DYNAMICS of CHROMOSOME MOVEMENT

By

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I. INTRODUCTION.

The cause of chromosome movements and the nature of the forces operating during cell division were one of the many interesting problems which engaged the attention of early cytologists. The resemblance of the processes of cell division to physical, physico-chemical and electrical phenomena led them into abstract speculation which took the form of vague theories that replaced each other in rapid succession. Their failure must be attributed chiefly to the fact that their data were scanty and their cytological observations mainly descriptive in nature.

The last decade, however, has seen the development of the new science of cyto-genetics in which genetical phenomena are dealt with from the cytological point of view. The carriers of heritable characters, the genes, have naturally attracted general interest during the last few years of development of genetics and data concerning chromosome behaviour during mitosis and meiosis are being rapidly accumulated. Important observations have thrown new

light/

light on the behaviour of chromosomes and it has become obvious that the chromosomes exhibit during their life cycle different movements and structural changes which are effective in their coordination and normally result in the formation of two cells that are quantitatively and qualitatively equivalent.

The time has arrived to analyse the movements of the chromosomes and to interpret them in terms of dynamic factors, in order to gain a better insight into the whole mechanism of karyokinesis and a better understanding of the relation which exists between mitosis and meiosis.

The only way of approaching such a problem is by inductive analysis. The analysis of chromosome movements and structural changes will enable us to infer the nature of the possible forces which operate in the mechanism of cell division and which, with the accomplishment of chromosome segregation, maintain the equilibrium of the entire system.

Since movements are coordinated changes in time and/

and space, they are detectable as modifications partly in the structure of chromosomes as a single unit of the complement, and partly in the relative position of chromosomes as members of the complex. Therefore these movements may be classified as (i) intra- and (ii) interchromosomal changes. This method of division will be adopted in the present paper.

The present analysis of chromosome movements in terms of dynamic factors is an outcome of cytogenetical investigations on several plants and animals in which the author has been engaged during the last few years, and although primarily a resumé, it will serve as an introduction to the study of chromosome behaviour in 'tissue culture' an investigation which it is the author's intention to carry out in the future.

II. MATERIAL and TECHNIQUE.

The following were the materials used : -

PLANTS.

Dicotyledons:

Vicia faba
Phaseolus vulgaris
Pisum sativum
Trifolium alexandrinum
Crepis rubra
Crepis aurea
Crepis incana
Delphinium consolida
Linum grandiflorum
Matthiola incana

Monocotyledons :

Festuca pratensis
Holcus mollis
Arrhenatherium avenaceum
Triticum durum
Triticum vulgare
Triticum alexandrinum
Allium cepa
Hyacinthus orientalis
Scilla sibirica
Tulipa potteriana
Fritillaria imperialis
Fritillaria Rhutenica
Tradescantia virginiana
Tradescantia virginiana var. *humilis*
Rhoeo discolor

ANIMALS/

ANIMALS.

Invertebrates:

Schistocerca gregaria
Locusta migratoria
Locusta migratoria var. danica
Stenobothrus parallelus
Chorthippus elegans
Metrioptera tessellata
Oedaleus decorus
Euchorthippus
Drosophila pseudo-obscura
Drosophila pseudo-obscura, race B.
Drosophila melanogaster

Vertebrates :

Salamandra maculosa
Batrachoseps attenuatus
Anas boschas
Cairia moschata
Gallus domesticus
Mus norvegicus
Dasyurus maculatus
Phascolarctus cinereus
Sarcophilus ursinus

Technique/

Technique. Perfect fixation of the chromosomes is a prerequisite for their correct description. The internal structure and the external appearance of the chromosomes in many cases were affected differently by the same fixatives, causing distortion in one or the other. Therefore several fixatives were used for the same material in order to discern every possible detail of structure under the best conditions

Smears and embedded material were used following the cytological technique as employed at the John Innes Horticultural Institution, London, and described in detail by La Cour (1931).

The following fixatives were used :

(1) Medium and strong Flemming to which usually 1% urea was added. Used for the study of meiotic divisions in animal material.

(2) 2B, 2BE and 2BD fixatives, the formulae given by La Cour.(1931). Used mostly for mitotic stages.

(3) Benda with Carnoy, for the study of the spiral structure in chromosomes.

(4) Navashin's fluid after Carnoy, for the study of ring/

ring formation in diakinesis.

(5) Hermann's fluid with chromic acid for chromosomes of Diptera.

(6) Allan's modified Bouin for avian chromosomes.

(7) Uranium tetroxide (2%) with chromic acid (10%) for spindle.

The clearing reagents were clove oil, cedarwood oil or cineole. The following stains were used : to a great extent gentian violet, iron haematoxilin, Giemsa, new magenta, new brazilin, the sections in every case being well differentiated and de-stained to render the chromosomes as transparent as possible.

The sections were cut at different thicknesses, plant material from 12-30 μ animal material from 8-20 μ.

All drawings except the diagrams were made with an H.I.90, 1.30 apochromate objective, a x18 and x30 compensating eye-piece and a Zeiss camera lucida; the initial magnification was 3500 and 4500 respectively. The microphotographs were taken with a cinema camera and by a Zeiss microphotographic apparatus/

apparatus. The method of chromosome measurement was that of Lewitsky (1931).

The terminology used in this paper is the same as that found in the literature dealing with cytogenetical investigations, invented and introduced by British geneticists.

III. CHANGES in STRUCTURE of CHROMOSOMES and their DYNAMIC INTERPRETATION.

A. MITOSIS.

(i) Morphological description.

(i) The cell and cell division. The structure of the cell is a result of its coordinated dynamic activity as a fluid colloidal system, with phases and interphases. This system is not an isolated one, for its destiny and behaviour are closely dependent on the surrounding medium. The function of the cell is an outcome of the entropy of the colloidal system which represents the structure of the cell. Thus there is the closest relationship between structure and function.

The cell is a centre of a multitude of reactions mostly chemical in nature and highly organised. They must be in a dynamic equilibrium as a whole and must return to it after disturbance if death of the cell is to be avoided. Any molecule which meets an adjusted enzyme within the cell will undergo changes and/

and will be directed into some one of the processes of metabolism and growth. Cell metabolism, however, is limited within a definite space, an increase or decrease of which leads to disturbance of equilibrium. In the case of increase, the dynamic continuity of cell activities will be restored by division, which is the readjustment of the colloidal system in size to maintain equilibrium.

The division is normally a complete distribution of the cell constituents into two derivatives. The most important of these constituents is the nucleus which, apart from the role which it plays in cell metabolism, is at the same time the carrier of the genom i.e. the gene complex on which the phaenotypic individuality of the organism primarily depends. The genes are localised in the chromatin material of the nucleus and transmitted into the next cell generation by the chromosome mechanism.

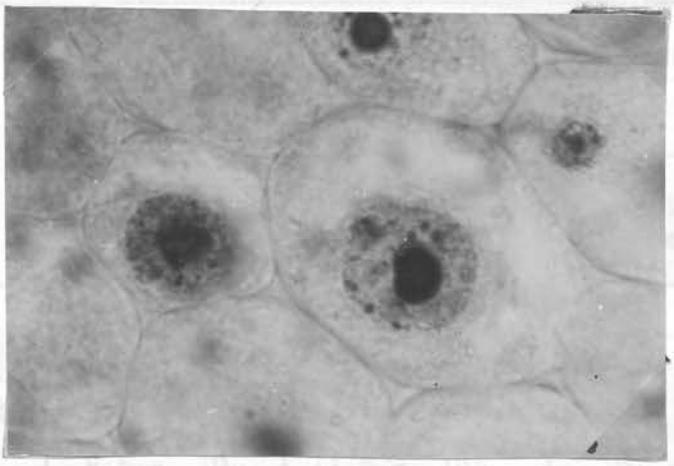
This brief introduction will aid us to visualize the pre-existing conditions of cell activity before division (Gray 1931).

(ii)/

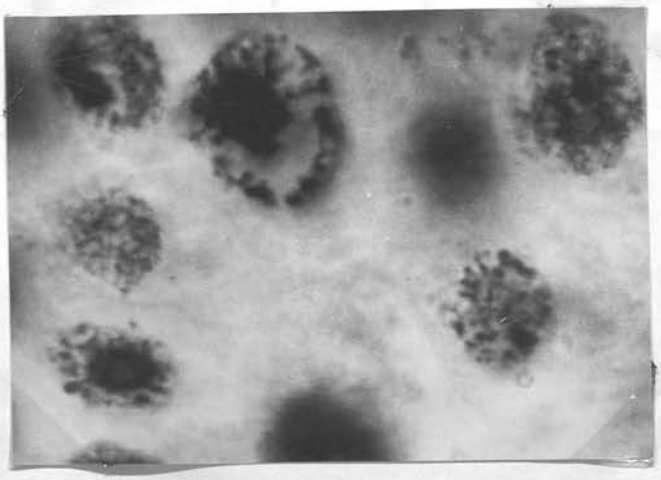
(ii) Structural changes during prophase. Mitosis is the process of cell division by which daughter chromosomes are separated into two groups and form two daughter nuclei containing the same genom complexes. The very early prophase of mitotic division is characterised by some minute changes in the nucleus. The granulation of the nucleus becomes more apparent, its chromacity is greatly increased as compared with the previous resting stage. In very good material (Vicia faba, Allium cepa, Tulipa potteriana) the first signs of the chromosomes can be recognised at this stage. They are closely associated and compressed into a mass within the nucleus, in which not only the chromosomes but one or more nucleoli as well are included (fig. 1).

Another phenomenon of mitotic prophase is the increase of nucleus in size. Measurements were taken (table I) which show the degree of relative increase that takes place in the nucleus at the beginning of division. This increase in volume is caused by the absorption of water from the surrounding cytoplasm. This can take place through the nuclear membrane which is semi-permeable. Analysis of the actual increase shows that it occurs in the inter-chromosomal substance/

Fig. 1.



Cells from the root-tips of Vicia . The nuclei are in the resting stage.



Beginning of mitosis. The spirals in the nucleus can be seen. The spreading towards the nuclear membrane is a result of fixation.

Table I.

Increase in the size of nucleus at the beginning of mitotic prophase.*)

Species	Size of Nucleus			
	Resting stage		First stage of Prophase	
	Shortest diameter	Longest diameter	Shortest diameter	Longest diameter
Vicia faba	10μ - 13μ	13μ - 15μ	12μ - 15μ	20μ - 21μ
Tulipa potteriana	9μ - 12μ	13μ - 16.5μ	12μ - 14.5μ	16μ - 22μ
Allium cepa	8.5μ - 10μ	11.5μ - 12μ	10μ - 12μ	13μ - 14μ
Festuca pratensis	4μ - 4μ	6μ - 7μ	5μ - 6μ	8μ - 8.5μ
Holcus mollis	5μ - 7μ	6μ - 8μ	6μ - 7.5μ	7μ - 9μ
Arrhenatherium avenaceum	7μ - 8μ	11μ - 12μ	12μ - 13μ	14μ - 16μ
Tradescantia virginiana	5μ - 6.5μ	10μ - 11μ	7μ - 8μ	13μ - 14μ

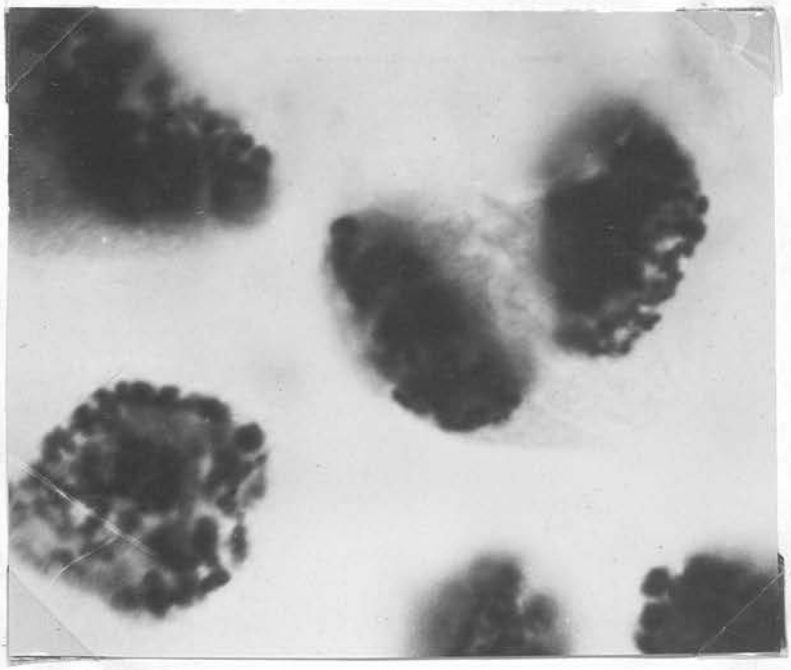
(* The number of nuclei measured was 100 in each species, the size of cell measured was approximately constant.

substance. The result is a separation of chromosome threads which become more distinct (Belar 1929a, b). The increase in the volume of the nucleus gradually proceeds, and sometimes just before the disappearance of the nuclear membrane a large space is provided for the chromosomes.

The chromosomes are fine convoluted zigzag and irregularly coiled threads (fig. 2) which soon become unravelled and uncoiled. This chromosome structure in early prophase was first observed by Grégoire (1905) and later found by several other workers, whose names together with the list of species was recently given by N. Shinke (1930) and Darlington (1932). The chromosome threads themselves are not even; they contain chromomeres, small aggregates of chromatin bodies with thin connective fibres between them. This is the cause of a more or less beaded appearance of the chromosomes during early prophase. Although the chromomeres may be regarded as 'artefacts' (Darlington 1932a), they are invariably present in the chromosomes (Wenrich 1916):,they must therefore represent a qualitative linear differentiation of chromosomes, and as such are characteristic.

The total length of early prophase chromosomes in/

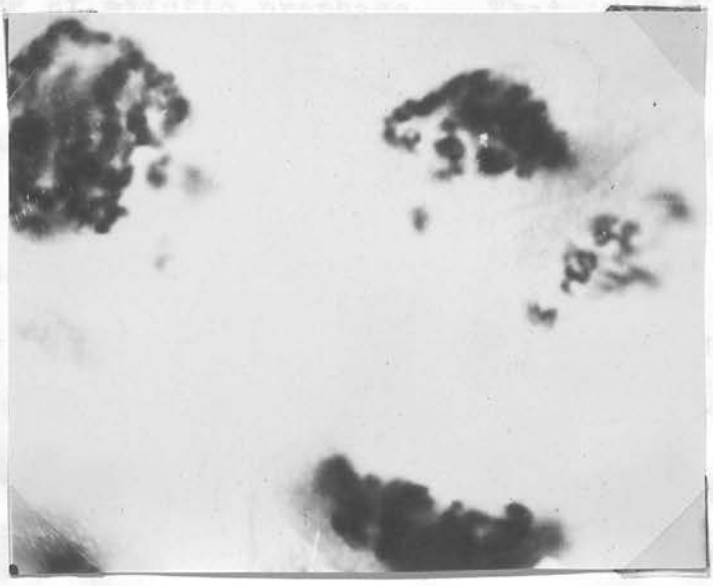
Fig. 2.



A

Chromosome-spirals at early prophase of mitosis in Tulipa potteriana. The diameter of the spirals indicates the presence of two chromatids in close association.

Fig. 2.



B

Chromosome-spirals in Tulipa potteriana showing clearly the association of sister chromatids.

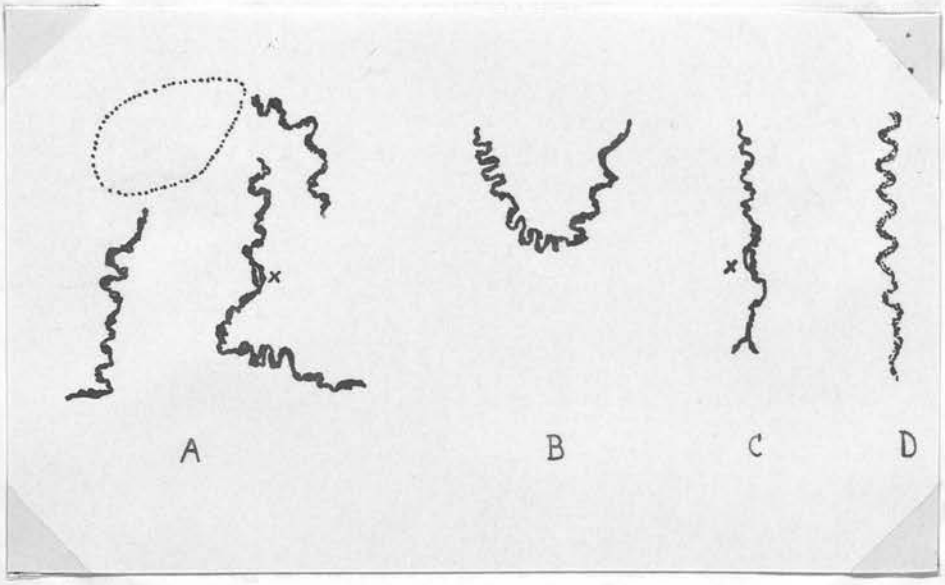
in the 'spiral' form can not be estimated. The threads of a single chromosome can be seen clearly only in small segments. The approximate number of coils per micron in Vicia faba is about 4-6 at the beginning of mitotic prophase. That in Tulipa potteriana is 6 when the spiral structure can be first seen, while in Euchorthippus the number was found to be 3. Fig. 3 illustrates the spiral chromosomes of those species.

The changes in structure are accompanied by gradual changes in length. The increase during the first half of the prophase has been estimated for Vicia faba chromosomes and is given in table II. The data show that a process of unravelling takes place but no actual increase in the chromosomes could be found.

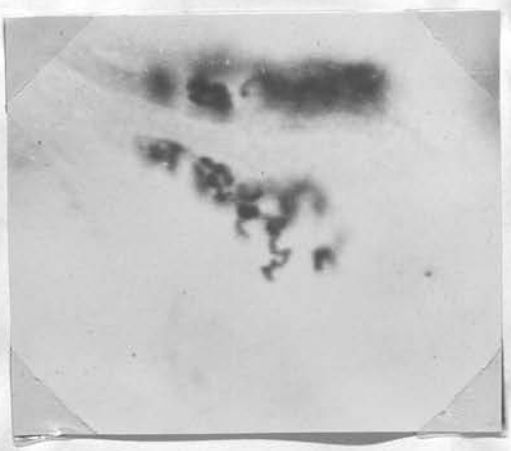
The unravelling of the spiral structure into a more or less straight thread is associated with the appearance of doubling in the single chromosomes (fig. 4). The two sister chromatids, derived from the same chromosome, can be seen first at the ends of the spirals, but sometimes the opening out of the 'secondary split' occurs interstitially. The chromatids are thin fine threads and their chromomeres can be seen much better than during the previous stage.

The/

Fig. 3.



Chromosome-spirals at early prophase; A: Vicia, B: Tulipa, C: Allium, D: Euchorthippus. The opening out of sister chromatids is indicated with an x. The coils of the spiral are uneven in A, B? C, but approximately even in D.



Tulipa potteriana. The spiral chromosome is separated into two sister chromatids.

Table II.

The length of chromosomes in Vicia faba *) during mitotic prophase.

Stage of prophase	M-chromosome		m-chromosome	
	Length of spiral	Absolute length (approx.)	Length of spiral	Absolute length (approx.)
1. Beginning	8μ - 10μ	20μ - 24μ	8μ	16μ - 17μ
2. Early prophase	12μ	20μ - 22μ	10μ	16μ - 16.5μ
3. Mid-prophase	13μ - 14μ	19μ - 23μ	10μ - 12μ	15μ - 16.5μ

*) Vicia faba has two long M-chromosomes with median and 10 shorter m-chromosomes with subterminal attachment constrictions. 30-50 single chromosomes were measured in each class.

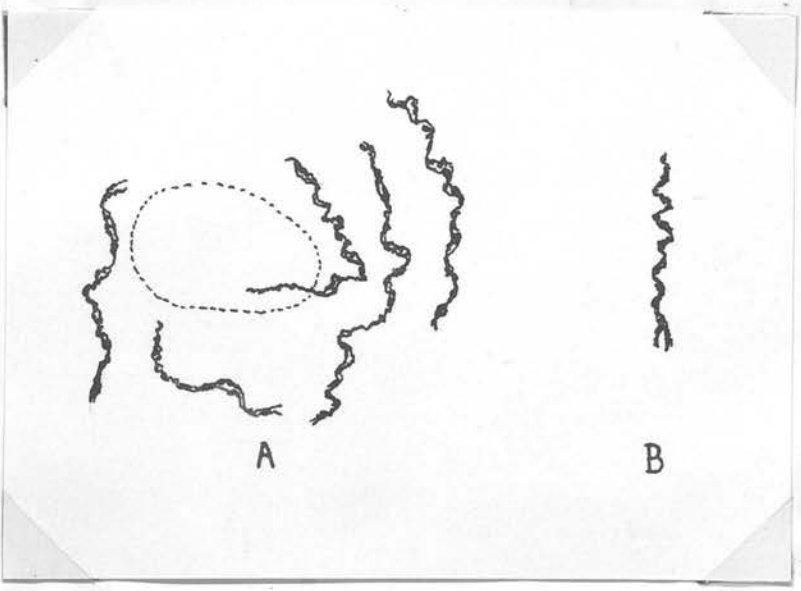
Table III.

Decrease in length of chromosomes in Vicia faba during prophase. *)

Stage of division	M-chromosomes	m-chromosomes
Mid-prophase	23μ	16.5μ
Late prophase 1	19μ - 21μ	14μ - 16μ
Late prophase 2	17μ - 19μ	10.5μ - 13μ

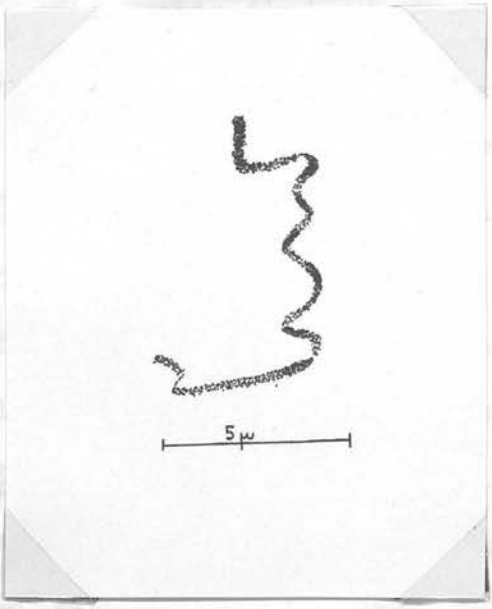
*) Estimated length of unravelled chromosomes.

Fig. 4.



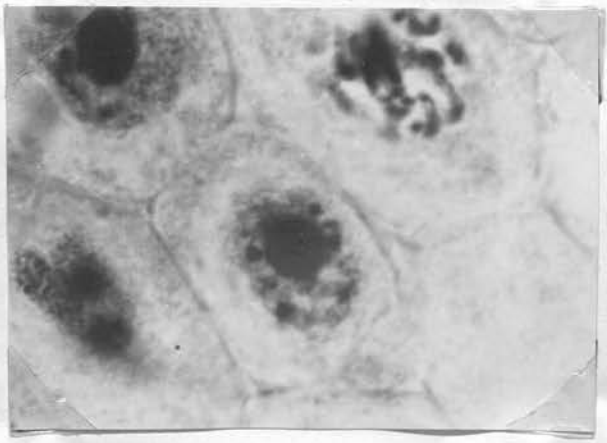
Unravelling of chromosome-spirals at early prophase of mitosis. A: Vicia, B: Tulipa. The sister chromatids open out at the end of the spiral in B, and at several loci in A.

Fig. 4.



C

Unravelling of spiral in Euchorthippus. The form of spiral indicates that unravelling is a process which is accomplished by rotation of the chromosome.



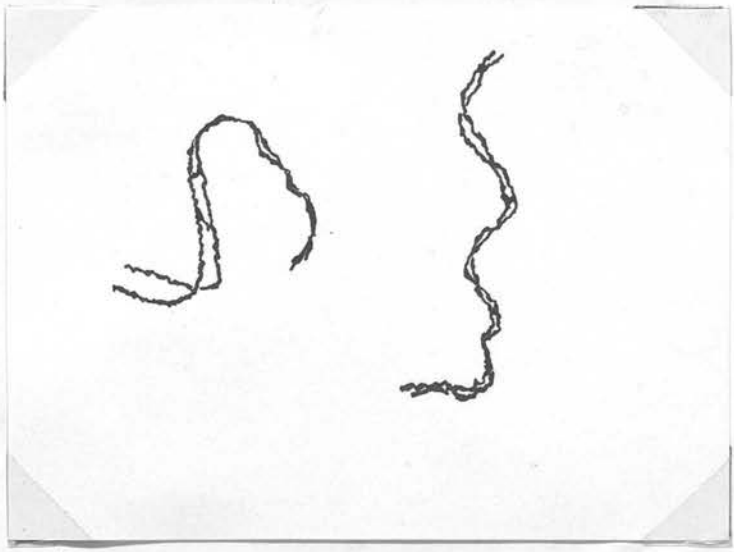
D. Microphotograph from Vicia showing the double spiral during unravelling.

The diameter of the apparently single spiral at early prophase is about twice that of one single chromatid. The unravelling of the spiral may result in arrangements where the sister chromatids lie parallel (Euchorthippus) (fig.5). When the unravelling is complete at mid-prophase, a relatively rapid decrease in length of sister chromatids can be found to take place; table III illustrates the decrease of chromatids in Vicia faba. The decrease in length is always accompanied by an increase in diameter (see Robertson 1915).

At the end of the prophase the sister chromatids are thick and lie either side by side in close association, or coiled one around the other with a few twists, which is very common if the chromosomes are long(fig. 6).

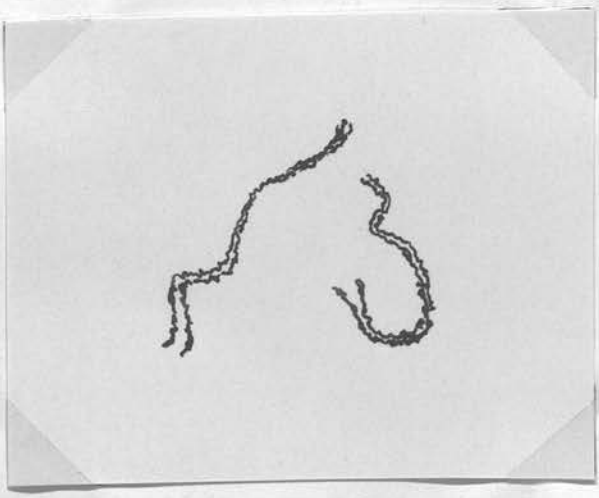
(iii) Metaphase, anaphase and telophase. Another characteristic change is the disappearance of the nuclear membrane and the formation of the spindle at the end of prophase. The chromosomes lie in the cytoplasm so as to be able to move freely towards the equatorial plate where they form an orderly arrangement as a result of dynamic equilibrium. The decrease of length of the chromosomes proceeds further during/

Fig. 5.



A

The unravelling of chromosome spiral completed. A: Vicia, the sister chromatids are coiled one around the other in several places; B: Euchorthippus, the sister chromatids lie parallel without twists.

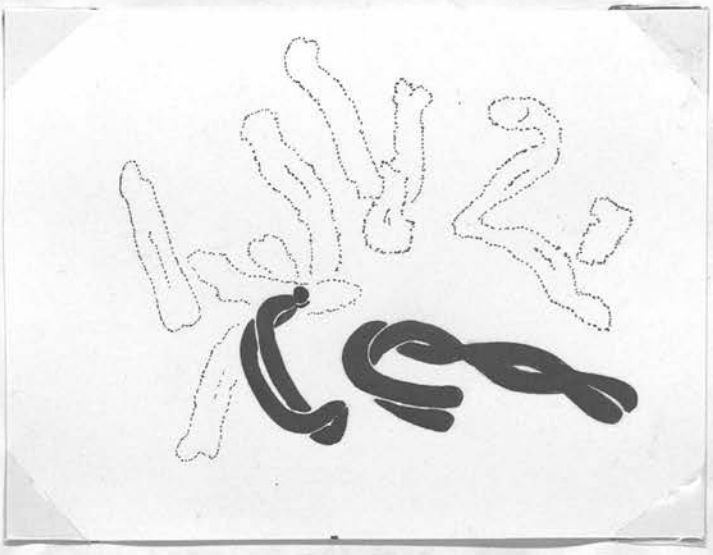


B

Fig. 6.

A

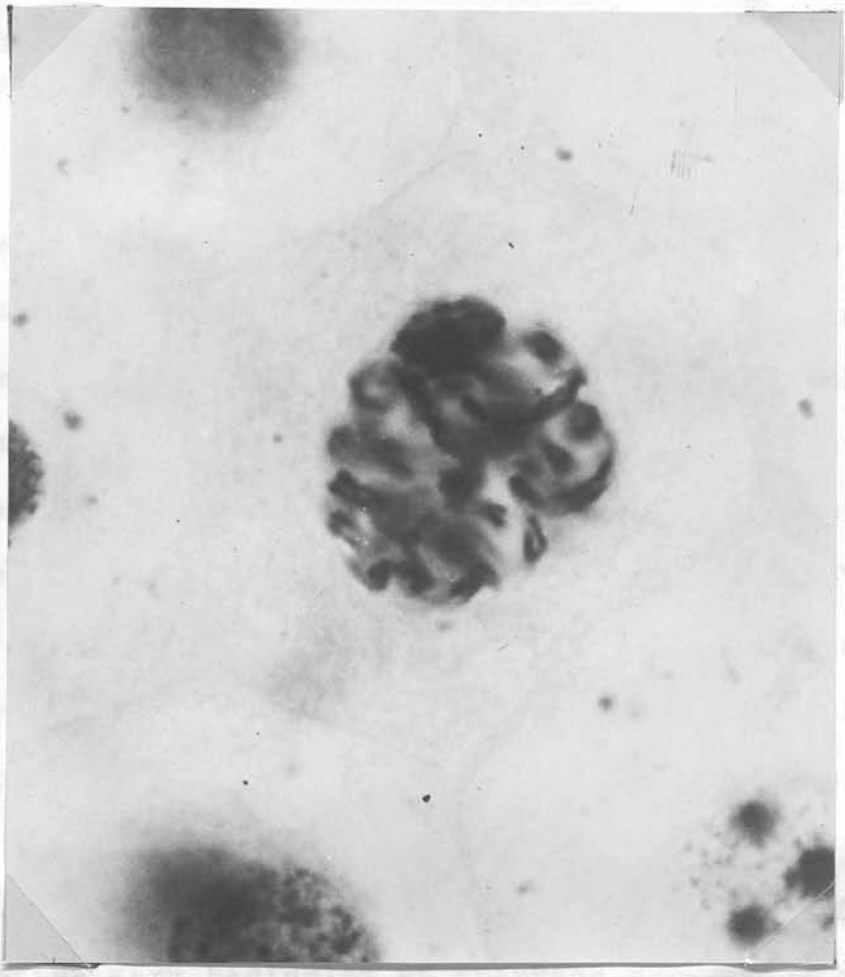
Late prophase of mitosis in Vicia. The building up of the matrix is already advanced, the sister chromatids are thicker.



B

End of prophase of mitosis in Vicia. The sister chromatids are held together at the attachment constriction.

Fig. 6.



C

Microphotograph from root-tip of Tulipa. End of prophase of mitosis. The sister chromatids twist one around the other.

during metaphase and the following anaphase but the decrease is slight as compared with that found in the previous prophase. The data in table IV illustrate the further decrease of chromosome length in Vicia faba at metaphase and the following stages.

The disappearance of the nuclear membrane is accompanied by the development of the spindle. The inclusion of chromosomes into this mechanism is a necessary condition of normal segregation, but the arrangement of chromosomes on the equatorial plate in relation to the spindle and the segregation of daughter chromosomes will be described in part IV.

At the end of metaphase the sister chromatids begin to separate, giving rise to two daughter chromosomes. This separation of daughter chromosomes always commences at the attachment or primary constriction of the chromosomes (Mather 1932). Fig 7 illustrates the very beginning of anaphase separation in Vicia, Tulipa, Salamander and mouse. The point of the attachment constriction is located differently in different chromosomes. The separation of the daughter chromosomes proceeds towards the ends if the attachment constriction is a median one, and towards the/

Table IV.

Length of chromosomes in Vicia faba.

