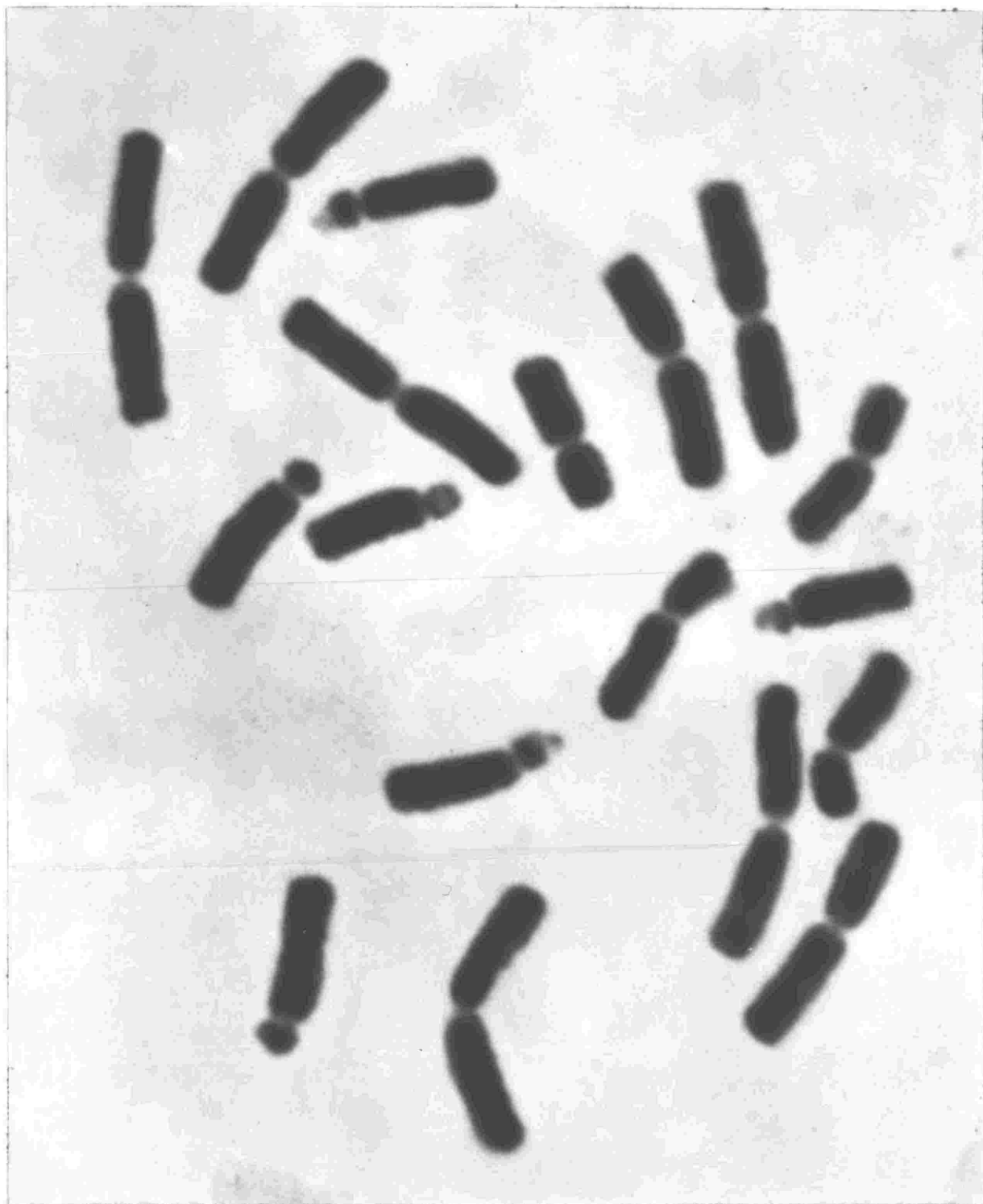# CYTOLOGICAL AND CYTOGENETICAL STUDIES ON

NORMAL AND INTERCHANGE ALLIUM TRIQUETRUM

Thesis submitted in partial fulfilment of the requirements for
the degree Doctor of Philosophy at the Botany Department,
Victoria University of Wellington, New Zealand.

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FRONTISPIECE : The normal somatic chromosome complement of <u>Allium triquetrum</u>.

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PREFACE

In biological research it is found not infrequently that if characteristics that arise when important processes become modified through certain upsets are investigated, these investigations can contribute much to an understanding of basic mechanisms involved. This is no more clearly demonstrated in the field of cytology than in the classical work of Creighton and McClintock (1931), which used a chromosome interchange in maize as a cytological marker, and which established a correlation between cytological and genetical crossing over. Similarly, the recent studies of Brown and Zohary (1955) on a paracentric inversion in _Lilium,_ and of interchange heterozygotes by Zen (1961), Noda (1961), Kayano (1960) and others, make it abundantly clear that chiasmata are indeed indications of genetic crossing over, as originally hypothesized by Janssens and Darlington. Today, gross chromosome mutations have become nearly as valuable to chromosome cytology as point mutations have to genetics.

The present studies, utilizing a group of _Allium_ _triquetrum_ plants that are heterozygous for an interchange, offer some contributions to cytological fact and theory.

INTRODUCTION

Interchanges (otherwise known as segmental chromosome interchanges
or reciprocal translocations), involving exchanges of segments of non-
homologous chromosomes, have been studied extensively in plants.
Probably the earliest observations were those of Gates (1908) on a
ring of chromosomes at meiosis in Oenothera rubrinervis. Belling's
reports of sterility in hybrids amongst certain velvet beans
(Stizolobium) were later attributed to an interchange of chromosome
segments (Belling, 1925). More clearly defined early cases were
provided by McClintock's (1930) cytological demonstrations of interchanges
in maize. Burnham's (1956) review indicates a sizable accumulation of
data in plants. The researches in maize by Brink, McClintock and Burnham,
and others, are by far the most extensive, and these data have
contributed much to our present understanding of many cytological
processes, particularly synapsis, chiasma formation and orientation
phenomena.

Few cases of interchange heterozygosity have been reported in
Allium, which is rather surprising in view of the large chromosomes
found in the genus, its readily observable meiotic divisions, and the
considerable amount of cytological work that has been done in many
species. Burnham (1956) briefly discussed six cases in his review,
the two most significant being those reported by Levan in A. ammophilum
and A. cernuum. In the former (Levan, 1935) a ring or chain of four
chromosomes, or a chain of three chromosomes plus a univalent, were
followed through meiosis I; and in the latter (Levan, 1939) both

meiosis I and pollen abortion were studied. Apart from my preliminary report on the present interchange (Rickards, 1964), two other accounts have come to my attention. Koul (1963) described an interchange in A. cepa, following meiosis through from diplotene to tetrad formation and estimating pollen viability; and in A. fistulosum Zen (1961) studied chiasma formation in interstitial segments in the interchange complex and resulting configurations at metaphase/early anaphase I. Apart from these clear cases of interchange heterozygosity a number of examples of associations of three or four chromosomes at meiosis (e.g. Battaglia, 1957, 1963) have been described in Allium, some of which may represent interchanges.

Following preliminary investigations on my own interchange material it became apparent that a detailed study of some aspects of its cytology and cytogenetics would prove informative. The major aspects studied include:

(a) Identification of the interchange chromosomes in somatic cells:

(b) Pachytene/early diplotene in the interchange complex.

(c) Chiasmata in the interchange complex from diplotene through to metaphase I, and chiasmata in interchange cells as a whole.

(d) Orientation in the complex at prometaphase and metaphase I.

(e) Relative position of the complex in the metaphase spindle.

(f) Mid-anaphase I and mid-anaphase II, including a detailed investigation on the developmental stages of pollen sacs in these phases.

(g) Sterility on both the male and female sides, together with information on seed setting.

(h) Pollen grain mitosis, including observations on delayed entry
of unbalanced pollen grains into mitosis.

BASIC CHARACTERISTICS AND BEHAVIOUR OF CHROMOSOME INTERCHANGES

An interchange is an exchange of segments of two or more non-homologous chromosomes arising by breakage and reunion. This general section will deal with interchanges involving only two chromosomes. More complex interchanges are basically similar in their general characteristics and behaviour.

Interchanges arise under natural conditions (spontaneously) or may be induced by X and other irradiations and other mutagenic agents. Spontaneous and induced interchanges are basically the same in characteristics and behaviour. They may arise during meiosis or in somatic tissues.

The interchange chromosomes may or may not be identifiable in somatic tissues, depending on the morphology of the chromosomes involved and the inequality of the interchanged segments. The interchange may or may not bring about an identifiable alteration in the phenotype of the individuals concerned.

The behaviour of the interchange chromosomes in meiosis permits ready cytological identification of individuals carrying such rearrangements in heterozygous condition. (They may of course be identified genetically through altered linkage relationships). Synapsis of homologous segments, shown at pachytene, results in the formation of a cross-shaped configuration in which normal and interchange chromosomes alternate with each other. The relative lengths of the arms of the cross vary according to the chromosomes involved and the lengths of the interchanged segments. The centre of the pachytene cross indicates the break points of the

interchange, so long as asynapsis or non-homologous pairing does not occur to cause misinterpretations. If pachytene stages cannot be examined with sufficient clarity, the innermost chiasma of the diplotene/diakinesis complex will give an estimate of at least the minimum lengths of the exchanged segments.

The nature of the complex at diplotene depends on the number of chiasmata present and their distribution. If chiasmata form in each arm of the pachytene cross a closed "ring" of four chromosomes will result.* Failure of chiasmata in one pachytene arm produces an open "chain" of four chromosomes (four types corresponding to the four arms); while failure in more than one arm produces trivalents, bivalents or univalents. Rings and chains are the commonest configurations. Different meiotic cells in the same individual may show different associations of the four chromosomes.

Chiasmata in the complex may arise in two distinct regions: (a) between the centromeres and the breakpoints (that is, in the interstitial segments), or (b) elsewhere in the pairing segments. Chiasmata in the interstitial segments give rise to special characteristics and results. Alterations in chiasma frequencies and distribution, and in crossover frequencies, may be brought about in the complex or elsewhere by the interchange.

At metaphase a ring of four chromosomes (without interstitial chiasmata) may be oriented in one of two basic ways: (a) in a zig-zag fashion, with alternate centromeres oriented to the same pole,

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*   The group of four chromosomes is sometimes referred to as a quadrivalent, but this term should probably be restricted to associations of four chromosomes in polyploid individuals (Burnham, 1956).

or (b) in an open fashion, with two ᵃ ᵈᵘᵖˡᶦᶜᵃᵗᵉᵈ adjacent centromeres oriented to reach a pole. There are two types of adjacent orientation: one where homologous centromeres pass to opposite poles, the other where non-homologous centromeres pass to opposite poles. The relative frequencies of these orientations depend on a number of factors, some of which are discussed in this thesis.

Of the three orientations above only the alternate one gives haploid nuclei that are fully balanced and viable. The adjacent orientations give rise to nuclei that are duplicated and deficient for certain chromosome segments and these spores usually abort. Thus spore abortion in interchange heterozygotes is governed largely by the frequency of adjacent orientations of the complex at metaphase I.

In chain forming complexes there is usually only one type of adjacent orientation that arises in any appreciable frequency, depending on which pachytene axis fails to form a chiasma.

The remaining stages of meiosis are generally normal in interchange heterozygotes.

In the progeny of selfed interchange plants three classes of individuals will be found; interchange heterozygotes, interchange homozygotes and normal homozygotes, and theoretically these will occur in a ratio of 2 : 1 : 1.

Interchange homozygotes are usually fully viable and phenotypically indistinguishable from normal homozygotes.

Burnham (1956 and 1962) has published two excellent reviews on the basic characteristics and behaviour of interchanges.

PREVIOUS WORK ON THE PRESENT INTERCHANGE

In September of 1962 three squash preparations of <u>Allium triquetrum</u> pollen mother cells at anaphase I showing an anomalous chromosome complex were brought to my attention. The following is a summary of the data and considerations that were recorded in my initial observations on the interchange material (section 3, Rickards, 1962).

From early and mid-anaphase I stages it was deduced that the meiotic cells were heterozygous for a chromosome interchange* between one small chromosome (st) with a subterminal centromere, and one long chromosome (1) with a median centromere. No conclusions were made as to which of the long and short chromosomes of the complement were involved in the interchange, though the approximate equality of the two st and 1 chromosomes of the complex was interpreted as indicating that the exchanged segments had been of approximately equal lengths.

The complex at anaphase I was always a chain of four chromosomes (no unequal bivalents etc. had been identified). From the regular occurrence of chiasmata in all homologous segments except the short arms of the st chromosomes, it was considered that the exchanged segments had involved the greater parts of the long arms of both the st and 1 chromosomes.

Scoring of cells at early and mid-anaphase I indicated that the chain complex was oriented in only two ways, (a) the alternate arrangement and (b) the adjacent type in which homologous centromeres

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*   The term "reciprocal translocation" was used previously; "interchange" is now preferred.

were oriented to the same pole. These two orientations were found to occur in a ratio of approximately 3 : 1 in the cells examined.

Chiasmata in the complex were described as being terminal or subterminal and appeared to show a lower number than expected from general observations on normal 1 and st bivalents (no details were recorded to support this conclusion).

A discussion was presented on the orientation types and frequencies found in this interchange. It was considered that in interchange complexes in general, pairs of linked, adjacent centromeres behave essentially as bivalents in their orientation. In a closed ring of four chromosomes in which there are four pairs of adjacent centromeres, it was considered that independent orientation of these pairs would produce a 2 : 1 : 1 ratio of the three orientation types seen. This was regarded as the basic ratio expected in complexes of four chromosomes. More particularly it was thought that it is this independent behaviour of adjacent pairs of centromeres that is the chief cause of non-disjunctional (adjacent) orientation, rather than these being the result of rigidity and hence "improper" orientation.

In the chain-forming complex examined it was suggested that the presence of two "adjacent" but non-linked centromeres, which are consequently unable to interact as those of a bivalent, modified the above expected ratio to 3 alternate to 1 adjacent type, and thus accounted for the frequency noted.

MATERIAL USED

The material from which initial observations on the interchange
were made (Rickards, 1962) had been lost, but it was known that one
of only a few local populations could have been its source. A search
during the winter flowering season of 1963 located a population of
plants showing a moderate incidence of pollen abortion. Though the
percentage abortion in these plants was less than expected, cytological
examination of the remaining young flowers (at anaphase I) identified
20 plants as interchange heterozygotes. These were separately potted
and labelled A, B, C etc. and their identification as interchange
heterozygotes was confirmed in the following flowering season. These
plants and their asexually produced progeny together represent the
interchange material used in these studies. Anaphase 1 cells in the
material now in culture are indistinguishable from those initially
examined.

Apparently normal plants were obtained from a number of localities
to provide material to be used as a control. Some populations of
Allium triquetrum show rather distinct phenotypic differences and some
of these have been shown to persist when plants are grown under approximately
the same conditions. Attention was therefore given towards obtaining
normal material that was essentially identical to that from which
the interchange material had been derived. Plants from three different
populations from the Wellington area and from three others (Auckland,
Palmerston North and Napier) were potted during 1963/64 and examined
phenotypically and cytologically (meiotic and mitotic). Of these,

plants from two populations proved to be heterozygous in satellite configurations and one other was rather distinct in floral characteristics. These plants were subsequently used only for certain comparisons in respect of general somatic chromosome complements and seed setting, but not for any detailed comparative work. The remaining samples were essentially identical as far as could be ascertained, and were indistinguishable phenotypically and cytologically from the normal plants obtained from the interchange population.

In addition to the above plants, two others, which did not show even moderate pollen abortion nor anaphase interchange configurations, were selected from the interchange population, potted, and some available flowering material (1963) from these plants was used in crosses with other normal plants obtained from elsewhere. The seed obtained from these crosses was sown and the F_1 plants examined meiotically in 1965 for interchange complexes at anaphase I. No such complexes were found and it was concluded that the parent plants from the interchange population were normal (rather than interchange homozygotes). These plants and their derivatives (control 9) were used in nearly all of the detailed comparative work done in this thesis.

METHODS

For root tip chromosomes. The basic technique used (2BD fixation and Feulgen staining) was the same as that recorded previously (Rickards, 1962). However, a number of modifications that warrant recording were introduced into this basic technique. The following summarises the schedule used.

1. Root tips were cut, washed, split lengthwise through the meristem and then pretreated in an aerated aqueous solution of 0.2% colchicine for $2\frac{1}{2}$ - 3 hours, or longer;

2. washed and fixed in La Cour's 2BD for $1\frac{1}{2}$ hours, followed by topping up with an equal quantity of 1% chromic acid for a further hour;

3. washed briefly in cold N HCl, and then hydrolysed for 25-45 minutes in N HCl at $60^{o}C$;

4. stained in freshly prepared Feulgen's stain for two hours in the dark;

5. washed, then bleached in 50% hydrogen peroxide for 15 minutes, transferred to 45% acetic acid and usually left overnight or longer (at $4^{o}C$).

6. Single root tips were macerated in a drop of 45% acetic acid on an albuminized slide and the cells spread out, gently heated and squashed under a coverglass.

7. After 20 minutes the coverglass was removed in 45% acetic acid and the material dehydrated through an alcohol series to absolute alcohol, cleared in three changes of eucalyptus oil and mounted.

The mitotic peak during the day in Allium triquetrum occurs around noon (as in Allium cepa; Jensen and Kavaljian, 1958), and cutting root tips into colchicine at about 10.30 a.m. blocks many divisions.

Root tips from out-door grown plants were used in preference to those grown indoors over water, since the latter accumulate quantities of fatty substances that stain heavily with osmium during fixation. The prolonged bleaching necessary to remove this discolouration adversely affects subsequent staining.

Pretreatment with 0.2% colchicine was found the most satisfactory for this material. For detailed chromosome analysis pretreatment was continued for no longer than three hours, so that the chromosomes were left in only a weakly contracted state, but for chromosome counts and general karyotype analysis where it is desirable to have near to maximum colchicine effect, this treatment was extended to 4 or 6 hours.

Bleaching to remove the bulk of cytoplasmic discolouration is a necessary procedure. Bleaching after staining to remove this fixation discolouration proved far superior to the more usual post-fixation procedure in being more effective and in having no adverse effect on staining.

Material that was not squashed immediately after bleaching was stored in 45% acetic acid at $4^{o}C$. Material may be left for at least one month in such a condition and possibly indefinitely (Ford and Hammerton, 1956; Battaglia, 1957; Wylie, 1963), giving much more satisfactory results than the more usual storage following fixation. Further, the prolonged treatment in 45% acetic acid greatly facilitates subsequent maceration, which otherwise often proves somewhat difficult

after 2BD fixation.

All permanent preparations were mounted in euparal.

Pollen mother cells. Two techniques were used with pollen mother cells, the standard Feulgen method and Snow's alcoholic carmine method.

The Feulgen method was the same as used previously (Rickards, 1962). Squash preparations were made from single anthers and were made permanent by the alcohol/eucalyptus oil method mentioned previously.

The alcoholic carmine method of Snow (1963) has some advantages over Feulgen's in this material, because of its ease, and it gives good, consistent results at all stages of meiosis, particularly at diplotene where the Feulgen technique is not fully satisfactory. The procedure is outlined below.

Inflorescences were fixed in acetic alcohol (1 : 3) for a minimum of three hours and then transferred to 70% alcohol for storage at $4^{o}C$. Material stored for 12 months still gives perfectly satisfactory results. When required material was transferred to a saturated solution of carmine in 85% alcohol and left to stain in the cold for 48 hours (minimum). Material left in the stain for three months is still in very good condition. Prior to making squash preparations the material was washed in 70% alcohol and transferred to 45% acetic acid.

Under this technique the chromosomes and nucleoli stain very well, but with only light colouration in the cytoplasm, even after prolonged staining, and thus this technique has considerable advantages over the standard aceto-carmine method.

Sectioned material. For certain studies whole inflorescences or dissected ovaries of appropriate age were fixed in Navashin's fluids,

dehydrated and embedded in wax by the standard TBA method, and sectioned longitudinally at 10μ. All sections were stained in Heidenhain's haematoxylin (rapid schedule, Darlington and La Cour, 1960).

Pollen grains. Snow's carmine method (above) was used throughout for pollen grain mitosis. It overcame the difficulty experienced to begin with in consistently procuring well stained preparations with contrasting chromosomes.

Pollen abortion. Fresh anthers were cut across into three approximately equal pieces and the middle section placed in a small drop of aceto-carmine on a slide. The pollen grains were washed out completely and the anther wall removed before applying a large coverslip. Further carmine was run in from the coverslip edges (the initial drop of stain was regulated to make this always necessary), the preparation warmed very gently (too much heat bursts the pollen grains), and then sealed with vaseline.

Abortive pollen grains are smaller than viable ones and become empty shortly after pollen grain mitosis I. When a coverslip is applied to a carmine preparation of abortive and good pollen the size and weight differences cause many of the abortive grains to move, very readily compared with good pollen, out towards the coverslip edges. Any sample scoring from such a preparation is bound to give inaccurate data.

Addition of glycerine to the carmine before applying the pollen reduces the amount of differential movement but not sufficiently to permit random sampling of pollen. Also, with the conventional way of applying a coverslip the pollen grains flow unevenly so that samples taken through the slide centre from edge to edge also give inaccurate results.

Thus it is necessary to dissect anthers prior to adding carmine (to stop differential flowing out of grains from cut edges), and to score the whole slide. A small drop of carmine was used in the early stages of preparation to reduce the amount of flow and keep all pollen grains well in from the coverslip edges. The whole preparation was scored by counting from successive strips, using a mechanical stage and a gratkicule blackened out partially to present a square rather than circular field of view in the ocular.

The above comments emphasize the precautions necessary when conducting pollen abortion studies. They will no doubt be necessary whenever there are any marked size and weight differences between abortive and good pollen. Burnham (1950) writes that in his pollen abortion studies, successive strips over the entire preparation were counted, suggesting an appreciation of the basic problem. Also Marks (1954) comments on the problem suggesting the use of glycerol jelly to avoid differential movement.

Pollination procedures. For artificial cross pollinations flowers were emasculated at partial anthesis, when both whorls of anthers were still undehisced. Inflorescences were then covered with polythene bags. When the stigmas became mature (judged by an outfolding of the stigma tips as three distinct lobes and accumulation on them of a glassy secretion), the required pollen was dusted on in ample quantities from a naturally dehisced anther, the tips of the perianth segments were cut to indicate pollination had been carried out and the

inflorescence then rebagged. All flowers in an inflorescence were
pollinated with the same parent pollen and appropriate records kept.

Ovaries begin to swell visibly two or three days after pollination.
They were left on the parent plant until ready to dehisce, when the
inflorescences were harvested into separate seed envelopes for future
use.

Synchrony in an anther: isolation of pollen sac contents. In
certain analyses conducted in this study it was necessary to assess the
developmental age of an anther from the stage of meiosis of its mother
cells. The accuracy of this assessment will depend, amongst other things,
on the pattern of synchrony within an anther and within individual pollen
sacs. Marked asynchrony in either would preclude any accurate
assessment. Some preliminary studies on longitudinally sectioned
anthers were carried out to determine the pattern of synchrony within
an anther. The following two basic facts were established.

(1) In the majority of cases the four pollen sacs comprising an anther
are at approximately the same stage of division, particularly when this
stage is a prolonged one (e.g. pachytene). In some cases, however, there
is a substantial difference between the pollen sacs, usually one though
sometimes two pollen sacs being out of step with the others. Thus while
three sacs might be at diplotene for example, the fourth might be at
anaphase I. Differences of this order of magnitude are not infrequent,
though they are seldom of greater magnitude.

(2) Within a pollen sac at more prolonged stages of division (prophase
and metaphase e.g.), pollen mother cells are completely synchronized.
There is no noticeable difference in timing of meiosis from top to

bottom of a pollen sac (cf. in tomato, Moens, 1964). In the shorter

division stages (e.g. anaphase) cells become a little asynchronized,

though not markedly so, and asynchronized cells are apparently scattered

at random through the pollen sac.

Point 1 clearly meant that whole anthers could not be used in

developmental analyses. Individual pollen sacs could legitimately be

used, however, and the following technique was employed to isolate the

contents of these sacs for separate analysis. Snow's alcoholic carmine

technique was used throughout these developmental studies. The

alcoholic base of the stain causes the anthers to become well hardened,

which is very necessary for successful dissection. Dissection, however,

cannot be done in alcohol because of rapid evaporation. Rather, anthers

were placed singly in a small drop of 45% acetic acid immediately prior

to dissection. It is essential to carry out dissection as soon as

possible after transfer to acetic acid since the acid rapidly softens

the anther wall, making subsequent handling considerably more difficult.

Anthers were bisected longitudinally along the groove between adjacent,

lateral pairs of pollen sacs, using a surgical scalpel under a binocular

microscope with x 25 magnification. Each half-anther was then

transferred to a new slide with a second drop of acetic acid. It is

a little too difficult to repeat this longitudinal bisection with

half anthers, since the groove separating the two pollen sacs is poorly

defined. Rather, a small snick was made along this groove at the very

tip of the half-anther, and then a second cut was made transversely

across one pollen sac so as to remove the top of the sac. The contents

of the pollen sac were then partially forced out with pressure from

behind and examined for their stage of division. If the stage of

division were appropriate to the analysis, the contents were fully

extruded (the meiotic cells usually adhere to each other during extrusion,

so the effect is much like squeezing from a tube of toothpaste!).

For further examination the pollen sac wall was removed, the contents

broken up and dispersed with a needle over the approximate area of a

large coverslip, a coverslip applied and the preparation squashed and then

sealed with vaseline.

After perfection of the above technique it is possible to examine

the contents of all the pollen sacs of an anther, though it is usual

to obtain only one or two per anther that are at the desired stage of

division (assuming the anther was approximately at the desired stage

in the first place).

Chromosome measurements. Initial measurements were made with

an ocular micrometer. For subsequent critical analysis the following

technique was used. Chromosome complements were photographed at

10 x 100 magnification. Where necessary two or three negatives were

taken of the same cell at different levels of focus or with different

groups of chromosomes in the centre of the field of view. Critically

developed prints were then obtained from the negatives, the final enlarge-

ment of these prints being approximately x 2,400. Arm, centromere and

total lengths of each chromosome were measured from these prints, using

a pair of dividers and a millimetre rule. Measurements were recorded

in quarter millimetres. When curves had to be taken into account the

chromosome was measured in sections along its mid-line.

Preparations were selected for measuring only when chromatids were

indistinct (i.e. not widely separated as after maximum colchicine action),
and where few if any curves or overlapping chromosomes were present.
Satellites were not included in the measurements.

Photography. All photographs were taken through a Leitz Laborlux
microscope with a x 10 photographic ocular, using an Asahi Pentax
Spotmatic camera. Agfa Agepe documentary film (very fine grain, slow
speed, high contrast) was used throughout and prints made through an
Ilfoprint machine. All mitotic/photographs shown (except the frontispiece)
are of approximately x 1,800 magnification, those of meiosis, x 1,200 Line drawings were
constructed from images projected from photographic negatives.

SECTION 1

SOMATIC   CHROMOSOME   STUDIES

1. THE NORMAL SOMATIC CHROMOSOME COMPLEMENT OF ALLIUM TRIQUETRUM

The normal somatic complement of Allium triquetrum was analysed
in some detail to provide a sufficiently broad basis for comparative
work with the altered interchange complements. Early studies in
interchange material indicated that at least one and perhaps both of the
interchange chromosomes could be identified consistently from cell
to cell and plant to plant. The following detailed studies were
conducted to permit a precise identification of both interchange
chromosomes, arms involved and break points.

I. Previous works

Levan (1932) and Kurita (1958) have published chromosome
morphological studies in Allium triquetrum.

