OBSERVATIONS OF SUPERNOVA NO. 3

PLATE	DATE	MAGNITUDE	PLATE	DATE	MAGNITUDE
AC 23756	June 4, 1921	[12.5	AI 21438	Dec. 19, 1921	[10.8
AI 21306	Oct. 29, 1921	[11.4	AI 21452	21	11.5
AI 21372	Dec. 5, 1921	11.1	AI 21461	27	11.8
I 40780	7	11.1	AC 24549	30	[12.1
AC 24449	7	11.1	AC 24553	31	[11.5
AI 21393	7	11.1	AI 21494	31	[12.6
AI 21404	8	11.1	MC 18301	Jan. 1, 1922	12.8
AC 24462	8	11.0	AI 21502	2	[12.6
AI 21416	11	11.0	AI 21551	23	[12.8
AC 24497	19	11.2:	I 40912	26	14.1

For more than two weeks in December, 1921, supernova No. 3 equaled or exceeded in brightness the whole spiral nebula in which it appeared.

The various position coördinates in NGC 3184 were measured by Mr. Cunningham and Miss Boyd; for No. 2, in agreement with Zwicky and Hubble, they obtain the coördinates 162″ and 34″.

To test the possibility that any one of these three stars might have been bright at some time other than indicated above, we have examined 1393 Harvard photographic plates distributed throughout the interval 1890 to 1939, with all the years represented except 1895. Thirty-one per cent of these plates show stars fainter than the thirteenth magnitude; seventy-one per cent, fainter than the twelfth magnitude. No further images of the three objects were found. The negative results of this detailed search, and the position of the images in the arms of the spiral, leave little doubt but that the objects are actual members of the spiral.

[1] *Harv. Ann.*, **88**, No. 2 (1932).
[2] Mt. Wilson Contr. 548 (1936).
[3] Letter from Zwicky and Hubble, July 31, 1939.

DISTRIBUTION OF INDUCED BREAKS ALONG THE X-CHROMOSOME OF DROSOPHILA MELANOGASTER[1]

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Introduction.—A study of the distribution of induced breaks along the chromosomes of *Drosophila melanogaster* was made by Bauer, Demerec and Kaufmann.[2] From the data accumulated at that time it appeared that break frequency was proportional to length within the limits of the euchromatic portion of each salivary chromosome limb, but that the frequency

was disproportionately high in the proximal heterochromatic regions. There was a close parallel, however, for the longer autosomes between frequency of observed breaks and length as measured in the late prophase or metaphase mitotic chromosomes. Break frequency in *X*-heterochromatin was much lower than expected on this criterion (about 22 per cent, including the correction for undetectable breaks, as compared with 33.3 per cent expected).

The collection of more extensive data seemed warranted, therefore, in order to measure the reliability of these and related findings. In securing such data, which form the basis of the present report, analysis was restricted to the *X*-chromosome because of the availability of Bridges' 1938 map,[3] and the possibility presented thereby of accurately localizing break position within the limits of the lettered subdivisions. Up to the present time more than 600 additional breaks in the *X*-chromosome have been mapped from a study of about 4000 pairs of salivary glands. Final determination of break position was made in each case by the writer so that greater uniformity of cytological analysis would be guaranteed.

About 44 per cent of all breaks here reported (278 out of 627) were in chromosomes carrying the delta 49 inversion, the remainder in chromosomes having the wild-type sequence of banding. Break distribution in and adjacent to inversion points was studied as a measure of the frequency of reinversion, inasmuch as Grüneberg[4] and Emmens[5] had reported a case of reinversion correlated with a change from the phenotypic effect "roughest" to wild-type. Moreover, since the present study was initiated, there has appeared the report of Sitko[6] of increased mutability in zones adjacent to the breakage points of inverted sections.

Break Frequency Adjacent to Limits of dl-49 Inversion.—A comparison of break distribution in the *dl*-49 chromosomes with those of the wild-type reveals no significant differences. In both cases many of the same sections conform closely to expected values, and others deviate sharply therefrom. The expected values here mentioned have been calculated from the number of bands per subdivision, since this criterion was regarded as a more reliable estimate of relative length than measurements made along the map. The numbers of observed breaks in subdivisions 4*D*, *E*, *F* and in 11*E*, *F*, 12*A* (the subdivisions closest to the limits of the inversion) were 1, 2, 4 and 1, 3, 0, respectively, as compared with expected values of 1.51, 0.65, 3.02 and 2.80, 1.72, 2.16. It is apparent that there are no constellations of breaks centering about the limits of the inversion. This finding, coupled with the fact mentioned above of general similarity of break distribution in both types of chromosomes studied, points to the conclusion that the *dl*-49 sequence is neither more fragile nor more labile in its response to x-radiation than is the control. Accordingly the data as a whole may be considered and general conclusions derived therefrom.

