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Present Day Concepts of the Gene with Special Reference to the Salivary Chromosome of Drosophila melanogaster

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Present Day Concepts of the Gene with Special Reference to the Salivary Chromosome of Drosophila melanogaster.

A gene is a unit of inheritance situated at some particular locus of a particular chromosome and transmitted according to the laws of Mendel. Thus it may be regarded as a physical entity, the unit material of heredity. For some time the chromosome was known to consist of functional units which were the actual transmitters of hereditary characteristics, but it was not until the last ten years that any results were achieved in the field.

The changes brought about by chromosomal aberrations, translocations and gene mutations were recognized but the nature of the gonial chromosomes prevented the actual location of gene loci. Observed under oil immersion, the gonial chromosomes appear as opaque bodies with their inner content not demonstrable. At the time of nuclear division, only deeply staining rods are visible and at earlier phases, such as the prophase, only knotted strands or threads are present.

Not until 1932 was the search for an organism with chromosomes large enough to have visible differences for gene location successful. It was a known fact among cytologists that the salivary glands of the Diptera larvae contained a "permanent spireme" and chromosomes large enough to show light and dark areas. In 1932 Theophilus S. Painter of the University of Texas began a series of investigations upon these structures in the larvae of Drosophila melanogaster.

He found that the chromosome consists of an elongated

cylindrical rod with alternating bands of light and dark staining material running across the long axis of the chromosome. The pattern of each chromosome was found to be remarkably constant in a great number of individuals. Thus, he proved that there was present a definite banding arrangement for each chromosome. Here a problem arose. Drosophila has eight chromosomes all arising from a common substrate, and consisting of four pairs of like elements. The salivary chromosomes, however, were only six in number. By further research it was found that the chromosomes had undergone a fusion process and the two arms of the II and III chromosomes appeared as separate elements. Thus, the number of gonial and salivary chromosomes was proven equal. Work was begun upon the actual location of the genes for individual characteristics.

The experimental work consisted of raising Drosophila larvæ, dissecting out the salivary glands, staining them, and relating their chromosomal structure to the characteristics of the adult flies. Various cultural media are in use, most of them with a basis of agar, bananas, and a syrup free from sulphuric acid. The formula I have found satisfactory consists of 30 grams of agar-agar, 400 grams of corn-meal, and 375 cubic centimeters of molasses. The agar is dissolved by boiling in 1725 cubic centimeters of water. To it is added the molasses in 600 cubic centimeters of water and the corn-meal mixed with another 600 cubic centimeters of water. After each addition the mixture is boiled. This media is placed in sterilized bottles to a depth of one-half inch, and a yeast suspension sprinkled on top. Then a piece of filter paper is inserted for pupation. A

wad of cotton forms a good cork. Six or eight flies are kept in a 250 cubic centimeter flask.

Eggs are soon laid and within a few days larvae are found crawling up the filter paper to pupate. These larvae being fully developed, are the best for experimentation. If placed in a cold blooded Ringer's solution, the glands may be dissected by grasping both ends of the larva with fine forceps and pulling. The larva splits just posterior to the head, and the salivary glands are revealed as glistening white bodies. There are transferred to a slide and spread out by bringing pressure to bear on the cover slip. Aceto-carmine, a saturated solution of carmine in 45% acetic acid, is then introduced beneath the cover slip. The glands are stained for approximately fifteen minutes. They are then flooded with fresh dye solution. The excess dye is removed with blotting paper, and the cover slip is sealed by applying melted paraffin to the edges. This produces a temporary mount which will last two or three days. Permanent mounts are not sealed at this point, but desiccated for twelve hours in 95% alcohol fumes and sealed with euparal. Various other techniques are used, but these are among the simplest and most efficient. In these preparations the bands described by Painter are clearly visible.

Dr. Bridges made a detailed study of the nature of these bandings. He found them to be solid discs composed of a bundle of small rods. These rods were continuous from disc to disc in the form of a cable consisting of spiral threads of which sixteen were found in each chromosome. Kostoff contends that these elements may be only discoidal light and dark components.

Other investigations found the discoidal elements present only on the surface with a deeper inner axis. Thus, there is still going on an argument between the exponents of the discoidal and those of the spiral strand theories. Actually there is no definite proof that the genes lie in these bands, whatever their structure, they may lie in the matrix.

More important work has been done on the location of the genes on the chromosomes. To do this it was necessary to develop a cytological map of each chromosome, by means of its normal morphology. From this, by comparison with abnormalities, the positions of genes are determined. I shall take up the normal morphology first.

The five main chromosomes are each divided into twenty sections, making 100 in all. Chromosome IV has sections 101 and 102. This gives us a key for position reference, particularly since each section begins with an easily recognized band. Each section is in turn divided into six subdivisions beginning with sharp bands and designated by capital letters A to F. Thus, we may easily refer to a band as located in 60A or 38D. This system eliminates the necessity for future changes which would have been required, had the bands themselves been numbered. The knowledge of band position has not been completed sufficiently to avoid possibilities of future changes. In fact, I shall later refer to some of the important changes already made in the map of the X-chromosome.

