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CHROMOSOME STUDIES IN ELEPHANTULUS WITH SPECIAL REFERENCE TO THE ALLOCYCLIC BEHAVIOUR OF THE SEX CHROMOSOMES AND THE STRUCTURE OF HETEROCHROMATIN.

by

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Thesis presented in fulfilment of the requirements for the degree of Master of Science in the University of the Witwatersrand.

January, 1947.

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"Now come: I will untangle for thy steps
Now by what motion the begetting bodies
Of the world-stuff, beget the varied world,
And then forever resolve it when begot .....
For far beneath the ken of senses lies
The nature of those ultimates of the world."

Lucretius.

ACKNOWLEDGEMENTS.

I wish to thank Professor R.A. Dart for providing the facilities for this research work in the Department of Anatomy and for the interest shown in the progress of the investigation.

Professor C.J. van der Horst kindly arranged for the trapping and delivery of the live animals used in this study.

Dr. Joseph Gillman is in many ways connected with the present investigation. It was begun under his instigation and carried safely to the end with his guidance. From a wealth of biological experience he offered assistance and constructive criticism which proved invaluable for the presentation of the thesis. It is a pleasure to extend my sincere gratitude and appreciation to him for all this, and also for instilling into me the spirit of scientific research.

I take pleasure in acknowledging my indebtedness to my colleagues, Dr. A.G. Oettlé, Phillip Tobias and Julien Hoffman for the many fruitful discussions on the problems of the chromosome.

My thanks are due to Mr. D.S. Dry for assistance with the photomicrography.

Finally, I wish to thank Miss J.M. de Saxe and Mrs J. Deacon for their patience and care in the typing of the manuscript.

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CHAPTER I.

GENERAL INTRODUCTION.

Every organism, whether it be plant or animal, worm or man, propagates itself with a definable degree of constancy. Such constancy cannot be entirely related to the ever-changing external environment; it becomes, of necessity, mainly an inherent function of the organism itself. Somewhere in the organism, there exists a system which determines, controls, or regulates the visible expressions of organismal constancy.

Mendel showed that the transmission of organismal characters could be assigned to a series of factors within the organism which segregated and recombined according to mathematical laws. Although these Mendelian factors governing heredity were completely hypothetical, they marked the advent of atomism to genetics. Organismal constancy was the function of a constant series of particles handed down from generation to generation in accordance with determined laws and ratios. This atomistic approach was the keynote of all the theories of heredity propounded by other nineteenth century

biologists. They all shared the common idea that particles transmitted by the germ cells, were ultimately responsible for the specific characteristics of the organism. The 'gemmules' of Darwin, Weismann's 'ids', 'biophores' and 'determinants' and the 'pangens' of de Vries are merely different trade-marks of the same product.

While Mendel's work lay forgotten in an obscure Austrian journal, Oskar Hertwig observed that penetration of the ovum by the sperm was essential for fertilization and in this way rejected the Animalculist-Ovist concepts of develpment. Flemming discovered the chromosomes and investigated the phenomena of mitosis, while Boveri, van Beneden and Strasburger described the structure and behaviour of the chromosomes at meiosis and fertilization. It did not take long to appreciate that, of all the cellular constituents, the chromosomes alone possessed that constancy of structure and behaviour necessary for inheritance. In this way, emerged the great achievement of nineteenth century cytology - the generalization that the <u>chromosome was the physical basis for heredity</u>.

At the turn of the century, Mendel's works were rediscovered. Equally important was the demonstration that the behaviour of the chromosomes at meiosis and fertilization could be correlated with the segregation and recombination of the Mendelian factors. Thus the particles, postulated by Mendel, received a material referent in the form of the chromosome. Since the organism has a large number of transmissible characteristics and only a limited number of chromosomes, the presumption was made that each chromosome was constituted of a large number of smaller particles which determined the specific characters of the organism and which showed Mendelian behaviour. This particle of inheritance is now called the gene.

Originally, the notion of the gene sprang from the need for a more intimate understanding of organismal variation and heredity. In the spheres of embryology, evolution and physiology, the gene has been of indisputable value as a biological concept, forming the basis for the interpretation of diverse biological phenomena. Thus, when Waddington (1940) states that the genes are undoubtedly the fundamental agents controlling the

processes of differentiation; when Dobzhansky (1941) claims that evolution is basically a change in gene constitution; when Haldane (1937) and Muller (1941) appoint the gene as the master-catalyst; in all three of these instances, we witness the reduction of biological concepts to a fundamental quantity - the gene. Differentiation, organismal change and intermediary metabolism all converge onto a single focal point.

The paradox is now fully established that while the gene provides a foundation for the erection of many important biological concepts, the gene itself still remains elusive. To Mendel, the gene, as we have already seen, was a purely hypothetical factor responsible for well-demarcated phenotypic expressions. After Morgan's work, the genes became units of crossing-over; for the cytologists Bridges and Painter, the gene was a morphological unit of the chromosome. Muller's work now establishes the gene as a unit of X-ray breakage; Schultz and Gulick consider it as a megamolecule of desoxyribonucleoprotein.

The attack on the properties of the gene was launched with a variety of different techniques. As a

consequence, the definitions became operational abstractions, necessitated by the technique employed. Each definition points to some facet of the gene, but the essential synthetic picture is still absent.

When a single facet of the gene, unrelated to its other attributes and to the biological system as a whole, becomes the basis for the definition of the particle responsible for heredity, the concepts developed in relation thereto, become inevitably restricted. It is therefore not entirely unexpected that such an orientation, although recognising the need for discovering the essential basis of heredity, still leads to a narrow alley, which, according to Dunn*, "although paved with good intentions, has at its end, a red light and a sign - The Gene: Dead End." The present application of the gene concept in biology therefore, casts a pessimistic reminder of the entelechy of Driesch and the reactions it produced in biological thought.

One fact is clearly evident, namely, heredity must

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*Dunn. L.C. Editor's preface to "Genetics and the Origin of Species" by T. Dobzhansky (1941).

have a material basis and, at any rate, the chromosome is one of the material components intimately connected with inheritance. If the particles related to heredity are lodged within the chromosome and are responsible for its structure, then the changes in chromosome morphology at the observable level, become a reflection of the component particles in the chromosome. In the same way as the investigation of the structure and function of complex multicellular biological systems proceeded historically from the organism to its component organs and tissues, from tissues to cells, from cells to nuclei and from nuclei to chromosomes; so too, the further elucidation of the problems of heredity will continue to develop through the chromosome to its submicroscopical constituents.

This profitable technique of proceeding from the known to the unknown in the understanding of the problems of heredity is evident from the following remarks of Dobzhansky (1941):

> "Biophysical and biochemical work of recent years has revealed an hitherto unsuspected complexity of cellular organization on the ultramicroscopical level of molecular aggregates. To a geneticist, it seems certain that the genes must at the same time be part of and determining agents of

> this 'molecular morphology', but the problems here involved have not been touched as yet."

This quotation provides the key to the attack on the morphological basis of heredity. The level of micromorphological organization includes not only the realm of paracrystalline aggregates, colloidal micelles and fibrous megamolecules but also the viruses and the cytoplasmic microsomes which seem to be closely related to the component particles of the chromosome. These particles are but micro-structural characteristics of the chromosome, and an investigation aimed at the micromorphology of the chromosomes is a logical approach to the problems of heredity. Moreover, the visible morphology and behaviour of the chromosomes are mainly expressions of reactions of their micromorphological substrata, and the elucidation of the latter will aid in the understanding of the overall structure of the chromosomes.

The morphological investigation of the chromosomes belongs clearly to the domain of the cytogeneticist, but to whom must fall the responsibility for the micromorphological problems of chromosome structure ? Not to the chemist, nor to the physicist; for, with few exceptions, these investigators have not

generally recognised the chromosomes as valid subjects of enquiry. The cytogeneticist, therefore, is still constrained to assume leadership in directing these investigations and to his already extensive vocabulary must be added the words 'micelles, macromolecules and monolayers.'

Thus the cytogeneticist is confronted by two main tasks. The first is concerned with the elucidation of the problems of the visible morphology and behaviour of the chromosomes, together with the correlative problems of cytotaxonomy and cytophysiology and the second, with the understanding of the mechanisms of heredity through an attack on the micromorphology of the chromosome.

The Presentation of the Thesis.

The present study is devoted to a cytogenetical investigation of Elephantulus myurus jamesoni. Besides the chapter on technical considerations, this thesis may be divided into two separate, though not wholly distinct parts. This is in accordance with the two above-mentioned tasks of the cytogeneticist. Thus, the first part of the thesis deals mainly with the visible

morphology and behaviour of the chromosomes at mitosis and meiosis in Elephantulus. The second is devoted to a discussion of the phenomena of charge and spiralization allocycly and the structure and genetical properties of heterochromatin.

In this way, it is hoped, firstly, that the present study will make a specific contribution to the biology of Elephantulus myurus jamesoni, an animal which has already been intensively investigated in the Departments of Zoology and Anatomy, and, secondly, that the peculiar behaviour of the sex chromosomes in Elephantulus will assist in the interpretation of chromosome structure both at the morphological and micromorphological levels.

CHAPTER II.

TECHNICAL CONSIDERATIONS.

All chromosomal studies are best conducted on living cells. The value of this procedure is illustrated by the recent contributions of Caspersson and his coworkers [Caspersson (1939); Brandt (1941); Caspersson and Brandt (1941)]. With the exception of these investigations and a few others (Lucas and Stark 1931), most cytogenetical studies are based on the observation of fixed and stained preparations of the chromosomes. Between the chromosomes as they exist in the living organism and those which appear under the microscope, there is interposed a series of treatments. It therefore becomes important to be able to control such treatments so that even if fixed chromosomes are not absolutely comparable with the living elements, at least the pictures can be constantly reproduced. Different observations made by two separate investigators attain significance only when the techniques used are similar. For this reason, all observations on chromosomal morphology and behaviour should always be prefaced by a description of the technical procedures.

Material.

The shrews used in this investigation were collected at Bronkhorstspruit in the Eastern Transvaal and sent alive

to the Department of Anatomy. All were killed with chloroform and their tissues removed and treated immediately after death. The material was prepared in a number of different ways depending on the particular staining method, applied subsequently.

Methods.

1. Squash Preparations.

Squash preparations are ideal for the enumeration of the chromosomes and for the characterization of the morphological attributes of the chromosome complement. Not only is the full complement of chromosomes preserved and visible in one plane, but also overlap and foreshortening are largely eliminated. Although this technique has been extensively used in plants and invertebrates, it has not been generally applied to mammals. The application of this technique in the present study has greatly facilitated the enumeration of the chromosomes in Elephantulus.

The original squash method was devised by Belling (1926) for the study of the chromosomes of pollen mother cells. This method consists of the simultaneous fixation and staining of teased pieces of tissue with iron-aceto-carmine. The same procedure with a few modifications was adapted to the chromosomes of other plant tissues and to the giant salivary chromosomes of the Diptera. This method provided flattened chromosome plates which permitted enumeration of the chromosomes. However, the carmine usually

stained the cytoplasm. This obscured the smaller chromosomes and thus interfered seriously with the accurate definition of the morphology of the individual chromosomes.

The acetic-orcein staining method of La Cour (1941) eliminates this disadvantage. Not only is it more specific for the chromosomes but also it requires no iron mordant. The acetic-orcein technique was the one initially used in this investigation. Although it was subsequently modified, it will be described in full, as it formed the basis for the new procedures introduced.

The acetic-orcein technique and its modifications.

The stain is prepared by dissolving one gram of orcein (Grüblers) in 45 ccs. of glacial acetic acid warmed to near boiling point. This solution is then cooled and 55 ccs. of distilled water are added, and the reagent is filtered.

Fresh tissue may be used for the squash preparation, but it is advisable to fix beforehand for 12 - 36 hours in a fluid containing 1 part of glacial acetic acid and 3 parts of absolute alcohol. This preliminary fixation prevents the cytoplasm from taking the stain, and consequently the chromosomes will be more sharply defined against a clear background.

A small piece of tissue, either fixed or fresh, is placed in a drop of the staining solution on a glass slide

for five or ten minutes. A coverslip is filmed with Mayer's Albumin and dried over a spirit flame. When a grey cloud issues from the albuminized surface of the coverslip, it is dry and ready for use. The coverslip is placed, filmed surface down, over the tissue in the drop of acetic-orcein and steady pressure is applied through several layers of blotting paper. Side to side movement of the coverslip should be avoided as it distorts the nuclei. The pressure disrupts the tissue and spreads out the cells in a single layer. At the same time, excess staining fluid is expelled. The slide is gently heated over a spirit flame to ensure the adhesion of the nuclei to the coverslip and to facilitate the flattening of the nuclei and their contents.

The preparation may be kept as a temporary mount in which case the coverslip is ringed with vaseline. If a permanent mount is required, the following method is recommended.

The slide is inverted in a covered dish containing acetic alcohol (1 part glacial acetic acid plus 3 parts absolute alcohol). In approximately twenty minutes the coverslip and the slide should separate, but if this does not occur, the coverslip may be gently prised off with a flat tipped dissecting needle.

The coverslip preparation is dehydrated in absolute alcohol (two changes) and cleared in thin cedar wood oil. It is then drained and mounted in xylol-clarite.

The method as described above has two disadvantages:

1. The orcein precipitates out of the solution on to the tissue, often obscuring important details,
2. The nuclei adhering to the coverslip are too lightly stained for study under high magnifications even with the appropriate filters.

The first difficulty may be avoided by filtering the acetic-orcein solution before use. The staining solution should be also continually renewed on the slide to prevent evaporation and subsequent precipitation.

The second disadvantage was remedied to some extent by restaining the preparations in acetic-orcein before making permanent mounts, but even after this second treatment the preparations were still unsatisfactory.

The best results were obtained when squash preparations were restained with Ehrlich's acid haematoxylin or with Heidenhain's iron haematoxylin. These stains are accepted as nuclear stains and although they have been widely used for sectioned and smeared material they have hitherto not been applied to squash preparations. Since differentiation with the retrogressive staining methods can be meticulously controlled under the microscope even

in squash preparations, it is possible to remove the background long before the chromosomes become decolourized. In this way, the separated chromosomes remain as intense black or blue bodies embedded in a colourless matrix. Intimate details of structure, such as size, shape and positions of constrictions are clearly displayed. Furthermore, the contrast and sharp definition of the chromosomes greatly facilitate photomicrography.

These haematoxylin procedures have been used to restain acetic-orcein preparations. It is possible to make the preparations with 45% acetic acid only, and then to proceed to staining with iron or acid haematoxylin. However, it is advisable to use the preliminary acetic-orcein treatment, to discover whether the number of metaphase plates in the preparation justifies further treatment.

The squash procedure is easily adaptable to a soft tissue such as testis where the packets of cells constituting the tubules are easily disrupted. Where the connective tissue forms a firm intercellular matrix, closely enveloping individual cells, satisfactory squash preparations are not readily obtained without preliminary maceration. This can be achieved without injury to the nuclei by immersing small fragments of the tissue in 45% aqueous acetic acid for 1 - 48 hours. Thereafter, the staining

technique is the same as that described above for testis.

2. Sectioned Material.

Sectioned material has its place in chromosomal studies. A general survey of the histological and cytological characteristics of the tissue is usually essential before the detailed study of chromosomal morphology may be attempted.

Fixation.

For bulk fixation, Darlington and La Cour (1942) recommend the use of the chromic-osmic-acetic acid fixatives. Although some of these fixatives such as the 2BD solution (La Cour 1931) can on occasion be useful, their penetration is greatly retarded in tissues such as the ovary, where excessive quantities of fat are present. Bouin's solution, for the most part, was entirely satisfactory as a fixative and was used throughout the investigation for bulk fixation.

The Bouin fixed material was dehydrated, cleared and embedded in the usual way. Paraffin wax sections were cut at a thickness of 12 - 15 μ on a rotary microtome. These were mounted on albuminized glass slides, usually a large number in serial order. Such sections were deparaffinized in xylol and brought to water through the down-graded alcohols.

Staining.

Some of the sections were stained with a modification of the Newton crystal violet method recommended by La Cour (1937). Although the results inclined to be erratic, the technique, when successful, distinctly displayed the leptotene and pachytene chromosomes.

For the more accurate study of chromatin in the interphase spermatogonium as well as for the investigations of chromosomal-plasmosomal relations, sections were stained with the Feulgen-light green procedure. The Feulgen reagent (Schiff's solution) was prepared according to the directions of de Tomasi (1936) who advocates the use of potassium meta-bisulphite for decolourizing the basic fuchsin solution. The light green was applied as a 0.5% squeous solution for a short period of time, and the preparation was subsequently differentiated in 70% alcohol. The counterstain recommended by Semmens and Bhaduri (1939, 1941) is unnecessarily complicated. All preparations after alcoholic dehydration were cleared in xylol and finally mounted in xylol-clarite.

Summary.

As a general routine for chromosomal studies the following methods are recommended.

1. Sectioned material fixed in Bouin's solution and stained with

(a) Crystal Violet,

(b) Feulgen light-green.

2. Acetic-orcein squash preparations restained with one of the haematoxylin methods.

The first method provides topographical relations of the cell types in the tissue under survey for the study of the chromosome cycle as well as the chromosomal-plasmosomal relations.

The second method is essential for the enumeration of the chromosome complement and for the characterization of the morphological attributes of the individual chromosomes.

CHAPTER III.

THE CHROMOSOME COMPLEMENT OF ELEPHANTUTUS.

"Search for measurable elements among your phenomena"
(A.N. Whitehead*).

Introduction.

The analysis of the chromosome complement of Elephantulus is the essential first step in the proposed cytogenetical investigation of this animal. Before attempting to describe the behaviour of the chromosomes at mitosis and meiosis, it is necessary to define the morphological characteristics of the chromosomes.

The chromosome complement is characteristic for each species and exhibits marked constancy within the individuals comprising the group. The same complement is found in somatic nuclei derived from the zygote during the ontogeny of the individual and in the nuclei of different individuals genetically related to form a species or race. These principles are embodied in the theory of chromosome permanency, reviewed in the books of Wilson (1937) and Darlington (1937). On the basis of this concept it may be stated that for the establishment of the chromosome complement of a species, justifiable generalizations may

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* Science and the Modern World, 1926.

be obtained from the study of the limited number of nuclei available in a few representatives of the species. Fortunately then, it is unnecessary to examine every single nucleus in every living Elephantulus to characterize the chromosome complement. Moreover, the definition of the characteristics of the chromosomes consists in the analysis of the constancies exhibited by the chromosome complement.

These constant features are: firstly, the number of chromosomes comprising the complement, and secondly, the size and shape of the individual chromosomes.

In the following account, these two aspects will be considered separately. Since the most favourable material has been obtained from testis, most of the observations have been made on the male complement, but wherever necessary, the nuclei of the female have been used to confirm and extend the findings in the male.

Observations.

The Chromosome Number.

Wilson (1937) states that for the establishment of the chromosome number in a species, the diploid number should be determined not only in germ cells, but also in somatic cells. Moreover, if the haploid number is also constant, and if, at the same time, the individual



Figure 1. Camera lucida drawing of a spermatogonial prometaphase plate showing the diploid number of fourteen chromosomes. The chromosomes are numbered.

12 μ section of testis stained with crystal violet.

X2400.

chromosomes can be consistently recognised in different cells, then there is a high degree of probability that the chromosome number has been accurately determined.

The metaphase of mitosis has been generally used for the enumeration of the chromosomes. Polar views of metaphase show the chromosomes arranged in one plane on the equatorial plate and therefore allow for precise observations.

In the testis of Elephantulus, such metaphase plates are rarely found in spermatogonia undergoing mitosis. In fact, in the large number of preparations examined only two such nuclei have been observed. Figure 1 is a camera lucida drawing of one of these nuclei which is at a stage just prior to metaphase. This pro-metaphase plate is complete with the exception of some foreshortening of chromosome pair 6. The complement is clearly diploid and contains fourteen chromosomes.

The second nucleus, at full metaphase, also contained the diploid number of fourteen chromosomes. The considerable underlay of the chromosomes in this case prevented accurate pictorial representation.

In addition to these plates, large numbers of nuclei containing twenty-eight chromosomes have been observed both in sectioned and squashed material. Figures 2, 3, 4, 5, 6 and 8 are microphotographs of such nuclei in squash preparations. The entire complement is clearly shown, and

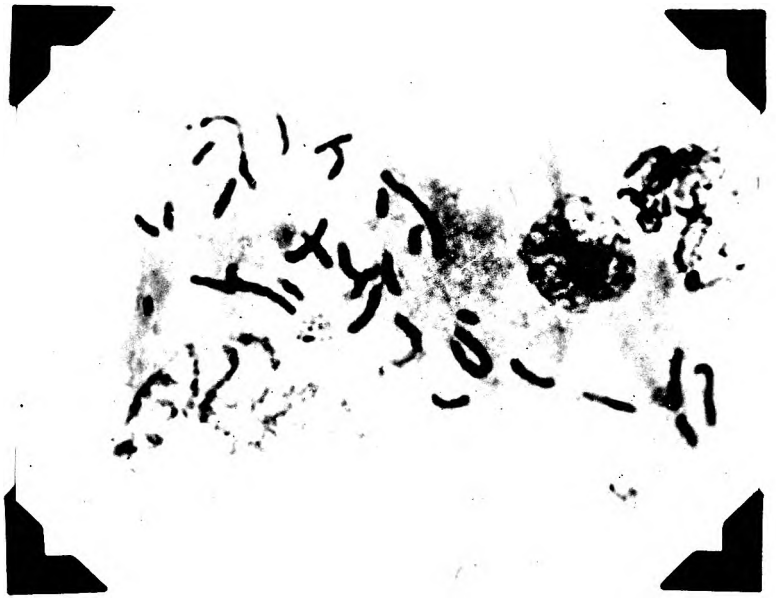


Figure 2. Photomicrograph of a spermatogonial mid-anaphase plate.

Acetic-orcein squash preparation restained with Ehrlich's acid haematoxylin.

X800.



Figure 3. Photomicrograph of a spermatogonial mid-anaphase plate.

Acetic-orcein squash preparation restained with Heidenhain's iron haematoxylin.

X2000.