Levan's studies were from the first pollen grain mitosis
in material from Sweden. He described the haploid complement of
$n = 9$ as comprising (a) four long (1) chromosomes, $13 - 14\mu$,
with approximately median contromeres, two of them $(1_1$ and $1_2)$ each with
a secondary constriction dividing one arm into approximately equal
parts:

(b) two smaller, $6 - 9\mu$, median - submedianally constricted chromosomes,
one, the smaller, without a satellite (sm), the other (S_1) with a
very small satellite on the short arm: (c) three subterminally
constricted chromosomes, two without satellites $(St_1, 7.5\mu;$ and $St_2,$
$6.1\mu)$, and the third $(S_4, 7.3\mu)$ with a conspicuous satellite on its
short arm. Levan described this S_4 satellite as a compound structure con-
sisting of up to five "chromatin globules of unequal size arranged in

a row". To reveal the full complexity of the satellite Levan applied pressure to the coverglass to unwind the large terminal lump that usually comprised the bulk of the satellite.

Fig. 1 (a - c) and Table 1 (a and b) are reproductions of Levan's complement and data. Note in particular in Fig. 1 b and c the compound nature of his S_4 satellite and presence of a second S_1 satellite and secondary constrictions.

Kurita (1958) has published a brief report on the root tip somatic chromosomes of a single specimen of Allium triquetrum from Portugal. His figures and measurement data are reproduced here in Fig. 1 d and Table 1 c. He classified the diploid complement of $2n = 18$, labelled a - i in decreasing order of size*, into two groups. One group (a - e, and g) included ".... 6 pairs of chromosomes with median or submedian constrictions, one of these pairs being nearly of the subterminal type." The second group (f, h and i) consisted of ".... 3 pairs of subterminal chromosomes, of them two pairs being furnished with small satellites on their short arm." Kurita compared his results with those of Levan and noted that his specimen is distinguished from Levan's" .... by (1) the absence of secondary constrictions on two large V-shaped chromosomes, (2) the lacking of satellite on one small V-shaped chromosome and finally (3) the presence of normal satellites on 2 of the J-shaped chromosomes". In his discussion Kurita concluded, on the basis of satellite and secondary constriction differences between his own and Levan's material, that ".... Allium triquetrum seems to be

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* His material was pretreated with 8-oxyquinoline; measurements excluded the centromeres (Kurita, 1952).

Table 1   a. Details of measurements from 5 pollen cells of
7 of the 9 _Allium triquetrum_ chromosomes studied by Levan (1932).

          b. Arm ratios (R_1 long/short, R_2 short/long) of the
satellited and other subterminal chromosomes of Levan (1932).

          c. Details from the one root tip cell of _Allium
triquetrum_ studied by Kurita (1958).

Table 1

C/s.	L.a.	S.a.	T.	R_1	R_2
S_1	4·8	3·4	8·2	1·46	0·708
S_4	6·2	1·2	7·4	5·38	0·194
St_1	6·6	1·1	7·7	6·09	0·167
St_2	5·2	0·9	6·1	5·97	0·173
I_1	6·4	5·9	12·3		0·922
I_2	6·4	6·4	12·8		1·000
Sm	3·7	3·4	7·1		0·919

<u>a</u>.   <u>b</u>.

Cs.	Arm lengths in μ	T.	R.
a	7·9 +7·7 8·0 +7·2	15·6 15·2	0·97 0·90
b	8·5 +6·5 8·0 +5·8	15·0 13·8	0·76 0·73
c	7·2 +6·9 6·9 +6·8	14·1 13·7	0·96 0·99
d	6·8 +6·6 6·8 +5·7	13·4 12·5	0·97 0·84
e	6·2 +3·5 6·3 +3·4	9·7 9·7	0·56 0·54
f	8·0 +1·4 7·3 +1·4	9·4 8·7	0·18 0·19
g	5·3 +3·8 5·1 +3·9	9·1 9·0	0·72 0·76
h	7·7 +1·4···t 7·3 +1·4···t	9·1 8·7	0·18 0·19
i	6·2 +1·0···t 5·8 +1·0···t	7·2 6·8	0·16 0·17

<u>c</u>.

polymorphic in karyotype".

A portion of the earlier work on Allium triquetrum by the present writer (1962), based on Levan's published work, was an attempt to establish the presence of the S_1 satellite and the compound nature of the S_4 satellite in some material available for study.* To summarise this work: the material studied differed from Levan's in that (1) the S_1 satellite was not present, as determined from direct observations on the relevant chromosomes and observations on the maximum number of nucleoli present in diploid (two) and haploid (one) tissues; (2) the conspicuous satellite (presumed S_4 - see later) was never compound in nature, being consistently observed as in Fig. 7f.

At the time of these (1962) studies I was unaware of Kurita's publication. The bulk of the present observations given below were also recorded prior to Kurita's paper coming to my notice, and hence appropriate comparisons and discussion are deferred until later.

II. Present Studies

Preliminary observations with an ocular micrometer were made from root tip complements of plants from a number of different populations. The details presented below were obtained from plants of one population (control 9 - see under materials), using the photographic measuring technique. Results from 10 cells selected on a basis of technical suitability are detailed in appendix table 1. Figs. 2 - 5 exemplify the complement and fig. 6 shows apparent photographic and diagramatic

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* Non-pretreated root tip complements were examined; chromosome lengths were not recorded.

idiograms with the chromosomes arranged to reveal relative lengths, arm ratios, chromosome groupings and future terminology.

The chromosomes can be separated very readily into three groups (I, II and III) according to sizes and centromere positions. These groups correspond to Levan's general classification. Groups I and II together correspond to Kurita's first group; group III to his second.

Group I chromosomes.

These comprise the four largest pairs of chromosomes in the complement which I have designated here as pairs 1, 2, 3 and 4. They have median - submedian* centromeres and are equivalent to Levan's l chromosomes and Kurita's a - d chromosomes. I found no secondary constrictions on any of these chromosomes in my material. Close examination of the chromosomes in the group (relative lengths and centromere positions; appendix table 1) showed, however, that one of the four pairs (number 4) can be distinguished very readily in all reasonably spread preparations. It has two markers. Firstly the two members of the pair are consistently somewhat shorter than all the others in the group. Associated with this size difference is the fact that members of the pair consistently have centromeres sufficiently off centre to be able to recognize short and long arms. It is the coincidence of these two features, relative length coupled with asymmetry in arms, that permits clear identification of the pair in all normal cells that are technically suitable for examination of all group

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* The terms median, submedian and subterminal are used here in the rather loose, general sense commonly encountered in plant cytology.

I chromosomes, even without resorting to actual measuring of the chromosomes (figs. 2 and 3 e.g.). Only in poorly spread cells with indistinct centromeres or badly twisted arms is any difficulty encountered in their recognition. Evidence given below on somatic association of pair 4 supports their recognition as a homologous pair.

The relative length of pair 4 members based on the total complement length was calculated from the data in appendix table I as 12.70 with long and short arm lengths of 6.69 and 5.57 respectively. The mean arm ratio (short/long) was calculated as 0.835 (1.22 for long/short) (tables 3 and 4).

In appendix table I the longer arms of members of chromosome 4 were paired as homologous in each cell, as were the shorter arms. In view of known variation in lengths of homologous chromosomes and arms such pairing conceivably might be introducing occasional arm reversals (Matern and Simak, 1968); i.e. the occasional pairing of what are in fact non-homologous arms. From a statistical analysis of this problem in the complement of Larix decidua Matern and Simak concluded that the risk of reversal of arms "cannot be disregarded if the average difference in length between long and short arms is less than 20 percent of their mean length."

From 10 measured cells the average difference in length between long and short arms of pair 4 in my material was found to be 18.23% (appendix table 1a). If technical procedures and patterns of variation in the present material are similar to those in Larix it is likely, therefore, that only rare if any instances of arm reversal occur in pair 4. This conclusion is supported by two other facts. Firstly,

Table 2   Details of measurements of chromosomes 4 and 6 from

four normal cells of _Allium_ _triquetrum_ , chosen from the complete

data of Appendix table 1 to exemplify interhomologous and

interkaryotypic variation in chromosome length.

Tables 3 and 4   Mean absolute lengths, mean arm ratios (table 3)

and relative lengths (table 4) of the somatic chromosomes from

10 normal cells (details in Appendix table 1). The data for

chromosomes 4, 6, 7 and 9 will be reasonably accurate, and

probably nearly as accurate for chromosomes 5 and 8, since

few if any instances of arm reversals and reversals of order

occur in these chromosomes. The certain occurrence of such

reversals, however, do not permit accurate data to be presented

for chromosomes 1 - 3, since these chromosomes have similar

lengths and arm ratios. The mean and relative total lengths

given provide some tentative information for these chromosomes.

Chromosome 4

Chromosome 6

Cell no.	Total	Long arm	Short arm	Ratio	Total	Long arm	Short arm	Ratio
2	38·50	20·25	17·25	0·852	27·25	22·75	3·75	0·165
	39·50	20·75	17·75	0·855	28·50	23·50	4·00	0·171
6	34·75	18·25	15·50	0·849	27·00	22·00	4·00	0·182
	36·25	19·25	16·00	0·831	26·75	21·75	4·00	0·184
9	30·00	16·00	13·00	0·828	22·00	18·00	3·25	0·180
	30·00	15·25	13·25	0·869	22·50	18·25	3·25	0·178
10	24·75	13·00	10·75	0·827	18·50	14·75	3·00	0·203
	25·25	13·25	11·00	0·830	18·25	14·50	3·00	0·207

Table 2

Chr. no.	Total	Long arm	C/m.	Short arm	Ratio	Chr. no.	Total	Long arm	Short arm
1	40·28					1	14·95		
2	39·58					2	14·69		
3	37·94					3	14·08		
4	34·15	18·01	1·14	15·00	0·835	4	12·70	6·69	5·57
5	26·18	15·40	1·13	9·65	0·627	5	9·72	5·72	3·58
6	25·15	20·50	0·95	3·70	0·182	6	9·34	7·61	1·38
7	23·06	18·79	0·92	3·35	0·179	7	8·56	6·98	1·24
8	23·03	12·68	1·09	9·26	0·731	8	8·55	4·71	3·44
9	19·96	17·09	0·82	2·05	0·120	9	7·41	6·34	0·76

Table 3   Table 4

in the data of appendix table 1 (and also in appendix tables 2 and 2a)
there are no instances in which long and short arms are not clearly
distinguishable in pair 4 members; and secondly, if frequent instances
of arm reversal occur, then the extent of variation in arm lengths in
pair 4 members must be considerably greater than that found in the
comparable long arms of pairs 6, 7 and 9, which is unlikely.

Any rare instances of arm reversal in the data of appendix table 1
will have exaggerated slightly the asymmetry calculated for pair 4 members.

Apart from pair 4 the remaining chromosomes of group I cannot be
identified clearly and consistently into homologous pairs. Some
suggestions are given by the data in appendix table 1 (see also fig. 6),
though in view of the magnitude of variation known to exist between
homologous chromosomes (Patau 1960, Matern and Simak 1968 e.g.),
arm reversals and reversals of order amongst these pairs cannot be
ruled out or reduced to a minimum, and hence exact characterisation of
these chromosomes in respect of relative length and arm ratios is not
possible.

There is no indication of an asymmetrical chromosome in Levan's
figures that would indicate a correspondence with pair 4 in my material.
Pair 4 here probably corresponds to d of Kurita's complement. It
agrees well in relative length, though only one of his pair corresponds
to my data in arm ratio, the other member has a nearly median
centromere. It is also interesting to note that the pairing into
homologues of the chromosome a - c of Kurita's specimen (table 1c and
fig. d here) gives a picture somewhat similar to that for group I

chromosomes of my material (fig. 6).

Group II chromosomes

This group comprises two pairs (5 and 8 here) with submedian centromeres. Neither carries a satellite, though they correspond clearly to Levan's S_1 (satellited) and Sm chromosomes. They correspond to Kurita's e and g pairs. They are distinctly smaller than pair 4 and generally a little more asymmetrical. Measurements of 10 cells (appendix table 1 and tables 3 and 4) suggested their classification into a smaller less asymmetrical pair (8) and a larger more asymmetrical pair (5). However, this classification is somewhat arbitrary, though some evidence given later on somatic association suggests that the pairs have been distinguished correctly with good consistency. Both Levan and Kurita recognized a smaller and larger pair in this group of chromosomes.

In view of their mean relative lengths the pairs have been designated 5 and 8, though in total relative length they are barely significantly different from pairs 6 and 7 (group III below). The possibility of occasional arm reversals and reversals of order amongst these chromosomes means that the characterisation in respect of relative lengths and arm ratios in table 3 are probably slightly inaccurate compared to certain other chromosomes.

Group III chromosomes

Chromosomes 6, 7 and 9. These chromosomes have subterminal

centromeres, and can be distinguished very readily from each other on size, arm ratios and, most obviously, by satellites. Their relative lengths and arm ratios are shown in tables 3 and 4.

Chromosome 6.

This is consistently the largest of the three group III chromosomes, in both long and short arm (and thus total) lengths. It has a mean arm ratio of 0.182 from 10 cells (5.54 for long/short) and does not carry a satellite. It must, correspond to Levan's largest subterminal st_1 chromosome, and to Kurita's chromosome f.

Chromosome 7.

This chromosome is usually slightly smaller than 6, mostly in the long but also a little in the short arm. It has, therefore, a very similar arm ratio (mean 0.179; 5.61 for long/short). The distinguishing feature, however, is the fact that the short arm bears a satellite, which is extremely variable in morphology. The satellite or satellite complex itself (excluding the attachment thread) is always very small. Most commonly it consists of a very slight swelling attached to the chromosome by a short, fine thread. At times, however, the satellite consists of a distinct linear series of up to four minute swellings; or the satellite may be absent altogether, there being simply a fine thread at the chromosome end. The attachment thread is usually about the same length as the arm bearing it, though at times it is much smaller, being barely visible; or it may be very long, extending in some instances up to two thirds the length of its chromosome. These latter cases are most remarkable and require very careful observation to

identify. Fig. 7 (a - c) illustrates the most common types of satellites observed. The satellite morphology appears to vary equally between cells from the same root tip and from different plants and populations. There is no simple relationship between length of attachment thread and size of the satellite though long threads have a greater tendency to bear complex satellites.*

Chromosome 7 compares well with Levan's S_4 chromosome in respect of relative length (no precise details given by Levan but cf. S_4 and St_1 e.g. in table 1a here), arm ratio (0.194 and 0.179) and presence of a satellite on its short arm. Also, Levan described his S_4 satellite as being compound in nature (fig. 1 b and c). However, the bulk of the satellite of Levan's chromosome is much greater than found in my material and it also appears rather less variable in morphology.

From relative lengths, arm ratio and presence of a satellite, chromosome 7 must correspond to Kurita's chromosome h. Kurita refers to both his and the other satellite in the complement (see below) as being "small" though they are differentiated in his drawings (see here Fig. 1d), that of chromosome h being noticeably the larger. Since Kurita's data covered only the one complement it is not possible to make comparisons with the complex morphology of this satellite in Levan's and my material. However, Kurita (p.5) does mention when comparing his complement with Levan's that both his satellites are "normal"

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*   Variation in morphological characters of satellites is well known. Koopmans (1955) e.g. recorded thread-like satellites of extreme lengths in trisomic Papaver.

which suggests that no compound morphology was noticed in his material.

Chromosome 9

This chromosome is the smallest of the complement. The short arm of the chromosome is consistently smaller than that of chromosome 6 and 7 by about 1/3 to 1/2. Its mean arm artio is 0.120; 8.34 for long/short. The short arm bears a very conspicuous satellite. The satellite is about 1/2 - 2/3 the length of the short arm bearing it and usually just a little less in breadth. It never shows a compound nature. The satellite attachment thread is sometimes very short so that the satellite is drawn into the chromosome arm and thus becomes a little difficult to delineate, though more often there is a conspicuous attachment thread of variable length. Fig. 7 f and g illustrates the two most frequently encountered types of satellite on chromosome 9.

In total relative length chromosome 9 must correspond to Levan's St_2 chromosome and Kurita's chromosome i. Levan's chromosome, however, carries no satellite and has a somewhat different arm ratio from that in my material (0.168 and 0.120 respectively) because of its relatively larger short arm. * Kurita's chromosome carries a satellite as in my material though his satellite appears somewhat smaller.

The earlier investigations on satellite morphology by myself (1962) were on the conspicuous satellite of the complement, i.e. of chromosome 9. The satellite of chromosome 7 was not identified in the material

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*   Arms of different lengths in a chromosome are distinguished as being either short or long. Short or long arms that differ amongst themselves are distinguished by the terms smaller or larger. Hence the descriptions, smaller and larger short or long arms.

then under study. As mentioned earlier it had been established that this
conspicuous satellite was not compound in morphology in comparison with
its assumed equivalent and conspicuous S_4 satellite of Levan. The
conclusion on the simple nature of this satellite on chromosome 9 has been
substantiated fully in the present studies. It is now not surprising
that no correspondence with the compound morphology described by Levan
for his S_4 satellite occurs, since the chromosomes do not appear to be
equivalent. Rather, chromosome 7 in my material approaches Levan's
S_4 chromosome in respect of size and satellite morphology.

Summarising the comparisons made above between the materials of
Levan, Kurita and myself, it can be seen that in general my material is
more akin to that of Kurita, though possible differences may be present
in arm ratios, relative lengths and satellite morphologies. Both my own
and Kurita's complements lack secondary constrictions on two of the
large chromosomes, bear no satellite on one smaller median - submedian
chromosome, and have satellites on two rather than one of the subterminal
centromered chromosomes.

The data presented above were obtained only from plants of one
population, though plants from other populations examined, including ones
from Palmerston North and Auckland, show essentially the same basic
complement. A number of variant individuals have been observed, however,
from different populations, the most frequent differences being those
associated with satellite numbers. Plants from one population in culture
appear to have consistent relative size and arm ratio differences in

in chromosome 6 and those from another are triploid. These have yet
to be studied in detail. No doubt other variations occur in different
populations.

III.   Association of Homologous chromosomes

Association of homologous chromosomes in mitosis, or somatic
pairing, is a well established feature in dipterous insects. A tendency
towards homologous association is also found to greater or lesser extents
in numerous other plant and animal materials (e.g. in human cells;
Schneiderman and Smith 1962 and in Ornithogalum (Liliaceae); Therman,
1951), and it is probably present to some extent in all mitoses though
requires statistical analyses of many cells to detect adequately. During
these investigations on normal and interchange root tip mitoses it became
evident that at least some homologous chromosomes of the complement tend
to lie closer together than expected with random distribution. A detailed
investigation of the phenomenon has been carried out in both colchicine
and non-colchicine treated cells. I shall describe certain aspects of the
phenomenon here as they give strong support to the identification of certain
chromosomes in normal and interchange complements. A much fuller account
is in preparation for publication.

In the studies using colchicine treated preparations the distances
between centromeres of homologous chromosomes were compared with distances
between one randomly chosen member of the homologous pair under study and
centromeres of randomly chosen non-homologous chromosomes. A total of
40 normal cells (and 35 interchange cells) were examined, initially from
the point of view of chromosome pairs 6, 7 and 9 (group III), but later
also for pairs 4, 5 and 8. Much care was needed in choosing cells
to be examined since in no way must the relative placement of homologous
members influence a decision to score a cell or reject it as unclear.
It was fortunate in this respect that 20 normal and 11 interchange cells

had already been selected and photographed for studies unrelated to the
present one, and these cells were found to show the same trends as cells
subsequently chosen specifically for the present analyses. The following
procedure, designed to remove any possible bias, was used to select cells
for examination.

(1) Only cells which were intact (cell wall apparently unbroken during
squashing) were selected for study. In such cells the 18 chromosomes
were usually distributed rather evenly throughout the cell cytoplasm in
a rough circle (fig. 2).

(2) All intact cells observed were examined under oil immersion for
identification of all three pairs of group III chromosomes, 6, 7 and 9.
If each member of these three pairs were clearly identifiable through
presence or absence of their characteristic satellites, then these cells
were selected for further study. If one or more of these pairs of
chromosomes were not clearly distinguishable (pair 7 satellites are sometimes
difficult to observe clearly) then the cell was rejected as unclear and
not subsequently examined.

(3) The chosen cells were then examined for identification of chromosomes
4, 5 and 8. In about 30% of cases recognition of chromosome 4, 5 or 8
pairs could not be made with absolute certainty, because of unclear
centromeres or chromosome ends or twisting of chromosome arms. These
30% of cells were not examined further for pairs 4, 5 or 8. It became
clear from a comparison of chromosome 4, 5 and 8 data from cells selected
for scoring in this way and those previously selected for reasons
unrelated to the present study that the selection is not a biased one.

The system of measurements and analysis used here followed that of

Schneiderman and Smith (1962) working with human chromosomes. The
selected cells were photographed and the 18 chromosomes randomly numbered
on drawings, made from the photographic negatives, from left to right of
the group using Fisher and Yates (1963) tables of random numbers.*
The distances between the central points of homologous centromeres of
chromosomes 6, 7 and 9, and 4, 5 and 8 when these were identifiable,
were recorded to the nearest millimetre. Then the distances between a
randomly chosen member of each pair and three randomly chosen non-
homologous chromosomes were recorded. The interhomologous distance for
each pair was then subtracted from the mean inter-nonhomologous distance.
If homologous chromosomes are distributed at random through the cell
then the difference (dx) will average out to approximately zero, while
dx will be positive significantly, on the average,if there is any tendency
for homologous chromosomes to associate with each other. The observed
dx values for each pair of chromosomes examined were tested statistically
by the standard t-test (mean dx for a particular chromosome pair over
all cells examined = Dx in centimetres.)

Results from 40 normal cells are given in table 5a. This number
of cells was regarded at the beginning of these studies as being appropriate,
since evidence from a similar study in human complements by Schneiderman
and Smith (1962) suggested that many fewer probably would have given a
sample toosmall to reveal any tendencies as being statistically
significant. Pairs 6, 7 and 9 were examined in all 40 cells; pairs
5 and 8 in 27 cells, and pair 4 in 30 cells.

Examining first the data for pairs 9, 7 and 6 (group III) it can be
seen that in all three cases there is a tendency for Dx to be positive

* Fisher, R.A. & F. Yates. (1963). Statistical Tables. Oliver & Boyd, London. 6th Edit.

Pair	Dx (cm)	No. of cells	S.E.	t
9	+0.84	40	0.38	2.21 *
7	+0.64	40	0.34	1.88
6	+0.67	40	0.31	2.16 *
5	+0.74	27	0.34	2.13 *
8	+0.68	27	0.40	1.70
4	+0.64	30	0.31	2.06 *

Table 5a   Summary of data on the association of homologous

chromosomes in normal cells.

Dx = mean dx values over all cells examined in centimetres.

S.E. = standard error; t = Dx/SE. * Significant at the 5% level

of probability.

All Dx values are positive, four significantly so, thus providing

evidence for association of homologues in these cells.

rather than zero, suggesting that homologous chromosomes of these pairs lie closer together than expected if they were randomly distributed through a cell. In pairs 6 and 9 the deviation from zero was significant at the 5% level of probability, while in pair 7 this level was almost reached.

Following the method of Schneiderman and Smith (1962) a series of control measurements were also recorded. Thus the distance between one random member of the chromosome pair under study (only pairs 9, 6, 5 and 4 were examined here) and one other randomly chosen non-homologous/pair was chosen and its distance from three other random
chromosome was chosen. Then one random member of this non-homologous chromosomes recorded. As above, the values were calculated and mean dx (Dx) tested statistically for deviation from zero. The data from the same 40 normal cells analysed previously are shown in table 5b.

The data show no deviation from null hypothesis, thus emphasizing the significance of the data obtained in table 5a.

Similarly, centromere distances between members of a random pair of chromosomes, one from each of pairs 9 and 7 were measured, and then those between a random member of this non-homologous pair and three other non-homologues. The data in table 5b show that pairs 9 and 7 are not associated as are homologous members of the two pairs.

Pairs 6, 7 and 9 can be identified unambiguously in a cell, there being no possibility of reversal of order within these pairs. As discussed previously, however, there is a possibility of reversal of order in pairs 5 and 8, and conceivably also for pair 4, though in respect of the latter it was shown that this is unlikely since it has two markers that together distinguish it clearly from all other group I chromosomes. If there is but little or no reversal of order in any or all of these pairs then it might be anticipated that identified pairs will show the same

Pair	Dx (cm)	No. of cells	S.E.	t
9/A	−0.29	40	0.47	0.62
6/B	+0.02	40	0.54	0.04
5/C	+0.19	27	0.58	0.30
4/D	−0.22	30	0.56	0.39
9/7	−0.36	40	0.30	1.20
5/8	−0.14	27	0.50	0.28
6/4	−0.44	30	0.40	1.10

Table 5b Summary of data on the association of non-homologous chromosomes.

A,B,C and D represent the non-homologous chromosome tested with a chromosome 9, 6, 5 and 4 member respectively. Other abbreviations as in table 5a. Dx values are both positive and negative round zero. t values are not significant. Compare with table 5a.

tendency for homologous association, if indeed they are genuinely homologous. This need not necessarily be so since association may c conceivably be restricted for some reason to subterminal type chromosomes of group III. But positive evidence for association will be strong support for correct or very nearly correct identification of homologues.

Table 5a shows that Dx for these pairs is also positive, to a degree very similar to that for group III pairs. In pair 5 the deviation was significant at the 5% level of probability, while for pair 8 the level of probability was between 10% and 5%. This evidence therefore suggests that pairs 5 and 8 are classified into genuine homologues with good if not perfect consistency. It is also informative to note from table 5b that random members one from each of pairs 5 and 8 show no tendency towards association as did members within each pair. Similarly for pair 4 in table 5a. The Dx value for this pair is very similar to that for other pairs and is significantly positive at the 5% level of probability. This evidence thus supports the contention made previously that pair 4 chromosomes can be readily recognized and correctly paired in normal cells.

$\overline{\text{IV}}$.　　　　Variation in chromosome lengths

Variation in chromosome lengths and arm ratios are clearly manifest
in the data obtained in the present studies (Table 2 and Appendix table 1).
It is necessary to recognize and possibly explain the types and extent
of these variations, particularly in certain chromosomes, in order to
interpret those differences found between normal and interchange chromosome
complements described later.

Battaglia (1957) recognized two types of variation in chromosome
lengths in his studies on Allium, viz.

(1)　between homologous chromosomes of the same nucleus - inter-
homologous variation;

(2)　between homologues of different nuclei (of the same or different
root tips or plants) - interkaryotypic variation. (In part, inter-
karyotypic variation covers variation in the relative lengths of
different chromosomes between different nuclei.)

Variations were attributed by Battaglia to both extrinsic (e.g.
different cytological techniques) and intrinsic (e.g. stage of
contraction) factors. Variations in arm ratios were also recognized
by Battaglia.

Bajer (1959) found possible variation between homologous chromosomes
of the one nucleus in his studies on living Haemanthus endosperm cells.

Sybenga (1959) in his studies on Secale cereale recognized "natural"
variations within a complement (see also Patau, 1960; Essad et. al.
1966) and "artificial" variations introduced by the experimenter. The
latter were clearly divided into: (a) variation in actual chromosome

lengths caused through cytological techniques, and (b) variation caused

through inaccuracies of measurements. Thus, for example, he found

differences in lengths between moderately and heavily squashed

preparations showing that the chromosomes become stretched under pressure.

He found some chromosomes of a set showed this stretching while others

did not, and, unexpectedly, that long arms are stretched proportionately

more than short arms. Secondly, Sybenga found in his detailed studies

that the main factors that introduce variability in repeated measurements

of the same chromosome are vagueness of chromosome ends and constrictions,

and errors when curves have to be taken into account.

Sybenga concluded that variability introduced by additional

squashing, though statistically significant, is appreciably smaller

than the variability already present (intrinsic variability). This will

probably also apply to the variability introduced by inaccuracies in

photographic procedure and actual measurements.

