur 50 times, but scarcely in mature individuals as many as 250 times, before it establishes itself in sufficient numbers for its future prospects to be secure . . . . Consequently the success of such a mutation must become established at a time when the mutation rate of the mutation in question is extremely low."

The important point for our discussion is that mutations conferring a selective advantage may be expected to survive and to increase in numbers when the mutation rate is very low and thus quite independently of the

increased rate due to heat. Natural selection determines the survival of advantageous mutations, and heating would not change the situation.

The second class of mutations are those which are neutral or slightly disadvantageous to their possessors in competition. The JOLLOS idea means here that such mutations appear with sufficient frequency to increase in numbers against selection. The mathematical situation involved was first adequately analyzed by HALDANE, and is summarized in his book *The Causes of Evolution* (1932). He shows that mutants which are even slightly disadvantageous will not increase unless the frequency of appearance exceeds the tendency of selection to favor the original type. He concludes (p. 109): "Even under the extreme conditions of MULLER'S X-ray experiments, when mutation was a hundred and fifty times more frequent than normal, a disadvantage of one in two thousand would have kept any of the new recessive types quite rare."

JOLLOS has given no data from which to calculate either the frequency of appearance or the increase in rate of mutation over the controls. Our summary in table 2 shows that frequency of appearance for all recessive autosomal mutations is in the neighborhood of 1 in 100, while no single mutation even approaches this rate. The general increase in mutation rate is between 5 and 6 times. Thus neither index shows an increase in mutation rate sufficient to overcome even a slight adverse selection.

If the increase in mutation rate has no influence on the survival of favorable mutations, and is insufficient to preserve neutral or unfavorable ones, the contentions of JOLLOS as to the directive effect of temperature on evolution are groundless. To quote from FISHER (p. 48): "It has been seen . . . that it is scarcely possible . . . to ascribe to mutations any importance in determining the direction of evolutionary change; their importance in evolution lies in playing the very different role of maintaining the stock of genetic variance at a certain level, which level in its turn *is a factor in determining the speed, though not the direction of evolutionary progress.*" (Italics ours.)

Precisely this conclusion may be applied to the effect of temperature. A general increase in mutation rate could increase the speed of evolution, but it can have no directive influence whatever.

Although it is apparent from the above discussion that the results which JOLLOS has observed cannot be expected to bring about the effects on evolution which he claims, it is reasonable to point out that our data do not bear out his conclusion that heating produces a step-by-step series of alleles. He cites in particular two cases (1930 and 1931). The first is the white series and the second the ebony alleles. In each case he found in heated stocks one of the "weaker" members of the allelic series, and following successive exposures to heat isolated successively "stronger" allelic genes in the series. Specifically, $W(+)$ gave successively w^e (eosin), then

"yellow" eye color, then "ivory" eye, and finally white, while "weakest sooty" gave "weak sooty" and so on to sooty or ebony. While it is possible that high temperature may tend to cause gene changes in a particular direction, in our cultures no evidence of such an effect was found. The one mutation in the *W* gene which appeared in a heated culture was *w* or white itself, while in a control culture we found w^{bl} (blood) which is a very "weak" member of the series. The work of MULLER (1920) and more recently of TIMOFEEFF (1932) has shown that mutations in the white locus tend to occur more frequently in the direction $W \rightarrow w$, and GOLDSCHMIDT'S original report lists white as one of the first heat-induced mutations. Since probability favors the finding of a series such as JOLLOS isolated, it seems more reasonable to suppose that heating simply speeds up mutation in a way already determined by the chemical architecture of the gene.

Without in any way questioning JOLLOS'S observations on the sooty series, we suggest that it is not an easy matter to determine the allelism of the intermediate conditions. We isolated two separate black trident stocks from heated lines exactly resembling the "weak sooty" of JOLLOS in appearance. The condition seemed to be caused by a multiple factor complex, at least one of the genes of which was located in chromosome III, but not at the sooty locus. Heating of these stocks during three successive generations gave no enhanced mutations, but selection of the darkest individuals gave a markedly darker average grade as long as selection was maintained. If a true sooty mutation had occurred in these stocks almost the same series described by JOLLOS would have been repeated. Actually, however, a black-bodied mutant appeared in an entirely different heated line. Finally we should emphasize that at the vestigial locus we found four separate mutations in heated lines, and one in the controls. MOHR (1932) has demonstrated the existence of a graded series of alleles for this gene. Nevertheless our mutants were all extreme alleles, and each was derived from normal parents. It is legitimate, in view of these results, to question the significance of JOLLOS'S findings in the sooty or similar series.

