for galactic obscuration, etc., give a slightly smaller displacement. These second-order corrections, together with the effect of magnitude errors, will be more fully discussed in a *Contribution* from the Mount Wilson Observatory.

Meanwhile it is evident that, within the small uncertainties, the isolated nebulae exhibit the same relation as the cluster nebulae and hence that their luminosity functions are closely similar. The important quantity M_0 is most reliably derived from the Virgo Cluster whose distance is indicated by stars involved in the later-type spirals. Reëxamination of data available at present suggests no significant revision of the earlier estimate,[2] -13.8, although the round number -14 might be used with about equal justification pending the detailed analysis of the Virgo Cluster.

The statistical correction, $M_0 - \overline{M}$, affects investigations of distribution by counts of nebulae and reduces the previously[3] estimated values of the density of matter in the observable region by a factor of the order of three. At great distances the correction diminishes as the red-shifts increase, and beyond about the nineteenth magnitude the variation cannot be ignored.

[1] *Mount Wilson Comm.*, No. 105; *Proc. Nat. Acad. Sci.*, **15**, 168–173 (1929).

[2] *Mount Wilson Contr.*, No. 427; *Astrophys. J.*, **74**, 43–80 (1931).

[3] Magnitudes are corrected for galactic latitude by the cosecant law $\Delta m = 0.15 (\text{cosec } \beta - 1)$. *Mt. Wilson Contr.*, No. 485; *Astrophys. J.*, **79**, 8–76 (1934). The corrections are required by the fact that most of the faint nebulae are in very low latitudes.

[4] K. G. Malmquist, "On Some Relations in Stellar Statistics," *Arkiv för Matematik. Astronomi och Fysik*, Stockholm, **16**, No. 23 (1921).

HEAT INDUCED MUTATIONS IN DROSOPHILA

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Although irradiation by x-rays remains the only reliable method for inducing mutations artificially, the discovery by Goldschmidt[1] that gene mutations were induced by exposing larvae of *Drosophila* for 24 hours to a sub-lethal temperature of 36° has stimulated interest in the problem of the origin of mutations in nature. The result had been suggested by the previous work of Muller and Altenberg[2] and of Muller,[3] but the results of Goldschmidt and more recently of his co-worker Jollos[4] constitute an important contribution to genetics which is the more interesting since there have been so many previous breeding experiments with *Drosophila* at

high temperatures. Since Muller and Mott-Smith[5] conclude that natural radiation is inadequate to account for mutations in nature, it seems possible to suggest that ubiquitous temperature variations may play that rôle.

Jollos has carried the work much further, and he believes that his data show the induction of specific and corresponding cytoplasmic and gene changes, as well as the calling forth of directed (gerichtete) mutations and their enhancement in a definite direction. His views are paraphrased by Goldschmidt ([6], 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 external conditions, but those within the protoplasm easier than those protected within the chromosomes."* Finally, he believes, successive allelomorphs of the same gene are produced by repeated exposures to high temperature which result in more and more extreme manifestations of the same characters. Jollos concludes not only that exposure to high temperature causes mutations, but that his results furnish the experimental basis for orthogenetic or straight line series in evolution in cases where the characters are favored by natural selection.

Such ambitious claims have naturally aroused much interest and a good deal of skepticism. Obviously it is desirable that the work should be repeated and the conclusions independently tested. This has been done by a number of Drosophilists with somewhat conflicting results. (Cf. Muller[7] for references.) Our own tests of the Goldschmidt method of treatment were begun by the senior author in 1930 and the junior author has borne the greater share of the work during the past two years. So far as possible we have followed the method of Goldschmidt and Jollos exactly, but we have kept somewhat more extensive records, at least than the latter has so far published. Certain of our results have been reported at intervals in abstract form to meetings of the Genetics Society,[8] and the full report will appear in *Genetics*. The publication date is, however, so far in the future that it seems desirable to make available a summary at this time.

In all, 10 separate experiments were run in which one or both parents were treated with high temperatures following the Goldschmidt method. These involved several wild stocks including the Florida culture, mutant stocks having gene mutations from Chromosomes I, II and III, and the X lethal testing stocks XX yellow and Cl B. In several experiments crossing-over data were assembled in addition to data on lethal recessive and dominant mutations. In several of the experiments the series were continued for as many as 10 generations after treatment. In every test all somatic variations of any sort were recorded. All were mated and their offspring followed for at least two generations. When a mutation occurred it was located by the usual methods, and if it appeared to be an allelomorph of a known gene, its allelomorphism was established by crosses to the proper stock when that was available. Controls were run for all the series, and

they were continued with the same tests of variations for the same number of generations as the similar heated stocks. We thus have a considerable body of data, from which are available comparisons of mutation rates for all types of mutations, and also comparisons of the relative numbers and kinds of somatic or non-inherited variations. Altogether the totals of all these experiments are in excess of 200,000 flies in heated lines and close to 100,000 control flies. These constitute a mass of data bearing on the effect of brief exposures to sub-lethal temperature on mutation which seems adequate for conclusive answers to the questions raised.