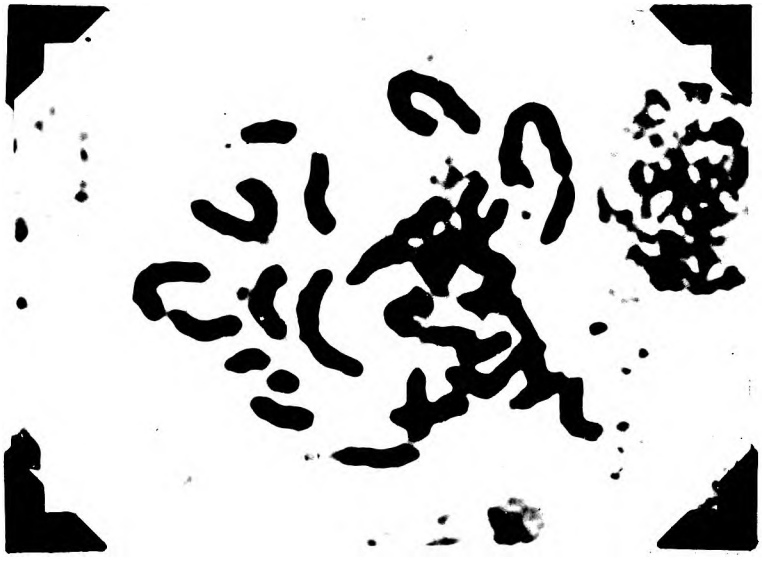


Figure 4. Photomicrograph of a spermatogonial mid-anaphase plate.

Acetic-orcein squash preparation restained with Heidenhain's iron haematoxylin.

X2000.



Figure 5. Photomicrograph of a spermatogonial mid-anaphase plate.

Acetic-orcein squash preparation restained with Ehrlich's acid haematoxylin.

X2000



Figure 6. Photomicrograph of a spermatogonial mid-anaphase plate.

Acetic-orcein squash preparation restained with Heidenhain's iron haematoxylin.

X 3200.

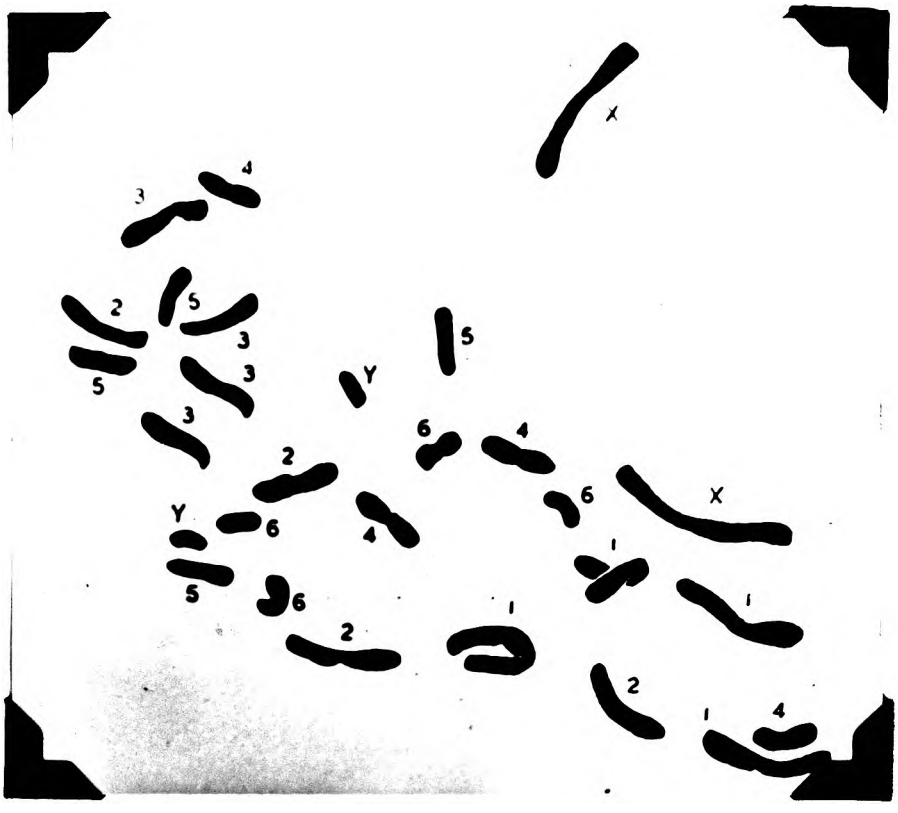


Figure 7. Drawing of the plate shown in Figure 6 with the chromosomes numbered.

X 3200.

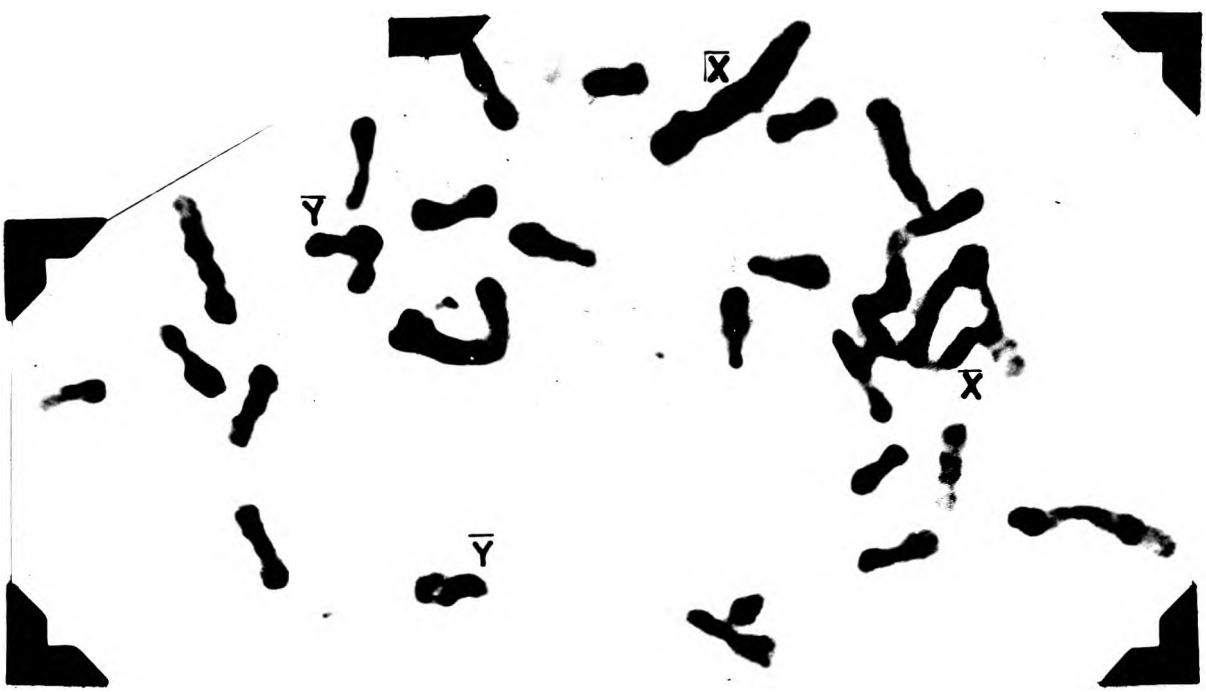


Figure 8. Photomicrograph of a spermatogonial mid-anaphase plate.

Acetic-orcein squash preparation.

X 3200.

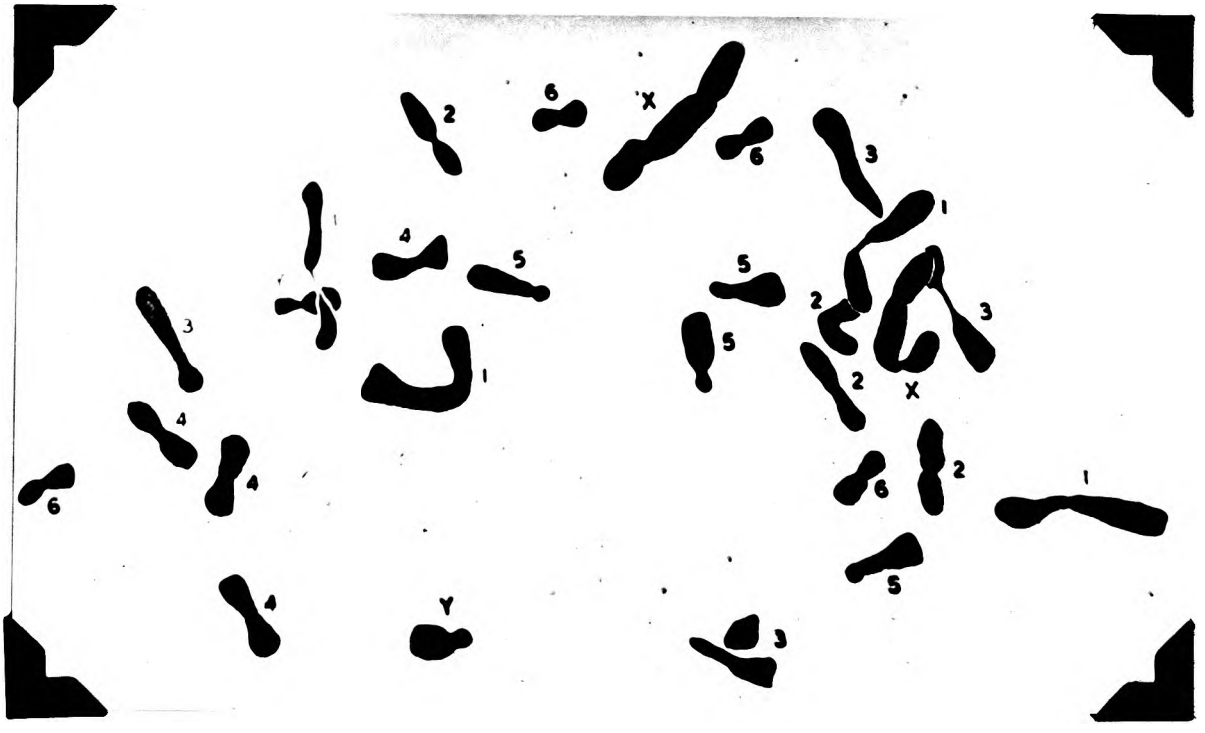


Figure 9. Drawing of the plate shown in Figure 8 with the chromosomes numbered.

X 3200.

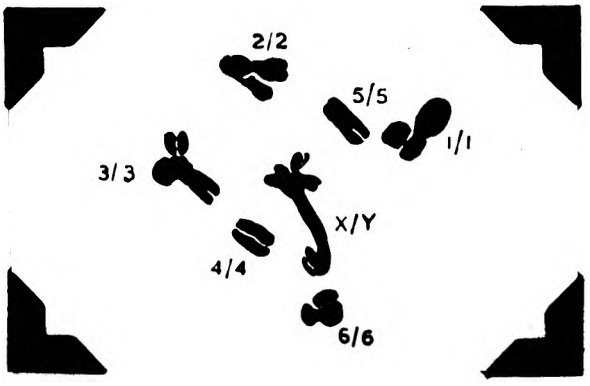


Figure 10. Camera lucida drawing of a first meiotic metaphase plate in a male germ cell. The chromosomes are numbered.

12 μ section of testis stained with crystal violet

X2400



Figure 11. Camera lucida drawing of an early first meiotic anaphase in a female germ cell. The chromosomes are numbered.

15 μ section of ovary stained with crystal violet.

X2400

all the chromosomes lie well separated and in one plane. An inspection of this complement shows that it is tetraploid i.e. four of each type of chromosome exist. Previous authors have often found tetraploid spermatogonia in the testes of other mammals, for example, in Man (Koller, 1937), but in these cases they exist as abnormalities and in small numbers. Because the vast majority of dividing spermatogonia appear to be tetraploid, it might be concluded that Elephantulus is a tetraploid animal. However, a few diploid spermatogonia have been found and these cannot exist in a tetraploid animal, because a meiotic division would be required for their production. As far as is known at present, meiosis is limited to the later divisions of germ cells. Moreover, from a study of meiotic stages in both the male and the female, the haploid number has been found to be seven chromosomes (Figures 10 and 11). This evidence indicates that the tetraploidy observed in dividing spermatogonia is only apparent and is due to the fact that such nuclei have already entered anaphase.

The tetraploid complement is therefore constituted by two diploid sets of chromatids. This is further supported by the fact that in some nuclei anaphase separation was incomplete, and the number of chromosomes found was intermediate between the diploid and tetraploid numbers.



Figure 12. Photomicrograph of a mid-anaphase plate of a stratum granulosa cell.

Acetic-orcein squash preparation restained with Ehrlich's acid haematoxylin.

X3200.



Figure 13. Drawing of the plate shown in Figure 12 with the chromosomes numbered.

X3200.

The chromosomes that had not separated were characteristically double in structure, indicating that they were composed of paired chromatids. If all the chromatids, both paired and separated, were counted, then the tetraploid number of twenty-eight was found.

In squash preparations of the ovary, mid-anaphase plates of dividing female somatic cells were extremely common. Here, too, very few metaphase plates were encountered. These mid-anaphase plates contained the tetraploid chromatid complement of twenty-eight (Figure 12).

The preponderance of such mid-anaphase stages, with the almost complete absence of metaphase plates in male germ cells and female somatic cells, indicates that metaphase is a uniformly rapid stage in Elephantulus. It appears that the chromosomes enter anaphase soon after arrangement on the equatorial plate.

The diploid number of chromosomes 2n = 14, is substantiated by the haploid number (x) of seven chromosomes and the tetraploid number of twenty-eight chromatids.

The size and shape of the chromosomes.

The size of the individual chromosomes as gauged by length and breadth, varies considerably in different prophase and prometaphase nuclei, but is remarkably constant at full metaphase or mid-anaphase. This fact has permitted identification of the individual chromosomes by mea-

surements.

Each chromosome usually contains also a well-marked constriction somewhere along its length. This is the centric constriction, the site of attachment of the spindle fibres to the chromosome. The position of this centric constriction is constant and determines the shape of the chromosomes at metaphase or mid-anaphase.

The two metaphase plates of dividing spermatogonia were totally unsuited for an analysis of the morphological characteristics of the chromosomes. In the prometaphase nucleus chromosome pair 3 had not spiralized to completion, while the other diploid nucleus showed considerable chromosome underlay.

For these reasons, the observations have been made on mid-anaphase nuclei. Since it is conventional to use the diploid complement for the purposes of description, reference will be made only to one of the two diploid sets in mid-anaphase nuclei.

On the basis of size and shape, the diploid chromosome complement may be separated into seven pairs (in mid-anaphase plates there are four of each type of chromosome due to the tetraploid character of the complement). Of these seven pairs, six are equal or homomorphic, while one pair is unequal or heteromorphic. Although this may be observed by an inspection of Figures 1, 2, 3, 4, 5, 6

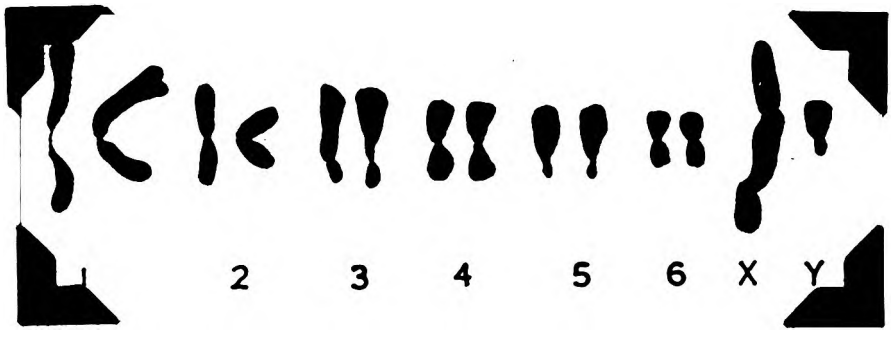


Figure 14. Drawing of one of the diploid sets of the mid-anaphase plate shown in Figure 8 with the chromosomes arranged in pairs according to size and shape.

X3200.

and 8, this feature of the complement may be brought out much better by arranging the chromosomes in order of pairs according to their size and shape relations. This is depicted in Figure 14, which represents one of the diploid sets of the mid-anaphase plate shown in Figure 8.

The two types of chromosome pairs will be discussed separately with regard to their shapes and sizes. Since the breadth remains constant for all chromosomes in a single plate, only the lengths will be considered.

The homomorphic pairs.

The homomorphic pairs range in length from 3 μ to 7.5 μ. Since, at present, there exists no standard nomenclature for chromosome pairs, in the present study they have been designated as 1 to 6 for descriptive purposes.

The two large chromosomes constituting pair 1, are 7.5 μ long, and a submedian centric constriction divides each of the chromosomes into two unequal arms of 3.5 μ and 4 respectively. Chromosome pairs 2 and 3 are both 5 μ in length; the chromosomes of the former pair contain median centric constrictions, while those of the latter pair have subterminal centric constrictions dividing each of the chromosomes into one 4 μ and one 1 μ arm.

The chromosomes of pair 4 with median centric constrictions have lengths of 4 μ . Pair 5 is constituted by two chromosomes which are also 4 μ in length. Although

some plates suggest the presence of a terminal spindle attachment, Figure 8 indicates clearly that this is not the case. The centric constriction is immediately subterminal, the smaller of the two unequal arms being represented by a relatively small portion of the chromosome. The smallest chromosomes form pair 6 and are 3μ in length. A median centric constriction divides each of the chromosomes into equal arms of 1.5μ in length.

These homomorphic pairs have been observed in all the mid-anaphase stages of dividing spermatogonia examined (Figures 7 and 9). Moreover, they have been identified in mid-anaphase plates of dividing female germ cells (Figure 13) and in the metaphase of the first meiotic divisions of both female and male germ cells (Figures 10 and 11). In all of these plates, the same chromosomal morphology was found.

The Heteromorphic Pair.

This pair is a constant feature of all spermatogonial cells examined and includes both the largest and the smallest chromosomes in the complement. The former has a length of 8.5μ while the latter is 2.5μ long. From their characteristic behaviour at meiosis it can be concluded that these are the sex chromosomes i.e. they constitute an X/Y pair.

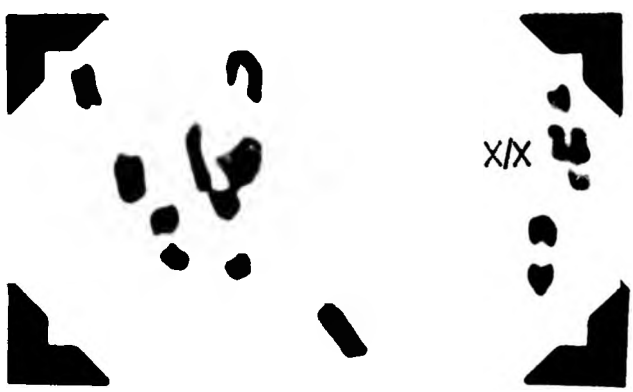


Figure 15. Photomicrograph of the first meiotic anaphase in a female germ cell. Note the terminal spindle attachments of the X chromosomes.

Acetic-orcein squash preparation restained with Ehrlich's acid haematoxylin.

X3200.



Figure 16. Drawing of one of the X chromosomes of the mid-anaphase plate shown in Figure 8. The secondary constrictions (I and II) and the regions of the X chromosome (a, b and c) are indicated. S denotes the position of the spindle attachment.

X3200.

The smaller chromosome has an immediately subterminal centric constriction, the shorter arm being represented by an exceedingly small portion of the chromosome.

The large chromosome contains two constrictions along its length. If both of these are centric constrictions, a dicentric chromosome would result, which, at anaphase would always appear M-shaped, and which would also occasionally undergo fragmentation. Neither of these two conditions has ever been observed in the large number of mid-anaphase plates examined. Therefore, only one of these constrictions may be a centric constriction, or neither is a centric constriction, but the spindle attachment is situated terminally on the chromosome. Inspection of side views of anaphase stages showed that the latter possibility is the more likely. The terminal spindle attachment is found not only at mitosis, but also at meiosis (Figure 15).

The two constrictions observed in this chromosome are therefore secondary constrictions. It will be seen that they divide the chromosome into three segments, which have been called a, b and c respectively. This is shown in Figure 16. Since the position of the secondary constrictions is constant in the chromosome, the lengths of the individual segments will be constant as well. Segment a is 2.5 μ long; segment b, which constitutes the major portion of the chromosome is 4 μ long; while segment c has a length of 2 μ. The secondary constriction separa-

ting segment a from segment b has been called constriction I, while the other secondary constriction between segment b and segment c has been termed constriction II.

The site of attachment of the spindle fibres is localized terminally in segment a. At mid-anaphase, the secondary constrictions assume the appearance of indentations on the surface of the chromosome (Figure 8). However, in late prophase stages they are represented as achromatic gaps in the chromosome.

As already stated, these two heteromorphic chromosomes constitute an X/Y pair. Although by analogy with the structure of the sex chromosomes in other mammals, the larger member of the pair was indicated as being the X chromosome, this could be substantiated only by the examination of the chromosome complement in dividing female germ cells.

In the mature ovary of Elephantulus, no mitotic stages of germ cells were encountered, and consequently, only first meiotic divisions could be studied. Examination of both metaphase and anaphase stages of such divisions clearly show the presence of two of the large sex chromosomes (Figures 11 and 15). No chromosome of the same dimentions as the smaller member of the X/Y pair in the testis could be found in the ovary.

In addition, numerous mid-anaphase stages of dividing

stratum granulosa cells were available in squash preparations of the ovary. A mid-anaphase plate of one such mitotic female somatic cell is depicted in Figure 12. Figure 13 is a drawing of the same nucleus in which the chromosome pairs have been numbered. This plate contains the tetraploid chromatid number of twenty-eight while four of the large sex elements are present. The diploid complement of such a nucleus would be fourteen chromosomes and would include two of the large sex elements.

From this evidence, it can be stated that the larger member of sex chromosome pair is the X chromosome. The smaller remaining member of the pair would therefore be the Y chromosome.

In Elephantulus, therefore, the sex determining mechanism is an X/Y pair in the male and an X/X pair in the female.

Comment.

It is evident from the above description that the diploid chromosome number of Elephantulus is fourteen. On a previous occasion van der Horst (1942) stated that he counted twelve chromosomes, but as his material was not specially prepared for chromosome studies, the smaller chromosomes may have escaped attention.

