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CHROMOSOMAL DEFICIENCIES AND THE EMBRYONIC DEVELOPMENT OF DROSOPHILA MELANOGASTER[1]

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Chromosome aberrations have marked effects upon the development of an organism. Many such aberrations are lethal. In *Drosophila* this is particularly true of chromosomal deficiencies, as first described by Bridges.[2] A more detailed study of the effects of such aberrations made by Li[3] shows that in all the cases he investigated deficiencies are lethal in the homozygous condition—the organisms dying in the egg or larval stages. Heterozygous deficiencies result in death in later stages, although many are not lethal. A few exceptional cases of viable homozygous deficiencies have been described.[4,5] In such instances the deficiencies are among the smallest known, probably for very few genes.

These facts emphasize the importance of the chromosomes in the developmental processes. Just what the chromosomal functions may be, however, is by no means clear; nor is the rôle of the individual gene evident. An ideal approach, such as the removal of one gene at a time, then of combinations of genes, to determine the part played by each and its interactions with others, presents many practical difficulties. The existence of numerous deficiencies, however, makes an approximation to this approach possible by the study of the effects of larger or smaller blocks of genes.

The present study is concerned with the effects of certain deficiencies upon the embryonic development of *D. melanogaster*. The deficiencies used involved greater or lesser portions of the X-chromosome, ranging from the total absence of the X to a small deficiency involving relatively few "bands" as seen in the salivary gland chromosome. The technique of Huettner and Rabinowitz[6] was used, in a somewhat modified form, for the observation of living eggs, and details of internal structure were studied by means of sectioned material. Only timed eggs were used. Usually females were allowed to lay for half an hour and the eggs allowed to develop to the desired stage at a temperature of 22–23°C.

Nullo-X Eggs: One-quarter of the eggs of $\widehat{XX}Y$ females fertilized by normal males receives no X-chromosomes. These YY or "nullo-X" eggs do not develop beyond the earliest stages. Observations on both living and sectioned and stained material show that cleavage proceeds in the absence of the X-chromosome. There is, however, no uniform distribution of the nuclei throughout the egg, and the normal migration of nuclei to the surface at the 256 nuclei stage does not occur.[7] Germ cells are rarely formed, for the nuclei seldom reach the polar region of the egg. Usually there are no nuclei in the posterior portion of the egg, which contains only coalesced protoplasm or yolk. The nuclei remain dividing in the anterior half. Between the fourth and fifth hours of development they form a more or less solid mass containing between 500 and 1000 nuclei. Cytoplasm does not collect around the nuclei and no cells are formed. Yolk spheres clump together in the central or posterior portions of the egg and become sharply separated from the material which would normally become cytoplasm. A few nuclei are usually included in this mass of cytoplasm. These are larger and less pycnotic than the other nuclei and seem to undergo fewer divisions.

In normal eggs the elapsed time up to the formation of the germ cells and the blastoderm is one hour and forty minutes at 24°C.,[8] and blastoderm formation is accomplished within two hours of fertilization at the temperature used (22–23°C.). The nullo-X eggs at this time contain nuclei only in the anterior half, chiefly in the region of the fusion nucleus. The separation of the yolk and cytoplasm begins at this time and is complete in six-hour eggs.

In eggs four to six hours old the nuclei are densely packed in the anterior portion of the egg. The nuclei are mostly of the same size, but scattered here and there are larger nuclei containing more chromatin. There is usually one such nucleus to every 15 to 20 smaller ones. Although the chromatin is usually clumped so that no chromosome counts can be made, the number of ends which appear in the larger nuclei may be several times that of the smaller nuclei. This indicates polyploidy. Disintegration of nuclei sets in by the sixth hour.

Thus in the absence of the X-chromosome mitosis proceeds, but there is no regular migration of nuclei. Formation of cells and subsequent morphogenesis fail.

Half-X Eggs: Eggs deficient for half of the X-chromosome were obtained by mating males carrying a translocation involving the X- and the fourth chromosomes to $\widehat{XX}Y$ females. In the translocation used, $T1$, 4-*CRB*, the X is broken near its genetic middle. One-half of the X (to the right of lozenge and including Bar) has been intercalated between the fourth chromosome and its spindle fibre, while the other half of the X including lozenge carries the X spindle fibre. The only zygotes from such a cross which fail to develop beyond the egg stage are those deficient for the entire X or one or the other of its halves. The nullo-X eggs are readily recognizable as described above. There remain (if the extra Y chromosome is neglected) only two other types of deficient eggs: those deficient for the left half of X, and those deficient for the right half. These are readily distinguishable, at the time of blastoderm formation, from the normal and nullo-X eggs. But no distinction can be made between the two half-X deficiencies.

The nuclei migrate to the surface, so that for a time a single layer of nuclei can be found on the surface of the egg. These nuclei, however, continue dividing and do not accumulate cytoplasm and produce cells. Together with the blastema they form a syncytial mass. In living eggs the regular pattern, which begins to appear on the surface as the nuclei reach it, becomes very irregular and forms a striking contrast to that of a normal incipient blastoderm. Thus no blastoderm is ever formed and all subsequent development of the half-X eggs is abnormal. The nuclei continue to divide and come to lie four or five deep over the central contents of the egg, chiefly the yolk with some protoplasm. The clumping of yolk and the coalescing of cytoplasm characteristic of nullo-X eggs does not take place.