Stages of division	M-chromosome	m-chromosome	Lewitsky's 1) data	Sakamura's 2) data
End of prophase	17μ - 19μ	10.5μ - 13μ		52
Metaphase	16μ	9.5μ - 8μ	15.9μ - 16.0μ 9.8μ - 7.9μ	50
Anaphase	14μ	7.5 (?)μ		44

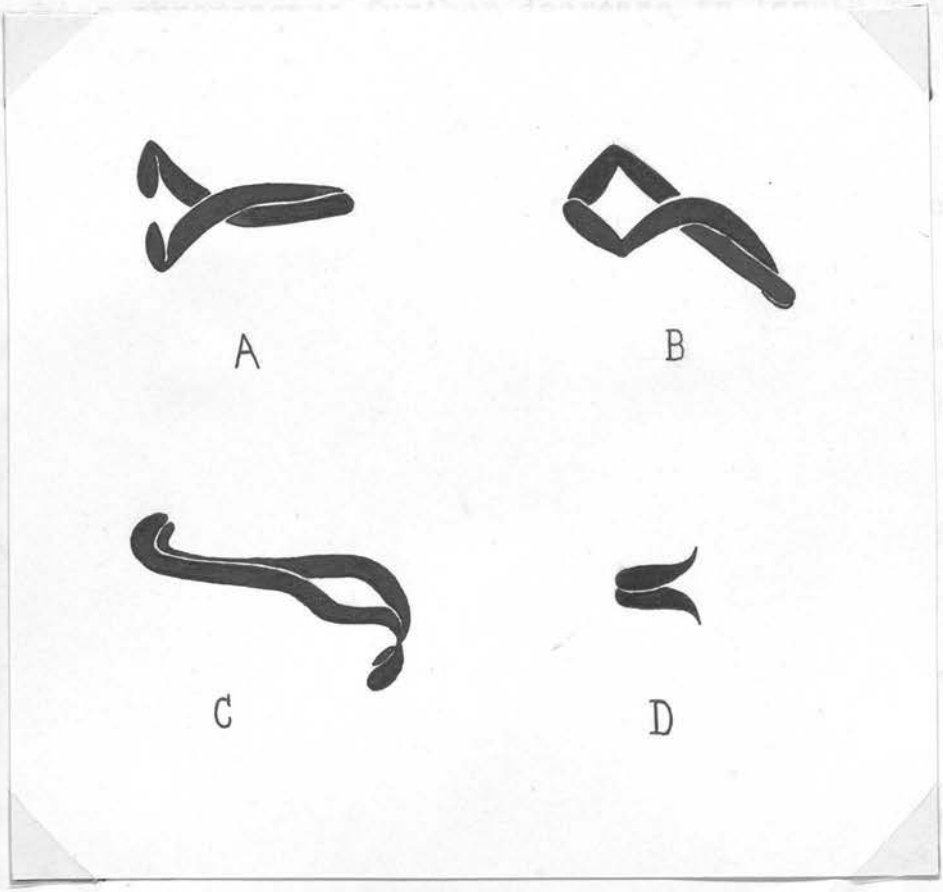
1)

Lewitsky used a different cytological method, hence the slight differences between his and our data.

2)

Sakamura (1915) gave the length of chromosomes, in mms., as measured by projection.

Fig. 7.



The commencement of anaphase. A: Vicia chromosome with subterminal, B: Tulipa, C: Salamander with submedian and D: chromosome in the mouse with terminal attachment constriction.

the distal end if the attachment is terminal or nearly so. During the anaphasic separation the daughter chromosomes further decrease in length but, as was mentioned before, the decrease is slight and not so easily measurable as in prophase.

At the following telophase the daughter chromosomes migrate to the opposite poles. The chromosomes of the same pole come into very close association and form the 'tassement polaire' (Wilson 1925) in which they appear to lose their individuality. Some chromosomes with long limbs will lie outside for a time, and in these limbs the spiral structure can be seen with the aid of a special fixation (fig.8).

The structural changes described above as the general process of mitosis may be classified as follows : -

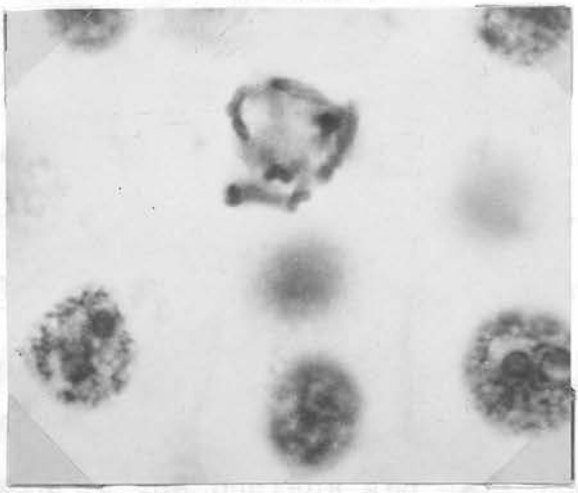
(a) Unravelling of the double spiral. Previously the chromatids were so closely associated that the spiral appeared as a single thread.

(b) Decrease in length with increase in diameter at the second half of prophase, which continues till the end of division.

(c) Separation of daughter chromosomes starting at the attachment constriction.

(2)/

Fig. 8.



A

Telophase of mitosis in Vicia. The limb of the long M-chromosome shows the spiral structure of the chromonema.

B

The structure of telophase chromosomes as revealed by Benda's fluid. The zigzag chromonema can be seen.

(2) Dynamic interpretation.

(i) The splitting of chromosomes. The first observable structural changes always occur in the nucleus at the beginning of mitotic division. It is not improbable that the whole process started with the division of the ultimate genetical units - genes- of the nuclear material, which are the most important constituents of the nucleus and cell (Gates 1932, Crew 1932). The existence of genes as fairly stable units, their transmission through generations of cells and individuals in general without change in their nature, are established facts finally settled by geneticists (Goldschmidt 1928). Correns (1919) assumes that the gene consists of a large molecule with side chains of atoms attached, variation in number giving allelomorphic series. The size of the gene was estimated in Drosophila (Morgan, Sturtevant and Bridges 1925) and found to be larger than the size of a haemoglobin molecule, while Belling claims to have observed the gene as the centre of the chromomeres (1931, 1932).

It/

It is highly probable that these ultimate units as a reaction to the limitation of growth, split into two quantitatively and qualitatively equivalent derivatives. This process must occur in the nucleus when there is as yet no other observable sign of the ensuing division. The stage is the 'resting stage', a term which refers only to the morphological structure of nucleus and cell and not to the physico-chemical process which takes place during the same phase.

The splitting of the genes is followed by the division of the structural units and elements of chromosomes, which are commonly called the chromomeres. Belling(1928 and 1933) has described how the actual splitting of chromomeres takes place in meiotic prophase and he suggests that this is the cause of the secondary split, and consequently of the division of chromosomes into two sister chromatids. Similar observations were made by the present author in Scilla and Dasyurus. These observations show that the division of chromosomes is preceded at mitosis/

mitosis by conditions similar to those that prevail at meiosis except that in the first case the process of division starts at a stage when the chromosomes can not be observed owing to their colloidal constitution being such as to render them invisible.

There are two other possibilities which must be considered when dealing with the time of the actual splitting of the chromosomes. This may happen in the previous division (metaphase, anaphase or telophase) or at later prophase. The latter opinion is held by many cytologists (Grégoire 1910, Gates 1912, Sharp 1920, Kuwada 1921 1926, Martens 1928). The telophasic splitting of chromosomes has recently been defended by Robertson (1931), while the existence of the metaphasic (tertiary) split is supported by Huskins (1933), Hedayetullah (1931) and Nebel (1932), who claim that a tertiary split occurs in the chromatids at metaphase and that this is primarily responsible for the anaphase separations.

The detailed study of the mitotic stages in Vicia, using several different methods and fixatives to/

to reveal the structure in the chromosomes, showed no evidence in support of the theories referred to above. The spiral structure of the chromosomes at telophase testifies against such split. Huskins' assumption is not confirmed by the analysis of the actual anaphasic separation, as will be seen later in Part IV. The actual splitting at later prophase was not observed in Vicia, but there is no indication that this coincides with the opening out of sister chromatids. The comparative study of mitotic prophase in Euchorthippus and Vicia faba shows that the spiral which appears to be single will, by special fixation reveal its double structure, the single chromosome threads being composed of two sister chromatids. Further details in favour of chromosome splitting during the resting stage were recently presented by Darlington (1932b).

(ii) Attraction and contraction. The apparent singleness of the chromosome spiral in spite of the presence of two sister chromatids must be a result of/

of the close association of sister chromatids and consequently of their ultimate structural and genetical units, chromomeres and genes. The corresponding chromomeres of sister chromatids which participate in a very effective association at the beginning of prophase, are similar in their structure in every respect being derived by division from one and the same unit. In this similarity, which is most probably chemical in nature, must be sought the ultimate cause of the association.

Observations made on mitosis and meiosis either in animals or plants, show that the association of two similar chromatids or chromosomes is a general phenomenon in the mechanism of division and is caused by the attraction which operates between any two homologous constituents. This attraction must be considered as a permanent inherent dynamic property of the genes and as a force operating effectively only between two homologous units, which may be chromomeres, chromatids or chromosomes.

At the beginning of prophase the attraction which/

which operates diagonally, associates the sister chromatids and produces an apparently single structure.

The spiral arrangement of the chromosomes at early prophase, however, is the outcome of another force which operates longitudinally along the axis of the chromosomes. The gene string (chromonema), the genetically effective part of the chromatids containing only genes, can not change the relative position of its constituents by yielding to any force which attempts to reduce its actual length. Genetical observations on crossing-over do not permit the assumption of such a change in the gene string. If, however, the actual distance between genes (genetically) and consequently between chromomeres (structurally) can not be decreased, a spiral adjustment of the string inevitably demonstrates the operation of an axial force. This force is contraction. The most effective arrangement of the chromonema under the influence of contraction/

contraction must be a spiral, if the gene string is limited to a definite space (the matrix).

Our observations on Vicia faba show that the actual lengths of coiled chromatids are approximately equal to those of the chromatids expanded and unravelled from the spiral at the early prophase.

It is not improbable that the contraction operates from the attachment constriction. Two observations favour this assumption : (a) the single structure of the attachment constriction at prophase; and (b) the reversed coiling of the twists at the attachment. The first instance can be seen in Vicia faba , the second in Euchorthippus.

Several observations on different plants (Vicia, Tulipa, Hyacinthus, Crepis) and animals (Dasyurus, Salamandra) show that the attachment constriction is undivided during prophase and its division takes place only at the end of metaphase, when the first sign of the presence of two attachment constrictions can be seen. No facts have been brought forward which would suggest that this undivided segment is not/

not the focus of the axially operating force of contraction. The behaviour of the attachment constriction at meiotic prophase strongly supports the view that this is the centre of contraction. If this were not the case, it would be difficult to explain the operation of contraction on two threads which are interstially connected at one point very intimately. The reversed twists of the spiral have been found in many other species and described by Kuwada (1927), Sakamura (1927) and Hsu Shiang (1932). It must be remembered that it is not the direction of the coiling but the plane that is altered, the direction remaining unchanged.

As prophase proceeds the spiral is unravelled. The formerly very intimately associated sister chromatids more or less separate during this process and the double nature of the chromosomes can be seen. This is caused by the decrease of contraction and probably to some extent by a certain degree of attraction as well. The unravelling of the spiral sometimes begins at the first appearance of chromosomes/

somes in which case (Locusta, Dasyurus) the contraction ceases to be operative at the beginning of prophase. There are species (Euchorthippus) in which the spiral structure persists for a considerable time. Similar observations were made by Sharp (1929) and Wilson (1925).

The spiral structure of the chromosomes at prophase is transmitted from the previous telophase where such a structure was found by the author and others in Vicia faba and other species. At telophase the bulk of the chromosomes perhaps obscures the spiral arrangement of the chromonema which is embedded in the matrix (vide infra), but such a structure is present and is revealed in the following prophase, where there is no bulk or matrix. During the resting stage the structure of the chromosome is rendered invisible owing to biochemical processes and reactions within the nucleus, but the chromosomes do not lose their individuality (Belar 1925, Schaede 1925-1931). The contraction, being an/

an inherent property of the chromosomes, most probably also persists during the resting stage and is primarily responsible for the maintenance of spiral structure which will appear at the beginning of the following prophase.

Soon, however, unravelling of sister chromatids takes place, which is a proof that there is either no more contraction present, or that it is rendered ineffective. The sister chromatids expand and coil round each other during the process of unravelling. The twisting is a result of the combined action of splitting and contraction, the latter operating axially on a double spiral. Such an arrangement, which was even more complicated in the compressed spiral, clearly shows that the unravelling is a necessary process to ensure an easy and successful separation of sister chromatids at the end of metaphase, and as such it is the most effective adaptation. Only after the unravelling of sister chromatids has the time arrived to build up the body/

body or bulk of the daughter chromosomes. A mechanically complete, orderly and successful segregation is most easily achieved when the body of the chromosomes is short and thick. This can be seen very well at meiosis. To arrive at such a condition, the unravelling and expansion of single sister chromatids are a prerequisite to the formation of a separate body for each.

The sister chromatids at late prophase are composed of a chromatic gene string and an achromatic matrix in which the chromonema is embedded (Bridges and Anderson 1928). This structure can be seen in Vicia faba at late prophase and telophase using Benda and Carnoy fluid as fixatives. Hsu-Siang (1932) found it at metaphase in Lilium tigrinum, and Frank Smith (1932) in Galtonia at telophase.

The origin of the matrix was discussed by several cytologists (cf. Dermen 1933). Without going into a more detailed discussion concerning this problem, it will be sufficient to show the correlation between the appearance of the matrix and the disappearance/

pearance of the nucleolus or nucleoli and vice versa. This suggests a very close relationship between the two. The gene strings build up a surrounding sheath of chromatin, most probably each gene building up its specific enzyme complex. This activity takes place in the resting nucleus. When the genes divide in response to the altered dynamic equilibrium in the activity of the cell, the chromatin sheath is differentiated into a more chromatic chromonema and a less diffuse achromatic matrix (Zirkle 1928, Fikry 1930).

At the very beginning of prophase the achromatic matrix can not be seen, the chromatids with their component chromomeres and connective fibres between them representing only the gene string. Sometimes in the twist of the spiral in Tulipa potteriana the author found that a small portion of the matrix is visible, but it is always discontinuous (Frank Smith 1932). The first sign of a continuous matrix is seen after the unravelling of sister chromatids only.

The gradual appearance of the matrix and of the body of the daughter chromosomes is accompanied by a continuous/

continuous decrease in length. This suggests that after the unravelling of the chromatids, when the matrix is formed, simultaneously contraction again sets in. It seems to us that the contraction operates upon the body, or rather upon the matrix, only and not upon the gene string. The early prophase shows that this assumption is a very probable one because at that stage the gene string without the matrix, although arranged in a spiral, soon untwists.

The result of contraction is different for the matrix and for the gene string. The latter is unable to contract and therefore must adjust itself in some other way to the rapidly decreasing length of the matrix. The pressure which the chromonema has to undergo necessitates its adjustment within the matrix into a spiral. This structure, however, soon becomes invisible owing to the strong chromacity of the matrix which stains deeply, and special technique is necessary to detect it. Sakamura (1927), who used boiling water as a fixative agent, succeeded/

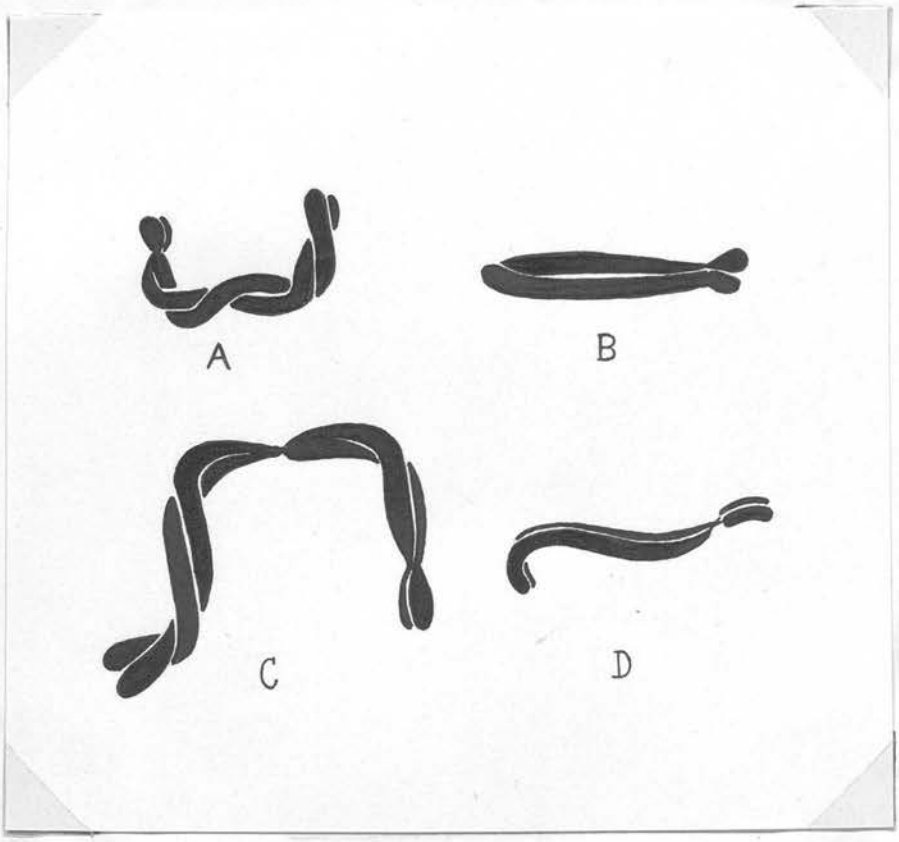
succeeded in revealing the spiral structure at metaphase, anaphase and telophase of Tradescantia.

The contraction determines not only the structure of the prophase chromosomes, but to some extent also the interrelation of sister chromatids. It counteracts partially the attraction and in some cases (Euchorthippus) the chromatids lie parallel, either separately or apparently held together by a few twists (Vicia) (fig. 9).

The most probable explanation of the decreased attraction is that contraction produces a zigzag arrangement of the chromonema. The genes and their relative position to homologous genes will be affected by the spiral arrangement. With increased distance the strength of attraction will decrease. Fig. 10 illustrates the process, which will result in a more or less loose association of the prophase chromatids.

In every division, however, the prophase chromatids remain in close association at the attachment constriction. Sometimes intimate association was found/

Fig. 9.



End of prophase of mitosis. The sister chromatids twist in Vicia (A and C), but lie parallel in Tulipa (B) and Euchorthippus (D).

Fig. 10.

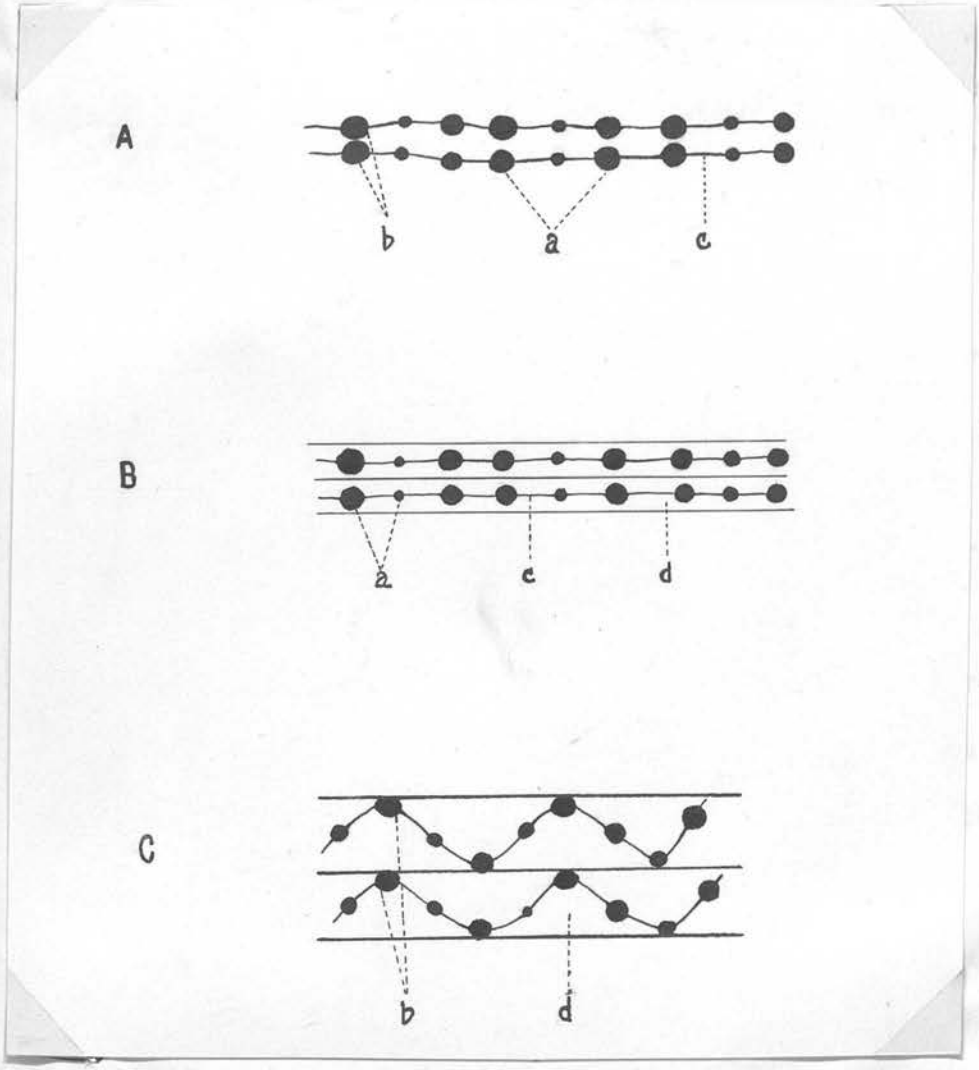


Diagramm illustrating the process of contraction. A: represents the gene-string without the matrix, B: the chromatids before and C: after contraction. The gene-string forms a spiral and the distance between homologous chromomeres is increased. (a: chromomeres, b: homologous chromomeres, c: connective fibre and d: matrix).

found at the secondary constriction, but this is accidental (Vicia and Tulipa).

During metaphase and post metaphase, further decrease of chromatids in length shows that contraction still remains in operation. Its action, however, is not so great as it was at the late prophase. Two probable explanations may be offered : (a) decrease in the strength of the force, or (b) greater resistance on the part of the daughter chromosomes which have already undergone contraction.

At metaphase the chromosomes arrange themselves at the equatorial plate. This arrangement, which becomes possible through the delayed division of the attachment constriction, will offer an easier and more successful segregation to the opposite poles. In Zea Mays (Beadle 1932) precocious splitting of the attachment constriction upsets the sequence in the mechanism of division and segregation is a disorderly one.

(iii) Repulsion at anaphase. At the end of metaphase the daughter chromosomes begin to separate and/

and the separation is always at the primary constriction. This is a characteristic feature of anaphasic segregation and for this uniformity the attachment constriction itself must be responsible. Several observations of similar nature, both in plants and animals, suggest that by the division of the primary constrictions a force of repulsion is released, which first repels the attachments and gradually proceeds towards the ends. It is interesting to note that at the beginning of anaphase, whilst the attachment constriction and the adjacent segments have separated, the distal ends of sister chromatids, specially if they are long, hold together for some time.

In the further segregation of daughter chromosomes the spindle plays an important part, but the relationship between spindle and chromosomes will be discussed in Part IV.

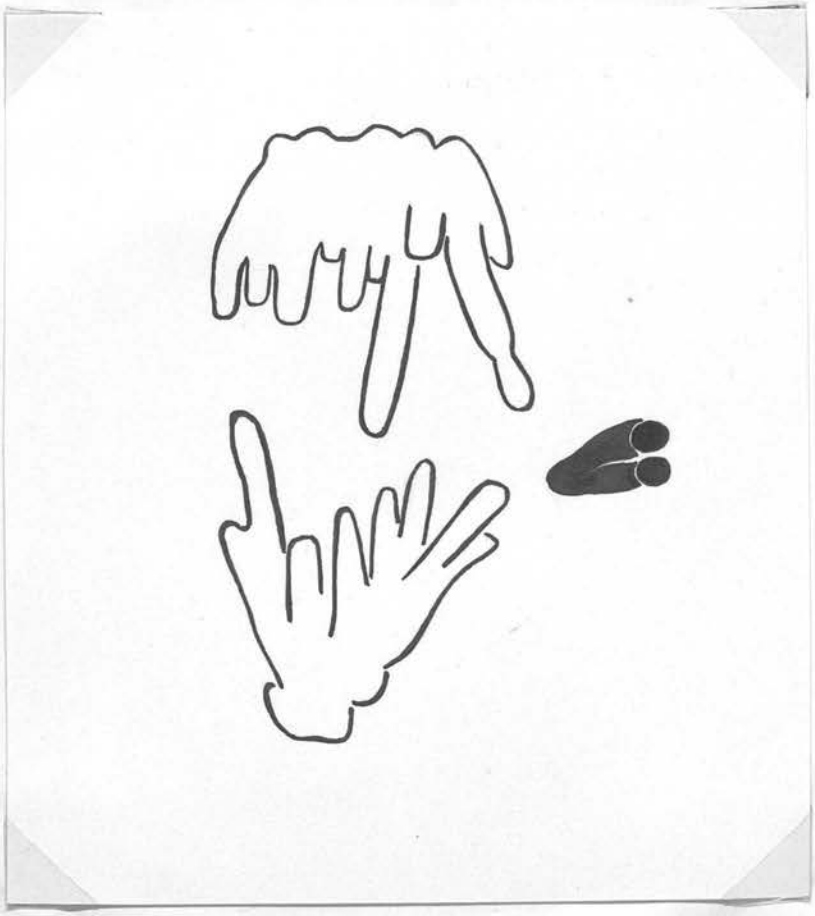
If the attachment constriction is absent (as may be produced experimentally in Vicia by X-rays), the sister chromatids remain together at anaphase in several/

several cases, a proof that repulsion is localised at the attachment. In other cases the chromatids fall apart; this is an accidental process resulting from the lack of the attachment which controls the association of sister chromatids by fusion and their separation by repulsion (fig.11).

In the anaphase separation the interaction of the three forces must be considered. The first is attraction which operates at prophase and metaphase between homologous chromatids. The second is the contraction which operates from late prophase to the end of division. It may suppress the effect of attraction to a certain extent and the association of the chromatids may become looser. However, this attraction will be completely overcome only at the end of metaphase by the third force, repulsion, which operates between two homologous attachments and results in anaphasic separation. Fig. 12 illustrates diagrammatically the interaction of these forces.

It has been shown that the division of the attachment constriction evokes a force of repulsion at/

Fig. 11.



Anaphase of mitosis in Vicia, treated with X-ray. The daughter chromosomes have segregated to opposite poles, except two, which remain associated near the equatorial plate. These chromosomes have lost the attachment constriction.

Fig. 12.

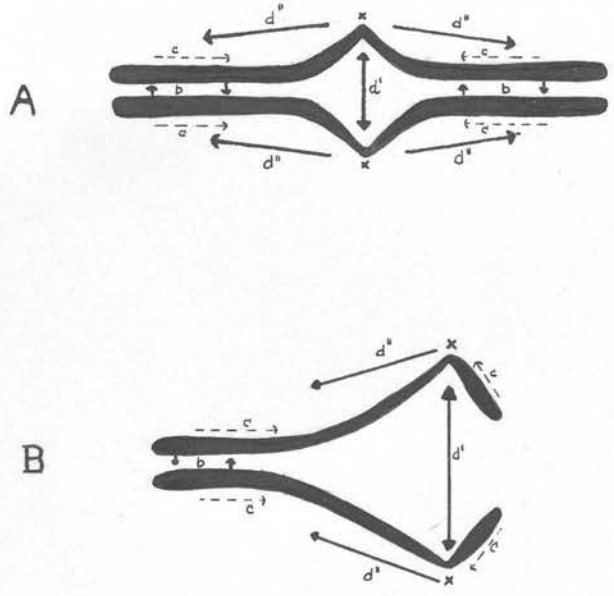


Diagramm illustrating the interaction of attraction, contraction and repulsion, operating at the beginning of anaphase. A: chromosome with median, B: chromosome with subterminal attachment constriction. (x: attachment constriction, b: attraction, c: contraction, d': special and d'': general repulsion.)

at mitotic anaphase. We must infer that two homologous primary constrictions have a general tendency to repel each other. In meiosis, however, two homologous attachments come into association at prophase and this tendency is not exhibited. This phenomenon will be treated in more detail in Part IV; here we must merely point out that the observations on meiotic divisions do not contradict the suggestions outlined above.

Huskins (1933) recently put forward another explanation of anaphasic separation. He assumes that there is a tertiary split in the sister chromatids at metaphase; this tertiary split will suppress the attraction between sister chromatids. Observations on mitosis and meiosis amply demonstrate that whilst attraction is present only between two homologous chromosomes (meiosis) or two homologous chromatids (mitosis), pairs of paired chromatids are invariably repelled. According to Huskins, each sister chromatid splits at metaphase: hence the anaphase repulsion, due to the present of two pairs of homologous/

homologues. If his assumption is valid, there is no attraction at metaphase but a general repulsion between daughter chromosomes, each composed of two chromatids through the tertiary split. The force of attraction would yield to the general repulsion and would result in separation of daughter chromosomes at any level. It was already stated, however, that the anaphase separation invariably starts at the attachment, - a proof that if the force of repulsion exists between the bodies of chromosomes or chromatids during metaphase, it is not effective except at one point, that of the attachment constriction. Furthermore, homologous chromatids in Vicia which have lost the attachment constriction by fragmentation remain in association at anaphase. These two facts are evidence against Huskins' hypothesis.

It has already been mentioned that at anaphase when the attachments were repelled, the distal ends sometimes remained in association. This can be seen in Vicia, Tulipa, Hyacinthus, and was observed by several cytologists. (cf. Wilson 1925). The probable cause of the delayed separation may be either the great/

great length of the distal limbs of daughter chromosomes, or a gradual decrease in repulsion away from the attachment, or else a stronger attraction at the terminal parts. The latter view is the most probable, as will be shown later.

The measurements of anaphase chromosomes suggest that there is a further decrease in length, caused by the contraction which is still in operation. It was already stated that in some chromosomes of Vicia faba and Tulipa during telophase the spiral structure has been observed, but the number of spirals per micron could not be estimated. It is resonable to assume that they are approaching the same condition as that which prevailed at the commencement of prophase.

(iv) Conclusions. The analysis of structural changes within the chromosomes during mitosis leads us to infer the presence of three forces : (a) attraction, (b) contraction, and (c) repulsion. Every structural change in the chromosomes can be explained in terms of these forces. They are general dynamic factors, acting and interacting in the mechanism of mitotic/

mitotic division. It must be noted that these forces are borne on the chromosomes as their inherent property, and as such they are subject to genotypic control (Darlington 1932). The operation of these forces may be arrested or altered, which would result in the appearance of irregularities, either cytologically or genetically detectable. We are, however, discussing only those mitotic processes and structural changes which are general and typical and will therefore avoid special cases. It should be remembered, nevertheless, that irregularities of mitotic division can always be explained by disturbances which inactivate or modify these three forces.

The table which follows (table V) illustrates intra-chromosomal changes in terms of forces.

Table/

Table V.

Dynamical interpretation of mitotic processes.

Stages of division		Operating forces	Structural changes
Resting stage		equilibrium (?)	Fission of genes, autocatalytic process
Prophase	I.	contraction (a)	Spiral chromosome
		attraction (b)	Single appearance of the spiral
	II.	decrease of (a)	Unravelling of the spiral
		decrease of (b)	Doubleness in the chromosomes appears
	III.	increase of (a)	Spiral structure of single chromonema in the matrix
Metaphase		contraction	Decrease in length of chromatids, which are held together at the attachment constriction
Anaphase		contraction	Decrease in length
		repulsion (c)	Separation of attachment constrictions and daughter chromosomes
Telophase		contraction	Spiral chromonema in the matrix
		repulsion (c)	Daughter chromosomes repelled towards the poles

B. MEIOSIS.

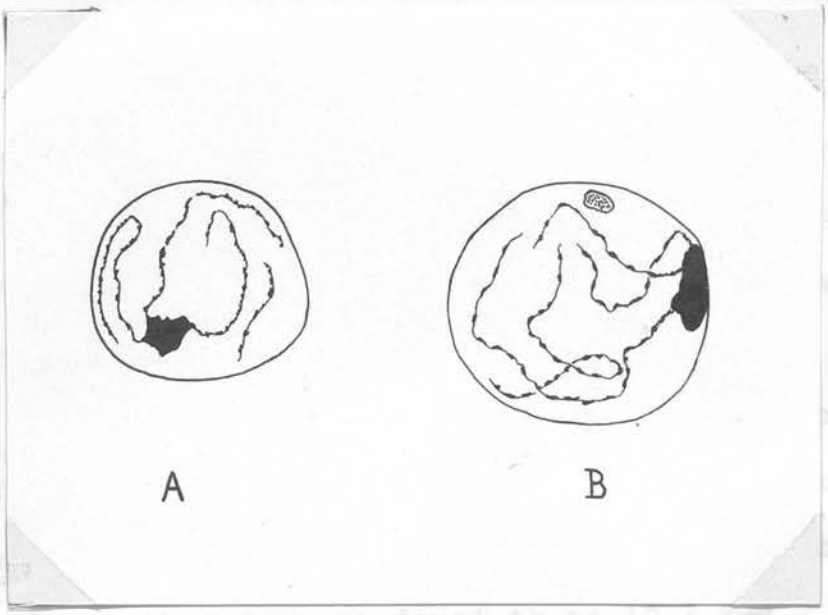
(1) Morphological description.