The older cytologists such as Wilson (1937) denied

the importance of chromosome number in evolution. This was understandable owing to the current concept that the chromosome was the algebraic sum total of its particulate, or genic components. It therefore was of no consequence whether the aggregates (or chromosomes) were few or many, as long as the sum total of genes remained the same. With the discovery of the 'position effect', came the realization that the chromosome was an entirely new level of organization with its own properties and bore more than an algebraic relation to its components. Genes were functionally related, and it became clear that changes in the chromosome number with resulting modification in gene sequences were of considerable significance in evolution. Furthermore, the fact that centromeres can arise only from other pre-existing centromeres showed that alterations in chromosome number could obtain only when a region containing a centromere was either duplicated or deleted. This again has significant consequences. It means that changes in chromosome number are accompanied not only by the appearance of new gene rearrangements but also by a material loss or gain of genic substance. If chromosome number has such evolutionary importance, it should then help in clarifying the taxonomical position of Elephantulus, a problem which has not yet been fully resolved.

Low chromosome numbers amongst mammals have been previously thought to occur only in the Marsupialia. A histogram showing the distribution of mammalian chromosome numbers has been constructed by White (1945) and demonstrates this fact clearly. The Marsupials with low chromosome numbers are separated by a sharp gap from the Eutheria which have large chromosome numbers. There is, however, a significant exception. The Striped Hamster, Cricetulus griseus, a member of the Rodentia has a diploid chromosome number of fourteen (Pontecorvo 1943) which places it well in the class of low chromosome numbers occupied by the Marsupials. The present investigation provides a further exception, showing that Elephantulus myurus jamesoni, an Insectivore, has a low chromosome number as well (2n = 14).

Previous investigations have revealed that Elephantulus has many morphological characters in common with the Marsupials. Thus Gilbert (1944) has identified a paired post-renal vena cava in Elephantulus, while Wright (1945) has pointed out the close resemblance that exists between the subcortical olfactory areas of the brain of Elephantulus and those of Marsupials. The nasal region of Elephantulus possesses numerous features in common with the Marsupials (du Toit 1942). The low chromosome number of Elephantulus might well be evidence of further affinities

with the Marsupials.

On the other hand, Elephantulus has a menstrual cycle which is typical of Primates, supporting the contention of previous investigators that the Macroscelididae, of which Elephantulus is a member, should be classed with the primates (see van der Horst and Gillman 1941). However, the low chromosome number of Elephantulus has nothing in common with the chromosome numbers of Primates. Those which have been determined, cluster around the diploid number of forty-eight.

The problem, then, is to determine what relation chromosome number may bear to the other morphological features which are used as criteria for determining the taxonomical status of an organism. In this connection, the evidence from Elephantulus is conflicting. On the one hand, the low chromosome number may indicate affinities with the Marsupials, a relationship which is favoured by some of the other morphological features of Elephantulus. Then, on the other hand, other morphological characters most certainly place it with the Insectivores, while still others support inclusion of Elephantulus with the Primates. In addition, the low chromosome number in Elephantulus cannot be correlated with the diploid number of thirty-eight found in Talpa europea, the other insectivore which has received cytogenetical characterization (Koller 1936b).

These are some of the problems facing the cytotaxonomist. Both chromosome number and the external morphological features of an animal are phenotypes. Without doubt, they stem from a common genotype, but their final expressions depend on environmental factors as well. Since such factors may be different for each of the phenotypes, it may well be that chromosome number may be used as a criterion of taxonomic status only with defined reservations. It has been found that members of the same family, and even variants of the same species may have widely differing chromosome numbers. Thus Sciurus carolinensis carolinensis has a diploid chromosome number of forty-eight (Cross 1931), while its close relative, Sciurus carolenensis leucotus, has a diploid number of only twenty-eight (Koller 1936a).

To the present author, however, it seems that these inconsistencies are due, for the most part, to the incomplete state of our knowledge concerning mammalian chromosome numbers. A survey of the available literature indicates that not more than a hundred mammals have received attention. Of these determinations, many cover species and subspecies of the same genus, so that out of the three thousand known genera of living mammals approximately 1 - 2% have been only sampled. This figure is even complimentary as it excludes the vast number of the members of each genus which await

investigation. Until further characterizations of the chromosomes of mammals become available, the presentation of a cytogenetical theory of taxonomical relations within the mammals is premature, as it would be based on insufficient empirical data.

CHAPTER IV.

THE BEHAVIOUR OF THE CHROMOSOMES IN THE GERM CELLS OF ELEPHANTULUS.

Introduction.

The morphological features of the chromosomes have already been defined in Chapter III. There now remains to be considered the behaviour of the chromosomes in the male and female germ cells during mitosis and meiosis. In the testis, where large numbers of cells are available, a complete investigation was possible, but the studies on female germ cells were confined to the first meiotic division for reasons to be detailed later.

Before proceeding to this analysis it is necessary to divert in order to consider some relevant aspects of terminology. The details of the various stages of mitosis and meiosis are defined in the books of Darlington (1937) and Waddington (1939). The descriptions provided by these two authors represent the modern view, and will be found to differ in some respects from the account furnished by some older cytologists such as Wilson (1937). The main differences are those of nomenclature, for the characterization of the overall pattern of chromosome behaviour is essentially the same in both cases.

The elaboration of the terminology of mitosis and meiosis is not only an indication of the growth of knowledge of these processes, but also the consequence of discoveries and advances in other fields, particularly biochemistry and biophysics. The older cytologists based their descriptions on chromosome preparations stained with the usual basic dyes. It therefore need hardly be mentioned, that they noted alterations in the staining intensity of the chromosomes at prophase and telophase. Discovering that the maximum intensity of the chromosome was attained at metaphase, they stated that during prophase "the chromatin substances rapidly increase in staining power", while the reverse, a loss of staining intensity, occurred at telophase.

With the application of the results of the investigations of the biochemistry of the nucleic acids, and with the advent of the specific Feulgen reaction to cytology, there has emerged an important concept of chromosome structure. Claims are now made that the variations in the intensity of the staining reaction of the chromosome depends on the amount of its attached desoxyribonucleic acid. This has been termed the nucleic acid charge of the chromosome. On this basis, a decrease in the intensity of the staining reaction of a chromosome with the Feulgen

reagent, is said to indicate a discharging of nucleic acid, while conversely, an increase in the intensity of the staining reaction denotes a charging with nucleic acid.

The older cytologists also observed modifications of chromosome length and breadth accompanying the variations in the staining reaction. It was noted that the chromosome shortens and thickens during prophase, while, at telophase, it becomes longer and thinner. The modern view, based on a concept of a spiral structure of the chromosome, substitutes a different interpretation. According to this view, the thread spiralizes at prophase to assume its presumptive metaphase spiral form, while the opposite effect of unspiralization occurs at telophase.

This new terminology has now almost completely replaced the older nomenclature. It attempts to denote in a more precise fashion the modifications of chromosome structure during the nuclear cycle. Whether or not the concepts of charge and spiralization are justified, and whether or not their definitions are both logically intact and universally applicable will be fully considered in a later discussion. For the purposes of the following description, this brief summary will suffice, and the terms will be used as defined above.

Having considered the terminology employed in the

description of chromosome behaviour, it is now essential to discuss the nomenclature relating to the interphase nucleus. Firstly, it is to be noted that the term 'resting nucleus' is a misnomer, for no nucleus ever enters into a permanent resting phase. The term interphase nucleus is useful in drawing attention to nuclear activity in the periods intervening between one mitosis and another. It should be remembered, however, that many cells emphasise a metabolism which does not usually manifest itself in repeated nuclear divisions, but instead is directed towards the realization of other aspects of cell activity. This applies particularly to the adult nerve cells. In this instance the events ensuing after a particular telophase cannot be regarded strictly as being enacted during an interphase period. Actually it is advisable in this case, to interpret the nuclear behaviour in terms of a post-telephase event.

In the past, there has been a divergence of opinion as to the use of the term nucleolus. Although it is generally agreed that a nucleolus is a body in an interphase nucleus, it appears that there exist a number of different types of nucleoli. The term nucleolus has been loosely applied to one or other of these types, and gives no indication whether the body is Feulgen-positive or

Feulgen-negative. Reserving nucleolus as a general term, the following nomenclature is recommended for the various types of nucleoli.

Any body (or nucleolus) in an interphase nucleus which stains positively with the Feulgen reagent will be termed a karyosome. If such a karyosome has structural characteristics which permit it to be recognised as a chromosome it will then be called a chromosome. Nucleoli, which are Feulgen-negative, and which stain with light green will be termed plasmosomes.

The following description will deal with chromosome behaviour in the germ cells of Elephantulus. Although the emphasis is placed on the nucleus, it must be remembered that the behaviour of the nucleus does not account for all the phenomena of cell division. This is a total cell reaction with the orderly segregation of the chromosomes as only an aspect. Any account of mitosis and meiosis without mention of the accompanying cellular modifications would be incomplete. The formation of the spindle, the gyrations of the centrosomal apparatus, the rhythmical appearance and disappearance of the plasmosomes, and, in fact, the movements of all the cytoplasmic components; all these must be at least recognised, if not fully discussed. However, the detailed inclusion of all these phenomena is beyond the scope of this investigation, which

primarily is directed to a study of the chromosomes. There has been selection of the subject matter, but its limitations are fully realized.

CHROMOSOME BEHAVIOUR IN MALE GERM CELLS.

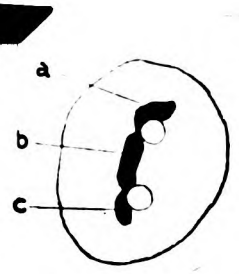
1. Mitosis.

For convenience, the description will be taken up at telophase. While most of the chromosomes unspiralize and discharge nucleic acid at this stage, there is one chromosome in the complement which departs from this standard behaviour. It remains unchanged during telophase, and even after the remaining chromosomes have completely disappeared, it persists fully charged and spiralized in the early interphase nucleus. This chromosome preserves all the characteristics it exhibited at the preceding metaphase or mid-anaphase and this has facilitated its identification. Because of its length (8.5μ) and the presence of two well defined secondary constrictions along its length it has been identified as the X chromosome. Towards the end of telophase a plasmosome appears in close relation with each secondary constriction. This relation is extremely intimate; in Feulgen-light green preparations the plasmosome is first noticed as a green staining mass embedded in the chromosome at the site of the secondary constriction. During the early part of interphase the two plasmosomes increase in size until they

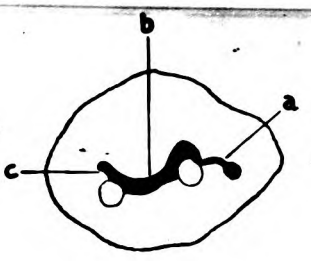
Figures 17 - 27. Camera lucida drawings of a series of spermatogonial interphase nuclei showing the behaviour of the X chromosomes and its associated plasmosomes.

12 μ section of testis stained with the Feulgen-light green method.

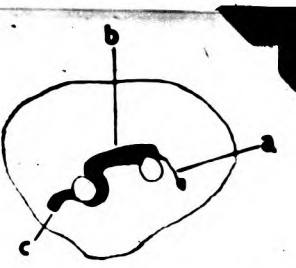
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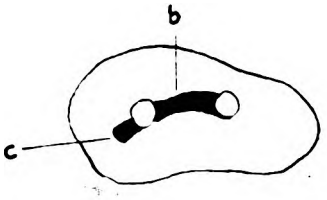
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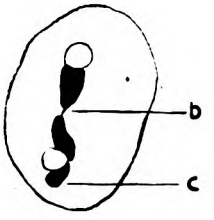
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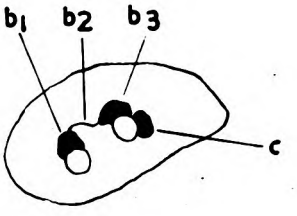
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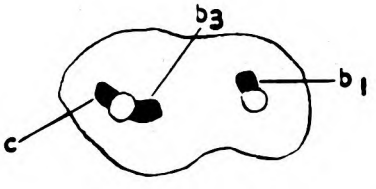
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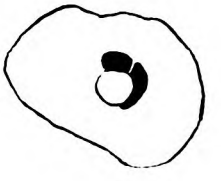
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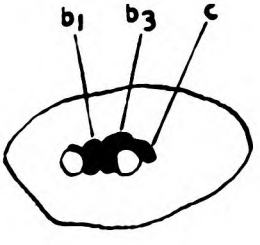
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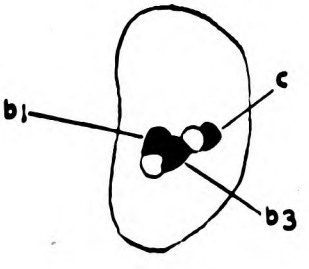
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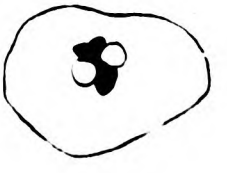
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attain a diameter of approximately 2 μ . Figure 17 depicts a typical early interphase nucleus of a spermatogonium. The X chromosome is easily recognised, and the two plasmosomes are seen to be closely associated with the two secondary constrictions.

The behaviour of the X chromosome and its associated plasmosomes in the interphase nuclei of spermatogonia.

The interphase nuclei of the spermatogonia exhibit a wide range of morphological features. It is possible to distinguish literally hundreds of types of spermatogonia. For example, some spermatogonial nuclei contain two karyosomes each associated with a plasmosome (Figure 23); others have the same features except that the karyosomes are connected to each other by a fine thread (Figure 22); while still other spermatogonial nuclei contain one large karyosome with two associated plasmosomes (Figures 25, 26 and 27).

After examining large numbers of these interphase spermatogonial nuclei, it became evident that each of the different types represented only a phase in a series of morphological modifications of the persistent X chromosome and its two associated plasmosomes. It has been possible to reconstruct the sequence of the process by arranging the numerous nuclear types in what seemed to be

the most logical order. In the description of this process the nomenclature proposed in Chapter III for the regions and the constrictions of the X chromosome will be used.

The complete X chromosome is only found in very early interphase nuclei. Soon after interphase has set in, segment a, the region of the X chromosome containing the terminal spindle attachment, begins to unspiral and discharge nucleic acid. Unspiralization commences at secondary constriction I and proceeds terminally. As is shown in Figures 18 and 19 the segment becomes thinner but does not alter in length. Finally, the fine thread formed disappears completely and secondary constriction I is obliterated. The plasmosome related to this constriction does not alter its position but remains attached to the terminal portion of segment b (Figure 20).

It is noteworthy that this type of unspiralization is not the same as that usually seen at telophase where the chromosome not only becomes thinner but also increases in length. In the case of segment a of the X chromosome, the absence of any alteration in length is probably a reflection of the structural differentiation of that segment, the significance of which is at present unknown.

The unspiralization and subsequent disappearance of

segment a is closely followed by an identical modification in the morphology of segment b, the mid-region of the X chromosome (Figure 21). The peculiar unspiralisation and discharge of nucleic acid commences in the centre of segment b and proceeds terminally in both directions towards both secondary constrictions. The area that unspirals is confined to centre portion of segment b, and therefore leaves two unaffected regions at both ends. These will be termed segment b_1, bordering on secondary constriction I which is roughly 1μ in length, and segment b_3 which is about 1.5μ long and which adjoins secondary constriction II. The centre segment b_2 is that portion of the X chromosome which exhibits the peculiar unspiralization. Its length is approximately 1.5μ.

Figure 22 depicts the penultimate stage of the process. Segment b_1 with its attached plasmosome is connected by a fine unspiralised thread (segment b_2) to segment b_3. The latter is separated from segment c of the X chromosome by secondary constriction II which is related to the second plasmosome. Finally, the thread disappears completely and leaves two separate karyosomes in the interphase nucleus, each with their accompanying plasmosomes (Figure 23).

These two karyosomes are liable to fuse to form a single large karyosome as shown in Figures 24, 25, 26 and

27. This structure will hereafter be termed the X karyosome. Furthermore, if the plasmosomes of two such fusing karyosomes come into contact with each other, they, too, will fuse. The product is a single large plasmosome, occasionally bilobed, but which is also generally twice the size of the original plasmosomes (Figure 24). For brevity, this structure will be named the fusion-plasmosomes.

Segment c neither unspirals nor does it discharge nucleic acid during the interphase. However, it may become more closely wrapped around the plasmosome at secondary constriction II.

To summarize:

1. Segments a and b_2 of the X chromosome unspiral and discharge nucleic acid in close succession.
2. After these segments have disappeared, the remaining portions of the X chromosome take up the following different arrangements:
   (a) Two Feulgen-positive karyosomes with accompanying plasmosomes may be seen in the nucleus, namely,
      (i) Segment b_1 with plasmosome,
      (ii) Segment b_3 with secondary constriction II, the satellite segment c and plasmosome.
   (b) These fuse later to form the single large X karyosome. The plasmosomes may take up two

different configurations.

(i) The two plasmosomes may remain separate,

(ii) The two plasmosomes fuse to form the fusion plasmosome.

The regionalization of the X chromosome.

In the characterization of the morphology of the chromosome complement (Chapter III) the X chromosome was divided into the three segments a, b and c. The morphological criterion for this division was the constant positions of the two secondary constrictions in the X chromosome.

The various modifications of the X chromosome in the interphase nucleus of the spermatogonium has permitted a further subdivision. Segment b was divided into three constant portions, b_1, b_2 and b_3, each approximately constant in length. This behaviour of the X chromosome also indicated that segment a was structurally different from the remainder of the X chromosome. Moreover, it was shown that segment b_2, which resembles segment a in many respects, diverged morphologically from the spatially related segments b_1 and b_3. The nature and significance of this structural differentiation of the X chromosome will be discussed more fully later.

The behaviour of the X chromosome at prophase.

The onset of prophase in the spermatogonial nucleus is accompanied by significant modifications in the morphology of the X karyosome. In Feulgen-stained material the previously lightly-stippled background of the nucleus becomes more chromatic, probably indicating a general charging of nucleic acid and the commencement of prophase. At this stage, the X karyosome discharges nucleic acid and unspiralization becomes evident. In this instance, the unspiralization is similar to that seen in telophase nuclei. It is expressed in the appearance of a number of definite coils, indicating a lengthening of the segments constituting the X karyosome. These coils appear to be very similar to those seen in the autosomes at prophase (vide infra) and have therefore been termed 'relic' coils.

Meanwhile, the remaining chromosomes of the complement are separating out in the nucleus. They seem to become distinguishable first in the region of the nuclear membrane where they appear as local condensations. Later, the easily recognisable prophase threads are formed and simultaneously they commence to charge with nucleic acid and also exhibit relic coils.

The movements attending the prophase unspiralization of the X karyosome are no different from those already observed in the case of the other chromosomes at telophase.

However, in the X karyosome, unspiralization is not continued to the stage of complete disappearance. The unspiralling X karyosome attains a structure similar to that of the other prophase chromosomes and promptly begins to recharge and respiralize. It is assumed here that segments a and b_2 of the X chromosome which disappeared during the early part of the preceding interphase reappear at the same time as the remaining chromosomes. These segments then join the now recharging and respiralizing portions of the X karyosome and the complete X chromosome is reformed.

Since the prophase nucleus is just a mass of spiral threads, difficulties have been experienced in observing the resynthesis of the X chromosome. The fact that a fully reconstituted X chromosome does appear at prometaphase indicates that there is undoubtedly a regular series of events which culminate in typical appearance of the X chromosome at prometaphase.

The behaviour of the plasmosomes at prophase.

During prophase the plasmosomes undergo no changes in structure or staining reaction but may alter their relation to the X chromosome. Such modifications in the positions of the plasmosomes may be reconstructed from observations of pro-metaphase nuclei.

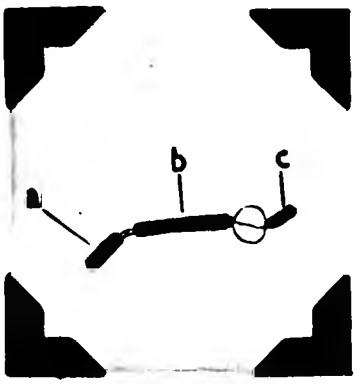


Figure 28. Camera lucida drawing of the X chromosome in an early prometaphase spermatogonial nucleus. The large fusion-plasmosome is located at secondary constriction II, while secondary constriction I appears as an achromatic gap.

12 μ section of testis stained with the Feulgen-light green method.

X2400.

Firstly, the plasmosomes often remain in close association with the X chromosome. Their exact location will depend on their morphology in the preceding interphase. If, at the previous interphase, the plasmosomes do not fuse, then one plasmosome will be found at each secondary constriction of the X chromosome at early pro-metaphase. On the other hand, if fusion has occurred then the large fusion plasmosome becomes related only to one secondary constriction, usually secondary constriction II. This latter relationship may be accounted for by the early obliteration of secondary constriction I in the preceding interphase. It is to be noted, that when the fusion-plasmosome is associated with secondary constriction II at prometaphase, secondary constriction I appears as a short achromatic gap in the X chromosome, (Figure 28).

Secondly, the plasmosomes do not always maintain contact with the X chromosome during prophase. Often prometaphase plates are encountered with the plasmosomes lying free in between the chromosomes. Occasionally the plasmosomes may appear to be loosely attached to one or more of the other chromosomes. This contact, however, is not as intimate as in the case of the X chromosome, where the plasmosomes appear to be actually embedded in the chromosome.

The behaviour of the chromosomes at metaphase and anaphase.

Between pro-metaphase and metaphase, the plasmosomes disappear and the chromosomes spiral and charge to completion. In this way, the characteristic metaphase morphology is attained by the chromosomes. Except for its previously mentioned uniform rapidity, metaphase presents no other striking features. Anaphase, too, exhibits no unusual characteristics, the chromosomes segregating in an orderly fashion on the spindle.

The above observations have shown that while most of the chromosomes follow a standard pattern of behaviour throughout mitosis, certain segments of the X chromosome depart from this standard. Nevertheless, the physiological conditions of the cell as a whole exerts control, and compensates for all divergent modifications. The whole process is accurately timed, so that ultimately the constant morphology of the chromosomes is maintained at metaphase.