Interhomologous variation

Interhomologous variation is clearly evident in the data of

Appendix Table 1 (see table 2 for some immediate details), as both

variations in total chromosome lengths (with and without centromeres)

and variations in arm ratio. These differences cannot be attributed

to inaccuracies in photographic and measuring procedures alone. To

what extent squashing during preparations is a cause of variation has

not been examined in this material. Undoubtedly some of the variations

are caused through natural differences in contraction patterns of the

chromosomes.

Table 2. Details of measurements of chromosomes 4 and 6 from
four normal cells of <u>Allium triquetrum</u>, chosen from the complete
data of Appendix table 1 to exemplify interhomologous and
interkaryotypic variation in chromosome length.

Tables 3 and 4. Mean absolute lengths, mean arm ratios (table 3)
and relative lengths (table 4) of the somatic chromosomes from
10 normal cells (details in Appendix table 1). The data for
chromosomes 4, 6, 7 and 9 will be reasonably accurate, and
probably nearly as accurate for chromosomes 5 and 8, since few
if any instances of arm reversals and reversals of order occur
in these chromosomes. The certain occurrence of such reversals,
however, do not permit accurate data to be presented for chromosomes
1 - 3, since these chromosomes have similar lengths and arm ratios.
The mean and relative total lengths given provide some tentative
information for these chromosomes.

Table 2

	Chromosome 4					Chromosome 6			
Cell no.	Total	Long arm	Short arm	Ratio		Total	Long arm	Short arm	Ratio
2	38·50	20·25	17·25	0·852		27·25	22·75	3·75	0·165
	39·50	20·75	17·75	0·855		28·50	23·50	4·00	0·171
6	34·75	18·25	15·50	0·849		27·00	22·00	4·00	0·182
	36·25	19·25	16·00	0·831		26·75	21·75	4·00	0·184
9	30·00	16·00	13·00	0·828		22·00	18·00	3·25	0·180
	30·00	15·25	13·25	0·869		22·50	18·25	3·25	0·178
10	24·75	13·00	10·75	0·827		18·50	14·75	3·00	0·203
	25·25	13·25	11·00	0·830		18·25	14·50	3·00	0·207

Table 2

Table 3

Chr. no.	Total	Long arm	C/m.	Short arm	Ratio
1	40·28				
2	39·58				
3	37·94				
4	34·15	18·01	1·14	15·00	0·835
5	26·18	15·40	1·13	9·65	0·627
6	25·15	20·50	0·95	3·70	0·182
7	23·06	18·79	0·92	3·35	0·179
8	23·03	12·68	1·09	9·26	0·731
9	19·96	17·09	0·82	2·05	0·120

Table 3

Table 4

Chr. no.	Total	Long arm	Short arm
1	14·95		
2	14·69		
3	14·08		
4	12·70	6·69	5·57
5	9·72	5·72	3·58
6	9·34	7·61	1·38
7	8·56	6·98	1·24
8	8·55	4·71	3·44
9	7·41	6·34	0·76

Table 4

An indication of the extent of interhomologous variation was sought in this material in respect of the recognizably distinct chromosome pairs 4 and 6, from the 10 cells fully measured. It will be shown later that the present interchange involved pairs 4 and 6, and hence it was desirable to obtain information on variation in lengths of normal members of these pairs, so as to interpret differences found between interchange and normal pairs in the interchange material. For this purpose differences in length between homologous chromosome arms were calculated as: length of larger arm minus length of smaller arm divided by the mean length of the two arms. Values were calculated in this way since cells showing different degrees of contraction were used in the analysis. Absolute differences are smaller in more contracted cells but proportionately much the same as in more weakly contracted ones.

Mean differences were calculated for each arm pair from the 10 normal cells analysed in detail. Deviations between homologous arm pairs were used in preference to deviations in total chromosome lengths as the former may be cancelled out and therefore unnoticed in total length differences. Also, arm differences proved useful in interpreting alterations in arm lengths caused through the interchange to be described.

Mean differences and standard deviations for the arms of pairs 4 and 6 are shown in table 6. There are no significant differences in the variances nor the mean deviations between the two arms of pair 4, nor between these arms and the long arms of pairs 6. The short arms of pairs 6 (and 7 and 9) appear to show a much lower degree of variation than their long arms. Of the 10 cells contributing to these data only two

Table 6.

Chromosome 4, Long arms.

Cell	dy	Mean length of arms	Dy = dy/mean	
1	1.25	21.375	0.058	
2	0.50	20.500	0.024	
3	0.75	19.375	0.039	
4	0.50	20.250	0.025	
5	0.25	18.125	0.014	
6	1.00	18.750	0.053	
7	1.00	16.750	0.060	
8	0.50	16.250	0.031	
9	0.75	15.625	0.048	
10	0.25	13.125	0.019	Mean Dy= 0.037
				S= 0.017

Chromosome 4, Short arms.

Cell	dy	Mean length of arms	Dy = dy/mean	
1	1.00	16.750	0.060	
2	0.50	17.500	0.029	
3	0.75	16.125	0.047	
4	0.75	16.625	0.045	
5	0.25	15.125	0.017	
6	0.50	15.750	0.032	
7	0.25	14.125	0.018	
8	0.50	14.000	0.036	
9	0.25	13.125	0.019	
10	0.25	10.875	0.023	Mean Dy= 0.033
				S= 0.015

Table 6 (Cont. on next page).

Chromosome 6, Long arms.

Cell	dy	Mean length of arms	$Dy = dy/mean$	
1	0.75	23.125	0.032	
2	0.75	23.125	0.032	
3	0.50	22.000	0.023	
4	0.75	22.375	0.034	
5	0.25	21.675	0.012	
6	0.25	21.875	0.011	
7	1.00	19.250	0.052	
8	0.75	18.875	0.040	
9	0.25	18.125	0.014	
10	0.25	14.675	0.017	Mean Dy= 0.027
				S= 0.014

Table 6 (Cont. from previous page). Interhomologous variation in the long and short arms of chromosome 4 (previous page) and the long arm of chromosome 6 in the 10 normal cells of Appendix table 1. dy = larger arm − smaller arm. S = standard deviation.

showed a detectable difference (0.25mm at approximately 2,400 magnifications) between the two homologous short arms of chromosome 6, for example. These two cells each had the relatively high Dy value of 0.-645 (relatively high because of the small total length of these chromosome arms), while the remaining eight cells showed no detectable difference in length of these arms, thus giving a mean Dy value of 0.013, which is somewhat lower than that of the long arms.

It seems probable that much of this apparent difference in variation between long and short arms of pairs 6 (and 7 and 9) is attributable to the measuring technique. If the short arms of chromosomes 6 say were to show a mean Dy of say 0.027, this would indicate an average difference in length between the two arms (of say 2.875 mm mean length at 2,400 magnifications) of approximately 0.1mm. This value would probably not be detected since lengths were recorded in 0.25mm's, there being little chance of procuring finer resolution in the face of inaccuracies in interpreting chromosome ends, actual measuring etc. Thus in measuring the short arms of chromosome 6 it is probable that only extreme differences (approaching 0.25mm and greater) have been recorded while somewhat smaller differences, though being present, have not been detected. Thus in part at least the low mean Dy value recorded for the short arm of chromosome 6 may be caused through the inability to record small differences which, because of the small total length of the arms involved, would contribute a relatively high dy value to the cal-culated mean per cell. This undoubtedly also holds for the short arms of chromosomes 7 and 9. Of course, these small differences will remain undetected in long arms as well, but they would, if detected, contribute

but little to the mean Dy value for these arms (because of the relatively large total length of the arms involved). The smaller the arm is, the more important these undetected differences become in assessing mean Dy values, and this may account for the observation that very small arms tend to show relatively lower Dy values than longer arms.

To some extent (to how much exactly is difficult to assess) the effect of inabilities to record small differences will be offset by overassessing detectable differences; i.e. where a difference is detected, recording it as a 0.25mm difference in cases will be an overassessment. Corresponding underassessment of detectable differences is unlikely to be important since when a difference is noted (> 0, $> 0.25, > 0.5$ etc.) it is recorded to the next unit of measurement, at this resolution one being unable to say with confidence which is the closest 0.25mm.

Any overestimation of differences as above will have a very noticeable effect on the assessment of Dy values in very short arms. This is probably the reason why, in some cells, comparisons of short arms in group III chromosomes sometimes show rather high values of Dy (e.g. 0.06) whereas these are seldom found in long arms.

Overestimation at least to some extent will balance out undetected differences. However, since only in two out of 10 cells was a difference in length noted for chromosome 6, this balancing out is probably not large compared to the effect of undetected differences. The mean difference and standard deviation shown for this pair is thus probably biased on the low side. It is most likely that all arms of the pairs of chromosome 4 and 6, (and other pairs) show approximately the same

pattern of variation. Maguire (1962) found this to be so in her detailed analyses of maize chromosomes.

In addition to the 10 cells fully measured a further 10 were examined in connection with interhomologous variation in the long and short arms of pair 6. In all 20 cells the mean Dy for the long arm was 0.029 with S = 0.015. For the short arm the mean Dy value was 0.017. These values are given since they will be compared later with values for chromosomes 6 and 4^6 in interchange cells.

Homologous chromosomes may sometimes show abnormally large differences in lengths. These extremes occur in isolated cells rather than being peculiar to groups of cells or particular root tips. The reason(s) for such large differences is unclear. In my analysis one cell was rejected from those to be measured since it showed extreme variation in lengths in a number of chromosomes, making it impossible to pair homologues with any accuracy.

Interkaryotypic variation

Interkaryotypic variation is likewise clearly evident in the data of table 2 (see also Appendix table 1. Compare e.g. figs. 2 and 4). To a very large extent this is a parallel variation of all the chromosomes of the complements compared, and the different patterns of variation are found between cells of the same root tip as well as between cells of different roots. Throughout these variations, arm ratios of particular chromosomes remain approximately the same (details below), showing that the two arms of a chromosome, as well as all the chromosomes, behave

in proportionately equivalent ways in interkaryotypic variation.

This large bulk of variation is generally attributed to the effect of colchicine or other analogous agents on the contraction patterns of chromosome complements (Levan, 1938; Levan and Ostergren, 1943; Ostergren, 1944). These agents are considered to induce an extra contraction on the chromosomes. Cells of a root tip may differ in their time of contact with colchicine and, probably more significantly, differ in their stage of division at the time of colchicine application (hence different _effective_ durations of the influence of colchicine on contraction in these cells*).

A second part of interkaryotypic variation is seen as differences in relative lengths of non-homologous chromosomes which, rather than being colchicine induced, is probably akin in extent and cause to inter-homologous variation within a nucleus. Though the data available have not been analysed in detail it can be seen that in general the range of variation in relative chromosome lengths is not greatly different from that expected from the known extent of interhomologous variation. Of particular note in this respect is the fact that chromosomes 4 and 6 are invariably distinguishable on relative lengths from other similar chromosomes (Appendix table 1). This is important for the distinction between F_1 interchange homozygous and normal homozygous plants (described later).

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* Some data available suggest that this is not the complete answer. Cells of different root tips obtained from the same plant and treated together appear to show different _ranges_ of interkaryotypic variation in this material: and prolonged treatment with colchicine will not necessarily induce maximum c-mitotic contraction. This problem has yet to be examined fully.

V.   Differential contraction of chromosomes

In recent years considerable data have suggested that contraction
may not affect small and large chromosomes in strict proportion to their
lengths. Conclusions obtained from cultured animal cells are somewhat
conflicting at present. Sasaki (1961) and Fitzgerald (1965) found that
at least in some different human cells longer chromosomes tend to contract
proportionately more than shorter ones, and that longer arms may contract
more than shorter ones. On the other hand, Levan, Hsu and Stikch (1962)
and Matthey (1962) found no such differential behaviour in other
cultured animal cells.

I have examined the available data of Allium triquetrum for the
possibility of gross differential contraction of large and small
chromosomes and chromosome arms, because of the influence such a phenomenon
might have on the identification of interchange chromosomes and chromosome
arms, and on the interpretations given to interchange pachytene
configurations presented later.

1.   Contraction in long and short chromosomes   Six strongly
contracted complements were compared with six weakly contracted ones.
Each set of six comprised those four normal complements and two interchange
complements that were in total chromosome length, (A) the shortest and
(B) the longest of the cells measured in detail (appendix tables 1 and 2).
(Figs. 2 and 4 e.g. were members of the strongly and weakly contracted
sets respectively.) The chromosome lengths (excluding the centromeres)
of (a) the large, group I chromosomes 1 - 4 and (b) the small, group III

chromosomes 6, 7 and 9 were summed for each of the six strongly and weakly contracted complements*. The summed lengths of the large chromosomes are denoted X hereafter and the small chromosomes Z.)

Mean X and Z values were obtained for each set of six complements. The mean values for each set were expressed as a ratio, mean X / mean Z. The two ratios obtained were compared.

If long and short chromosomes contract in strict proportion to their lengths, then the above ratios from strongly and weakly contracted complements should be approximately equal. On the other hand the ratio obtained from the highly contracted complements should be significantly lower than that of the weakly contracted complements, if larger chromosomes in general contract more than shorter ones.

The data are shown in table 7a. The difference between the mean X / mean Z values for highly and weakly contracted complements (0.014) is in the initially expected direction but is not significant ($p > 0.8$). Long group I and short group III chromosomes thus contract in proportion to their lengths.

It might be argued that the above is not as critical a test as desirable. The long arms of group III chromosomes are roughly the same size as each of the two arms of group I chromosomes, and thus the two groups may for this reason be behaving similarly in contraction patterns. A more critical test perhaps is that which compares the long group I chromosomes with the short group II chromosomes, the latter being similar in length to group III but with median-submedian centromeres as

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*   In the interchange complements the actual interchange chromosomes were not included in the summations, their places being taken by a double scoring of the appropriate normal chromosome. A slight error will be introduced here but this will have no significant effect on the general conclusions to be made.

Table 7.

	X	Z	Mean X	Mean Z	Mean X/ Mean Z
Weakly contracted complements	1961.50	861.75	326.917	143.625	2.276
Strongly contracted complements	1407.25	622.25	234.542	103.708	2.262

Table 7a

	X	Y	Mean X	Mean Y	Mean X/ Mean Y
Weakly contracted complements	1961.50	633.50	326.917	105.58	3.096
Strongly contracted complements	1407.25	444.50	234.542	74.08	3.166

Table 7b

Table 7   Summary of data on differential contraction of long and short chromosomes. For X, Z, and Y values from weakly and strongly contracted complements see text.   The two ratios obtained in each table do not differ significantly from each other, indicating no differential contraction of long and short chromosomes.

in group I. The data for this comparison are given in table 7b, Y here representing the summed lengths of group II chromosomes for the same strongly and weakly contracted sets as used above.

It can be seen here also that there is no significant difference between the two ratios obtained. Indeed, the difference (0.07) is in the wrong direction if longer chromosomes contract proportionately more than shorter ones.

It can be concluded, therefore, that in Allium triquetrum long and short chromosomes contract uniformly within the range of lengths studied here. This conclusion vindicates the calculation of standard, relative lengths of the distinct chromosomes in this species (table 4).

11 Arm ratios in long and short chromosomes   Arm ratios were also compared in strongly and weakly contracted complements. Even though long and short chromosomes have been shown above to have proportionately equivalent patterns of contraction, long and short arms within a chromosome might still show differential behaviour; relatively greater contraction in one arm being offset by relatively less in the other arm.

The summed arm ratios for (1) all nsix (smaller) group III chromosomes - those with very asymmetrical arms - (designated W), and (2) the two (larger) chromosome 4 members with only moderately asymmetrical arms (designated V) were obtained for each of the six strongly and weakly contracted complements used above. Complement mean values for each set (W and V) were then calculated and compared. If long and short chromosome arms contract in proportion to their lengths then the summed and mean values from the two sets of complements should not be significantly different; whereas if long arms contract to a greater extent than smaller arms, then the summed and mean values from highly contracted complements

should be significantly greater

than those of weakly contracted complements.

The data obtained are shown in table 8.

(a) Chromosome 4. The difference between the weakly and strongly contracted mean values 0.033 is in the expected direction if long arms contract proportionately more than short arms in a chromosome, but this difference is not significant statistically (compare with (b) below). Thus in this chromosome pair over the range of contraction examined there appears to be no clear tendency for differential contraction of arms within a chromosome. The result from this analysis was not unexpected since in a very strongly contracted chromosome complement as that in fig. 5, chromosome 4 and indeed also the suggested pairs 5 and 8 retain clear asymmetry in their two arms.

(b) Group III chromosomes. Here the difference is also in the expected direction if long arms contract proportionately more than shorter ones. This difference (0.114) is statistically significant ($t=4.0$ $p.$ $<0.05>0.01$), though on a per chromosome basis it is very small and imperceptible without these detailed measurements. Thus visually both long and short arms of these chromosomes contract proportionately, as suggested from e.g. fig. 5; though on a finer analysis some indication of disproportionate contraction is given.

Some observations were made on chromosome 9 in non-colchicine preparations at metaphase. In three chromosomes measured the arm ratios were 0.120; 0.109; and 0.118* (cf. for chromosome 9 in table 3). Thus even over this extended range of contraction both small and long arms behave very similarly.

The significant difference detected in the above analysis thus required some further consideration. The difference may be the result of

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* It is somewhat more difficult to delineate centromeres in non-colchicine preparations.

Table 8.

Arm ratios

Chromosome 4	Totals	Mean per cell	
Weakly contracted complements	9.975	1.662	
Strongly contracted complements	10.168	1.695	Difference (strong – weak) = + 0.033.

Chromosomes 6, 7 and 9 together						Totals	Mean per cell
Weak	0.902	0.872	0.921	0.965	0.942	0.964	5.566	0.928
Strong	1.046	0.931	1.043	1.058	1.107	1.067	6.252	1.042

Difference (strong – weak) = + 0.114.

Table 8   Differential contraction of long and short arms of
chromosomes 4 (top table) and chromosomes 6, 7 and 9 together. For
chromosome 4 the totalled and mean arm ratios in weakly and strongly
contracted complements are not significantly different. For chromosomes
6, 7 and 9 together the details from each cell, totals and mean per cell
are significantly larger in strongly contracted complements than in
weakly contracted ones, suggesting differential contraction of long and
short arms of these group III chromosomes. See, however, explanation
in text.

sampling error. On the other hand there may be a real, though very slight, tendency in these chromosomes for long arms to contract a little more than shorter ones. Thirdly, however, and most likely to me, the difference detected may be caused through extrinsic errors in measurements.

In measuring chromosomes some difficulty is encountered in delineating arm extremities because of diffraction fringes. Further, because of this, there is in the measuring technique used here, I feel, a tendency to over measure chromosome arms by a slight amount, rather than under measure them. In long arms this error will be very insignificant, though in small arms it could assume important proportions, and the smaller an arm becomes during contraction the more significant this error will be. In calculating the arm ratios of group III chromosomes in weakly and strongly contracted preparations, the above error introduced will have most effect at levels of extreme contraction, and I feel, has been revealed in the above analysis as statistically significant differences between the two sets of complements. In chromosomes with both arms moderately long the error will not be revealed without more extensive data than used here; and thus remains statistically undetected in chromosome 4 (though suggested, note - see table 8). This will likewise account for the fact that though differences in arm ratios in group III chromosomes were detected, no significant difference was noted in comparing whole chromosome contraction patterns between groups I and III chromosomes.

General conclusions and comparisons on contraction patterns

In general it is evident that in Allium triquetrum there are no major differences in contraction patterns in long and short chromosomes and

chromosome arms over the range of lengths studied here. What very slight differences have been detected are probably artefacts of measuring.

These results contrast sharply with those obtained e.g. from human chromosome measurements.

The summed lengths of the group I chromosomes in my material range from 98-110 μ in the strongly contracted complements, and from 142 - 150 μ in the weakly contracted ones; there is thus a reduction in length from one to the other of about 30%. Sasaki (1961) studied the ratio of the longest human chromosome over the shortest, in preparations treated for two and five hours in colchicine, these showing approximately the same degree of reduction in length as in my complements (see his figs. 6 and 8). Mean long/mean short values showed a very significant reduction from 5.72 - 3.76 over this range of contraction, showing clear differential contraction patterns for these long and short elements. Similar data were obtained by Fitzgerald (1965), also in human chromosomes.

Similarly, Sasaki (1961) found a significant difference ($p = 0.3$) of 0.38 (approximately 20% reduction) between mean arm ratios of the submedian chromosome 2 in cells treated for two and five hours in colchicine. This is a considerably greater difference than detected in my group III chromosomes (11% reduction), and in my material three pairs of chromosomes were jointly examined, whereas Sasaki examined only one.

It seems unlikely that the larger, significant differences in contraction patterns between long and short chromosomes and chromosome arms observed by Sasaki and Fitzgerald, amongst others, are attributable to measuring errors as in my material. Rather, they strongly suggest

some form of differential contraction in these complements. Why different materials should show such marked differences in this respect must remain unexplained at present. Clearly more investigations are needed in other materials to clarify the phenomenon.

2. SOMATIC CHROMOSOMES OF INTERCHANGE PLANTS

As indicated previously the interchange material studied here is indistinguishable from that which provided the meiotic cells studied previously (Rickards, 1962). Also, all the wild interchange plants examined show the same basic irregularity and are undoubtedly clonal and possibly F_1 or other derivatives of one original plant in which the interchange arose.

Anaphase I preparations had previously established and have subsequently confirmed here (details later) that the interchange involved one chromosome from each of groups I and III. The somatic chromosomes of interchange plants were studied to establish more precisely if possible the chromosomes and chromosome arms involved. Full detailed measurements were recorded from eight root tip complements from seven different plants (Appendix table 2). In addition measurements of 3 of the four interchange chromosomes that are always clearly identifiable were recorded from 8 other cells (Appendix table 2a).* Relative lengths and arm ratios of recognizably distinct chromosomes are summarized, from the full detailed measurements, in tables 9 and 10.

Figs. 8 - 11 exemplify the interchange complements, and apparent diagrammatic and photographic idiograms are shown in Fig. 12. Comparisons are made below with the observations already reported on normal plants. The interchange chromosomes are described in the order in which they were first recognized.

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* These additional 8 were a random selection from 35 which had been selected previously on a basis of recognition of pairs 7 and 9 (and thus also the other two group III chromosomes 6 and 4^6) for purposes unrelated to obtaining measurements of particular chromosomes. In all 8 the relevant interchange chromosomes were clearly recognizable and easy to measure.

Tables 9 and 10   Mean absolute lengths, mean arm ratios

(table 9) and relative lengths (table 10) of the somatic chromosomes

from eight cells from interchange plants (details in Appendix

table 2). Comments here as in caption to tables 3 and 4.

The data for chromosome 6^4 are probably somewhat incorrect

because of likely instances of confusion with asymmetrical

members in pairs 1 - 3.

Chr. no.	Total	Long arm	C/m.	Short arm	Ratio
1	40.28				
2	40.20				
3	38.00				
5	26.00	15.31	1.19	9.50	0.620
7	22.64	18.41	0.92	3.31	0.181
8	22.75	12.53	1.19	9.03	0.719
9	19.84	16.94	0.85	2.05	0.122

Chr. no.	Total	Long arm	Short arm
1	14.96		
2	14.94		
3	14.11		
5	9.66	5.69	3.53
7	8.41	6.84	1.23
8	8.45	4.66	3.35
9	7.37	6.29	0.76

↕ Table 9   ↕ Table 10

Chr. no.	Total	Long arm	C/m.	Short arm	Ratio
4	34.47	17.97	1.41	15.09	0.839
4^6	21.44	17.25	1.03	3.16	0.184
6	24.63	19.94	1.06	3.63	0.184
6^4	38.38	20.69	1.35	16.34	0.788

Chr. no.	Total	Long arm	Short arm
4	12.81	6.68	5.61
4^6	7.96	6.41	1.17
6	9.15	7.41	1.35
6^4	14.26	7.69	6.07

I. The chromosomes involved in the interchange.

Group III chromosomes

Chromosomes 7 and 9 agree well with those of normal plants in both relative lengths and arm ratios.

The two members (6 and 4^6 in tables and figures - symbolism of 4^6 explained later), superficially identifiable through arm ratios and absence of satellites as being akin to chromosome 6 members of normal plants are, however, very different from each other, in both long and short arms, and in total lengths.

(a) The long arms. The lengths of these two arms relative to the whole complement are 7.41 and 6.41 for chromosomes 6 and 4^6 respectively. In the 16 cells examined (appendix table 2 and 2a) the mean Dy value for these arms is 0.140. This value is very much larger than that found between the long arms of pair 6 in normal cells (table 6) and the difference is clearly very significant. The important points to note are that in all cells a difference was noted, and the smaller of the two arms (that of 4^6) is always borne by the shorter chromosomes (table 11).

(b) The short arms. Similarly the relative lengths of the short arms of these two chromosomes are very different, being 1.35 and 1.17 for 6 and 4^6 respectively. In the 16 cells of appendix table 2 and 2a the mean Dy value is 0.134. This value again is very much larger than that between the short arms of pair 6 in normal cells (0.013 see p. 41) and is clearly very significant. In every case a difference was detectable, and the smaller chromosome always carried the smaller short arm.

Table 11 compares the long and short arms of chromosomes 6 and 4^6 in all 16 cells in which these chromosomes have been measured. When compared to the values for the long and short arms of members of pair

Table 11.

Chromosomes $6/4^6$

	Long arms				Short arms	
Cell	dy	Mean of arms	Dy	dy	Mean of arms	Dy
1	3.25	21.375	0.152	0.25	3.625	0.069
2	2.50	20.750	0.120	0.75	3.625	0.207
3	3.50	20.750	0.169	0.25	3.625	0.069
4	3.00	19.250	0.156	0.50	3.500	0.143
5	2.75	19.625	0.140	0.50	3.500	0.143
6	3.25	17.125	0.190	0.25	3.125	0.080
7	2.00	15.500	0.129	0.50	3.250	0.154
8	1.25	14.375	0.087	0.75	2.875	0.261
9	3.00	21.750	0.138	0.50	3.750	0.133
10	2.75	19.875	0.138	0.75	3.625	0.207
11	2.75	19.875	0.138	0.25	3.625	0.069
12	2.75	17.875	0.154	0.50	3.500	0.143
13	2.50	17.750	0.141	0.50	3.500	0.143
14	2.25	15.625	0.144	0.25	3.125	0.080
15	1.75	15.375	0.144	0.25	2.875	0.087
16	2.00	16.250	0.125	0.50	3.250	0.154

Table 11 Comparison of the long and short arm lengths of chromosomes
6 and 4^6 in 16 cells in which these chromosomes were measured (Appendix
table 2 and 2a). Abbreviations as in table 6. In all cells the
chromosome 6 arms were the longest. Mean Dy values; 0.140 (long arms)
and 0.134 (short arms); compare with table 6.

6 in normal cells it is clear that the differences found in interchange cells cannot be attributed to interhomologous variation alone.

Chromosome length as a whole.

As indicated above the shorter chromosome bears both the smaller long and the smaller short arms. On the average the total length of the shorter chromosome is smaller than the larger by about 1/3th.

Arm ratios.

There is virtually no difference between the mean arm ratios of the two chromosomes (0.18412 and 0.18400 for 6 and 4^6 respectively - table 9). This identity in arm ratios in the face of differences in chromosome size arises because the differences were present, and proportionately nearly the same, in both the long and short arms.

The normal and abnormal chromosomes

These can be identified through relative length comparisons with other normal chromosomes in the interchange complements and with the relevant chromosomes of normal cells. In relative length chromosome 6 falls above chromosome 7 in interchange cells and agrees well with the relative length of chromosome 6 in normal cells (9.15 and 9.34). The difference is within the range of variation met with in comparisons with other chromosomes of normal and interchange cells. Chromosome 4^6 on the other hand falls well below chromosome 7 and a little above chromosome 9 in both normal and interchange cells, and the relative length difference (1.38) between 4^6 of interchange cells and 6 of

normal cells is clearly significant.