(i) Early prophase. Meiosis is a form of mitosis and differs from it in that the nucleus divides twice and the chromosomes only once, the result being a numerical reduction of chromosomes in the gametes.

The very beginning of meiotic prophase is characterised by the appearance of thin beaded threads, —(leptotene). They represent the undivided chromosomes with chromomeres. Their number is the same as that of the chromosomes in the diploid nucleus. In Crepis aurea (2n = 10) and other plants and animals with small chromosome number (e.g. Dasyurus, 2n = 12 + XY), the individual single threads can be followed along their whole length. Fig. 13 illustrates the leptotene chromosome threads of Crepis aurea. The chromomeres in each thread can be seen very well, with the fine connective fibre between them. They are uneven in their structure.

Sometimes/

Fig. 13.



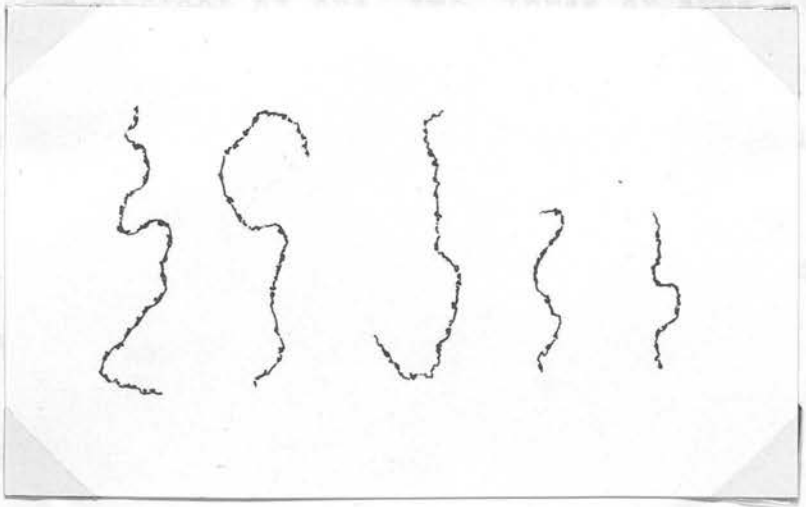
Leptotene chromosomes in Crepis aurea (A) and in Locusta migratoria (B). The threads are single with uneven chromomere-arrangement.

Sometimes the leptotene threads are polarized, one or both ends of the chromosomes being turned towards one side of the nucleus near to the centrosome (Gelei 1921). This phenomenon during leptotene is found in Stenobothrus parallelus. In many instances, however, the polarization is obscured owing to fixation which causes the polarized leptotene threads to clump into a darkly stained knot on one side of the nucleus.

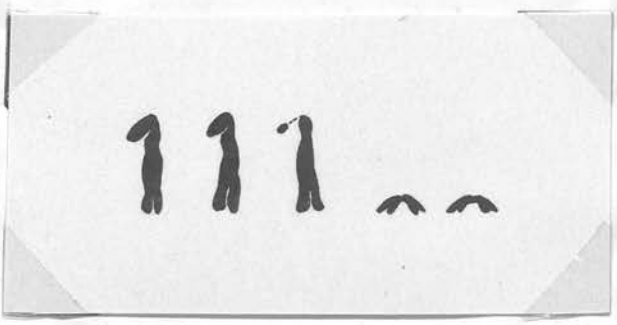
The length of the mitotic metaphase chromosomes in Crepis aurea was found to be 5 - 2.5 micron, the chromosomes being of different sizes. At leptotene the single threads were found to be 12.3 and 7.5μ respetively, representing the longest and the shortest chromosomes. Fig. 14 illustrates the relation between the mitotic chromosomes and leptotene threads in Crepis aurea.

As the prophase proceeds, the leptotene threads pair at different sections (zygotene). The commencement of pairing was studied in Marsupials and Tettigidae and it was found that the chromosome threads come/

Fig. 14.



A



B

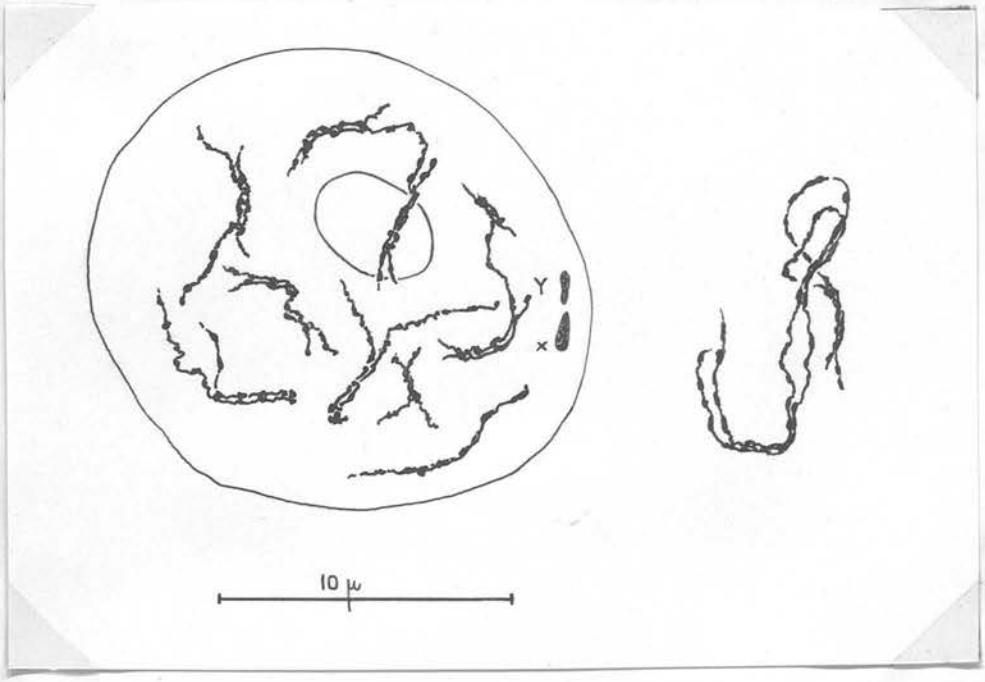
The chromosomes of Crepis aurea at leptotene (A) and mitotic metaphase (B). Note the different lengths of chromosomes in the two divisions.

come into contact at one, two, three or even more loci. Two characteristic features of zygotene pairing must be mentioned, as observed in Marsupials : (a) the loci of pairing are at random, and (b) the association or pairing is never restricted to one chromomere but always to a rather long segment (fig. 15).

In most cases the zygotene stage is difficult to follow and study because the great length of leptotene threads, even when their number is small, always complicates and obscures the loci of pairing. This difficulty is partly overcome at the following stage when the number is reduced to half owing to the completion of pairing (pachytene). In Crepis aurea at this stage only five threads are present, the diameter of which is increased. In Phascolarctus cinereus it could be observed that pairing took place between threads which showed the same chromomere arrangement (fig. 16).

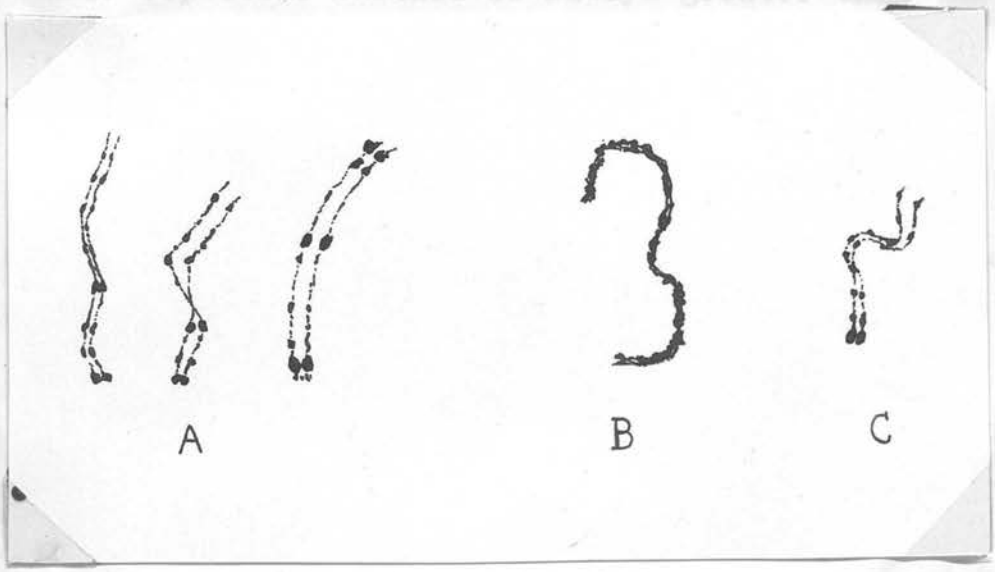
In comparison with the single leptotene chromosomes, the paired pachytene threads are shorter, thicker/

Fig. 15.



Pairing of chromosomes at zygotene in Dasyurus. The chromosomes are associated in sections of appreciable length. The X and Y sex-chromosomes are precociously condensed. Only one pair of chromosomes - illustrated separately - has been followed in its entire length, showing three pairing blocks.

Fig. 16.



Pairing of chromosome-threads at pachytene. A: Locusta, B: Crepis and C: Sarcophilus. The association of homologous chromomeres can be seen in Locusta and Sarcophilus. In Crepis, as the pairing of homologues is completed, contraction comes into being, which increases the diameter and decreases the length of the paired chromosomes.

thicker and stain better. The diameter of two paired leptotene threads is always greater than the sum of those of two unpaired threads at leptotene or zygotene. The decrease in length and increase in diameter could be very clearly observed in Schistocerca gregaria at the beginning and end of pachytene stage. This stage is the longest in meiotic prophase and the paired threads may become very thick. They represent two completely associated single chromosomes. In few cases (Tulipa and Scilla) sometimes opening out was found at some loci. This might be either the primary or the secondary split, as a more exact determination was impossible. Only in Marsupials was the author able to observe the actual opening out of the secondary split.

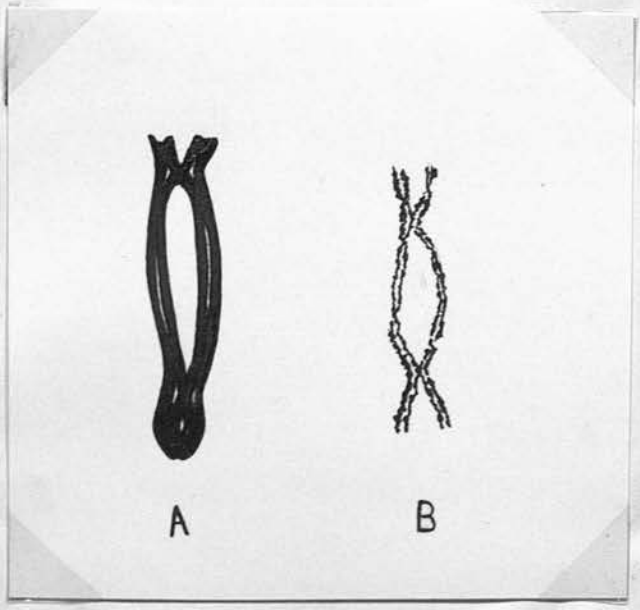
(ii) Chiasma formation. The next stage of meiotic prophase is characterised by the loops or nodes and internodes between paired chromosomes (diplotene). At this stage each chromosome is composed of two chromatids, each pair of chromosome being/

being represented by four chromatids. The chromatids derived from the same chromosome are called sister chromatids, those derived from homologous chromosomes are called partner chromatids. The double structure of the threads was seen by Janssens (1924), Newton (1927) and described by Belar (1928). Similar double structure was observed by the author in Batrachoseps, Schistocerca and Locusta (fig.17). If the paired chromosomes are very short there is no loop but a form of a cross is visible in most cases. Such an arrangement was found in Stenobothrus and Chorthippus.

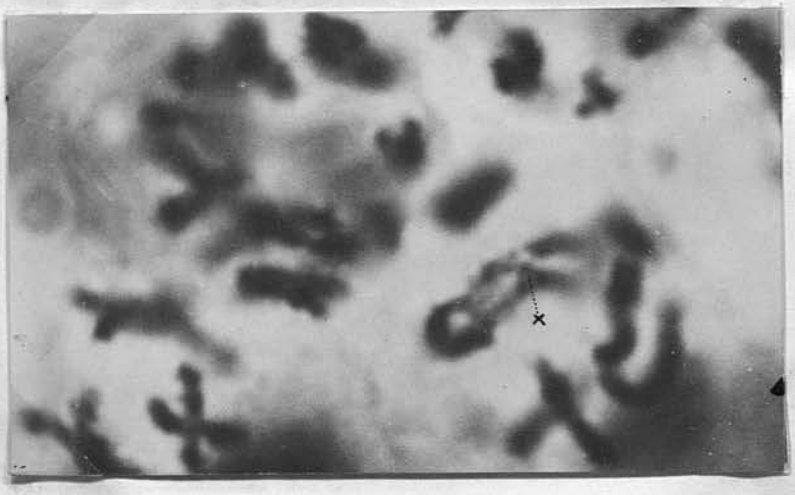
The loci of meeting of partner chromatids is a place of exchange between them, described and interpreted by Janssens as chiasmata (1909, 1924). It was possible to see the detailed structure of diplotene bivalents (i.e. associated homologous chromosomes) in some very favourable material, e.g. Orthoptera, and to follow the mode of association of partner chromatids at the chiasma or chiasmata (fig. 18).

The/

Fig. 17.



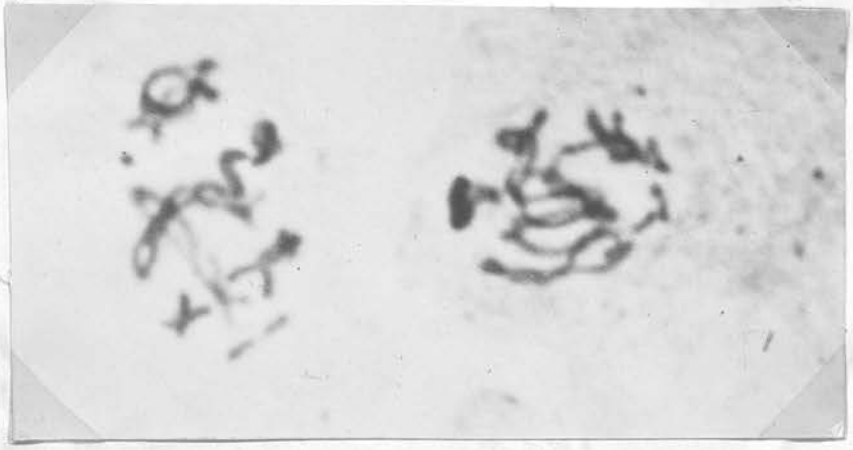
Bivalents at diplotene held together by chiasmata. A: Batrachoseps and C: Crepis. The quadruple structure of paired chromosomes can be seen at the chiasmata.



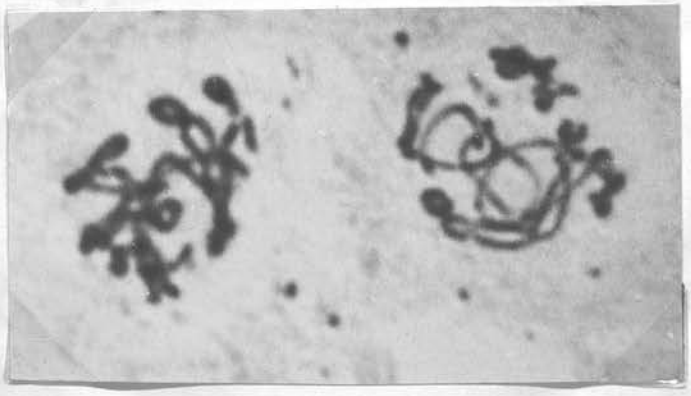
C

Diplotene bivalents in Schistocerca. The actual exchange between partner chromatids is indicated by an x.

Fig. 17.



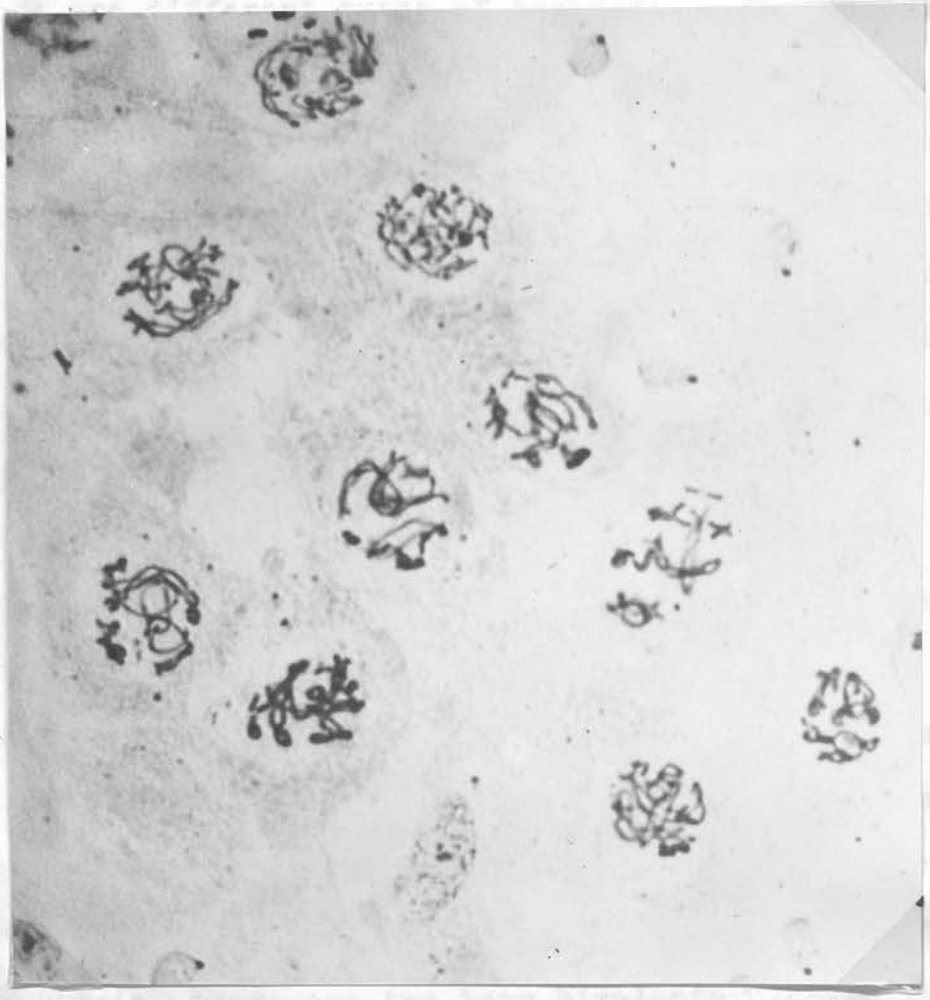
D



E

Microphotograph of bivalents at diplotene in Stenobothrus parallelus.

Fig. 18.

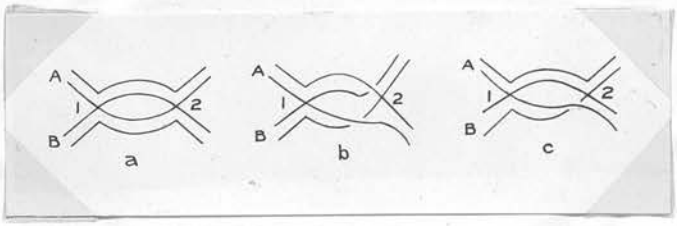


Diplotene stage in Stenobothrus. The nodes (chiasmata) and internodes can be clearly seen, indicating the repulsion between pairs of paired chromatids.

The observations on chiasmata demonstrate that there are different types of exchange. Those cases where the same sister chromatids are in association after the seond chiasma as before it, are called the compensating type; and those in which the second chiasma does not restore the original chromatid arrangement are termed non-compensating. These types and their genetical significance have been described and illustrated by Sansome and Philp (1932) (fig. 19).

The number of the loops and the chiasmata are correlated to a certain extent with the length of the paired chromosomes, long chromosomes being capable of forming more chiasmata than short ones, but no close correlation could be found (O'Mara 1931, Darlington 1932c). The small bivalents usually have a higher chiasma frequency than the longer ones. In Locusta migratoria there are two long bivalents usually with three or four chiasmata, while the shorter ones are associated by only one chiasma. A similar condition was found in Chorthippus, in which the long bivalents have four, the small ones only one, chiasma. The latter/

Fig. 19.



Types of chiasmata: (a) reciprocal, (b) complimentary and (c) diagonal. (a) and (b) are compensating, (c) non-compensating type. The sister chromatids designated A and B respectively.

latter were 4-6 times smaller than the former. The following table and graph (fig. 20) will illustrate the relation between length and chiasma formation in Locusta migratoria.

During diplotene the length of the bivalents decreases further and is accompanied by an increase of diameter. The loops which were of different size usually become equalized and more uniform in distribution and appearance.

In diakinesis following the diplotene stage, the bivalents are thick and short, the lateral expansion having reached its maximum, as observed in Dasyurus (fig. 21). The adjacent loops of the bivalents are placed at right angles. Very commonly the bivalents at diakinesis in the first spermatocytes are extruded to the periphery (fig. 22). The first signs of spindle formation are observed.

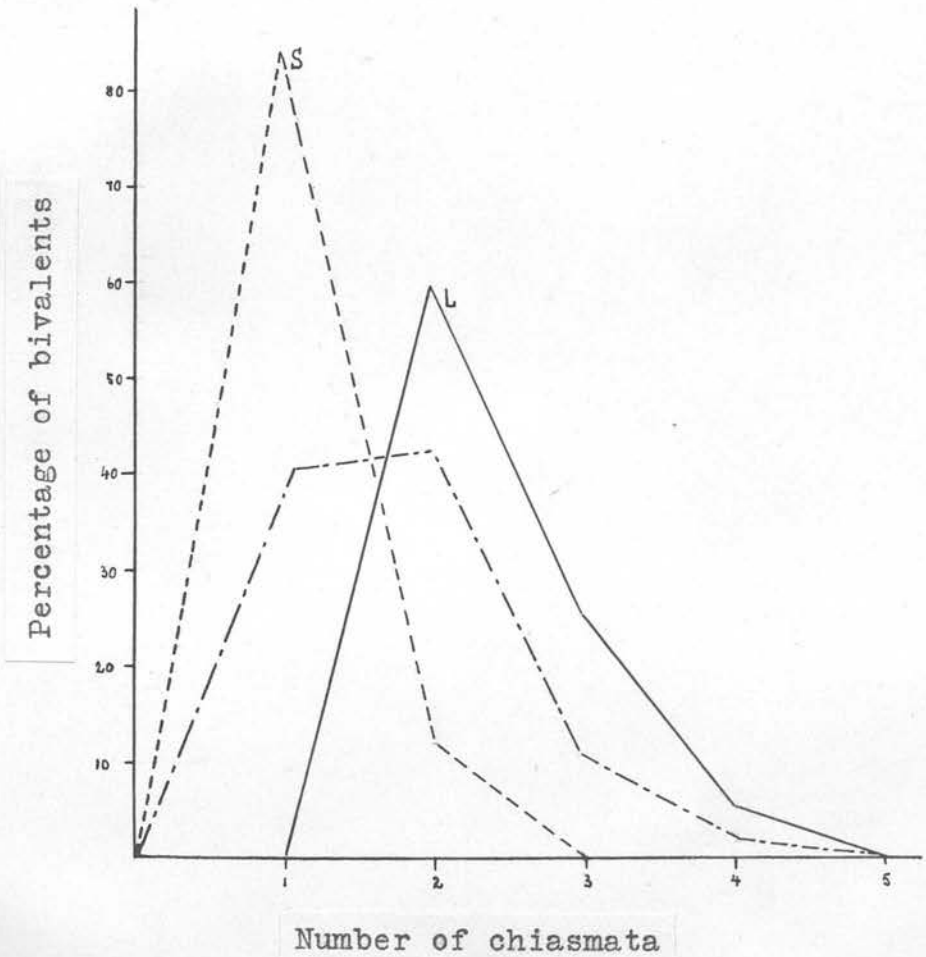
At this stage the decrease in size of the bivalents is greatest and the number of chiasmata also is usually smaller than before. This reduction is a very common phenomenon in the first meiotic prophase/

Table VI.

Chiasma frequency at diplotene in long and short bivalents of Locusta migratoria.

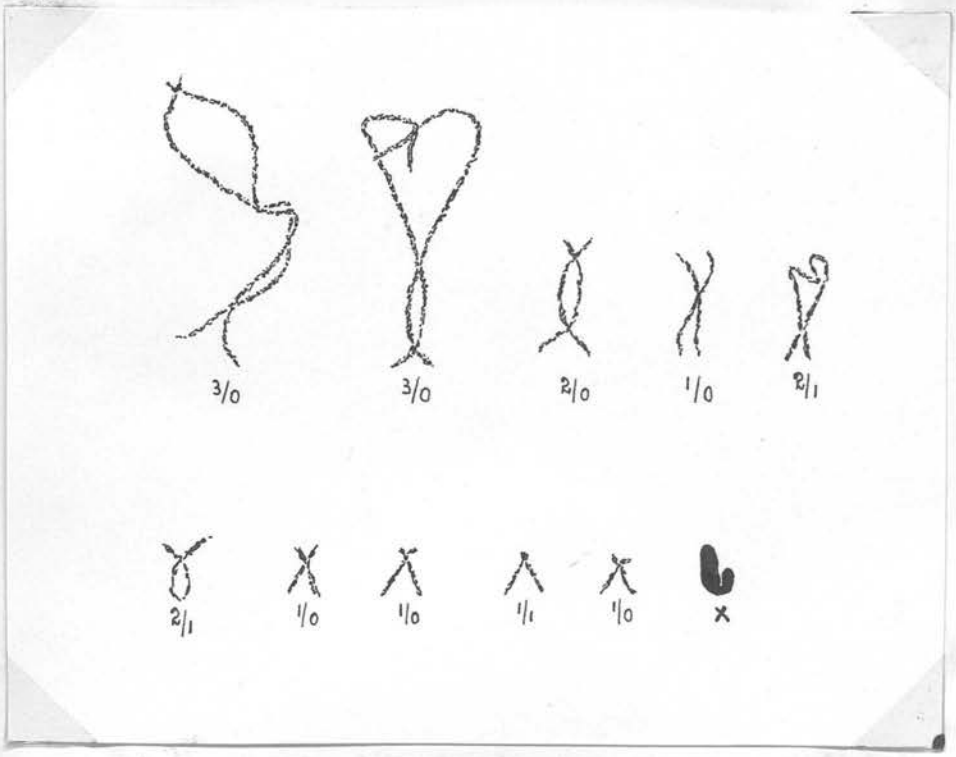
Number of chiasmata per bivalent						
Long bivalents				Short bivalents		
1X	2Xta	3Xta	4Xta	1X	2Xta	3Xta.
-	53	19	4	54	8	-
Total number of chiasmata			179	70		
" " " terminal chiasmata			20	14		
Chiasma frequency per bivalent			2.3	1.1		
Terminalisation coefficient			0.11	0.20		

Fig. 20.



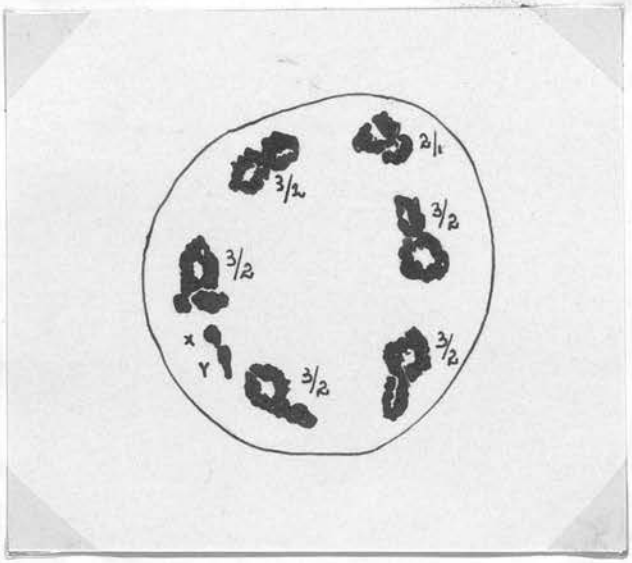
Graph illustrating the chiasma frequency at diplotene in Locusta migratoria. S: the curve of short, L: that of the long bivalents. The third curve represents the chiasma frequency when all the bivalents are counted together.

Fig. 20.



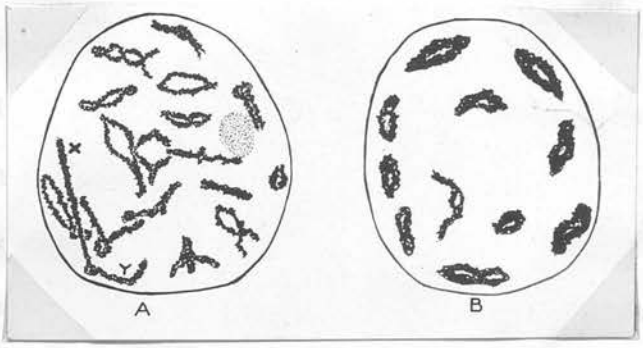
Diplotene bivalents in Locusta migratoria. The total number and the number of terminal chiasmata is given for each bivalent.

Fig. 21.

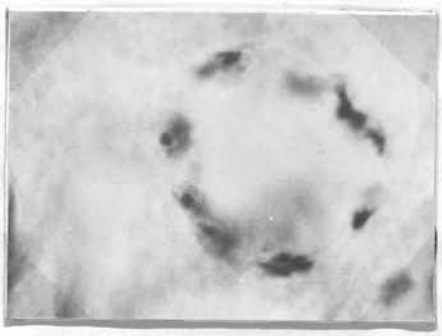


Diakinesis in Dasyurus. The whole complement is represented. The lateral expansion is very great, the bivalents are short and thick. The adjacent loops are arranged at right angles.

Fig. 22.



Diakinesis in the mouse. A: early, B: late diakinesis. The number of chiasmata decreases, the bivalents become shorter and thicker.



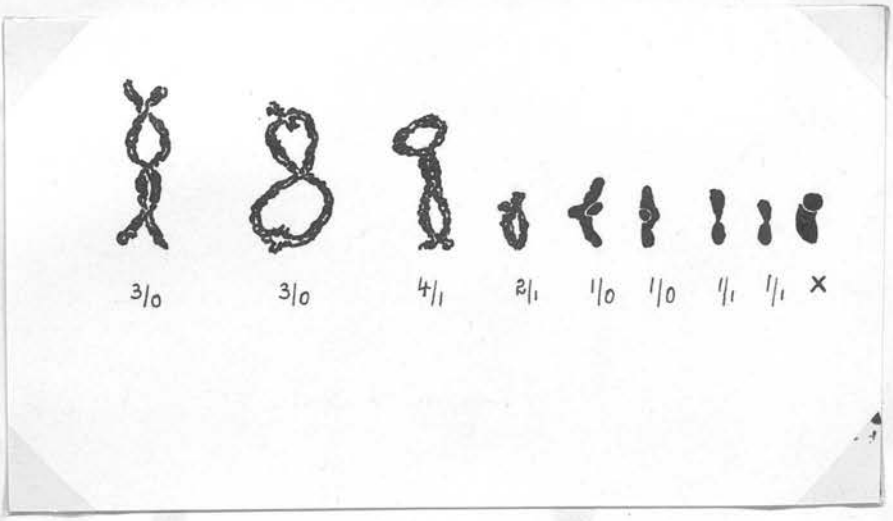
C

Microphotograph of diakinesis stage in the rat. The bivalents are repelled to the periphery of the nucleus.

phase both in animals and plants. It was studied by the author in Crepis and Locusta. In the first a great reduction was found between diplotene and metaphase, while in the second it was only slight. Very often the chiasmata are grouped at one end away from the attachment constriction. If the primary attachment is localised at the middle, the loops lie on both sides of the attachment, the largest loop always including the attachment constriction. (Fig. 23)

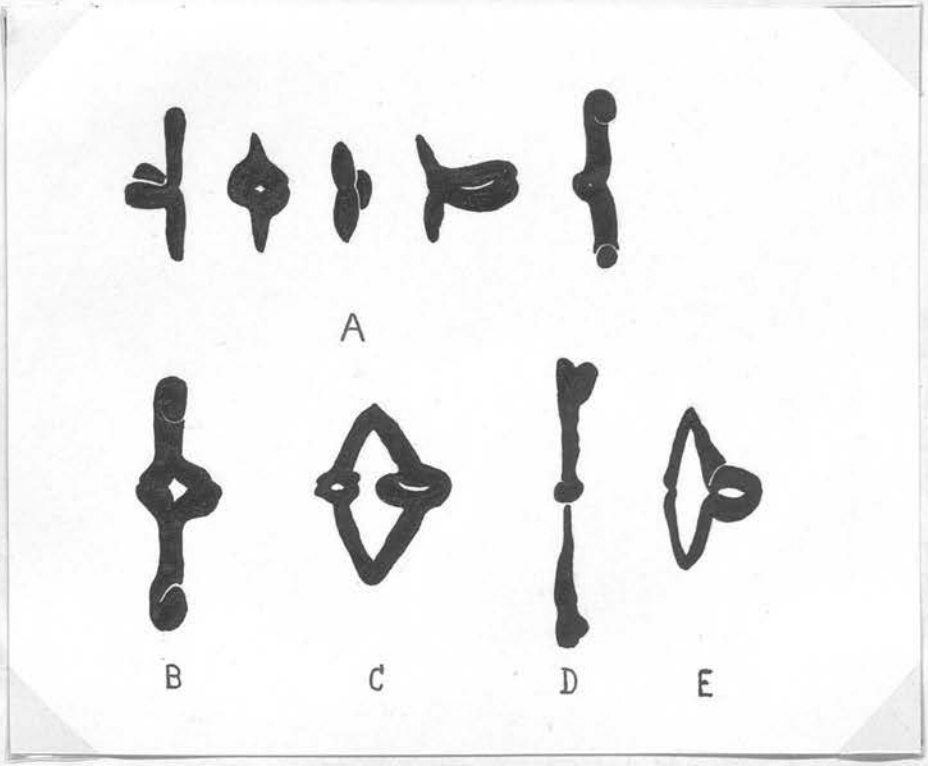
(iii) Metaphase. At metaphase the bivalents may be divided into two groups : (a) those with a terminal association only; and (b) those with one or more subterminal, or interstitial chiasmata. Fig. 24 illustrates some metaphase bivalents in various plant and animal species. In many cases, namely where bivalents belong to the first group, the number of chiasmata is reduced. Chiasma frequency during diplotene and metaphase was calculated for Locusta and Mus and the degree of decrease is illustrated in table VII. In other species the reduction is slight. Fritillaria imperialis may serve as an example, as it has/

Fig. 23.