2. Meiosis.

The numerous accounts of meiosis in the literature all point to the primary spermatocyte as the cell which undergoes the first division of meiosis in the testis. In Elephantulus, it has not been possible to draw any distinctions between interphase spermatogonia and primary spermatocytes. The interphase nucleus of the primary spermatocyte exhibits all the modifications of the X chromosome already described for the spermatogonia. Since the interphase nuclear morphology of both cell types is identical, a primary spermatocyte could easily be mistaken for a spermatogonium and vice versa. Thus, for the purposes of the present study, the term primary spermatocyte has been discarded and hereafter both cell types will be regarded as spermatogonia.

The presumptive fates of these spermatogonia fall into two distinct categories. There is one type which will enter into mitotic division, while the other type is destined to undergo meiosis. Only at the beginning of prophase is it possible to predict the prospective behaviour of a spermatogonium, and only at this stage, then, may spermatogonia be first designated as premitotic or premeiotic.

The various configurations assumed by the nuclear

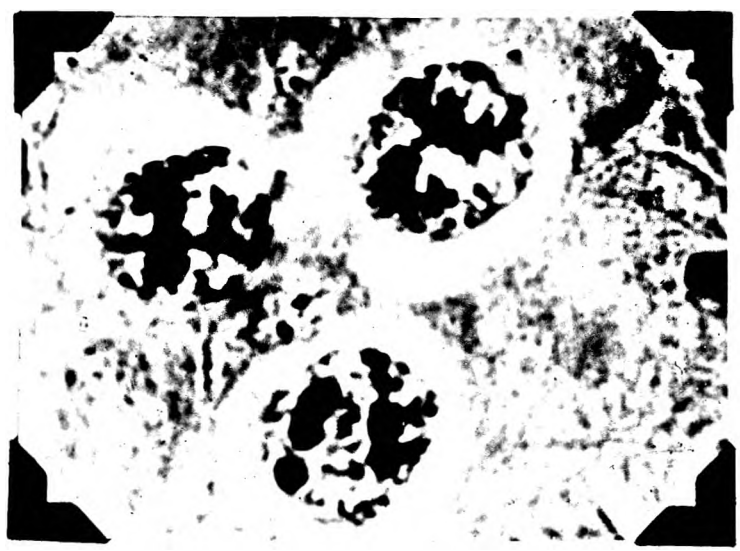


Figure 29. Photomicrograph of a group of pre-meiotic spermatogonia in the earliest recognisable phase of the leptotene stage of meiosis. The X karyosome is still clearly visible and small karyosomes are present in each nucleus.

12 μ section of testis stained with crystal violet.

X2000.

constituents of premitotic spermatogonia at early prophase have already been fully considered. The object of the following account is to define the nuclear events of premeiotic spermatogonia at early prophase, and to follow this by a characterization of the subsequent behaviour of the chromosomes at meiosis. In this account, too, the sequence of events has been reconstructed from numerous cells observed in fixed and sectioned material.

The nuclear events during the early part of the meiotic prophase.

The appearance of a number of minute Feulgen-positive granules in the nucleus of a premeiotic spermatogonium is the first indication that meiotic prophase is commencing (Figure 29). Simultaneously the X karyosome releases its associated plasmosomes which lie free in the nucleus for some time. These then disappear rapidly. This is followed by the rounding off of the X karyosome and the assembly of the granules into threads. Thread formation makes its first appearance in the vicinity of the X karyosome and subsequently radiates throughout the nucleus. The leptotene threads thus formed are therefore polarized in the region of the X karyosome. In contradistinction to the zygotene polarization characteristically manifest at a subsequent time, it is advisable to term

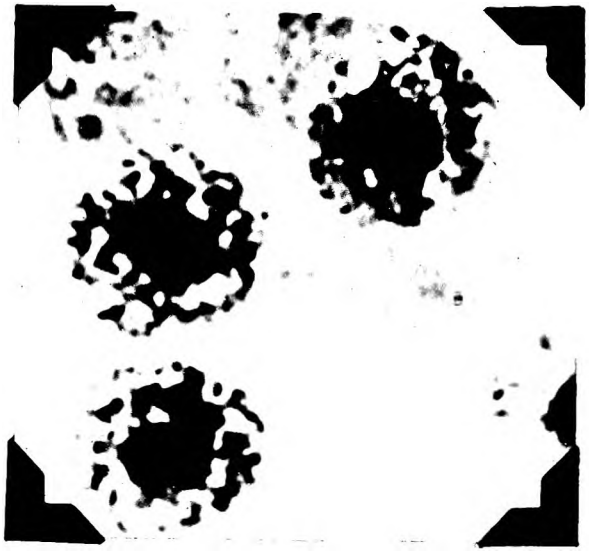


Figure 30. Photomicrograph of three nuclei in a later phase of the leptotene stage of meiosis. Each nucleus contains a mass of threads which are polarized in the region of the X karyosome.

12 μ section of testis stained with crystal violet.

X2000.

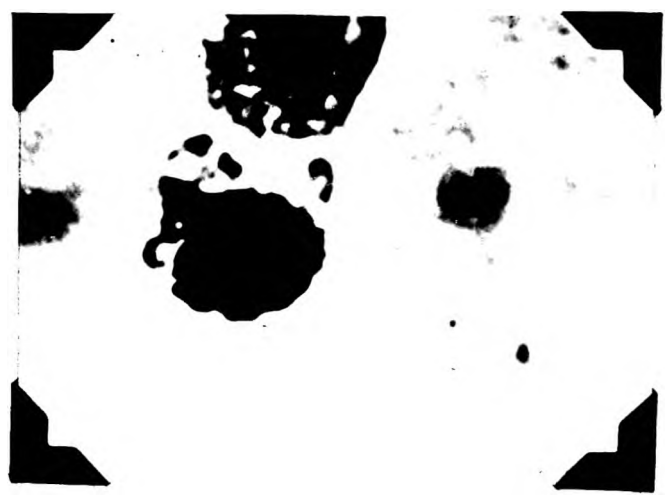


Figure 31. Photomicrograph of a nucleus in the zygotene stage of meiosis. Note the synezesed chromosomes collapsed to one side of the nucleus and presenting the typical bouquet appearance.

12 μ section of testis stained with crystal violet.

X2000.

the polarization of the leptotene threads in the region of the X chromosome as the primary polarization of the leptotene threads. Figure 30 depicts this stage of the meiotic prophase. The rounded X karyosome occupies the centre of the nucleus and is surrounded by a mass of fine long leptotene threads.

While the leptotene threads have been increasing in the nucleus, the X karyosome has all the time been discharging nucleic acid. Eventually the nucleus is occupied by a tangled mass of these threads which obscure the X karyosome and further observations become impossible.

The subsequent modifications in the X karyosome at meiotic prophase are very probably analogous to those occurring in the prophase of mitosis. The X karyosome would discharge nucleic acid and unspiral, but, again, not to completion. It would then respiral and recharge together with the other portions of the X chromosome (segments a and b_2). These would have reappeared in the nucleus at the same time as the remaining threads. In this way, a leptotene thread representing the complete X chromosome would be reconstituted.

The somewhat heavily charged leptotene threads now undergo synizesis, clumping on to one side of the nucleus. The characteristic bouquet stage thus formed is depicted in Figure 31. It is very likely accentuated by fixation,

but the structure is constantly recurring which indicates that the threads have in some way undergone polarization. This then, is the secondary polarization of the leptotene threads. At this stage, too, the onset of zygotene pairing may be observed.

The essential features of the leptotene stage of the meiotic prophase in Elephantulus may be summarized as follows:-

1. The plasmosomes are released from the X karyosome and disappear very early in the prophase. Only rarely, are plasmosomes observed in leptotene nuclei.
2. Coincident with the disappearance of the plasmosomes the leptotene threads exhibit primary polarization on the X karyosome.
3. Secondary polarization of the threads to one side of the nucleus occurs at the end of leptotene.

The behaviour of the chromosomes during the later stages of prophase.

The next observable stage in the meiotic prophase is that of pachytene, characterized by the completion of the pairing of the chromosome threads. The sex chromosomes at this stage could not be distinguished from the remaining autosomes. There is no precocious condensation of the sex chromosomes as reported by previous workers in other mammals (For example the Striped Hamster [Ponte-

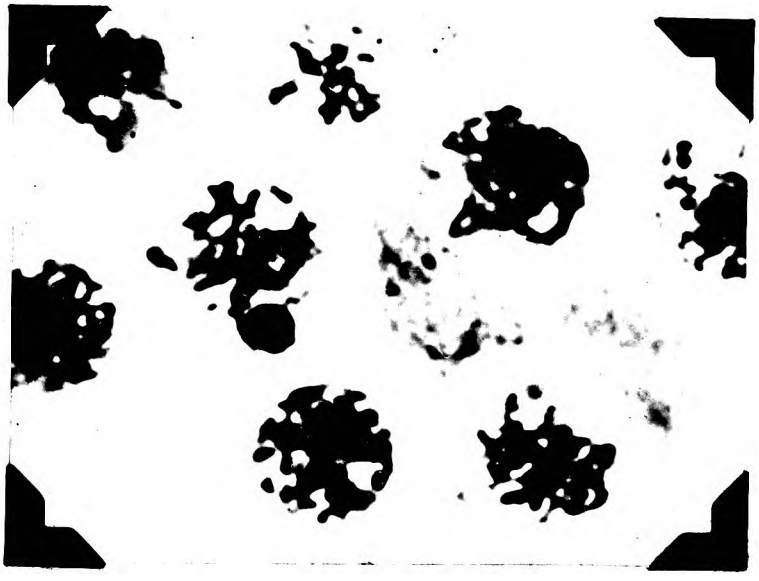


Figure 32. Photomicrograph of a group of early and late pachytene nuclei.

12 μ section of testis stained with crystal violet.

X2000.

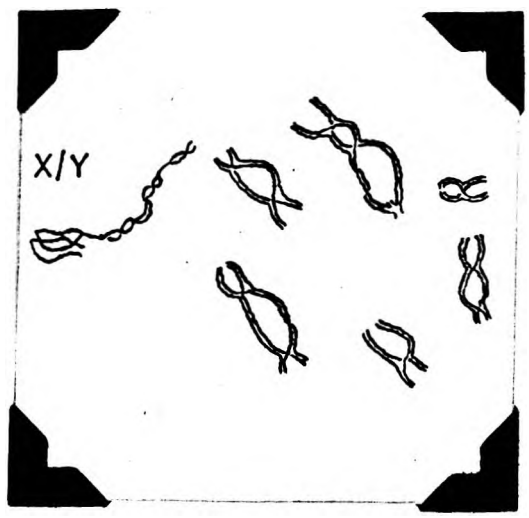


Figure 33. Camera lucida drawing of a diplotene nucleus. The chromosomes are thin and threadlike and they are associated in pairs to form tetrads. Both interstitial and terminal chiasmata are present in the autosomes. In this instance a chiasma has failed to form between the pairing segment of the X chromosome and the Y chromosome.

12μ section of testis stained with crystal violet.

X1700.

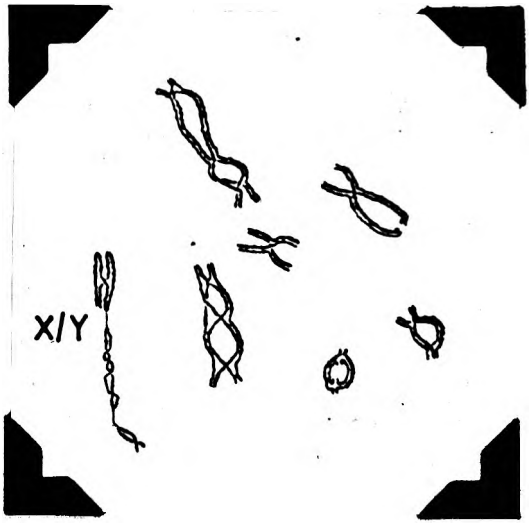


Figure 34. Camera lucida drawing of a diplotene nucleus. The appearance is similar to that of the previous nucleus (Figure 33) except that, in this case, an interstitial chiasma is present between the pairing segment of the X chromosome and the Y chromosome.

12 μ section of testis stained with crystal violet

X1700.

corvo 1943]). Although the individual chromomeres were recognised on the threads without any difficulty, no clear evidence of relational coiling of either chromatids or chromosomes could be found. Figure 32 depicts typical pachytene nuclei in the testis of Elephantulus.

Diplotene stages are shown in Figures 33 and 34. Each of the chromosomes have divided into chromatids and crossing over has occurred. Both interstitial and terminal chiasmata are present, the smaller chromosomes containing one or two, while up to three chiasmata are found in the larger chromosomes.

The sex chromosomes may be now recognised as a sex bivalent. The Y chromosome is paired off with only a portion of the X chromosome. Measurements indicate that the pairing segment of the X chromosome is segment a. The remaining portion of the X chromosome which includes segments b and c has no homologue on the Y chromosome and therefore constitutes a differential segment. In this segment the chromatids are relationally coiled around each other.

Chiasma formation between the pairing segment of the X chromosome and the Y chromosome is variable. In some diplotene nuclei the Y is only loosely held in contact with the pairing segment of the X chromosome (Figure 33). In other nuclei, chiasma formation does occur and an



Figure 35. Photomicrograph of a first meiotic metaphase in a male germ cell. This plate does not show all the chromosomes but demonstrates the structure of the sex bivalent. The thick condensed region represents the pairing segment of the X chromosome and the Y chromosome. The thin tail-like structure is constituted by the two relationally-coiled chromatids of the differential segment of the X chromosome.

12 μ section of testis stained with crystal violet.

X2000.

interstitial chiasma is found in the pairing segments of the X/Y bivalent (Figure 34).

Diakinesis is not very much different from diplotene. The same characteristic configuration of the chromosomes is found. These chromosomes have now become spiralized and charged with nucleic acid. Some of the chiasmata show terminalization. This stage continues directly into metaphase.

The morphology of the chromosomes at the metaphase of the first meiotic division.

At metaphase, the morphological unit is a quadripartite structure. These tetrads, as they are called, are constituted by four chromatids. At the first metaphase of meiosis in Elephantulus, the number of tetrads is seven (Figure 10), demonstrating that reduction of the chromosome number has been initiated.

The rate and degree of spiralization is constant for all the autosomes and for the pairing segments of the X and Y chromosomes. A departure from this standard behaviour is generally exhibited by the differential segment of the X chromosome. In Figure 35 which is a microphotograph of the sex bivalent at metaphase, the thick pairing segments contrast markedly with the thin differential segment. The latter is composed of two underspiralled



Figure 36. Photomicrograph of a first meiotic anaphase in a male germ cell. The X/Y bivalent has undergone a pre-reductional division as is witnessed by the distribution of the differential segment of the X chromosome to only one of the daughter nuclei.

Acetic-orcein squash preparation restained with Ehrlich's acid haematoxylin.

X2000.

thread-like chromatids relationally coiled about each other. These chromatids are usually undercharged. It must be mentioned that this reaction is variable, the differential segment of the sex bivalent in Figure 10 shows no sign of underspiralization and undercharging. Such bivalents exhibiting standard behaviour of the differential segment of the X chromosome are only rarely encountered; the vast majority of first meiotic metaphase plates show the departure from standard behaviour in the differential segment of the X chromosome.

This peculiar behaviour of the sex bivalent at the first meiotic metaphase provides further confirmatory evidence that the various segments of the X chromosome are not all the same. Segment a, which pairs with the Y chromosome, is obviously different from segments b and c which have no homologue on the Y chromosome. The structural differentiation underlying these morphological differences in behaviour will be fully examined later.

The behaviour of the sex bivalent at the anaphase of the first meiotic division.

The tetrads separate into diads at the first meiotic anaphase. At this stage, two arrangements of the sex bivalent are encountered. The first may be seen in Figure 36. This shows both chromatids of the Y chromosome moving to one pole of the spindle while the other pole receives

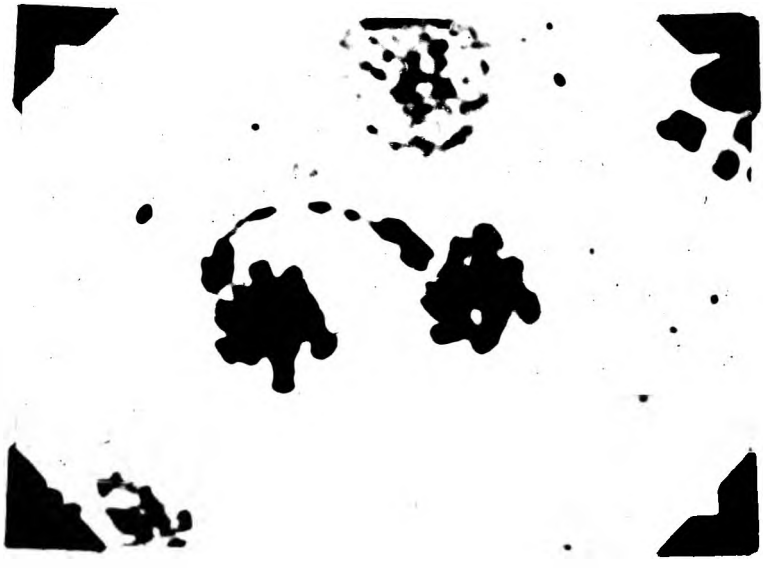


Figure 37. Photomicrograph of a first meiotic anaphase in a male germ cell. In this case, the X/Y bivalent has undergone a post-reductional division. Each daughter nucleus receives one chromatid of the X chromosome together with one chromatid of the Y chromosome.

Acetic-orcein squash preparation restained with Ehrlich's acid haematoxylin.

X2000.

both chromatids of the X chromosome. This anaphase arrangement is a direct consequence of the type of diplotene association of the sex chromosomes shown in Figure 33. Since no chiasma was formed, the X and Y chromosomes merely separate at anaphase.

The other arrangement depicted in Figure 37 shows one chromatid of the X chromosome associated with one chromatid of the Y chromosome, moving to one pole of the spindle and the other chromatids of each sex chromosome moving to the opposite pole. This is the result of the diplotene association shown in Figure 34, where a chiasma was formed between the X and Y pairing segments. Subsequent terminalization proceeded through the differential segment.

With the first type of anaphase arrangement segregation of the differential segment of the X chromosome takes place at the first meiotic division; this then corresponds to a pre-reductional division of the sex bivalent. The second type of configuration is not associated with a segregation of the differential segment, and is therefore a post-reductional (equational) division for the sex bivalent.

The chromosomes at the second meiotic division.

No breakdown of first meiotic telophase nuclei into interphase nuclei has been observed. That is to say, no stage corresponding to an interphase secondary spermatocyte was found. Either this stage is extremely rapid, or the chromosomes of the first meiotic telophase pass directly into the metaphase of the second division with no intervening interphase.

The second meiotic division completes the segregation of the differential segment of the X chromosome. If the first division was post-reductional (equational) then the second division will be reductional.

The telophase of the second division passes into the characteristic interphase stage of the spermatid nucleus. These interphase nuclei show a fine stippled background staining lightly with the Feulgen reagent. No karyosomes were detected.

Meiosis has resulted in the reduction of the chromosome number. The spermatid now develops into the sperm, and although the nuclear reactions of this process are significant, a description of spermiogenesis is far beyond the scope of this investigation. The object of this section was to characterize the behaviour of the chromosomes at meiosis. This has been accomplished.

CHROMOSOME BEHAVIOUR IN FEMALE GERM CELLS.

In the previous section, considerable emphasis has been placed on the peculiar behaviour displayed by the X chromosome in male germ cells. Particularly important, was the unusual modification in the morphology of the differential segment of this chromosome at the first division of meiosis. This region does not pair at all with the Y chromosome and this suggests that its departure from the standard behaviour may be a consequence of the absence of a homologue on the Y chromosome.

It is evident that the above possibility may be tested only by the observation of chromosome behaviour in female germ cells. In such cells, two equal X chromosomes are found and these pair together throughout their whole length at meiosis. The purpose of this description, then, is to obtain information about the behaviour of the X chromosome in female germ cells.

The material used for this description imposed severe limitations on the scope of this study. Unlike the testis, the mature ovary of Elephantulus does not contain a large number of cells in every stage of mitosis and meiosis. Mitotic activity of the female germ cells has not yet been observed in the material available. It appears that continuous proliferation of the female germ cells by mitosis does not occur in the ovary of Elephantulus, and

that the number of cells is limited and laid down some time prior to maturity. Furthermore, the investigations of van der Horst (1942) have revealed that the second division of meiosis does not take place in the ovary at all, but in the uterine tube at the time of fertilization.

For these reasons, the investigation was confined to the first division of meiosis in the female germ cells. Even here, a complete analysis was not possible, because the significant stage of diplotene was not encountered. While these limitations will necessarily detract from the completeness of the study, sufficient stages have been observed to provide information regarding the behaviour of the X chromosomes in female germ cells, and these will be described. At the same time, wherever it has been possible, reference will be made to the characteristics of the plasmosomes and their relations to the chromosomes.

The behaviour of the chromosomes in the first meiotic division of female germ cells.

The early stages of the first meiotic prophase are found in germ cells situated in the cortex of the ovary. These primary oocytes together with a single surrounding layer of stratum granulosum cells constitute a primordial follicle. In the preparations examined, the majority of the nuclei are in the leptotene stage of meiosis. These

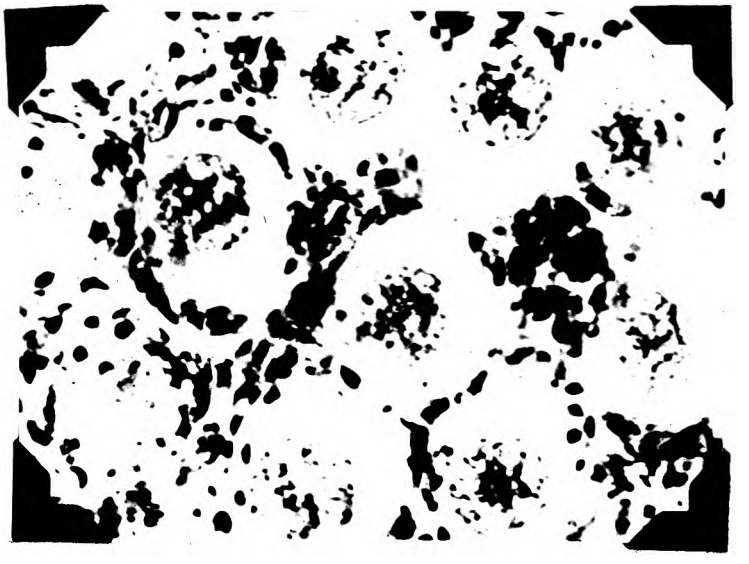


Figure 38. Photomicrograph of a group of primordial follicles showing the primary oocytes in the leptotene stage of the first meiotic division.