Chromosome 6 of interchange cells must therefore be the normal member whilst 4^6 must be the abnormal one.

Group II chromosomes.

The measurements obtained for the apparent chromosomes 5 and 8 in interchange cells agree well with those of normal cells.

Group I chromosomes.

In interchange cells there is only one chromosome that in relative length and asymmetry can be identified clearly as chromosome 4 of normal cells. The data for this chromosome agree well with those for chromosome 4 of normal cells (compare tables 4 and 10). The remaining seven chromosomes fall essentially into one group comprising pairs 1 - 3 of normal cells plus the 'partner' of chromosome 4. In the cells examined at least one, though usually two or three, chromosomes somewhat approaching member 4 in respect of asymmetry, but always larger than it, can be identified (see 6^4? in figs. 8 - 11 e.g.). It is probable that one or other of these chromosomes is the 'partner' of member 4. It is not possible to identify and characterise the 'partner' chromosome because of confusion with other group I members, particularly those tentatively regarded as pair 2 of normal cells (compare 6^4? with the top, left hand, horizontal group I chromosome in fig. 9 e.g.). The measurements and apparent idiograms for chromosome 6^4 (tables 9 & 10; Fig. 12) are only suggestive. The conclusion made below regarding the arms involved in the interchange requires the second interchange chromosome to be a large, asymmetrical member (Fig. 19 and discussion below).

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It is concluded from the observations above that one of each of

chromosomes 4 and 6 are involved in the interchange. This conclusion is

reinforced from further observations given below. In each of the two

pairs of chromosomes in interchange cells the apparently normal chromosome

is the unaffected member while the abnormal one represents the interchange

chromosome. The two pairs of abnormal and normal members complement each

other quite accurately in total length in each cell, within the limits

imposed by the inability to be fully certain of the identification of

chromosome 6^4 - e.g. in cell No. 1 of appendix table 2 where the total

length of 4 plus 6 is 65.00, and for 4^6 plus 6^4, 66.75. Thus in a broad

analysis, what has been gained by one of the interchange chromosomes has

been lost by the other, as theoretically expected.

II. The arms involved in the interchange.

From the general nature of the interchange complex at anaphase I

it had been concluded in my previous work (1962) that the interchange

involved the long arm of the group III chromosome (6) and one or other of

the group I chromosome (4). It is now recognized that this does not

necessarily follow (see Rickards, 1964, p. 141). The exchange might have

involved the short arm of the group III chromosome. Fig. 13 (1) and (2)

illustrates the two general types of interchange involving a group I and

group III chromosome that can be expected to give rise to anaphase I

configurations of the types that are seen. No attention has been paid

to exact relative chromosome lengths in this figure, nor to actual break

points. In (1) the interchange involved the long arm of the group III

chromosome, and in (2) it involved the short arm. Both of these types are likely, theoretically, to produce alternate and adjacent orientations at metaphase I when involved with either arm of the group I chromosome. In (1) the general group classification of the resulting chromosomes remains the same as the unaffected ones. In (2) however, it is important to note that the centromere of the normal group I chromosome has become that of a group III type, and that of the normal group III chromosome become of a group I type. Also, in (1) the interchanged segments are roughly equal; while in (2) the interchanged segments are grossly unequal. (Important differences in (1) and (2) concerning pachytene centromere positions and adjacent orientation at anaphase I will be discussed later).

The problem of which of types (1) and (2) occurred in the present interchange material was initially answered from studying the lengths of the short arms of the normal and interchange group III chromosomes involved. If there are consistent and significant differences in the sizes of these two arms, then this can be taken as direct evidence that the interchange involved the short arm of 6 and was thus of type (2). If no differences can be detected then a conclusion either way is not possible.

As indicated above, consistent and significant differences in the short arms of chromosomes 6 and 4^6 in interchange cells have been established (table 11). These differences cannot be attributed to natural interhomologous variation or measuring techniques alone; they must be attributed very largely to the interchange, and it is concluded from this evidence that the interchange break occurred in the

short arm of chromosome 6. The break was such as to produce a group III chromosome (4^6) from chromosome 4, having a slightly smaller short arm than that of the normal chromosome 6. The interchange was of type (2).

From a general point of view as in Fig. 13 it is possible for the interchange to have involved either arm of the group I chromosome. However, since the two arms of the involved chromosome 4 are asymmetrical in length and since chromosome 6 has lost a noticeable length in the interchange, then it should be possible also to conclude which arm of chromosome 4 was involved. Bearing in mind that only slight differences occur between the short arms of chromosome 6 and 4^6, the following points arise from the two possibilities -

A. If the interchange involved the <u>long</u> arm of chromosome 4 then

    (a)   the long arm of the resulting chromosome 4^6 should be approximately equal to the <u>short</u> arm of 4, and

    (b)   the resulting group I type chromosome (6^4) should be almost symmetrical in its two arms (since the long arms of 4 and 6 are similar in length).

B. If the interchange involved the <u>short</u> arm of chromosome 4, then

    (a)   the long arm of the resulting chromosome 4^6 should be approximately equal to the <u>long</u> arm of 4, and

    (b)   the resulting chromosome (6^4) should be very noticeably asymmetrical (since the long arm of 6 is considerably larger' than the short arm of 4).

In the eight interchange cells fully measured the relative lengths of the short arm of 4 and the long arm of 4^6 show a considerable difference

(5.61 and 6.41). In all 16 preparations examined only in none was the long arm of 4^6 equal to the short arm of 4, but in all 16 cases it was clearly larger. Overall these two arms show a systematic pattern of variation and show a much greater difference than expected from normal interhomologous variation (table 12a). Mean Dy for these arms is 0.146 which is much greater than in table 6. Hence homology between these two arms is very unlikely. On the other hand, the relative length of the long arms of 4 and 4^6 are approximately the same, (6.68 and 6.41). Over all 16 cells examined, in five the long arm of 4^6 was shorter than that of 4, in two the arms were of equal length, while in nine, that of 4^6 was larger than that of 4. The mean Dy for these arms (0.042) is comparable to that for the long arm of 4 in normal cells (table 6). Statistically the differences met between these two arms are not significant and are no greater than expected from known interhomologous variation of homologous long arms of pair 4 in normal cells. Thus these long arms of 4 and 4^6 are considered to be homologous, and therefore the interchange must have involved the short arm of chromosome 4. This is supported further by the fact that the odd group I chromosome that can be identified occasionally in interchange cells is noticeably asymmetrical rather than symmetrical, as expected on the above ground.

It is evident from the above, then, that B is the correct interpretation. The interchange involved the short arm of chromosome 4 as well as that of 6.

Two other expectations follow from these considerations.

(a) The long arms of chromosomes 6^4 and 6, being homologous and unaffected by the interchange should be approximately equal.

Table 12.

Chromosomes $4/4^6$

	(a) Short arm 4/long arm 4^6			(b) Long arm 4/long arm 4^6		
Cell	dy (4^6-4)	Mean of arms	Dy	dy (4^6-4)	Mean of arms	Dy
1	+3.50	18.000	0.194	+0.25	19.625	0.013
2	+2.00	18.500	0.108	−0.75	19.875	0.038
3	+2.00	18.000	0.111	−1.25	19.625	0.064
4	+1.25	17.750	0.070	−2.25	18.875	0.119
5	+3.00	16.750	0.179	+0.25	18.125	0.014
6	+1.50	14.500	0.103	−2.00	16.250	0.120
7	+1.75	13.625	0.128	−0.50	14.750	0.034
8	+2.25	12.625	0.178	+0.50	13.500	0.037
9	+3.50	12.675	0.189	+0.50	20.000	0.025
10	+2.00	18.500	0.114	−1.50	19.250	0.078
11	+3.25	16.875	0.193	+0.25	18.375	0.014
12	+2.75	15.125	0.182	0	16.500	0
13	+2.50	15.250	0.164	−0.50	16.750	0.030
14	+1.50	13.750	0.109	−0.75	14.875	0.050
15	+1.75	13.625	0.128	−0.50	14.750	0.034
16	+2.75	13.875	0.198	0	15.250	0
		Mean = 0.146			Mean= 0.042	

Table 12   Comparison of the long arm of chromosome 4^6 with the long
and short arms of chromosome 4 in 16 measured cells (Appendix table 2 & 2a).
In (a) the dy values are all positive and mean Dy is
much greater than expected if these arms are homologous. In (b) the dy
values are positive, negative and zero and mean Dy is as expected
if these two arms are homologous.

(b) The short arm of 6^4 should be consistently larger by a small amount than the short arm of 4 (since what has been lost in the interchange from 6 must have been gained by 4). It is not possible to verify these points fully since an accurate characterization of chromosome 6^4 is not possible. However, in those cells where chromosome 6^4 appears to stand out in the complement as somewhat atypical as compared with normal cells, then appropriate comparisons show the points to be borne out in these cases (e.g. cell 1 in appendix table 2).

In conclusion it is worth rementioning that the two main points:

(a) significant differences in the short arms of chromosomes 6 and 4^6, and

(b) the short arm of 4 being consistently smaller than the long arm of 4^6,

make it very unlikely that the interchange involved any combination of arms other than the short arms of both chromosomes 4 and 6.

III. Symbolism of interchange chromosomes.

The interchange chromosomes have been symbolised 4^6 and 6^4. The reason for this is now clear from the considerations on the arms involved in the interchange. The bases refer to the non-interchanged segments that carry the centromeres, while the superscripts refer to the segments that were interchanged and indicate the chromosomes from which they were derived. This symbolism is most commonly used (Burnham, 1956, 1962 etc.).

IV. The interchange break points.

The interchanged segments must have been grossly unequal. The exact

lengths of the segments cannot be ascertained from somatic data alone, though since the short arm of chromosome 6 was involved, narrow limits can be placed on the location of the break points.

(a) <u>Distal limits</u>. For chromosome 6 this is at the distal end of the short arm. This would mean that this arm contributed nothing to the formation of the short arm of chromosome 4^6. Hence the corresponding break point in chromosome 4 would have been at a position the length of the short arm of chromosome 4^6 from the centromere.

(b) <u>Proximal limits</u>. On the other hand the short arm of chromosome 6 could have contributed all to the formation of the short arm of 4^6, the break point in this arm being at a point the length of the short arm of 4^6 from the arm end. The corresponding break point in chromosome 4 would have been at the proximal end of the arm (at the centromere).

Fig. 14 illustrates these points. The main feature to note is that the breaks must have been fairly close to the centromeres of both chromosomes.

The evidence presented above indicated that the interchange was of type 2 in fig. 13, involving the short arms of both chromosomes 6 and 4. Chromosome 4^6, therefore, though superficially very similar to chromosome 6, is homologous with it in respect of only a small piece of chromosome on the short arms of the pair, at the most. On the other hand 4^6 is largely homologous with the long arm of chromosome 4 and carries a chromosome 4 centromere.

Members of pair 6 in normal cells have been shown to exhibit a degree of somatic association (table 5a) as do members of pair 4. No somatic

association is shown however, between members representative of each
of these two pairs (6/4 in table 5b). If, as suggested above, chromosome
4^6 is largely homologous with the long arm of chromosome 4 and carries
a chromosome 4 centromere, rather than being largely homologous with
chromosome 6, then it will be expected that 4^6/6 members in interchange
cells will show no somatic association, but rather that chromosomes 4^6
and 4 will. Data on somatic association have been obtained from 35
interchange cells; a portion of this information is presented here since
it is directly relevant to the above suggestions.

The 35 preparations examined were selected on a basis of technical
suitability and recognition of all group III type chromosomes, as in the
40 normal cells discussed previously. In all of these cells chromosomes
6 and 4^6 were clearly distinguishable; in 30 cells chromosome 4 was clearly
identified, in the remaining five this chromosome being indistinct because
of unclear centromere and/or chromosome ends. Measurements were
conducted as described previously for normal cells. The relevant data
are presented in table 13. Firstly it will be seen from these data
that homologues of pairs 7 and 9 show association as they did in normal
cells. But there is no evidence of association between chromosomes
4^6 and 6. These two chromosomes are separated on the average at least
by a distance expected if they are non-homologous and randomly distributed
in respect of each other. Secondly, the data show evidence of association
between chromosomes 4^6 and 4 in these interchange cells.

The above observations provide good supporting evidence (a) for the
identification of chromosome 4 as one of the two chromosomes involved
in the interchange, and (b) for the nature of the interchange as involving
the short arm of chromosome 6.

Table 13.

Pair	Dx (cm)	No.of cells	S.E.	t	
9	+0.80	35	0.369	2.168	*
7	+0.78	35	0.360	2.167	*
$6/4^6$	−0.37	35	0.264	1.402	ns
$4/4^6$	+0.63	30	0.308	2.045	*

Table 13 Summary of data on association of homologues and of chromosomes $6/4^6$ and $4/4^6$ in interchange cells. Abbreviations as in table 5a. For pairs 9, 7 and $4/4^6$ Dx values are significantly positive. For $6/4^6$ the Dx value is negative, thus showing an absence of association between these two chromosomes.

V. Normal and interchange homozygotes. A number of normal and interchange homozygotes have been produced in culture from selfed interchange heterozygotes. The somatic root tip complements of these have been examined and compared with the normal and interchange heterozygote complements described already. Some information is presented here to reinforce conclusions made previously.

Normal homozygote complements

Three complements have been examined critically. Fig. 15 illustrates the complement and fig. 16 is an apparent photographic idiogram from a second cell. Mean relative lengths and arm ratios of chromosomes 4, 6, 7 and 9 from the three cells measured are shown in table 14. When these data and figures are compared with tables 3 and 4 and figs 2 - 4 of normal cells it will be seen that there are no basic differences between these complements and those of normal plants examined previously. Of particular note is the presence of two normal members of chromosomes 4 and 6.

Interchange homozygote complements.

Three complements have been examined here also. The complement is illustrated in Figs. 17 and 18 and table 15 summarizes relative lengths and arm ratios of the appropriate chromosomes from the three preparations measured. The homologous pair of interchanged chromosomes 4^6 is readily identifiable on relative sizes and arm ratios. Its values agree very well with those of chromosomes 4^6 from interchange heterozygotes (compare with Figs. 8 - 12 and tables 9 and 10). Worthy of emphasis here

Table 14   Mean absolute lengths, mean arm ratios and relative

lengths of the somatic chromosomes from 3 cells of a normal

plant derived from selfing an interchange plant.   Compare with

tables 3 and 4, noting in particular the occurrence of two

normal members of chromosomes 4 and 6.   Comments in respect

of chromosomes 1 - 3 as in caption to tables 3 and 4.

Table 14.

Chr. no.	Total	Long arm	C/m.	Short arm	Ratio	Chr. no.	Total	Long arm	Short arm
1	41·88					1	14·94		
2	41·92					2	14·95		
3	38·75					3	13·83		
4	35·79	18·92	1·49	15·38	0·818	4	12·77	6·75	5·45
5	27·13	16·17	1·29	9·67	0·600	5	9·68	5·77	3·45
6	25·42	20·46	1·08	3·88	0·190	6	9·07	7·30	1·37
7	23·67	19·17	0·87	3·63	0·189	7	8·45	6·84	1·30
8	24·83	13·63	1·28	9·92	0·729	8	8·85	4·86	3·54
9	20·92	17·96	0·88	2·08	0·117	9	7·46	6·41	0·74

Table   14

Table 15   Mean absolute lengths, mean arm ratios and relative

lengths of the smatic chromosomes from 3 cells from interchange

homozygous plants derived from selfing interchange heterozygous

plants.   Measurements of the interchange chromosomes are given

separately in the lower portions of the table.   Compare with

table 14 on one hand and with tables 9 and 10 on the other,

noting in particular the absence of normal chromosome 4

and 6 members.   Comments for chromosomes $1 - 3$ and 6^4 as

in caption to tables 9 and 10.

Table 15.

Chr. no.	Total	Long arm	c/m.	Short arm	Ratio
1	41·13				
2	40·54				
3	37·21				
5	25·71	15·00	1·04	9·67	0·644
7	23·29	18·63	0·99	3·67	0·198
8	23·29	13·21	1·04	9·04	0·682
9	20·21	17·29	0·88	2·04	0·118

Chr. no.	Total	Long arm	Short arm
1	15·12		
2	14·92		
3	13·70		
5	9·47	5·52	3·56
7	8·57	6·86	1·35
8	8·57	4·86	3·33
9	7·44	6·37	0·75

Table   15

4^6	21·96	17·71	0·92	3·33	0·188
6^4	38·38	20·63	1·37	16·38	0·795

4^6	8·08	6·52	1·23
6^4	14·13	7·60	6·03

is the size of the short arm of chromosome 4^6 members which is very noticeably smaller than that of chromosome 7 (cf. in normal plants, in figs. 2 - 4). Also in figs. 17 and 18 two chromosomes can be identified which are somewhat larger and more asymmetrical than expected from normal cells. These **possibly** represent chromosomes 6^4, though their confusion with apparent chromosome 2 members cannot be ruled out.

Altogether the information on these normal and interchange homozygotes fits expectation very well, thus supporting the identification of the interchange chromosomes.

1. A GENERAL SURVEY OF MEIOSIS

Meiosis in normal and interchange material was studied in pollen mother cells. Some caution is necessary in making detailed extrapolations from pollen meiosis to ovule meiosis in Allium (and other) species since differences are known to exist e.g. in chiasma frequencies (Ved Brat, 1964).

Feulgen and alcoholic carmine preparations were used for the majority of these studies. Some haematoxylin stained sections were employed for certain aspects.

In describing the results obtained a general account of meiosis will be given first for both normal and interchange cells, followed by a fuller account of certain aspects that were studied in detail.

(a) Early stages of prophase to pachytene. The early stages of meiosis (leptotene - pachytene) are difficult to study in detail in Allium triquetrum. The general course of events over these phases, however, appears to follow closely that described by Oksala and Therman (1958) as being representative of liliaceous type plants in general, with pollen mother cells at the early stages of meiosis showing the chromatin clumped together on one side of the nucleus as the so called "synizetic knot", as most commonly found in plant material.

The number and lengths of the chromosomes make detailed studies at pachytene very difficult in Allium triquetrum. Synapsis is virtually complete in normal cells, although occasional short, intercalary or terminal, unpaired segments may be seen. I have been unable to identify

centromeres positively at this stage, and no pronounced chromomeres

are visible. It is only rarely possible to follow chromosomes

throughout their lengths at this stage. One or two large nucleoli

are present.

Parts of the complex association of four chromosomes have been

identified at pachytene in interchange material by first looking for

the large unpaired segments that characterize the configuration. Details,

however, were obtained only from very late pachytene/early diplotene stages

where the chromosomes are considerably more contracted and thus more

accurately studied and measured. Extra caution is needed in using these

late pachytene/early diplotene stages since desynapsis may confuse

some interpretations. Desynapsis begins at the chromosome ends in

this material, when the chromosomes have become considerably contracted;

and there is little or no difficulty in distinguishing between non-

synapsed portions of late pachytene and desynapsed portions of diplotene

in the interchange complexes described.

Fig. 13 (1) and (2) shows the pachytene configurations expected

from each of the two general types of interchange that were originally

considered possible for this material. In each configuration chiasmata

can be expected to arise in the three long pairs of chromosome arms;

a failure of chiasmata in the short arm pair will lead to chains of four

chromosomes being produced at diakinesis, and these will give the

alternate and adjacent orientations that characterize anaphase I cells.

In the type (2) interchange, involving a break in the short arm of

the subterminal contromered chromosome, the centromeres at pachytene are

on the horizontal axis; while in the type (1) interchange, involving a

break in the long arm of this chromosome, the centromeres are on the vertical axis. In each case the "centre" of the configuration, where homology changes, represents the break points of the interchange.

The studies reported on somatic chromosomes in interchange cells showed that the interchange was of type (2) above, involving the short arm of the subterminal chromosome 6. The short arm of the other, median-submedian chromosome 4 was also involved in the interchange. Fig. 19 more precisely illustrates this interchange and its expected pachytene configuration, using the most proximal break points that are compatible with the somatic chromosome data.

Fig. 20 illustrates the interchange complex as it commonly appears at late pachytene-early diplotene. Accompanying the photograph is an outline diagram and an interpretative figure. In the latter particular portions of the complex are labelled as follows: the two portions of the vertical axis as 1a, 1b; and of the horizontal axis, 2a, 2b. Bracketed portions in axis 1, labelled x, x'; y, y', are unpaired segments. Arrows in the outline diagram indicate attenuated segments. Centromeres are not included as these are not visible in the preparations.

The interchange configuration at this stage (and later at diplotene) is somewhat difficult to interpret at first because of the unusual features of asynapsis and attenuation in certain segments of the complex. The general appearance of the configuration is that of a "T", as is usual with chain forming interchanges, rather than a "cross" as with ring

forming ones (Burnham, 1962). The T is rather "open", however, appearing more like a "Y" because of the large unpaired segments along axis 1. The four chromosomes in the complex can be identified from the clear distinction in length between the two short chromosomes; the larger chromosome 6 and the smaller interchange chromosome 4^6. The other two chromosomes (4 and 6^4) are thus also identifiable, as shown in Fig. 20. Identification of chromosomes 4 and 6^4 through their size differences alone proves impossible because of attenuation in some regions of the chromosomes.

Towards the ends of the non-synapsed portions of each of chromosomes 6 and 4^6 (y, y') is a conspicuously less chromatic band, which can be identified in the majority of complexes examined.

Further details on the interchange complex at this stage, particularly those concerning its asynapsis and attenuation, are described later in a special section.

(b) <u>Later prophase stages to diplotene/diakinesis.</u>   Diplotene/ diakinesis stages are reasonably favourable for study in <u>Allium</u> <u>triquetrum</u>. The chromosomes fix well in acetic alcohol and good results can be obtained with Feulgen and alcoholic carmine stains.

Commonly one or two, rarely three, nucleoli remain through to the very end of these stages, though they have disappeared by the first general signs of prometaphase. The nucleoli are often free in the nucleus (some detachment from organizing chromosomes may be induced through squashing), though one is frequently attached to the end of a pair of chromosome satellites on one pair of small chromosomes (Figs. 28 and 29). This

association must represent the nucleolus and satellites of bivalent 9.
Two closely associated satellites, one joined to each organizing
chromosome by a moderately thin thread, can be distinguished usually,
each being very similar in general form to the chromosome 9 satellites
of somatic cells (compare Fig. 28 with Fig. 7f). It is interesting that
the attachment point of the nucleolus, presumably at its organizing locus,
is at the satellite end. This is somewhat different from the situation
in maize and some other plants, where the nucleolus is attached to a
deeply staining, heterochromatic region at the base of the satellite
stalk (McClintock, 1934; Stewart and Bamford, 1942; Brown, 1949).
Probably similar (though less clear) associations of nucleoli and
satellites have been noted at diplotene in other Allium species (e.g. Levan
1935).

The nucleolus and satellite described above must belong to the
chromosome 9 pair, since the satellite of chromosome 7 would not show
such a large bulk of chromatin at diplotene. Occasionally a second
nucleolus appears associated with the end of another chromosome pair,
though the supposed attachment here is difficult to distinguish from
a fortuitous juxtapositioning of nucleolus and bivalent. It may represent
the nucleolus and organizer of the chromosome 7 bivalent in some instances.

The diffuse outline of the chromosomes that usually characterizes
diplotene is still prominent at a stage when the bivalents apparently have
reached their maximum state of contraction (Fig. 23). For this reason,
and because of the long persistent nature of the nucleoli, diakinesis stages
are difficult to distinguish precisely from diplotene ones. Judging
from illustrations published by other writers (e.g. Levan, 1935) stages

as those of Fig. 22 and 26 are virtually diakinesis, showing maximum

or near maximum contraction and strong desynapsis; while those as Fig. 21

and 24 e.g. with weaker contraction and desynapsis are diplotene. On

this basis the studies that complement those of pachytene are largely from

mid-late diplotene stages; while the chiasma studies presented later

are from late diplotene/diakinesis stages (as for other published works

on chiasmata in Allium).

At diplotene/diakinesis stages in normal cells two classes of

chromosomes can be distinguished (Fig. 26):   (a) four large chromosomes,

usually with two or three chiasmata, representing group I (chromosomes 1 -

4) of somatic cells; and (b) five smaller chromosomes, with one or two

chiasmata, representing groups II and III combined (chromosomes 5 - 9)

of somatic cells. Centromeres cannot be identified at these stages so

it is impossible to recognize individual chromosomes consistently.

However, chromosome 9 can often be clearly identified because of its association

with a nucleolus and/or because of its satellite. Also chromosome 4

sometimes can be identified tentatively from other group I chromosomes,

because of its smaller size (e.g. middle bivalent in Fig. 26). The

possible attachment of chromosome 7 to a nucleolus sometimes suggests

its identification. The submedian chromosomes 5 and 8 and the

subterminal ones 6, 7 and 9 cannot be distinguished since they all

are similar in length and chiasma frequency.

Relational coils of homologous chromosomes are still evident in

mid-diplotene stages (when contraction and desynapsis are not very marked)

(Fig. 21). Hence chiasmata cannot be identified clearly. At late

diplotene/diakinesis, when contraction and desynapsis are marked, clear

loops between successive chiasma nodes are revealed (Fig. 26). Twists between the homologous members of a bivalent occasionally persist from earlier stages. Although these superficially look like chiasmata they can usually be distinguished, because of the two distinct levels of focus necessary to study them under the oil immersion. In normally squashed preparations it is possible only occasionally to observe chiasma structure. However, observations on nodes after excessive squashing show that the clear majority of these are true chiasmata and only a relatively low proportion are twists, most of which appear to be recognized. It is probable that occasional misinterpretations occur in scorings for chiasma frequencies, but since these will occur in approximately the same frequency in normal and interchange cells they will not seriously affect the comparisons made. They would, however, probably cause a slightly greater degree of terminalization to be recorded than actually obtained (when chiasma frequency at early stages is compared with that of later diakinesis/metaphase stages).

Chiasmata appear to be distributed randomly along the chromosomes. The large bivalents have a greater number of chiasmata, on the average, than the smaller ones. Thus chiasma distribution is similar to that found in the majority of Allium species, rather than being one of the more unusual types with extreme proximal or distal localization (Ved Brat, 1965). Further data on chiasmata are given later.

The complex can be distinguished in interchange cells, at early stages (Figs. 20 and 21) and at later stages (Fig. 22 and 25), most often occurring as a chain of four chromosomes with usually three or four chiasmata, though occasionally unequal bivalents are formed

(Fig. 30). The remaining bivalents comprise three rather than four group I pairs, and four rather than five group II plus III pairs. This is expected since the interchange involved one group I and one group III chromosome. The nucleolus is not associated with the interchange complex, again as expected since the non-satellited chromosome 6 of group III was involved in the interchange.

(c) Diakinesis/Metaphase I stages. Cells showing centromere activity in some or all of the chromosomes (bivalent or complex), but still pre-anaphase I, were recorded as diakinesis/metaphase I for chiasma analyses. Metaphase I stages usually have been used in chiasma studies in Allium (e.g. Maeda, 1942; Cochran, 1953; Ved Brat, 1965).