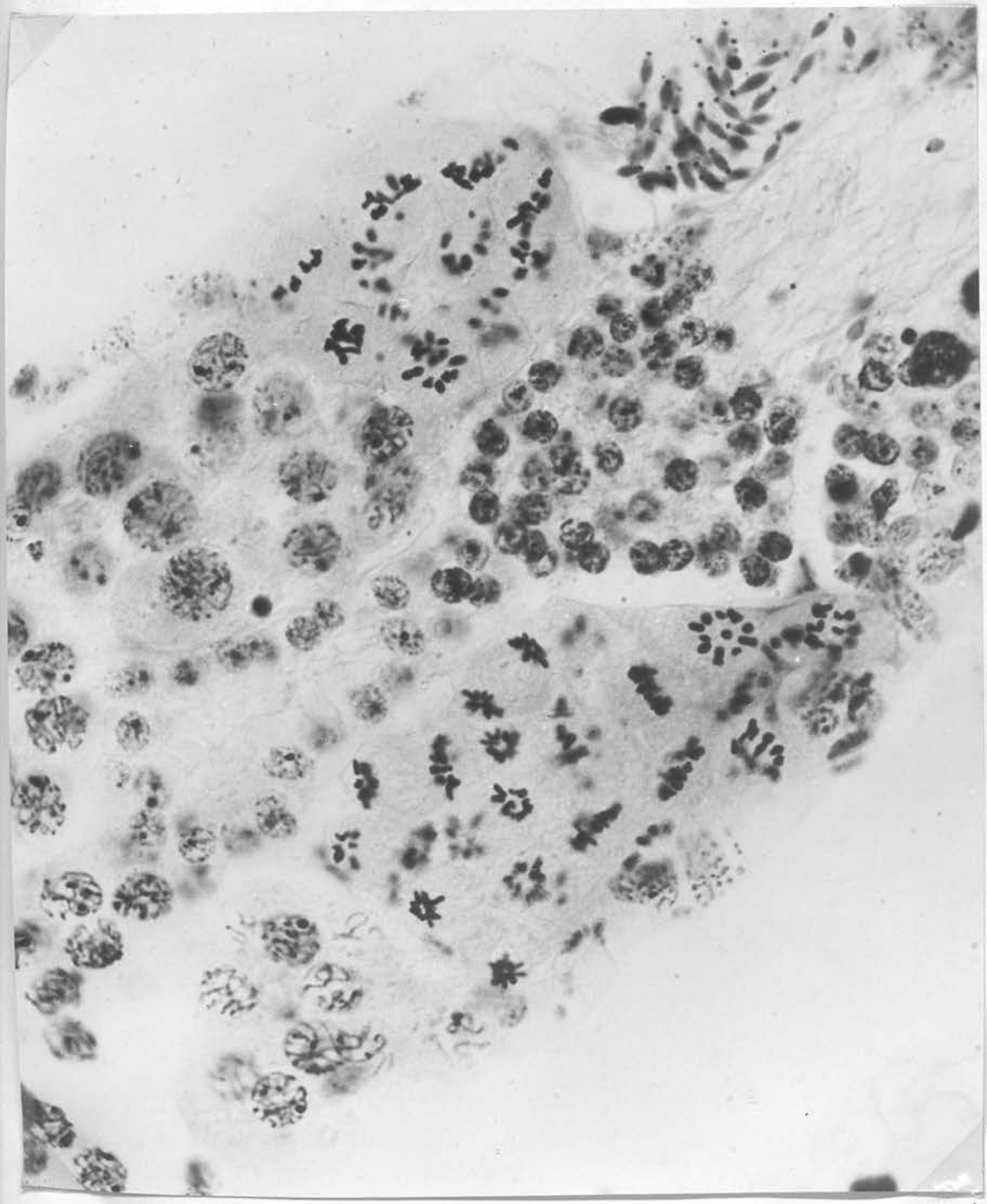
Diakinesis in Chorthippus. Bivalents are contracted further and show less chiasmata than at diplotene. The largest loop includes the attachment constriction. The number of total and terminal chiasmata is given for each bivalent.

Fig. 24.



Bivalents at metaphase. A: Crepis aurea (the whole nucleus is represented), B: Scilla, C - E: Triticum.

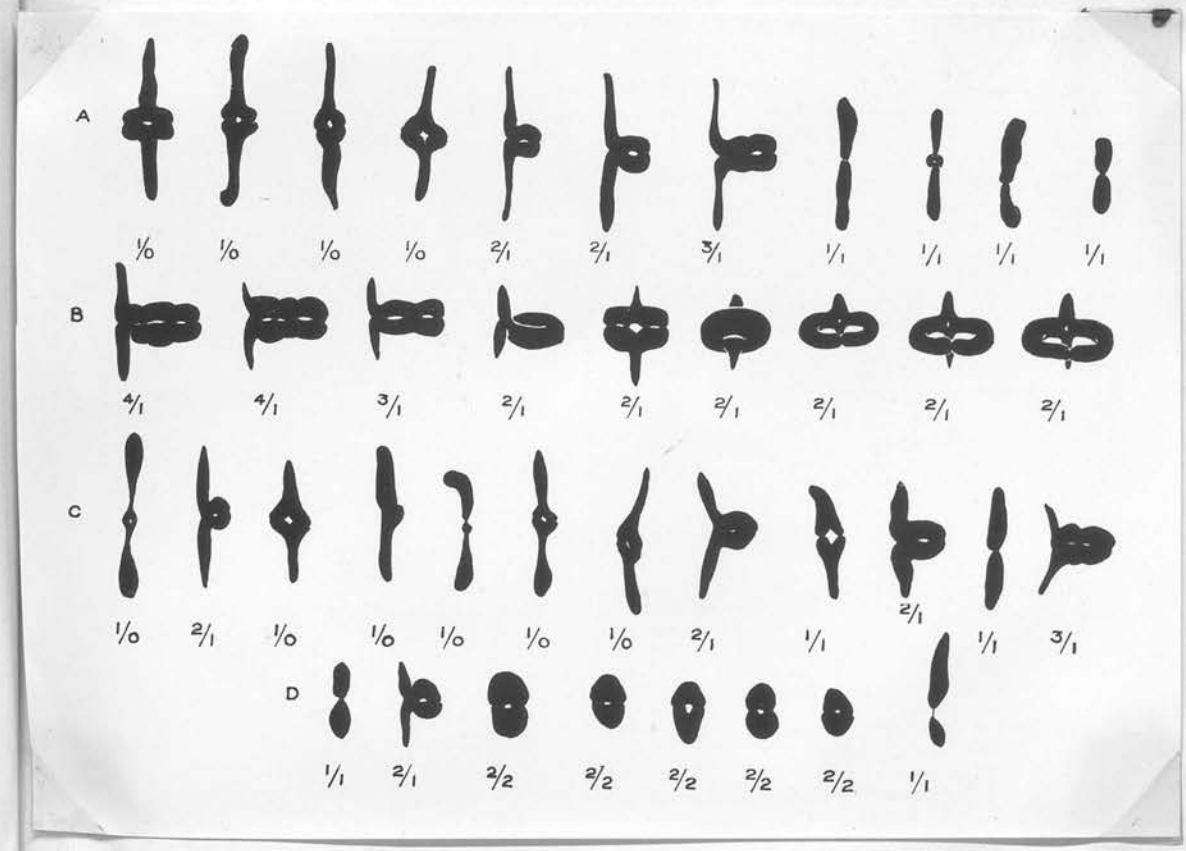
Fig. 24.



Microphotograph of meiotic stages in

Schistocerca gregaria.

Fig. 24.



Bivalents at metaphase in the female (A-B) and in the male (C-D) mouse. The number of total and terminal chiasmata is given for each bivalent.

Table VII.

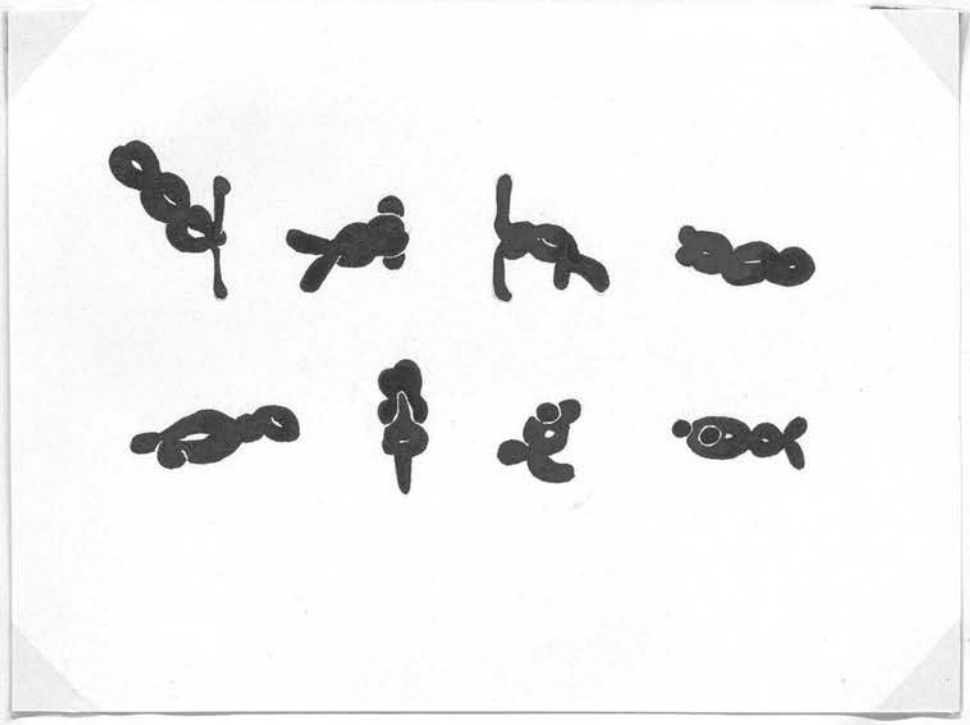
Chiasma frequency in diplotene and metaphase.

Species	Diplotene		Metaphase	
	Chiasma frequency	Terminalisation coefficient	Chiasma frequency	Terminalisation coefficient
ocusta migratoria	1.8	0.13	1.0	0.76
ocusta migratoria ar. danica	1.7	0.14	1.0	0.44
louse (female)	2.8	0.32	1.9	0.43
louse (male)	2.4	0.33	1.4	0.67

has many interstitial chiasmata at metaphase (fig. 25). In other species, e.g. Scilla and Fritillaria Rhutenica, some bivalents retain the chiasmata, while others show great reduction. Both types of bivalents are present in the same complex (fig. 26). In the case of great size differences between bivalents within the same complex, the shorter will often separate precociously at metaphase. Such bivalents have only terminal association, while the others are held together by one or more interstitial chiasmata. Fig. 27 illustrates such a case in Delphinium.

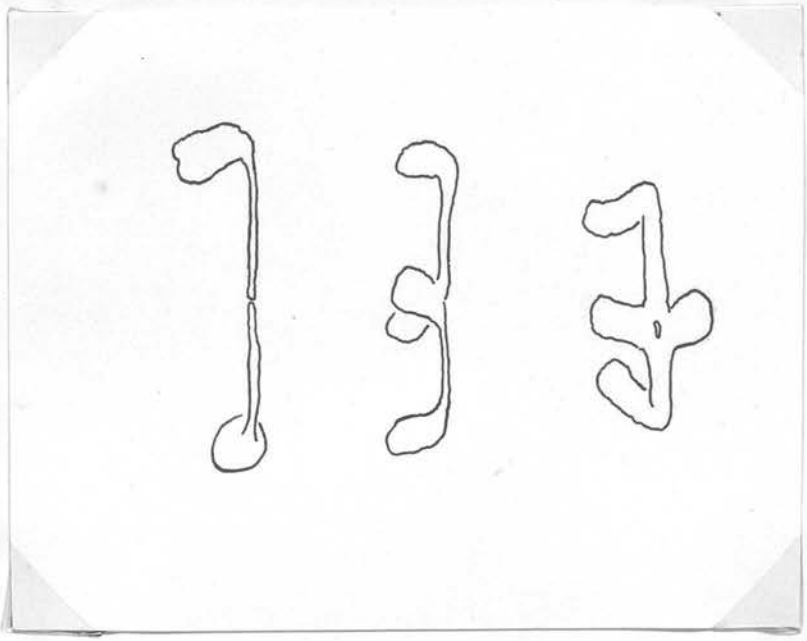
The spindle is fully developed at metaphase and by using uranium tetroxide as fixative agent, the author was able to demonstrate its fibrous nature (fig. 28). The metaphase bivalents are normally arranged in the equatorial plate, their attachment lying axially on the spindle. If the primary constriction is subterminal or median, at the end of metaphase only the attachments are repelled and become widely/

Fig. 25.



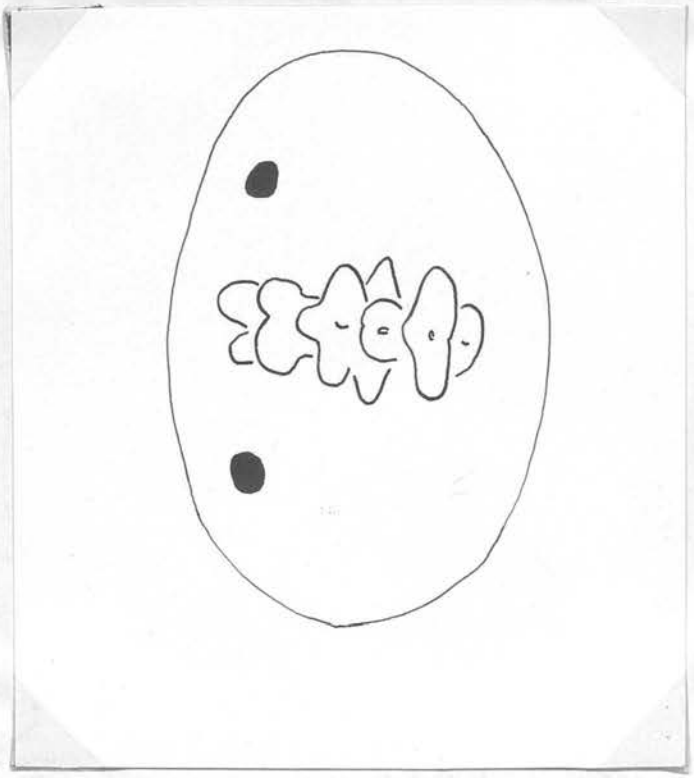
Bivalents at metaphase in Fritillaria imperialis. Interstitial chiasmata are still present.

Fig. 26.



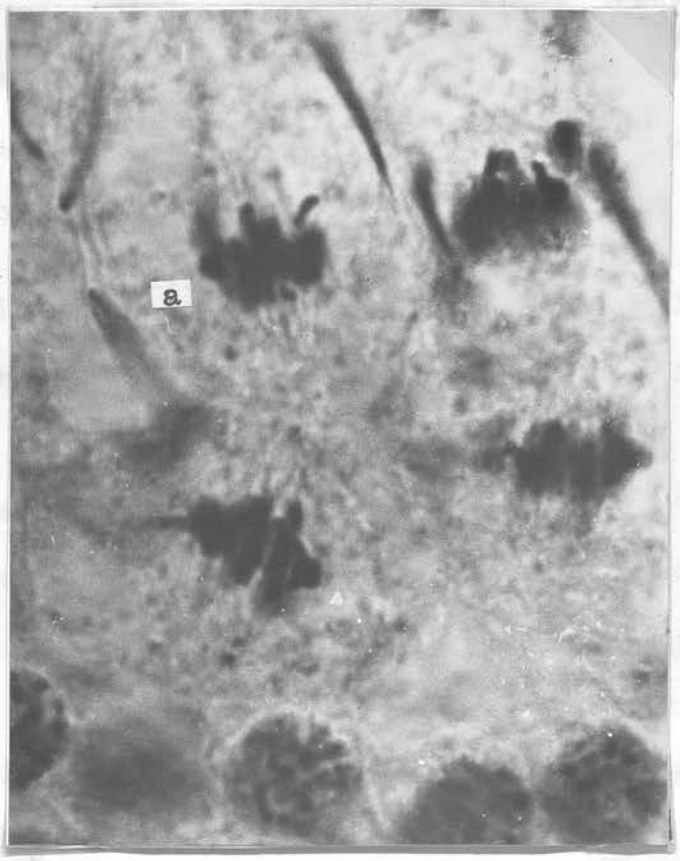
Metaphase bivalents in Scilla. The bivalents have one chiasma only, but its type is different.

Fig. 27.



Precocious separation of bivalents at metaphase. These bivalents were short and associated only by terminal chiasma. (Delphinium)

Fig. 28.

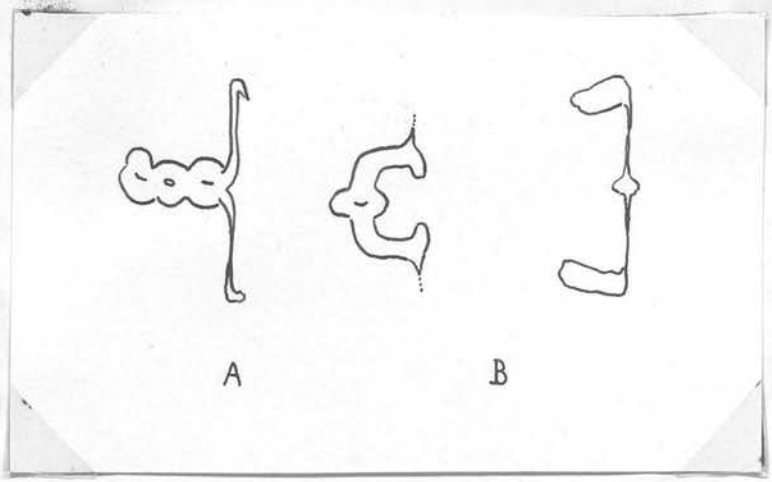


The spindle mechanism at meiotic metaphase in the rat. The X-Y bivalent can be seen in cell indicated by a.

widely separated; this can be seen very well while the interstitial chiasmata are still present. --- The opposite case, is when the bivalents have only one chiasma and their great bulk is separated, (they are held together at the chiasma) (fig. 29).

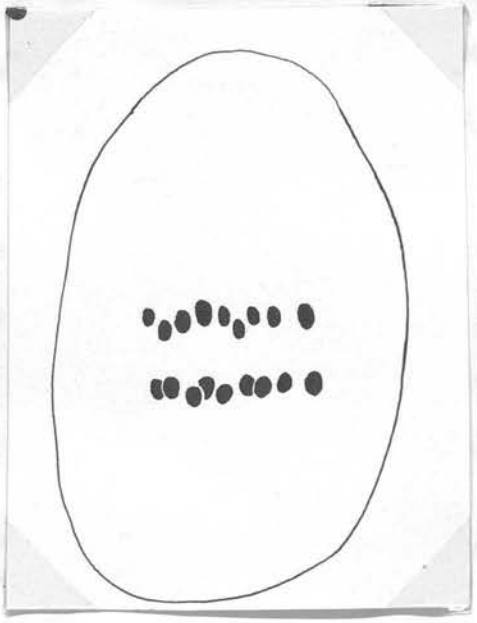
(iv) Anaphase. At anaphase the bivalents migrate to the opposite poles. The separation begins at the spindle attachment. This can be observed where the primary constriction is located subterminally and there are no chiasmata at the proximal side, as is the case in Fritillaria meleagris. If bivalents at metaphase were associated only terminally, the separation is easily achieved (fig. 30). If, however, the bivalents were held together interstitially by one or more chiasmata at metaphase, the separation is more difficult, in accordance with the number and type of chiasmata (Moffett 1932a). If every bivalent of the same complex has the same chiasma arrangement and is of the same size, the mechanical difficulty can not be detected because the bivalents separate simultaneously/

Fig. 29.



Bivalents at metaphase in Fritillaria imp. (A) and in Scilla (B-). The attachment constrictions show repulsion.

Fig. 30.

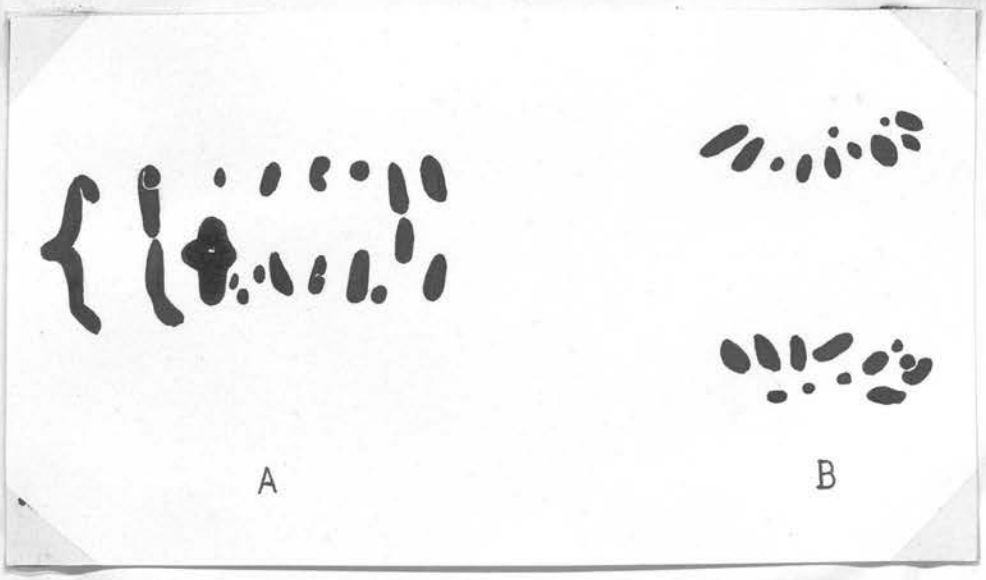


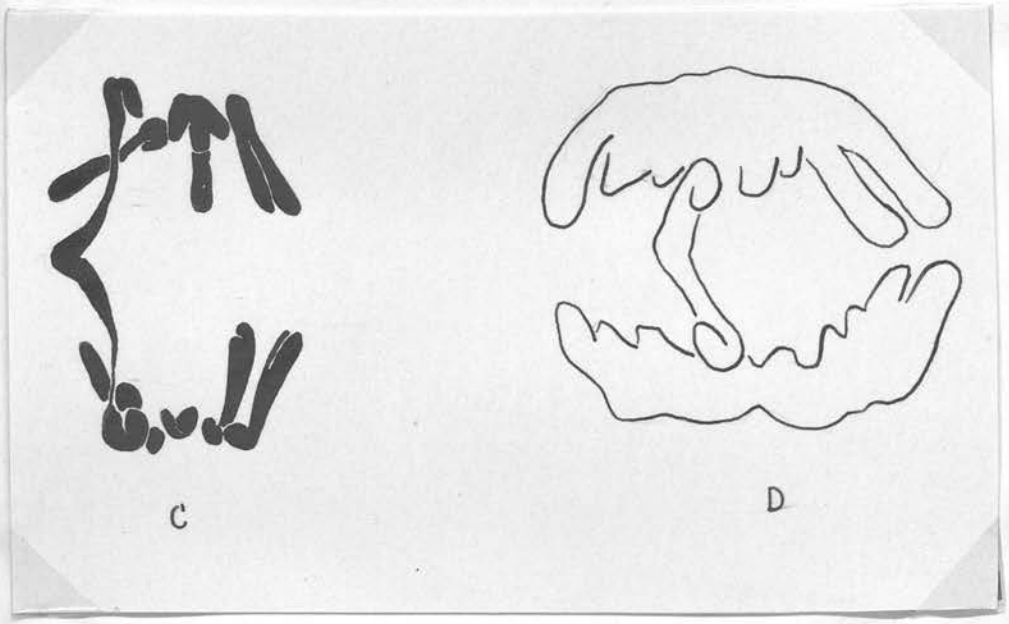
Small, terminally associated bivalents of Linum separate easily at anaphase.

ly. If among the bivalents one or more have only terminal association, while others have several chiasmata, they will separate precociously, as shown in fig. 27; while in the opposite case, when interstitial chiasmata are present only exceptionally, these bivalents will be left behind the others (Sansome 1932) (fig. 31).

The first separation of bivalents at anaphase produces a certain distance between the bivalents and they will remain in this position for some time. After that, the separation continues until they are completely segregated. This process was observed very well in the rat and mouse, in which the chromosomes are of about equal size. In some cases the separating bivalents will reveal their structure. In longer ones the presence of two chromatids in each chromosome is seen very well, but in small ones it is easily obscured. From the appearance of the separating bivalents, it is possible to infer the nature and type of chiasmata in the previous metaphase. Fig. 32 illustrates the compensating and non/

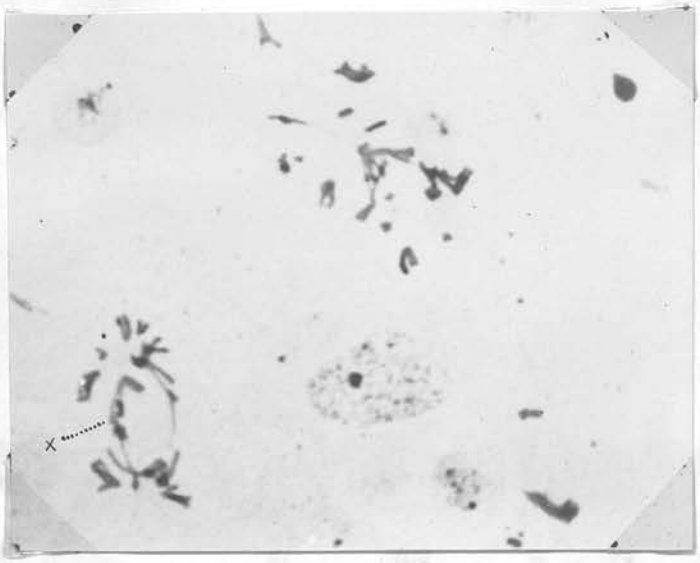
Fig. 31.





Separation of bivalents at anaphase. A: Locusta, B: Schistocerca, C: Crepis, D: Batrachoseps. Long bivalents with interstitial chiasmata lag behind the others.

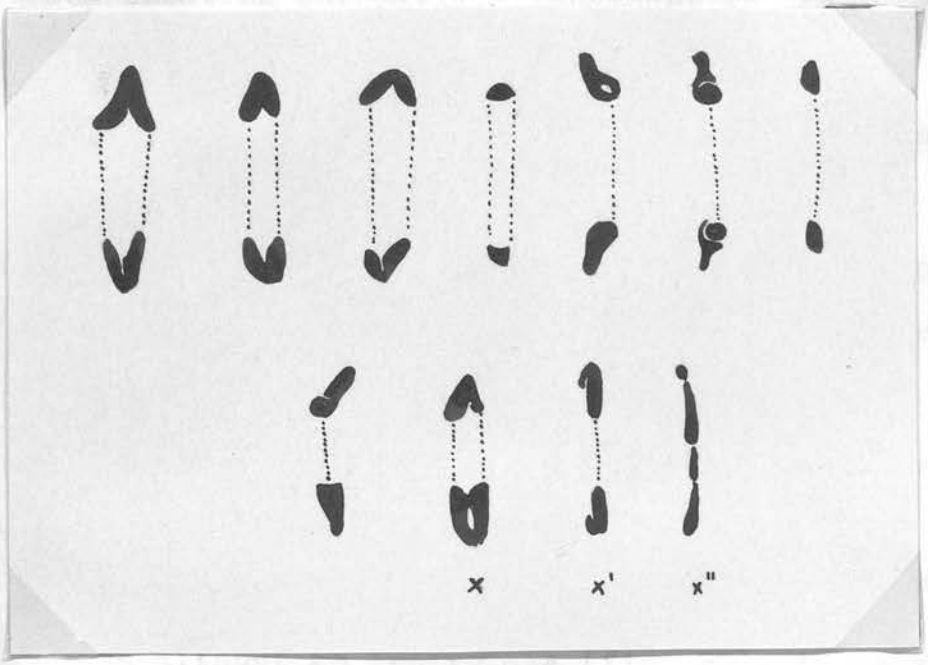
Fig. 31.



E

Microphotograph of meiotic anaphase in Stenobothrus. The long bivalents lag. They have had many interstitial chiasmata at metaphase and in consequence lag at anaphase.

Fig. 32.



Bivalents during anaphase separation in the mouse. The type of compensating and non-compensating (x) chiasmata can be seen. The X-Y chromosomes are indicated by an x''.

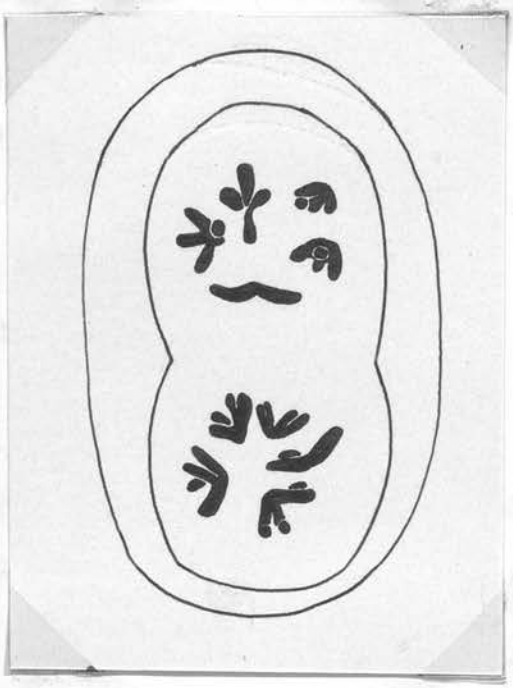
non-compensating chromatid arrangement in the separating bivalents of the mouse.

The two chromatids of the separating bivalents are held together very closely at the attachment constriction. This was found in the secondary spermatocytes of Marsupials and could be followed very well in Crepis. The possible cause of such chromatid behaviour at the first meiotic anaphase will be dealt with in the following part.

The chromosomes usually pass into the second metaphase without an interphase (fig. 33), in which case the length of the chromosomes remains the same as it was during the first anaphase. If, however, an interphase follows, the chromosomes in the next division will be of normal mitotic length. (Tulipa)

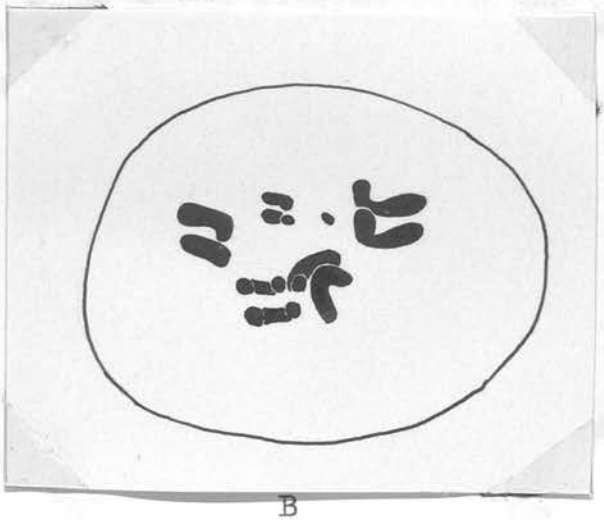
By the completion of the second division the numerical reduction of chromosomes is achieved. This is normal mitotic division, except that the prophase is omitted when there is no interphase. Numerically the division is an equational one, but genetically it may be reductional for segments of chromosomes. It should/

Fig. 33.



A

First anaphase stage in Crepis. The chromatids are held together at the attachment constriction only.