In recognizing positions on chromosomes, the bands are oftimes not easily identifiable and a series of landmarks has been adopted. Of these the "puff" in 2B, the "four brothers" in

9A, the "weak spot" in 11A, the two "chains" in 15, the "turnip" in 16, and the "offset" in 19E distinguish the X-chromosome. I have, by the technique previously described, developed a series of slides in which these characteristics have been the main identification clues. By their use I have found the X-chromosome readily identifiable. IIL is distinguished by the "dog-collar" in 21CD, the "shoe buckle" of 25A, the "shield" in 30A, the "goose-neck" in 31BF, the "spiral loop" of 32-35, a "turn-back" in 36, and the "basal loop" in 37-39. Recognition of 2R occurs by its thick "onion" base and "huckleberry tip". IIIL has a "barrel" at 61CF, a "ballet skirt" in 68BC, "chinese lanterns" in 74-75, and "graded capsules" in 79CDE. The longest limb, IIIR, has a clear "cucumber" base 81-83D, a "duck's head" at 89E to 91A, and a "goblet" tip. Chromosome IV is recognized by its very short length.

The total length of moderately stretched salivary chromosomes was found to be approximately 150 times that of the gonial chromosomes. Of this length the X = 220u; II = 215 - 245 = 460u; III = 210 - 275 = 485u; IV = 15u; with the total length being 1,800u. Total gonial length equals 7.5u. This large size of the salivary chromosomes is attributed to their compound nature. As previously stated, each of the fused maternal and paternal homologues consists of eight chromonemata giving a total of sixteen strands. These homologues are quite evident in the slides I prepared.

Dr. Bridges has given us his conclusions on the location of the gene upon the banded areas. He believes that the sixteen dots seen in cross-section of a band correspond to the

loci of eight maternal and eight paternal sister genes. A count of the bands resulted in 537 for X, 1,032 for II, 1,047 for III, 34 for IV, or a total of 2,605. This agrees very well with the approximation of 1,500 to 3,000 genes per animal. Whether the genes actually lie enclosed within the walls of the strands, in the walls, or in the vesicular portion, is at present undeterminable. They have found, however, that bunching of the genes is due to reduced crossing over as that near the end of X. The strands have a tendency to slip toward the end rather than to break.

Calvin Bridges has recently revised the map of the X-chromosome. By use of permanent preparations and camera lucida drawings, he has increased the number of known bandings from 725 to 1,024. He has discovered a number of reversed repeats and done considerable work upon the important 3C region which is the loci of white and a region of high crossing-over.

Having considered the normal morphology, I shall now present a discussion of the methods of gene location. In order to do this, one must first understand the process which Painter calls "somatic synapsis". In somatic synapsis two homologous chromosomes, one maternal and one paternal, unite into one apparently single structure.

Painter lists three general methods of determining the position of gene loci. I shall take them up separately. The first method is a study of simple mutual translocations or inversions in which we know genetically between which genes the break or breaks have occured. In larvae heterozygous for a normal and a broken chromosome, synapsis takes place up to the point of

the break, where the aberrant diverges. A comparison is then made of the two elements and the two genes separated by the break determined on each side of the point of breakage. The missing bands are then easily found. Painter has located genes on the X-chromosome to within the area of two or three bands. (Fig. 1.)

Professor Patterson, of the University of Texas, has contributed some very interesting studies on the production of translocations in Drosophila. He first crossed an X-rayed wild-type male with a female having an X-chromosome, homozygous for mutant gene yellow, and three pair of autosomes, homozygous for brown, ebony, and eyeless, respectively. The F_1 heterozygous, wild-type males were backcrossed with the same type of female. This produced an F_2 generation showing eleven types of translocations as follows:

(X-II) males non-brown; females brown.

(X-III) males non-ebony; females ebony

(X-IV) males non-eyeless; females eyeless.

(II-III) flies either non-brown, non-ebony, or else brown, ebony.

(II-IV) flies either non-brown, non-eyeless, or else brown, eyeless.

(III-IV) flies either non-ebony, non-eyeless, or else ebony, eyeless.

(X-II-III) males non-brown, non-ebony; females brown, ebony.

(X-II-IV) males non-brown, non-eyeless; females brown, eyeless.

(X-III-IV) males non-ebony, non-eyeless; females ebony, eyeless.

(II-III-IV) flies either normal or else brown, ebony, eyeless.

(X-II-III-IV) males normal; females brown, ebony, eyeless.

While carrying on this study, Mr. Patterson discovered that the breaks occur at the free or spindle-fiber ends of the chromosomes. In the X-chromosome the majority of breaks occur between scute and ocelliless or forked and carnation. In the II-chromosome the majority were determined between black and purple in the left arm and between purple and curved in the spindle fiber area. The break loci lie between scarlet and pink on the III-chromosome and between sooty and claret in the right arm. The various types of translocations are found to be due to four things: (1) Length of the chromosome involved, (2) position of the chromosomes within the cell, (3) regional affinity either for breakage or for a non-homologous chromosome, (4) size of the cell. Another interesting result of this investigation was the placement of hyper- or hypoploidy absence in thses translocations in the autosomes, not in the X-chromosome.