In my own studies I found that some caution was necessary in scoring late diakinesis/metaphase I cells, in order to be sure these were not in very early anaphase (and thus perhaps had lost some of their chiasmata through anaphase movement). Two observations proved very useful in selecting the correct cells to score. In squashed cells late metaphase/anaphase I stages retain a precise linear orientation of their bivalents in the equator; while in late diakinesis/early metaphase I cells the bivalents most frequently are not placed in, or have been displaced from, this strict orientation so that the chromosomes at these latter stages are placed much more irregularly, even though their centromeres may show pronounced co-orientation (cf. figs. 34/43). The photographs of Ved Brat (1965 e.g.) show this same characteristic in cells used for chiasma studies. Secondly, and more importantly, in cells in which anaphase I has been initiated (barely), the distal chiasmata are readily

terminalized and lost even with only moderate squashing. In earlier cells in which anaphase presumably has not been initiated, even heavy squashing was found only rarely to cause separation of a terminal chiasma. Thus cells were scored for chiasmata when they showed no precise equatorial orientation and no evidence from successive squashings that anaphase I had been initiated. The general procedure was to squash a preparation mildly at first, examine under low magnification (X 100) and select cells where all bivalents were clearly seen. These cells were then examined and scored tentatively for chiasmata, noting particularly the occurrence of terminal or near terminal chiasmata. Cells were then resquashed and examined for the evidence cited above for anaphase initiation, and then finally scored for chiasma frequency if appropriate.

Twists are absent at these stages. Loops between successive chiasmata commonly though not always are placed at right angles to each other following squashing.

Centromere activity makes it possible to recognize the bivalents formed by group I, II and III chromosomes, whereas groups II and III were indistinguishable at earlier stages. Within groups it is not possible to identify individual chromosomes consistently, though size differences and centromere locations sometimes give suggestions. The nucleolus of bivalent 9 is no longer present and its pair of satellites are undetectable; presumably they have merged with the rest of the chromosome through further chromosome contraction.

In interchange cells the complex can be identified readily at these stages, usually as a chain of four chromosomes (fig. 33), though occasionally unequal bivalents are found as at diplotene (fig. 32). The remaining

bivalents can be placed into three groupings, comprising (a) group II as in normal cells, (b) group I with three instead of the normal four bivalents, and (c) group III with two (7 and 9) pairs instead of three.

Detailed studies were made at these stages in respect of chiasma frequencies in normal and interchange cells.

(d) <u>Prometaphase/metaphase I and early anaphase I stages.</u>   Metaphase/ early anaphase I stages have been studied most frequently in interchange materials, from the point of view of identification of the chromosomes involved, general form of the interchange complex, and, in particular, of the types of orientation shown by the interchange complex. Knowledge on the latter has been particularly useful in furthering an understanding of the general process of prometaphase orientation in meiosis.

Prometaphase is the phase following diakinesis and leading to metaphase I, in which the chromosomes undergo their characteristic movements, giving rise to a precise orientation of chromosomes along the metaphase equator. Studies at this stage in meiosis, particularly when involving complex associations of chromosomes, have been singularly sparse, undoubtedly because, to be of most value, living material must be used, and this of course presents great difficulties in most plant and in many animal meioses. The studies of Bauer, Dietz and Röbbelen (1961) in animal spermatocytes stand out as being of immense value, and it is unfortunate that the culture of pollen mother cells has not progressed to the finesse necessary to allow similar studies to be carried out on the abundant, good material available in plants. However, I have found that static studies on fixed and stained material at this stage

in my material have been far from uninformative, and indeed have some advantages over studies on living materials, as shown later.

Metaphase/early anaphase I stages are very amenable to study in Allium triquetrum. The pollen mother cells at least at this and later stages have a preferred plane of lying, so that almost invariably the most useful, lateral aspect of the metaphase plate is presented for observation. In squashed preparations the bivalents separate well for close study, but do not become radically displaced from their linear orientation unless sudden pressure is applied during preparation. In "unsquashed" preparations referred to later, where only pressure from the weight of the cover slip is applied, the cells remain intact, with no disruption of chromosome alignment (though there is some lateral compression of the plate.)

Prometaphase stages were studied in unsquashed and subsequently squashed preparations so as to be fully aware of what effect squashing has on the distribution of chromosomes in the cells studied. This is very important, as will be realized later. Chromosomes oriented in the equator in unsquashed cells are of course difficult to study, but some of the characteristics of prometaphase in themselves enable quite clear pictures to be obtained without necessarily having to resort to squashing. However, in all these studies subsequent squashing was
                   to
carried out/make a check on some structural interpretations initially made.

Prometaphase stages are undoubtedly of short duration; they are particularly difficult to obtain in adequate numbers. However, in Allium triquetrum the mother cells in a pollen sac are closely synchronized so that one preparation at the desired stage permits quite an extensive

analysis. Most difficulties arise when studying development sequences' of this stage.

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In normal cells. At metaphase/anaphase the four group I chromosomes stand out because of their size and chiasma numbers. The other two groups of bivalents can also be distinguished readily, even when all have a single chiasma, since the short arms of group II bivalents, compared with those of group III, are much larger and thus always conspicuous.

At complete metaphase I all the bivalents are co-oriented along the equator. However, in what are undoubtedly earlier stages, instances of incomplete orientation in one or a few bivalents frequently can be seen in squashed and unsquashed preparations. These incompletely oriented bivalents most frequently occupy positions at or near one of the two spindle poles, instead of along the equator (Figs. 40 and 41). These cells are regarded as representing prometaphase stages.

In interchange cells. Seven bivalents and the interchange complex of a chain of four chromosomes can be identified readily at metaphase/early anaphase I (Figs. 42-44). As at diakinesis there are three rather than four group 1 bivalents, and two rather than three group III bivalents, with the normal two group II members. These findings agree precisely with those made from similar cells examined in my original study on the abnormality, from which I concluded that the interchange had involved "one of the four 1 chromosomes" (groupI) and "one of three st chromosomes" (group III) (Rickards, 1962). There is little doubt that the interchange material I have in culture was obtained from the same

population from which the original slides had been prepared. And the studies already reportd here have confirmed and extended the identification of the chromosomes involved in the interchange.

The two fully co-oriented arrangements in the complex of figs. 42 and 43 correspond to the stable co-oriented arrangement in normal bivalents. In prometaphase I stages unstable orientations in the complex can be identified, paralleling the prometaphase polar orientations of normal cells (figs. 46 and 47). These are described in detail later.

Of the two basic orientations of the complex (fig. 13 and 42/43) the first is the alternate arrangements the second the adjacent arrangement. The overall relative frequency of these two types is approximately 3 : 1. A detailed investigation of this frequency is reported later.

In addition to these two basic orientations those involving unequal bivalents are also observed (figs. 53/54), as well as 3 : 1 and other discordant types (figs. 57/58). These are described later. They occur in very low frequencies.

(e) <u>Mid-late anaphase I stages</u>. Chromosomes of groups I, II and III are clearly recognizable at mid-anaphase I, according to size and presence of two (only) or four long chromatid arms (fig. 66), the two short chromatid arms of group III chromosomes being very inconspicuous.

From the alternate arrangement (Fig. 42) all chiasmata in the complex are "actively" separated by polar movement of the centromeres. In the adjacent orientation, however, the chiasma in the bridging 1a segments is lost "passively", since its two adjacent centromeres are

moving to the same pole. Cases often can be seen at early anaphase
of complexes showing adjacent orientation in which the la chiasma is still
intact, while most other chiasmata in the cells have been lost (Fig. 56).
By later stages the la chiasma has always been lost.

Mother cells within a pollen sac are not completely synchronized with
each other in respect of entry into anaphase I, though at the correct
stage of development up to about 85% of cells can be seen in mid-anaphase
at one time. There is no clear relationship between asynchrony and
position of a cell within the pollen sac, (observations from longitudinal
sections of anthers).

The two basic concordant orientations in the complex, alternate and
adjacent-1, produce very distinct segregation complements at mid-anaphase
I. From an alternate orientation each polar group contains four group
I and three group III chromosomes, as well as the two group II
chromosomes (Fig. 66). Both are cytologically and genetically balanced
complements; one contains the normal chromosomes (4 and 6) while the
other contains the two interchange chromosomes (4^6 and 6^4). The two
complements cannot be distinguished. From an adjacent-1 orientation one
polar complement contains three instead of four group I and four instead
of three group III chromosomes, while the other contains five group I and
two group III chromosomes - plus two group II chromosomes in each case
(Fig. 67). The former complement contains both the normal and interchange
group I chromosomes (4 and 6^4) and neither of the relevant group III
chromosome; while the latter contains both normal and interchange group
III's (6 and 4^6) and no relevant member of group I. Both complements are
duplicated and deficient (to different degrees) and are thus unbalanced

cytologically and genetically.

Previous work (Rickards, 1962) had suggested that these two patterns of segregation occurred in an approximate ratio of 3 : 1, corresponding to a similar ratio of alternate and adjacent orientations at early anaphase I. Further studies have shown that such an analysis may be misleading, and this has led to a detailed analysis of the developmental stages of anaphase I in a pollen sac.

In addition to the usual 9 : 9 segregation of anaphase I occasional cases of numerical non-disjunction have been identified. These are of two types, (a) in which one polar group of the cell contains 10 and the other only 8 chromosomes (fig. 68), and (b) in which one or two chromosomes from either polar group remain lagging near or on the previous metaphase equator (fig. 69). Detailed studies on these segregation types are given later.

(f) <u>Meiosis II stages</u>. Studies here were confined almost entirely to mid-anaphase II stages, centred round chromosome complement types and pollen sac developmental stages. General observations on other stages showed that meiosis II is visibly quite normal in this interchange material, as is generally so. The usual three groups of chromosomes can be distinguished readily in mid-anaphase II cells (fig. 75). No satellites are visible on chromosomes 7 and 9 at mid-anaphase II, this probably being related to the fact that no nucleoli form in the meiotic interphase nucleus between the two divisions (Rickards, 1962). But the group III chromosomes can be distinguished sometimes on size differences in both short and long arms.

The alternate and adjacent type complements at mid-anaphase II are shown in figs. 75 and 76. In the former (Fig. 75) all four complements show 4 group I, 2 group II and 3 group III chromosomes. In the adjacent type (Fig. 76) two sister chromosome complements have a 5, 2, 2 composition; the other two 3, 2, 4.

The frequencies of alternate and adjacent type tetrads are described later in connection with developmental studies in anaphase II pollen sacs.

As well as complements from alternate and adjacent segregation those resulting from numerical non-disjunction are sometimes observed (figs. 74 and 77). These parallel the unusual complements observed at anaphase I and are discussed in detail later.

2. ASYNAPSIS, ATTENUATION AND SEPARATION OF PARTS OF THE PACHYTENE INTERCHANGE COMPLEX

As already pointed out briefly the interchange configuration observed at pachytene/diplotene in this interchange is markedly different from that expected, and is hence somewhat difficult to interpret at first. The difference is brought about by three features, seen clearly in fig. 20:

(a) asynapsis in proximal portions of axis 1a/b

(b) wide separation of the complex 'halves' (to the right and the left of axis 1 in fig. 20), and

(c) attenuation in unpaired segments of 1a.

These are consistent and characteristic features of the present interchange and are described in some detail below.

(a) Asynapsis in 1a/b.

The apparently homologous segments y and y' of chromosomes 6 and 4^6 respectively that comprise axis 1b never show synapsis with each other. When it is possible to measure accurately both these portions in one cell it is found that they are of approximately the same length; and in different cells they have approximately the same length relative to the other chromosomes of the complex (details from 4 cells in table 18). This constancy in the length of segments y and y' suggests

(a) they are homologous segments (the less chromatic band towards the end of each (fig. 20) provides morphological evidence for this conclusion);

(b) the junction of y or y' with axis 2a or 2b indicates a change in homology; and thus (c) the junctions represent the points at which the interchange breaks arose in the affected chromosomes 4 and 6. The constancy in size points to an absence of the variably expressed, non-homologous pairing that has been reported in some maize interchanges (McClintock, 1932; Burnham, 1950) and which, if present in my material, would have confused the above conclusion on break points.

The proximal segments x and x' of axis 1a also consistently show asynapsis. In contrast to the previous case, however, the unpaired segments are of varying lengths, with a minimum approximately as in Fig. 20. A measure of asynapsis cannot be determined directly from chromosome measurements, because attenuation in these segments (see below) complicates interpretation.

The asynaptic segments are always continuous, never broken up into shorter lengths with interspersed paired segments.

(b) Partial separation of the complex "halves".

The two halves of the complex invariably are separated from each other as shown in Figs. 20 and 21, giving the complex the appearance of a Y rather than the expected T. A portion of this separation is probably induced in some cells (reduced in others) through squashing during preparation. Also, complexes with well separated halves are more likely to be chosen for critical study, since often they will be clearer. However, it is clear from the consistent nature of the phenomenon that it is largely a natural feature. This conclusion is reinforced by the observations given below on attenuation and by diplotene studies

reported later. This spatial separation of the halves together with the long chromosomes in the species are the major reason why clear preparations of the complex are very difficult to obtain at this stage.

Observations suggest that the spatial separation of the halves and asynapsis in x and y are related phenomena. Complexes with greater degrees of asynapsis in x have their halves more distantly separated.

(c) Attenuation of x and x'.

The unpaired segments x and x' at these late pachytene/ early diplotene stages never appear "loose", as if they should be showing synapsis (or representative of desynapsed segments). Rather, these segments usually give the impression of being under tension and therefore apparently incapable of synapsis, because of the separation of the two complex halves. Also, they regularly show varying degrees of attenuation or "stretching" along their lengths (arrows in outline diagram of Fig. 20). This impression of attenuation is obtained from comparisons in the density of staining and thickness of the chromosome threads between affected and non- affected segments (e.g. between and y and x in Fig. 20). The attenuated segments are less strongly stained and thinner than the normal segments. The interpretation receives added support from the observations given later on chromosome size.

The attenuation in x and x' is considerably variable, in both extent and location. In Fig. 20 x and x' are approximately equal in length, and since these must be homologous segments and therefore expected to be of equal lengths they must have become attenuated to approximately equal extents. However, whereas the attenuation in x

is roughly uniform along the whole length of the segment, that of x' is localized in two main regions that are separated by a less attenuated segment.

The majority of complexes examined show patterns and degree of attenuation similar to those of fig. 20. In a few cases either or both x and x' show little or no clear signs of attenuation.

In fig. 20, the proximal portion of the paired segments of axis 1a also shows some evidence of attenuation. Other than this the paired segments of axis 1a, axis 2a and 2b, and y, y' never show attenuation, and the phenomenon has been seen only rarely in other bivalents (see arrow in Fig. 21).

The tension and attenuation in segments x and x' are probably related to the separation of the complex halves and asynapsis that were described above.

Diplotene observations. Two of the above features - wide separation of the complex halves and attenuation in some of its segments - were studied further at diplotene. Both these features are evident in a very clear majority of cells examined. Fig. 21 illustrates the wide separation of the complex halves at early diplotene, and figs. 23a and b both separation and attenuation at later diplotene stages. And the two features are evident still though to a lesser extent, at late diplotene/ diakinesis stages (figs. 25 a - c).

A number of studies were made on cells after successive squashings to ascertain that the wide separation of the complex parts and attenuation in some of its segments were not induced through squashing

during preparation.

The regularity in distribution of the parts of the complex and consistent attenuation both suggest that the phenomena are natural. But also, these two features can be seen regularly in unsquashed, only slightly compressed cells (fig. 24), and squashing was found to have very little effect on the spatial separation of the complex parts, and heavy squashing breaks rather than stretches the chromosomes (fig. 24c).

The three undoubtedly related phenomena described above are relevant to studies presented later on chiasmata in the complex, and the description above gives an appreciation of the interchange complex as seen at pachytene/early diplotene. A detailed examination of the above mentioned phenomena is at present being conducted, including an analysis of the frequency of the ways in which the complex parts are distributed in relation to each other, and the frequency, extent and location of attenuation. The phenomena are of great interest, since firstly they suggest some form of specific placement of at least certain chromosomes in the premeiotic or early meiotic nucleus; and secondly they probably have an influence on the patterns of orientation that the chromosome complex shows at anaphase I. A full discussion on these aspects is not warranted at present because of the need for more information and hence will be submitted later for publication.

3.   CHROMOSOME MEASUREMENTS AT PACHYTENE

Measurements of chromosomes at pachytene present more difficulties
than those of somatic chromosomes. Not only are the chromosomes longer
(late pachytene chromosomes in _Allium triquetrum_ are about twice the length
of weakly colchicine-contracted somatic chromosomes), and thus only
occasionally found in an unobscured, easily measured condition. But
also, paired chromosomes often are coiled round each other, and their
limits are more difficult to ascertain owing to the "woolly" appearance of
chromosomes at this stage. Further, the possibility of non-homologous
pairing in interchange cells must be borne carefully in mind, and
confusion also arises if synapsis is incomplete round the centre of the
interchange complex (Burnham, 1950). Moreover, in the present interchange
the fact that the complex parts are usually stretched across the nucleus
in characteristic fashion makes it difficult to obtain clear preparations
to observe and measure. Also, the phenomenon of attenuation mentioned
above will cause difficulties in interpreting chromosome lengths. Finally
the centromeres are not identifiable at these stages in _Allium_
triquetrum. These difficulties will cause considerable inaccuracies in
chromosome measurements and interpretations, and hence the data obtained
are somewhat restricted in value.

Theoretically the lengths of chromosomes from ends to centre of
the configuration should provide evidence on the location of the
interchange break points (Burnham, 1956, 1962). This will hold true only
if non-homologous pairing does not occur, for otherwise the centre of

the complex will be confused. The evidence presented above on the equality and relative constancy in lengths of y and y' indicates that

(a) non-homologous pairing does not occur between these segments and adjacent segments of axis 1a; and

(b) the junctions of y, y' with axis 2a, 2b represent the centre of the configuration and thus the interchange break points.

Measurements of axis 2a and y and/or 2b and y' can be used, therefore, to locate the interchange break points and the position of the centromeres on the pachytene configuration. However if long arms of chromosomes contract somewhat more than short arms, then the positioning of the centromeres on an axis, or the location of break points relative to lengths of somatic chromosomes, using relative lengths of short and long axes as 1b, 2a above as a guide, will give rise to incorrect interpretations. However, the data on somatic chromosomes in Allium triquetrum have shown the possibility of a slight differential arm contraction in only group III type chromosomes, and this is probably only an artefact of measuring. The point, however, should be borne in mind.

Data have been obtained from four cells in which one of the axes 2a or 2b were reasonably straight and relatively easily measured. In all cells data were obtained on the lengths of ȳ and/or y'. Where 2a or 2b in a configuration was difficult to measure accurately because of curves or folds (e.g. 2b in Fig. 20), then an estimate of its length was calculated from the measured total length of one chromosome in the complex and the known relative lengths of these chromosomes in somatic cells.* The data are presented in table 13. Fig. 20 is from cell 1 of this table.

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* This procedure is justified below.

Table 16.

		Diplotene/ diakinesis	Diakinesis/ metaphase I
	Type 1	63 (54%)	72 (82%)
	Type 2	47 (41%)	12 (13.5%)
	Type 3	2 (1.5%)	0 -
	Type 4	4 (3.5%)	4 (4.5%)

Table 16   Frequencies of the different complex types, based
on chiasma conditions, at diplotene/diakinesis (116 cases) and
diakinesis/metaphase I (88 cases). Type 4 includes all unequal
bivalents, including those with three chiasmata (fig. 31).

Table 17.

		Interchange cells	Normal cells
Diplotene/ diakinesis	Group I	11.23 (8.74 + 2.49)	11.28
	Group II + III	8.42 (6.59 + 1.83)	8.32
Diakinesis/ metaphase I	Group I	9.00 (6.90 + 2.10)	9.26
	Group II	3.30	3.09 *
	Group III	4.01 (2.47 + 1.54)	4.18

Table 17   Group comparisons of chiasma frequencies in normal

and interchange cells. The values in interchange cells for

groups I, II + III and III are the sums of those actually

obtained (table 19) plus the values theoretically expected for

chromosomes 4 or 6. Group II chromosomes at diakinesis/metaphase I

in interchange and normal cells are directly comparable and show

no statistically significant difference.

* difference = 0.21, S.E. 0.17, t = 1.235, p > 0.25.

   F = 0.543/0.375 = 1.45, p > 0.25.

Table 18.

Cell No.	y	y'	2a	2b	y/2a	y'/2b
1	7.5	7.5	50.00	(42.00)**	0.150	(0.179)
2	6.0	6.0*	42.00	(35.75)	0.143	(0.168)
3	7.25	7.0	(50.25)	43.00	(0.144)	0.163
4	7.0	7.25	47.00	40.25	0.149	0.180

Table 18   Measurements and ratios from parts of the complex
from four pachytene/early diplotene interchange cells.   Refer to
Fig. 2D for meaning of the abbreviations.

* Estimated from y.
** Figures in brackets indicate calculated or partly calculated
values as against measured values (see comments in text).

2a/2b - centromere location

Using measured values of y and 2a etc., the ratios y/2a and y'/2b
calculated and are shown in table 18. For the four cells,
an y/2a value of 0.147 was obtained, and for y'/2b, 0.173. These
ues were compared with the mean arm ratios for chromosomes 6 (0.184) and
(0.185) respectively.

The mean y/2a value for chromosome 6 is considerably lower than its
ratio, and this is so for all four individual cell comparisons. The
ference supports the conclusion already deduced from somatic cells, that
centromere of chromosome 6 is not included in segment y but located,
th that of 6^4 on axis 2a - in other words, that the interchange is of
pe (2) rather than type 1 (fig. 13). Admittedly differential
ntraction of long and short arms could invalidate this evidence, but it
uld need to be greater than that suggested for individual group III
romosomes of somatic cells.

In contrast, the y'/2b value for chromosome 4^6 is only a little lower
an the arm ratio for this chromosome. Thus again, the most likely
nclusion is that the centromeres of 4 and 4^6 are on axis 2b, though closer
the proximal end of this axis than those of axis 2a. The conclusion
hat 4 and 4^6 centromeres are on axis 2b of course follows if those of 6
nd 6^4 are on 2a; it is noteworthy, though, that the separate data
omplement each other in these respects, though the y'/2b data from these
our cells would hardly be convincing on their own.*

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* It is worth noting that the y/2a and y'/2b ratios are, if anything a
little <u>high</u>, since 2a and 2b arms are likely to be undermeasured because
of unclear relational twists, twists that of course do not occur in
y or y'.

The measurements of both chromosomes 6 and 4^6 in cell number 4 made
it possible to check the validity of estimating the length of a
chromosome from the known length of another chromosome and the known
relative lengths of these chromosomes in somatic cells. Using the
total length of chromosome 6 in cell 4 (54.0) as a basis of calculation,
an estimate of chromosome 4^6 of 46.9 is obtained. This is very close to
the measured value for this chromosome (47.50).

Measurements from three of the four cells examined were also obtained
for chromosome 4 and/or 6^4 in the interchange complex. However, these
measurements are very limited in their value, firstly because of likely
inaccuracies in measuring, but also because of the attenuation of x and
x' segments of axis 1a. In fig. 20 the length of the portion of
chromosome 4 contributing to axis 1a is somewhat _longer_ than the axis 2b,
though this portion is the short arm of the normal chromosome and the centromere
is included in axis 2b. Also, the portion of chromosome 6^4 contributing to
axis 1a is approximately the same length as axis 2a, yet this portion
theoretically should be somewhat _shorter_ (compare with the expected pachytene
configuration in fig. 19). The reason for these discrepancies clearly lies
in the attenuation of segments x and x' which makes the whole 1a axis appear
too long compared to expectation. Indeed the abnormal length of axis 1a can
be adduced as evidence in support of the claim that x and x' are in fact
attenuated.*

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*   The other possibility that would account for the lengths of axis 1a is
that the centromeres are really on axis 1a/1b (and that the interchange
was of type 1 in fig. 13 involving the _long_ arm of chromosome 6 and
the long arm of chromosome 4). But this possibility must be discounted
since it is incompatible with all the data obtained from somatic chromosomes
in respect of both measurements and association of homologues, and it
would then be introducing discrepancies in the measurements of y and 2a
for example in fig, 20 that would be very hard to explain. It would also
be ignoring the direct evidence for attenuation in x and x' cited
previously.

Interchange break points.

The y/2a ratios in table 18 are but moderately lower than the arm ratio of chromosome 6 in somatic cells (0.185). Hence the interchange break point in this chromosome must have been close to the centromere. The pair of interchange break points are thus probably close to the proximal limits rather than the distal limits illustrated in fig. 14. The main point to note then in relation to following studies is that the segments between the centromeres and break points must be very small.

4.   CHIASMA STUDIES AT DIPLOTENE - METAPHASE I

These studies compare chiasma frequencies and distributions in normal and interchange cells, firstly at diplotene/diakinesis and secondly at diakinesis/metaphase.   Interchromosomal distributions of chiasmata are investigated.

Data on chiasma frequency and distribution in normal cells were obtained from the same control plants as used in obtaining details of chromosome lengths. Different populations of Allium triquetrum are likely to show small but significant differences in chiasma frequencies, as found in numerous other species (Rees, 1961). Records were not kept of the plants from which scorings were obtained, either in normal or interchange cases, since individuals in the two cultured populations are mostly if not entirely clonal and thus genetically very homogeneous. Any variations in chiasma frequencies that do occur between different normal plants will be largely environmentally induced. Material for these studies was collected round mid-day, from plants _on the same day,_ grown in the same culture habitat: minor fluctuations in chiasma frequency probably occur with daily changes in the environment (Rees, 1961).

Chiasma frequencies were obtained only from pollen mother cells. Ved Brat (1964) has shown that in at least some Allium species significant differences in chiasma frequency occur between pollen and embryo sac mother cells.

In scoring for total chiasmata in a cell some caution was found necessary in the selection and rejection of cells for analysis. Cells with higher frequencies are more difficult to analyse correctly; the more chiasmata in a bivalent, the more difficult is the analysis. It was realized early in this study that such cells tended more often to be rejected as "unclear" compared with those with lower chiasma frequencies. If uncorrected this would clearly

give too low an estimate of chiasma frequency over a number of cells. To overcome this bias, cells were selected for scoring under the low power magnification (x 100) when all the bivalents and complex were found to be reasonably well separated with little or no overlapping. Only these cells were scored for chiasmata under the oil immersion magnification. This stringent selection of cells reduced the number of cells rejected as unclear to very few. All the selected cells were scored unless breaks, confusion with other cell bivalents or other similar features introduced the possibility of error; even if considerable difficulty was encountered when chiasma frequency was high. In some of the latter cases cells had to be resquashed and re-examined a number of times to ascertain their chiasma number. This procedure may have introduced occasional errors, but it is considered to have given a more accurate analysis in the end.

The problem of twists being confused with chiasmata has been discussed already. When occurring, twists will have been identified as such in the large majority of cases.

Terminal chiasmata were noted during examination. In the figures illustrating chiasmata in normal and interchange cells the total number of chiasmata scored is followed by a bar and then the number of chiasmata that were classed as terminal; the normal procedure (e.g. Darlington, 1937).

I.   Chiasmata in normal cells

A. General

Synapsis is complete at pachytene and this is reflected in a roughly random distribution of chiasmata at diplotene. From general observations as well as detailed analyses below, the number of chiasmata per bivalent is

approximately proportional to the length of the chromosomes involved. Thus the larger group I bivalents show a proportionately higher chiasma frequency than the smaller ones. At least one chiasma always arises in a bivalent so that univalents do not form. Also, only bivalents have been found in the normal material used. In all these studies no case of interlocking bivalents has been found, though it should be noted that the stringent method of selection of cells for scoring would greatly reduce the likelihood of detecting these if they do occur.

(a) <u>Diplotene/diakinesis stages</u>. Data in left hand portion of table 19 (A), see Appendix table 3 for details.

<u>Group I: chromosomes 1-4.</u> These form 1-4 chiasmata usually 2 or 3, with a mean frequency of 2.82 per bivalent in this analysis. Terminalization coefficient (number of terminal chiasmata/total number of chiasmata), 0.131. The larger chromosomes in this group are probably responsible for the majority of cases with 3 and 4 chiasmata, and the smallest chromosome 4 in the group for the majority of cases with 1 or 2 chiasmata. However, observations on interchange cells have shown that the occurrence of 1 and 2 chiasmata is not confined to chromosome 4 bivalents; and likewise cases have been observed in which all four chromosomes have 3 chiasmata. It is uncertain that chromosome 4 can form 4 chiasmata. Fig. 27 shows a group I bivalent with a single chiasma; for other examples see Fig. 26.