In a recent very thought-provoking discussion WRIGHT (1932) indicates "that evolution depends on a balance among its factors," among which are a certain rate of mutation, a moderate amount of selection, a certain ratio of inbreeding. Disturbance of this balance by increasing any one factor would not, he believes, lead to more rapid evolution. Since frequent fluctuations in temperature are normal in the environment of a species like Drosophila, it may reasonably be assumed that they constitute an important factor in maintaining a certain mutation rate in nature. Apparently the only significant fact for evolutionary theory suggested by these studies of JOLLOS, and ourselves, is that one element in the equilibrium required for evolutionary change, namely a certain mutation rate, may be maintained by temperature variations in the natural environment.

SUMMARY

1. Ten separate experiments were run to test the GOLDSCHMIDT-JOLLOS method for the induction of mutations in *Drosophila melanogaster* by brief exposures of larvae to sub-lethal high temperature. The results definitely confirm the findings of GOLDSCHMIDT and JOLLOS that the treatment increases the number of mutations.

2. A total of 186,000 flies from five generations of lines heated once yielded 44 mutations, while 73,000 control flies from the same lines gave only 3 mutations. The rate of mutation was thus increased about sixfold.

3. Classification of the different kinds of mutations from the heated lines showed in comparison with the number of tests made that sex-linked lethals were the most frequent, then autosomal recessives, next chromosomal aberrations and finally sex-linked recessives and visible dominants.

4. Several new visible mutations were found, including Glued-eye, a chromosome III dominant lethal when homozygous; and a new vestigial allele, vg^{N^o}, a dominant notch, which is wingless in homozygous condition.

5. Of 34 loci showing mutations, 10 gave 2 or more re-mutations, 3 occurred 3 times, and 2 appeared 4 times. This indicates that the heat tends to induce mutations in some genes more than others. None of these genes were the same as those found by JOLLOS to give recurrent mutations.

6. Non-inherited somatic variations among the offspring of heated females were 2.5 more frequent than in the control lines. When only the male parent was heated, however, no increase in somatic variations was found. The increase thus appears to have been due to inherited cytoplasmic effects.

7. Tabulation of the mutations by classes and generations showed that at least one-third must have occurred later than the time of exposure to high temperature. This suggests some effect on certain genes which is inherited and later results in mutations. An equal number of mutations appeared among the offspring of heated males and heated females.

8. No cases of Dauermodifikationen were observed, nor was there any significant correspondence between non-inherited variations and the mutations which appeared. This fact makes doubtful JOLLOS's conception that cytoplasm and the "corresponding" genes react in the same way to external conditions. Rather, high temperature appears to produce an independent increase in rate of both cytoplasmic modifications and mutations.

9. The inherited effect of temperature in producing modifications was transmitted through females only (6) and that producing mutations through both males and females (7). These facts disprove JUST's contention that external agents act first on the cytoplasm and the changed cytoplasm in turn on the chromatin.

10. The brief treatment with sub-lethal high temperature caused a marked increase in crossing over in certain regions of chromosome II which showed itself among the offspring for ten days. In this it resembled the effect of X-radiation. The increased crossing over caused by heat treatment showed a tendency to be carried over for one generation, as in the case of modifications and mutations.

11. Treatment of successive generations with high temperature gave no evidence of the production of a step-by-step series of alleles as reported by JOLLOS.

12. The increase in the rate of mutation found in these experiments is entirely inadequate to cause survival of any particular mutation if it is even slightly disadvantageous in competition. Thus the claim of JOLLOS that high temperature would bring about orthogenetic evolution is without foundation.

13. It is suggested that temperature variation may be one cause of natural mutations.

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