B

Second meiotic metaphase in Dasyurus. The daugther chromosomes are already separated.

should be mentioned that at the point of attachment the second division is always equational, showing that the first division at this point is always reductional (Sansome and Philp 1932).

(2) Dynamic interpretation.

(i) Attraction. At the beginning of meiotic prophase the chromosomes are present as single threads. They are fine and very thin in structure and represent the gene string alone without the matrix. Having no bulk, they are exempt from the action of contraction if such is present during leptotene and zygotene. At pachytene there is complete association, which was studied by the author in Crepis and Dasyurus (vide supra). The cause of this association is attraction which operates between the corresponding constituents of a pair of chromosomes. The linear differentiation of the chromosomes was studied by the author in Marsupials and Locusta , where it is very well marked by the differences in the chromomeres. The corresponding chromomeres/

chromomeres of the single leptotene threads come into contact at zygotene and pachytene (fig. 14). The chromosomes which yield to the force of attraction are of the same length and have the same chromomere arrangement exhibiting the same linear differentiation. This is a proof that the chromosomes which are represented twice in a diploid complement have come into contact. These chromosomes are homologous, their structural constituents and chromomeres being similar. The force of attraction can operate only between chromosomes which are identical in every respect, including the physico-chemical constitution of the genes. Their homology causes them to associate in pairs. In a normal diploid always two and only two single chromosomes will exhibit such conformity of their internal and external structure (Darlington 1932).

In structural hybrids one or more chromosome segments are inserted by translocation within a non-homologous chromosome. At this segment the chromosome may associate, but the mode of association will be/

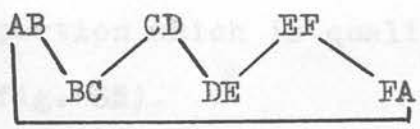
be altered. The chromosome which contains the translocation will associate with its normal partner up to the point of translocation, and at that segment it may associate with a third chromosome which is the 'donor' containing a segment of similar constitution. Such a case was found in Rhoeo discolor. By segmental interchange (reciprocal translocation) the entire chromosome complement underwent no structural changes. If the original complement be represented so

AA BB CC DD EE FF

by segmental interchange it will become

AB BC CD DE EF FA

Such a structural change would result in an association of chromosomes at metaphase into a closed ring :



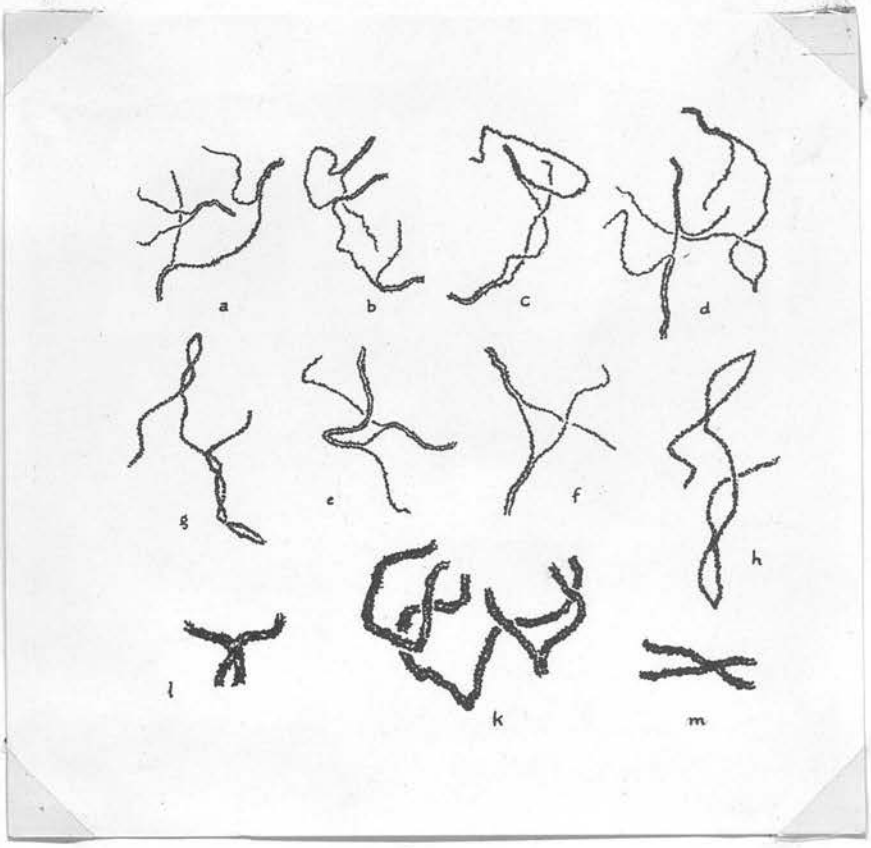
The ring arrangement was seen in Rhoeo discolor (Kato 1931, Sax 1931, Koller 1932). Similar cases were found in Oenothera (Gates 1928), Datura (Blakeslee 1929), Pisum (Sansome 1932), Drosophila (Dobzhansky 1931) and other species. At pachytene parasynaptic/

synaptic pairing of three chromosomes was observed by the author in Rhoeo (fig. 34). The length of the paired segments was seen to be different, a proof that parts of different length may participate in segmental interchange.

Further proof that only homologous segments are capable of coming into association is the pairing of unequal chromosomes. In Tradescantia some chromosomes include segments the length of which is equal to that of a short chromosome. Such a short chromosome will associate along its entire length with the segment of a long chromosome which is homologous with it. Similar cases were observed in the mouse, in which X and Y chromosomes, although differing greatly in size, yet pair at pachytene; the X must therefore contain a portion which is qualitatively equivalent to the Y (fig. 35).

In structural hybrids (e.g. Tradescantia) fragments are sometimes found in the complement. They are derived from chromosomes and will associate laterally with the long chromosomes. Such pairing of fragments/

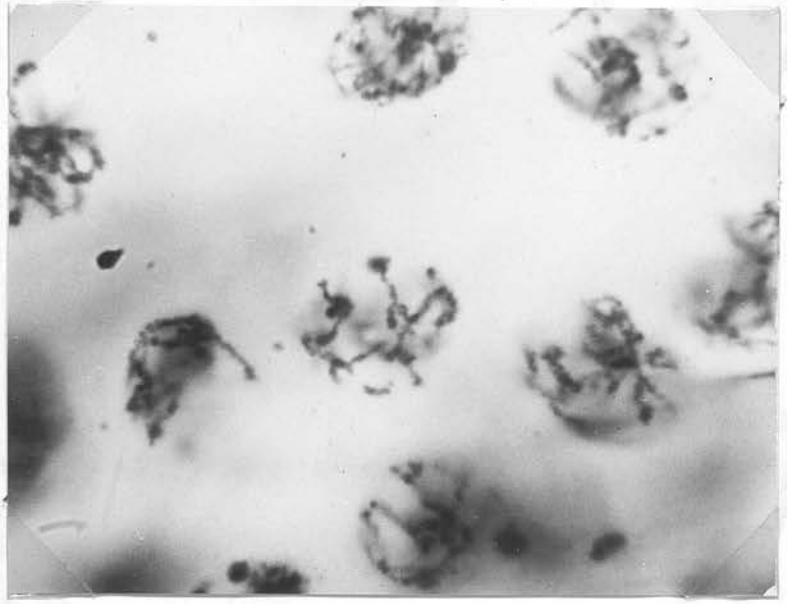
Fig. 34.



A

Pachytene pairing in Rhoeo. Three chromosomes are associated owing to segmental interchange between non-homologous chromosomes.

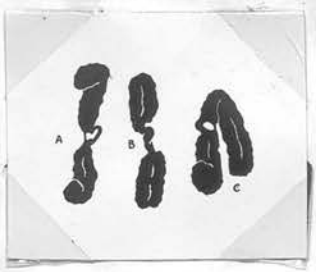
Fig. 34.



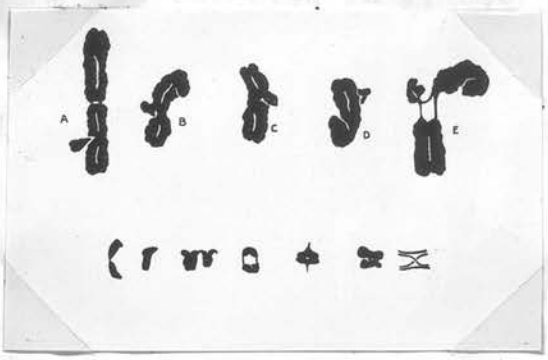
B

Microphotograph of pachytene stage in Rhoeo. The association of three chromosomes can be seen.

Fig. 35.

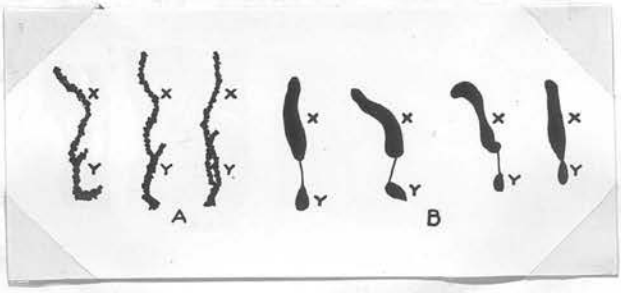


1



2

Bivalents at metaphase in Tradescantia.
1. Bivalents with unequal chromatids, 2. Pairing of fragments with bivalents. The loci of association are different.



3

3. Pairing of the unequal X and Y chromosome in the mouse. A: pachytene - diplotene, B: metaphase.

fragments with chromosomes was observed in Tradescantia virginiana var. humilis. The place of pairing is usually interstitial and the same fragments were observed to participate in pairing with different chromosomes. This latter fact shows that they are aggregates of pieces derived from more than one chromosome. (fig. 35).

The facts just stated provide evidence that pairing is effected only between homologous constituents. If two single chromosomes are similar in their entire constitution, they will associated completely along their whole length: internal homology is a condition sine qua non for chromosome pairing. The association of two chromosomes either totally or partially can be arrested when there are differences in their internal structure. This is mainly due to some new arrangement in the chromosomes brought about by translocation, inversion or by segmental interchange; discussed in detail by Beadle (1933) and Emerson and Beadle (1933).

The differences in character expression must undoubtedly/

undoubtedly be correlated with some structural differences in the constitution of the gene. These differences are most probably molecular in nature and therefore they must represent only very minute dissimilarities in the constitution of the chromosomes, for although allelomorphic genes should not pair, they will nevertheless associate owing to the homology of the adjacent loci on either side (Catcheside 1932). If these minute differences are enhanced by the introduction of other allelomorphs at the adjacent loci, the pairing may be disturbed; this can be detected genetically, as in the case of Drosophila (Serebrovsky, Ivanova and Ferry 1929).

It is clear from the above discussion that when two single chromosomes associate in pairs in a diploid, they are yielding to the force of attraction which operates between homologues, whether they be genes, chromomeres or chromosomes. Hence, the association of sister chromatids during mitotic prophase, and that of homologous chromosomes during meiotic/

meiotic prophase are both produced by the same force.

(ii) Polarisation. The leptotene threads are sometimes polarised (Dendrocoelum, Stenobothrus), i.e. they turn one or both ends towards one side of the nucleus. Such direction is always found in the neighbourhood of the centrosome and it is highly probable that polarisation is an outcome of a special attraction between the nuclear pole, or centrosome, and chromosome ends. The attachment constriction is located at the end which usually turns first towards the nuclear pole (Gelei unpublished, Wenrich 1916, 1917). Some observations made by the author on Stenobothrus supply evidence, however, that the attachment constriction is not responsible for polarisation, because the loci of the attachment constriction are median or subterminal and it is the chromosome ends, and not the segments carrying the attachment, which turn towards the nuclear pole. Such orientation of the chromosomes shows that there exists a special relation between the ends of the chromosomes and the centrosome. Most probably it is an/

an adaptation to secure effective pairing between homologous chromosomes. In Stenobothrus the nucleus is small, containing 16 homologous leptotene threads some of which are very long, representing chromosomes which during mitotic metaphase are 13, 11.8, and 9,2 μ long respectively (Darlington and Dark 1933).

The pairing of homologous members at meiotic prophase is made certain by such an arrangement as polarisation. This property is peculiar to the species and must have been evolved by selection, being most probably genetic in origin. There are many other species, however, in which, though the conditions are very similar, there is no polarisation of the leptotene threads. Therefore the attraction which operates between the ends of chromosomes and the nuclear pole is not a general but a specific dynamic factor.

(iii) Pairing blocks. The association of two single homologous chromosomes at zygotene was traced step by step in Dasyurus and Sarcophilus. It was found that pairing commences first at one locus and/

and always includes a short section of the chromosome. The ultimate unit of pairing is not one chromomere, and consequently not only one gene, but a group of chromomeres and genes. The force of attraction located in the homologous genes and chromomeres immediatly spreads to a number of neighbouring chromomeres, until a'block'is formed. The process may be repeated but the number of such blocks along the chromosome is limited. In Dasyurus the leptotene threads usually associate in one, two or three short sections and from these centres pairing proceeds in both directions, until the association between the entire chromosomes is completed. It has not been possible to obtain more details concerning the mode of pairing, owing to the difficulty of discriminating homologous leptotene threads along their entire length.

The fact that the homologous chromosome threads are associated at two or even more short sections simultaneously excludes the a priori assumption that pairing must be due to the special force of attraction located at the attachment constriction. The behaviour/

iour of the attachment constriction demonstrates its importance as a kinetic factor and it represents the focus of manifold dynamical activity, while the attraction is dispersed throughout the whole body, including homologous constituents.

The ultimate structural unit of pairing is the chromomere, but when one chromomere comes into association with its homologue, the adjacent chromomeres at once participate in the pairing; hence, in suitable material the zygotene association was always seen to include a longer segment. The chromomeres during zygotene pairing act in groups, as could be observed in Dasyurus and Sarcophilus. This method of pairing is probably primarily responsible for chromosome behaviour not only in diploids but also in polyploids. Darlington and Mather (1933) put forward the hypothesis of 'pairing blocks' from a study of chiasmata in polyploids. They discuss the conditions which control the number and extent of the pairing blocks in triploid Hyacinthus and demonstrate that chiasma formation in polyploids depends/

depends on zygotic pairing.

If the chromosomes pair at different and several sections during zygotene, their movements will be greatly hindered if they are long and the nucleus relatively small. The homologous chromosomes will find mechanical difficulties in pairing since other chromosome threads may lie in their way, and this will result in interlocking during pachytene and the following stages. Interlocking, however, is comparatively a very rare phenomenon in normal meiotic division of diploids, and polyploids. To explain the rarity of interlocking in plants when there are numerous long threads in zygotene, we must assume that the number of pairing blocks is inversely proportionate to chromosome length; pairing commences at a few sections only, perhaps three, two or even one, if the chromosome is very long. Another factor which will eliminate interlocking is repulsion, which comes into being at the point of interlocking and prevents the completion of pairing at the distal ends (Darlington and Mather 1933). This phenomenon was actually observed by the author in Dasyurus.

(iv)/

(iv) Attachment constriction and association.

In mitosis during prophase sister chromatids, derived from one chromosome, are in association. The dynamical cause of this association is the attraction between the homologues. At zygotene and pachytene the same force of attraction is in operation, causing a complete association of homologous chromosomes. There is, however, a great difference between these two types of association. In the first case, the force of attraction operates between sister chromatids and only one attachment constriction is present; in meiosis the force of attraction operates between two homologous chromosomes and two attachment constrictions are present. It was already pointed out that the splitting of a single attachment constriction into two homologous ones at the end of mitotic metaphase evoked a strong force of repulsion which was primarily responsible for the separation of daughter chromosomes.

In meiosis there is present an association of two homologous attachment constrictions, but this fact/

fact does not constitute a contradiction, since it may be explained by other differences in association.

The prophase chromosomes at mitosis are already divided and consist of two chromatids, while the attachment constrictions, as was shown, remain undivided. This is a characteristic phenomenon at mitosis, both in plants and animals and was clearly traced by the author in Vicia, Tulipa, Euchorthippus , and Locusta.

The repulsion at the attachment appears only at the end of metaphase where the force of contraction rises nearly to its maximum and chromosomes are thick solid and more or less rigid. After metaphase there is no great contraction, as was already stated, and this suggests that contraction is not needed after metaphase, or at least is not so important as it was before. Therefore it is not improbable that repulsion, although conditioned by the splitting of attachments, can operate only upon a definite structure produced by contraction, and we may assume that the attachment/

attachment does not split because the degree of contraction is as yet insufficient for the operation of repulsion.

In meiosis two single chromosome threads, each with an attachment constriction, will pair at zygotene-pachytene, while at leptotene, as was already mentioned, there is no contraction. The threads are long and fine, and although two homologous attachments are present at pachytene, repulsion can not operate since the required size and structure of the chromosome body has not yet been attained. When the paired chromosomes become thicker through contraction and only then, will the force located at the attachment constriction be able to come into action. At the commencement of pachytene contraction is below the required level, but at the end of that stage it is increased and splitting takes place. This process profoundly affects the interrelation of the paired chromosomes. Each of the paired prophase chromosomes is composed of two sister chromatids which must repel each other in accordance with the general law of pairing/

pairing outlined above. The specific repulsion between two attachment constrictions perhaps also comes into operation at the same time, since, as a result of contraction, the chromosomes have attained that bulk upon which it can most efficiently act. However, there exists evidence to show that if this forces is in operation, it is not very powerful. At early diplotene the loops are of the same size in several cases while one would expect the attachment loops to be larger since they are subjected to the influence of two forces, viz. repulsion between pairs of chromatids and that between two attachment constrictions. Such size variation will be seen only later, in diplotene and diakinesis. Before, however, the separation of homologous chromosomes can be completed as a result of the two forces mentioned above, an exchange of partner chromatids will take place and will prevent their complete separation.

The difference between pairing during meiotic and mitotic prophase may also be explained on the basis of the differences between the paired units.

In/

In mitosis two chromatids, in meiosis, two chromosomes are in association. Apparently in meiotic pairing attraction is a stronger dynamical factor because it operates between two single chromosomes and therefore it is easily able to overcome the repulsion between the two attachment constrictions which perhaps already exists at this stage. On the other hand, the elements upon which the force of attraction acts during meiosis and mitosis are entirely equivalent in their internal genetic constitution and therefore such a difference in the strength of attraction does not appear very probable. All evidence seems to favour the former assumption.

(v) Contraction. Pairing is completed at pachytene as a result of attraction acting between homologous chromosomes. The paired chromosome threads decrease in length and become thicker. The decrease in diameter is not merely the outcome of the association of two threads but is caused by contraction, which reduces the length and simultaneously increases the thickness of the chromosomes. At zygotene, there are/

are paired or partially paired segments of chromo - somes, but they never show the same appearance as the threads of late pachytene. At the commencement of this stage contraction is very slight (Belling 1931) and not always easy to detect. Towards the end of pachytene, the length of the paired threads decreases considerably; this decrease has been estimated in Dasyurus and Sarcophilus :

Table VIII.

Leptotene threads (approximate)	17-20 μ
Pachytene (early)	14-16 μ
(late)	10-12 μ
Metaphase bivalents	4- 8 μ

Another proof of the operation of contraction is given in fig. 24b, where the different stages of pachytene in Schizocerca may be discerned. The paired threads stain much better in late pachytene, as a result of greater condensation which is correlated with contraction. The pachytene stage is the longest during meiotic prophase, and if the associated chromo- somes/

somes undergo profound changes or become temporarily invisible, they do so during this stage (Wilson 1925). Such accessory changes have been observed during oogenesis of the fowl. The paired chromosomes in the developing egg assume a characteristic shape and remain in this condition for a long time until the egg has greatly increased in size.

Other phenomena have been observed during pachytene in the Marsupials and in Crepis. The paired chromosome threads are obviously twisted one around the other at various points. This must be a consequence of zygotic pairing, association having taken place at opposite sides of the chromosome threads. This condition is not very easy to detect during early pachytene because the chromosome threads are very thin, but as contraction advances, the twisted arrangement can be seen much more clearly.

Though the force of contraction operating during meiosis is the same as that which operated during mitotic prophase, nevertheless in the latter case it is acting upon chromosomes already doubled by the secondary/

secondary split, whereas during meiosis they are undivided. In both cases it is most probably the attachment constriction which is the focus of this force. The result of the contraction is a coiled spiral structure, both in chromatids and chromosomes.

In plants (e.g. Tradescantia) the spiral structure was observed at meiotic prophase and later at metaphase. The author, however, found in Marsupials that the spiral structure was definitely a result of zygotic pairing and was merely accentuated by contraction. Therefore the structure can not be equivalent to the spiral of the chromonema described in mitosis. Signs of spiral structure have been observed in Tulipa at pachytene, but its origin is doubtful.

The great decrease in length and increase in diameter of the paired threads are sufficient evidence for the assumption of the existence of contraction, a force which operates axially and independently in the paired chromosomes.

(vi)/

(vi) Splitting. The secondary splitting of paired chromosomes commences at the end of pachytene. This assumption is based on the fact that in the following diplotene stage, the chromosomes reveal a quadruple structure, each being composed of two sister chromatids. In Scilla at late pachytene the opening out of the secondary split was clearly visible. Belling (1933), from observations on most carefully prepared material and living cells, showed that the splitting must have taken place at late pachytene, and a similar observation was made by Gelei (1922) in Dendrocoelum.

Dasyurus, Sarcophilus and Scilla are very suitable material to study the conditions at late pachytene and at the beginning of diplotene. The opening out of the secondary split could be seen to occur at several points along the chromosomes; where the chromomeres are sufficiently large, it can be seen that their number is three or four instead of the normal two. The opening out of the secondary split extends to three or four chromomeres at least. The bilobe/

bilobe nature of the divided chromomeres could not be detected, most probably through fixation and staining obscuring them, if present. The split proceeds from several centres along the chromosome and most probably at the end of pachytene the entire chromosome or chromomere complex has divided into two.

Only by this secondary splitting of the chromosomes into two sister chromatids is the same condition which was present at the beginning of mitosis attained. There are two possibilites as to the cause of the difference between the two divisions. (a) Either the contraction is a precocious process during meiosis and precedes the secondary split; or (b) Splitting is a delayed process which allows the force of contraction to exercise its action first. The former theory was elaborated by Darlington (1930) in his 'Precocity Theory of Meiosis', while the second is upheld by Huskins (1932).

The splitting is essentially a process of growth, both in mitosis and meiosis. The disturbed physical and chemical equilibrium which reacts upon the whole activity/

activity of the cell can be restored only by the division of the cell and its constituents. Whilst in mitosis the splitting and division of chromomeres takes place at the resting stage long before contraction occurs, during meiosis the splitting is preceded by contraction and pairing of homologous chromosomes. The ultimate cause of this change in time relation between mitotic and meiotic divisions is most probably a genetical adaptation, brought out in the process of evolution and associated with sexual reproduction. The discussion of this process would lead us upon very hypothetical grounds and we will therefore not deal with it here.

As a result of the splitting, the chromosomes have to separate. The previous pairing of homologous chromosomes, however, will complicate the simple process of chromosome and chromatid separation The association and pairing during division is always between only two homologous elements and never more, even in polyploids (Darlington 1932). This law of pairing was deduced from several observations on/

on meiosis in diploids and polyploids, which permit of no other interpretation. Therefore, when the longitudinal secondary splitting is completed, four chromatids are present, but, in accordance with the law, only two sister chromatids can remain in association. The observations which were recorded in the previous section illustrate clearly that the pair of paired chromatids repel each other and separate at diplotene. In the small bivalents of Chorthippus and Stenobothrus repulsion is very effective and separates the chromosomes, now each composed of two chromatids. The result is that the small bivalents are widely separated at the distal segments.

(vii) The cause of chiasma formation. The bivalents will obey the innate force of repulsion operating between pairs and repel each other. A complete separation, however, can not be achieved by repulsion, for they are held together at one or more points called chiasmata. The chiasmata are responsible for the diplotene and post-diplotene association Without chiasma the chromosomes would fall apart, as usually/

usually do those segments which have no chiasma. If two or more chiasmata are formed, the pair of chromatids between them will be repelled and form loops.

There are two hypotheses which offer an explanation of chiasma formation : (a) the chiasmatype theory of Janssens (1909 1924), and (b) the classical theory (Wenrich 1917, Sax 1930, 1932). The former assumes that chiasmata are the loci of actual exchange between partner chromatids and represent genetical crossing-over. On the classical theory, chiasmata are formed by the opening out of chromatids in different planes which gives rise on one side to an equational and on the other to a reductional loop. On this hypothesis chiasma precedes genetical crossing-over.

The origin of the chiasma can be explained more easily and more probably on the first hypothesis, as follows. The force which precedes chiasma formation is torsion. This must be produced by the attachment constriction and is related to contraction

The/

The ultimate cause of torsion is the operation of two contractions, one in each paired chromosome, acting independently of the attachment constriction. The chromosomes offer a resistance to the force of torsion, although they will yield to it to some extent but only by twisting. The torsion is unable to break the chromosomes, which would release the strain, for the chromosomes have accumulated sufficient strength in their body by contraction to resist such damage. At the end of pachytene, when the time to divide has arrived, the paired chromosomes are under the dynamic influence of attraction and independent contraction, which cause torsion. The thin chromatids derived from the secondary split will suffer under the influence of torsion, being incapable of offering the same resistance as the chromosomes since they are very delicate (as seen in Scilla and Hyacinthus).

At the actual moment of the split the force of torsion will overcome the resistance and break the chromatids at several loci. A slight twist at the breaking/

breaking point will relieve the strain and the threads will move in the direction of the torsion. The result will be that partner chromatids will come into contact and will reunite by fusion. Meanwhile the repulsion which operates between pairs of paired chromatids will come into action and repel the chromosomes. The repulsion, however, can be effective only at segments where there was no break and no subsequent fusion of partner chromatids. At the point of actual exchange the chromosome bivalents will be held together by chiasma or chiasmata which represent the break and consequently the genetical crossing-over.

The classical hypothesis postulates that the strain caused by torsion will be relieved by the opening out of the sister chromatids. The break occurs later when other strains will be imposed upon the chiasmata either by further opening out of the internodes or by unequal contraction of the chromonemata, or by a slight amount of twisting of the chromonema as it coils. The break occurs at the s

same/

same locus of homologous partner chromatids owing to their close association. The free ends of broken chromatids then pair gene by gene with the intact chromatids until the broken segments of the different chromatids are brought into contact. With this assumption Sax (1930) explained the origin of nodes and inter-nodes found at diplotene between paired chromosomes, and the decrease or disappearance of chiasmata from diplotene towards metaphase.

A hypothesis which was recently elaborated by Belling (1933) to explain the origin of crossing-over, is closely related to the chiasmatype hypothesis; it is based on direct observation. He suggests that the opening out at early diplotene is always at the primary split and first at many points. The seondary split occurs at pachytene, giving rise to two equivalent chromioles from one chromomere. Belling argues that the supposed break and fusion of partner chromatids do not occur because the breaks in the two chromatids ought to correspond with an accuracy of less than half a micron, which is the distance between/

between two chromomeres from centre to centre. The breaks are due to tension of torsion. This force would make a simultaneous fusion after the break very improbable. The chiasmata are due to an overlapping of the chromatids and not to the twist. The crossing-over is produced by the development of new connective fibres between chromioles which, owing to the overlap, come to lie near each other.

As a working hypothesis the chiasmatype theory is the most useful and on this hypothesis it is possible to correlate directly cytological and genetical observations. Janssens' and Belling's hypotheses assume that the post-diplotene association of bivalents is due to chiasmata and that crossing-over is a prerequisite for such an association. On Sax' hypothesis the post-diplotene association depends on chiasmata which do not break, i.e. crossing-over is a consequence of chiasma formation.

The theories outlined above give a more or less definite answer to the question as to why the homologous chromosomes remain further in association aft after/

after they have split into two chromatids. The general repulsion separates two bivalents as far as this is possible. The force which operates between the body of the bivalents will be accompanied by another force of repulsion which operates between the two attachments, because at the end of pachytene the chromosomes have attained the optimum degree of contraction and the specific force of repulsion between the two attachments can be released. The interaction of this force causes a difference in the size of the loops formed, the largest (usually at late diplotene or diakinesis) always being those which include the attachment constriction.

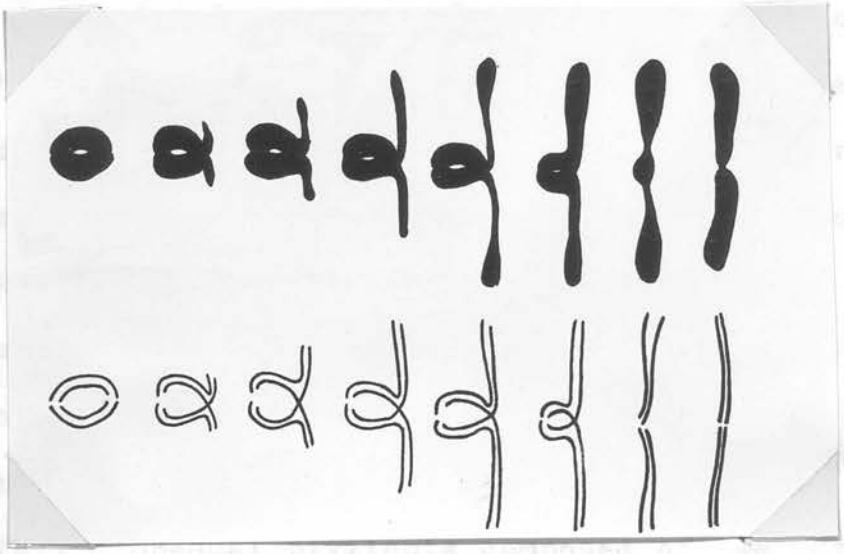
The double structure of attachment constrictions of the separate bivalents at diplotene and metaphase was not found; they exhibited only a single undivided attachment constriction : whilst four chromatids are present, the number of attachment constrictions is only two. The single structure of the primary constriction may be seen very well at the first anaphase when the bivalents separate; the sister chromatids/

atids lie apart except at the attachment, where they are held together in an intimate association.

(viii). Terminalisation. The force of repulsion which acts between pairs of paired chromatids is a general one. The other specific repulsion, as was already described, is located at the attachment and operates between the corresponding primary constrictions. The chiasmata will offer a resistance to the two types of repulsion. They may overcome them entirely and remain stationary, as in the case of Fritillaria meleagris; or they may yield to them, as a result of which the chiasmata will move towards the distal end of the chromosome. This movement of chiasmata away from the attachment is termed terminalisation (Darlington 1932a). It explains the decrease in the number of chiasmata from diplotene to metaphase. The chiasmata do not cancel each other by fusion but the distal chiasma slips off at the end first, while the second remains but moves towards the end. This movement was observed in the mouse (fig. 36). The origin of the different forms of bivalents at metaphase can be explained by terminalisation.

On/

Fig. 36.

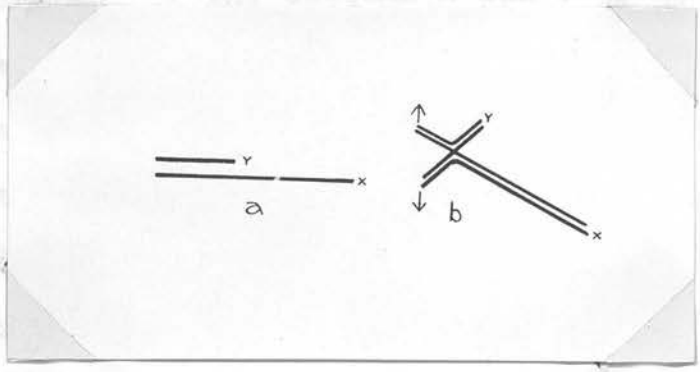


Different types of metaphase bivalents in the mouse as resulting from terminalisation. Under each bivalent is given the probable interpretation of chromatid constitution.

On Darlington's hypothesis, terminalisation is a consequence of the repulsion between attachments and pairs of paired chromatids. On Sax' hypothesis, the decrease in the number of chiasmata is attained by their breakage. In the following part further proofs will be presented by the author to show that the chiasmatype hypothesis of Janssens as elaborated by Darlington, can explain the cytological phenomena, in particular the decrease in the number of post-diplotene chiasmata.

(a). Unequal bivalents composed of two chromatids different in size were found at metaphase in Tradescantia, and pairing of unequal sex-chromosomes was observed in the mouse at diplotene (fig. 35). The X and Y chromosomes pair and associate sometimes by chiasmata; the chromatids exchange at the chiasmata and the prophase pairing gives rise at metaphase to bivalents consisting of unequal chromatids. (Fig. 37 illustrates the pairing and interchange in the case of unequal chromosomes. Commonly in metaphase of the mouse, where the number of bivalents is twenty the/

Fig. 37.