The distribution of the nuclei is fairly constant. In older eggs (10–16 hours) they continue to lie around the yolk entirely filling the region normally occupied by the germ band at the time of its greatest extension.[9] Division of the nuclei continues slowly in this period; only a few divide at a time. The nuclei continue even at this late stage to be fairly uniform in size. There are few polyploid nuclei. Nuclear degeneration has not set in. A few division figures were found in sections of eggs 16 hours old. Older eggs were not examined.

In a smaller proportion of these older eggs the beginning of a constriction or furrow around the anterior end can be seen in the position at which the cephalic furrow forms in the normal embryo during the third hour of development.[9] This is the only observational indication of two types of deficient eggs. It seems entirely possible that eggs lacking one of the half-X chromosomes exhibit the furrow, while those lacking the other half do not.

Thus it is seen that in the absence of either half of the X-chromosome development becomes abnormal because of the failure of cell formation and growth, and the subsequent failure to produce a blastoderm.

Notch-8 Eggs; Notch-8 is a deficiency extending from just to the left of the white locus (1.5) to, but not including, the locus of echinus (5.5) in the X-chromosome. Mohr[10] who found this deficiency was unable to demonstrate it cytologically, but studied it in considerable detail genetically. It has since been described in the salivary gland chromosomes by Mackensen.[11] One-fourth of the expected offspring of Notch-8 females fail to appear. These are the Notch-8 males which Li[8] found to die in the egg stage after some development had taken place.

Observations made on living eggs show that the pole cells and blastoderm form normally and that no externally apparent irregularities occur in the early stages. Hence normal and deficient eggs are indistinguishable until somewhat later. The most marked irregularity which is noted in the living egg appears in the middle period of development (11–12 hours) when the germ band normally contracts and the larval segmentation becomes pronounced.[9] Only the faintest signs of segmentation appear in the Notch eggs and contraction does not occur.

The details were followed in sections. The most striking feature of such eggs is that they contain very little or no endoderm or mesoderm. The proctodaeal and stomodaeal invaginations occur, the ventral blastoderm elongates, apparently without the formation of the underlying layers, and the embryo looks superficially normal. The yolk is never enclosed, for the mid-gut fails to form. The hind-gut and its diverticula, the Malpighian tubes, never attain their normal position. The development of the hind-gut is abnormal; it comes to fill a larger portion of the posterior part of the egg than normal. It is possible that some of this material represents endoderm. The fact that no endoderm can be found united with the stomodaeum, however, indicates that the process of germ layer formation has been interfered with seriously. Nothing which can be interpreted as mesoderm has been found in these embryos. The ectoderm proliferates especially along the ventral mid-line and produces what appears to be a semblance of the early nervous system. There is no very sharp demarcation between this nervous tissue and the outer ectoderm. The cells are more densely packed in this region; in the median part clear areas which resemble the nerve fibre portions of the normal nervous system become more prominent in 14- and 16-hour eggs. There is no shortening of the embryo and the hind-gut remains with its opening in the dorsal side. In eggs 20 to 22 hours old the abnormal development is still pronounced, but there is as yet no sign of disintegration or cellular breakdown. The ventral nervous tissue and the hind-gut remain the most characteristic features. In the case of normal eggs of this age the young larva is complete and actively moving preparatory to the escape from the egg membranes.

In the case of the Notch-8 male it is clear that while the missing part of the chromosome is not essential for the formation of cells and the production of a blastoderm, it must be present if the inner germ layers are to be produced normally and take part in subsequent morphogenesis.

From the evidence which has been presented it is clear that certain, if not most, sections of the X-chromosome are essential for specific processes in the embryonic development of *Drosophila*. The larger the section of the chromosome missing (i.e., the more genes missing) the earlier and more general are the causes of abnormal development, while the smaller the missing piece the more specific the effects. Thus in the region at the left end of the X-chromosome Muller[4] has found "a viable two gene deficiency," and Demerec and Hoover[5] have found a deficiency which survives in homozygous condition. In this same region many small lethal deficiencies are known, one of which (Deficiency scute-8) has been found to cause death very late in the egg stage just before hatching (Poulson, unpublished). Also small lethal deficiencies are found scattered throughout the chromosome complement. Hence it would appear that genes which play very definite parts in the embryonic developmental processes are distributed quite as much at random throughout the X-chromosome as are genes which affect only the most superficial characters.

A more detailed account of the behavior of these deficiencies, together with some further data, will be presented elsewhere.

[1] Work carried out at the Wm. G. Kerckhoff Laboratories, California Institute of Technology, Pasadena.

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DEFICIENCIES AND STRUCTURAL VARIATIONS WITHIN THE GIANT CHROMOSOMES IN RELATION TO THE PROBLEM OF GENE STRUCTURE

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The giant chromosomes in salivary and certain other glands of dipteran larvae are characterized by transverse chromatic discs or "bands," separated from one another by intervening achromatic material. Since one of the primary aims of studies on these chromosomes is to identify the genic as distinguished from non-genic materials, and to interpret the visible structures in terms of individual genes, attention naturally becomes focused