Breaks in Proximal Heterochromatin.—The first consideration relates to the proportion of breaks which occur in the proximal heterochromatic region, subdivisions 20*A*–*F*. The value obtained, based on observed breaks, ranges between 21.6 per cent and 24.2 per cent, depending on the distribution of certain cases which are regarded as questionable because the single slide available for each rearrangement did not afford a sufficiently large number of nuclei to permit accurate diagnosis. If these questionable cases are apportioned among the chromosomes on the basis of the relative mitotic lengths of their proximal heterochromatic regions, the percentage of breaks within the *X*-heterochromatin rises to a value of about 23 per cent.

In addition to these observed breaks there are others which delimit alterations restricted to the proximal heterochromatin. Such breaks usually remain undetected, primarily because of the close pairing which obtains within the chromocentral region. Some adjustment should be made,

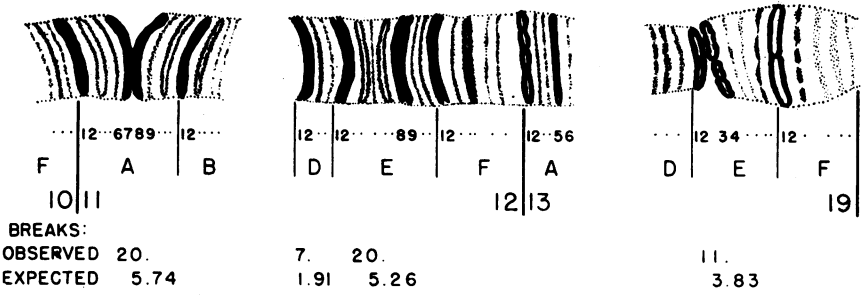

FIGURE 1

Intercalary regions of *X*-chromosome of *D. melanogaster* with highest break frequency. Comparison of numbers of observed and expected breaks shown below. Drawing and lettering from Bridges' 1938 map.

therefore, so as to incorporate an estimate of the frequency of such cytologically undetected changes. When this adjustment is made, using as a basis for the calculation the proportions of euchromatin to heterochromatin found in mitotic chromosomes, the percentage of breaks in *X*-heterochromatin reaches a value of 28.4 per cent. This is somewhat short of the expected 33.3 per cent but nevertheless does not exclude the possibility that break frequency is proportional to mitotic chromosome length.

Distribution of Breaks within the So-called Euchromatic Limb.—The positions of 475 breaks have been determined within the limits of the 114 subdivisions 1*A* to 19*F*. Certainly there are no long regions of the *X*-chromosome which are immune to induced breakage, since only 3 (2*D*, 6*B*, 15*B*) of the 114 subdivisions failed to show any breaks. At the other extreme, 2 subdivisions (11*A* and 12*E*) each were broken in 20 cases (Fig. 1). If break frequencies are plotted against values expected on the basis of the

number of bands per section, several striking departures from randomness are apparent, and a consideration of the entire chromosome shows that the values obtained are significantly different from expectancy (as computed by the χ^2 method, the difference equals about 7 times the standard error). The most striking differences between expected and observed frequencies were found in sections 11*A*, 12*D*, 12*E* and 19*E* (Fig. 1). Other regions with a break frequency that appears to fall outside the limits of chance distribution are 1*F*, 4*E*, 7*B*, 7*C*, 8*B*, 16*E*, 16*F* and 19*F*. It is quite probable that the accumulation of more extensive data will reveal the presence of other similar regions along the chromosome.

Discussion.—It is apparent that the findings of the present study do not support the assumption that break distribution is entirely at random along the length of the so-called euchromatic portion of the *X*-chromosome. Rather it is clear that there are subdivisions with a break frequency so high as to simulate thereby the behavior of the proximal heterochromatic region. This suggests therefore that these intercalary regions also contain heterochromatin. Certain cytological observations support this interpretation. The intercalary regions with highest break frequency, namely 11*A*, 12*D*, 12*E* and 19*E*, occasionally pair with each other, or with the chromocenter, a type of affinity that is characteristic of the heterochromatic chromocentral material. It follows, therefore, that the properties of heterochromatin may account for such pairing as well as for the high incidence of induced breakage occurring within these subdivisions. Moreover, as figure 1 shows, subdivisions 11*A*, 12*D*, 12*E* and 19*E* are characterized by repetition of sequences of bands, the so-called "reverse repeats." Conflicts in pairing between the bands of the repeat sequences of one chromosome and those of the homologous chromosome may lead to distorted or diagonal synapsis, and frequently the salivary chromosome appears to be broken completely across (occasional pairing within the repeat is found in the unsynapsed or haploid strand). Such behavior may likewise be attributable to the presence of heterochromatin. It seems unnecessary to regard these regions as "weak" spots in the sense that they represent portions of the chromosome axis in which there exists primarily a reduced longitudinal cohesion.