TABLE 1

TOTAL NO. FLIES	NO. MUTATIONS FOUND (A) X LINKED LETHALS	(B) X LINKED RECESSIVES	(C) AUTOSOMAL RECESSIVES	(D) VISIBLE DOMINANTS	(E) CHROMOSOMAL ABERRATIONS	TOTAL NO. MUTATIONS / TOTAL NO. FLIES
186,426	13	7	9	13	2	$\frac{44}{186,426} = 0.000236 \pm 0.0000240$
73,221	1	1	0	1	0	$\frac{3}{73,221} = 0.000195 \pm 0.0000283$

In table 1 are summarized the mutations found in the first five generations of the lines heated once and the corresponding controls. The difference is here 6.7 times the probable error of the difference, which makes the chance that the two values are identical of the order of 1:250,000. Since the data indicate that the number of mutations in the heated lines is nearly six times greater than in the controls, this constitutes clear confirmation of the Goldschmidt-Jollos results. Taking into account the number of tests of each of the various kinds of mutations, we find that the visible dominants (d) show the lowest frequency of mutation and that autosomal recessives (c) and X linked lethals (a) show a frequency about 150 times greater. In these totals no mutations are included which appeared in both heated and control lines, nor are doubtful or irregular cases. The totals therefore show minimal rather than maximal values.

In all, visible mutations were found in 24 loci in all chromosomes, and X linked lethals in 10 loci. Most of these loci were known, but in several cases new allelomorphs were found. The most striking of the latter, which occurred 3 times in heated lines and once in the controls, was a dominant vestigial allelomorph called vg^{No}. It has been identified by Dr. O. L. Mohr as being next in the series of vestigial allelomorphs to "no wing." In heterozygous condition it shows a notch in each wing, and in homozygous state it is wingless and the females are sterile. Another striking dominant is Glued eye (Gl), which is a new chromosome III locus close to Dichaete. At least two mutants to the dominant Star eye (Chromo-

some II) were found, while of the known recessive mutations garnet (Chromosome I) occurred in heated lines 3 times, brown (Chromosome II) twice and among others white (from normal) once. There is no evidence whatever of the appearance of allelomorphs in a step-by-step series as recorded by Jollos, even when mutated stocks were given several successive treatments. In general, the results resemble those shown in spontaneous or natural mutation except that the rate of mutation is increased about six times.

It is of interest, however, that the number of recurrent mutations found is considerably in excess of those found spontaneously. The 49 mutations which appeared in the complete series of heated lines gave a total of 34 different loci. Ten of these gave 2 or more mutations in the same locus, 3 gave 3 or more and 2 gave 4. Using the formula for calculating mutation rate (Muller[3]) these figures might be expected to fit into a simple proportion. As a matter of fact they indicate a much increased tendency for remutation in certain loci. This result indicates that the treatment with high temperature causes certain genes to mutate with a greater frequency than others. However, the mutations which have recurred most frequently in our experiments do not correspond with any of those reported as recurring by Jollos, nor indeed do his results correspond from one experiment to another. Until it can be shown that the same treatment produces consistent remutations in certain particular loci, the only safe conclusion is that exposure to high temperature increases the general mutation rate as already indicated.

Since every anomaly of any sort which occurred in the heated and control lines was tabulated and bred for two generations, accurate data are available on the number and kinds of non-genetic or somatic variations (modifications). Such variations occurred in a large percentage (often 100%) of the first generation flies directly subjected to heat. In successive generations they continue to occur in heated lines for five or six generations at a rate at least three times greater than in the control lines. Although they are in greater numbers in the heated lines, these non-inherited anomalies are not different in kind from those in the controls. In each the most common anomaly was one or another type of rough eye—the facets irregularly disarranged—and each showed a number of irregular, reduced or unsymmetrically strap-like wings. It is of interest, though probably not significant, that these correspond with the most frequently occurring mutations. While a few of these somatic variations reappeared in an occasional fly in later generations we found no single case of an "enduring modification" in the sense of Jollos. We did find clear evidence that the increased tendency to produce somatic modifications was inherited, but through the female line only. The offspring of heated males gave no increase in the number of non-genetic variations over the controls. It is of great interest

that the mutations themselves show no such relation. The number of mutations among the offspring is approximately the same whether the male or the female parent has been heated, and is approximately doubled when both parents have had the heat exposure. A portion of the data showing this contrast is summarized in table 2. These and other similar data