Centromeres cannot be identified at these stages of division, though studies at later stages show that there is probably a maximum of two chiasmata per arm in these chromosomes. The chiasmata vary in position throughout

Table 19   Chiasma frequencies, terminal chiasma and terminalization
coefficients.

A. From normal cells; diplotene/diakinesis 25 cells,
                        diakinesis/metaphase I 34 cells.

B. From interchange cells; diplotene/diakinesis 27 cells,
                        diakinesis/metaphase I 30 cells.

Details of chiasma scorings are given in appendix table 3.

Table 19.

A. NORMAL CELLS

	Diplotene/diakinesis				Diakinesis/metaphase I			
	No. of XO	Term XO	Mean per cell	Term. coef.	No. of XO	Term. XO	Mean per cell	Term. coef.
Total	490	79	19.60	0.161	562	187	16.53	0.333
Group I	282	37	11.28	0.131	315	96	9.26	0.305
Group II					105	47	3.09	0.448
Group III					142	44	4.18	0.310
Group II + III	208	42	8.32	0.202	247	91	7.26	0.368

B. INTERCHANGE CELLS

Total	509	68	18.85	0.134	472	144	15.73	0.305
Group I-4	236	30	8.74	0.127	207	60	6.90	0.290
Group II					99	40	3.30	0.404
Group III -6					74	20	2.47	0.270
Group II + III - 6	178	30	6.59	0.169	173	60	5.77	0.347
Complex	95	8	3.52	0.084	92	24	3.07	0.261

the length of the bivalent, and where 3 or 4 chiasmata occur they are approximately evenly distributed along the bivalent.

Group II and III; chromosomes 5 - 9. These form 1 - 3 chiasmata, usually 1 or 2 with a mean frequency of 1.66 per bivalent. Terminalization coefficient, 0.202. As above the longer chromosomes 5 and 6 are probably responsible for the majority of cases with 3 chiasmata, and the others for cases with 1 chiasma. However, bivalent 9 has sometimes been observed to form two chiasmata. Later studies show that the short arms of the group III bivalents occasionally form a chiasma. See Fig. 26 for the two main types of bivalents in this group.

Total chiasmata per cell. A total of 25 cells were scored. The total chiasma frequency per cell ranged from 15 to 23 with a mean in the sample of 19.60. Terminalization coefficient 0.161

(b) Diakinesis/metaphase I stages. Data in right hand portion of Table 19.

Group I; chromosomes 1 - 4. As in earlier stages these show 1 - 4 chiasmata, usually 2 or 3, though with a mean frequency here of 2.32 per bivalent. Terminalization coefficient, 0.305 (cf. 2.82/0.131 at diplotene/diakinesis). There is a maximum of two chiasmata per arm, and where two do occur they are either proximal and distal or medial and terminal in approximate positions. A single chiasma in an arm is variably placed. See Fig. 34 for the two main types of group I bivalents. Fig. 35 shows one of the smaller group I bivalents with a single chiasma.

Group II; chromosomes 5 and 8. These are readily distinguished from group I bivalents by their size, and from group III by their centromere position (Fig. 34). They have one or two chiasmata per bivalent with a mean frequency

of 1.55 per bivalent. Terminalization coefficient, 0.448. An arm shows only one chiasma, positioned in a medial or distal/terminal site. The larger bivalent 5 is probably responsible for a greater proportion of cases with two chiasmata.

Group III; chromosomes 6, 7 and 9. These show one or two, rarely three chiasmata; mean frequency of 1.39 per bivalent. Terminalization coefficient, 0.310. Mostly the one or two chiasmata occur in the long arms; when two occur they are usually proximal and distal/terminal; when one occurs it varies from proximal to terminal. Occasionally the short arm of these bivalents shows a single chiasma (Figs. 36 and 37). I have been unable to observe details of chiasma structure in these cases since they are always terminal or very nearly so. Initially I wondered whether these chiasmata were the satellites of bivalent 9, associated at their ends as they often are at earlier stages (see Fig. 28). However, these structures are no different from terminal chiasmata in other bivalents, and their frequencies (see below) are incompatible with their being associated satellites, but fully in line with their identification as chiasmata. Similar chiasmata in very short arms are evident in the studies of Ved Brat (1965), and Levan (1934).

A maximum of two of these short arm chiasmata has been noted in a cell, so it is uncertain that all three chromosomes can form them. The most likely exception is bivalent 9 since its short arm is considerably smaller than those of the other two group III chromosomes and also it carries a large satellite. The evidence given below, however, suggests that these bivalents do form their share of short arm chiasmata.

The mean frequency of these chiasmata in a cell is approximately 0.50. It is probable that bivalent 6 with its longer pair of short arms has the

highest frequency of short arm chiasmata.

The mean frequency of chiasmata per bivalent in groups II + III combined is 1.45.

Total chiasmata per cell. A total of 34 cells were scored. The frequency of chiasmata per cell ranges from 13 to 21 with a mean frequency of 16.53. Terminalization coefficient 0.333.

The reduction in mean chiasma frequency and rise in terminalization coefficient in these stages compared with earlier ones is clear from table 19. This is evident also for the bivalent group coefficients. Both these characteristics can be accounted for by general chiasma terminalization between these stages, during chromosome contraction (Darlington, 1937; Swanson, 1960 etc.), two or more chiasmata being terminal and coincident. Two other possibilities, however, should be mentioned. Firstly, a proportion of distal/ terminal chiasmata may be lost during these stages, a situation that, though frequently said not to occur (Darlington, 1937; John and Lewis, 1965), evidently occurs in some plants, e.g. Lilium (Lawrence, 1961). Secondly, any slight over-estimation of chiasma frequency at earlier stages caused through occasional scoring of twists as chiasmata would not be present at diakinesis/ metaphase I stages. This would cause a slightly greater drop in mean chiasma frequency at these later stages than actually obtains. This latter possibility is probably of very little significance.

B. Chiasma frequency/chromosome length relationship.

It was indicated earlier from general observations that the frequency of chiasmata per bivalent varied approximately proportionally with

chromosome length. It is possible indirectly to test this conclusion in
part by calculating the expected mean frequency of chiasmata in groups I and
II + III in early stages and I, II and III in late stages, from a basis
of the known, summed, relative lengths of these chromosomes; and then to compare
the calculated frequencies with the observed ones. More specifically, the
calculated and obtained values for chromosome 9 in early stages (the only one
clearly recognizable) can be compared.

(a) <u>Early stages.</u> The summed relative lengths of group I and group II + III
chromosomes (centromeres included) were used to calculate the total and mean
chiasma frequencies per group, in the 25 cells analysed, that would be expected
if frequencies follow in strict proportion to chromosome length (table 20).
It can be seen that there is very close agreement between calculated and
observed frequencies.

Secondly, in 9 cells in which chromosome 9 could be identified,
positively, a total of 12 chiasmata were observed in this bivalent, giving
a mean frequency of 1.33 per cell. On the basis of its relative length a
frequency of 1.45 per cell is expected. The observed is a little lower than
expected though not significantly so. (I have no direct evidence that the short
arm of bivalent 9 forms a chiasma at times; possibly it is too small to do
so in an easily detectable frequency; or perhaps the nucleolus interferes
with synapsis and/or chiasma formation in this short arm, as found by Zen
(1961) ain <u>A. fistulosum</u>.)

These findings on the close relationship between chiasma frequency and
chromosomes length, reinforced below from later stages, were very useful in
assessing the expected chiasma frequency in the complex of interchange cells
at diplotene/diakinesis. This is not possible directly since the precise chiasma

Table 20 Expected distribution of 490 chiasmata from 25 normal cells at diplotene/diakinesis and 562 chiasmata from 34 normal cells at diakinesis/metaphase I, based on the relative lengths of the chromosomes within groups. The observed distributions are also given and show close agreement with the expected values.

* The data from chromosome 9 were obtained from 9 of the 25 diplotene/diakinesis cells in which this chromosome could be identified.

The data for chromosome 9 and the short arms of the group III chromosomes are included in the totals of their appropriate groups.

Table 20.

	Rel. length	Diplotene/Diakinesis				Diakinesis/Metaphase I			
		Expected XO cell exp.	Observed XO cell obs.	Mean per cell exp.	Mean per cell obs.	Expected XO cell exp.	Observed XO cell obs.	Mean per cell exp.	Mean per cell obs.
Group I	56.42	276	282	11.04	11.28	317	315	9.32	9.26
Group II	18.27					103	105	3.03	3.09
Group III	25.31					142	142	4.18	4.18
Group II + III	43.58	214	208	8.56	8.32				
C/some 9*	7.41	13	12	1.45	1.33	19	17	0.56	0.50
Short arms of group III	3.38								

frequency of the interchange chromosomes 4 and 6 cannot be determined directly from normal cells. But an expected value for these chromosomes can be obtained from the mean chiasma frequency in normal cells and the known relative lengths of these chromosomes. Thus on this basis chromosome 4 would show a frequency of approximately 2.49 chiasmata per bivalent, and chromosome 6, 1.83 per bivalent.

(b) Late stages. The ability to recognize three groups of bivalents at metaphase I permitted a further test of this conclusion that chiasma frequency in Allium triquetrum follows in strict proportion to chromosome length. Expected frequencies for each group were calculated and compared with the obtained values (table 20). It can be seen that there is very good agreement between calculated and obtained values in all three groups.

Included in table 20 is the calculated and observed chiasma frequency for the short arms of group III chromosomes. The agreement is also good, showing that even these small arms form chiasmata in a frequency proportional to their lengths. The data suggest that bivalent 9, as well as the others in group III, forms a chiasma at times. If in calculating expected chiasma frequencies for the short arms of group III bivalents the short arm length of chromosome 9 is not included, then in the 34 cells analysed a total of 14 short arm chiasmata would have been expected. This is considerably lower than the 17 that were actually observed. The data conform better to the hypothesis that bivalent 9 does indeed form chiasmata at a frequency proportional to its length rather than to a hypothesis that it does not.

On a basis of their relative lengths chromosomes 4 and 6 at diakinesis/ metaphase I stages would show chiasma frequencies of 2.10 and 1.54 respectively.

The conclusions above on the direct relationship between chiasma frequency and chromosome length are in agreement with similar observations in many other

species that have been examined (Mather, 1937, 1938), and in particular in
Allium macranthum where Levan, (1934) conducted an analysis very similar
to my own.

II   Chiasmata in interchange cells

(a) Diplotene/diakinesis stages.   Data in left hand portion of table 19 (B).
Chiasmata in the complex.   The number of chiasmata in the interchange complex
varies from 2 to 5 at these stages, usually 3 or 4, with a mean frequency of
3.519. Terminalization coefficient of 0.084. The distribution of the
chiasmata in the complex parts is very characteristic and will be described
in detail. In this description I have used the axis terminology introduced
in the report on pachytene studies: though the exact proximal limits of the
axes cannot be distinguished their general identification presents no difficulty.
Axis 1a and 1b. Axis 1b never forms a chiasma (Figs. 23 - 25). This clearly
is to be expected since these regions are never synapsed. (It is clear from
the fact that short arms of group III chromosomes form chiasmata that if
synapsed these segments would be expected to form chiasmata in some cases).
The absence of chiasmata in these segments means that chains of four chromosomes
(occasionally unequal bivalents - see below) rather than closed rings are formed.

Axis 1a most often shows but a single medial - distal chiasma (Figs. 23 and
24); a proximal chiasma never forms, as expected since these proximal regions
are never synapsed. (Since the centromeres cannot be recognized at these
stages it is possible to estimate chiasma position relative to these regions
only roughly. Careful examination of the relative lengths of the chromosomes
in the complex, however, makes it very clear that proximal chiasmata do not
form in 1a).

In 27 cells fully scored no complex showed more than one chiasma in 1a,
though in one case outside this sample two chiasmata were identified as probably
occurring in this segment (Fig. 25 c). Occasionally chiasmata fail altogether

in these segments, giving rise to characteristic unequal bivalents (Figs. 30 and 31). This is the only type of unequal bivalent that forms as the 2a and 2b segments always form chiasmata. (Thus also, univalents do not arise.) The occasional failure of chiasmata in 1a is notably different from that of 1b, Whereas that of 1a follows synapsis(all cells at pachytene show some synapsis though this is occasionally over very short lengths in 1a), that of 1b follows a failure of synapsis.

Axis 2a and 2b. Axis 2 as a whole always forms at least 2 chiasmata to give chains of four chromosomes or unequal bivalents. It often is possible on size differences to distinguish 2a and 2b (Fig. 23). Both form one or two chiasmata, though 2a shows a greater frequency of two chiasmata than 2b (9 : 3 in 12 cells in which the distinction was very clear), undoubtedly because of its greater length.

The frequency of the four basic types of complex and unequal bivalents are shown in table 16/from 116 scorings. Only approximately 4% of unequal bivalents arise, and types 1 and 2 together comprise 95% of cases.

(P.88)

Interstitial chiasmata. No evidence from diplotene through to anaphase I has been obtained for the occurrence of interstitial chiasmata.* The interstitial segments between centromeres and break points are very small

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* The term "interstitial" is used here to refer to chiasmata arising in the segments between centromeres and break points in the complex (Burnham, 1956, 1962). The term has frequently been used also to indicate a chiasma occurring well in from the chromosome ends of a bivalent etc. (Darlington, 1937; Swanson, 1960).
    The cytological and genetical effects of crossing over in the interstial segment will not be discussed here since they are unimportant in this interchange. See Burnham loc. cit. for relevant information.

in both 2a and 2b (see p.66), and if chiasmata do arise in these segments (they probably pair quite regularly) they do so very infrequently.

Total chiasmata per cell. A total of 27 cells were fully scored. The total chiasmata per cell ranged from 14 to 23 (lower value one chiasma less than in normal cells; higher value the same) with a mean frequency in this sample of 18.85. Terminalization coefficient 0.134.

In the description given above on the chiasma frequency and distribution in the interchange complex it was indicated that chiasmata fail totally in 1b and in proximal regions of 1a, and occasionally in the whole of 1a. This failure of chiasmata was expected to cause an overall reduction in chiasma frequency for the complex and thus for the cell as a whole. As far as the cell as a whole is concerned the comparisons between the mean frequency per cell in normal and interchange material shows a slight reduction (19.60 for normal and 18.85 for interchange cells),though a t-test on the two sets of data shows that the difference between mean values is not significant at the 5% level of probability (table 21). In the face of the considerable variability in total chiasmata per cell (range 14 to 23) a some-what larger sample would have to be scored to bring the probably real difference to a statistically significant one. However, further data from metaphase studies given below reinforce the impression gained here.

It will be useful to examine further what chiasma frequency should be expected in the complex, what magnitude of reduction occurs, and thus what magnitude of reduction should be expected in cells as a whole. A good indirect estimate of the chiasma frequency in the complex can be obtained from

Table 21.

	Mean XO per normal cell	Mean per inter. cell	diff.	S.E.	t	d.f.	p
Diplotene/ diakinesis	19.60	18.85	0.75	0.564	1.33	50	< 0.2 > 0.1
Diakinesis/ metaphase I	16.53	15.73	0.80	0.442	1.81	62	< 0.1 > 0.05

	Expect ed mean in complex	Obser ved mean					
Diplotene/ diakinesis	4.32	3.52	0.80	0.112	7.14	26	< 0.001
Diakinesis/ metaphase I	3. 64	3.07	0.57	0.082	6.95	29	< 0.001

Table 21   Comparison of mean chiasma frequencies in normal and
interchange cells and the interchange complex at diplotene/
diakinesis and diakinesis/metaphase I. The expected mean for the
complex was calculated from the relative lengths of the chromosomes
in the complex and the mean total number of chiasmata in interchange
cells, assuming chiasma frequency is proportional to chromosomes
length (table 20).

diff. = difference, S.E. = standard error, d.f. degrees of freedom,
p. probability.

the calculated expected frequencies for chromosome 4 and 6. These values, calculated on the basis of relative chromosome lengths and mean chiasma frequency per cell in normal material, are 2.489 and 1.830. Assuming complete synapsis and unaffected chiasma relationships, the expected frequency for the complex will be the sum of these two values i.e. 4.319. The value obtained for the complex was 3.519, showing a difference of 0.800 which is statistically very significant (table 21).

It is worth noting also that the reduction in chiasma frequency in the complex has led to a similar sized difference in mean cell frequency between normal and interchange material (difference 0.75).

The difference between expected and observed frequencies in the complex, from the considerations given earlier, should have been caused through a reduction in axis 1 (a and b). Since axis 2 shows complete synapsis it can be expected to show no reduction in chiasma frequency (unless the interchange itself has affected chiasma formation in some way). If the method used above for calculating expected frequency for a chromosome is valid (see also later), then the calculated frequency for 2a + 2b (basically equal to the long arms of chromosomes 6 plus 4^6) and the observed frequency should be roughly the same. Expected frequency: 2.80 per complex; observed frequency: 2.52 per complex; difference: 0.28 which is statistically not significant. The data show satisfactory agreement.

(b) <u>Diakinesis/metaphase I stages</u>. Data in right hand portion of table 19 (B).

<u>Chiasmata in the complex</u> The total of chiasmata in the complex ranges from two to four, usually three or four; mean frequency 3.067; terminalization

coefficient 0.261. The distribution of chiasmata in the complex segments closely follows that expected from the distribution seen at earlier stages with ensuing terminalization. The frequency of unequal bivalents at this stage (Fig. 32) is a little higher, but not significantly so, than that of earlier stages (table 16), *p. 88* showing that few if any chiasmata in 1a are lost during terminalization. The single chiasma that usually occurs in 1a is either distal or terminal in position. As in earlier stages univalents do not arise. One or two chiasmata occur in each of 2a and 2b (Fig. 38 and 39), giving the two main complex types most frequently observed. Compared with earlier stages there is a marked reduction in type 2 complexes and a corresponding marked increase of type 1 at metaphase I (table 16), undoubtedly due to terminalization of chiasmata in the 2a and 2b segments. In type 1 the single chiasma in 2a or 2b is medial to terminal in position.

Total chiasmata per cell. A total of 30 cells were scored. The range of chiasmata per cell (13 - 21) is virtually the same as for normal cells though with a stronger concentration of cases round 15 and 16 (see appendix table 3). Mean frequency per cell, 15.73; terminalization coefficient 0.305.

As with earlier stages there is a slightly lower mean chiasma frequency per cell in interchange cells compared with normal cells (table 19) and the difference at these stages with this sample is approaching significance at the 5% level of probability (table 21).

As before, it will be useful to examine further what magnitude of reduction should be expected and what occurs in the interchange complex and cells. (1) Expected frequency in the complex; 3.64 (2.10 + 1.54) observed frequency; 3.07.

As found at earlier stages the reduction in chiasma frequency (0.57) is statistically very significant (table 21) and the percentage reductions at early and late stages are approximately the same (18.5% and 16% respectively).

(2) Since the short arm of chromosome 6 sometimes shows a chiasma at diakinesis/metaphase I in normal cells but does not in the interchange complex, the frequency of group III short arm chiasmata per interchange cell should be proportio̊nately lower than that of normal cells. Such a reduction is found. No case of two of these chiasmata in one interchange cell has been seen (cf. maximum of two in normal cells), and their mean frequency in the 30 cells analysed was 0.367 per cell (0.5 in normal cells).

(3) For segments 2a plus 2b of the complex a chiasma frequency of 2.25 per cell is expected. The obtained value was 2.133 per cell, thus showing close agreement, as in the earlier studies.

The remaining chromosomes in interchange cells appear to be behaving as they do in normal cells. The only direct comparison in this respect that can be made is between group II's in normal and interchange cells at diakinesis/metaphase I. Neither the variances nor the mean chiasma frequencies of the two are at all different statistically (table 17 *p. 88*) indicating comparable behaviour in normal and interchange cells. The other groups cannot be so compared. However, if the expected frequencies in chromosomes 4 and 6 are added to the appropriate group frequency in interchange cells, then similar comparisons can be made. These are shown in table 17 and indicate no essential differences between normal and interchange chromosomes. Hence it is apparent that all the chromosomes except 4 and 6 are behaving identically in normal and interchange cells.

III   Interchromosomal distribution of chiasmata in normal and interchange cells.

It has been shown above that in interchange cells chiasma frequency in the complex is reduced as a result of localized asynapsis at pachytene, while no significant change was recorded in chiasma frequency in other chromosomes. These two facts were further revealed in an overall reduction in mean chiasma frequency in interchange cells compared to normal ones, a reduction which, though not statistically significant at the 5% level of probability with the rather small samples of cells used here, gives the impression of being a real one, in the light of all the related information.   The implication is that in these cells there was no balancing out of chiasma frequency in the chromosomes not involved in the interchange.   However, a more critical test of interchromosomal correlations seems desirable in view of the accumulating evidence for such correlations, both positive and negative, in various plant and animal species.   Since individual bivalents are not recognisable in the present material, correlation analyses are limited to the groups of bivalents shown in the following table (I-4 e.g. means group I minus chromosome 4;   4/6 represents the chromosomes of the complex):

	Normal cells	Interchange cells
Early	I, II + III	I-4, II + III - 6, 4/6
Late	I, II, III	I-4, II, III-6, 4/6

To test whether chiasma frequencies between groups within pollen mother cells are correlated, both correlation coefficients and analyses of variances were used. Correlation coefficients were calculated by use of the standard formula, $r = \sqrt{\dfrac{\text{covariance } xy}{\text{variance } x \cdot \text{variance } y}}$ where x and y denote the chiasma frequencies of the two groups of chromosomes under study. This method was used for the two groups of chromosomes that can be recognised at diplotene of normal cells, and also to compare the chiasma frequencies of the interchange complex and the rest of the bivalents in interchange cells. Complete and incomplete analyses of variances were employed following the general technique used by Mather (1936), Elliot (1958), Basak and Jain (1963) etc.. The complete analysis of variance permits a comparison to be made between the internuclear variance, which measures the variation between nuclei of the sample of cells, and the inherent variation, which measures the variation of the groups within nuclei after allowances have been made for differences between nuclei and between groups. A larger internuclear variance is evidence for positive correlation while a larger inherent variation is evidence for negative correlation. No correlation is present if the internuclear and inherent variances are the same. (Mather, 1936, Elliot, 1958). Positive correlation can be expected to arise frequently since the environment and mixed material will tend to influence chiasma frequencies equally in all bivalents. Negative correlation, however, suggests an interaction or competition between bivalents for chiasmata, irrespective of outside factors (Mather, 1936).

The analysis of variance can be taken a step further by examining correlation within a group.   Since the individual bivalents cannot be recognized consistently within a group, the analysis here must be by the incomplete method, comparing the total variation within nuclei with that between nuclei, a larger within variation again indicating negative correlation.   Mather has shown that this incomplete method of analysis is valid and useful for detecting correlation so long as there are no great differences between bivalents within a group in respect of size and chiasma frequencies (so that the contribution of variation between bivalents to the total within nuclei is relatively small).

The complete analysis of variance was used for all four samples of cells examined.   The incomplete method was used in diplotene cells of normal material to examine for correlation within groups I and II + III.

Since there is a certain amount of terminalization of chiasmata between diplotene and metaphase the analyses at early stages will be somewhat more informative.   Terminalization tends to dampen the detection of correlation (Mather, 1936).

Analyses of variances in the four samples of cells examined are shown in table 22 and summarized in table 23.   Table 24 shows the results of the incomplete analysis of variances within groups I and II + III at diplotene/diakinesis, and table 26 shows the three correlation coefficients that have been calculated.   The results can be stated as follows.

(1) The three correlation coefficients show no significant deviation from zero, thus showing an absence of correlation in chiasma frequencies between the groups I and II + III bivalents, and between

Table 22 Analyses of variances of chiasma frequencies at
diplotene/diakinesis (A & B) and diakinesis/metaphase I (C & D),
in normal (A & C) and interchange (B & D) materials. In all
cases the internuclear variance is greater than the inherent
variance. In one case the difference is significant at the
5% level of probability. The data show no evidence of negative
correlation in chiasma frequency. See table 23 for summary of
data.

A.

Item	SS	d.f.	Variance	f	p
Between groups (b/g)	109.52	1	109.520	56.54	< 0.001
Between nuclei (b/n)	52.00	24	2.167	1.119	ns
Remainder = inherent (i)	46.48	24	1.937		
Total (T)	208.00	49			

B.

Item	SS	d.f.	Variance	f	p
b/g	372.03	2	186.015		
b/n	33.80	26	1.300	1.749	ns
i	38.64	52	0.743		
T	444.47	80			

C.

Item	SS	d.f.	Variance	f	p
b/g	749.71	2	374.855		
b/n	42.00	33	1.272	1.939	< 0.05
i	43.29	66	0.656		
T	835.00	101	0.656		

D.

Item	SS	d.f.	Variance	f	p
b/g	363.14	3	121.047		
b/n	17.47	29	0.602	1.029	ns
i	50.86	87	0.585		
T	431.47	119			

Table 23.

| | Variance | | | |
	Inter nuclear	Inherent	F	p
Normal (E)	2.167	1.937	1.119	ns
Interchange (E)	1.300	0.743	1.749	ns
Normal (L)	1.272	0.656	1.939	< 0.05
Interchange (L)	0.602	0.585	1.029	ns

Table 23   Summary of analyses of variances of chiasma frequencies in normal and interchange material at diplotene/diakinesis (E) and diakinesis/metaphase I (L). The data show no evidence of negative correlation in chiasma frequency. Further details of analyses in table 22.

Table 24.

1. Group I chromosomes

Item	SS	d.f.	Variance	F	p
Internuclear	16.26	24	0.678	1.66	< 0.05
Intranuclear	30.50	75	0.407		
Total	46.76	99			

2. Group II + III chromosomes

Item	SS	d.f.	Variance	F	p
Internuclear	6.69	24	0.279	1.047	ns
Intranuclear	29.20	100	0.292		
Total	35.89	124			

Table 24   Incomplete analysis of chiasma variances in groups I
and II + III at diplotene/diakinesis. The differences between
internuclear and intranuclear variances are not significant, thus
indicating an absence of correlation in chiasma frequency between
bivalents within these groups.

Material	Diplotene/ diakinesis	Diakinesis/ metaphase I	F	p
Normal	2.167	1.272	1.704	$< 0.1 > 0.05$
Interchange	1.300	0.602	2.159	< 0.05
F	2.113	1.667		
p	< 0.05	$< 0.1 > 0.05$		

Table 25   Internuclear chiasma variances at diplotene/diakinesis and diakinesis/metaphase I in normal and interchange material. The data show reductions of variances between the two stages (due to terminalization) and between normal and interchange material (due to reduction in chiasma frequency).

Comparison	r	d.f.	p
Group I v. II + III diplot./diakin.	+ 0.060	23	ns
Complex v. rest diplot./diakin.	− 0.102	25	ns
Complex v. rest diakin./met.I	+ 0.312	28	< 0.05

Table 26   Chiasma frequency correlation coefficients (r)

the complex and the remaining bivalents.

(2) In all four samples of cells the internuclear variance was larger than the inherent variance. In one case the difference was significant at the 5% level of probability while in the others it was not significant. Thus there is no evidence of negative correlation between groups of bivalents. The fact that the internuclear variance is the larger of the two in all cases, and in one case significantly so, is not surprising since environmental differences and perhaps also mixed material were undoubtedly present to some extent. *

(3) In the incomplete analysis of variance there is also no significant difference between internuclear and intranuclear variances, neither in group I nor group II + III bivalents, thus showing an absence of correlation in chiasma frequency within these two groups of chromosomes.