Some of the other regions with a break frequency that seems to fall outside the limits of chance distribution appear cytologically to represent repeats (as, for example, 8*B*); others show no conspicuous repetition of banding. There is no *a priori* reason why intercalary heterochromatic regions should be associated with repeats, except, as Muller[7] has pointed out, duplications within "inert" material would cause less functional disturbance than in "active" regions, and therefore they would have greater survival value. On the other hand, certain regions which appear cytologically to represent repeats, such as 2*B* and 3*C*, have not shown a high break frequency in the present experiments. That 2*B* and 3*C* contain

heterochromatin has been reported recently by Prokofyeva-Belgovskaya,[8] who bases her diagnosis on the cytological interpretation of pairing phenomena arising from "the capacity of inert regions to conjugate with one another." This cytological method of analysis and the break frequency approach of the present paper both lead to the conclusion that regions 7*C*, 11*A*, 12*E*, 19*E* and 20*A*–*F* contain heterochromatic material. On the other hand, several of the subdivisions which Prokofyeva interprets as heterochromatic have shown no increased break frequency in the present study, although high frequency may occur in an adjoining region. Thus, Prokofyeva reports an inert region in 17*A*, whereas in the present study this subdivision showed fewer breaks than expected (1 as compared with 5.74). Subdivision 16*F*, abutting on 17*A*, was broken, however, in 9 different cases, in comparison with the expected 3.83. One other region which may be mentioned as showing strikingly different behavior in the two studies is the tip of the *X*. Subdivisions 1*A*–1*F* all show break numbers higher than expected values, but not deviating sharply therefrom except in the case of 1*F*. Prokofyeva, however, has presented evidence that 1*A* is heterochromatic and that the presence of inert regions at the distal ends of chromosomes is a fundamental feature of their structure.[9]

In order that such differences may be appraised more critically and that pronounced trends in break frequency may be distinguished from chance deviation, the procuring of additional data seems warranted. It is apparent that in the study of Bauer, Demerec and Kaufmann,[2] divisions 11 and 12 showed the trend toward high break frequency that can now be clearly demonstrated, although with the limited number of breaks then available, the distribution for the entire chromosome gave no significant deviation from randomness. It seems best, therefore, to extend the present study before considering the apparent differences in pairing phenomena and response to irradiation shown by heterochromatin in various parts of the *X*.

The distribution of heterochromatin throughout the *X* in the course of its evolution probably has involved rearrangement primarily through inversion and the production of reverse repeats, types of changes both of which have been induced experimentally. In the *Y*-chromosome of *Drosophila miranda* as MacKnight[10] has recently reported there exists an alternating series of euchromatic and heterochromatic segments, probably arising in a similar manner.

Such evidence for the *X* suggests the possibility of the existence of similar heterochromatic regions along the limbs of the longer autosomes of *D. melanogaster*. From the standpoint of the confirmatory cytological evidence, attention is directed to the foremost of the areas of confused pairing which exist in the left limb of the second chromosome, forming the "loops" and "turn back" of Bridges' original description.[11] It seems quite probable that the production of mottling which may follow transfer of sensitive loci

to distal autosomal regions may involve juxtaposition to such intercalary heterochromatin.

Likewise, if higher break frequency occurs in certain sections of the chromosome the possibility of inversion and reinversion between two such heterochromatic regions increases. Location of original break positions in heterochromatin may account for the relative instability of some inversions as compared with the stability of those with breaks in euchromatin. In addition, the existence of intercalary heterochromatin may explain certain regional differences in distance as measured along the salivary chromosome between pairs of loci with similar crossing-over frequencies. Genes which are relatively close in the salivary chromosome may be more widely spaced in the meiotic prophases. Moreover, inert regions may have specific effects in reducing crossing-over in regions near them, probably through conflicts in pairing.