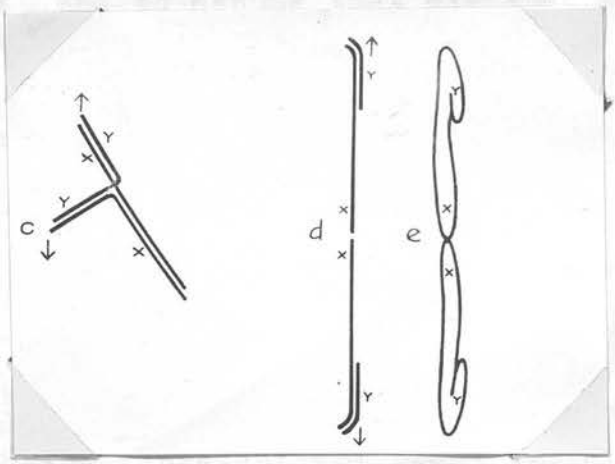


Diagram illustrating the pairing of unequal chromosomes (X-Y) in the mouse by interstitial chiasma.

the association of the unequal X and Y could not be recognised. In several first spermatocytes, however, bivalents with one long and one short limb were observed at the commencement of anaphase (fig. 32). These two facts together with the observation that precocious separation and lagging of the unequal sex chromosomes is relatively infrequent (Cox 1926) rather permit one to assume that exchange of partner chromatids takes place during prophase and obscures the great structural inequality at metaphase. At the second anaphase the chromosomes with unequal chromatids will divide and give rise to two types of spermatids, one with the long X and the other with the shorter Y, in equal numbers. In this case the second anaphase represents the reductional division for the X and Y.

The exchange between the unequal sex chromosomes eliminates the qualitative differences in the pairing segments, though they do not destroy the quantitative dissimilarity. It may be concluded from the observations that a quantitative difference between sex/

sex chromosomes exists in the mouse, a view which is in close agreement with the genic balance theory of Bridges (1925). If the X and the Y differed in their internal structure, i.e. were non-homologous, then the maintenance of this qualitative dissimilarity would be a prerequisite of sex determination and they would not pair during meiosis. Since, however, the association of X and Y by chiasmata was observed, this proves that Y is homologous with a particular segment of the X.

The association of unequal chromatids at metaphase and the previous chiasma formation between unequal chromosomes is a proof that the breakage or crossing-over precedes chiasma formation. The first fact was observed in Tradescantia (fig. 38), the second in the mouse (figs. 35 and 37).

(b). Another proof was found in the interlocking of bivalents during diakinesis in Tulipa and the rat, the latter having been subjected to X-rays. Such interlocking at diakinesis or metaphase requires a break between partner chromatids and can not be explained/

Fig. 38.

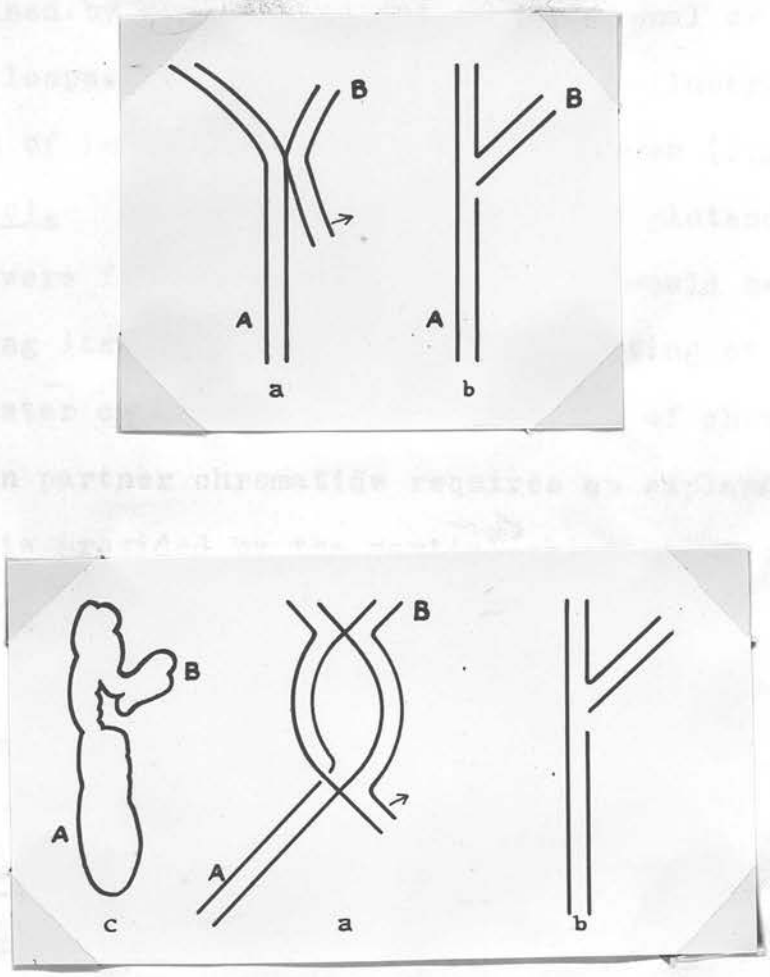


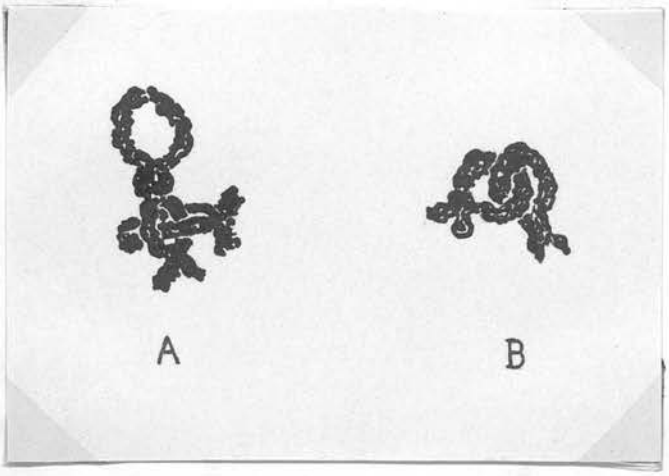
Diagram illustrating the pairing of a/ fragment with a/ chromosome at diplotene and metaphase in Tradescantia. By the formation of one or two interstitial chiasmata, chromosomes containing unequal (c) chromatids will result. Type c was illustrated in fig. 35. B.

explained by the opening out of equational or reductional loops. The following diagrams illustrate the method of interlocking on both hypotheses (fig. 39).

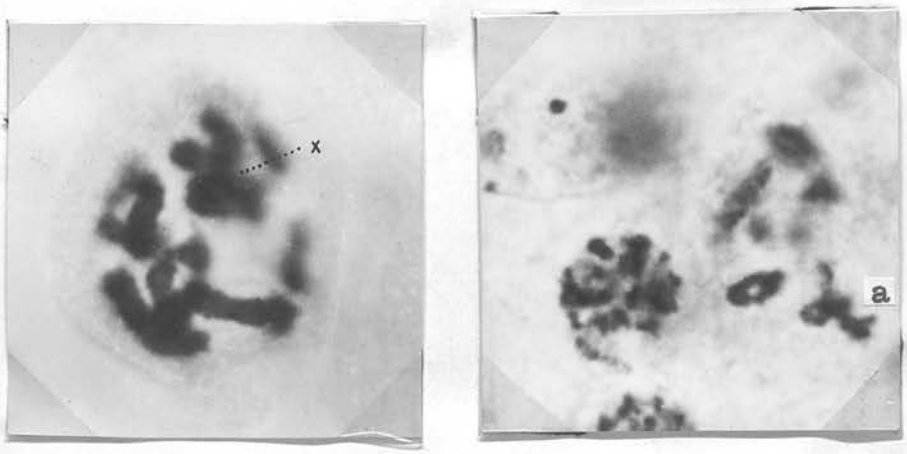
(c). In Schistocerca gregaria diplotene bivalents were found where each chromatid could be followed along its entire length. The twisting of associated sister chromatids and the presence of chiasmata between partner chromatids requires an explanation, which is provided by the partial chiasmatype hypothesis. The opening out of chromatids into different planes where there are twists of sister chromatids would meet with a great mechanical difficulty, as illustrated in fig. 40.

(d). A further proof is the exact correlation of chiasma frequency at diplotene and metaphase with that of crossing-over, as found in the male and female mouse. The following table illustrates chiasma frequency at diplotene and metaphase in the female and male mouse respectively (table IX). The animals were selected from the same stock and were of the same genetic constitution. Chiasma frequency of 6 weeks old/

Fig. 39.



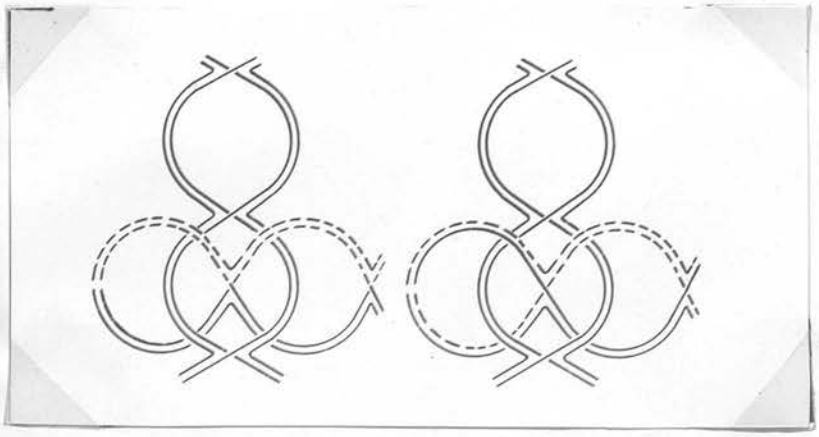
Interlocking of bivalents at diakinesis in Tulipa (A) and in the rat (B).



C D

Microphotograph of diakinesis in Tulipa (C) and in rat (D) showing interlocking of bivalents.

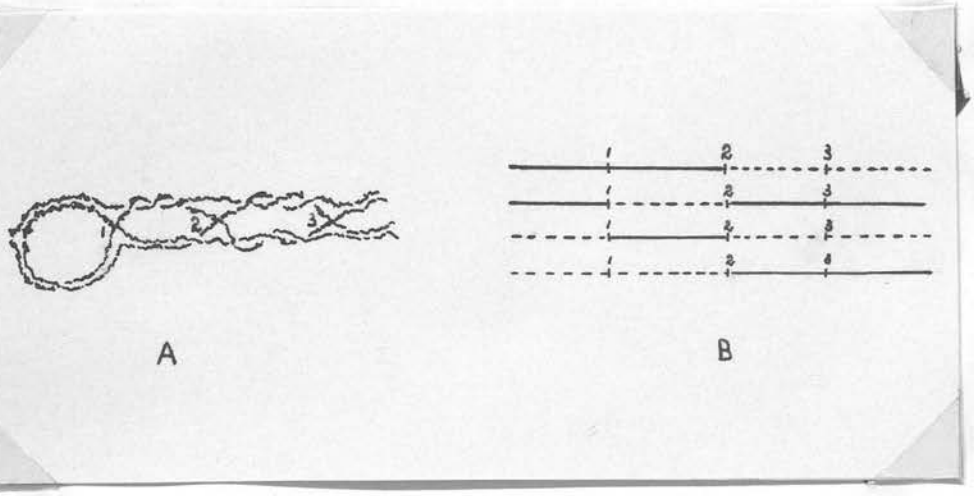
Fig. 39.



E F

Diagram illustrating the method of interlocking on Janssens' (E) and Sax's(F) hypothesis. The latter interpretation is inadequate since it requires a special break, occuring before interlocking.

Fig. 40.



Diplotene bivalent in Schistocerca, kept at low temperature. The sister chromatids twist around each other rendering an opening out mechanically difficult or impossible. The constitution of chromatids resulting from crossing over is illustrated.

Table IX.

Chiasma frequency of the female and male mouse at diplotene and metaphase.

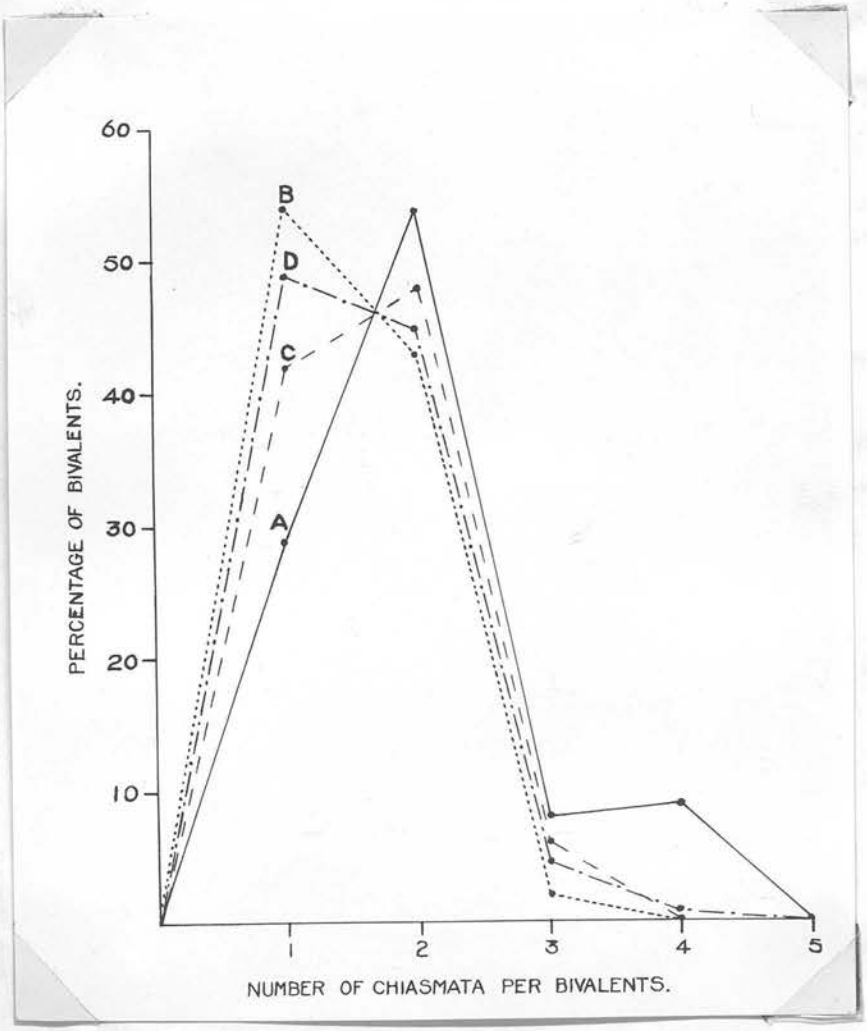
Sexes	Number of bivalents with						Total No. of chiasmata	Total term. chiasmata	Mean X/biv.	Term. coeff.
	1 X	2 Xta	3 Xta	4 Xta	5 Xta	6 Xta				
Female	8	10	11	8	3	2	120	39	2.8	0.32
Male	7	38	35	5	-	-	208	78	2.4	0.33
Female	29	54	8	9	-	-	197	86	1.97	0.43
Male	55	43	2	-	-	-	144	100	1.47	0.67

I = chiasma; Term. = terminal; X/biv. = chiasma per bivalent; Term. coeff. = terminalisation coefficient.

old male was found to be 1.64, the terminalisation coefficient being 0.61. The percentage of bivalents with two chiasmata in the young is higher than in an older one. This is what would be expected from the observations made by Dunn (1920), who reported slight decrease of crossing-over with advanced age in the mouse. Cytologically this may be explained on the assumption that the two chiasmata are of the reciprocal type and give rise to two recurrent double cross-overs and two non-cross-overs, that is, the second chiasma restores the original constitution (fig. 19). Chiasma frequency in the female and male mouse is illustrated in fig. 41.

The difference found in chiasma frequency is seen to correspond very closely to the sex differences in respect of crossing-over. It was found that the crossing-over is more frequent in the male than in the female and Dunn (1920) suggested that the explanation of this sex difference must be sought in the structure and function of chromosomes. Table X gives the data concerning crossing-over between pink eye and albinism in/

Fig. 41.



Graph showing the chiasma frequency at metaphase in the mouse. A: represents the female, B: the male with the same genetic constitution, C: is the curve of the chiasma frequency of a very young male, D: represents the combination of B and C.

Table X .

Illustrating the difference of crossing-over value of the albinism and pink-eye factors in the male and female mouse. (From Castle and Wachter, 1924).

Sex	Gametes tested	Cross-over gametes	Crossing-over value	Authors
Female	2.789	444	15.92±0.90	Dunn (1920)
Male	3.683	503	13.65±0.78	"
Female	0.556	106	19.06±2.02	Castle and Wachter (1924)
Male	3.374	462	13.89±0.82	-
Total: Female	3.345	550	16.44±0.82	-
Male	7.057	965	13.77±0.57	-

in the female and male mouse respectively.

On the partial chiasmatype hypothesis it could be predicted that more chiasmata would be found in that sex in which crossing-over was most frequent. The figures given above show that sex differences in crossing-over are equalled by sex differences in chiasma frequency. On the alternative hypothesis, where crossing-over is regarded as being conditioned by chiasmata, the sex with increased crossing-over should exhibit a reduced, rather than a higher, chiasma frequency.

The evidence described above, as observed by the author, together with other proofs presented by Darlington (1932a), Stern (1931) and others, demonstrates that the chiasmata at diplotene are the actual loci of genetical crossing-over and the decrease in their number towards metaphase is the result of the force of repulsion. This force operates partly between the pair of paired chromatids and partly between the two attachment constrictions and causes the chiasmata to move towards the distal end in such a/

a way that in many cases the bivalents are held together at metaphase only by terminal association.

The general repulsion acts on every loop of a bivalent and operates equally along the bivalent. The specific repulsion is a localised force, causing an increase in the size of the loop which includes the attachment, as compared with loops without attachment. The second force of repulsion, therefore, operates somewhat against the first. The balance between the two forces determines the degree of terminalisation. If the force of repulsion at the attachment is arrested, either by environmental or by genetical agents, there will be no, or very slight, terminalisation, but if repulsion at the attachment is strong and acts freely, complete terminalisation may result.

The present analysis shows that the operating forces are : (a) attraction, (b) two types of repulsions, and (c) permanent contraction. The latter will reach its maximum at diakinesis where the bivalents show the presence of a strain (Keunecke 1923) caused/

caused by the interaction of contraction and repulsion. The loops lying on both sides of the chiasma will under the influence of these forces arrange themselves at right angles; such structure of the alternate loops is clearly visible at diakinesis in Tulipa, Batrachoseps and Chorthippus.

The chiasma frequency from diakinesis to metaphase decreases only slightly, while it is usually greatest before diakinesis. The cause is either a strong resistance offered by the more rigid bulk of the bivalents, or the contraction operating in some way against repulsion which by increasing the diameter decreases the distance between the pairs of paired chromatids.

(ix) Terminal association. Bivalents at metaphase, in spite of the operation of repulsion resulting in complete terminalisation, will remain associated terminally. There are several observations both on plants and animals, which show that at meiotic metaphase the terminal association of bivalents is a very common phenomenon (Tradescantia, Linum, small bivalents/

bivalents in Locusta). The chiasma yields to the operating repulsion and moves away from the attachment constriction until every sign of it disappears. The same forces, however, are unable to interrupt the association of terminal chromomeres, which strongly suggests that those terminal particles must possess some special property which is absent in the other intercalary chromomeres. The terminal chromomeres have a general attraction between two homologues and this force is satisfied by the association of the two chromomeres located in the sister chromatids. A similar force exists between the intercalary chromomeres but there the attraction will be overcome by terminalisation and therefore the terminal chromomeres should separate also.

Contrary to expectation, however, our observations show that in most cases terminal chromomeres remain in association longer than the intercalary. We must assume, therefore, that the terminal particles are endowed with a special affinity, if we want to give an explanation of the terminal association of bivalents/

bivalents during metaphase. Sometimes this terminal affinity may be affected by either environmental or genetical factors (Anemone, Moffett 1932b),which will alter the behaviour of the chiasma and the mode of terminalisation.

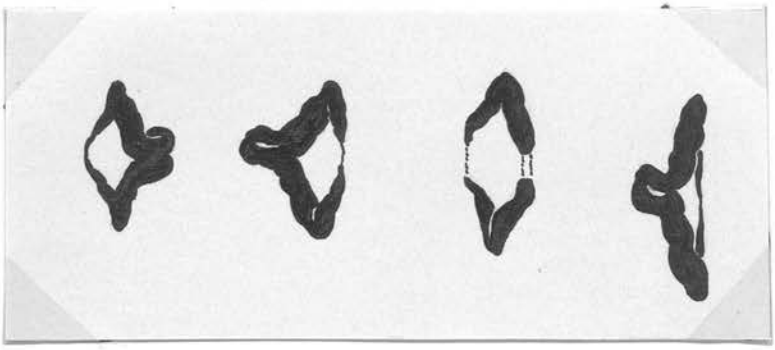
Another explanation may be offered to account for terminal association. It is obvious from many observations that at the end of diakinesis and during metaphase the repulsions between the attachments and that between pairs of paired chromatids weaken. This weakening may be inferred from the fact, that the degree of terminalisation is lower from diakinesis to metaphase than before diakinesis. Therefore it is not improbable that the attraction at the terminal segments will overcome those weakened forces and the bivalents will be held together at the distal ends.

If the attachment is located in the middle of the chromosome, then in some cases both ends of the bivalents may remain in association, on the same grounds as this was explained above. If, however, on one side of the attachment constriction there are formed/

formed more chiasmata than on the other, or if the attachment has a submedian locus in the chromosome, then by terminalisation one end of the bivalents will come into a terminal association sooner. The repulsion can be still in operation and try to break the terminal association on this side. This process can be seen in Triticum and is illustrated in fig. 42. In many cases the break will not occur and the two ends remain in terminal association, connected by a very thin thread.

(x) Repulsion and ring formation. In Rhoeo there is complete terminalisation and no interstitial chiasmata have ever been observed either in diakinesis or in metaphase. At diakinesis the ends of the chromosomes are terminally associated and arranged in a closed ring. Repulsion operates not only between homologous attachments, the ring formation showing that it exists between any attachments which are dissimilar, being greatest between the adjacent attachments and decreasing with the increase of distance. The closed ring is a most effective temporary equilibrium at diakinesis, where repulsion is operative within/

Fig. 42.



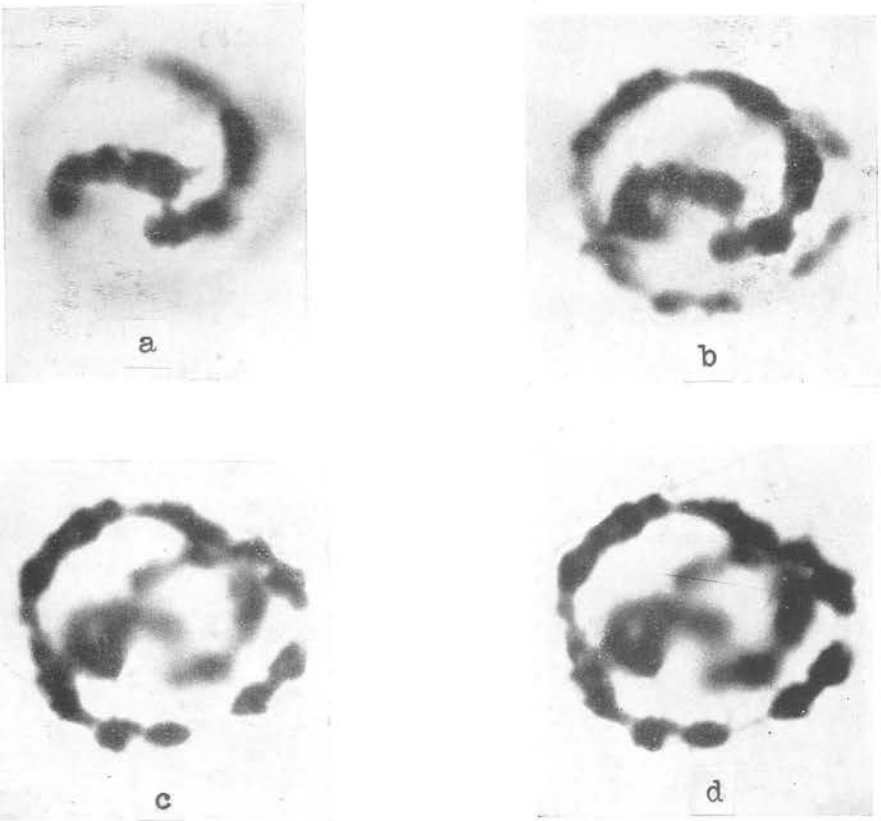
Bivalents at metaphase in Triticum. The interaction of repulsion between the two attachment constrictions with terminal affinity is shown in the form of the bivalents.

within the nucleus, and will not be disturbed by other forces (fig. 43). The terminal affinity of chromosomes in a ring-forming species, e.g. Rhoeo or Oenothera (Gates 1928, Gates and Catcheside 1932) is very strong, for in spite of the combined effort of the two repulsions, they remain in terminal association; the number of breaks is very small, the chiasma frequency at metaphase being 0.96.

Ring formation will undoubtedly meet with many mechanical difficulties mostly due to the small space within the nucleus in which the chromosomes are confined. Fig. 44 illustrates some complicated ring arrangement with interlocking. In such cases the operating repulsions will increase in their action and break the terminal association, the result being one or more chains. Tradescantia as a structural hybrid shows multivalent chain association in which the arrangement of chromosomes is different from that found in Rhoeo. (fig. 45).

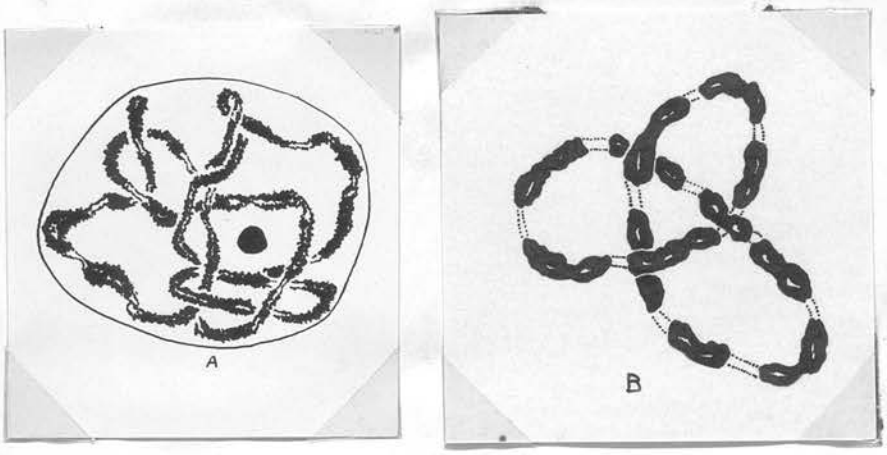
The structural changes in the bivalents produced by the forces operating before and during metaphase may/

Fig. 43.



Diakinesis in Rhoeo. The chromosomes are associated terminally forming a ring. Only repulsion operating between attachment constrictions is present.

Fig. 44.



Interlocking in a diakinesis ring of Rhoeo. A: illustrates interlocking, which prevents the formation of a close ring, B: double interlocking.

Fig. 45.

Meiotic metaphase in Tradescantia virg. var. humilis. The whole nucleus is represented. A: pairing of fragment with trivalent, B: chain of five chromosomes. They are associated terminally and the adjacent attachments are repelled to opposite directions. C: fragment, D: bivalent illustrates torsion.

may perhaps influence the anaphase separation. The number and type of chiasmata may provide mechanical obstacles against a free chromosome separation but their effect is only temporary. The dynamic forces which come into being at the commencement of anaphse sooner or later will overcome the purely mechanical difficulties and complete the segregation of chromosomes to the opposite poles. The description and analysis of this process will form the subject of the following part.

(xi) Conclusions. The forces which operate during meiosis and determine the behaviour of chromosomes are the same in effect and probably in nature as were the forces operating at mitosis, namely : (a) attraction; (b) repulsion; and (c) contraction. The interaction of these forces, however, is profoundly changed by the fact that at meiotic prophase the chromosomes are single at the beginning of division and two homologous chromosomes pair in a diploid. Chiasma formation is a consequence of genetical crossing-over, i.e. exchange between partner chromatids produced by the simultaneous operation of splitting/

ting, repulsion and contraction (torsion). Decrease in number of chiasmata in the post-diplotene stages is a result of a general repulsion, operating between any pair of paired chromatids, and that of a special repulsion which operates between the corresponding attachment constrictions. The terminal association of metaphase bivalents demands a special property of terminal chromomeres in the so-called terminal affinity. The forces described above are the characteristic dynamic factors which determine the general process of meiosis in diploids. These factors are under genotypic control in such a way that in some cases the whole mechanism of meiosis may be altered. The pairing of homologous chromosomes can be arrested or the loci and number of chiasmata may be influenced by genetical (and environmental) factors. The ultimate causes of such and similar change in meiotic processes must be sought in the disturbance of those forces (Darlington 1932b).

Table XI illustrates the forces operating during meiosis, their effects and result of genotypic control of their operation.

Dynamical analysis of meiotic processes.

Stages	Forces	Structural changes	Some examples of genotypic control
Leptotene	Attraction (?)	Single chromosome thread	Polarisation (Stenobothrus)
Zygotene	attraction (a)	commencement of pairing	Arrest of pairing (Perla marginata)
Pachytene	attraction (a) contraction (b)	complete pairing decrease in size	Delayed contraction (Allium triquetrum)
Diplotene	splitting attraction (a) contraction (b) torsion (a-b) repulsion (c)	chiasma formation decrease in length	Localised chiasma formation (Fritillaria meleagris)
Diakinesis	attraction (a) contraction (b) repulsion (c)	association of pairs of chromatids decrease in length separation of pairs of paired chromatids	Stationary chiasmata (Fritillaria imperialis) Terminalisation
Metaphase	attraction (a) repulsion (b)	equilibrium at the equatorial plate	Secondary pairing (Pomoidae)
Anaphase	attraction (a) repulsion	chromatids held together at the attachment separation of bivalents	

Table XI

IV. CHANGES in POSITION of CHROMOSOMES WITHIN the CELL and their DYNAMIC INTERPRETATION.

Besides the changes in internal and external structure which the chromosomes undergo during mitosis and meiosis, they also exhibit a series of positional changes within the cell after the disappearance of the nuclear membrane, when they move more or less freely in the colloidal cytoplasm. Briefly, the direction of their movements is first towards the equatorial plate where equilibrium is maintained for a short time, after which they separate into two groups, one group migrating towards each pole where fusion takes place, the result being the formation of two daughter nuclei. In these daughter nuclei complete equilibrium is maintained until the next division. Though the chromosomes are invisible during this stage, the spiral chromonema may be observed if special methods of fixation are used (Belling 1933).

(i)/

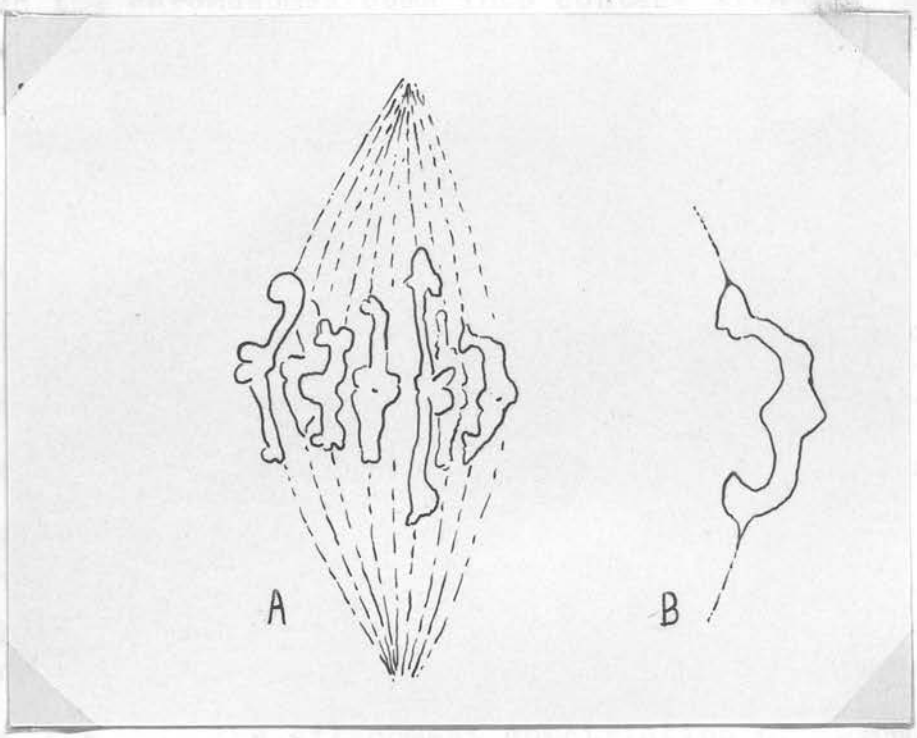
(i) The spindle. The disappearance of the nuclear membrane, which permits the chromosomes to lie in the cytoplasm, is normally accompanied by the development of the spindle mechanism from the opposite poles. (Several hypotheses of the origin of the spindle are discussed by Wilson (1925) and Sharp (1926).) The spindle persists from the time when the chromosomes cease to be confined within the nucleus until the formation of the 'tassement polaire'. As will be seen later, there exists a close relationship between chromosome movements and the spindle, and the latter is not without its proper functions in the mechanism of cell division.