All the evidence above as well as that presented earlier on the reduction of chiasma frequency in the whole interchange cell and the normal behaviour of group II bivalents in normal and interchange cells, points strongly to an absence of any clear interbivalent correlation of chiasma frequencies in this material. Rather, the different groups appear to show autonomy at this level of chiasma formation. The different combinations of chromosome groups in the four types of cells

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* It is worth pointing out here that in both internuclear and inherent variances there is a significant drop between E and L stages, (table 26). This must be brought about by chiasma terminalization between the two stages. Also, there is a significant difference between variances of normal and interchange material at each of the two stages. This is clearly the result of the lowered frequencies of chiasmata in the complex and thus in the entire cell, as discussed previously.

examined and the incomplete analysis of variance strongly suggest that this autonomy extends to the level of individual bivalents.

This conclusion agrees in part and is at variance in part with the results obtained by Mather (1936) on chiasma correlation in two Allium species. In A. macranthum (n = 14 with long, medium and short classes of chromosomes) Mather found no correlation between classes of bivalents as in my material. He also found no intraclass correlations for the medium and short classes, but found a strong negative correlation between bivalents within the long class of bivalents. The long bivalents in my material showed no such negative correlation. In A. zabdanense (n = 9) Mather found on the other hand that long bivalents tend to be uncorrelated amongst themselves or positively correlated, but the short chromosomes tend to be negatively correlated.

IV   Discussion

Mather (1936) in his studies on numerous plant and animal materials
was the first to study the problem of interbivalent control of chiasma
frequencies in detail, though earlier both Darlington (1933) and Sax (1935)
had investigated the problem briefly in rye and Vicia faba respectively.
Mather showed clearly that negatively correlated chiasma frequencies may
occur between and within groups of bivalents within a nucleus, a low
frequency of chiasmata in a bivalent or group of bivalents being accompanied
by a higher frequency in others.   However, Mather did find that the occur-
rence of negative correlation is very variable, many species showing little
or no evidence of the phenomenon, others showing it very clearly.
Variations in this respect were found even between different individuals
of the same species, and when present only some of the bivalents may show
correlations.   In general Mather found that species with a low number of
bivalents or low frequency of chiasmata per bivalent rarely show the
phenomenon, while those with higher numbers of bivalents or higher chiasma
frequency per bivalent more often do.

The phenomenon of negative correlation in chiasma frequencies has
recently been examined extensively in Delphinium and Chrysanthemum by
Jain and co-workers (Jain and Bose, 1960; Jain and Maherchandani, 1961;
Basak and Jain, 1963, 1964; Bhatnagar and Jain, 1963) and by Rowlands
(1958) in Vicia faba. Delphinium in particular has been shown to exhibit
negative correlations very consistently, and with the aid of interchanges

it has been shown that the interchromosome effects are manifest between individual bivalents, not simply between different groups of bivalents. It was noted, however, that nuclei with very low or very high chiasma frequencies showed a considerable relaxation of interchromosome effects (Basak and Jain 1963). In Vicia faba Rowlands (1958) found that a wide range of correlations could be demonstrated between and within groups of bivalents in both related and unrelated plants of the same species. Elliott (1958) showed that in Endymion even different anthers in the same plant may show different types of correlation amongst bivalents, and in Hyacinthus that inherent and internuclear variances may be altered separately through temperature influence for example, thus causing the detection of very different correlations. Elliott also illustrated in Endymion that significant correlation is expected only if the variance for both bivalents or groups of bivalents under study is high, no correlation being expected between different bivalents if one or both of them has a low variance of chiasma frequency. On the other hand Southern (1967) found no evidence of interbivalent correlation of chiasma frequency in his detailed study in grasshoppers.

It is clear from the above comments then that the occurrence of correlations in chiasma frequencies is very variable and depends on both intrinsic and extrinsic factors for its expression.

Interchromosome effects are also well known from the point of view of crossing-over frequencies. Both inversions and interchanges have been

shown in some cases to cause not only an increase in crossing-over
in distal, non-rearranged portions of the altered chromosomes themselves,
but also in other bivalents of the complement (e.g. see Schultz and
Redfield, 1951; Ramel et al., 1964; Susuki, 1963 on inversions, Hinton,
1965 on interchanges).   Cytological parallels have been demonstrated for
the above in some cases, both intrachromosomally and interchromosomally.
Thus e.g. White and Morley (1955) have shown that pericentric inversions
in Trimerotropis and Moraba species cause an increase in the chiasma
frequency in regions distal to the re-arrangements.   And Hewitt (1967)
found that an interchange in Cibolacris parviceps raises considerably
the chiasma frequency both in the complex and in all the other chromosomes
in the complement.   In this respect also, Jain and Bose (1960) found that
an X-ray induced reduction of chiasma frequency in one group of chromosomes
in Delphinium led to an increased frequency in the other group.   But such
cytological parallels are also variable.   Hewitt and John (1965) found
that at least some interchanges in Chorthippus do not alter chiasma
frequencies in other bivalents in the nucleus.   And White and Morley (1955)
noted that inversions in some cases do not reveal interchromosome effects
on chiasma frequency in the cell.

Chiasma frequency and distribution in Allium triquetrum are almost
certainly under genetic control as in other Allium species (see e.g.
Emsweller and Jones, 1945) and in other organisms (Rees, 1961).   The mean
chiasma frequency at diplotene and metaphase I is not high, there being
2.18 and 1.84 chiasmata per bivalent respectively.   Since in this
complement there are four large bivalents that frequently form three

chiasmata as well as five smaller ones often with two chiasmata, it is evident that the mean chiasma frequency in the cell is not distributed at random amongst the bivalents.   If it were so at least occasional univalents should be formed when some bivalents fail to form chiasmata.   Univalents have never been seen in my own studies of this material.   Rather, some control is evidently exercised to ensure that every bivalent forms at least one chiasma (Jones and Rees, 1964).   However, no interchromosome effects of the type investigated here have been detected in either normal or inter-change material.   The number of chromosomes in the nucleus is not as small as in many of those organisms in which Mather found no evidence of interbivalent correlation, and the number of bivalents and chiasma frequencies here are similar to A. zabdanense which showed evidence of some though variably expressed correlation between bivalents.   On this ground it is perhaps a little surprising that no correlations were observed in my material.   Also, the chiasma frequency per nucleus is somewhat higher than in say Delphinium where at metaphase there are 10 or 11 chiasmata per cell and eight bivalents to share them, and Delphinium consistently shows negative correlation amongst its bivalents.

On the other hand, while the variance in chiasma frequency of group I bivalents is considerable (1 - 4 chiasmata per bivalent) that of the smaller bivalents and the complex is considerably lower.   On the basis of Elliott's (1958) considerations in Endymion then it is perhaps not surprising to find no correlation between groups of chromosomes in this material.

Recently, Sybenga (1967) considered that the presence of genuine negative correlation in chiasma frequency between bivalents has not as yet been proven or disproven satisfactorily. He points out that when not observed, negative correlations may be present but covered up by between cell heterogeneity causing positive correlations.[*] This is conceivable in my present material, since all analyses in table **22** show suggestions of positive correlation between groups of bivalents, indicating between cell heterogeneity. The present material might be inadequate for the detection of small negative correlation in chiasma frequency amongst the bivalents. On the other hand it is interesting to note that dispite this between cell heterogeneity a reduction in mean chiasma frequency in interchange cells was observed in the present material. This emphasizes that the interchange has not altered chiasma frequency in the other bivalents in any radical way.

Sybenga also suggests that where negative correlations have been identified, these may in fact be due to "similar within cell heterogeneity in plasmatic conditions affecting chromosome behaviour", though as he points out this cannot account for negative correlations of the type where a reduction in one chromosome causes an increase in specific regions of another chromosome, as has been reported in some cases (Schultz and Redfield, 1961).

The chiasma relationships in this Allium interchange are very similar to those in the L_1 - M_5 interchange in Chorthippus brunneus described

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[*] Evidence to support this suggestion has been documented recently by Dryansagar & Sudhakaran (1968).

by Hewitt and John (1965).   Here when L_1 and M_5 chromosomes are associated in an interchange multiple they form significantly fewer chiasmata than when occurring as separate bivalents in normal cells. This reduced chiasma frequency did not cause any alteration in chiasma frequencies in other bivalents, and thus caused a slight lowering of the overall cell frequency.

In their discussion Hewitt and John briefly considered the possibility that rather than being a system of control in itself, chiasma competition, as suggested from correlation studies, might be a manifestation of _deranged_ control.   Under optimal conditions chiasma control may be such as to permit complete autonomy in chiasma formation within certain limits.   Under abnormal conditions or stress, as for example those brought about by a re-arrangement of genetic material (e.g. in an interchange), the system of control that regulates chiasma properties in a cell may be deranged giving rise to correlations amongst the bivalents in such material.   Rowlands (1958) inferred this too in his discussion, accounting for the possibility on a basis of an upset in chromosome pairing and reproduction, by enviromental or other factors, which might differentially affect certain chromosomes (long versus short e.g.). This could be manifest in different chiasma frequencies amongst bivalents and thus significant negative correlation, if there is any tendency to control chiasma frequency within fairly close limits, as there is in _Vicia faba._

This interpretation of correlation amongst bivalents has similarities with the within-cell heterogeneity suggestions of Sybenga and is attractive in the light of the very varied expression of the phenomenon amongst different individuals, and its different manifestations under different conditions. It implies that while some conditions, external or internal, may derange control so as to produce apparent correlations between bivalents, others may not do so. The latter might apply to the present interchange in Allium triquetrum, both normal and interchange materials here working under optimum conditions allowing considerable autonomy in chiasma formation amongst the bivalents.

<u>V</u>   Terminalization coefficients of normal and interchange cells.

The terminalization coefficient for interchange cells is somewhat
lower than that for normal cells at diplotene/diakinesis stages (table 19). *p. 94*
Without the complex the other 7 bivalents show a value of 0.14**5** in inter-
change cells, a value closer to that for normal cells (0.161).   The value
for the complex itself, however, is 0.084, which is considerably lower than
expected for a group I plus group III bivalent combined.   Hence the low
total interchange value appears to be due largely to the low value in the
complex.   At later stages the same tendency is also suggested, though to
a lesser extent.

Theoretically, this apparent lowering of the terminalization coeffic-
ient in the complex could be brought about in two ways : (a) an actual
reduction in the degree of movement of chiasmata, (b) a reduction in
frequency of origin of distal/terminal chiasmata.   Since we do not at
present fully understand the process of terminalization and its control
(Swanson, 1960; John and Lewis, 1965) it is difficult to comment meaning-
fully on possibility (a).   On the other hand, a reduction in the frequency
of origin of distal/terminal chiasmata is to be expected because of the
reduction in overall chiasma frequency in the complex.   Thus (i), the
terminal chiasma, expected from a bivalent basis to occur sometimes in
segments 1b, does not (cannot) arise.   Further (ii), it should be recalled
that when two chiasmata arise in an arm pair they are not positioned in-
dependently of each other, but rather are located in proximal and distal,
or medial and terminal positions.   The implication of this fact is that

one chiasma interferes with and inhibits the formation of another in its near neighbourhood, a well known chiasma phenomenon (Henderson, 1963; Southern, 1967 e.g.). But with only one medial-distal (not proximal) chiasma arising in 1b in the very large majority of complexes, chiasma position here could not be influenced in a similar manner. The chiasma arising in 1b would not be forced to take up a more distal position as it would be in a normal situation when a proximal chiasma is also present in the arm pair. Thus the absence of proximal chiasmata in 1b would permit more random location of the one chiasma that does arise, lowering the frequency of it being positioned near terminally.

This second explanation receives some support from recent chiasma studies in grasshoppers and Drosophila. In four species of Truxaline grasshoppers Southern (1967) found that the general tendency for one chiasma in a bivalent to lie in a distal position was more accentuated when a proximal chiasma near the centromere also formed. Thus a proximal chiasma restricts the distribution pattern of the chiasma that forms distally, causing it more frequently to be positioned near to the end of the bivalent. In the X chromosome of Drosophila Stephens (1961) found an equivalent genetical effect, one crossover normally being located medially but double crossovers formed proximally and distally. The situation in the interchange complex I describe appears to be simply a converse of the above. Finally the suggestion is supported by the fact that in the 27 diplotene/diakinesis interchange cells fully scored for chiasma frequency all the eight terminal chiasmata recorded in the complex occurred in 2a and 2b

segments, none in axis 1 (2a and 2b together had a terminalization coefficient of 0.116 which is approximately as expected from the coefficient in normal cells). Also, in the one complex in which a terminal chiasma was recorded in axis 1b (outside the sampled 27) this axis also had a second more proximal chiasma (fig. 25c).

5.   CENTROPHILIC BEHAVIOUR OF BIVALENTS AND COMPLEXES AT PROMETAPHASE

The following studies concern unstable, centrophilic bivalents and interchange complexes at meiotic prometaphase.   The observations are divided into those from normal and those from interchange cells.

I.   In Normal Cells.

At complete metaphase I all the bivalents are co-oriented along the equator.   However, as pointed out briefly already, instances of incomplete orientation in one or a few bivalents frequently can be seen in squashed and unsquashed preparations.   These incompletely oriented bivalents most frequently occupy positions at or near one of the two spindle poles, instead of along the equator (Figs. 40 and 41).   Such polar oriented chromosomes have been referred to as being "centrophilic" (Bloom et al., 1955*) and have been noted previously in both mitotic and meiotic prometaphase (Bajer, 1958;   Bauer et al., 1961).   It is frequently impossible to ascertain how the centromeres of these bivalents are oriented in relation to the poles (observation must be made on unsquashed preparations to be certain of the correct interpretation), though in a number of instances this has been possible.   The group III bivalent in Fig. 40 is a clear example.   The two homologous centromeres, near the chromosome ends, are oriented to the same pole, while the chromosome arms and chiasma are

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*   The "centro" in centrophilic refers to the centrosome (or centriole)
     adjacent to which the centromere)= kinetochore) lies in the cells
     studied by Bloom et al. (1955).   Although I use the term centromere
     rather than kinetochore this is no justification for introducing a
     new term to avoid possible confusion, and hence the term centrophilic
     is retained in its original meaning, even though no centrosomes or
     centrioles are present at the poles in my material.

directed backwards towards the equator. In Fig. 40 spindle fibres were observed to connect the centromeres of the centrophilic bivalent to the one pole; the centromeres are slightly drawn out towards the pole because of activity on the spindle. It is clear, therefore, that the two centromeres in this centrophilic bivalent are oriented to the same pole, as in others where the centromeres were equally clear. Their polar position is undoubtedly brought about through their unipolar orientation.

It is not possible to be certain that this orientation (both homologous centromeres to one pole) applies to all centrophilic bivalents. It is most often seen in those of group III, though it is clear why this should be so, for with only a very small short arm to these chromosomes, and most frequently with only chiasmata in the long arms, the centromeres of these bivalents are visible more frequently than those in groups I and II bivalents, where both arms are relatively long and where chiasmata usually occur in both arms. In a number of cases of centrophilic bivalents only one centromere could be identified, the other being hidden from view; the one was always oriented to the nearest pole. It is probable that the majority of the centrophilic bivalents seen are of the type described for Fig. 40. Three other possibilities should be mentioned, however. In some cases only one centromere of a centrophilic bivalent may be active and oriented to the near pole; the other centromere may be inactive as yet. Secondly, both centromeres of the particular bivalent concerned may be inactive as yet, the bivalent merely lying near one pole fortuitously, prior to its orientation. Most observations reveal at least one active centromere in a centrophilic bivalent so that these latter cases,

if arising, would be infrequent.   Also, all centromeres in a cell
appear to become active close in time to one another, so that instances
of the former case are probably infrequent as well. Thirdly, in what could
only be a few cases, homologous centromeres may in fact be oriented to
opposite poles, the centrophilic bivalent being in the process of movement
onto the equator.

In the analysis below centrophilic bivalents were identified as
such only when they were clearly lying away from the equator towards one
pole as in Fig. 40.

All groups of bivalents show centrophilic behaviour at prometaphase,
so such behaviour is not characteristic of any one bivalent.   In one
pollen sac in which 72 cells with centrophilic bivalents were scored,
38 centrophilics were of group I, 18 of group II and 19 of group III, these
data* suggesting that different types of bivalents show such behaviour in
a frequency roughly proportional to the number of bivalents in the group;
or in other words, that each bivalent appears to have the same tendency
towards centrophilic orientation (the expected numbers on this basis are
33, 17 and 25 for groups I - III respectively).   Further data are desirable
on this point, particularly since observations reported later suggest that
a particular bivalent may show centrophilic behaviour with changing
frequency during development of metaphase I in a pollen sac.

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* Some misinterpretations possible occur, since it is often difficult
to distinguish a centrophilic group II bivalent from a group III one
when both have chiasmata in both arms.   Group III bivalents rarely
have short arm chiasmata.

Some cells in a pollen sac showing many prometaphase stages have no centrophilic bivalents. These are of two types [1] those showing no clear signs of orientation (diakinesis), and [2] those showing complete bivalent orientation (metaphase). The other cells designated prometaphase here, show up to three centrophilic bivalents, distributed at one or both poles. The other bivalents in cells showing centrophilic ones are co-oriented along the cell equator (Figs. 40 and 41).

The frequency of cells showing centrophilic bivalents in a pollen sac varies with what must undoubtedly be different stages of development of prometaphase/metaphase in the sac. The highest frequency observed out of five pollen sacs examined was 39%, while the lowest (excluding those where orientation was complete) was 2%. The cases with high frequencies must represent relatively earlier stages in the development of prometaphase/metaphase in the pollen sac. Sufficient analyses have not been carried out as yet to obtain an indication of the maximum percentage at very early stages, nor the overall frequency. Centrophilic bivalents must eventually achieve equatorial co-orientation along with the other bivalents. This is evident from the facts that pollen sacs in early anaphase I show no cells with centrophilic bivalents, and no cases of numerical non-disjunction have been observed in normal cells. Nor have cells been observed in mid-anaphase I with lagging bivalents still co-oriented in the equator. It is evident, therefore, that cells achieve complete orientation in all bivalents prior to anaphase, which is not initiated while some bivalents remain in a centrophilic condition. This same conclusion has been reached from studies on centrophilic chromosomes

in living cells (e.g. Bloom et al., 1955; Bajer, 1958).

It is not possible from static studies to decide whether bivalents
that have already become co-oriented in the equator can later become
centrophilic, or whether only non-oriented bivalents can become so.
One would expect only the latter, and the studies of Bauer et al. (1961)
and Nicklas (1967) on living cells have shown this to be so.   Thus
centrophilic behaviour is an unstable event that gives rise to stable
co-orientation in the equator.

The occurrence of centrophilic bivalents during prometaphase
probably should be regarded as a normal event rather than as an abnormal
situation that is ultimately corrected.   The polar location of bivalents
having both centromeres oriented to one pole can be expected on the basis
of centromere/spindle activity leading to chromosome movement.   Conversely,
bivalents located in the equator are in an apparent equilibrial position
by virtue of the fact that their centromeres are co-oriented to opposite
poles (Rickards, 1965 for references and discussion).

II   In interchange cells.

The two stable, fully co-oriented, alternate and adjacent orient-
ations in the complex (Figs. 42-44) correspond to the stable co-oriented
arrangement in normal bivalents.   In prometaphase I unstable orientations
in the complex can be identified which parallel the unstable centrophilic
orientations of bivalents.   Since there are four centromeres in the inter-
change complex, compared with two in a bivalent, four basic types of
arrangements in the complex occur that can be referred to as being fully

or partially centrophilic. These are illustrated diagrammatically in Fig. 45a - d (e and f here are the stable orientations; a' described later). In a one of the four centromeres as yet has failed to achieve co-orientation in respect of its adjacent one; in b there are two non-cooriented centromeres (though reorientation in one is sufficient to achieve full co-orientation, and hence from this point of view type b has only one non-cooriented centromere); in c there are two non-cooriented centromeres, and in d no pair of centromeres has achieved co-orientation. Type d is the one that most obviously parallels centrophilic bivalents since the whole complex is polar oriented; but types a - c are cases that arise because there are four rather than two centromeres in the complex, two or three of which have already become co-oriented in respect of each other. The latter cases may be referred to more correctly as being partially centrophilic, though I shall only make this distinction where necessary; when reference is made to centrophilic complexes it refers to the four different types in general.

Two different subtypes of a and b will occur, depending on which end centromere is non-cooriented. Since individual chromosomes of the complex cannot be recognized clearly at these stages it will not be possible to distinguish these subtypes. They will be treated as one.

All four types of centrophilic complexes have been identified, originally from squashed preparations but subsequently from unsquashed ones (Figs. 46 - 50 and description below). As with centrophilic bivalents it is usually not possible to identify all the centromeres of a centrophilic

complex, though the location and orientation of unclear ones can be established with reasonable certainty from the way the complex is lying in relation to poles and equator. In the line drawings accompanying these figures the centromeres are drawn only when their location and orientation could be established reasonably clearly. Otherwise they are merely arrowed to indicate their approximate position.

Sometimes it appears from direct observations and inference from the way chromosomes are lying that some of the centromeres of the complex are inactive and unoriented (Fig. 51). In these cases there is no attenuation of chromatin at the centromere region(s) and the chromosomes involved appear to lie haphazardly, with no tension round the chiasma. This phenomenon appears to be peculiar to centromeres of the complex, not bivalents as well. In the analysis below these types were not classified into any of the four centrophilic complex types (Fig. 45). They are infrequent compared to cases with all centromeres of the complex apparently oriented. They will be considered again later.

The position of the various chromosomes of centrophilic complexes in relation to the poles and equator depends on (1) the type of centrophilic complex, and (2) its centromere/proximal chiasma relationships. Pairs of co-oriented centromeres and their chromosome arms are positioned roughly mid-way between the poles along the equator, as in co-oriented bivalents. In centrophilic complex type d (Fig. 50) the whole complex is positioned at or very near one of the spindle poles, with the centromeres oriented to the one pole and the chromosome arms and chiasmata lying indifferently in relation to one another. In Fig. 50 only two of the four centromeres could be identified positively, though the position of the

complex as a whole testifies to the probability that all four are oriented to the one pole. In centrophilic complex type a (Fig. 46) the centrophilic chromosome arms and centromere are positioned towards the one pole to a degree permitted by the position of the associated, co-oriented centromeres of the complex and, in particular, by the position of the "centrophilic" chiasma(ta). The non-cooriented centromere approaches the pole most closely when this latter chiasma is distal/terminal in position (Fig. 46). When there is a proximal chiasma the appropriate centromere is positioned some distance from the pole towards which it is oriented (Fig. 45 a'). These latter cases are difficult to identify at first, since the complex superficially appears to be of a fully co-oriented type. These types are uncommon; they are discussed in greater detail later.

The same relationships determine the location, in relation to poles and equator, of non-cooriented centromeres and chromosome arms in centrophilic complexes of types b and c. For type b the non-cooriented end centromere is usually positioned close to the spindle pole, while its adjacent one is usually not so close (Fig. 47 and 48). The occasional exception to this arrangement is that in which it appears as if the end one or two centromeres had oriented to but not yet moved to the opposite pole (Fig. 52.)

In type c the two end, non-cooriented centromeres take up positions close to the poles to which they are respectively oriented (Fig. 49).

The location of the whole complex near to one pole as in type d probably is due to the fact that all the centromeres are oriented to this pole. Orientation involving the spindle leads to movement to the pole, as in the case of a centrophilic bivalent. The same reasoning will apply to the location of non-cooriented centromeres and chromosomes in the other types of centrophilic complexes.

As expected, centrophilic bivalents also occur in interchange cells that show centrophilic complexes (Fig. 51a).

In a pollen sac showing a predominance of prometaphase stages not all cells show centrophilic complexes. Paralleling normal cells those showing no centrophilic complexes or bivalents are of two types: (1) those in which no clear evidence of orientation is present (diakinesis), and (2) those in which coorientation in both bivalents and complex is complete (metaphase). Some pollen sacs show only prometaphase and metaphase cells, while others show a predominance of cells at diakinesis. These undoubtedly represent relatively later and earlier developmental stages of prometaphase/metaphase in a pollen sac. In pollen sacs in early anaphase I cells with centrophilic complexes and /or bivalents are not found (the special 3 : 1 orientations and other discordant types are treated later; they are quite distinct from the usual type of centrophilic complex). It is clear then, as with bivalents in normal cells, that centrophilic complexes represent unstable stages in the development of prometaphase in these cells, and that these orientations are converted to stable co-orientations of the alternate or adjacent I types before the onset of anaphase. As with bivalents these centrophilic complexes

represent orientations that can be expected and do arise in the normal process of prometaphase mechanics.

An attempt was made to obtain information on the relative frequencies of the four types of centrophilic complexes in a pollen sac, particularly in view of their possible connection with events described later in the developmental sequences of anaphase I. In obtaining such information it was found necessary to take into account the stage of development of prometaphase in the pollen sac being examined, i.e. whether it was early (many cells still at diakinesis) or late (most cells fully co-oriented.) The two analyses necessary, however, have been confronted with numerous obstacles that have precluded a sufficiently detailed assessment at present. Firstly, with the short duration of prometaphase in a pollen sac, correct stages for examination are difficult to come by, and the searching procedure requires much laborious effort. Secondly, unsquashed preparations must be used at least for initial identification of cells with centrophilic complexes, so as to be absolutely certain of their orientation. This probably leads to non-recognition of some centrophilic types, particularly of type a' with/proximal chiasma in the non-cooriented chromosome arm pair. The other types of complexes are readily recognizable for obvious reasons, though only about 70% of centrophilic complexes are classifiable with full assurance, the other 30% being unclear, mainly because of overlapping chromosomes. It is possible that a slight bias occurs in the rejected 30%, since type a complexes are generally a little more difficult to observe fully. This

possible bias introduced into scoring must be borne in mind when inter-
preting the results of any analysis. Two features, however, probably
reduce any extra error here to a reasonable minimum. First, the inter-
change complex is positioned very frequently at or near one side of the
metaphase plate (data and discussion presented later), thus permitting
clearer examination in many cases. Also, the frequency of proximal
chiasmata throughout the whole complex is low, so that centromeres are
usually well separated from each other, this again being most favourable
for examination.

Possibly the most significant problem associated with the desired
analysis is the difficulty in unsquashed preparations of distinguishing
between cells at diakinesis with a few scattered bivalents on one hand,
and prometaphase stages with numerous centrophilic bivalents on the other.
(This distinction is necessary for determining developmental age in
a pollen sac.) Prior to the onset of orientation the bivalents appear
to become congressed towards the cell centre (evidence for this from
living cells of Haemanthus was noted by Bajer, 1958), so that super-
ficially at least this stage in unsquashed cells appears somewhat like a
fully oriented one. This probably means, however, that there are but
few scattered bivalents that might be confused with centrophilic bivalents.
Also when a cell enters prometaphase it is probable that most of the
bivalents become co-oriented immediately along the equator, and only up
to three bivalents per cell go through centrophilic orientation prior
to stabilizing in the equator. If these above two indications are correct

then there should be a reasonably clear distinction between diakinesis and prometaphase cells. Since appreciating this point I have analysed only two pollen sacs with a reasonably high proportion of cells still in diakinesis. While the distinction between diakinesis and prometaphase cells in these cases appeared to be reasonably accurate, some further investigations are desirable along these lines.

I have analysed only ten pollen sacs so far for their frequency and types of centrophilic complexes. This analysis is clearly very incomplete and the considerations given are tentative, but the data are worthy of presentation because of their apparent significance to the interpretation of some anaphase I data.

There is no clear evidence that different stages round diakinesis/ metaphase are related to cell position within a pollen sac. Individual pollen sacs were isolated according to the technique described already. Unsquashed preparations were scored under high magnifications (10 X 40) for (a) number of cells at diakinesis and metaphase I and of cells with centrophilic bivalents and/or complex (prometaphase); and then under oil immersion magnification for (b) the frequencies of the four types of centrophilic complexes.