TABLE 2

DATA FROM EXPERIMENT X ONLY

TREATMENT	GENERATIONS NO.	NO. FLIES	NO. SOMATIC VARIATIONS	% NO. SOMATICS / NO. FLIES	DIFFERENCE (FROM CONTROL) / P.E. OF DIFFERENCE
♂ + ♀ heated	2	6,702	42	0.00627 ± 0.000650	5.0
♀ heated	2	7,407	50	0.00675 ± 0.000642	5.8
♂ heated	2 + 3	45,761	116	0.00253 ± 0.000050	−1.2
Control	2 + 3	24,197	68	0.00281 ± 0.000230	

TREATMENT	GENERATIONS NO.	NO. FLIES	NO. MUTATIONS	% NO. MUTATIONS / NO. FLIES	DIFFERENCE (FROM CONTROL) / P.E. OF DIFFERENCE
♂ + ♀ heated	2 − 5	11,220	5	0.000446 ± 0.0001344	2.9
♀ heated	2 − 5	15,755	4	0.000254 ± 0.0000854	2.2
♂ heated	2 − 5	75,449	20	0.000265 ± 0.0000400	2.3
Control	2 − 5	36,024	2	0.000055 ± 0.0000265	

apparently leave no doubt that mutations result from an effect of the heat on the chromatin of either male or female germ cells, but that non-inherited somatic variations result from an effect of the heat on the cytoplasm which is carried over in the egg only and not in the sperm.

These results appear to throw doubt on the correctness of Jollos's view that external conditions like temperature react in the same way upon the same stuffs in cytoplasm and chromatin. Rather the effect seems to be non-specific, and to induce a general increase in the "natural" rate of spontaneous cytoplasmic and gene changes.

Finally a tabulation by generations of the mutations from heat treated lines indicated that 67% of them might have been produced by direct effect of the heat on the genes at the time of application. At least 33%, however, must have occurred later than the time of treatment. Since even this fraction appears to constitute a significant increase over the number of mutations in the controls, this result indicates that the heat produces changes in certain genes which only later manifest themselves as mutations. This delayed effect has not been noted following x-radiation.

While our data confirm the observations of Goldschmidt and Jollos that brief exposure to sub-lethal high temperature brings about a marked increase in mutation rate, we cannot agree that this can be expected to bring about orthogenetic evolution. As Haldane[9] has shown by mathematical analysis, mutations that are only slightly disadvantageous could

survive against selection only as a result of much higher mutations rates than are here found. On the other hand advantageous mutations would be selected without regard to this slight increase in rate.

These results suggest, however, that temperature variations in nature may be an important cause of spontaneous or natural mutations.

[1] Goldschmidt, *Biol. Zbl.*, **49**, 437–448 (1929).

[2] Muller and Altenberg, *Proc. Soc. Exp. Biol. Med.*, **17**, 10–14 (1919).

[3] Muller, *Proc. Int. Congr. Plant Sci.*, **1**, 897–921 (1928).

[4] Jollos, *Biol. Zbl.*, **50**, 542–554(1930); Suppl. Bd., *Zool. Anz.*, **5**, 252–295(1931); *Naturwis.*, **21**, 831–834(1933).

[5] Muller and Mott-Smith, *Proc. Nat. Acad. Sci.*, **16**, 277 (1930).

[6] Goldschmidt, *Science*, **78**, 539–548 (1933).

[7] Muller, *Proc. Sixth Int. Congr. Genetics*, **1**, 213–255 (1932).

[8] Plough and Ives, *Proc. Sixth Int. Congr. Genetics*, **2**, 156–158(1932); Plough, *Amer. Nat.*, **57**, abstr. (1933).

[9] Haldane, *The Causes of Evolution*, Harpers (1932).

* In a recent abstract of our work (Plough and Ives, *Amer. Nat.*, **58**) we appear to have misstated Jollos's views due to a mistranslation of his conclusions. We took issue with his supposed belief "that induced somatic variations subsequently become genetic," when in fact his view as stated several times in his papers is that of a parallel effect on genes and similar gene products in the cytoplasm. Our data agree much more closely with this interpretation as the subsequent summary shows. Our error, which we regret, was kindly called to our attention by Professor A. H. Sturtevant, and more recently by Professor Jollos himself.

BLOOD-GROUP INCOMPATIBILITY IN RABBIT EMBRYOS AND IN MAN

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It is known that transfusion of blood from one individual to another may have disastrous results if donor and recipient belong to incompatible blood-groups. It is known, also, that blood-groups are determined by inheritance from both parents. If husband and wife happen to belong to different blood-groups, is it possible that the child may have blood incompatible with that of the mother and thus be in danger of having its blood agglutinated prior to birth? This question has often been raised but seems not to have received a conclusive answer. It is known that mothers of a particular blood-group may produce living children of any blood-group whatever, so that it seems improbable that any harmful effect follows a difference in blood-group between mother and child. The question then arises, how are harmful effects, such as may occur in