The classical observations of Martens (1927-30) and Belar (1919a,b) on living cells, cleared up a great deal of misunderstanding and brought forward much evidence concerning the nature of the spindle mechanism during cell division, whether mitotic or meiotic. Although the achromatic figure as observed in the cell may be an artefact, or result of fixation, this artefact must be a morphological expression of/

of internal differentiation in the colloidal cytoplasm. It is not improbable that certain plasma colloids under the influence of special forces aggregate at the attachment constriction and the path of the migration of these colloidal particles from the chromosomes towards the poles is rendered visible under fixation (Alexander and Bridges 1926, Gray 1931) In Scilla by the use of special methods of fixation, the fibrous appearance of the spindle becomes very pronounced (fig. 46).

All chromosomes have a locus in the attachment constriction where the association of spindle and chromosomes is most intimate and at which the pellicle of the chromosomes is weakened, as can be demonstrated by micro-dissection (Chambers, unpublished). Belling (1933) actually saw the 'traction fibre' in Lilium. The intimate association between chromosomes and spindle at this locus led Belar (1929a) to formulate his 'Zugfasern' hypothesis. He assumes that at the attachment the chromosome has a polar granule which develops a mechanism by means of which/

Fig. 46.



The spindle mechanism at meiotic metaphase in Scilla. A: the fibrous nature of the spindle is rendered visible by using uranium tetroxid as the fixative agent, B: the 'traction fibre' and attachment constriction.

which the chromosomes come into contact with the spindle. The 'traction fibre' becomes merged into the mechanism developed from the polar granule and the chromosome movements are directed along the spindle. Belar and Huth (1933) accumulated much evidence in support of this hypothesis. The work of Belar, Hughes-Schrader (1924, 1931) and others suggests that a very close relation exists between chromosome movements and the spindle.

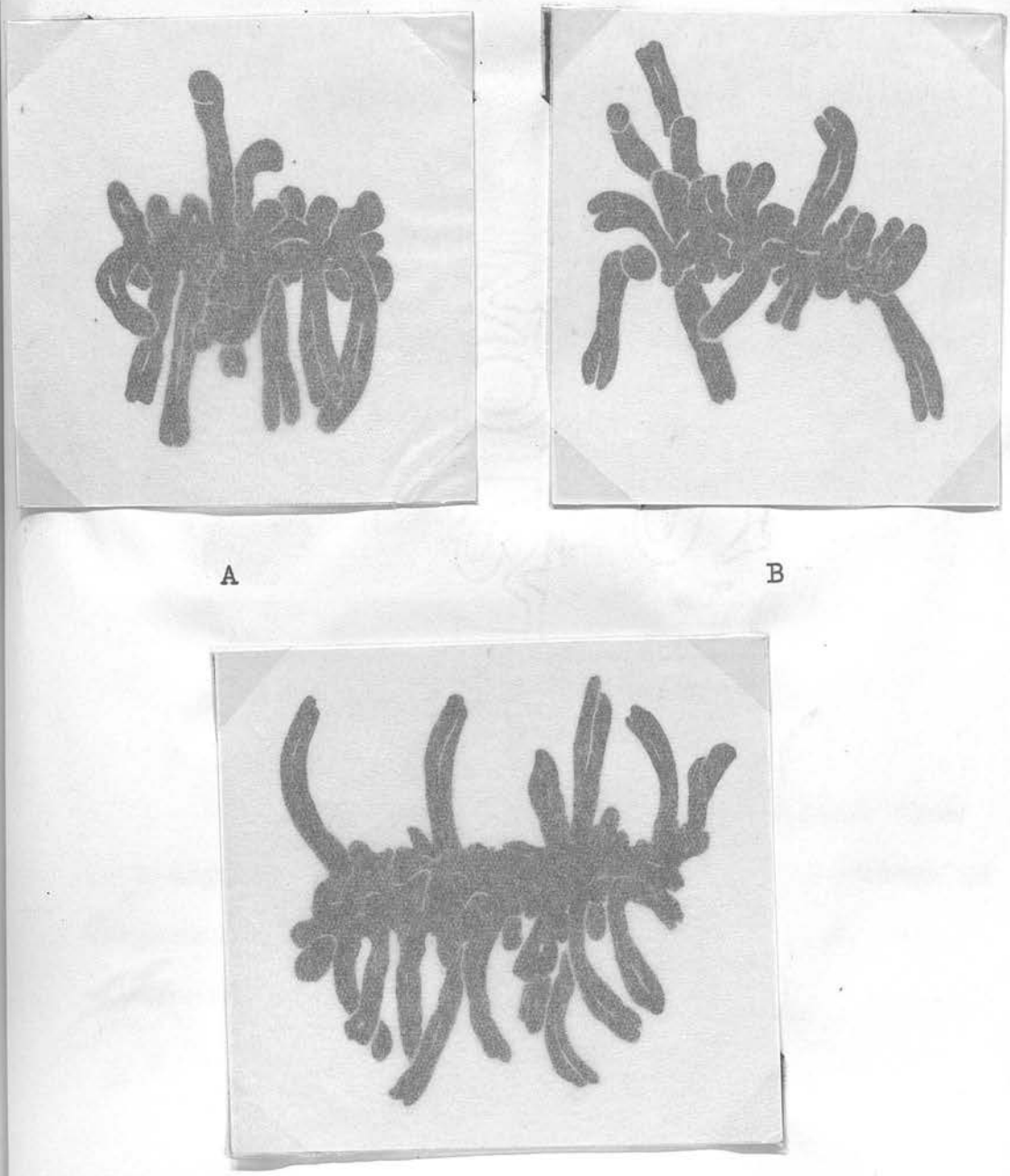
During mitotic prophase the chromosomes move towards the equatorial plate. It was already stated in Part III that the attachment constriction of chromosomes composed of two sister chromatids is undivided and single and the point of attachment is turned towards the centre of the cell. If the locus of primary constriction is a median or submedian one, and if the limbs of the chromosomes are long, these limbs usually lag behind the segment where the attachment constriction is located. This arrangement is more pronounced in side view during metaphase, as seen in Vicia/

Vicia, Hyacinthus, Tulipa and Allium (fig. 47).

The attachment constrictions of the whole complement usually lie in one plane and may be seen in polar view (fig. 47 D). The limbs of the long chromosomes are not necessarily included within the spindle; in many instances they lie outside and are moved as inert bodies in the cytoplasm.

The characteristic arrangement of the chromosomes at metaphase indicates that it is not the chromosomes but the attachment constrictions which show a tendency to come into equilibrium under the influence of dynamic factors operating upon them uniformly from both poles, namely the force of repulsion. The equatorial plate offers a position where temporary equilibrium is attained. Most commonly only the attachment and neighbouring sections of the chromosomes are included within the metaphase plate, the distal limbs being turned either upwards or downwards, in accordance with the previous position of the chromosomes, and lying outside the spindle. This arrangement strongly supports the view that the force/

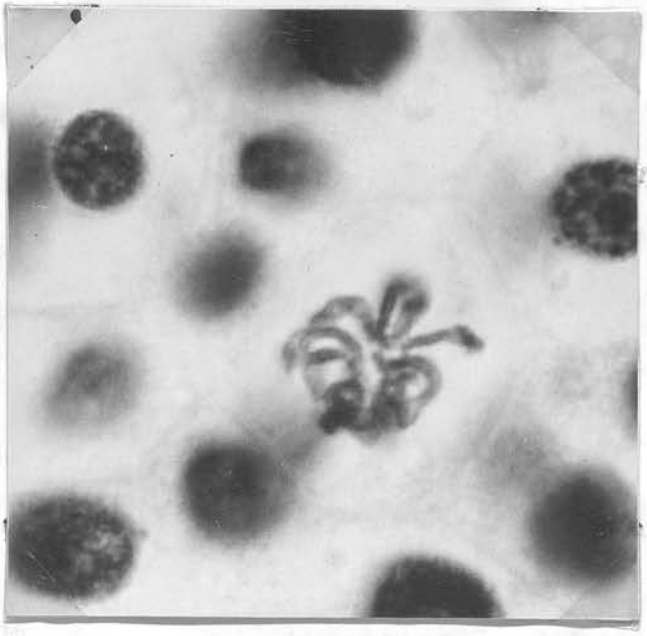
Fig. 47.



A B C

Metaphase of mitosis in Vicia (A), in Allium (B) and in Hyacinthus (C). The limbs of long chromosomes lag behind and are not included in the equatorial plate.

Fig. 47.



D

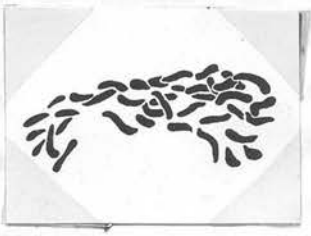
Microphotograph of mitotic metaphase from root-tips of Vicia. The attachment constrictions of chromosomes are turned towards the centre of the equatorial plate.

force of repulsion operates only between the poles and the attachment constriction and not between the poles and the whole chromosome.

The presence of a fully developed spindle before the metaphase arrangement suggests that the chromosomes even before metaphase depend in their movements on the spindle mechanism. Observations on division where no spindle is present show that, although the chromosomes move towards the metaphase plate, their movements result merely in a disorderly arrangement. This suggests that the spindle fibre plays the part of a directing line along which the attachment proceeds towards equilibrium. In several tumour cells in the mouse it was found by the author that the chromosomes became scattered in the cytoplasm after the disappearance of the nuclear membrane, or if they moved, their movements were at random. Thus, in spite of the presence of the attachment constriction, they are unable to move normally towards the equatorial plate, probably owing to the absence of the spindle mechanism (fig. 48).

From/

Fig. 48.



Mitotic metaphase of a tumor cell in the mouse. The chromosomes are scattered on the equatorial plate.

From these observations it is clear that the chromosomes are directed towards the metaphase plate by the spindle fibre. The movement is due to repulsion acting between attachment and poles which forces the chromosomes towards the centre of the cell where equilibrium is reached. The final arrangement of chromosomes at the equatorial plate, however, is an outcome of this and another repulsion, that operating between the attachment constrictions themselves, the existence of which has been proved in the preceding part. Before analysing the dynamical factors of metaphase patterns, it is necessary to discuss the effect and interaction of the force which is constantly present in the divided chromosome during prophase and metaphase. This force is the attraction which operates between homologous chromosomes (at meiosis) or sister chromatids (at mitosis) respectively.

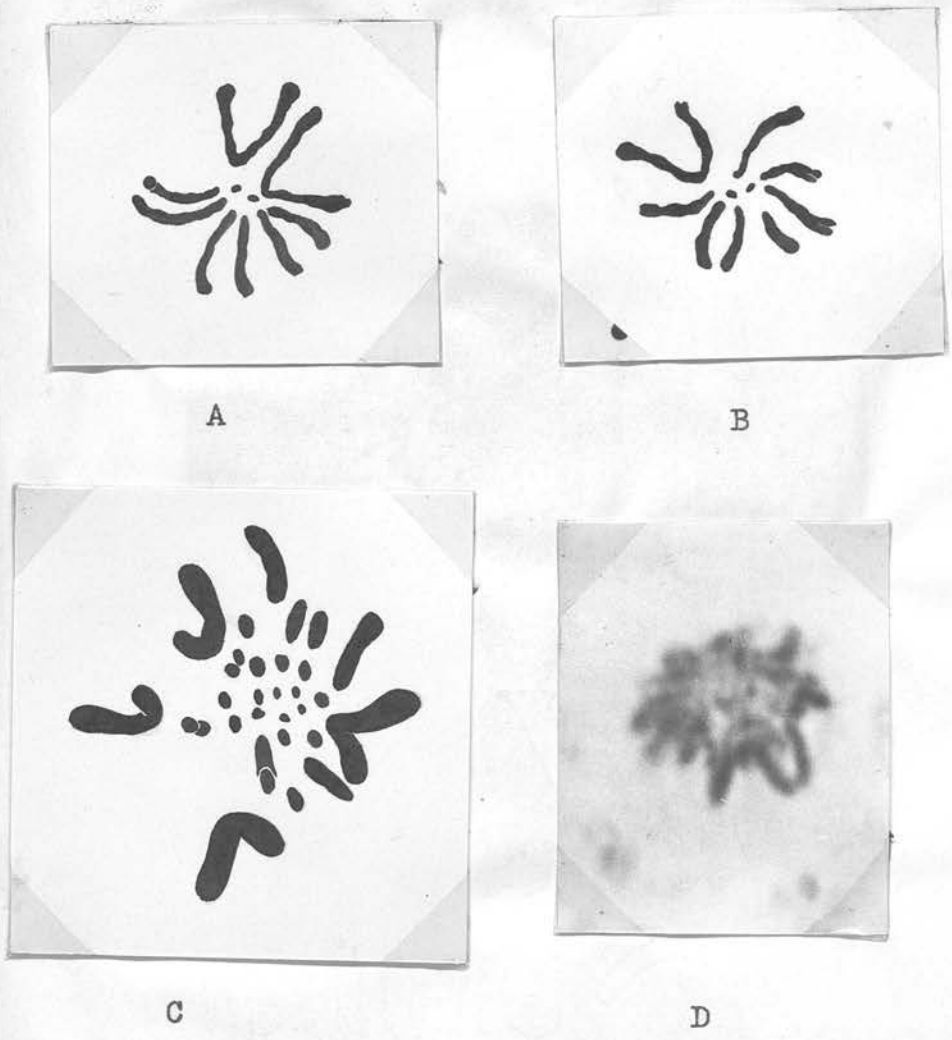
(ii) Somatic pairing. There are many instances where the metaphase pattern is very characteristic, the/

the homologous chromosomes being sorted out in the complement and lying near each other but without coming into contact. Such cases can be found during mitotic metaphase in Diptera (Metz 1916), Hemiptera (Wilson 1932) and were described by several cytologists as gonomeric grouping (O. Werner 1931) (fig.49).

In Drosophila obscura five pairs of chromosomes are present. The small dot chromosomes are invariably arranged in the centre of the metaphase plate, as illustrated in fig. 49, while the V-shaped X and the rod-shaped Y chromosomes and the autosomes are located at the periphery. The chromosomes of the same length and structure are lying side by side : these are the homologous chromosomes.

This very characteristic arrangement can not be an accidental one : its constancy suggests that it is the result of the action of dynamic factors which cause the metaphase plate to assume this arrangement. Such arrangement is termed 'somatic pairing' and its cause is ascribed to the homology in the internal structure of chromosome pairs in a diploid. In such somatic pairing the homologues lie parallel or radiate/

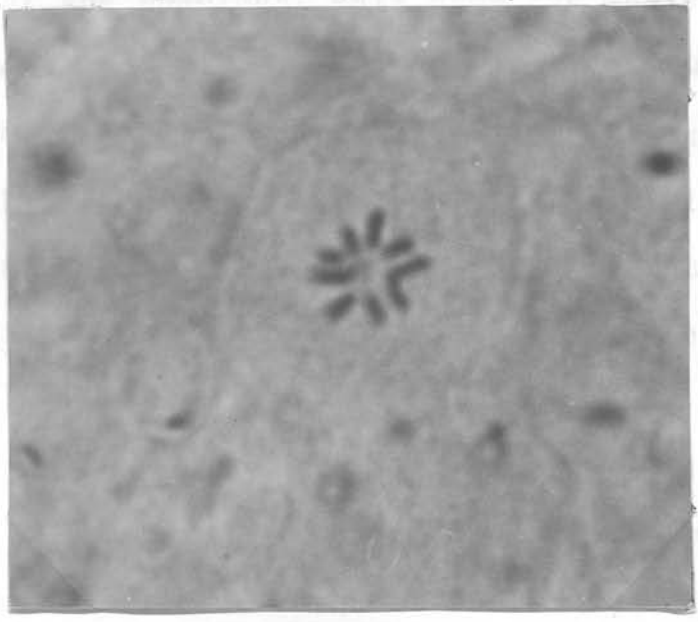
Fig. 49.



A B C D

Metaphase of mitosis. A: female, B: male Drosophila pseudo-obscura. The homologous chromosomes lie side by side. C: 'gonomeric grouping' in the mitotic chromosome complement of the fowl. D: microphotograph of mitotic metaphase in the fowl, showing the characteristic chromosome arrangement.

Fig. 49.



Microphotograph illustrating somatic pairing during mitotic metaphase in Drosophila pseudoobscura, Fallen. male ?

radiate like the spokes of a wheel, the inner ends where the attachments are located, pointing towards the centre. The force of attraction, which is an outcome of the homology, acts upon the whole body of the chromosomes and is responsible for the sorting out of the corresponding pairs during late prophase. Somatic pairing, however, requires very definite conditions. First of all, the number of chromosomes must be small; where the complement is large it would be impossible for all the chromosomes to be arranged in this manner, for they would interfere with one another. It is also necessary that the chromosomes should be small in size. In such plants as Vicia or Tulipa, where the chromosomes are very long (cf. Part III), their length would obscure any somatic pairing if any were present.

The force of attraction which is responsible for such an association is, however, unable to withstand the repulsion which operates simultaneously between the attachment constrictions and the result is equilibrium, the homologous chromosomes lying within/

within a definite distance of each other.

It should be mentioned that during somatic pairing each chromosome is composed of two sister chromatids. During meiosis it was seen that strong repulsion exists between the pairs of paired chromatids. On the same principle, during mitotic metaphase (showing somatic pairing,) the two homologues which lie side by side ought to repel each other. Therefore we must assume that somatic pairing is a special case of metaphase pattern, where, besides the general forces of repulsion, characteristic of other metaphase arrangements, another factor must be operative, probably a force of attraction based on homology, which helps to direct the movements of the chromosomes towards the metaphase plate.

The insertion of non-homologous segments into chromosomes is reponsible for the arrest of pairing during meiotic prophase and will disturb the arrangement of somatic pairing. This is best illustrated in metaphase plates taken from Drosophila obscura carrying translocations in the complement (fig.50).

A/

Fig. 50.

Mitotic metaphase in Drosophila pseudo-obscura. The complement contains chromosomes with translocated segments from non-homologous chromosomes, which obscures the somatic pairing.

A similar phenomenon found in meiosis during metaphase and anaphase is described as 'secondary pairing.' (Lawrence 1931). Like somatic pairing, it is caused by the general affinity of homologous chromosomes. Secondary association of metaphase bivalents and somatic pairing are evidence that affinities between chromosomes exist though in a lesser degree than that expressed by primary association when pairing is complete. The conditions required to detect secondary association are similar to those mentioned above in the case of somatic pairing, i.e. small number and small size of chromosomes.

(iii) Repulsion between attachments. The instances of metaphase arrangement, i.e. somatic and secondary pairing which have just been described, are the combined result of the general forces, namely two repulsions operating at every metaphase and a special force of attraction the action of which is restricted to definite conditions.

The general forces which are primarily responsible for the metaphase arrangement of chromosomes either/

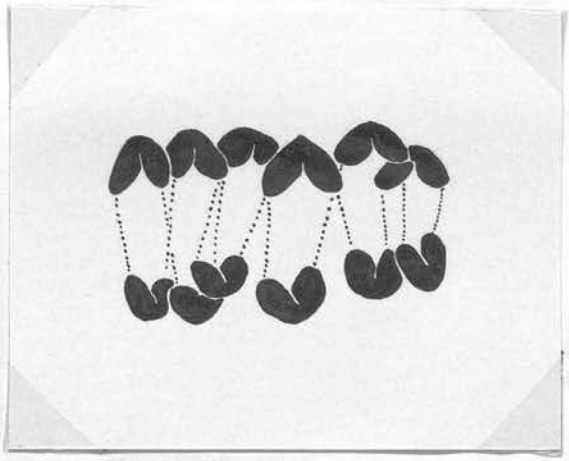
either in mitosis or in meiosis are (a) repulsion between poles and attachments, and (b) repulsion between the attachment of homologous and non-homologous chromosomes. The existence of this second force of repulsion can be clearly seen in multivalent associations (Tradescantia and Rhoeo). In the chain or ring there are more than two attachments. At diakinesis, as a result of repulsion between attachments, the chromosomes are arranged in a circle within the nucleus (fig. 43), but after the disappearance of the nuclear membrane they are exposed to another repulsion, that operating between poles and attachment. Under the influence of the repulsions operating between similar and dissimilar attachments and between attachments and poles, the chromosomes will arrange themselves in a zigzag ring. The adjacent attachments, being nearer, repel each other to a greater extent and simultaneously yield to the other force. The number of non-disjunctional arrangements in large and small rings is the same (Gairdner and Darlington 1931), which is a proof that the zigzag arrangement is/

is the most effective. (Fig. 51).

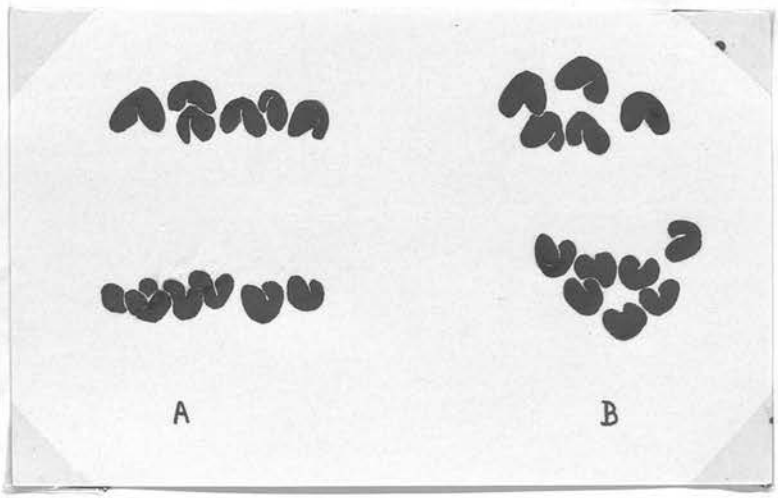
Another instance was found in irradiated Vicia faba, where it was possible to study the behaviour of attachments in relation to the poles at metaphase. In some cells, as a result of irradiation, chromo - somes were found without attachment constriction, usually lying outside the metaphase plate (fig.52). If the repulsion between chromosomes and poles acted upon the whole body, the chromosome without attachment should move towards the equatorial plate as a result of the forces operating. But, as has been shown, chromosomes which have lost their attachment are unable to respond not only to the repulsion between poles and chromosomes, but to the force of repulsion between chromosomes themselves. Hence we must assume that the effective repulsion operates upon the attachment only.

The arrangement of the chromosomes at metaphase, especially if they are small, indicates that possibly some kind of repulsion may exist between the bulk of the chromosomes as well. This repulsion is a much weaker/

Fig. 51.

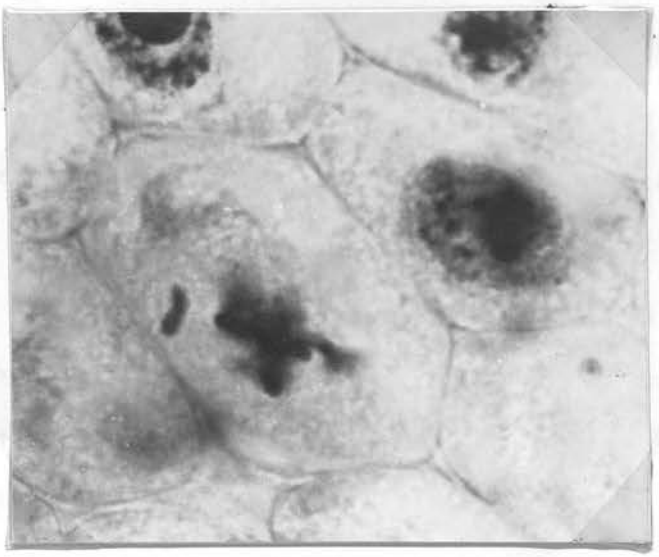


Meiotic metaphase in Rhoeo. The ring is arranged in zigzag formation as a result of repulsion between poles and attachment constrictions.



A: disjunctional separation is a result of zigzag arrangement. B: non-disjunctional separation is due to interlocking within the ring during premetaphase.

Fig. 52.



Microphotograph of mitotic metaphase in *Vicia* treated with X-rays. A chromosome, which has lost the attachment constriction lies outside the equatorial plate.

weaker force compared with that existing between the attachments themselves or between poles and attachments, and is unable by itself to direct the movements of the chromosomes. If the chromosomes are long, the limbs of homologous chromosomes at mitosis ought to be arranged parallel as a result of the force of repulsion upon the bulk, but this arrangement is nearly always obscured by the viscous nature of the cytoplasm. A high viscosity is bound to cause differences in the speed of chromosome movement in accordance with their size, even if the other forces are uniform for all attachment constrictions.

(iv) Cytoplasm as a factor in chromosome movement. Among the factors which influence and determine the movements of chromosomes either pre- or post metaphase, we must consider the part played in the mechanism of division by the cytoplasm (Chambers 1919, and recently). Heilbrunn (1928) proved by his ingenious centrifuge method that the changes in the nuclear material, especially the disappearance of the nuclear membrane, are accompanied by a great increase/

increase in viscosity within the cytoplasm and the formation of the spindle is a result of the gelation or coagulation of the interior of the cytoplasm. If this process is arrested or disturbed by applying anaesthetics (e.g. ether) (Nemec 1926), the spindle is not formed. In the cytoplasm a process of liquefaction ensues, illustrating that the spindle formation is conditioned by a high viscosity in the cytoplasm.

In the dividing tumour cells of the mouse several instances were found where the whole mechanism of division was greatly affected. The chromosomes were scattered, the anaphase separation was disturbed, and aneuploid or polyploid cells were observed (Winge 1930). In several cells the chromosomes were arranged more or less in the centre but lying very close to each other, similarly to somatic pairing described above. They were 'double' in appearance and assumed to be daughter chromosomes not yet segregated towards the poles. A similar case was described by Ludford (1930). The spindle of the dividing tumour cell was absent/

absent or very irregular. The absence and other irregularities of the spindle are primarily responsible for the aneuploid and polyploid chromosome number in the tumour cell; the segregation of the chromosomes could not be completed for they were unable to migrate towards the poles without the spindle mechanism (fig. 53).

In the tumour cell it is very obvious that the normal cytoplasmic environment is profoundly altered, and this must be either primarily or secondarily responsible for the arrest of spindle formation. The irregularities in chromosome movements within the tumour cells are reflections of an unusual cytoplasmic viscosity. The observations on tumour cells prove that certain biochemical changes occur during division; that a special cytoplasmic environment is a prerequisite for the formation of the spindle; and that chromosome movements are determined and influenced by the spindle.

(v) Segregation of the daughter chromosomes.

At the end of metaphase the temporary equilbrium which/

Fig. 53.

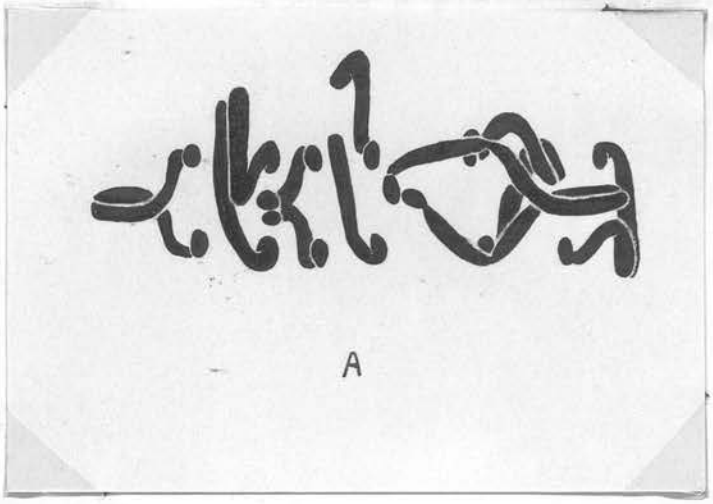
Mitotic metaphase of a tumor cell in the mouse. The daugther chromosomes lie side by side, unable to move towards the pole; the spindle has not been formed.

which was reached by the chromosomes in mitosis and by the bivalents in meiosis will be disturbed and the position of the chromosomes will be changed again. This may be produced by (a) the division of the attachments in mitosis; or (b) a definite increase of repulsion between attachment constrictions of the same bivalents in meiosis; or (c) a decrease in repulsion between poles and chromosomes. Another possible cause is the fact that at the end of metaphase the optimum degree of contraction is reached which permits the repulsion between corresponding attachment constrictions to come into operation.

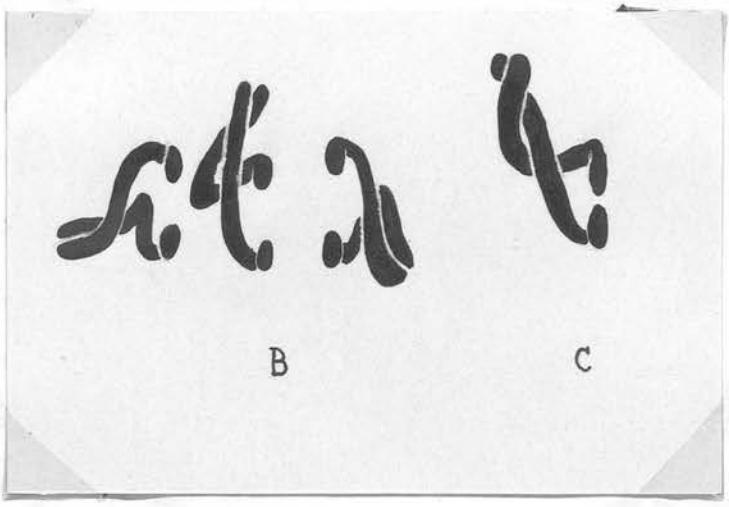
At mitotic anaphase the daughter chromosomes, as a result of one of the causes enumerated above, are repelled to a very definite distance, where a second period of equilibrium is attained between poles and attachments. In Vicia faba the distance between the two corresponding attachments during this stage is about half the distance between the equatorial plate and the poles, while in Allium and Crocus the distance is much smaller (fig. 54).

During/

Fig. 54.



A



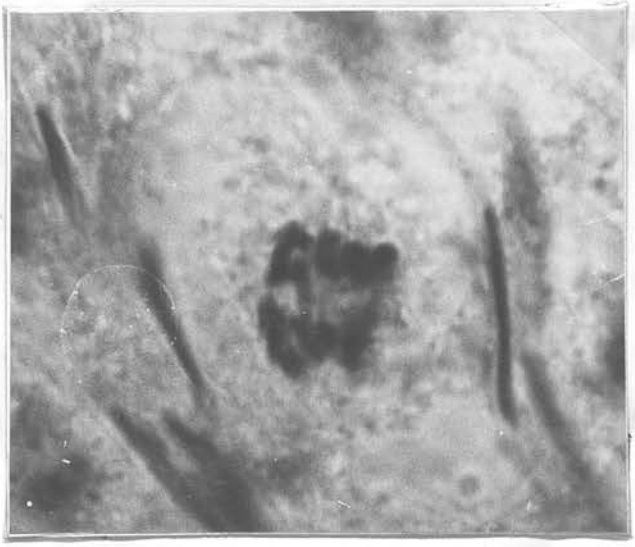
B C

Anaphase of mitosis; A: Vicia, B: Tulipa and C: Crocus. The daugther chromosomes are repelled to a definite distance where a second period of equilibrium is attained.

During meiotic anaphase a similar process takes place (fig.55). In many instances the first separation of bivalents can not be detected. The long distal part of the bivalents may remain in terminal association, only the segments near the attachment participating in the first separation until the second period of equilibrium is reached and further movement of the attachments towards the poles will be temporarily arrested. Therefore it is not justifiable to speak of 'lagging of bivalents' or to infer special chromatid interlocking which would be responsible for the association of longer bivalents at the beginning of anaphase, since these phenomena can be seen only at late anaphase.

From the second period of equilibrium the daughter chromosomes gradually migrate towards the poles where they form the 'tassement polaire' and fuse together to form the new daughter nucleus. The half-spindle (between chromosomes and poles) decreases in size; at telophase it will be represented only as a small polar cap, and very soon disappear entirely. The/

Fig. 55.



The second period of equilibrium at meiotic anaphase in the rat.

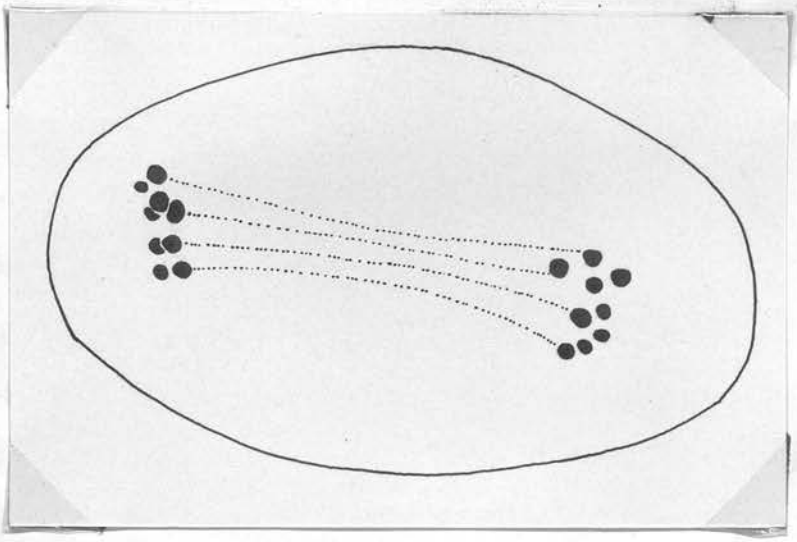
The interchromosomal part of the spindle (i.e. that between the two separating daughter complements) increases and extends; the length of the chromosomes may decrease further and the spiral structure of the chromonemata within the matrix may be seen in favourable material (cf. Part III).

