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The Cytogenetics of the Salivary Gland Chromosomes of Three Wild Type Strains of *Drosophila melanogaster*

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THE CYTOGENETICS OF THE SALIVARY
GLAND CHROMOSOMES OF THREE WILD TYPE
(TITLE)
STRAINS OF *Drosophila melanogaster*

BY

Rosemary Ann Harris

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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DEPARTMENT HEAD

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I would like to dedicate this thesis to my husband, John R. Harris, for all of his help during its preparation; and to my parents, Mr. and Mrs. Warren E. Driskell, who impressed upon me the importance of an education.

INTRODUCTION

This investigation involves the cytogenetics of three strains of field-collected, wild-type Illinois Drosophila melanogaster. These three strains were collected from Charleston, Channahon, and Olney, Illinois, in 1967.

The purpose of this study was to compare cytogenetically these three strains and to determine if there exists differences among them. First, the chromosomes themselves were studied for the purpose of identifying the four chromosomes of D. melanogaster by their characteristic banding patterns. Then, the chromosomes of the three strains were examined for chromosomal aberrations, principally inversions. The cytogenetics of the chromosomes of the three strains were then compared and contrasted.

Photographs were taken of the chromosomes, the aberrations, and other chromosomal structures of cytogenetical significance to illustrate these similarities and dissimilarities, if any, among the three strains of Drosophila.

LITERATURE REVIEW

History of Polytene Chromosomes

The giant polytene chromosomes of the salivary glands of Dipteran larvae are one of the best tools the geneticist has to work with in estimating the linear arrangements of genes on chromosomes as well as structural changes in chromosomes. According to Gardner (1968), E. G. Balbiani is credited with their discovery in 1881. However, their cytogenetic importance was not realized until a much later date, beginning in the 1930's. Others that contributed to the knowledge about these chromosomes included J. B. Carnoy, who made further observations on their structure in 1884; and F. Alverdes, who in 1912 traced the development of these chromosomes from the early embryo stage to the late larval stage. In 1930, D. Kostoff suggested a relationship between the bands of these structures and the linear sequence of genes. E. Heitz and H. Bauer, working with March flies (Biblio), in 1933, identified these as giant chromosomes occurring in pairs. They also described the morphology in detail and discovered the relation between the salivary gland chromosomes and other somatic and germ cell chromosomes. In addition, these two men demonstrated that comparable elements occurred in the giant chromosomes and in chromosomes of other cells of the same organism (Gardner, 1968).

Through the work of T. S. Painter in 1933, Drosophila salivary gland chromosomes were first used for cytological verification of genetic data. Painter stated the following:

Each of the chromosomes has a definite and constant morphology and is made of segments, each of which has a characteristic pattern of chromatic lines or broader bands, which appear to run around the achromatic matrix. The same chromosomes, or characteristic parts thereof, may easily be recognized in different cells of an individual, or in different individuals of a species. If the position of one or more segments is shifted, by some forms of dislocation (translocation, inversion, etc.), the exact morphological point (or points) of breakage can be determined for the segments identified in their new position. This discovery places in our hands, for the first time, a qualitative method of chromosomal analysis and once the normal morphology of any given element is known, by studying chromosomal rearrangements of known genetic character, we can give morphological positions to gene loci and construct chromosomal maps with far greater exactness than has been heretofore possible. (Painter, 1933)

Thus, Painter related the bands to genes; but he was interested in the morphology of the chromosomes and the implications concerning speciation rather than the association of sections of the chromosome with genes, according to Gardner (1968).

Features of Polytene Chromosomes

Except for lampbrush chromosomes found in the oocytes of amphibians (newts), the giant chromosomes of the larval stages of certain Diptera are the largest chromosomes. (Cohn, 1969). These chromosomes are found only in certain tissues, including the salivary glands, Malpighian tubules, epithelial lining of the gut, and some fat bodies. The organisms in which they occur include Drosophila, Chironomus, Sciara, and Rhynchosciara.

According to Cohn (1969), the size of the giant chromosomes is a function of the large number of chromosome strands in each chromosome, many more than are present in the chromosomes of other somatic cells. The giant chromosomes are called polytene because of their numerous chromonemata. To achieve this condition, several duplications of the chromosomes have taken place without the usual accompanying cell division. Thus, all of the chromosomes remain together in the same cell. The homologous chromosomes remain together in somatic pairing. The number of chromonemata in a single giant chromosome varies from 1024 in Drosophila to 4096 in Chironomus. In contrast to normal somatic chromosomes which have a total length of 715 microns, the polytene chromosomes of D. melanogaster have a total length of 2000 microns. The cells are in G₁, S, or G₂ (Growth period I, Synthesis, Growth period II) of interphase or undergoing endomitosis.

In the nuclei of Drosophila larval salivary glands, as an adjunct to the somatic pairing of the chromosomes, the kinetochore (centromere) regions of all of them are in association, producing a chromocenter that is heteropycnotic, or exceptionally deep staining, during interphase and mitotic prophase (Cohn, 1969). Chromocenters are absent in Chironomus and Sciara.

When polytene chromosomes are heated with certain stains, some regions on the chromosome appear as dark bands while others appear as light interbands. Cohn (1969) states that the bands are composed of

chromomeres of individual chromonemata in a linear array perpendicular to the axis of the chromosome. Therefore, they are most likely due to a tighter coiling of the chromonemata in certain regions than in others. Observations of interbands with the electron microscope bears this out. In the interbands, there is little or no coiling, and the chromonemata are arranged parallel to the chromosome axis. In addition to coiling differences, there are chemical and functional differences between the bands and interbands. The bands undergo morphological and biochemical changes related to their genetic activity, known as "puffing." The interbands, however, represent largely structural nucleoprotein, and are less active genetically; they are not involved in RNA synthesis. As a result of the greater concentration of coiled chromonemal strands in the bands, the ratio of DNA content between bands and interbands may be as high as 60:1. Differences in protein content also indicate that interbands have a relatively high content of non-histone proteins, and bands a higher content of histone proteins (Cohn, 1969).

Regardless of their large size and unique characteristics, polytene chromosomes are like the other chromosomes of the same species, according to Gardner (1968). Because of the distinct banding, they correspond in linear structure with other chromosomes of that species. They are constructed of DNA and protein like the other chromosomes. The only difference between these and other chromosomes is that the duplicated strands are held together in bundles instead of separating out to new cells during cell division.

Gardner (1968) states that these chromosomes are valuable for genetic study for three main reasons, all concerned with the unique features listed above. First, they are the largest chromosomes known that are readily available for genetic analysis. Their size is over one hundred times the length of somatic metaphase chromosomes. When prepared for study and stretched by a smear technique, the chromosomes in an aggregate reach a length of 1180 to 2000 micra. Secondly, the bands differ in thickness and other structural features. This fact, coupled with the large size, permits accurate mapping of each chromosome throughout its length. Also, gene loci can then be identified with certain bands (Swanson, et al., 1967). Thirdly, the continuous state of somatic synapsis makes them valuable for study because they are thereby comparable to the chromosomes of meiotic prophase. If one member of a pair is altered by a deficiency, duplication, inversion, or translocation, an irregularity occurs in the pairing. Observable irregularities such as these make possible the recognition of different kinds of chromosome modifications and their location on the chromosome (Gardner, 1968). The banding phenomenon is also important in conjunction with the synapsis, since structural changes occur for the purpose of keeping the bands of the two homologous chromosomes lined up precisely (Swanson, et al., 1967).

Applications of Polytene Chromosomes

Applications of the polytene chromosomes include four major possibilities (Gardner, 1968):

- 1) Comparison of cytological chromosome maps and linkage maps.
- 2) Location of genes and identification of structural changes in chromosomes.
- 3) Study of the effects of environmental agents on chromosomes.
- 4) Physiological studies of gene action (mRNA synthesis).

This paper will be concerned principally with the latter part of the second application, the identification of structural changes in polytene chromosomes of Drosophila melanogaster.

History of Chromosomal Aberrations

The history of chromosomal aberrations within the polytene chromosomes is fairly recent. According to Gardner (1968), a series of events led to their discovery. In 1917, C. B. Bridges observed that in a presumably heterozygous sex-linked trait, a recessive gene was expressed. He postulated that a section of the homologous chromosome containing the dominant gene was missing; a deficiency had occurred in the chromosome. Conversely, when a recessive gene, presumable homozygous, was not expressed, Bridges hypothesized that a dominant allele was present in the chromosome set; that is, a duplication of a section had occurred. To explain various genetic irregularities, A. H. Sturtevant and Bridges postulated that rearrangements in chromosomes (inversions) and exchanges between chromosomes (translocations) occurred. It was several years later that these predicted changes could be actually observed through the microscope.

Gardner (1968) continues to state that in 1928 H. J. Muller and Edgar Altenburg induced structural changes in the chromosomes of Drosophila with X rays and detected translocations. The first cytological demonstration of the chromosomal aberrations in plants was made by Barbara McClintock in 1930 in maize. She demonstrated all four aberrations by studying the large pachytene chromosomes. Demonstrations were made later in Drosophila using the giant polytene chromosomes.

Chromosomal Aberrations--Introduction

The constancy of a chromosome as a structural entity lies in its capacity to reproduce itself at each cell division with extraordinary precision (Swanson, et al., 1967). However, chromosomes can undergo change spontaneously and the newly constructed chromosome, like its original counterpart, is replicated exactly at each cell division thereafter. Under natural conditions, such changes are rare; they can be induced with relative ease, however, by ionizing radiation and by chemical mutagens.