Finally some attention should be directed to the cytological implications of these findings. One of the properties of the proximal heterochromatin is its heteropyknosis in resting nuclei of the mitotic cell. If intercalary heterochromatin possesses this property, and if the frequency of breakage is a measure of the proportional length which the region concerned occupies in the mitotic chromosome, some of the intercalary heterochromatic materials should be visible as deeply staining granules in the resting or interphase nucleus. This poses the question whether some, at least, of the small spherical bodies of very early prophase chromosomes, previously described as chromomeres (cf. Kaufmann[12]), may not represent intercalary heterochromatin. Obviously the validity of such homologies can be tested only by painstaking cytogenetic analysis. Nevertheless they serve to show the various ramifications arising from the break frequency approach to the problem of the organization of the chromosome.

Summary.—The positions of 627 x-ray induced breaks have been plotted within the limits of the lettered subdivisions of the *X*-chromosome. The distribution of breaks in chromosomes carrying the *dl*-49 inversion was similar to that in chromosomes bearing the wild-type sequence of banding. About 28 per cent of breaks (including the correction for undetected breaks) occurred in the proximal heterochromatic region, 20*A*–*F*. Certain subdivisions along the so-called euchromatic portion of the chromosome, particularly 11*A*, 12*D*, 12*E* and 19*E*, showed a break frequency higher than expected on random distribution, which may be interpreted as resulting from the presence of heterochromatic material in these intercalary regions.

[1] Paper presented August 26, 1939, at Seventh International Congress of Genetics, Edinburgh.

[2] Bauer, H., Demerec, M., and Kaufmann, B. P., *Genetics*, **23**, 610–630 (1938).

[3] Bridges, C. B., *Jour. Heredity*, **29**, 11–13 (1938).

[4] Grüneberg, H., *Jour. Genetics*, **34**, 169–189 (1937).

[5] Emmens, C. W., *Ibid.*, **34**, 191–202 (1937).

[6] Sitko, N., *Memoirs on Genetics, Acad. Sci. Ukrainian S. S. R.*, **2**, 3–30; 31–49 (1938).

[7] Muller, H. J., *Collecting Net* (Woods Hole), **13**, 181, 183–195, 198 (1938).

[8] Prokofyeva-Belgovskaya, A. A., *Comp. rend. acad. sci. U. R. S. S.*, **22**, 270–273 (1939).

[9] In a more recent publication which was received subsequent to the presentation of the present article, A. A. Prokofyeva-Belgovskaya and V. V. Khvostova (*Comp. rend. acad. sci. U. R. S. S.*, **23**, 270–272, 1939) have likewise reported the use of the break frequency method as a measure of the location of inert regions. Of the total of 141 breaks reported for the entire X, 37 were in divisions 11 and 12, a proportion much higher than that of the present study. The difference may be attributable in part to the fact that the data of Prokofyeva and Khvostova are based largely on a cytogenetic analysis of lethals and not on a study of F_1 larval progeny of irradiated fathers as has been employed in the present study.

[10] MacKnight, R. H., *Genetics*, **24**, 180–201 (1939).

[11] Bridges, C. B., *Jour. Heredity*, **26**, 60–64 (1935).

[12] Kaufmann, B. P., *Jour. Morph.*, **56**, 125–155 (1934).

A THEORY OF THE COLOR OF DYES[1]

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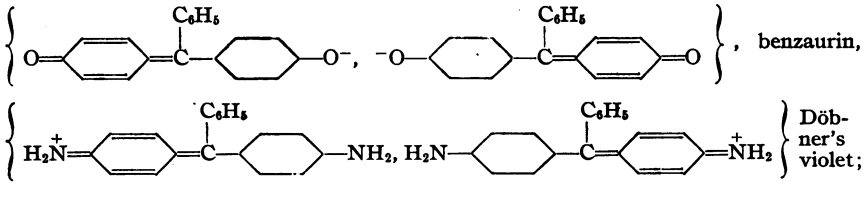
Communicated September 18, 1939

It has become recognized during recent years that the color of dyes is associated with the resonance of electric charge from atom to atom of the dye molecule.[2, 3, 4, 5, 6] Because of the complexity of the problem, however, it has not been easy to expand this idea into a theory of color permitting the rough quantitative calculation of the frequencies and intensities of the absorption bands of dyes. I have now developed a theory of this nature; the theory and some of the results of its application are described briefly in the following paragraphs.

The long-wave-length absorption band of a dye such as benzaurin or Döbner's violet has been associated with resonance of the type



the normal state involving the symmetric combination of the structures and the upper state their antisymmetric combination. It is obvious that