12 μ section of ovary stained with crystal violet.

X800.

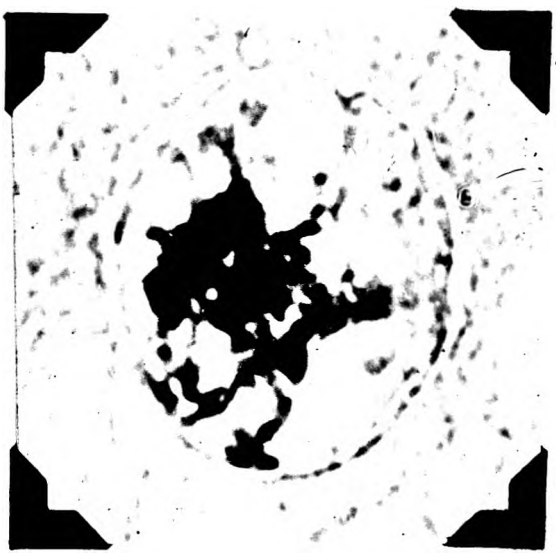


Figure 39. Photomicrograph of the zygotene stage of meiosis in the primary oocyte. The synezesis is not very marked, but some unpaired chromosome ends are in evidence.

12 μ section of ovary stained with crystal violet.

X2000.

nuclei contain a mass of intertwining leptotene threads which are long and fine in structure (Figure 38). It is surprising that no interphase stages of the female germ cells have been observed. In the cells which were not at the height of leptotene, the leptotene stage was still being enacted. In such nuclei, the threads were incompletely formed. These nuclei contained two condensed chromatic bodies which possibly represent the X karyosomes. Two of these structures would be present in female germ cells owing to the existence of an X/X pair of sex chromosomes.

These leptotene nuclei contain a variable number of plasmosomes; usually one is found, occasionally two. No quantitative relations could be found between the plasmosomes and the chromosomes, probably because plasmosome fusion is liable to occur.

With rare exceptions, the nuclei of the primary oocytes persist in the leptotene stage until proliferation of the stratum granulosa and growth of the oocyte cytoplasm have taken place. At this stage, the leptotene threads, now fairly heavily charged, undergo synizesis clumping on to one side of the nucleus. Figure 39 shows one such bouquet stage with the chromosomes polarized. Synizesis of the chromosomes is accompanied by the onset of zygotene pairing, which begins in the chromosomes ad-

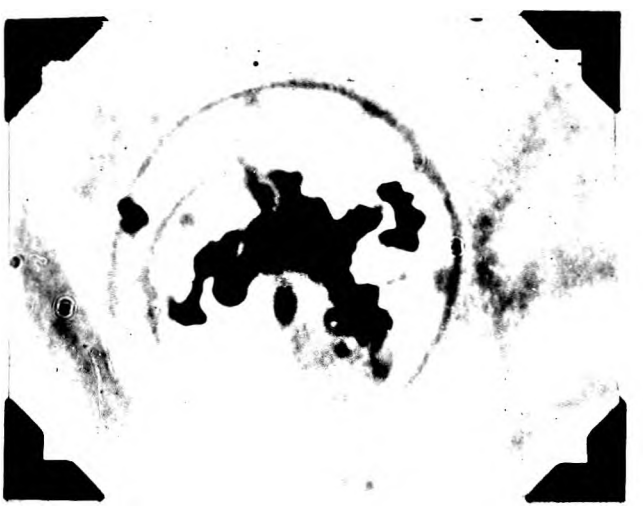


Figure 40. Photomicrograph of the pachytene stage of meiosis in the primary oocyte. Note the large plasmosome attached to one of the chromosomes, probably the X/X pair.

12 μ section of ovary stained with crystal violet.

X2000.

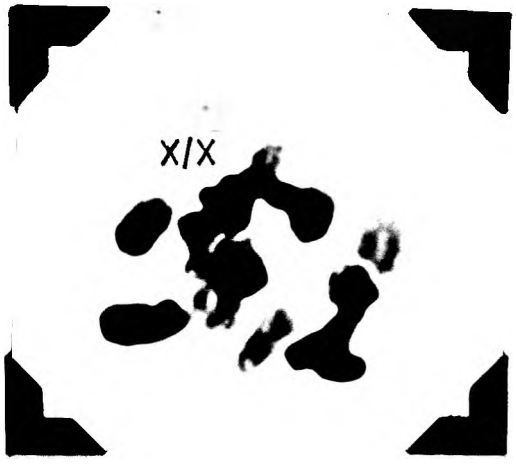


Figure 41. Photomicrograph of the diakinesis stage of meiosis in the primary oocyte. The plate is incomplete, but interstitial chiasmata are present in the X/X bivalent.

12 μ section of ovary stained with crystal violet.

X3200.

jacent to the nuclear membrane. This pairing later progresses over the whole nucleus. The pachytene stage is attained when the pairing of the chromosomes is completed. In pachytene nuclei, one large plasmosome is found lying closely applied to a condensed region of one of the paired chromosomes (Figure 40). This large plasmosome is occasionally bilobed, but generally it is twice the size of the original plasmosomes in leptotene nuclei. The condensed chromatic region may represent a portion of the X/X bivalent.

As already stated, the significant diplotene stage was not encountered. However, the observations recorded on a late diakinesis stage indicate the possible arrangement of the chromosomes at diplotene. Persistent chiasmata in a number of chromosomes as well as their quadripartite structure at diakinesis suggest that at diplotene the chromosomes had split into chromatids and that crossing over had occurred.

Figure 41 is a microphotograph of some of the chromosomes at diakinesis. The configuration of the sex bivalent is significant. The two X chromosomes are paired throughout their whole length and interstitial chiasmata are present. The regions corresponding to the differential segment (segments b and c) are as fully charged and spiralized as segment a, the pairing segment in the male.

In the case of female germ cells, then, the morphological regionalization of the X chromosome is not attended by differential behaviour.

The absence of differential behaviour in the X/X bivalent is confirmed by a study of metaphase and anaphase stages (Figures 11 and 15). At anaphase the X/X bivalent appears to show some sluggishness in separation. As is shown in Figure 15 the two X chromosomes are still peeling off from each other while most of the remaining chromosomes are already far apart on the spindle.

At telophase, a large fusion-plasmosome appears in close association with the constrictions of the X chromosome. The chromosomes clump together, discharge nucleic acid and unspiral. When they have disappeared all that remain in the nucleus are a few extremely fine chromatic threads and the large well defined fusion-plasmosome.

From the foregoing, it is evident that, with the exception of a lack of differential behaviour in the X/X bivalent, the events of meiosis in female germ cells are essentially similar to those in male germ cells.

CHAPTER V.

ALLOCYCLIC BEHAVIOUR OF THE X CHROMOSOME IN ELEPHANTULUS AND THE STRUCTURE OF HETEROCHROMATIN.

The preceding observations have shown that the X chromosome in Elephantulus has two distinctive properties.

1. In male germ cells, the X chromosome shows departure from the standard behaviour of the remaining chromosomes.
2. In interphase spermatogonia, the X chromosome presents specific relations to the plasmosomes. These two features are important, and therefore merit detailed and individual consideration. Each will be discussed in turn.

1. The behaviour of the X chromosome in Elephantulus.

The irregular behaviour of the X chromosome in male germ cells is registered on two different occasions. Firstly, at mitosis, when segments b_1, b_3 and c persist fully charged and spiralized into the interphase, and secondly, at the first metaphase of meiosis, where the differential segment appears underspiralized and less heavily charged than the remaining chromosomes. If this behaviour of the X chromosome is compared with that of the other chromosomes, then the disturbance is seen to be one

involving a change in rate, timing and intensity of the various reactions. To this typical differential behaviour, Darlington (1942) has applied the term allocycly.

Up to this stage, chromosome behaviour, both standard and allocyclic, has been characterized in terms of observable modifications in chromosomal morphology. Change has been recognised, but such recognition has so far penetrated no further than the cytological level. It is now clear from recent investigations (to be detailed later) that all these morphological changes are expressions of the physico-chemical reactions of an underlying minuter structure within the chromosome. That is to say, there exists within the chromosome a micro-morphological organization, which plays a causative role in determining the morphological manifestations of chromosome behaviour. All present day notions of allocyclic and standard behaviour are based on the concepts of the organization of a micro-morphological chromosome structure. To obtain a more intimate understanding of chromosome behaviour, it will be necessary to discuss the micromorphological organization of the chromosome. This, then, is the first task of this section.

The micromorphological organization of the chromosome as a basis for chromosome behaviour.

> "Without in any way denying that the morphological level is subjected to complicated laws of its own, which may be stated without explicit reference to physicochemical processes, we cannot but admit that the morphological changes are the outward and visible signs of what is going on in the inward and chemical places." (Joseph Needham*)

The recent advances in biochemistry, more especially those concerning protein and nucleic acid chemistry have already registered their major impact in the field of cytogenetics. From these researches emerged the modern view of the micromorphological structure of the chromosome. The chromosome is now thought to be composed of an extensible protein framework which provides a locus for chemical reactions, the best known of which is the specific modification in the amount of attached desoxyribonucleic acid. It is claimed that mechanical gyrations of the protein framework, together with the changes in the quantity of desoxyribonucleic acid provide the micromorphological explanation for observable modifications of chromosome morphology. The modern approach to the pro-problems of chromosome behaviour is through the concepts of spiral rearrangements of the protein framework and

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* Proc. Roy. Soc. Med. 29:1577 (1936).

nucleic acid charge.

Brief definitions of charge and spiralization were presented at the beginning of the previous chapter. It is now necessary to consider these notions in more detail; to discuss the origin and content of their terminology and to study their importance for cytogenetics.

CHARGE.

The historical background.

It was not until after Miescher appeared on the scene of biochemistry that the nucleic acids were recognised as materials worthy of chemical investigation. The intensive researches thus launched, showed that nucleic acid was a widely distributed constituent of living matter. These earlier chemical studies also revealed the existence of two essentially different types of nucleic acids. The one, isolated from the thymus of the calf became known as thymo-nucleic acid, while the other, prepared from plant and yeast sources was called phyto- or zymo-nucleic acid. Although the work of Kossel (see Jones [1920]) showed that both the nucleic acids were basically composed of a purine or pyrimidine base, a sugar and phosphoric acid, there was some doubt regarding the nature of the suggr component. It was thought to be a hexose in thymonucleic acid and a pentose in phytonucleic

acid (Kossel and Neuman 1894). With the techniques available to these earlier biochemists, thymonucleic acid could be obtained in recognisable quantities only from animal tissues, and the same applied to the extraction of phyto- or zymonucleic acid from plants and yeast. This led to the then universally accepted view that these nucleic acids were specific to plant and animal nuclei respectively.

This theory was disproved in 1924. In that year, Feulgen (1924) introduced a specific histochemical test for thymonucleic acid. The use of the Feulgen reaction, as the test was later called, permitted the identification of thymonucleic acid in sectioned material. In the hands of the cytologist, the test became an efficient tool and an analysis was undertaken of the distribution of thymonucleic acid in different types of tissues. The intensity of these investigations can be judged by the fact that up to 1938 the bibliography of the Feulgen reaction fills eighteen pages (Milovidov 1938). This application of the test proved conclusively that both plant and animal nuclei as well as the nuclear-like bodies of yeasts and bacteria all contained thymonucleic acid. It became clear that thymonucleic acid was previously recognised only in extracts of animal tissues, because of the relatively greater preponderance of nuclei in such tissues. When

more delicate tests for thymonucleic acid were applied to plant tissue extracts, its presence could be demonstrated, but the quantities were never comparable to those obtained from animal tissue extracts. These investigations realized a fundamental principle: that thymonucleic acid is a universal constituent of the chromatin of all cells.

At the same time, further advances in nucleic acid chemistry necessitated a change in nomenclature. Precise investigations showed that both types of nucleic acid contained a pentose sugar; but, whereas the pentose in thymonucleic acid was desoxyribose, that in phytonucleic acid was ribose. The two different nucleic acids were accordingly renamed desoxyribonucleic acid and ribonucleic acid respectively.

The concept of nucleic acid charge and the nucleic acid cycle.

Feulgen's investigations had even a greater importance than that of elucidating the overall distribution of desoxyribonucleic acid in living matter. The application of the test to dividing nuclei showed that the 'stainable chromatin substance' of the older cytologists was identical with desoxyribonucleic acid. All the observations made by these workers on the decrease at telophase and increase at prophase of 'stainable chromatin substance' could be interpreted in terms of the quantity of desoxy-

ribonucleic acid attached to the chromosome. This, then, is the basis for the concept of nucleic acid charge.

Charge is defined as the amount of desoxyribonucleic acid attached to the chromosome at any one stage of the nuclear cycle. During this process the charge is variable, so that underlying the observable behaviour of the chromosomes at mitosis and meiosis is a cycle of increase and decrease of the nucleic acid charge. The maximum degree of attachment is attained at metaphase; prophase is characterized by the charging of the chromosome with nucleic acid, while at telophase, the chromosome discharges nucleic acid.

This nucleic acid cycle has been demonstrated in many plants and animals. Although most of these studies have been based on Feulgen-stained material, they have received confirmation from the work of Caspersson (1939). This worker has used an ultraviolet light microspectrophotometric procedure, which permits the quantitative estimation of the nucleic acid charge at different stages of the nuclear cycle.

In general, these concepts have been accepted by most cytogeneticists. However, they have been recently challenged by a number of workers. Should these criticisms be upheld then a further revision of these concepts will become inevitable. The challenges are based on:

1. Criticism of the specificity of the Feulgen reaction.
2. Criticism of the existing concepts of chromosome structure.

1. The Specificity of the Feulgen Reaction.

The main denial of the specificity of the Feulgen reaction is based on the fact that the Schiff's reagent used in the test can stain other aldehyde containing substances such as fats and polysaccharides. This is perfectly true for in vitro reactions, but the conditions of the test limit the staining reaction to potential aldehyde groups of the desoxyribose in the nucleic acid. Semmens (1940) has further suggested that the resultant reaction may be due to the purine and pyrimidine components of the nucleic acid, but Barber and Price (1940) have dispelled this view. Further detailed evidence for the specificity of the reaction has been presented by Stowell (1945) in a comprehensive review of the subject. The general conclusion is that the Feulgen reaction is wholly specific for desoxyribonucleic acid, provided it is carried out with strict adherence to all the conditions originally laid down.

2. Concepts of chromosome structure.

The more serious challenge to the role of nucleic acid in chromosome behaviour has emerged from the work of Stedman and Stedman (Stedman and Stedman [1943a, 1943b,

1943c, 1944]; Stedman 1944). These investigators claim to have discovered a new protein 'chromosomin' which they believe to be the most important component of chromatin. They raise no doubts as to the specificity of the Feulgen reaction, but state that the aldehyde-leucobasic fuchsin is watersoluble. It could therefore be formed anywhere in the nucleus and could diffuse to become adsorbed on to the chromosomin. The Feulgen reaction, in their opinion, does not indicate the distribution of desoxyribonucleic acid at all; it only demonstrates the localization of chromosomin, which has adsorbed the aldehyde-basic fuchsin stain. As evidence, they cite the experiments of Choudhouri (1943) who was able to stain chromosomes with the Schiff's solution 'developed' with formaldehyde. Moreover, they believe that the acidic properties of chromosomin are responsible for the reactions of the chromosomes with basic dyes such as crystal violet.

In this way, by elevating chromosomin to first place in the structure and function of the chromosome, Stedman and Stedman have at the same time relegated nucleic acid to a component of the nuclear sap. Furthermore, they suggest that the physico-chemical properties of desoxyribonucleic acid such as its aniso-metric molecular form, and its capacity for orientation, provide evidence for its organization into the spindle at cell division. This view,

if correct, would nullify the concept of nucleic acid charge. However, objections can be and have been raised to the chromosomin hypothesis. These have been fully expressed by Callan (1943), Barber and Callan (1944) and Caspersson (1944). Only the more important criticisms will be considered in this discussion.

Firstly, with regard to the suggested role of desoxyribonucleic acid in the formation of the spindle, it may be stated that the spindle is achromatic and never Feulgen-positive. It remains clearly visible after acid hydrolysis, and is stainable with acid dyes. Darlington (1937) has also shown that the spindle is a cytoplasmic component, organised by the interaction of the centromeres of the chromosomes and the centrosomes of the cytoplasm. As further evidence in support of this view, the spindle never forms until the nuclear membrane disappears. The investigations of Schmidt (1937) with polarized light have demonstrated that the birefringent properties of the spindle are markedly different from those of desoxyribonucleic acid. The former is positively-birefringent, the latter, negatively-birefringent. All these studies indicate that the construction of the spindle from desoxyribonucleic acid is unlikely.

Secondly, the fact that desoxyribonucleic acid is localized on the chromosomes as visualized by the Feulgen

technique, has been confirmed by the investigations of other workers. Caspersson (1936) has arrived at this same conclusion by using the ultra-violet light micro-spectrophotometric method, which detects the purine and pyrimidine components of the desoxyribonucleic acid. Mörberg (1942), tracing the phosphorus component of the nucleic acid with a micro-incineration photometric procedure, is in complete agreement with the findings of Caspersson. A final proof has been supplied by the differential digestion experiments of Mazia (1941).

From the analysis of the above findings, it is clear that whichever way the desoxyribonucleic acid is detected, whether by its sugar, purine and pyrimidine or phosphorus components, all the results strongly suggest that desoxyribonucleic acid is localized on the chromosome. Nevertheless, even with this convincing evidence at hand, it cannot be claimed that the findings of Stedman and Stedman are unimportant. Their hypotheses are revolutionary, and cannot be dismissed only because they clash with orthodox views. If the existence of chromosomin can be confirmed, then it will call for a complete reassessment of present day concepts of the protein structure of the chromosome. In this lies the importance of their work, but on the basis of other evidence, their views on the distribution of desoxyribonucleic acid are unacceptable.

The concept of nucleic acid charge, then, has a well established basis, and this justifies its continued use by the cytogeneticist. It provides an efficient micro-morphological basis for understanding the modifications in the staining intensity of the chromosomes during the nuclear cycle. The fact that there exists a causal link between nucleic acid charge and its regular variability on the one hand, and modifications in chromosome morphology on the other, fully justifies the inclusion of this discussion on biochemistry into an account of morphology.

SPIRALIZATION.

The previous discussion has shown that the regular variability in the staining reaction of the chromosome may be successfully interpreted in terms of the concept of nucleic acid charge. Although desoxyribonucleic acid plays an important role in chromosome behaviour, all of its reactions occur on a morphological substratum. Thus, besides desoxyribonucleic acid, there exists, as a critical component of the chromosome, the protein thread or chromonema. The number of chromonemata within the chromosome is still uncertain, but this problem has no bearing on the following discussion. The chromonema, whether numerically simple or numerically complex still behaves as a single unit.

The older cytologists observed alterations in the

dimensions of the chromosomes at different stages of the nuclear cycle. It is to their credit, that, even with their unrefined technical methods, they were able to recognise such morphological modifications. What is more, they were able to define these changes, even though their nomenclature was limited. Thus, the old books on cytology mention that 'the chromosomes become shorter and thicker during prophase and longer and thinner at telophase'.

The provision of a micromorphological basis for this type of chromosome behaviour will be the main task of this section. It is clear that reactions of desoxyribonucleic acid no longer enter directly into the picture, and attention must be now focussed on to the protein framework, or chromonema, as a possible agent in the production of changes in the dimensions of the chromosome. Into this setting, may be introduced the theory of spiralization. This theory claims that the transformations in the chromosome during the nuclear cycle are expressions of a series of spatial rearrangements of the underlying protein framework. More specifically, it states that these spatial reorganizations take the form of a spiralization during prophase and an unspiralization at telophase. The theory therefore distinguishes two mechanically stable landmarks: the metaphase chromosome which has a coiled protein thread or chromonema, and the interphase

nucleus in which the chromonema is relatively uncoiled.

There can be no doubt that the chromosome has a spiral structure at metaphase and that this is attained during prophase and unravelled during telophase. A brief review of the evidence available will demonstrate that this is indeed the case.

The evidence for spiralization and unspiralization.

(a) Evidence from the metaphase chromosome.

Since a spiral structure of the metaphase chromosome is a corollary of the theory of spiralization, one means of vindicating the theory would be to prove such a structure in the metaphase chromosome. Usually these bodies are uniformly cylindrical and appear completely homogeneous. Special treatments are required to demonstrate the spiralized chromonema lying within the chromosome. Geitler (1935), Upcott (1935) and Coleman (1940) claim to have revealed the spiral structure in the metaphase chromosome under experimental conditions. However, since the treatments used may have produced artifacts, this evidence is not entirely convincing.

(b) Evidence from mitotic telophase and prophase chromosomes.

A more elegant proof for the existence of a spiral structure is provided by the work of Darlington (1935,

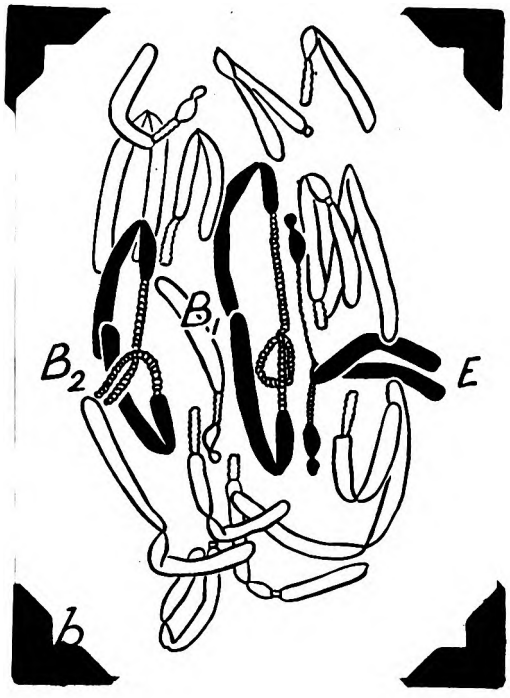


Figure 42. Anaphase stage in Trillium showing the consequences of chromosome division at low temperature. Reproduced from Darlington and La Cour (1940).