Structural changes presuppose breaks in the chromosomes, according to Gardner (1968). More than one break can occur in a single chromosome or set of chromosomes, and the broken parts may then reunite in new arrangements. The loss or addition of chromosome segments may also occur. In addition, more than one type of structural modification may occur at the same time. Swanson, et al. (1967) state that chromosomal aberrations leading to rearrangements in the linear

order of genes may thus be grouped into four classes: deficiencies and duplications, which are additions or deletions from the chromosome; and inversions and translocations, which represent rearrangements within the chromosome.

Deficiencies or Deletions

Deficiencies or deletions involve the detachment and loss of a block of chromosome from the remainder of the chromosome. The deleted portion of chromatin will not survive if it lacks a centromere because it cannot move in anaphase (Swanson, et al., 1967). It lags and is excluded from the rest by the nuclear membrane of the new cell (Gardner, 1968).

Strickberger (1968) states that deficiencies were the first chromosomal aberrations indicated by genetic evidence. This evidence was provided by C. B. Bridges in 1917 when he showed a deletion of the X chromosome in Drosophila that included the Bar locus. Since then deficiencies have been located in Drosophila, corn, man, and other organisms.

Deficiencies may be of two types, terminal or interstitial (Swanson, et al., 1967). A terminal deficiency arises by a single break in a chromosome followed by a healing of the broken end. The formation of a terminal deficiency leads to the loss of the normal end, or telomere of the chromosome. According to Strickberger (1968), Muller hypothesized that the tip has a unique function in preventing adhesion between chromosome

ends which would otherwise stick together. Muller states that all deficiencies that are capable of being maintained in stocks must therefore be interstitial and cannot involve the loss of the telomere. The relative rarity of terminal deficiencies seems to support this hypothesis; however, the absence of telomeres in some terminal deficiencies may indicate that the sticky end can "heal."

The second type of deficiency, called an interstitial or intercalary deficiency, results from two breaks followed by a reunion of the broken ends (Swanson, et al., 1967). This type is much more common than the terminal type. When an intercalary part is missing, a buckling effect occurs in the chromosome as shown below:



A deficiency involves the loss of genic material; therefore, it would be expected to have a deleterious effect on organisms. Homozygous viable deficiencies would be expected to be rare (Swanson, et al., 1967). Homozygous deficiencies are usually lethal; and heterozygous deficiencies lower viability (Gardner, 1968). Deficient gametes can survive to take part in fertilization in animals, but not in plants (Swanson, 1957). If a gamete with a deficiency is fertilized by a gamete with a non-deficient homologue, the resulting cells will carry the deficiency in the heterozygous condition. In addition, recessive genes in the region of the deficiency on the nondeficient chromosome may express themselves. This is referred to as pseudodominance (Gardner, 1968).

In Drosophila melanogaster, homozygous deficiencies involving the tip of the X chromosome are viable provided they are very tiny (Swanson, et al., 1967). A loss of more than fifty bands in D. melanogaster is usually considered lethal even when the homologous chromosome is intact. The X chromosome has over 1000 bands; hence, the physiological balance can readily be upset.

Since deficiencies do result in a loss of genes, Swanson, et al. (1967) state that they give rise to recognizable genetic consequences and may act as recessive lethals. They also produce detectable morphological changes that are inherited as dominant characters, according to Swanson. For example, characteristics in Drosophila associated with deficiencies include blond, pale, beaded, carved, snipped, and plexate. In Drosophila, there is also a correlation between phenotypic effect and the number of bands deleted. For example, deletions in the vestigial region behave as dominant characteristics; and the greater the loss of chromatin, the more severe the phenotype in its departure from normal.

In man, many congenital abnormalities have been traced to chromosomal deficiencies in the heterozygous state (Swanson, et al., 1967). Chronic myeloid leukemia is associated with the Philadelphia chromosome, Ph^1 , identified as chromosome 21 minus a large part of its long arm. The cri-du-chat syndrome, or cat-like cry, which is characterized by severe mental retardation, physical abnormalities, and a cat-like cry, results from the loss of the short arm in the number 5 chromosome.

Other abnormalities involve deficiencies in X, Y, and chromosome 18.

W. H. Gates discovered a deficiency in another mammal (Gardner, 1968). A recessive gene was known to produce a peculiar nervous abnormality in mice, and they were therefore called "waltzing mice." Those carrying the gene in homozygous condition move about erratically until they become exhausted. When Gates crossed homozygous normal female mice ($v+v+$) with homozygous waltzer male mice (vv), all of the F_1 progeny were normal (heterozygous). However, one waltzer female appeared in one litter out of seven. * Gates explained the results on the basis of a deficiency including the v locus, which originated in the female of the original cross mentioned above and was transmitted to her daughter. (*This waltzer female produced normal progeny when mated with normal males and waltzer progeny when mated with waltzer males. When mated with a male carrying one $v+$ gene, five normal and two waltzers were produced.)

Deficiencies are used to locate genes on salivary gland chromosomes in Drosophila. (Swanson, et al., 1967). The principle used is as follows: a correlation is made between the absence of a particular band in the chromosome and the presence of a particular morphological phenotype. Therefore, they provide a method for chromosomal mapping.

According to Swanson, et al. (1967), deficiencies are not very significant to the course of evolution. It is unlikely that they would affect it since they diminish metabolic controls.

either "bucklings" in the duplication heterozygote or as cross-pairing between sections of different chromosomes. According to Swanson, et al. (1967), they may also be recognized by the absence of recessive phenotypes.

Perhaps the most common and best studied example of a duplication is the Bar phenotype in Drosophila. Bridges (1936) states that S. C. Tice in 1913 found the reduced-eye mutant as a single male in a progeny of normal-eyed parents. According to Swanson, et al. (1967), this represents a tandem duplication which involves four or five bands in region 16A of the X chromosome. Normal males have the 16A region repeated one time, Bar males twice, and double (ultra) Bar three times. Individuals have been obtained with as many as eight regions in tandem sequence. With each addition, the number of facets in the eye is reduced. Bridges (1936) states that eye-reduction behaves as a sex-linked dominant, with a locus at 57.0, and has been one of the most important of all sex-linked characteristics of D. melanogaster. Bridges also states that the change from bar to double bar represents a single gene duplication; and the converse change from bar to bar-reverted involves a one gene deficiency.

A duplication found in humans is one that involves the four polypeptide chains composing the protein hemoglobin (Swanson, et al., 1967). Humans designated Hb-A have hemoglobin molecules composed of two alpha and two beta chains. Those designated Hb-A₂ have two alpha and two delta chains. Beta and delta are closely linked and are thought to be

duplicate loci. An abnormal hemoglobin (Hb-Lepore) studied by Baglioni, has two normal alpha chains, but the other two chains have amino acid sequences characteristic of both beta and delta chains. Baglioni suggested that unequal crossing over has taken place between the beta and delta (duplicate) loci, giving a deleted segment in one chromosome and a duplicated segment in the other.

The frequency of duplicate banding patterns, or "repeats," in salivary gland chromosomes indicates that duplications have not been uncommon in the past and have occasionally been incorporated in the homozygous condition as part of the normal chromosome (Strickberger, 1968). It has also been suggested that all cases of "complementary" genes or "multiple factors" in which different gene pairs affect the same character in a similar fashion arose initially as duplications of single genes.

Swanson, et al. (1967), state that the differences in gene number must have arisen through a variety of mechanisms during the course of evolution, since all organisms do not have the same number of genes. (Different amounts of DNA indicate this.) The abrupt loss of material through deficiency is an unlikely evolutionary event because it reduces the potential of the gene pool. Duplications, however, do not possess evolutionary limitations. The duplication of loci would appear to provide a feasible method for the acquisition of new genes, and therefore of new physiological functions. In addition, two genes identical in function and origin can diverge through mutation to the extent that they may control

different and separate functions. If a mutated gene is present as a duplication with a normal gene, the possibilities of its retention and continued mutation, possibly in new directions, becomes considerably greater.

As compared to deficiencies, duplications are observed more frequently in nature. They are also less likely to be lethal than are deficiencies (Swanson, et al., 1967).

Inversions

Dobzhansky (1951) states that in an inversion, the location of a block of genes within a chromosome may be changed by a rotation through 180 degrees. The resulting chromosome carries the same genes as the original one, but in a modified arrangement; for example, from sequence ABCDEFG to AEDCBFG. Some inversions result from entanglements of threads during meiotic prophase causing chromosomal breaks (Gardner, 1968). These are then perpetuated in the pairing process at meiosis and segregated into viable gametes.

Inversions are the most frequently encountered aberration in wild populations and probably the one most useful to the geneticist. They were first detected by Sturtevant in ~~Drosophila~~ through the altered order of genes in linkage groups (Swanson, et al., 1967). Sturtevant and Plunkett worked with third chromosome maps for two species, D. melanogaster and D. simulans, based on tests for third chromosome genes and on linkage studies performed with each species (Strickberger, 1968).

They found the gene sequences to be as follows for each species:

D. melanogaster se st p D1 H ca

D. simulans se st H D1 p ca

They thought the maps could most reasonably be explained as an inversion, a reversal in the order of the gene sequence p D1 H, between the two species.