The analysis of the processes described above in the anaphase separation after the second period of equilibrium leads us to assume the operation and interaction of the following forces : (a) decrease of repulsion between attachment and pole; (b) expansion of the inter-chromosomal spindle; (c) contraction within the daughter chromosomes. It is certain that in anaphasic separation of the second stage, the expansion of the inter-chromosomal spindle is the most visible result of the operation of dynamic factors. By special fixation the inter-chromosomal spindle can be studied in divisions in plant and animal species. During the first separation and at the time of the second period of equilibrium it has a concave appearance, but later, when the equilibrium is/

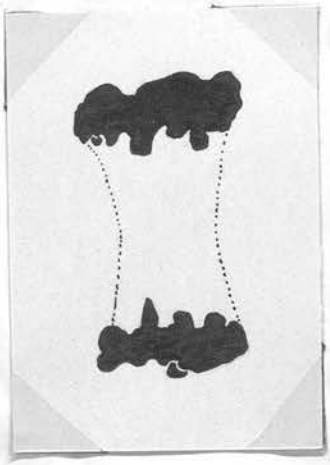
is again upset, it becomes elongated and convex (fig. 56).

Belar (1930) studied the behaviour of the inter-chromosomal spindle (Stemmkörper) in living cells and was able to prove its autonomous expansion and its resistance in different osmotic environments. Further evidence was found by the present author in favour of the expansion of the inter-chromosomal spindle in the meiotic division of the rat. The animal was treated by X-rays, as a result of which it showed in the chromosome complement fragmentation. At meiosis the fragment or fragments were lying outside the equatorial plate, having no attachment constrictions and hence unable to orientate themselves in the equatorial plate. When the second period of equilibrium is disturbed and the chromosomes move towards the poles, the fragments drift into the equatorial plate and may move even further towards one or other pole. A similar case was found in Tradescantia, where univalents entered into the metaphase plate after the bivalents had moved towards the poles. Here/

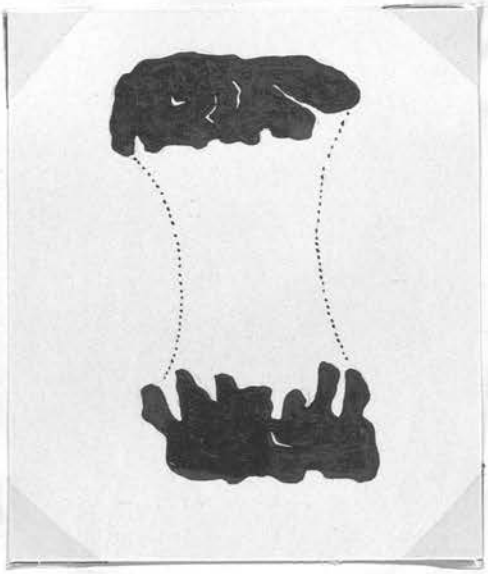
Fig. 56.



A



B



C

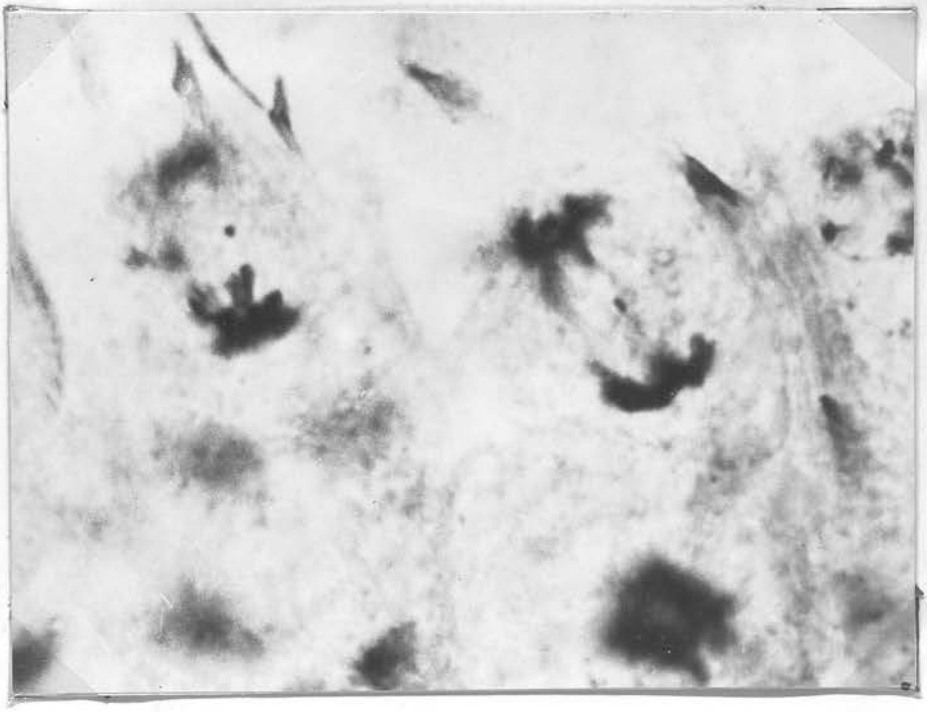
Anaphase of meiosis in Linum (A), Delphinium (B) and Scilla (C). The interchromosomal spindle shows expansion.

Here, however, the univalents possess an attachment and therefore their movements can not be considered merely as a passive result of the expansion of the inter-chromosomal spindle as is the case in the rat, where the passive movement of fragments into the centre of the cell provides a proof of such an expansion. (Fig. 57).

(vi) Attachment at anaphase. Although the expansion of the inter-chromosomal spindle is the most important dynamic factor in chromosome segregation at anaphase, the movement of chromosomes towards the poles are not the result of this expansion alone. The attachment also plays a part during the migration towards the poles. The primary constriction acts either as a guide for the chromosomes, directing them towards the pole along the spindle fibre; or else exhibits a special attraction which operates at the end of anaphase between pole and attachment.

Chromosomes which have lost their attachment constriction are in many instances included in the metaphase/

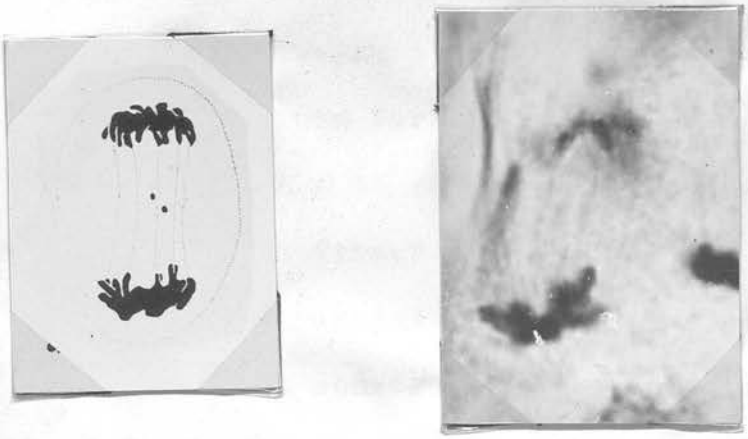
Fig. 57.



A

Anaphase of meiosis in the rat treated with X-rays. The fragments drift into the equatorial plate by the expansion of the interchromosomal spindle.

Fig. 57.



B C

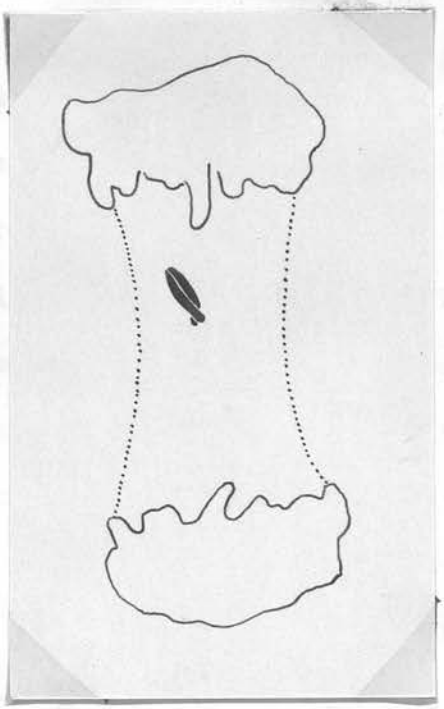
Meiotic anaphase in the rat, treated with X-rays. B: two fragments are carried into the centre of the cell as a result of the expansion of the interchromosomal spindle. C: illustrates the interchromosomal spindle.

metaphase plate, but they will remain there at anaphase too, lagging behind the other segregating chromosomes. The expansion of the inter-chromosomal spindle will move them further towards the pole but they will not be able to reach the pole where the daughter nucleus is formed (fig. 58).

In Vicia faba treated with X-rays chromosomes with two attachment constrictions were found as a result of fragmentation and translocation induced by irradiation. Such chromosomes provide further evidence on the importance of spindle attachment during anaphase. Two types were observed : (a) the two attachments were median and near to one another; or (b) the attachments were located at the opposite ends subterminally.

In (a), the chances are equal that both attachments of the same chromatid will be directed towards one pole, or that one of them will be repelled to the opposite pole. In the second instance the resulting anaphase configuration shows that there is a repulsion which operates only between corresponding homologous/

Fig. 58.



Mitotic anaphase in Vicia. The chromosome without attachment constriction is moved towards one pole by the expansion of the interchromosomal spindle.

homologous attachments (fig. 59A.). In such cases a break will occur which will eliminate chromosomes with two attachments. In (b) a metaphase overlap of sister chromatids, which is very common in the long chromosomes of Vicia faba, will cause an interlocking when both attachments of the same chromatid will be repelled towards the same pole (fig.59 B).

The chromosomes with double attachment constrictions illustrate that there is repulsion during late anaphase which operates between the corresponding attachments, but no repulsion between other attachments. If the repulsion between dissimilar attachments of the same complement would remain in force during anaphase, the arrangement of chromosomes into a 'tassement polaire' would remain unexplained.

The behaviour of univalents provides another piece of evidence to show that the attachment constriction is a kinetic body of the chromosomes. In most cases univalents are a result of heterology, for the chromosomes have no partners and therefore do not pair, and, although they have an attachment constriction/

Fig. 59.

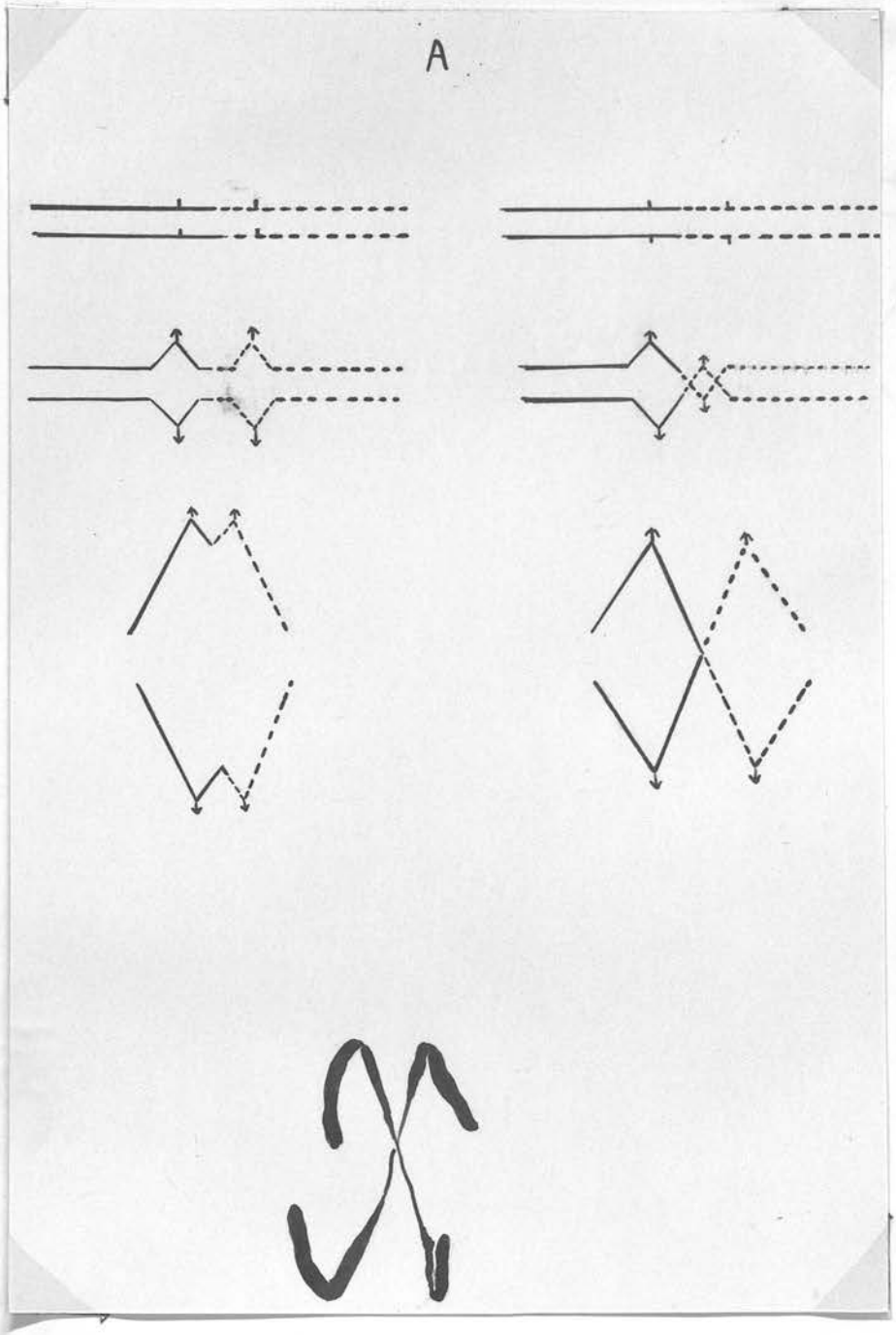


Diagram illustrating the behaviour of two attachment constrictions, located in one chromosome during anaphase of mitosis.

Fig. 59.

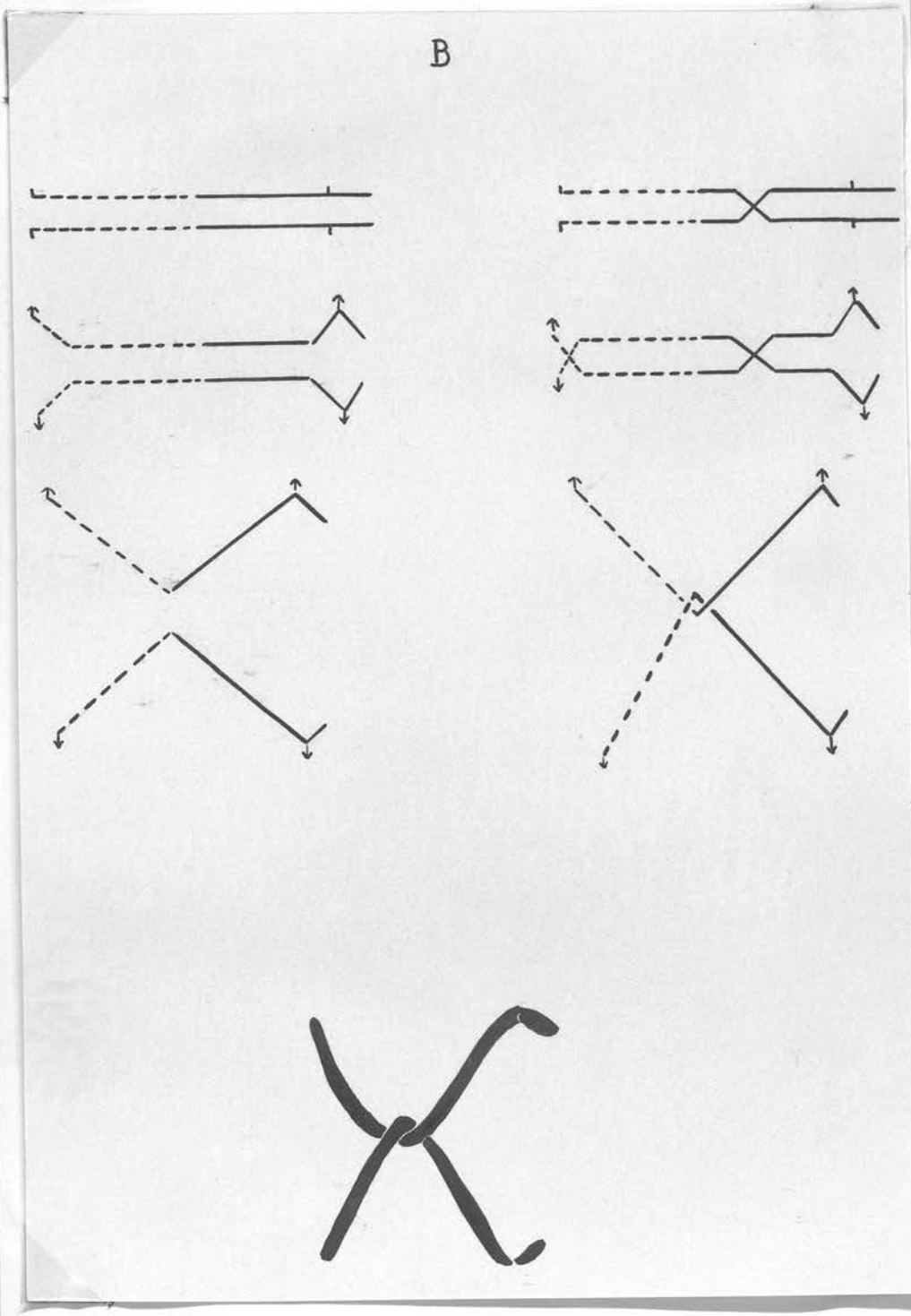
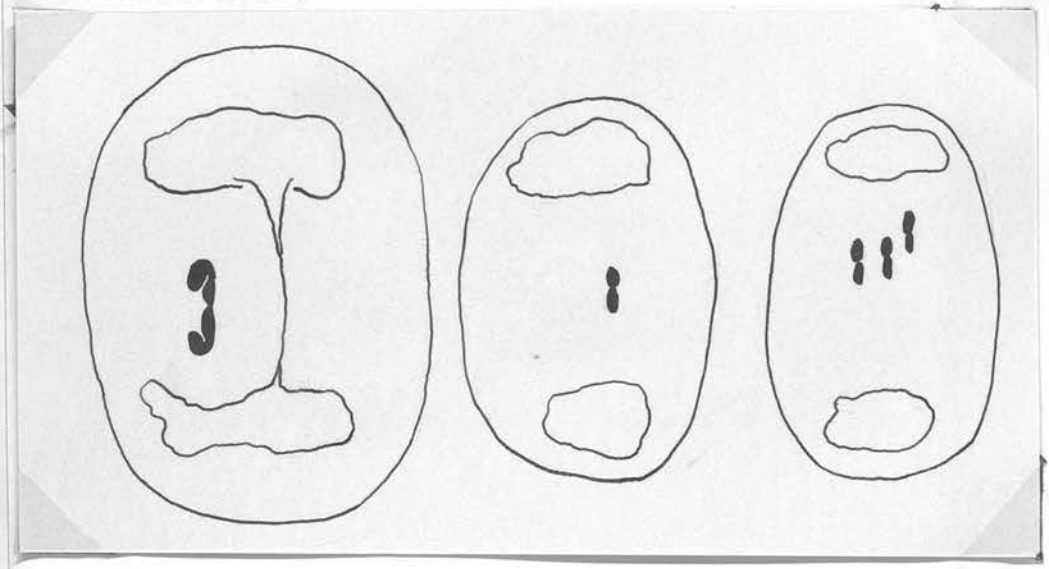


Diagram illustrating the behaviour of two attachment constrictions, located in one chromosome during anaphase of mitosis.

constriction they are usually scattered in the cytoplasm and lie on the periphery. This metaphase arrangement must be due to the fact that they are single in their structure and the forces which operate upon the bivalents uniformly will have a different effect on these unpaired chromosomes. They move into the equatorial plate just after the separation of the bivalents and arrange themselves axially and divide. This was observed in Triticum-Aegilops hybrids (Kihara 1931) and also described by Catcheside (1932). Univalents are very common in Tradescantia virginiana var. humilis, owing to its structural hybridity, and their behaviour at anaphase can easily be followed. (fig. 60).

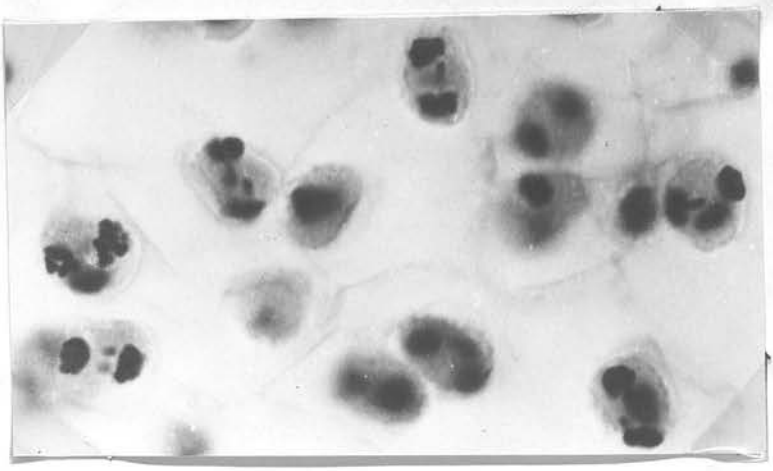
The daughter univalents produced by equational divisions will lag behind the other chromosomes during the second meiotic metaphase and anaphase. This is due to their delayed division in the first anaphase. At the time of their split the interchromosomal spindle has already expanded and the repulsion between the attachment constrictions is unable alone/

Fig. 60.



A B

Univalents at meiotic anaphase in Tradescantia (A) and in Matthiola (B)



Microphotograph illustrating univalents in pollenmothercells of Matthiola.

alone to force the daughter univalents into the second metaphase plate. This shows that during anaphase after the second period of equilibrium the attachment constriction acts only as a guide; the repulsion between the homologous attachments or the attraction between pole and attachment are also secondary agencies. The most important factor in segregation after the second period of equilibrium is the autonomous expansion of the inter-chromosomal spindle.

In the author's opinion, based upon the observations presented and discussed above, both the spindle mechanism and the attachment constriction are equally necessary for a complete anaphase separation and segregation of the chromosomes. The facts presented strongly suggest that chromosome movements can not be merely passive, as believed by Wassermann (1926) and Schaede (1925, 1927), who assume that chromosome movements at anaphase are due either to currents produced by changes in viscosity or by vortical currents of the spindle alone. Similarly, the hypothesis of Bleier (1931, 1933) and/

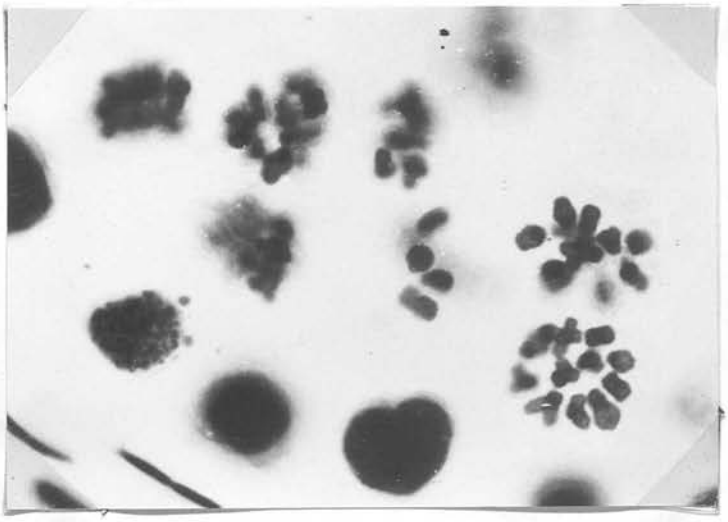
and Korperich (1930), according to which the spindle is only necessary for the poleward orientation of the chromosome, can not be upheld in view of the observations presented above. It is reasonable to assume from the chromosome behaviour that the spindle substance and the chromosomes are very closely associated and form an integral organic unit in the mechanism of division, both being equally necessary in the determination of chromosome movements. A similar opinion was expressed by Professor Ruggles Gates in his presidential address to the Royal Microscopical Society, 1932, in which he presented in a masterly way our knowledge of nuclear structure.

(vii) The nature of the forces determining chromosome movements. The analysis of the forces which determine chromosome movements in the cell after the disappearance of the nuclear membrane leads to various interpretations concerning the nature of these dynamic factors. It is highly probable that the repulsion and attraction which act upon chromosomes are electro-magnetic forces. This view is/

is
supported by a great deal of evidence and several observations described in the previous parts of this paper are in favour of it. Lillie (1905), more recently Cannon (1923) and Kuwada (1929) carried out experiments using magnetic needles in floating corks, and compared their distribution in an electromagnetic field with that of chromosomes in the metaphase plate. These experiments suggest that chromosomes have an electric charge which is localised at the centre, i.e. the attachment constriction; the polar granules are probably equivalent to the magnetised needles in the floating corks. In some cases there is no marked difference in the magnitudes of the charge between chromosomes of different size. In this case the large chromosomes will arrange themselves on the periphery (fig. 24 B). In other cases, however, the large chromosomes will be situated in the centre (fig. 61), the magnitude of the charge being proportional to the size. Smaller chromosomes have a smaller charge, and vice versa. The large chromosomes in this case will be able to move/

Fig. 61.

Meiotic metaphase in Locusta. The bivalents are arranged in a circle on the equatorial plate.



Microphotograph of meiotic anaphase in Tulipa. The large bivalents are arranged in the centre of the metaphase pattern.

move through the cytoplasm with a velocity which is about equal to that of the small, and so are able to overcome the difficulties due to the viscous nature of the cytoplasm.

On this hypothesis, the behaviour of the univalents is determined by differences between charges carried by bivalents and univalents respectively. The univalents are able to acquire the same charge as the bivalents only later.

The chromosomes are all repelled from the poles at prophase, which suggests that they are similarly charged. Wilson's (1925) cataphoresis experiments show that poles, nucleus and prophase chromosomes while they are enclosed within the nuclear membrane, are all negatively charged. The physical and chemical conditions within the nucleus being very different compared with those in the cytoplasm, it is not improbable that the sign of electro-magnetic charge will be changed in the cytoplasm. Ions may be taken up on an oppositely-charged surface to such an extent that the charge of that surface may be actually/

tually reversed in sign. Kuwada and Sugimoto (1928) were able to demonstrate by using neutral violet extra that the chromosomes stained differently at diakinesis and in metaphase, in accordance with their charge.

If we assume that the chromosomes are negatively charged in the nucleus (Wilson 1933), they must repel each other, being of the same charge. The arrangement at diakinesis is most probably the result of such a condition. After the disappearance of the nuclear membrane the poles or centrosomes with the same charge will repel the chromosomes, forcing them towards the equatorial plate, which has a positive charge and so will attract the chromosomes. The direct migration of chromosomes towards the equatorial plate shows that either there is a repulsion between pole and attachment constriction, or an attraction, due to the opposite charge of the chromosomes and the equatorial plate.

At the metaphase plate the repulsion between poles and attachments and that between attachments themselves/

themselves act on similarly charged elements and the result will be an arrangement which is most effectively in equilibrium, namely a circle. The arrangement of the chromosomes on a metaphase plate is similar to that of floating magnets and is produced by the same forces as that of the magnetised needles in an electro-magnetic field.

At metaphase the chromosomes change their charges, probably because they lie freely in the cytoplasm and not in the nucleus. The change in sign of electrical charge will cause an attraction of the chromosomes towards the poles. At telophase the chromosomes come into very close association at the 'tassement polaire'; this is possible only, if they are discharged towards the end of anaphase. They will become re-charged only at the beginning of the next division, probably soon after the development of the new nuclear membrane.

From the above-given description it is obvious that the forces which operate upon chromosomes and determine their movements in the cell are to a great extent/

extent comparable with the electro-magnetic forces, since the effects which are produced by those forces are very similar. The explanation outlined above is based upon such similarity. However, it must be remembered that the chromosomes and cytoplasm are highly organised constituents of the cell; their activities include autonomous processes unknown in their origin and detectable in their effects only. The internal constitution of the chromosomes as expressed by somatic and secondary pairing must be taken into consideration when analysing their movements. Therefore, the electro-magnetic interpretation of chromosome behaviour must be accepted only as a very useful working hypothesis, which emphasises the similarity between those forces. The great value of this hypothesis lies in the uniform classification of the dynamic factors involved in the movements of chromosomes.

(viii) Conclusions. The analysis of chromosome movement as coordinate changes in the cell during either mitotic or meiotic division, leads us to assume the operation/

operation of forces between poles and attachments and between the attachment constrictions themselves. Those forces are repulsions and are primarily responsible for the first period of equilibrium, resulting in the characteristic metaphase pattern of mitotic and meiotic divisions. The first anaphase separation is produced by increase in repulsion operating between the corresponding or adjacent attachment constrictions (if the chromosomes are arranged in a ring). This repulsion will cause a temporary second period of equilibrium, after which the segregation will be completed through the autonomous expansion of the inter-chromosomal spindle. The attachment constriction in this stage is necessary only for orientation, similarly to the part it played in directing the chromosomes towards the equatorial plate before metaphase.

V./

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VI. SUMMARY.

(1) At mitotic prophase unravelling of the chromosome spiral occurs without actual increase in length.

(2) The opening out of the secondary split is at mid-prophase. The actual split most probably does not coincide with the opening out of the sister chromatids during mitosis.

(3) The commencement of mitosis is the fission of genes and this must occur during the resting stage.

(4) The single structure of prophase spiral at mitosis is due to the close association of the homologous genes, chromomeres, and chromatids. The cause of the association is homology, most probably physico-chemical in nature.

(5) Unravelling of the chromosome spiral is a condition sine qua non for the building up of the matrix of each chromonema separately.

(6) Contraction operates from the undivided attachment constriction. This force acts upon the matrix, causing it to decrease gradually in length. The chromonema does not shorten but adjusts itself by forming a spiral. This structure can be recognised in/

in the prophase of the following division (persistance of chromosome individuality).

(7) The tertiary split at metaphase was not found in Vicia, Tulipa and Allium. Anaphase separation is due to repulsion which operates between two homologous attachment constrictions.

(8) The loci of pairing at zygotene between homologous chromosomes are at random, but always include groups of chromomeres. Polarisation is caused by special attraction between the ends of chromosomes and centrosomes or nuclear pole. It is most probably genetical in its origin.

(9) At pachytene the homologous chromosomes twist around each other.

(10) The opening out of the secondary split is at the end of pachytene.

(11) The general rule of pairing - that association always occurs between pairs of homologues, and repulsion always between pairs of paired homologous constituents, - is demonstrated by several observations.

(12)/

(12) At the end of pachytene, attraction and contraction produce a torsion. The secondary split introduces the repulsion and as a result of the interaction of these forces, breaks occur. The fusion of partner chromatids produces the chiasma.

(13) Chiasma frequency is not related to the size of the bivalents.

(14) The decrease in the number of chiasmata from diplotene to metaphase is caused by two repulsions . The first is general, operating between pairs of paired chromatids; the second is specific and acts between two corresponding homologous attachment constrictions. If the latter is greater, the result of interaction is movement of the chiasmata towards the distal end.

(15) The following data supply evidence in favour of Janssens' chiasmatype hypothesis :

(a) Pairing of unequal chromosomes;

(b) Interlocking of bivalents at meiotic prophase.

(c) Twisting of sister chromatids on both sides of chiasmata;

(d) Decrease in genetical crossing-over parallel to a similar decrease in chiasma frequency.

(16)/

(16) The terminal association of bivalents depends upon a special affinity between terminal chromomeres. If intercalary chromomeres become terminal by translocation, they attain this special affinity.

(17) The movement of chromosomes towards the equatorial plate is a result of repulsion operating between poles and attachment constrictions only.

(18) Metaphase equilibrium is a result of repulsion between poles and attachments and between attachments of similar and dissimilar chromosomes.

(19) In some cases the internal affinity of chromosomes will interact with the other forces and determine the mitotic or meiotic metaphase pattern, as it is the case in secondary association and somatic pairing.

(20) The spindle mechanism is necessary for normal chromosome movements before and after metaphase. It guides the chromosomes by their attachment constriction towards equilibrium either at the metaphase plate or at the poles. The spindle can be formed only in a normal cytoplasmic environment.

(21) At anaphase there is a second period of equilibrium where the repulsion between the corresponding attachment/

attachment constrictions and poles is equal. Further separation is due to the expansion of the inter-chromosomal spindle, for which new evidence is put forward.

(22) At anaphase there is no repulsion between the similar or dissimilar attachments migrating towards the same pole. Repulsion exists only between the corresponding homologous attachment constrictions.

(23) The similarity between effects of forces operating at mitotic and meiotic division and those which act in an electro-magnetic field indicates a close relationship in the nature of those forces.

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