1937). Darlington has studied the unspiralization of the metaphase chromosome which occurs at telophase. Furthermore, he has found that telophase unspiralization may be postponed for completion until the next prophase. Here it becomes superimposed on the newly developing spiralization process. This delayed unspiralization reveals itself in prophase nuclei as the 'relic' coils of the prophase chromosomes. It is obvious that the appearance of uncoiling phenomena, either at telophase or at the following prophase, necessarily presupposes the previous existence of a spiral structure in that chromosome at metaphase. Therefore, such relic coils demonstrate not only unspiralization but also the metaphase spiral structure. The latter, in turn, provides evidence for spiralization during prophase.

(c) Evidence from the consequences of anaphase bridges at mitosis.

Further evidence is available from the experimental work of Darlington and La Cour (1940). Chromosomes entering anaphase at low temperature show defects in separation. Adhesion of sister chromatids occurs with subsequent formation of anaphase bridges. This is clearly seen in Figure 42, which is reproduced from the article of Darlington and La Cour. It is to be noted that in

the case of chromosome E the traction imparted by the separating chromatids has become sufficiently powerful to draw out the spiralized chromonema into an unspiralized thread. This provides evidence for a spiral structure of the metaphase chromosome, from which may be derived both spiralization and unspiralization.

(d) Evidence from relational coiling and chiasma formation in meiotic chromosomes.

The coil of mitotic chromosomes at metaphase is known as the minor or internal spiral. The same coiling system develops in meiotic chromosomes. However, the peculiar behaviour of meiotic chromosomes results in several modifications of the spiralization cycle.

The first of these modifications is the phenomenon of relational coiling. According to Darlington (1935), delayed unspiralization of the minor spiral occurs in the meiotic prophase as well as in the prophase of mitosis. In the mitotic prophase threads, it will be remembered that delayed unspiralization was related to the production of 'relic' coils. In the case of the meiotic prophase, the individual chromosomes become associated in pairs at zygotene. The stress of longitudinal cohesion is now superimposed on the torsion imparted by the relaxing minor spiral. The two forces, coupled, produce chromosome relational coiling. Furthermore, when chromo-

some division occurs at pachytene, the stresses in the chromosome become transmitted to the chromatids. Consequently, these, too, become relationally coiled.

Secondly, there is the formation of chiasmata, which Darlington relates to the spiralization process. Chiasma formation is said to be a consequence of the relational coiling system, in that the tension is relieved when these coils become replaced by chiasmata.

It is evident that relational coiling and chiasma formation in the meiotic prophase chromosomes are the counterparts of the relic coils of the prophase chromosomes of mitosis. As already shown, the appearance of relic coils at mitosis permits the inferences of spiralization and unspiralization. In the same way, these may be derived from relational coiling and its consequence, chiasma formation.

Enough evidence has been presented above to demonstrate that unspiralization and spiralization are empirical data both for mitotic and meiotic chromosomes. There can be no doubt, therefore, that these processes play a decisive role in determining the modifications in the dimensions of the chromosome during the nuclear cycle.

The problem can be approached from a different point of view. If the changes in the dimensions of the chromo-

somes are analysed theoretically, then at least two different interpretations become evident. Firstly, it may be assumed that material is lost from the chromosome during prophase, such material being resynthesized at telophase. Or, secondly, interchanges in material may be regarded as impossible, whereupon, the only geometrical changes which can explain chromosome behaviour are those of prophase spiralization and telophase unspiralization.

The crucial difference between these two approaches is that while the first considers the possibility of a changing protein thread, the second asserts a material constancy of the chromonema throughout the nuclear cycle.

This notion of a permanent chromonema may be derived in two different ways. Firstly, investigations in experimental genetics have shown that the loss of even a small region of the Dipteran giant salivary chromosomes may be associated with disastrous phenotypic effects. Therefore, it may be argued that loss of material from the chromonema during the nuclear cycle is wholly impossible, especially in those cases where it accounts for the shortening of a chromosome to the extent of 18% of its prophase length. The validity of this inference depends on whether the chromosome has uniform gene activity, and also whether such activity is exerted during nuclear division. At present, there is no information available

regarding these aspects of chromosome function. Therefore, the inference is doubtful, and not entirely acceptable.

The second path of derivation of a constant chromonema issues from the work of Kuwada and Nakamura (1934). These investigators transformed a metaphase nucleus into a structure similar to that of an interphase nucleus by treatment with ammonia vapour. According to Darlington (1935):

> "This experiment solves three important problems at once. First, it shows the structure both of the resting nucleus and of the active chromosomes in terms of a permanent* element, the chromosome thread. The resting nucleus consists of chromosome threads with their spirals more or less relaxed or uncoiled, the active chromosomes consist of the same* threads with their spirals compact or coiled. The experiment shows therefore, secondly, that the thread structure of the chromosomes is maintained* from one division to the next. It thus vindicates the theory of permanence and continuity."

The idea of permanence in cytogenetics has assumed various expressions. There is permanence implied in:

(a) Constancy of the number of chromosomes and their individual morphology.

(b) Constancy of the linear arrangement of genes along the chromosome.

(c) Continuity, implying a permanence in the dimension

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* The italics are mine. S.B.

of time.

(d) Constancy in the structure and volume of material of the chromonema.

It is clear that the permanency referred to by Darlington is exemplified in the instance (d) above. In other words, there is postulated a constant structure and volume of material of the chromonema persisting through all stages of the nuclear cycle.

The problem now arises as to the relation between spiralization and unspiralization as empirical data and the assertion of the chromonema as a constant structure. At the outset a few general statements will be necessary. It is evident that if the chromonema has a constant length during the nuclear cycle (as implied in its postulated permanence), then spiralization and unspiralization become inevitable consequences and do not require empirical proof. Furthermore, the validity of the assertion of a permanent chromonema would provide an unequalled vantage point for the elucidation of chromosome behaviour. For, knowing the length of the chromonema, the length and diameter of the chromosome, and applying the mathematical formulae of three dimensional spiral geometry, it would be possible to calculate the internal structure of the chromosome at any one of its visible stages in the nuclear cycle! The mechanics of chromosome behaviour could

be given precise mathematical expression. All this depends on the validity of the postulate of chromonema permanence, and therefore it is necessary to discover whether this assumption is justified or not.

Darlington obtains evidence for the postulate from the investigations of Kuwada and Nakamura. However, his conclusions are not entirely justified for a number of reasons.

Firstly, the fact that a group of metaphase chromosomes can be transformed into a structure resembling an interphase nucleus only demonstrates that it is possible to prepare with special treatments an artificial interphase nucleus which presents morphological similarities with the natural one. It does not necessarily prove that the underlying structures of both are identical. The natural interphase nucleus arises under totally different conditions from the artificial one, and therefore the latter may be of the nature of a 'phenocopy'.

Secondly, and apart from the above mentioned theoretical objections, there are several empirical deficiencies in the Kuwada and Nakamura experiment. Darlington's conclusion could only be valid if the experiment fulfilled two requirements.

1. Establishment of constant chromonema length by measurements, in both the artificial and the natural interphase nucleus.

2. A metrical proof to test the identity of the chromonemata in the artificial interphase nucleus with those in the original metaphase chromosomes. This is essential to eliminate the possibility of the treatment removing portions of the chromonemata.

Since chromonemata have not yet been observed in the interphase nuclei, this makes their measurement impossible. Still, the experiment requires metrical proofs if Darlington's conclusion is to be valid. Because the experiment does not provide such proofs, its results are disputable and therefore Darlington's conclusion is not substantiated.

Until techniques are elaborated permitting the study of chromonemata at all stages of the nuclear cycle, the postulate of chromonema permanence is unjustified.

The crucial point that emerges from the above discussion is that the theories of spiralization and unspiralization can be framed independently of a premise of chromonema permanence. The former are empirical data of cytogenetics, the latter an unjustified assumption. Not only is the inclusion of a postulate of chromonema permanence unnecessary in the formulation of the theory of spiralization but also it can be a very dangerous procedure. It may lead to the erection of a mathematical system of chromonema mechanics which is totally unjustified.

In fact, the author had previously constructed such a calculus, but in the light of the above discussion, it has been abandoned.

So far, the discussion of spiralization and unspiralization has been limited to mechanical changes in the chromonema. It is quite possible that spiralization of the chromonema may be accompanied by a loss of material, while unspiralization may be associated with a resynthesis. Caspersson (see Caspersson and Santesson [1942]) has demonstrated a loss of protein from the chromosome during prophase, which renders likely the possibility of interchange of chromosomal material during the nuclear cycle.

Another possibility is that the chromonema may undergo contraction during prophase and elongation during telophase. If the chromonema does consist of histones and other fibrous proteins (see Mirsky 1943), then such micelles would exist in a highly oriented state. Elasticity and molecular contractility are striking features of highly oriented anisometric molecules such as the keratins and myosins (Astbury 1943), and would be a property of the chromonema protein structure. Such changes in the elasticity of the chromonema may accompany spiralization and unspiralization. Thus in the meiotic prophase of Trillium, Huskins (1941) claims that:

> "Throughout the spiralization cycle changes are occurring in the length of the chromonema. To what extent these are true elongations and contractions of the material constituting the chromonema and to what extent they are due to the formation of and stretching out of a waviness or a minor coil with a diameter near the limits of microscopical resolution cannot be determined by present methods. Both types of change seem to be involved."

If we are seeking for generic theories of micromorphological chromosome structure, it is not useful to consider the chromonema as a permanent structure. Instead, the attitude should be that of Ris (1945) when he says

> "The chromonema is not uniform in length, but it can vary greatly from cell and cell in the same organism as well as in the same cell in different metabolic states."

It is essential to change some of the emphasis from the gross mechanics of spiral formation and breakdown to the metabolic changes in the chromonema during the nuclear cycle.

Just like any other biological system, the chromonema is not a rigid structure but is in a continual state of flux and flow. Its physiology is not a physiology apart, but is an aspect of a total cell reaction, and because of this, it interacts with its surroundings, and changes accordingly.

Allocyclic behaviour of the sex chromosomes in Elephantulus.

With the concepts of charge and spiralization formulated, it is now possible to consider the allocycly of the sex chromosomes in Elephantulus. It will be, however, first necessary to discuss what is meant by allocyclic behaviour in general.

The nature and consequences of allocyclic behaviour.

It has long been known that the cytological behaviour of certain chromosome segments, or, in some cases, even complete chromosomes, is different from that displayed by the rest of the complement. There are therefore chromosomes with standard behaviour and those with differential behaviour. Heitz (1935) was the first to draw a sharp distinction between these two types of chromosomes, and decided that the structure of each was different. He assigned the term euchromatin to the material composing the standard chromosomes, and heterochromatin to that of the aberrant chromosomes. In general, the most striking cytological distinction between euchromatin and heterochromatin is that of a difference in staining reaction at various parts of the nuclear cycle. Differential staining of a heterochromatic segment, with consequent departure from the standard of euchromatic cy-

cle, has already been defined as allocyclic behaviour. Since it has been shown that the staining reaction of a chromosome is a morphological expression of its nucleic acid charge, it is possible to reframe the definition of allocycly in these terms. In this way, allocycly has been defined as a 'variability and irregularity in the rate and/or extent of nucleic acid charge' (Darlington and La Cour 1940). This variability is manifested in two different ways: either the heterochromatic segment in question is undercharged or it is overcharged*. Further, it is found that the same segment may be overcharged at one stage of the nuclear cycle and undercharged at another stage. Such reversibility seems to be a general feature of allocycly. Thus Darlington and La Cour (1940) have shown in plant chromosomes that segments which are overcharged in the interphase, become undercharged at metaphase when such plants are exposed to low temperatures. The same is true for heterochromatic segments in Triton (Callan 1942). White (1945) has demonstrated the same reversibility in the X chromosomes of grasshoppers and crickets.

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* In White's terminology (1945) heteropycnosis is equivalent to allocycly, while positive and negative heteropycnosis are respectively synonomous with overcharging and undercharging.

Besides an apparent disturbance in nucleic acid charge, allocycly may reveal itself in aberrations of spiralization and chromosome division. Whether the disturbance in nucleic acid charge is primary and produces the other aberrations is uncertain at the moment, but all the phenomena are closely related. Thus Darlington (1939) has shown that the overcharged sex and M chromosomes of Heteroptera divide precociously at the metaphase of meiosis; overdosage of the heterochromatic supernumerary chromosomes in Zea Mays produce "morbid mitosis" in pollen grains (Darlington and Upcott 1941); while the undercharged segments of frozen Trillium chromosomes are incompletely spiralized at metaphase (Darlington and La Cour 1940). Finally, the formation of anaphase bridges with subsequent chromosome breakage is a consequence of reproductive aberrations associated with undercharged heterochromatic segments (Darlington and La Cour 1940).

To summarise the general aspects of allocyclic behaviour: firstly, heterochromatic segments do not necessarily exhibit a constant allocyclic behaviour at all stages of the nuclear cycle.

Secondly, such behaviour will be manifested not only in disturbances of nucleic acid charge but also in aberrations of spiralization and chromosome division.

Recently, there have been some attempts to clarify

the problem of allocycly in more detail. Broadly speaking, allocyclic behaviour presents three separate aspects for analysis. Firstly, there is the structural differentiation of the heterochromatic segments. In these regions are located micromorphological mechanisms which determine allocyclic behaviour. Such mechanisms will differ considerably from those found in euchromatin. Secondly, there are the total physiological conditions of the cell, which permit the emergence of allocyclic behaviour and which may modify its final expressions. Finally there is allocycly, the result of the interaction of the intrachromosomal mechanisms with the extrachromosomal conditions. The latter has already been previously examined; allocyclic behaviour has been defined and the means for its detection have already been formulated. The following discussion will deal with the first two aspects of allocycly, namely, the nature of the heterochromatic segments and the physiological conditioning of allocycly by the cell.

<u>The micromorphology of allocyclic behaviour: the structure of heterochromatin and the cellular timing of allocycly.</u>

Darlington (1942) has explained allocyclic behaviour by suggesting that heterochromatin has a lower reactivity of desoxyribonucleic acid synthesis than euchromatin. This accounts only for the particular type of allocycly

exhibited by undercharged chromosome segments at the metaphase of mitosis and meiosis. According to Darlington, heterochromatin displays such allocyclic behaviour when the amount of nucleic acid precursors is so reduced that heterochromatin cannot compete equally with euchromatin for the diminished supply. Heterochromatin will then show defective nucleic acid charge.

Darlington's thesis has emerged from his low temperature experimental investigations in plants, and is open to two main criticisms.

Firstly, with regard to its usefulness in interpreting allocyclic behaviour in the sex chromosomes of Elephantulus, it cannot explain at all the full nucleic acid charge preserved by segments b_1, b_3 and c of the X chromosome during interphase. The concept may account for the low charge of the differential segment of the X chromosome at the metaphase of meiosis. However, since Elephantulus is a homiothermic animal, reduction of the supply of nucleic acid precursors needs to be attained by some mechanism other than the lowering of temperature.

Secondly, that low temperature does in fact lower the supply of nucleic acid precursors, is only postulated by Darlington. It is quite conceivable that the lowered nucleic acid charge of heterochromatin is brought about by a differential inhibition of its constituent enzyme systems,

those on euchromatin remaining unaffected by the decreased temperature. Still, Darlington's concept of allocycly is a significant contribution, for it recognises possible physico-chemical differences between euchromatin and heterochromatin.

Pontecorvo (1943, 1944) has recently suggested another explanation of allocycly. While he is not in disagreement with Darlington as to the role of the cell in conditioning allocycly, he elaborates on the structure of heterochromatin.

The first important point made by Pontecorvo is that heterochromatin has less 'internal differentiation' than euchromatin. At first, following the discovery of the so-called genetically 'inert' segments by Muller and Painter (1932) and their subsequent identification with heterochromatin by Heitz (1933) it was thought that such heterochromatic segments contained very few or no genetical loci at all. Recent work, however, suggests that this is not the case. It has been shown by Mather (1944) that heterochromatin contains the full quota of loci, which are, however, expressed only quantitatively in the phenotype. Mutations or changes in quantity, such as duplication and deletion of heterochromatin leave no qualitative impression on the overall phenotype. They are

therefore, difficult to detect. This genetical evidence indicates that heterochromatin does in fact contain loci and that the number of these loci are no different from that in euchromatin.

This is further supported by the work of Darlington and his collaborators on the supernumerary chromosomes of plants (Darlington and Thomas [1941], Darlington and Upcott [1941]). Such chromosomes are completely heterochromatic, and genetical activity, although present, is strictly local and difficult to detect in the phenotype.

Pontecorvo then proceeds to explain what he means by the 'lesser differentiation' of heterochromatin and its relation to allocyclic behaviour. He considers first the factors underlying the uniform behaviour of euchromatic regions during the nuclear cycle. During a short period of prophase, it is found that the chromomeres of euchromatic regions differ from each other in the extent of their nucleic acid charge. This is even better demonstrated in the giant salivary chromosomes of Diptera. Here, the longitudinal differentiation is in the form of bands, each with a different nucleic acid charge. Obviously then, each chromomere is allocyclic in relation to others. At metaphase, where it is virtually impossible to recognise the individual chromomeres, all that an euchromatic region presents is an aggregation of its compo-

nent chromomeres and their individual charges. The uniformity of such regions at metaphase is therefore only a statistical consequence of the charges of numerous and different chromomeres.

This is sharply contrasted to heterochromatin, where as Pontecorvo suggests, the chromomeres are all, or nearly all of a single type. The array of chromomeres, all identical with each other is reflected in the lesser 'internal differentiation' of heterochromatin. This is supported by the genetical evidence mentioned above.

The consequences of this concept are important. It explains, for example, the non-homologous pairing of heterochromatic segments. In this instance, the 'type chromomere' of one of the segments is the same as that found in the other. Moreover, the different allocyclic behaviour of a number of heterochromatic segments in the same nucleus (and therefore presumably under the same physiological conditions) would appear to be a consequence of different 'type chromomeres' in each segment. Each 'type chromomere' determines a particular pattern of allocyclic behaviour.

Finally, in commenting on the origin of heterochromatin, Pontecorvo suggests that heterochromatin would arise when euchromatic chromomeres undergo longitudinal duplication and remain permanently associated in linear order.

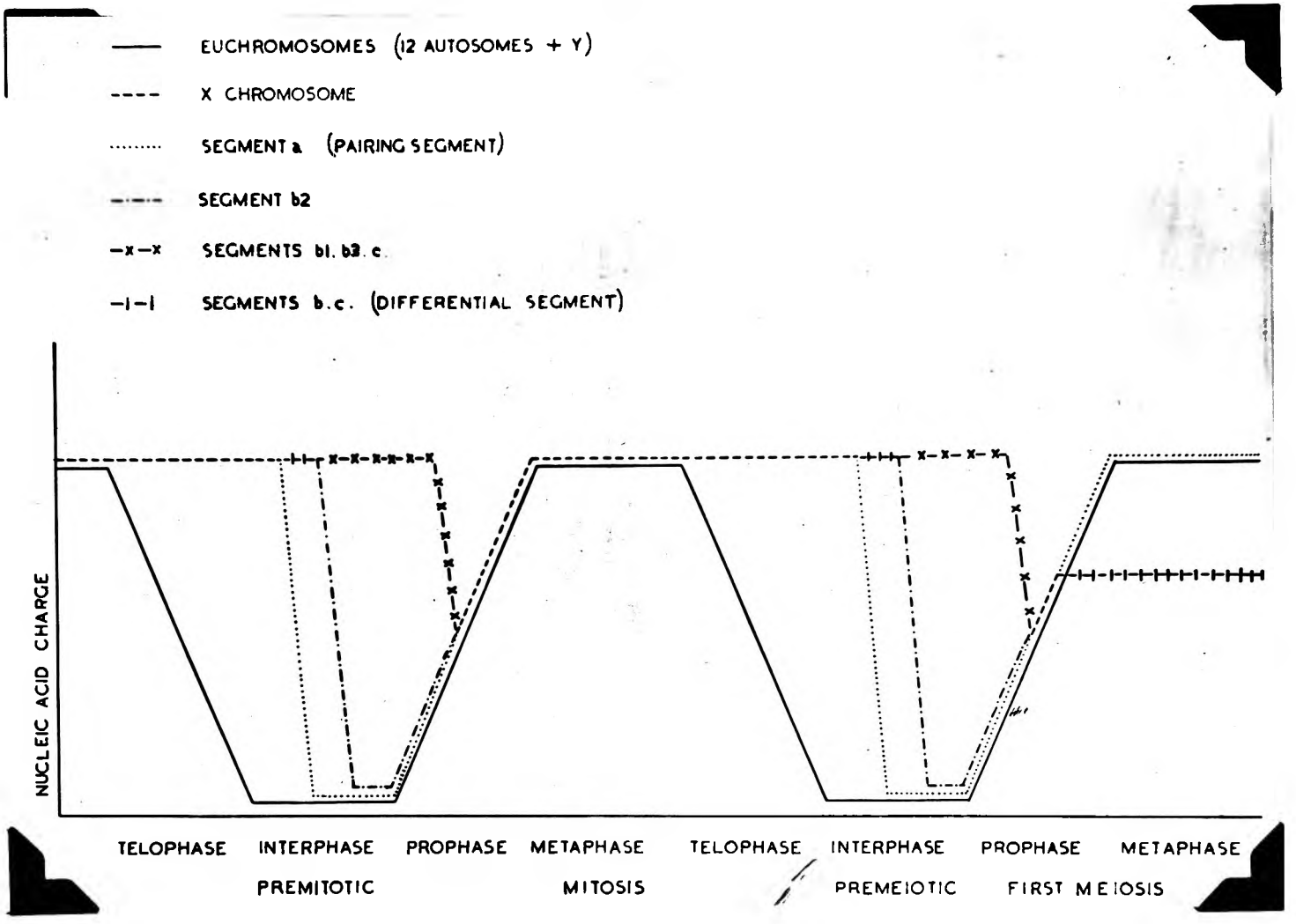
While Pontecorvo has advanced present views on allocyclic behaviour and the structure of heterochromatin, a great deal still eludes clear explanation. Now that allocyclic behaviour can be interpreted as an expression of the constituent chromomeric physiologies of heterochromatin, the physico-chemical attributes of such chromomeres become relevant. Apart from its particular genetic action and the mechanisms responsible for the execution of such action, it can be deduced, that each chromomere has an enzyme system for the synthesis and breakdown of nucleic acid. The reaction rates of the particular enzymes in such a system, as well as the physiological relations between synthetic and depolymerising enzymes, will largely determine the nucleic acid cycle of that chromomere. Thus, if synthetic enzymes act slowly, undercharging will occur at metaphase, and if intervention of depolymerizing enzymes is postponed at telophase, then the full nucleic acid charge will be maintained during interphase. Since the behaviour of each segment is the expression of its individual chromomeric physiologies. the segment will exhibit allocyclic behaviour only when its constituent chromomeres have 'allocyclic' enzyme systems. This concept provides an explanation for Darlington's observations on frozen chromosomes other than the

postulated reduction of nucleic acid precursors. The decrease in temperature may differentially slow the reaction rates of the enzyme systems on heterochromatin, leaving those in euchromatin unaffected.