A better and more exact method of studying inversions is by observation of polytene chromosomes (Dobzhansky, 1951). Heterozygous inversions can be detected in salivary chromosomes by inversion loops which form when all portions of the two homologous chromosomes synapse. This may be diagrammed as follows:



(Gardner, 1968)

Inversions may be present in the homozygous or heterozygous state. Inversion homozygotes are those in which both chromosomes of the pair have the same gene arrangement (Dobzhansky, 1951); that is, the same inversion is present in both chromosomes. Homozygous inversions may lead to inviability as a result of alteration in protein synthesis (Swanson, et al., 1967). Inversion heterozygotes are those in which the two chromosomes of the pair have different gene arrangements (Dobzhansky, 1951); that is, only one contains the inversion. In Drosophila, no serious reduction in gamete viability is encountered as a result of inversion heterozygosity (Swanson, et al., 1967).

Inversions may be classified in two ways. First, if a single inversion is present, its location on the chromosome in relation to the centromere is used to classify it (Swanson, et al., 1967). Two types are recognized, paracentric and pericentric inversions. Paracentric inversions are confined to a single arm of the chromosome. It produces no visible change in chromosome morphology in somatic cells since the centromere is not involved. For example:

Normal chromosome A B C D E F G H

Inverted chromosome A B F E D C G H

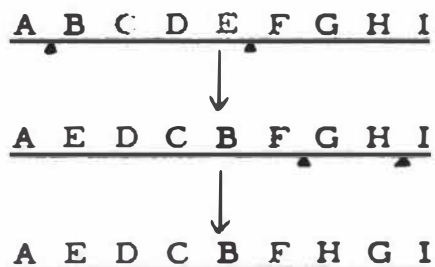
In pachytene or salivary gland chromosomes, these can be recognized by the inversion loop which forms when all parts of the two chromosomes synapse in the homologous fashion. Pericentric inversions involve the centromere and may alter chromosome morphology. If two breaks occur equidistant from the centromere, the chromosome would be unchanged morphologically. If the breaks occur at different distances from the centromere, a shift in the centromere occurs. For example, an acrocentric chromosome may be converted to a metacentric type. In some species of Drosophila and some members of the order Orthoptera, pericentric inversions causing centromeric shifts may have been influential in producing new karyotypes, according to Swanson, et al. (1967).

The second method of classification involves principally the ways in which two inversions in the same chromosome occur in relation to one another. By this method, Dobzhansky (1951) recognizes four types of inversions. The first type is a single inversion and may be represented

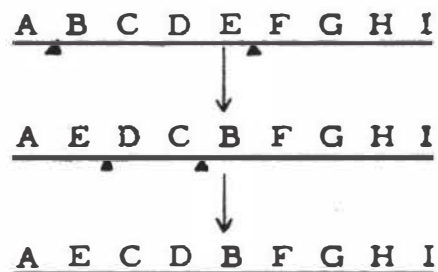
diagrammatically as follows:



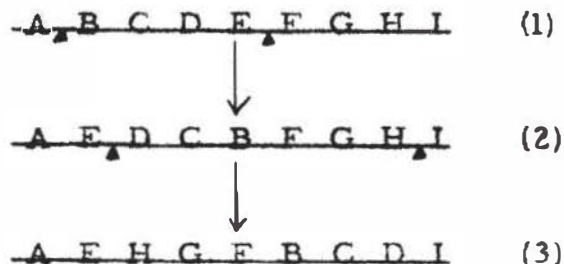
The homologous chromosomes will pair by forming a loop as stated above. The second type involves two inversions of the same chromosome independent of one another. This may be represented as follows:



The homologues in this case will pair by forming a double loop. The third type occurs when the second inversion forms inside the first and is termed an included inversion. This may be diagrammed as follows:



The fourth and final type of inversion is termed an overlapping inversion and occurs when the second inversion has one end inside and one end outside the limits of the first. A diagrammatic representation of this would be:



In this case, the first (1) can arise from the second or the second from the first through a single inversion. The same is true for the second and third chromosomes. But the third cannot arise from the first without first going through the second one. Therefore, the phylogenetic relationship is $1 \rightarrow 2 \rightarrow 3$, or $3 \rightarrow 2 \rightarrow 1$, or $1 \leftarrow 2 \rightarrow 3$, but not $1 \rightleftharpoons 3$ (Dobzhansky, 1951).

Inversions are the most important aberration of the four types when evolutionary significance is considered. Evolution in this group centers around the paracentric type. Dobzhansky's work with inversions in D. pseudoobscura is the classical example of inversions as well as their effect on the evolution of a species (Swanson, et al., 1967). Dobzhansky found that even though many inversions are present in the same natural populations, when their relative frequency is plotted on a regional or seasonal basis, certain patterns emerge. For example, the standard inversion in D. pseudoobscura, ST, was more frequent at lower altitudes in the Sierra Nevada mountains than the Arrowhead inversion, AR, but a reversal in frequency took place at high altitudes. The Chiricahua inversion, CH, was also present in the same population but showed no altitudinal variations. In addition, the ST inversion at any altitude was higher in frequency when the temperature was warm than when it was cool. Conversely, CH increased in frequency as cool weather approached. The conclusion derived from this was that inversions may be selected for in natural populations. Seasonal and regional studies show that such inversions have definite selective

values, which in Drosophila are sufficient to cause the population to respond, in terms of frequency of individuals, to the changing characteristics of the local environment (Swanson, et al., 1967).

Translocations

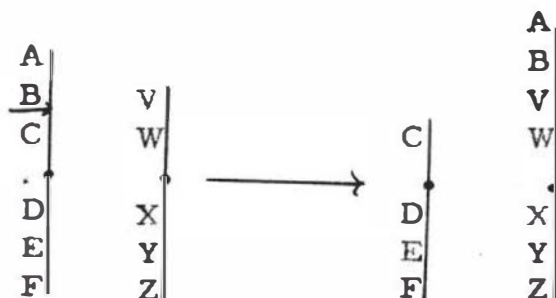
Translocations are chromosomal aberrations which occur when parts of chromosomes become detached and reunited with non-homologous chromosomes. The term also includes exchanges between different, nonhomologous parts of the same chromosome pair; for example, between the X and Y chromosomes (Gardner, 1968).

In Drosophila, translocations were first recognized genetically by the unusual behavior of a part of the second chromosome, a gene known as Pale, which had the phenotypic effect of diluting certain eye colors (Strickberger, 1968). Pale was lethal in the homozygous condition. Bridges found that its lethality as well as its phenotypic effect could be suppressed by the presence of another gene discovered at the same time on the third chromosome, which was also lethal in the homozygous condition. The lethality of the latter, in turn, was suppressed by the presence of the former. He found that Pale was caused by a deficiency for a small section of genes on the tip of the second chromosome. These genes, from the deficiency in one chromosome, had been translocated to another chromosome.

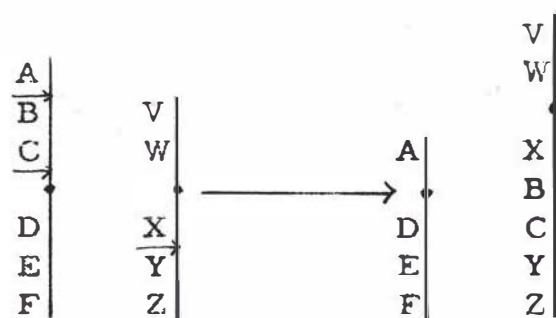
Swanson, et al. (1967) state that translocations may be homozygous or heterozygous. Homozygous translocations behave as the

normal chromosomes from which they arose except that new linkage groups are formed. If they persist, they can give rise to a new chromosome race. This type is common in plants such as Oenothera, Paeonia, and Datura; in animals it is common in scorpions and roaches. Strickberger (1968) adds that translocation homozygotes form the same number of pairs as normal homologues as long as the centromeres have not been lost. Meiotic disjunction between homologous translocation chromosomes is normal, and each gamete receives a full complement of genes, according to Strickberger. The second type, translocation heterozygotes, are easily recognized by their pairing configuration during prophase and metaphase of the first meiotic division. Complete pairing requires that the chromosomes form a typical cross-like figure at pachynema (Swanson, et al., 1967). In Drosophila salivary gland chromosomes, one can see the crosses in great detail because of the close somatic pairing between homologous sections (Strickberger, 1968).

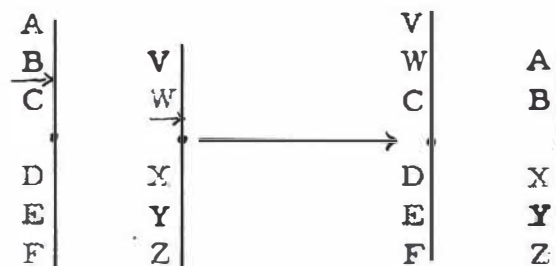
A variety of translocation types are known. Strickberger (1968) discusses three major types: A simple translocation involves a single break in a chromosome and the transfer of the broken piece directly onto the end of another chromosome. This type, which is rarely found, can be diagrammed as follows:



The second type, the shift, involves three breaks. A two-break section of one chromosome is inserted within the break produced in a non-homologous chromosome. An example would be the Pale translocation inserted within another chromosome as described previously. This kind of translocation is more common than the simple one. It may be diagrammed as follows:



A reciprocal translocation, the third type, results when two chromosomes are broken and then mutually exchange blocks of chromatin (Swanson, et al., 1967). The two will function normally in division if each possesses a single centromere. If dicentric or acentric chromosomes are produced, they will be eliminated because they will fail to segregate properly at anaphase. Strickberger (1968) diagrams this situation as follows:



In dipteran salivary gland chromosomes, translocations are easily recognized, and the banded structure permits the exact points of breakage to be determined with certainty (Swanson, et al., 1967). Gardner (1968) states that they are also detected by noting altered linkage arrangements brought about by exchanges of parts between different chromosomes.