A second and better method was developed by Otto Mackensen under Professor Painter's direction. It consists of a study of short deletions where we know what genes are missing. A deleted area is one in which a portion of the chromosome is missing. In the synapsis of a deleted homologue with a normal, fusion takes place on both sides of the deleted area and the normal elements corresponding to the deletion are pushed out in a loop to the side. (Fig. 2.) Such deletions have been very successfully produced by irradiation. Otto Mackinsen irradiated normal male flies and crossed them with females carring a series of recessive genes along the X-chromosomes. Thus, he produced in F_1 females a pseudo-dominance of a female recessive characteristic due to deletion of the normal gene in the male. Larvae heterozygous for

this deletion were studied.

It was found that if both ends of the deletion occured in clear areas, homologies were easily studied. Difficulty was encountered in exact determination when the breaks occured in one or both ends in banded areas. In this case synapsis of non-homologous parts may seemingly occur. The most exact determinations were made when two slightly overlapping deletions removed the same locus. By this means, genes were located between two definite bands.

By the use of this method, Otto Mackensen has located eight gene loci whose position I shall give as found on Dr. Bridges' map of the X-chromosome. The prune locus lies between 2D6 and 2F2 and was determined by a long deletion in the X-chromosome. Here the deleted section was inserted into the right arm of the II-chromosome. A deletion in 3C2 removes white but not prune, split, or facet. The loci of facet, split, and Notch must lie between 3C3 and 3C6. In experiments in white, white-mottled, and echinus, the echinus loci was found to lie between 3E2-3E4 and white-mottled to lie in the same band as white, 3C2. The cut locus has been deleted from the X and inserted into the right arm of the II-chromosome. This piece lies between 7A3 and 7B4, and does not include singed, ocelliless, or carmine, as shown by genetical tests. Vermilion was found by a case of inversion with a short deletion at each end of the inverted area. It was placed in a wide band, 10A1, to the right of the left point of breakage. Study of a break in X between forked and Bar loci in females has placed the forked condition within the "Bar" band of 15E1-15F1. The last characteristic determined was Beadix in 17C1-17D1. This latter one is a tentative location assumed from

the study of the forked locus.

The third method consists of the study of a number of breaks which have occured between genetically adjacent gene loci.

I cannot close this discussion without some reference to the important work of Dr. H. S. Müller on "position effect". While working with translocations of the scute genes of the X-chromosome, he found that when they lie next to new gene neighbors the flies produced are so different as to constitute mutations. The effect has been proven to be due to the propinquity of the genes and not to differences in genetic material. If this principle is found to have general application, it may give us a clearer understanding of the way the gene operates in controlling development of morphological characters.

Illustrations

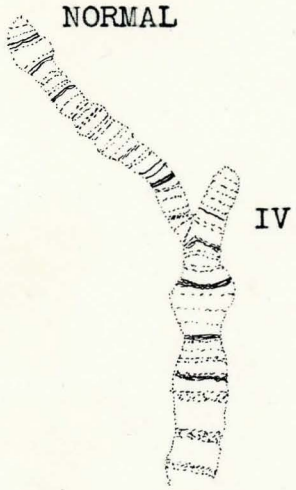

Fig. 1. The normal element lies on the left and the aberrant one to the right. The aberrant element synapses with the normal right up to the point of breakage. This case involves a mutual exchange with a fourth chromosome. (Painter)

Fig. 2. Deleted and normal X-chromosomes after they have undergone somatic synapsis. (Painter)

Summary

1. The importance of the salivary chromosomes lies in the fact that they have allowed the tracing of the genes to definite regions of the chromosomes. This is the outstanding feature of the work to date.

2. The deletion method is valuable because it proves that the genetic deficiencies may be accompanied by cytological deficiencies and, also, because by it we are able to determine the cytological locus of genes more exactly than by the usual translocation method.

3. When a translocation or deletion is isolated, a cytological examination of heterozygous larvae reveals immediately where the break has occured with reference to known gene loci or to what genes have been deleted.

4. The salivary chromosome maps have been found useful in the study of all types of chromosome rearrangements. In fact, it has already been proven that complex rearrangements, such as those of Patterson's experiments, are much more numerous than was suspected from breeding experiments.

5. Through Patterson's experiments with X-ray stimulation, it has been proved that in many cases new species are preceeded by a chromosomal rearrangement which sets up a physiological isolation within the parent species.

6. It has been possible by these methods to determine the nature of aberrations on the individuals.

7. Painter's discoveries in somatic synapsis have been of great usefulness in the work in crossing-over. He also expects the work unfinished to throw light upon the nature of the mitotic spindle as well as on chromosomes and speciation.

8. While great advances have been made in the field of the gene, it is the aim of the workers to give every characteristic a definite gene loci on the chromosome. When this is done, we may be able to control and predict more accurately and with more certainty. This would prove of enormous value in both plant and animal breeding, particularly if we were enabled to produce varied characteristics by gene manipulations such as those of the X-ray.

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