But more important, realization of this significance of chromomeric physiologies in determining chromosome behaviour, permits elaboration of Pontecorvo's hypothesis of the formation of heterochromatin. If he is correct in postulating that heterochromatin arises from euchromatin by repeated linear reproduction (as seems to be the case in the 'repeats' of Drosophila) then it is evident that for heterochromatin to be allocyclic, the euchromatic chromomeres selected to give rise to heterochromatin must be those in which 'allocyclic' enzyme systems have already made their appearance. The mode by which such selection is attained and controlled cannot be indicated at present, but is probably adaptive in the species. Such intrachromosomal modifications are expressions of evolutionary change and account for the specific pattern of heterochromatin of any species (White 1943).

It is evident from the foregoing, that in order to obtain an intimate understanding of allocyclic behaviour it is necessary to seek adequate micromorphological bases for such reactions. Only when these are formulated, do the morphological modifications begin to take on some significance.

Figure 43. Graph demonstrating chromosome behaviour in Elephantulus. The allocyclic behaviour of the various segments of the X chromosome may be compared with the standard behaviour of the remainder of the chromosome complement. The co-ordinates are entirely arbitrary.


EUCHROMOSOMES (12 AUTOSOMES + Y)
X CHROMOSOME
SEGMENT a (PAIRING SEGMENT)
SEGMENT b2
SEGMENTS b1. b3. c.
SEGMENTS b.c. (DIFFERENTIAL SEGMENT)
NUCLEIC ACID CHARGE
TELOPHASE
INTERPHASE
PROPHASE
METAPHASE
TELOPHASE
INTERPHASE
PROPHASE
METAPHASE
PREMITOTIC
MITOSIS
PREMEIOTIC
FIRST MEIOSIS

The allocyclic behaviour of the X chromosome in Elephantulus.

Four different cycles of nucleic acid charge have been distinguished in the X chromosome at mitosis, and two separate cycles at the first meiotic division in male germ cells. The relations between each of these cycles of the X chromosome and their relation to the nucleic acid cycle of the remainder of the complement is expressed in Figure 43. This is in the form of a graph with arbitrary co-ordinates of time intervals and degree of nucleic acid charge.

It will be noted that the X chromosome remains completely charged in the interphase stage of mitosis, while the other chromosomes (twelve autosomes and the Y chromosome) have already discharged nucleic acid at the telophase. Interphase is characterized by further changes in the X chromosome. Soon after interphase has commenced segment a of the X chromosome discharges nucleic acid and disappears. This is succeeded by the disappearance of segment b_2. The remaining segments of the X chromosome, namely segments b_1, b_3 and c remain fully charged throughout the interphase stage, and although they undergo fusion, only at the following prophase do they discharge nucleic acid. The four cycles of the X chromosome in the male complement at mitosis are, in order, the cy-

cles of:

1. The complete X chromosome
2. Segment a
3. Segment b_2
4. Segments b_1, b_3 and c.

At the first division of meiosis, the pairing segment of the X chromosome is as heavily charged as the euchromatic chromosomes (twelve autosomes and the Y chromosome), while the differential segment appears undercharged. The two distinguishable cycles are, then the cycles of:

1. The pairing segment (segment a)
2. The differential segment (segments b and c).

The Y chromosome is completely euchromatic at mitosis and at the first meiotic division.

In addition to the obvious disturbances of nucleic acid charge, the allocyclic behaviour of the X chromosome is characterized by aberrations in spiralization. These are different at mitosis and meiosis. At mitosis, the a and b_2 segments undergo unspiralization during the interphase, but this unspiralization is certainly not of the telophase type. Whereas telophase unspiralization is characterized by the appearance of relic coils, segments a and b_2 only become thinner, they neither elongate nor show relic coils. Similarly, at meiosis, the differential segment of the X chromosome is thinner than the pairing

segment, although its length is the same as that at mitotic metaphase.

In both of these instances, it is certain that a peculiar spiral structure is present. An analysis of this spiral form had been attempted by the author, but the conclusions were based on the postulate of a permanent chromonema length. Since this assumption is not substantiated by existing evidence, the interpretation has been abandoned.

The above analysis of the allocyclic behaviour of the X chromosome in Elephantulus raises several important problems. It is evident that the nucleic acid cycles of the various segments of the X chromosome differ from each other as much as the cycle of the X chromosome differs from that of the euchromosomes. The only segments exhibiting allocycly which is constant for mitosis and meiosis are segments b_1, b_3 and c. To these, a heterochromatic structure may be undoubtedly assigned.

The difficulty arises in the interpretation of the structure of segments a and b_2. The former, although euchromatic at meiosis is slightly heterochromatic at mitosis. The latter, which is heterochromatic to some extent at mitosis, is strongly heterochromatic at meiosis. There seems to be no constancy in the allocyclic behaviour of these two segments of the X chromosome.

The observations have shown that segment a pairs with the euchromatic Y chromosome at meiosis. The fact that crossing over can occur between segment a and the Y chromosomes, shows that they are strictly homologous. Therefore segment a is undoubtedly euchromatic as well. This is compatible with its euchromatic nucleic acid cycle at meiosis, but cannot account for its heterochromatic properties at mitosis. Another explanation needs to be invoked.

The genetical 'position effect' has already been previously mentioned in connection with the cytotaxonomy of Elephantulus. The cytochemical counterpart of this phenomenon was first demonstrated by Caspersson and Schultz (1938). These authors have shown that euchromatic segments exhibit an approximation to a heterochromatic nucleic acid cycle, when they become translocated to the vicinity of heterochromatin. This phenomenon has been more recently confirmed by Prokofyeva-Belgovskaya (1945).

Such a cytological 'position effect' may well account for the slight heterochromatic properties exhibited by segment a at mitosis. This segment adjoins a block of heterochromatin in the form of segment b1, which may modify its nucleic acid cycle in favour of that of hetero-

chromatin. In this way, segment a will exhibit a partially heterochromatic cycle at mitosis. It is to be noted, that such an explanation, of necessity, postulates the absence of such a 'position effect' at meiosis, when segment a behaves as euchromatin. This may be related to the vastly different conditions of the meiotic nucleus, or, it is conceivable that a 'position effect' may be exerted by the Y chromosome itself. This would neutralize the heterochromatic 'position effect' of segment b_1 on segment a. The latter possibility is most unlikely, as 'position effect' exerted by euchromatin has not yet been demonstrated, nor has it been shown that a position effect can occur between two different chromosomes.

An explanation based on the 'position effect' breaks down completely when applied to the allocyclic behaviour of segment b_2. A 'position effect' could account for the behaviour of this segment, only if it were euchromatic, but this is discounted by its completely heterochromatic properties at meiosis.

However, what does seem clear is that segments a and b_2 represent intermediate forms between eu- and hetero-chromatin. In this the author is in full agreement with White (1945) who says: (p.313).

> "It is probable that too much stress has been laid on the antithesis between euchromatin and heterochromatin. It is at any rate certain that

> "in most organisms there are several different kinds of heterochromatin, and it is possible that what we ordinarily call eu- and hetero-chromatin are merely the end terms of a series that includes a number of intermediate types of protein framework [as suggested by Poulson and Metz (1938) and Pontecorvo (1943)]."

If such a 'spectrum' of chromosome structure is to be accepted then segment a would appear either at or near the euchromatic end, while segment b_2 belongs more or less to the heterochromatic end. It is further possible that the heterochromatic end of the 'spectrum' is characterized by a greater proportion of 'allocyclic' chromomeres and that these become more and more diluted as the euchromatic end is approached. This would correlate the idea of a chromosome 'spectrum' of structure with Pontecorvo's hypotheses (vide supra).

In this light, segments a and b_2 assume a new significance in the understanding of the evolutionary importance of heterochromatin. If it is shown that euchromatin does mutate to heterochromatin, as Pontecorvo has suggested, then the intermediate forms are crucial for obtaining information about the mechanisms and features of such changes.

The above analysis has indicated some aspects of the heterochromatic segments partaking in allocyclic behaviour, and has indicated some of the problems of the nature of segments a and b_2 of the X chromosome of Ele-

phantulus.

The investigations on the ovary throw more light on the physiological conditioning of allocycly. The absence of such behaviour in the differential segments of the X chromosomes at the first meiotic division in the female indicates that the physiological conditions here are different from those in the male. Since allocyclic behaviour was recognised in early leptotene nuclei in the form of the X karyosomes, the absence of such behaviour at metaphase may be due to the X chromosome being paired throughout its length with an equal homologue. Another possibility is that the allocyclic behaviour of the single X chromosome in the male is partly a consequence of conditions peculiar only to the male. White (1945) has demonstrated this in grasshoppers, where the male has a single allocyclic X chromosome, while no such behaviour is observed in the two X chromosomes of the female. That this is not due to the mere presence of two X chromosomes is suggested by observations on tetraploid male cells. Here, two X chromosomes are present, and contrary to expectations, both are allocyclic. However, it is to be remembered that these two X chromosomes still remain unpaired.

This analysis of the allocyclic behaviour of the X

chromosome in Elephantulus has permitted a further morphological regionalization of this chromosome. The distribution of heterochromatin and euchromatin are now known. The genetical consequences of this regionalization will be discussed later.

2. The relation of plasmosomes to the X chromosome.

de Mol (1927) was the first to stress the existence of a relationship between the number of plasmosomes and the number of chromosomes. Later Heitz (1931) showed a strict numerical relation between the plasmosomes and secondary constrictions. Working mainly with Vicia, he found that the plasmosomes were closely related to the secondary constrictions of the chromosomes. Such constrictions are always undercharged, and therefore, Heitz designated chromosomes which contained secondary constrictions as S-A-T chromosomes (sine acide thymonucleique). McClintock (1934) extended these studies and showed plasmosomal-secondary constriction relations to hold good in Zea Mays. This worker, by a series of ingenious genetical experiments, also succeeded in demonstrating that blocks of heterochromatin adjoining the secondary constrictions were related to the production of plasmosomes. These heterochromatic regions she termed 'nucleolar organizers'.

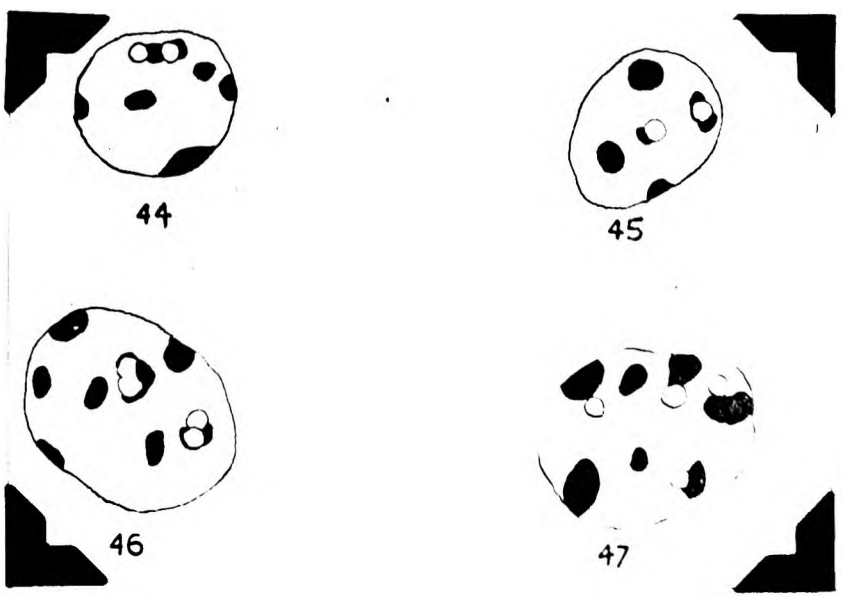
Although the relation between plasmosomes and secondary constrictions is very common in plants, it is not widely known in animals. Coonen (1939) in a review of the subject lists only six animals: Mosquito, Drosophila, Bibio, Opalina, Amblystoma and Salmo. Recent additions to this list are Triton (Callan 1942) and Vulpes vulpes (Wipf and Shackleford 1942), the latter being the only mammal in which, strictly speaking, this relation has so far been found. In the literature, there are numerous references to the relation of plasmosomes to chromosomes, but the exact association of such plasmosomes with defined secondary constrictions has not been observed. Thus the plasmosome is associated with the sex chromosomes in the Rat (Rattus Norvegicus) [Koller and Darlington 1934], the ferret (Putarius furo) [Koller 1936b], the Golden Homster (Cricetus auratus) [Koller 1938], the Striped Hamster (Cricetulus griseus) [Pontecorvo 1943] and various marsupials [Koller 1936c].

In Elephantulus, two plasmosomes arise in close association with the two secondary constrictions of the X chromosome in late telophases of both premitotic and premeiotic spermatogonia. During the interphase stage, secondary constriction I becomes obliterated by the disappearance of segment a of the X chromosome. However,

the plasmosome associated with that constriction remains attached to the heterochromatic segment b_1. This fact permits the suggestion that segment b_1 acts as a nucleolar organizer for that plasmosome. Similarly, it may be suggested that either segment b_3 or segment c or even both act as nucleolar organizers for the plasmosome at secondary constriction II.

The male and female cells of Elephantulus differ in their dosage of X chromosomes. Theoretically, then, it is to be expected that female cells with two X chromosomes would contain four plasmosomes, as opposed to the two plasmosomes found in the male cells with only one X chromosome. The information obtained from a study of the chromosome cycle in the ovary was unsatisfactory in this respect, as only the haploid state could be investigated. Here, it will be remembered, two plasmosomes arose in association with the secondary constrictions of the single X chromosome in the haploid complement.

For this reason, an ad hoc study was carried out on the interphase nucleus of the liver cell, to ascertain the existence of any plasmosome differences between the male and female nuclei. In the male nucleus, two plasmosomes were found associated with a number of blocks of heterochromatin. These were identified as the heterochromatic regions of the X chromosome. Thus, one plasmo-



Figures 44 - 47. Camera lucida drawings of interphase liver nuclei.

Figure 44. Diploid nucleus of female with two plasmosomes.

Figure 45. Diploid nucleus of male with two plasmosomes.

Figure 46. Tetraploid nucleus of female with four plasmosomes.

Figure 47. Tetraploid nucleus of male with four plasmosomes.

Alcohol fixed smear of liver stained with methyl green-pyronin.

X1700.

some was attached to a single block of heterochromatin representing segment b_1 of the X chromosome, while the other plasmosome was related to segments b_3 and c of the X chromosome (Figure 45). A number of large nuclei were found, which were shown to be tetraploid, and these contained four plasmosomes (Figure 47).

The female nuclei, as far as could be detected were no different from those of the male (Figure 44). These nuclei contained two plasmosomes associated with heterochromatin. The expected number of four plasmosomes was not found in any diploid liver nucleus. However, just as in the male, large tetraploid female nuclei contained four plasmosomes (Figure 46).

It appears that there exists some genotypic control of the number of plasmosomes in the female nucleus. Whether the nucleus is haploid or diploid, only two plasmosomes were found. Such genotypic mechanisms are probably located in the autosomes, and therefore, it is possible that only when the autosomes increase together with an increase of X chromosomes, will the plasmosome number increase correspondingly.

It is further evident that the location of nucleolar organizers on sex chromosomes will have important repercussions on the autosomes. In Elephantulus, the evolution of the X chromosome with secondary constrictions

imposed a parallel evolution of autosomal mechanisms which keep the plasmosome number constant in both the male and female cells.

If this should prove to be the case, then the genetical consequences of the evolution of sex chromosomes with secondary constrictions are different from that line of evolution where secondary constrictions were confined to the autosomes. There are thus two lines of sex chromosome evolution in mammals. On one line is Elephantulus with sex linked secondary constrictions and on the other Vulpes with secondary constrictions on an autosomal pair (Wipf and Shackleford 1942). It will be necessary to classify the sex chromosomes of all the mammals in this manner, but this is at present not possible owing to the paucity of information regarding the secondary constrictions of mammalian chromosomes.

CHAPTER VI.

THE GENETICAL PROPERTIES OF THE SEX CHROMOSOMES IN ELEPHANTULUS.

The genetical properties of sex chromosomes in general may be attributed to their structural differentiation. Since this also affects the distinctive behaviour of such chromosomes at meiosis, it is desirable to examine in more detail the behaviour of the sex chromosomes in Elephantulus.

The behaviour of the sex chromosomes in Elephantulus.

So far, according to Pontecorvo (1943), regional differentiation of the sex chromosomes has only been found in twelve species of mammals. To this list, may be added Elephantulus.

The observations recorded above have shown that the X chromosome in the male complement is divided into a pairing and a differential segment. The pairing segment, with a counterpart on the Y chromosome, includes the terminal centromere and segment a. The differential segment, which comprises segments b and c and both secondary constrictions, has no pairing homologue on the Y chromosome.

The Y chromosome is composed largely of a pairing segment, but the presence of a differential segment may

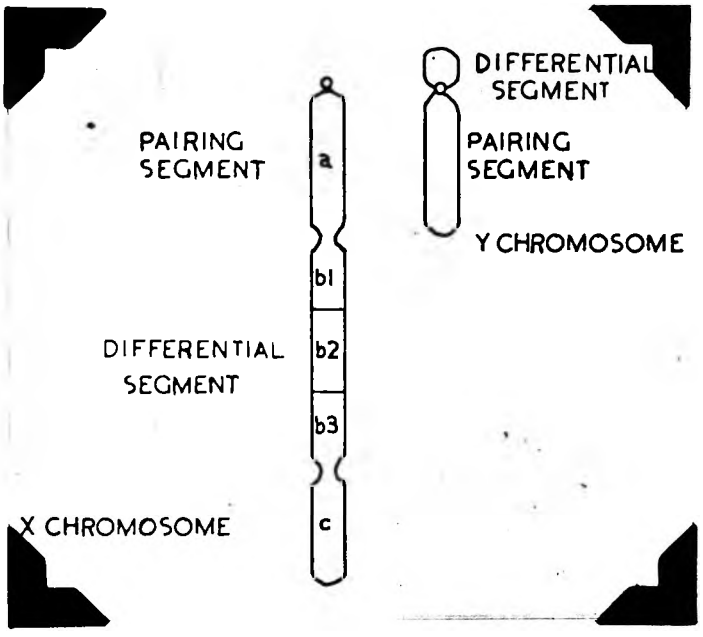


Figure 48. Diagram depicting the regionalization of the sex chromosomes in Elephantulus.

be inferred. Since pairing at meiosis is due to the attraction of like chromomeres, the centromeres, being special types of chromomeres will become associated. The X chromosome which is either telocentric or approximately telocentric will therefore pair in this fashion, with the Y chromosome which contains a subterminal centromere. It is evident that the small region of the Y chromosome terminal to the centromere, will have no counterpart on the X chromosome. This region will therefore constitute a differential segment. It is exceedingly small, measuring well under 0.5μ at the metaphase of mitosis; and this probably accounts for it not being consistently seen at meiosis. Nevertheless, indications of the presence of such a differential segment on the Y chromosome may be seen in the post reductional sex bivalents in Figure 37. Figure 48 depicts diagrammatically, the regional differentiation of the sex chromosomes.