According to Swanson, et al. (1967), translocations lead to changes in gene linkage and consequently easily incurred meiotic abnormalities. For example, Down's syndrome, which is the condition of trisomy in chromosome 21, results in a diploid number of forty-seven chromosomes. Sometimes, however, the same phenotype is encountered in some individuals with the normal number of forty-six. Two kinds of translocations are involved, both involving chromosome 21. In the first kind, a translocation between chromosomes 15 and 21 gives a karyotype that includes a normal 15, and a translocated 15-21 chromosome, and a pair of 21 chromosomes. The segment of 21 represented in triplicate results in the syndrome. The second kind, a translocation between the two 21 chromosomes, produces a new chromosome with both of its arms similar in genetic content. When present in an individual with a normal 21, the phenotype is again seen because of a triplicated region. Gardner (1968) gives a second consequence of translocations: the reduction of crossing over by interfering with chromosome pairing.

Translocations are more common in plants, and therefore more important evolutionarily, than they are in animals.

Chromosomal Puffs in Polytene Chromosomes (Beerman and Clever, 1964)

Another structural modification that occurs on the polytene chromosomes is known as chromosomal puffs or Balbiani rings. These enlarged regions on the giant chromosomes found in some insect cells, such as those of Drosophila and Chironomus, have been shown to be active genes and probably produce the nucleic acid that translates the genetic information.

The coherence of the chromosome filaments is loosened at the puffed regions. The loosening always starts at a single band. In small puffs, a particular band simply loses its sharp contour and presents a diffuse, out-of-focus appearance in the microscope. At other loci, or at other times, a band may look as though it had "exploded" into a large ring of loops around the chromosome. These donut-like structures are called Balbiani rings after E. G. Balbiani who first described them in 1881.

Puffing is thought to be due to the unfolding or uncoiling of individual chromomeres in a band. On observing that specific tissues and stages of development are characterized by definite puff patterns, Beerman in 1952 postulated that a particular sequence of puffs represents a corresponding pattern of gene activity. If differential gene activation does occur, one would predict that genes in specific types of cells will regularly puff whereas the same gene in another type of cell will not. A gene of this kind has been discovered in Chironomus. A group of four cells near the duct of the salivary gland of C. pallidivittatus produces

a granular secretion. The same cells in the closely related species, C. tentans, gives off a nongranular fluid substance. Also, the size of the puff is positively correlated with the number of granules. This reveals the association between a puff and a specific cellular product.

Chromosome puffs contain significant amounts of RNA. Normal, unpuffed bands contain chiefly DNA and histone. The amount of these compounds remains relatively unchanged in the transition from a band to a puff, whereas the amount of RNA increases greatly. C. Pelling demonstrated that RNA is the major puff product by using the autoradiography technique. He used uridine labeled with radioactive hydrogen (tritium), working with Chironomus larvae. Only the puffs and nucleoli were labeled. Also, when the preparations were treated with an enzyme that decomposes RNA, the label was absent. Pelling also demonstrated that the rate of RNA synthesis is closely correlated with the relative size of the puffs. Administering the antibiotic Actinomycin D, a specific inhibitor of any RNA synthesis that depends directly on DNA, stopped the formation of RNA, thereby proving that the synthesis was taking place at the site of the DNA in the chromosome. It is believed that the RNA is of the messenger type.

The authors also found that puff formation is associated with metamorphosis in insects, governed by the hormone ecdysone. The time relation between the changes in puffing of individual loci and the metamorphic process in the larva indicated phases in which a

puff is produced alternating with phases in which a puff is absent. Some puffs have no apparent connection with molting; others, however, appear regularly only after the molting of the larva has begun, some at the start of molting, others later. Apparently, these chromosomal sites participate in metabolic processes that take place in the cell only during the molting phase. Thirdly, another group of puffs, which are found the larvae of all ages, become particularly large during metamorphosis. This indicates that some components of the metabolic process not specific to molting are intensified at that time.

MATERIALS

The following materials were needed for this investigation:

Sterilized Drosophila culture bottles with cotton plugs

Instant Drosophila medium

Yeast

Fourth instar larvae of three strains of D. melanogaster, wild-type, from: Olney, Illinois; Charleston, Illinois; and Channahon, Illinois

Stock cultures of the three above-mentioned strains of flies

Two dissecting tools (insect pins placed in pieces of balsa wood cut to the size needed for the person dissecting. The pin point was sharpened.)

Small watchglass

Glass slides and coverslips (both square and round)

Two siliconized slides

Lacto-aceto-orcein stain (2%)

Carnoy's fluid (Fixative)

Glacial acetic acid - 20.0 ml

Absolute ethyl alcohol - 60.0 ml (Humason, 1967)

Dipteran ringer solution

5.5 g NaCl

0.22 g KCl

0.44 g CaCl_2

Dissolved in one liter distilled water (Buck, 1942)

Dissecting scope with light source

Light microscope with phase contrast microscopy

Dry ice

Razor blades

Forceps

70% ethyl alcohol

95% ethyl alcohol

Xylene

Kleermount mounting medium

Dissecting needles (Probes)

Slide warmer

Camera

Black and white film - Kodak Panatomic X-High Contrast 32 ASA

Chemicals for film development:

Microdol X

Stop bath

Rapid fixer

Hypoclearing agent

Photoflow

Dektol

METHODS

Three cultures of Drosophila melanogaster, which were field-collected in 1967 from Olney, Charleston, and Channahon, Illinois, were subcultured. These three stocks were then subcultured about every two weeks for two reasons: to perpetuate the strains; and to obtain continuous fourth instar larvae from each strain.

The two percent lacto-aceto-orcein stain was filtered prior to using it. The Ringer solution was made up according to the procedure used by Buck (1942). The fixative was prepared as stated in Humason's technique book (1967). These were placed under refrigeration and kept cool until time for use. After each use, they were returned to the refrigerator.

The fourth instar larvae were removed from the culture bottle and placed in Dipteran ringer solution. A drop of the Ringer's solution was placed on a siliconized slide to keep it from spreading out, and a larva was placed in this drop. Under the dissecting scope, the salivary glands of the larva were dissected out by placing one dissecting probe in the anterior end just behind the mouth hooks, the other probe in the mid-body region, and pulling gently. The salivary glands emerged from the anterior end when this procedure was done correctly. If not, they could be dissected out from the mass of internal organs. The glands were then moved to a clean part of the Ringer's drop and the fat was removed from them. Following this, the glands were placed in a drop of Carnoy's fixative on another siliconized slide

for three minutes. They were then transferred to a non-siliconized, clean slide upon which a drop of lacto-aceto-orcein had been placed. Slides made prior to June 17, 1970, were left in the stain for three minutes; after this date, they were left in the stain for five minutes. A clean coverslip was then placed over the stain, and the slide was placed between paper towels. The preparation was smashed by rubbing the thumb in a circular motion over the coverslip area. Several times, pressure was applied in the center of the coverslip. Care was taken not to move the coverslip when smashing the preparation. The slides were labeled with the strain of fly and date of preparation. The temporary slides were placed in the freezer of a refrigerator until they could be made permanent.

The slides were made permanent using the dry ice method. The temporary slides were placed on a block of dry ice for approximately thirty minutes. A slide was then removed and the coverslip was "snapped" off with a razor blade. The tissue was affixed to the slide as a result of the freezing action of the dry ice. The slide was then placed in a series of alcohol solutions, 70% ethyl, 95% ethyl, and 95% ethyl for two minutes each. It was then immersed in xylene for about thirty seconds. The slide was placed on end on a towel to absorb the excess xylene. A drop of Kleermount mounting medium was placed over the tissue and a glass coverslip (round) was lowered into place with a probe. The slides when completed were placed on a slide warmer for at least forty-eight hours. Approximately one hundred

slides were prepared for each of the three strains of fruit flies in this manner.

The slides were permanently labeled and viewed using phase contrast microscopy as well as light microscopy. Photographs were taken of well-spread chromosome preparations and the photos were developed and printed using the following technique:

Development of film:

- 1) Microdol X
Dilution 1 Microdol 3 Water
6 minutes at 70° F
- 2) Rinse
- 3) Stop bath 15 seconds
- 4) Rapid fixer
Undiluted 2-4 minutes
- 5) Hypoclearing agent
Undiluted 1-2 minutes
- 6) Rinse in photoflow

Development of paper:

- 1) Dektol
Dilution 1 dektol 2 water
- 2) Stop bath
- 3) Rapid fixer
5-10 minutes paper
- 4) Rinse 30 seconds
- 5) Hypoclearing agent 2 minutes
- 6) Photoflow rinse

RESULTS AND DISCUSSION

The giant polytene chromosomes of the salivary glands of Drosophila melanogaster serve as an excellent means for studying the cytogenetics of these three strains of flies. Their large size and stainability enable comparisons to be made easily.

This species is ideal to work with in investigations of this nature not only because of the giant chromosomes but also because of its rapid multiplication rate and the ease with which it can be maintained in the laboratory.

The salivary glands of D. melanogaster are paired, grayish organs found only in the larval stage. Just prior to pupation, they degenerate and are therefore not present in the pupa nor in the adult stages. The best larval stage for optimum chromosomes in both size and clarity is the fourth instar larva approximately eight hours prior to pupation.

The chromosomal complement of D. melanogaster consists of four pairs of chromosomes in the synapsed condition. The centromeres of all these chromosomes are joined at the chromocenter. What is seen, then, is the X chromosome, the right and left arms of the second chromosome, the right and left arms of the third chromosome, and the very short fourth chromosome radiating out from this chromocenter. This is illustrated in Figure 1.

The chromosomes were studied and identified from among the three strains by studying and using a system devised by C. B.

Bridges in 1935 and by the use of a chromosome map which illustrates this system.

Bridges (1935) established a system of cataloguing the bands of the salivary chromosomes which divides the five main chromosome limbs (1=X, 2L, 2R, 3L, 3R) each into twenty sections, one hundred in all:

X = 1 - 20
 2L= 21 - 40
 2R= 41 - 60
 3L= 61 - 80
 3R= 81 - 100
 4 = 101 - 102

Therefore, the number of a section is a key to the chromosome limb and to the relative position along that limb.

In this system, each section begins with a conspicuous and easily recognized band since sharpness and definiteness are essentials. Also, since the 102 divisions average over twenty-five bands each, six subdivisions were established by Bridges for each division. Each of these also begins with a sharp band and is designated by the letters A - F. Therefore, a band could be referred to as 17B3, for example.

Some of the "landmarks" used by Bridges and subsequently by the author to identify the chromosomes include the following:

The X Chromosome:

- a. "Puff" in 2B
- b. "Four brothers" in 9A
- c. "Weak spot" in 11A
- d. 2 "chains" in 15
- e. "Offset" in 19E
- f. The huge, lightly staining nucleolus of the salivary cell nucleus is attached to the base of the X chromosome at bands in 20 C and D.

Chromosome 2L:

- a. "Dog collar" in 21CD
- b. "Shoe buckle" of 25A
- c. "Shield" in 30A
- d. "Goose-neck" in 31BF
- e. "Spiral loop" of 32-35

Chromosome 2R:

- a. Thick "onion" base
- b. "Huckleberry" tip

Chromosome 3L:

- a. "Barrel" at 61CF
- b. "Ballet skirt" at 68BC
- c. "Chinese lanterns" in 74-75
- d. "Graded capsules" in 79CDE

Chromosome 3R:

- a. Large, clear "cucumber" base 81-83D
- b. "Duck's head" at 89E to 91A
- c. "Goblet" tip

Chromosome 4:

Short length

Some of these bands identified by Bridges could be seen in the chromosomal preparations. Many, however, were obscured by the characteristic coiling of the chromosomes. In some instances, the squash preparation yielded almost straight chromosomes; and these were the ones studied most closely for chromosomal identification. Therefore, a few key markings were used for the identification of each chromosome.

The X chromosome was identified almost exclusively by the very obvious "puff" in region 2B. In some preparations, it could also be identified by the large, lightly-staining nucleolus attached to its base.

Chromosomes two and three were most easily recognized by their characteristic tips. Some other structures used for chromosome three included notably the "ballet skirt" in region 68BC; the "Chinese lanterns" in 74-75; and the "duck's head" extending from regions 89E to 91A. (As mentioned previously, these were the terms used by Bridges to describe these bands and areas on the chromosomes.)

Chromosome four was recognized by its very small size as compared to the others. In most preparations, it was obscured by the larger chromosomes, but was found in one smear.

The lengths of the chromosomes can be measured by means of a slide micrometer. A photograph of it was also taken and included to compare the sizes with those in the literature. (Fig. 2)

According to Bridges (1935), the lengths of the chromosomes are as follows:

X = 220 micra

2L= 215 micra

2R= 245 micra

3L= 210 micra

3R= 275 micra

4 = 15 micra

Total =1180 micra

The chromosome map included in the appendix illustrates Bridges' system of chromosome map nomenclature and shows the landmarks of the chromosomes as well as their comparative lengths.

The photograph which follows illustrates a well-spread chromosome and the detail which can be seen with a light microscope. (Fig. 3)

Several examples of chromosomes undergoing "puffing" or the formation of Balbiani rings were observed. This would indicate

that the synthesis of RNA was occurring in these regions. These were observed in all three strains of flies and was an expected occurrence. A photograph illustrating a large ring follows. (Fig. 4)

Another cytogenetical structure that was observed was the presence of interchromosomal connectives. These are thin threads that appear to connect one chromosome with another or one part of one chromosome with another part of the same chromosome. According to Keppler (Pers. Comm., 1970), the function of these connectives is unknown; but they perhaps indicate a form of communication between two chromosomes or between distant parts of the same chromosome. These were observed in the Charleston population of flies and are illustrated in the photographs that follow. Their function and significance need further investigation. (Fig. 5)

Areas of asynapsis were found in some of the chromosome preparations. Since it is a characteristic of these polytene chromosomes to occur in the synapsed condition, the presence of an area along the chromosomes that is not synapsed would indicate a change in the gene structure or arrangement preventing these two chromosomes from pairing as usual. These were not considered true inversions since they did not follow the looping pattern that an inversion shows. It was thought that perhaps these areas were the beginning of inversions or some other aberration. The most prominent one appeared in the Channahon strain of flies and was photographed. Regretfully, only a small portion of the chromosome was present and represented an insufficient amount for identification. Other areas of asynapsis were

found in the other strains as well. The most prominent one is illustrated in Figure 6.

Finally, the purpose of this study was to try to determine if these three strains differed by comparing the frequencies of ~~inversions~~ and the other aberrations. Only one true inversion was identified, and this occurred in the Olney population. Upon close examination, this inversion was found to be located in the left arm of the third chromosome. It was a heterozygous inversion which involved the chromosomal bands located between 70CD and 74CD. Although part of the chromosome was faded in the preparation, certain landmarks were well-established and verify that it was in this region. The band at region 75BC was very obvious as was the puff in region 68BC. (Fig. 7)

Many loops were found in all the the strains; but when they were enlarged and their structure studied, they were all found to be intricate coils in the chromosomes instead of inversion loops. Thus, only one true inversion was found; and no other aberrations were apparent in any of the strains at the time the study was made.

The fact that only one inversion was found in the Olney strain out of all of the chromosomal preparations made indicates that it was most likely a mutation in the chromosome rather than indicating an evolutionary trend in this strain. Had many such inversions occurred in this population and in the same chromosome, a statement could be made

concerning the trend of this group. However, this was not the case.

Therefore, these three strains seem to be cytogenetically identical with the exception of a few mutated areas. The observations indicate that these few areas are not of great of significance to justify stating that the strains are dissimilar. The chromosomes, in masse, look identical and it would take a person with a great deal of experience with chromosome identification to be able to distinguish the three strains of flies on the basis of their chromosome appearance. Therefore, the distances that separate these three strains of D. melanogaster in the state of Illinois do not affect their cytogenetics. They represent common, similar examples of this species.

Some of the problems encountered during this investigation included the following: First, the larvae had to be obtained at just the right time in order to obtain optimum chromosomal preparations. Secondly, the squash preparation must be employed correctly to obtain good spreads. It was found that a slight tap on the coverslip produced better spreads. It also appeared that the time of the year affected the quality of the chromosomes as well as their spreading patterns. The spring season yielded optimum chromosomes, and this might be attributed to the biological clock mechanism that operates in all animals during this season. Thirdly, overstaining and understaining obscured the chromosome bands. Lastly, the coiling nature of the chromosomes, even in good spreads, made identification of the chromosomes difficult.

CONCLUSIONS AND SUMMARY

Based upon the information obtained from this investigation, the three strains of Illinois Drosophila melanogaster are cytogenetically identical. There appears to be no major difference in the structure of the chromosomes of these three strains, although a few minor differences were found to exist. These were assumed to be nothing more than mutated areas in the chromosomes and not an evolutionary trend in the direction of different chromosomal structure for the strains involved.

It is the opinion of the author that more work needs to be done in this area of studying the similarities and dissimilarities between different strains of one population of any species of organism. Perhaps greater geographical isolation of the strains used would produce more aberrations. Also, a larger survey of the strains would perhaps yield more aberrations with which to work. Finally, the employment of specific stains to the chromosomes might enhance the results by showing greater detail in chromosomal structure.