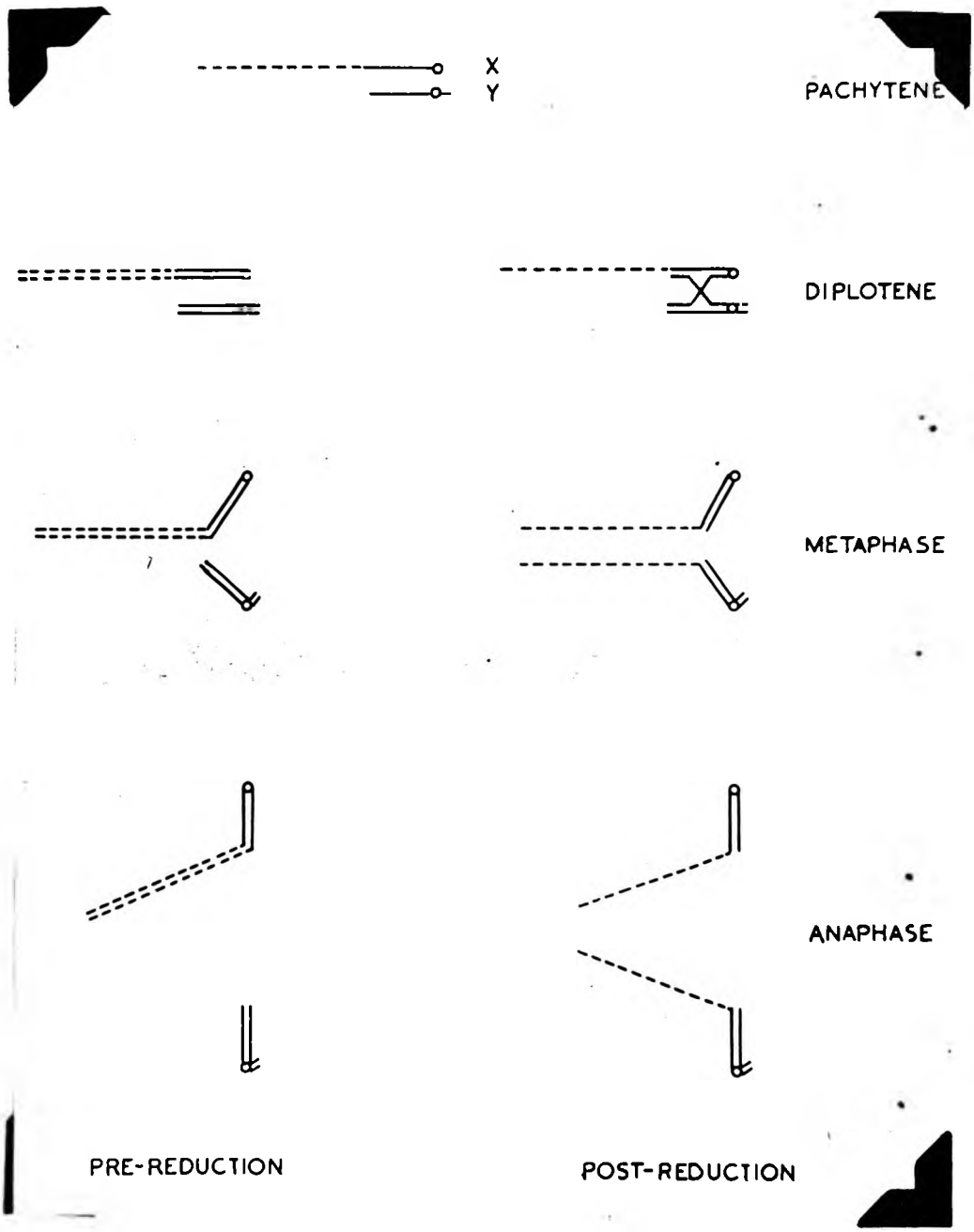
The segregation of the sex chromosomes at the meiotic anaphase depends largely on the type of association found at the preceding diplotene. In Elephantulus, the main variable is the presence or absence of a chiasma in the pairing segments of the sex bivalent. Thus, if chiasma formation occurs at diplotene, the resulting anaphase segregation will be post-reductional (equational)

Figure 49. Diagram depicting the structure and behaviour of the X/Y bivalent at the first division of meiosis.

_____ Pairing segment.

------ Differential segment.

o Centromere.


X
Y
PACHYTENE
DIPLOTENE
METAPHASE
ANAPHASE
PRE-REDUCTION
POST-REDUCTION

for the differential segment of the X chromosome (Figure 49). If, on the other hand, a chiasma does not form in the pairing segments, then the differential segment of the X chromosome will segregate pre-reductionally at anaphase (reductional division) (Figure 49). In Elephantulus, therefore, there is a facultative post-reduction of the differential segment, i.e. it depends on the presence of a chiasma, but should that chiasma not form, a pre-reductional division will result.

It is to be noted that the first division of meiosis is always pre-reductional for the differential segment of the Y chromosome, as chiasmata can never form terminal to the centromere, there being no homologous region on the X chromosome.

The first division of meiosis is always balanced by the second division in order to ensure complete genetic segregation.

The genetical interpretation of sex chromosome behaviour in Elephantulus.

The consistent manner in which the sex chromosomes undergo pairing and association permits genetical interpretation. In Elephantulus, it is now possible to assign the localization of genetical loci to the various regions of the X and Y chromosomes.

The pairing segments of both chromosomes contain loci which may undergo genetical interchange, by chiasma formation. Such loci would only exhibit partial sex-linkage, providing they showed phenotypic effects.

Loci on the differential segment of the X chromosome if associated with phenotypic expressions, would be completely female sex-linked. Similarly, loci on the differential segment of the Y chromosome would be completely linked with the male sex phenotype.

In Elephantulus, therefore, the suppression of crossing over or genetical interchange between completely sex linked loci is attained by isolation on differential segments.

Further genetical considerations.

It was possible in the previous section to determine the distribution of genetical loci on the sex chromosomes of Elephantulus. This localization was based on the cytological investigations of the sex chromosomes at meiosis.

The genetical localization may be now indicated in more detail. The studies on the allocyclic behaviour of the sex chromosomes have provided a further regionalization of these chromosomes in the form of the distribution of euchromatin and heterochromatin. It is however, essential first to examine the information dealing with the

genetical differences between eu- and hetero-chromatin.

Mendel's original experiments clearly demonstrated the relation of genes to the gross phenotypic manifestations of the organism. This has since been confirmed for the association of many genes, with the phenotype, whether the latter was morphological or physiological. Clear indications of the essential nature of such genes have been provided by the experimental geneticists. Alterations in the chromosomes, such as the characteristic duplications, deletions, translocations and inversions, as well as gene mutation itself, are often associated with gross phenotypic modifications. In many cases death of the organism results, and the essentiality of the chromosome locus concerned may then be judged by its lethality on mutation.

However, not all chromosome loci have such well-defined associations. Some loci have been thought to bear no relation to the phenotype, as mutations in such loci resulted in no detectable phenotypic modifications. In the past, chromosome segments of this type have been termed 'inert', implying that their component loci carried no genetic activity. Cytologically, these segments correspond to heterochromatin. 'Inert' segments are therefore heterochromatic as opposed to the 'active' euchromatic regions.

Recent cytogenetical investigations have shown that this is not the case. There exists a cytological difference between eu- and hetero-chromatin; but whereas the genetic properties of euchromatin may be termed 'active', those characteristic of heterochromatin are certainly not 'inert'.

In Zea Mays and Sorghum, Darlington and his associates have found a number of wholly heterochromatic chromosomes over and above the standard chromosome complement. (Darlington and Upcott 1941, Darlington and Thomas 1941). Such 'B' or supernumerary chromosomes vary in number in different cells of the same organisms and even more frequently in different organisms of the same species. A balance is set up in the population, and on this basis, Darlington has assigned such chromosomes some genetical activity. The variation in number of such chromosomes shows that as far as the viable phenotype is concerned they are dispensable. Nevertheless, they have genetical activity, and this is demonstrated in pollen grains where the number of supernumeraries may become abnormally increased owing to centromere defects. In these cases, an abnormal mitosis sets in; the abnormality being one in the timing of division in that an unorganized polymitosis occurs (Darlington and Thomas 1941). On this basis, Darlington differentiates between eu- and hetero-chromatin, by stating that the activity of the former is wide-

spread and 'high specific', while that of the latter is local and 'low specific'.

These investigations were extended by Mather, who, working with Drosophila has also come to the conclusion that heterochromatin has some genetical activity (Mather 1943, 1944). However, Mather's interpretation of the genetic features of heterochromatin differs from that proposed by Darlington. Mather points out that while Mendel selected 'tallness' as a genetic feature in his experiments on Pisum Sativum, he was not concerned with the actual degree of tallness. Tallness in the pea may vary from six to seven feet. Mather's experiments demonstrate clearly that such 'discontinuous variation' is controlled by heterochromatin. Continuous variation, viz. tallness as opposed to dwarfness, is a function of euchromatic loci. The latter Mather has termed <u>oligogenes</u>, the former, <u>polygenes</u>. As corollaries, Mather maintains that the genes in heterochromatin need not differ qualitatively from those found in euchromatin. But whereas the genes in euchromatin are markedly heterogeneous in character, those in heterochromatin are all identical. In other words, heterochromatin contains polygenes, formed by the reduplication of a single type of gene. On the other hand, genes which exist in the genotype in the single form are oligogenes. While the loss

of oligogenes would be certain to be associated with phenotypic modifications, that of polygenes would not result in any qualitative changes in the phenotype. Such genes exist in the genotype in the form of identical replicas and loss on mutation would only show quantitative changes, and these have hitherto not been easily detectable in the phenotype.

This concept of the oligogenic action of euchromatin as opposed to the polygenic character of heterochromatin may now be applied tothe genetical localization of the sex chromosomes in Elephantulus. The hypothesis, presented below, is based on the assumption that the genetical properties of heterochromatin are identical in all types of living matter. In other words, the postulate claims that heterochromatin detected cytologically in a mammal, may be assigned the same genetical properties as has been demonstrated for the heterochromatin of a plant or Drosophila.

The peculiar allocyclic behaviour of the X chromosome in Elephantulus demonstrates that it is constituted largely of heterochromatin. This is predominantly localized in segments b_1, b_3 and c. Segment a appears to be completely euchromatic or nearly so, while it seems possible to assign to segment b_2 both eu- and heterochromatic properties.

The differantial segment of the X chromosome in Elephantulus is therefore comprised mainly of polygenes with some oligogenic loci in segment b_2. All these loci are completely linked to the female sex. There are, therefore, female sex polygenes, and female sex-linked oligogenes in the differential segment of the X chromosome.

The pairing segments of both the X and Y chromosomes contain oligogenes which are only incompletely sex-linked as they are liable to interchange by crossing over. If any polygenes exist in segment a they would be male sex polygenes as they have homologous loci on the Y chromosome.

These conclusions on the genetical localisation of the sex chromosomes in Elephantulus are essentially similar to those of Pontecorvo (1943). However, in the Striped Hamster (Cricetulus griseus) Pontecorvo finds no female sex-linked oligogenes in the differential segment of the X chromosome and a greater number of male sex polygenes in the pairing segments of the X and Y chromosomes. Since the pairing segments show allocycly in the meiotic prophase (precocious condensation) the latter conclusion is justified.

It is to be remembered that none of these interpretations have been proved; they are only inductions and

should be considered in this light. However, they demonstrate the results that can be achieved using the cytological procedure only. Moreover, they indicate the need for more detailed studies of the sex chromosomes. These are, at present, rare in the literature, and they will be essential not only for the understanding of the evolution of the sex chromosomes, but also for the chromosomal mechanisms of sex mediation.

CHAPTER VII.

SUMMARY AND GENERAL CONCLUSIONS.

The cytogenetical study of the chromosomes in Elephantulus was undertaken for two main reasons. In the first place, in view of the intensive investigations conducted on this animal by workers in the Departments of Zoology and Anatomy, the elucidation of the structure and behaviour of the chromosomes in the germ cells of Elephantulus was essential for a more intimate understanding of the biology of this insectivore. Secondly, the peculiar behaviour of the sex chromosomes in Elephantulus necessitated an analysis of the micromorphological attributes of chromosomes in general.

The diploid chromosome number of Elephantulus myurus jamesoni is fourteen. The enumeration of the chromosome complement was greatly facilitated by the use of squash preparations which provided flattened chromosome plates. Very few metaphase plates were found, and therefore most of the determinations were made from mid-anaphase stages, in which the tetraploid number of twenty-eight chromatids was encountered. Meiotic stages of both male and female germ cells contained the haploid number of seven chromosomes. Since the number of chromosomes in Elephantulus

is small, it was possible to characterize each chromosome individually. In the male, the chromosome complement could be separated into six homomorphic pairs and one heteromorphic pair. The lengths of the homomorphic pairs range from 7.5 to 3μ. Three of the pairs have median centric constrictions, two have subterminal constrictions, while the remaining pair contains a submedian centric constriction. The heteromorphic pair is composed of a large chromosome of 8.5μ, and a smaller element with a length of 2.5μ. It was shown by comparison with female nuclei that the former is the X chromosome. The smaller chromosome with a subterminal centric constriction in male nuclei, is therefore the Y chromosome. Both of the constrictions in the X chromosome are secondary constrictions. The constant position of the latter in the chromosome permitted the regionalization of the X chromosome into three segments: segment a (2.5μ), segment b (4μ) and segment c (2μ). The spindle attachment of the X chromosomes is situated terminally in segment a.

As far as Elephantulus is concerned, its chromosome number does not help to resolve the problem of its taxonomical position. Until a few years ago, it was thought that low chromosome numbers were characteristic

only of the Marsupials; now, however, two exceptions to this rule have been found. The one is the Striped Hamster (Cricetulus griseus), a Rodent with a diploid number of fourteen chromosomes, the other is Elephantulus in which the diploid number of chromosomes is also fourteen. In fact, from this evidence, and from the anomalous case provided by the squirrels, Sciurus carolinensis carolinensis (2n=48) and Sciurus carolinensis leucotus (2n=28), it may be concluded that, at present, it is not possible to formulate a cytogenetical theory of taxonomical relationships within the mammals. Most certainly, such a generalization will have to await further determinations of mammalian chromosome numbers.

At mitosis, in male germ cells, the X chromosome remains fully charged and spiralized in the interphase nucleus; all the remaining chromosomes disappear at telophase. At each secondary constriction of the X chromosome a plasmosome makes its appearance, and thereafter, the X chromosome undergoes a series of peculiar reactions. First, segment a disappears, and then the mid-region of segment b. This latter reaction permitted a further regionalization of the X chromosome, in that segment b could be subdivided into

three separate regions: segment b_1 adjoining secondary constriction I; segment b_2, which undergoes the interphase spiralization; and segment b_3, bordering on secondary constriction II. Both segments a and b_2, when disappearing become thinner but maintain a constant length; at present, this type of unspiralization remains unexplained. The remaining segments of the X chromosome, i.e. segments b_1,b_3 and c persist in the interphase nucleus with their accompanying plasmosomes until the next prophase. They may, however, undergo fusion in the interphase nucleus to form a single large body, which has been called the X karyosome. During prophase, these segments undergo an incomplete telophase type of unspiralization, but before they disappear altogether, they recharge and respiral with the reappearing segments a and b_2. In this way, a fully reconstituted X chromosome is seen at prometaphase. The mitotic prophase is further characterized by the appearance of 'relic coils' in the chromosome threads.

It was not possible to differentiate between the interphase nuclei of premitotic and premeiotic spermatogonia. Both show the same interphase behaviour of the X chromosome. The onset of the meiotic prophase, however, is different from the

prophase of mitosis. In meiosis, first a number of small karyosomes appear in the nucleus. Later these karyosomes become assembled into the leptotene threads. The stages of zygotene and pachytene were similar to those occurring in other mammals, but diplotene showed several interesting features. This was the earliest stage of meiosis when the sex bivalent could be recognised without any difficulty. In such stages, the Y chromosome was found paired with segment a of the X chromosome. The remainder of the X chromosome i.e. segments b and c constitutes the differential segment. Chiasma-formation between the pairing segment of the X chromosome and the Y chromosome is variable.

The sex bivalent has a typical structure at the metaphase of the first meiotic division. Generally, this takes the following form: A thick condensed region which represents the X pairing segment and the Y chromosome, and a thin tail-like portion which is composed of the two relationally-coiled chromatids of the X differential segment. In exceptional instances, the differential segment of the X chromosome is as thick as the pairing segment. However, in most cases, the differential segment of the X chromosome is undercharged and underspiralized.

Two types of first meiotic anaphase configurations have been found. The first shows both chromatids of the X chromosome moving to one pole of the spindle and both chromatids of the Y chromosome moving to the other. This pre-reductional type of anaphase could be correlated with the diplotene figures, in which a chiasma failed to form in the sex bivalent. The second type of anaphase configuration is post-reductional, each daughter nucleus receiving one chromatid of the X chromosome together with one chromatid of the Y chromosome. Such anaphases are the result of those diplotenes where an interstitial chiasma formed between the Y chromosome and the pairing segment of the X chromosome.

The material available for the investigation of the chromosome cycle in female germ cells was unfortunately incomplete, but a fairly detailed study of the first meiotic division was made. The features of meiosis in the female are similar to those in the male, in that the same sequence of events is followed. The equal X/X bivalent, however, shows no differential behaviour at the metaphase. The differential segments of the X chromosomes in the female are as fully charged and spiralized as the pairing segments.

Two significant features of the X chromosomes in Elephantulus warranted detailed and individual discussion. These were: 1. The departure of the X chromosome from the standard behaviour of the rest of the complement, during the interphase of mitosis and at the first meiotic metaphase.

2. The precise relation of the secondary constrictions of the X chromosome to the formation of the plasmosomes.

It was decided that the strange behaviour of the X chromosome could be classed together with the group of phenomena termed allocycly by Darlington. Recent investigations have made it clear that allocyclic and standard behaviour are the cytological expressions of reactions occurring within a micromorphological structure of the chromosome. It therefore became necessary to discuss the micromorphology of chromosome structure and behaviour.

As far as is known at present, the chromosome is made up of two major components, the protein thread, and desoxyribo-nucleic acid. The regular variability in the staining reaction of a chromosome may be successfully interpreted in terms of its attached

desoxyribo-nucleic acid i.e. its nucleic acid charge. The Feulgen reaction which is specific for desoxyribo-nucleic acid may be used as a precise indicator of such modifications. According to Stedman and Stedman, however, desoxyribo-nucleic acid plays a role of no importance in chromosome behaviour; all the reactions are centred about the newly discovered protein 'chromosomin'. Sufficient evidence was presented to show that the objections of Stedman and Stedman are irrelevant, and that the concept of nucleic acid charge is fully substantiated not only by the results of the application of the Feulgen reaction but also by the work of Caspersson, Nörberg and Mazia.

It is generally believed that the changes in the dimensions of the chromosomes during the nuclear cycle are consequences of spiral transformations of the protein chromonema. Evidence in support of spiralization and unspiralization was accumulated from such diverse sources as the relic coils of mitotic prophase chromosomes, relational coiling and chiasma-formation at meiosis and the consequences of anaphase bridges. The relation of these two phenomena to the postulate of chromonema permanence was then examined. It was shown that the notion of a constant chromonema, as deduced by Darlington from the experiments of Kuwada and

Nakamura, was totally unnecessary in the formulation of the theories of spiralization and unspiralization. A note of caution was sounded in accepting such a postulate, especially when it leads to the erection of a system of chromonema mechanics with unsubstantiated premises. Furthermore, experimental evidence shows that spiral modifications are not the only phenomena which lead to changes in the dimensions of the chromosomes. Active loss and resynthesis of chromonema material, occurring during the metabolic upheavel of nuclear division, as well as longitudinal contractions and expansions of the protein framework comprising the chromonema, are other reactions which require to be taken into account in arriving at an assessment of all the factors regulating the behaviour of the chromosomes.

In the section on allocyclic behaviour, the theories of Darlington and Pontecorvo were discussed at some length. As far as Elephantulus is concerned, Darlington's theory of nucleic acid'starvation' of heterochromatin is not applicable to the allocyclic behaviour of the X chromosome, especially at the mitotic interphase. In the discussion of Pontecorvo's theory it was shown that if euchromatic chromomeres were selected to give rise to heterochromatic blocks by repeated linear

reproduction, then such euchromatic chromomeres are already endowed with the property of allocycly. This point served to emphasize the importance of chromomeric physiologies in allocyclic behaviour.

In the male Elephantulus, the X chromosome presents four different cycles of nucleic acid charge at mitosis and two cycles at meiosis. These are: at mitosis, the cycles of 1. the complete X chromosome;

2. segment a;
3. segment b_2;
4. segments b_1, b_3, and c;

at meiosis, the cycles of

1. the pairing segment (segment a);
2. the differential segment (segments b and c).

Since segments b_1, b_3 and c showed a constant allocyclic behaviour at mitosis and meiosis, their structure is undoubtedly heterochromatic. Difficulty was experienced in assigning a definite structure to segments a and b_2. An explanation for the behaviour of these segments was attempted in terms of the cytochemical 'position effect', but this proved a failure. It was then concluded, in agreement with previous authors such as White, that euchromatin is not to be regarded as the complete antithesis of heterochromatin. Instead, heterochromatin and euchromatin

are merely the end extremes of a series of chromosomal micromorphologies. They form, as it were, the red and blue of the chromosome 'spectrum'. In this light, it became clear that segments a and b_2 were intermediate forms between eu- and hetero-chromatin, and, as such, assume new importance for the understanding of the evolution of heterochromatin. It was further decided that while segment a exhibited features characteristic of the euchromatic extreme, segment b_2 verged in the direction of heterochromatin.

No ready explanation was available for the absence of allocyclic behaviour in the X/X bivalent of the female. Two possible interpretations were offered. In the first place, the absence of allocyclic behaviour in the female might be related to the fact that the X chromosome is paired completely with an equal homologue. Or, secondly, the allocyclic behaviour of the X chromosome in the male might be related to a physiological effect imparted by the Y chromosome.

The observations on the chromosome cycle in the male established that each of the secondary constrictions of the single X chromosome was related to a plasmosome. In view of the limited material available, the studies on female germ cells remained inconclusive in elucidating

the chromosomal-plasmosomal relations in the female. An ad hoc study of interphase liver nuclei showed that, contrary to theoretical expectations, the female nuclei contained only two plasmosomes. To explain this phenomenon, autosomal genotypic control of plasmosome production was postulated. This was discussed in relation to the directions of sex chromosome evolution in the mammals.

From an analysis of the structure and behaviour of the sex chromosomes in Elephantulus the following genetical interpretations were made: (a) loci, completely linked to the male sex are located in the inferred differential segment of the Y chromosome; (b) incompletely sex-linked loci are found in the pairing segments of both the X and the Y chromosomes; (c) loci, completely linked to the female sex are situated in the differential segment of the X chromosome.

These conclusions were extended by deductions from the distribution of eu- and heterochromatin in the sex chromosomes. According to Mather, euchromatin contains oligogenes which are related to qualitative phenotypic expressions, while heterochromatin contains polygenes

which exert quantitative effects in the phenotype. The final conclusions on the genetical properties of the sex chromosomes in Elephantulus were as follows:

(a) the pairing segments of both sex chromosomes are composed almost exclusively of incompletely sex-linked oligogenes;

(b) the differential segment of the X chromosome contains female sex polygenes, with a few completely sex-linked oligogenes in segment b_2.

From a general review of the study as a whole, the conclusion becomes inevitable that the future advance of biology depends, in part, on the bridging of the gulf between biochemistry and morphology. Joseph Needham, in his book "Biochemistry and Morphogenesis has already demonstrated the nature of the synthesis which can be expected when the biochemical events of morphogenesis are correlated with the major morphological achievements of embryonic development. Another common meeting place for biochemistry and morphology unquestionably resides in the submicroscopical realm of the chromosome. The co-ordinated efforts of morphologists and biochemists in attacking the micro-morphological level of organization will lead to those perspectives essential for the synthetic outlook in genetics, in particular, and biology, in general.

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