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A P P E N D I X



Figure 1. The chromocenter of the chromosome complement of Drosophila melanogaster. (1000x)

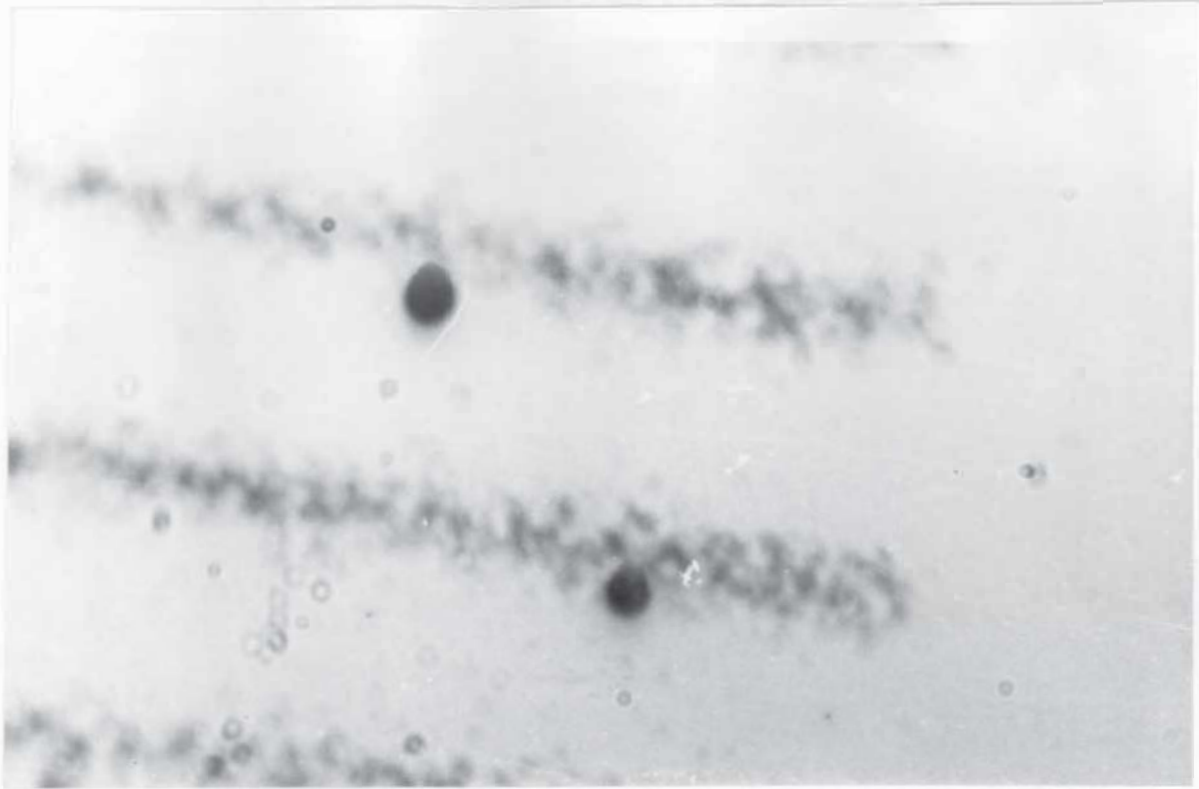


Figure 2. Slide micrometer. (1000x)

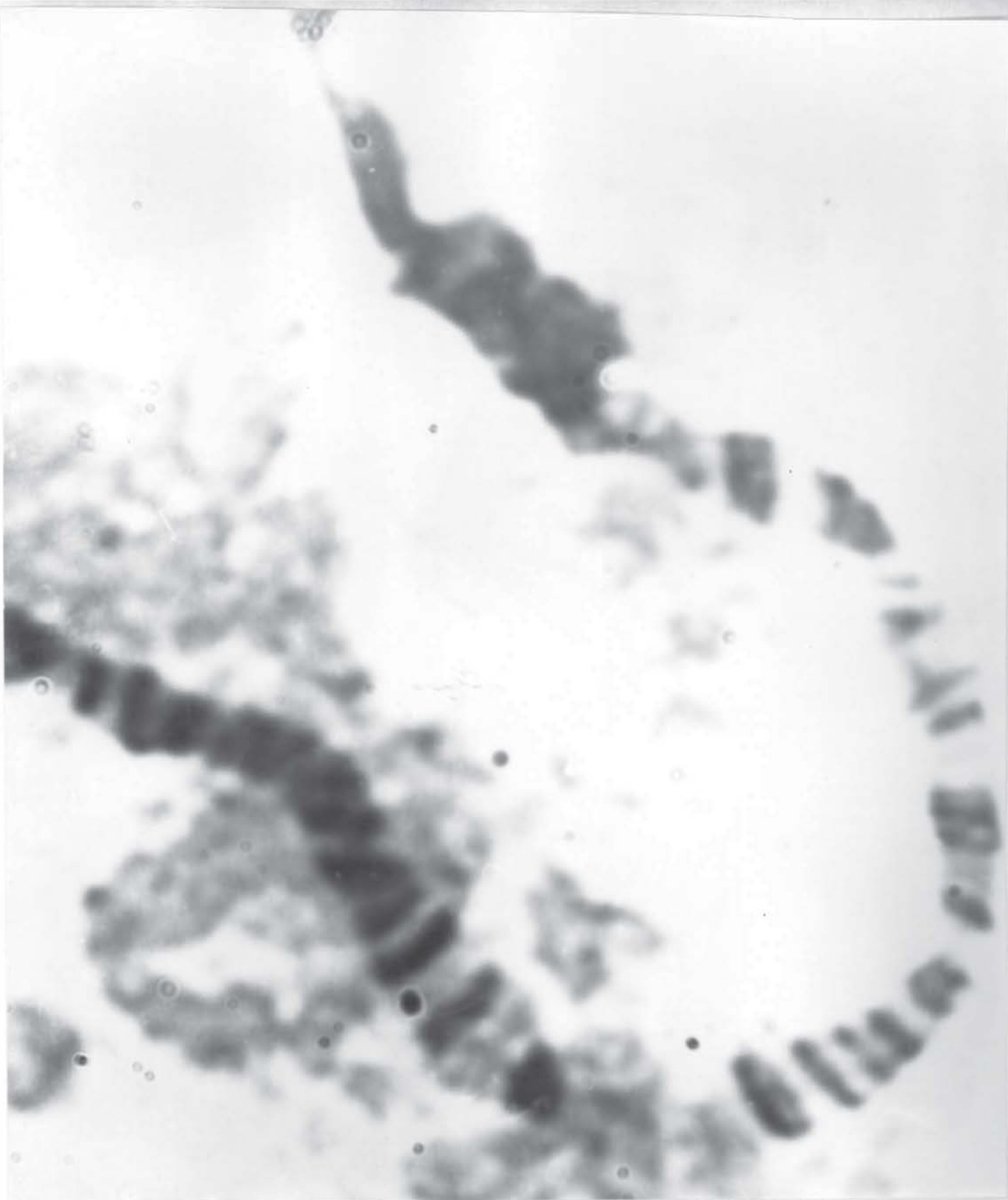
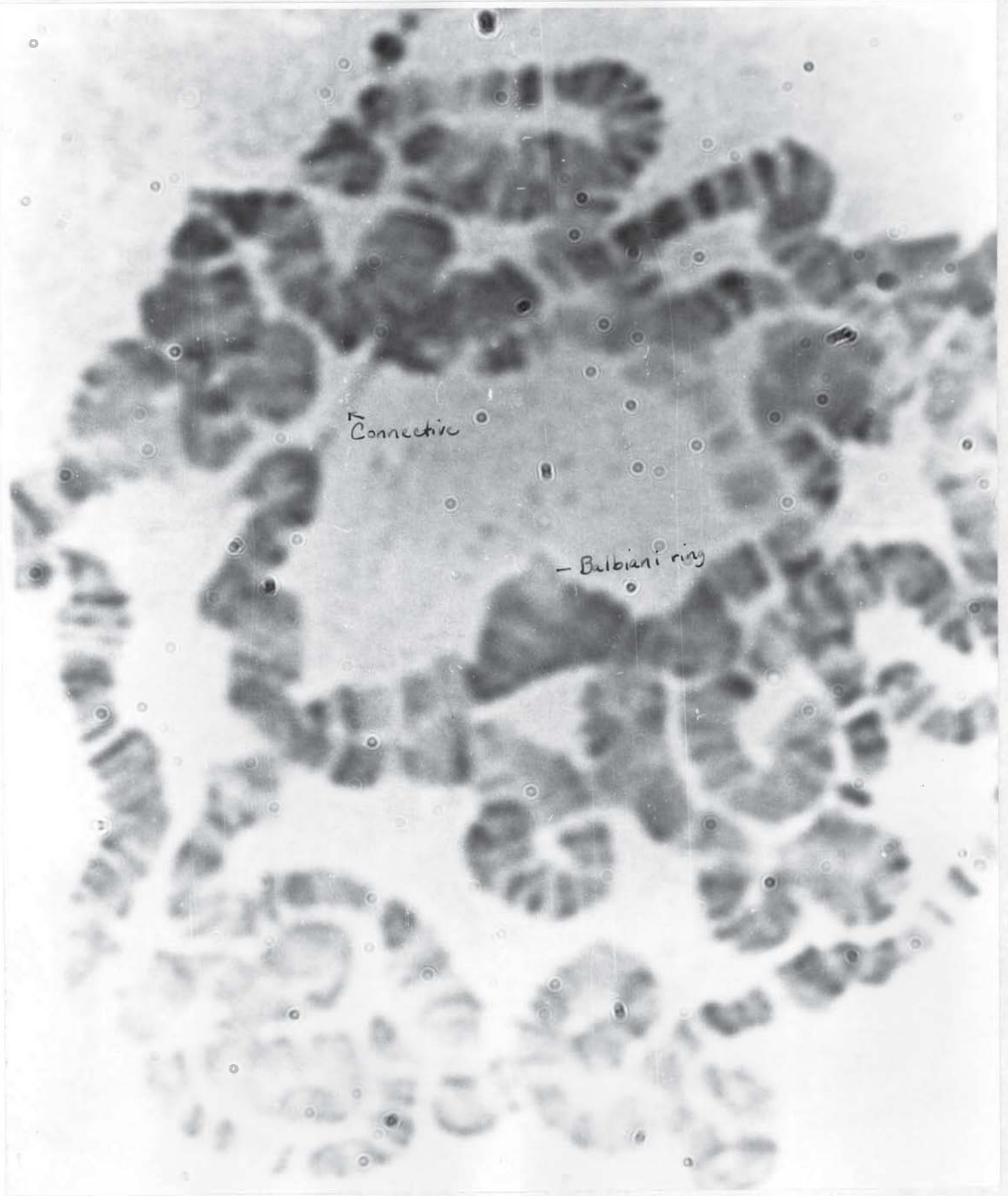


Figure 3. A portion of chromosome 3 showing cytological detail. (1000x)



Figures 4 and 5. A chromosomal smear showing both a Balbiani ring and the chromosomal interconnective. (1000 x).

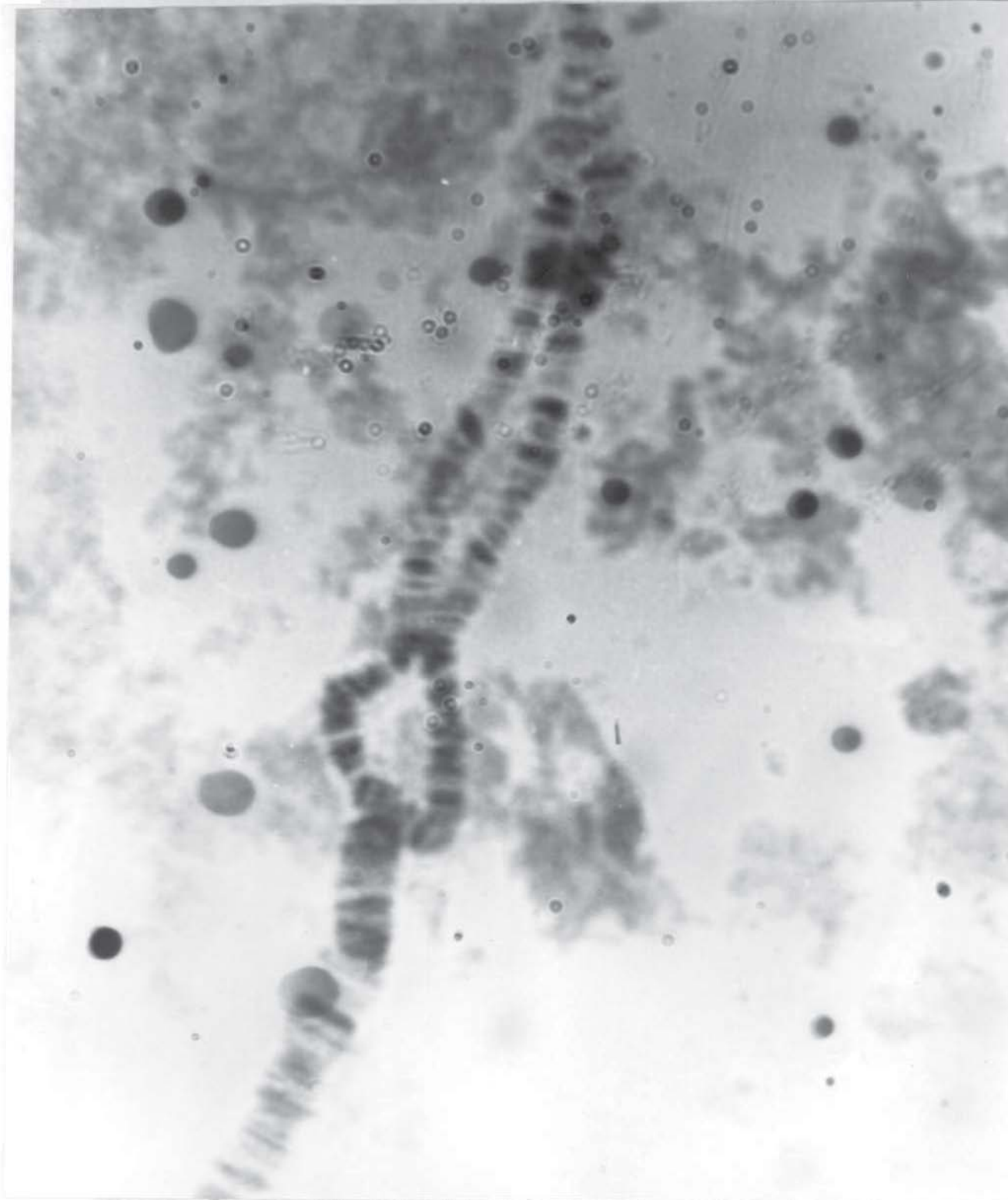


Figure 6. An asynaptic portion of a salivary gland chromosome. (1000x)

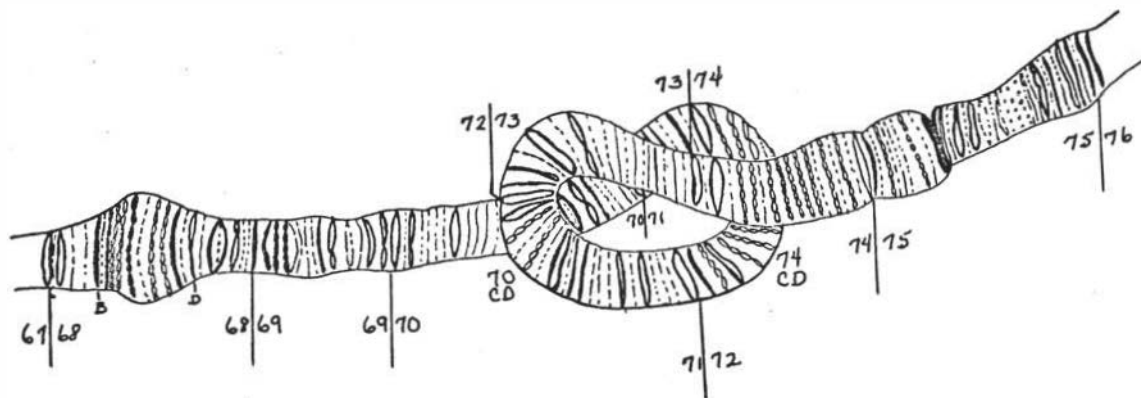
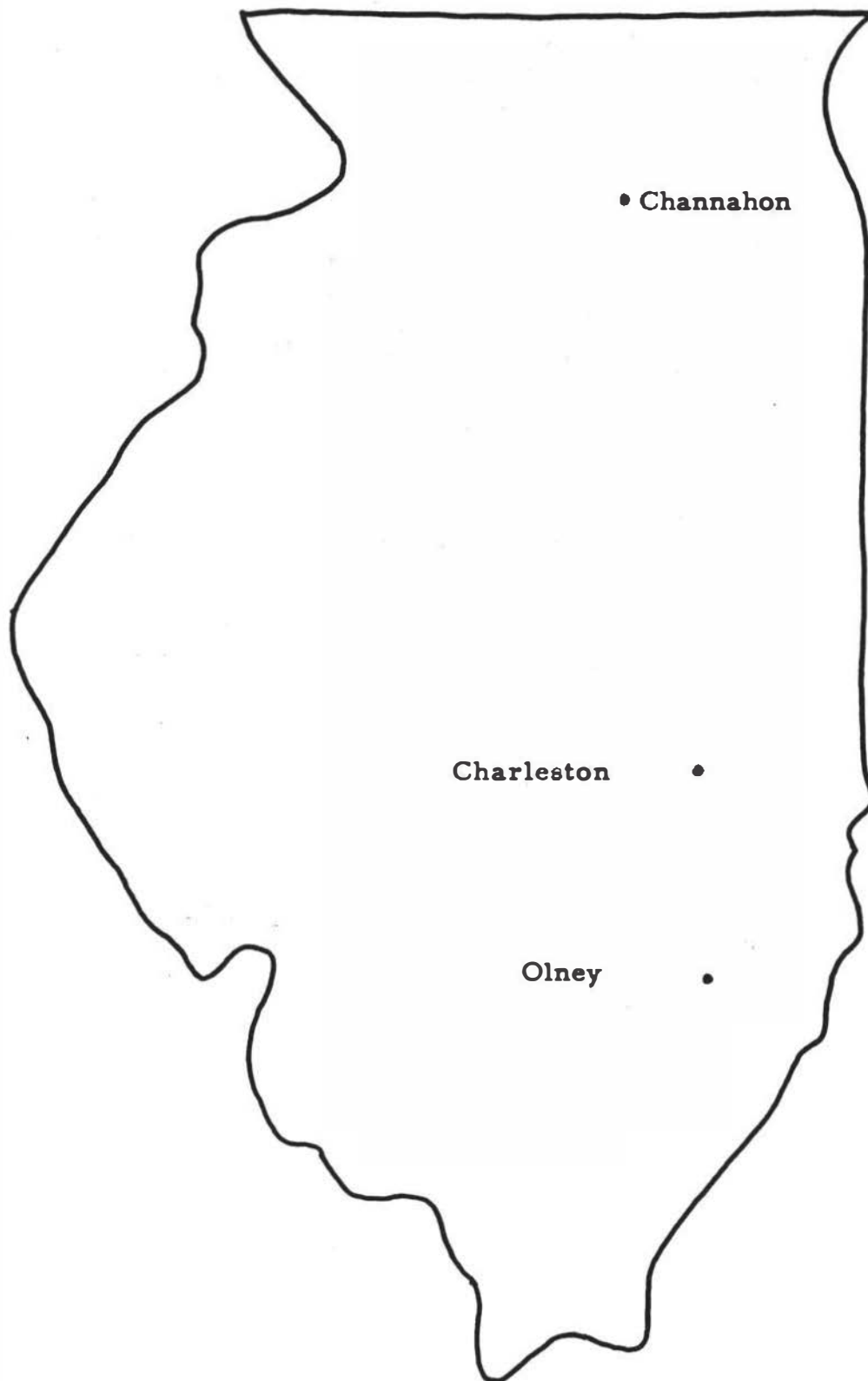
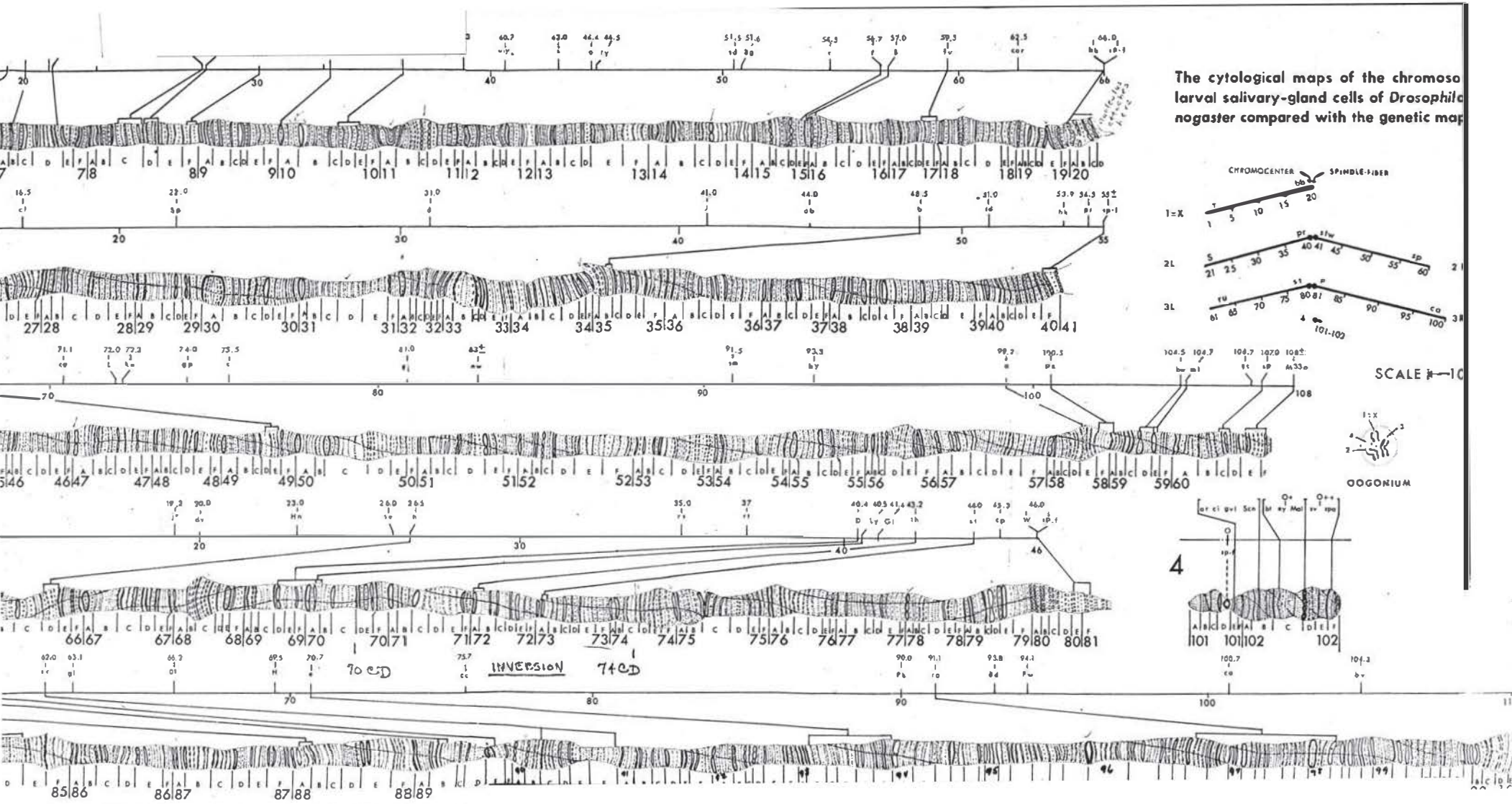


Figure 7. A detailed diagram of the heterozygous inversion present in the Olney strain of Drosophila melanogaster.

MAP OF ILLINOIS

Location of Collection Sites





The cytological maps of the chromosome larval salivary-gland cells of *Drosophila melanogaster* compared with the genetic map

Figure 10-4 The cytological map is subdivided into 102 divisions each of which is further divided into six subdivisions lettered in groups of six (A-F). The sub-

Chromosome map used for chromosomal identification.

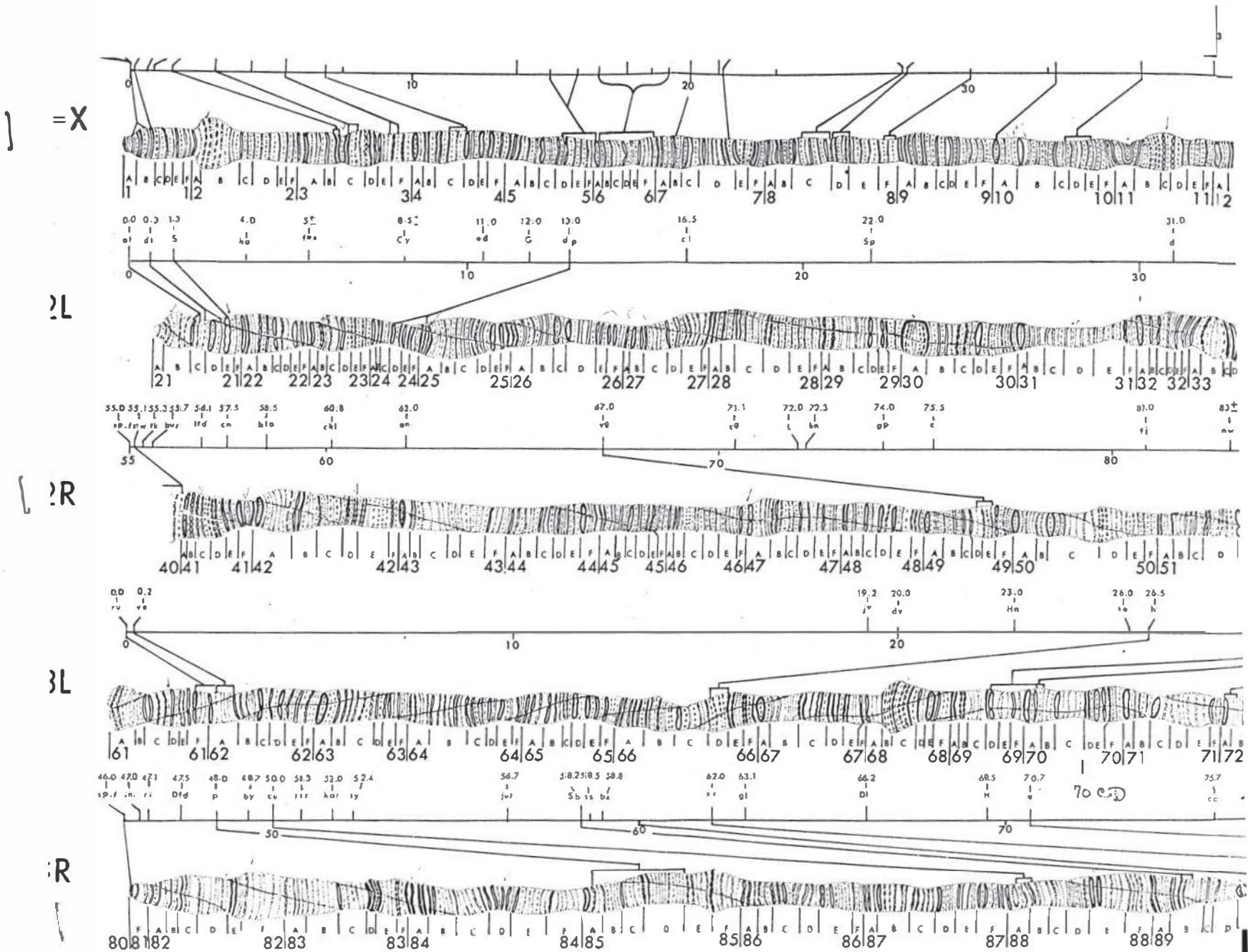


Figure 10-4 The cytological map is subdivided into 102 divisions each of which is further divided into six subdivisions lettered in groups of six (A-F). The sub-