Two developmental stages of pollen sacs were recognized: early (E) stages with a high proportion of cells still in diakinesis (pre-prometaphase); some in prometaphase and a few in metaphase (past-prometaphase); and late (L) stages with few or none in diakinesis, a number in prometaphase but most in metaphase. The evidence suggests that true mid developmental

stages have yet to be examined.

The data for the ten pollen sacs analysed are presented in tables 27 and 28. The following tentative conclusions are made from these data. (a) One of the striking points in the data as a whole concerns the overall frequency of cells with centrophilic complexes compared to those with centrophilic bivalents. Of the total of 403 cells scored at pro-metaphase I, 205 showed only centrophilic bivalents, 190 only centrophilic complexes while 8 showed centrophilic bivalents and complex (table 28). Taking into account the <u>number</u> of centrophilic bivalents in each cell (table 28), the total of 403 cells showed 248 centrophilic bivalents and 198 centrophilic complexes. This is a ratio of 7 : about $5\frac{1}{2}$; i.e. for every 7 centrophilic bivalents there occur 5 - 6 centrophilic complexes. Since there are 7 bivalents in interchange cells, all of which show approximately the same tendency towards centrophilic behaviour at pro-metaphase, the data suggest that the interchange complex has a centrophilic tendency about five to six times as great as that of the bivalents.

As pointed out in (b) below, the complex also appears to take a somewhat longer time to reach ultimate full co-orientation than expected on a bivalent basis. This slow co-orientation of the complex would prolong its centrophilic behaviour and so increase the frequency of centrophilic complexes relative to centrophilic bivalents in later stages. More informative figures on relative frequencies of centrophilic complexes and bivalents will thus be obtained from pollen sacs 1 and 2, since these

Table 27 Summary of data from ten prometaphase I pollen

sacs at two major developmental stages, early (E) and

late (L). Pollen sacs 1 and 2 were from the same

anther, as were 6 and 8. For complex types a - d see

Fig. 45. Pre, in and past mean pre-prometaphase

(i.e. diakinesis), in prometaphase (with centrophilic

bivalents and/or complex), and past-prometaphase

(i.e. metaphase) respectively. (See table 28 for further

data on these ten pollen sacs).

Table 27.

Pollen sac	D/ment stage	%pre	%in	%past	No. cells with centrophilic		Cells with o/philic complexes					
					bivs.	complex.	Total scored	Type a	b	c	d	
1	E	55	30	15	57	38	33	8	16	5	4	
2	E	50	29	21	52	45	39	10	21	5	3	
3	L	0	19	81	36	17	16	9	5	1	1	
4	L	2	12	86	16	24	11	9	2	0	0	
5	L	0	11	89	23	19	15	10	4	0	1	
6	L	0	9	91	9	14	12	10	2	0	0	
7	L	0	7	93	10	13	10	8	2	0	0	
8	L	0	6	94	7	13	8	8	0	0	0	
9	L	0	2	98	2	14	10	10	0	0	0	
10	L	0	0.5	99.5	1	1	1	1	0	0	0	

Table 27

Table 28   Distribution of centrophilic bivalents and complexes

in 403 prometaphase I cells scored from ten pollen sacs

(see also table 27).

Table 28.

Table 28

Pollen sac	Total prom. cells examined	cells with centrophilic bivs. only				cells with centrophilic complex			
		Total	1	2	3	Total	only	plus 1 biv.	plus 1 plus 2 bivs.
1	93	55	48	6	1	38	36	2	0
2	97	52	39	10	3	45	45	0	0
3	50	33	29	4	0	17	14	3	0
4	38	14	12	1	1	24	22	1	1
5	41	22	20	2	0	19	18	1	0
6	23	9	9	0	0	14	14	0	0
7	23	10	9	1	0	13	13	0	0
8	20	7	7	0	0	13	13	0	0
9	16	2	2	0	0	14	14	0	0
10	2	1	1	0	0	1	1	0	0
Totals	403	205	176	24	5	198	190	7	1

are young stages of prometaphase development, in which probably few if
any bivalents or complexes have as yet passed through events leading to
ultimate co-orientation.   The equivalent ratio obtained for these two
pollen sacs is 133 : 88, or 7 to a about 4·6;   i.e. a centrophilic
tendency about four to five times as great as that of the bivalents.
It is worth noting also that in centrophilic complexes c and d both
pairs of homologous centromeres are oriented as they are in centrophilic
bivalents.   Thus from this point of view c and d should each be classified
as equivalent to two centrophilic bivalents rather than one, and therefore
the above comparative estimate of controphilic tendency in the complex
will be low.

These figures are somewhat surprising on first thought, since from
general considerations on the chromosome composition of the complex one
might expect it to show a frequency equivalent to that of about two
bivalents.   On the other hand, one might expect an increase over basic
expectation because of the increased chance of disagreement between the
centromeres.

(b)   While the number of cells with centrophilic bivalents is somewhat
greater than those showing centrophilic complexes in early pollen sacs,
the reverse appears to be so in very late ones (table 27).   It does not
seem likely that the later cells entering prometaphase in a pollen sac
show a greater tendency towards centrophilic behaviour in their complexes
than do earlier cells.   Rather the data suggest that at least some
centrophilic complex types take somewhat longer to achieve eventual co-
orientation than the bivalents.   This phenomenon is probably related to

(a) above.

(c)   Within a pollen sac at either developmental stage the frequency
of centrophilic types <u>a</u> and <u>b</u> is always greater than that of types <u>c</u>
and <u>d</u>. Now in each of types <u>a</u> and <u>b</u> basically only one of the four
centromeres is considered to be abnormally oriented in relation to
complete co-orientation;   whereas in type <u>c</u> two are, and in type <u>d</u>
all are abnormally oriented.   Since the frequency of centrophilic
bivalents in a normal cell at prometaphase is always less than the
frequency of fully co-oriented ones it appears that it is more usual
for pairs of centromeres to become initially co-oriented at the beginn-
ing of prometaphase than initially centrophilic.   On this basis the
relative frequencies mentioned above of the centrophilic complex types
in a pollen sac can be explained; the complex types with the greater
number of non-cooriented centromeres (<u>c</u> and <u>d</u>) will be less frequent in
occurrence, relative to types with the lower number of non-cooriented
centromeres.

(d)   The frequencies of the types of centrophilic complexes change
during the development of prometaphase in a pollen sac.   This is
evidenced indirectly by comparing frequencies in early and late develop-
ment stages from different sacs.   Types <u>c</u> and <u>d</u> mostly occur in young
pollen sacs.   This almost certainly is due to the fact that these types
are converted to stable co-oriented or other centrophilic types during
progression through prometaphase.   The same will apply in part at least to
the gradual reduction in the frequencies of types <u>a</u> and <u>b</u>.   However, the

striking feature about a and b is their relative frequencies in the two development phases. In early pollen sacs type b predominates, while in late sacs type a does.

A number of possible reasons can be visualized to account for this latter observation. For example, the last cells to enter from diakinesis to prometaphase may show a predominance of type a over type b, when their complexes become centrophilic. I have no evidence for such a contention and it does not appear very likely. But secondly, type b might achieve ultimate co-orientation more rapidly than type a. A precedent for this possibility will be presented later. Thirdly, during development of prometaphase in a pollen sac, and in individual cells, types b and c, and d may be converted, at least in part, to type a before they achieve complete co-orientation. This will continue for some time to maintain a relatively high frequency of type a while the frequencies of the other types gradually drop off.

It will become evident later that the change in relative frequencies of types a and b during development of prometaphase is in agreement with interpretations made from developmental phenomena established for anaphase I; and might also be expected on theoretical ground.

6.   BASIC ORIENTATION TYPES AND FREQUENCIES OF THE COMPLEX AT METAPHASE I.

The two basic types of orientation expected in a chain forming complex of four chromosomes are diagrammed in Fig. 13 and examplified in Fig. 42 and 43, obtained from my own culture material (cf. Fig. 3:1 and 3:6 in Rickards, 1962).   The first is the alternate, zig-zag or disjunctional arrangement in which alternate, non-homologous centromeres are oriented to the same pole.   The second is the open, adjacent or non-disjunctional arrangement.   In ring forming complexes (chiasmata form in all four limbs at pachytene) two distinct types of adjacent orientations occur;   adjacent-1 (=adjacent non-homologous) in which adjacent, non-homologous centromeres are oriented to the same pole (homologous centromeres to opposite poles); and adjacent-2 (=adjacent homologous) in which adjacent, homologous centromeres are oriented to the same pole (non-homologous centromeres to opposite poles) - see McClintock, 1945;   Burnham, 1956, 1962;   Lewis and John, 1963.   These three concordant orientations account for nearly all those seen in ring forming complexes.   In chain forming complexes, on the other hand, only one type of adjacent arrangement arises in any appreciable frequency (Burnham, 1956), this type depending on which pachytene axis the centromeres are located on relative to the axis that does not form a chiasma. In the two types of interchange originally considered in relation to the present material (Fig. 13) the centromeres are situated on different axes, so that in the first interchange type the adjacent arrangement that occurs is adjacent-2 (no adjacent-1), while in the second type it is adjacent-1 (no adjacent-2).   In my original study on this interchange material the

adjacent arrangement was designated adjacent-2, the interchange being considered to have been of type (1) (Fig. 13). Since then, however, it has been realized that this conclusion was not necessarily correct, with the information then available, and in fact the conclusion was incorrect since the interchange has now been shown to be of type (2), and hence the adjacent arrangement is adjacent-1, not adjacent-2. This fact also negates some of the considerations given earlier on orientation in the complex (e.g. p.36 in Rickards, 1962).

In Fig. 42 and 43 the three chiasmata of each complex are situated well towards the chromosome ends. This is the most common situation. The main variation that occurs is where either of segments 2a or 2b has a medial chiasma or a proximal as well as a distal one. Configurations of the type shown in Fig. 44 are so produced.

Frequencies of alternate and adjacent orientations

Considerable caution, from two points of view, has been found essential in scoring cells for frequencies of orientation types at metaphase/anaphase I. Firstly, cells should be scored only when all chromosomes can be clearly delineated. In cells with overlapping, unclear bivalents and complex the adjacent orientations are more clearly recognized than the alternate ones, because the 1a segments usually lie well above the equator, across the bivalents, and are thus more readily observed (see e.g. Fig. 60). Thus if one scored cells with unclear, overlapping bivalents and complexes, even though the complexes were clear, then there

would be a distinct tendency for cells rejected as indecisive to contain an unnatural bias towards alternate types, thus making the frequency of adjacent types unnaturally high in the scored cells. (Burnham, 1962 has briefly pointed out this difficulty in scoring for orientation frequencies).

In the analysis presented below only cells in which all seven bivalents and the complex could be delineated clearly (e.g. Figs. 42 - 44) were scored. When some bivalents could not be recognized clearly because of overlapping then the cell was rejected as indecisive, even though the complex may have been clearly seen and classified.

The necessity of analysing preparations in which a high proportion of cells can be scored will become evident from data presented in the following section. It has been found that if for reasons of clarity cells in which the complex is situated at or near one side of the metaphase equator are preferred for scoring, then an unreal frequency of orientation types will be obtained in this material. To overcome this it is imperative that only preparations in which nearly all cells can be scored are used in this type of analysis.

Secondly, observations on developmental sequences of anaphase I have shown that it is essential to score only pollen sacs in which all cells are visibly synchronized at metaphase/ early anaphase I. At the correct stage of development of a pollen sac in <u>Allium</u> <u>triquetrum</u> all cells are seen in such a synchronized state, with no cells still in prometaphase or into mid-anaphase I (chiasmata lost). These are the correct pollen sacs to score. If pollen sacs possess some cells either still in

diakinesis/prometaphase, or in mid-anaphase I, or late stages, as well as some in metaphase/early anaphase I, then an incorrect overall picture of the frequencies of alternate and adjacent orientations will be obtained, if such preparations are analysed in this material. The reason for this will become evident following the account of anaphase developmental sequences.

In my previous analysis of this interchange material a total of 161 cells were scored at early anaphase I, and these showed 122 alternate and 39 adjacent orientations, giving a ratio of a little more than 3 : 1 (Rickards, 1962 p.27). The precautions mentioned above had not been followed in this initial analysis, so further probably more accurate and more extensive data were obtained from my own culture material.

Two fully synchronized pollen sacs at metaphase/early anaphase I from different plants were scored. A total of 516 cells were observed, 35 of which were rejected as indecisive on the grounds of inability to recognize all chromosomes in the cell because of overlapping, or because orientation in the complex was of the infrequent discordant types. Both pollen sacs showed virtually the same relative frequency of alternate and adjacent arrangements, giving an overall frequency of 358 : 123 or 2.91 : 1 (table below). The data show a slightly lower ratio than previously observed.

Pollen Sac	Observed	Indecisive	Alternate	Adjacent-1
No. 1	219	15	150	54
No. 2	297	20	208	69
Totals	516	35	358	123
			2.91 :	1

A well as the above two orientation types, certain others occur in low frequencies. These include orientations of unequal bivalents and 3 : 1 and other discordant types. These and their anaphase I and anaphase II complements are described in a later section.

7.   POSITION OF THE COMPLEX IN THE EQUATORIAL PLATE.

I.   Observations

Mostfrequently the two co-oriented halves of the complex, whether in alternate or adjacent orientation, are positioned close together in the equatorial plate, so that in flattened cells the complex lies free from or across only a few bivalents (Figs. 42 - 44 and data given later). This is to be expected since the two pairs of centromeres are not entirely independent of each other, but reasonably closely tied together. Occasionally, particularly when in adjacent orientation (more clearly recognized), the complex halves may be well separated, the complex as a whole bridging most of the bivalents (e.g. Fig. 60 from an unsquashed preparation). These cases may result from random positioning of the two pairs of co-oriented centromeres along the equator, or they may arise as an after effect from some prophase cells which themselves showed extreme separation of the complex halves (Fig. 24 e.g.). One unusual case has been noted (Fig. 61) in which the bridging la segments of the complex had been drawn out at the chiasma into a fine thread, presumably because of the wide separation of the two pairs of co-oriented centromeres.

General observations at metaphase/early anaphase I suggested that the interchange complex was most frequently positioned at or near one end of the flattened metaphase plate (Figs. 42, 44 and others). Caution clearly was necessary in assessing these impressions, however, since complexes in such end positions are the ones most likely to be chosen for examination, because they are likely to be clearer. Critical analysis was therefore

carried out on a large number of suitable cells. A total of 300 cells were observed under . x100 magnification from two well suited preparations from one flower. In these preparations nearly all cells showed a lateral aspect of the metaphase plate, with all the chromosomes in a reasonably straight line and with but little complete overlapping of chromosomes. Of the 300 cells scored 20 only were not classified for position of complex, not because the complex occupied a central position and was thus perhaps more difficult to observe, but in all cases because the chromosomes had been squashed out of line and were thus difficult to classify accurately, or because the complex was of an unusual orientation type (other than alternate or adjacent chain of four).

Cells were classified according to the following scheme. Nine positions were recognized along the equatorial plate, seven occupied by the seven bivalents and two by the two halves of the complex. In assigning two positions to the complex no confusion arose in classifying cells containing either of the two orientation types, even in cells where the complex overlapped some of the bivalents. In classifying cells the two ends of the equatorial plate were not distinguished. The nine positions were numbered consecutively 1 - 9 starting from the end to which the complex was nearest . In scoring cells each metaphase plate was drawn roughly for positions of bivalents and complex and then appropriate position numbers allotted to the complex. The complex position was determined by the position of its two co-oriented halves, whether or not the middle 1a segments linking the complex halves straddled one or a number of bivalents (diagonally in alternate or horizontally in adjacent orientations). Fig. 64 illustrates

the scheme of classification.

(a)   In the first set of data, obtained from preparation 1, only those cells were recorded in which the complex did not straddle any of the bivalents - i.e. occupied positions 1/2, 2/3, 3/4 and 4/5; and no record was kept of the type of orientation in the complex in each case.   A total of 80 cells were classified.   In 35 (44%) the complex lay in positions 1/2; 21 (26%) in positions 2/3; 13 (16%) in 3/4 and 11 (14%) in 4/5. The data suggest a preference for the complex to lie towards the ends rather than the centre of the flattened equatorial plate.

A more detailed analysis was conducted on preparation 2, where all possible cells were classified, whether or not the complex straddled a number of bivalents.   Also, a record was kept of the type of orientation shown for each classification.   The data obtained are shown in table 29.

Column 2 shows the data that are directly comparable with those from preparation 1, showing, as previously, a strong preference for the complex to lie in end or near end positions in the equator.   Nearly half of the 94 cells where the complex did not straddle any bivalents showed the complex in positions 1/2, and another 25% or so in positions 2/3; while the inner positions 3/4 and 4/5 are represented together by only about 25% of cases.   With due recognition of likely variation caused by smaller sample sizes, approximately the same trends can be seen in comparable positions in columns 3, 4 & 5, where the complex straddles 1, 2 & three bivalents respectively.   The totals in column 6 for the four comparable groups (bracketed) also reveal the same phenomenon, as expected.

Table 29   200 metaphase I cells from one pollen sac

classified according to position of the complex and its

orientation types and frequencies. See Fig. 64 and text

for interpretation of complex positions in column 1. The

data are separated into columns 2 - 5 to facilitate

desired comparisons, and summarised into four groups in

column 6.

* Figures in brackets indicate the numbers of cells

scored as alternate or adjacent orientation at the various

complex positions; thus e.g. (29 + 17) indicates 29

alternate and 17 adjacent orientations from 46 cells in

which the complex was in position 1/2.

**Theoretically half of these values should be assigned

to each of their equivalent but indistinguishable

position 4/7 (or 4/8), though since the values are so

small this would have negligible effect on the conclusions

drawn from the data.

Table 29.

1	2	3	4	5	6	
1/2	46 (49%)(29 +17)*				Gp.1	93 (47%)
1/3		37 (25+12)				(59+34)
1/4			7 (4 +3)			
1/5				3 (1 + 2)		
2/3	25 (27%)(22 + 3)				Gp.2	55 (28%)
2/4		23 (19 + 4)				(45+10)
2/5			6 (4 + 2)			
2/6				1 (0 + 1)		
3/4	9 (10%)(8 + 1)				Gp.3	31 (16%)
3/5		18 (14 + 4)				(25 + 6)
3/6			4 (3 + 1)**			
3/7				0		
4/5	14 (14%)(13 + 1)				Gp.4	21 (9%)
4/6		7 (7 + 0)				(2 0 + 1)
(=3/6)4/7			—			
(=2/6) 4/8				—		
	94 (100%)(72+22)	85 (65+20)	17 (11+6)	4 (1 + 3)		200 (100%) (148+52)

Table 29

(b)   The totals at the base of each of columns 2 - 5, as well as the
individual values within each bracketed group, are an indication of the
conclusion made previously, that most frequently the complex halves are
positioned relatively close together so that none or only a few bivalents
are straddled.

(c)   The table also shows the frequencies of the two basic alternate
and adjacent orientations of the complex observed in each of the equatorial
positions.   The total at the base of column 6 shows an overall frequency
of 148 alternate to 52 adjacent, very close to the 2.91 : 1 ratio reported
earlier.*   The ratios obtained from the totals of columns 2 - 5 are
3.27, 3.25, 1.83 and 0.33.   The small sample sizes at least of columns 4
and 5 are undoubtedly responsible in part for the sharp drop shown in the
ratios.   Despite this, however, the drop is probably partly real and
predictable, since the central segment of the complex could bridge further
across the metaphase plate when horizontal than when oblique. **

On the other hand, when equivalent positions within a column are
compared there are suggestions that complexes in end positions have a lower
than expected ratio of alternate to adjacent orientations; while complexes
in middle positions have a higher than expected ratio. In column 6 the
total frequencies for the four equivalent groups can be compared.

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*   The data of table 29 are from the same pollen sac as No. 1 that was
      analysed in connection with alternate:, adjacent orientation frequencies.
      The reason for the slight differences in totals scored and rejected, and
      in orientation ratios, stems from the fact that the two procedures of
      analysis, conducted together, did not necessarily lead to common rejection
      of a cell as indecisive.

**   Professor Gordon (see acknowledgments) kindly pointed out the significance
      of these data to me.

Wheras in groups 2 and 3 the deviation from expected is reasonably small
and statistically not significant, in group 1 there is an excess over
expected of adjacent types which is statistically significant ($x^2 = 4.88$,
p.< 0.05) while conversely in group 4 there is a statistically significant
excess of alternate types ($x^2 = 3.87$, p = 0.05). Thus the data suggest
that there are differences in alternate : adjacent frequencies in the two
extreme positions occupied by the complex.

Whatever the cause of this phenomenon it indicates that caution is
necessary when scoring cells for frequencies of alternate and adjacent
orientations. If, for reasons of clarity, cells with complexes positioned
at one end of the equator are preferred for scoring, then an unreal overall
frequency will be obtained. To overcome this it is necessary, as indicated
previously, to score pollen sacs in which a very high proportion of cells
can be classified in respect of orientation in the complex.

II. Three dimensional aspects of the metaphase plate

Difficulty is encountered in examining polar aspects of metaphase
in Allium triquetrum since the pollen mother cells have a very strong
preference for lying so that lateral aspects of the plate are almost
invariably seen. Polar aspects are sometimes seen before a cover-slip is
applied to the preparation. It is obviously not possible to examine these
in any detail and application of a cover-slip has the annoying effect of
rolling these occasional cells over to present a lateral aspect of the
metaphase plate. A cavity slide was used to overcome this difficulty, to
which a cover-slip could be applied without affecting all of the few cells

that showed polar aspects of metaphase. These were examined under 10 x 40 magnification. Fig. 62 shows a cell at metaphase I in polar view from normal material. The nine bivalents are readily observed since almost invariably they lie a little apart from each other. The equatorial plate in this view is roughly circular with usually two, rarely 1 or 3 bivalents occupying the centre of the spindle, the remaining 7 bivalents arranged peripherally. It is impossible to distinguish groups of chromosomes with any satisfactory degree of accuracy.

In interchange cells the polar aspect at metaphase can seldom be distinguished from that of normal cells. Identification of the complex cannot be achieved with certainty because of difficulties with depth of focus, except on occasions when the complex is oriented in an adjacent way and the middle 1a segments positioned towards the "upper" pole. Observations on the position of the complex in this view are thus impossible. In these interchange cells 9 "bodies" usually can be distinguished in the equator, corresponding to the seven bivalents and the two co-oriented halves of the complex (Fig. 63). Two of these occupy central positions as in normal cells.

In carefully flattened preparations (no lateral movement) virtually all cells show a two dimensional, lateral view of the metaphase plate, with the chromosomes in a rough line and usually showing only partial overlapping of bivalents. Occasional cells show the bivalents considerably more out of line than usual, these probably representing the few cells where the plane of squashing had not been so strictly at right angles to

that of the equator.   There is considerable broadening of the metaphase

plate on squashing, though observations on unsquashed and subsequently

squashed cells showed that there is little or no lateral displacement or

alteration of relative positions of bivalents and complex during

preparation.

In a flattened metaphase plate the two bivalents that occupied

the central positions in the three dimensional spindle (Fig. 63) no doubt

come to occupy near-to-central positions in the two dimensional lateral

aspect, since there is very little relative displacement of chromosomes

in squashing and hence very little chance of their coming to occupy outer

positions.   Likewise bivalents positioned relative to the plane of

flattening as 3 and 7 with a/a' in Fig. 65 would, in most cases, come to

occupy more central positions in a lateral aspect; while those as 2 and

5 would tend to occupy the outer positions.   These probabilities can be

demonstrated by constructing polar models as in Fig. 65 and converting

these to flattened lateral models using a number of different planes of

compression, as illustrated.

III   Discussion

Two features have been revealed from the analysis presented in

this section.   (A) The complex is most frequently positioned at or near

one end of the flattened lateral aspect of the metaphase equator, and less

frequently occupies the more central positions.   (B) Adjacent orientation

is over-represented in end positions, and alternate orientation in central

positions.

The first phenomenon is interpreted as indicating that the complex

becomes located in the three dimensional spindle equator in regions that most often become end positions in the two dimensional equator. It is apparently not an artefact of preparation as observations already mentioned show that there is little significant alteration of relative positions of bivalents and complex during squashing. And if there were, the larger complex would probably be expected to be displaced less than the smaller bivalents, thus leading to a tendency for it to occupy more central rather than peripheral positions. It seems equally unlikely that the preferential positioning of the complex might have been induced through fixation or other preparatory procedures. It also does not appear likely that the results might be an artefact of the scoring or grouping procedure. Further, the preferential location of the complex after squashing seems incompatible with random, location during prometaphase. This can be verified by constructing a number of polar models and converting these to lateral ones (Fig. 65), varying either the position of the numbered bivalents or the plane of flattening to produce the effect expected on the basis of random positioning of bivalents etc.

From the point of view of the two dimensional lateral aspect of the metaphase plate alone, one would expect the complex to occupy each of the positions 1/2, 2/3, 3/4 and 4/5 in approximately equal proportions (25%). This clearly is not so. It will be more correct, however, to consider positioning in the three dimensional plate in assessing expected frequencies.

With random positioning and nine available positions, a particular

bivalent in normal cells will have 2/9 or about a 22% chance of achieving a location that would give an end position in the flattened equator. This chance is raised to about 30% (2/7) if the particular bivalent under consideration is excluded from the two central positions as seen in polar view. Now, the simplest case that can be considered in respect of interchange cells is for the complex to be strictly equivalent to <u>one</u> bivalent. Thus if the two halves of the complex behaved strictly as a unit in positioning its chance of achieving end positions in a lateral aspect, with random location, is 2/8 or 25%; or 33% if the complex is excluded from the central locations of a polar aspect.

The behaviour of the complex as one unit, equivalent to one bivalent, is the most favourable condition for producing the highest possible frequency of end positions on the basis of random location. Even this frequency is only about half that actually attained, and is still low if the complex were to be excluded for some reason or other from the central positions of the three dimensional spindle. And any tendency for the complex halves to behave independently of each other, as they would be expected to do, to some extent at least, will lower the chance for the complex to come to occupy end positions in lateral metaphase plates.

It is thus very clear that the complex is preferentially located in the three dimensional spindle in positions that most frequently become the ends or near ends of the flattened, two dimensional, lateral plate.

It is well known in many metaphase plates, especially in meiosis,

that the arrangement of the chromosomes, relative to each other, is not
necessarily random, but rather certain chromosomes may occupy certain
relative positions in the metaphase equator.   In the plant genus Yucca
e.g. (O'Mara, 1932), where the metaphase complement comprises both large
and small chromosomes, the former occupy the periphery of the three
dimensional spindle, while the latter lie in the centre.   However, this
is by no means a universal character of large and small chromosomes; and
in some other cases the relative distribution appears to involve only
sex chromosomes versus autosomes (Schrader, 1953; Swanson, 1960).
Various hypotheses have been suggested to account for some of the available
facts but the problem still remains largely unexplored and unexplained
(Schrader, Swanson, loc. cit.).

More recent evidence on the specific, relative location of certain
chromosomes at metaphase has come from the studies of flattened metaphase
figures obtained from colchicine/hypotonic treated human leucocytes
(Miller et al., 1963 a, b; Barton et al. 1965.)   These studies have
shown that there is a statistically significant tendency for certain
morphologically different chromosomes to occupy peripheral locations in
the flattened figures, and others to show tendencies towards central
location.   The fact that morphologically similar chromosomes show different
distributions indicates that specific, relative positioning does not rest
on chromosome size differences (cf. Yucca); and cannot be caused through
colchicine treatment, squashing or other preparatory procedures.   Rather,
the distribution after squashing probably reflects non-random distribution
in the unsquashed metaphase plate; and that relative positions may remain

constant throughout successive mitoses (Miller et al., 1963b).

Two distinct phenomena must be considered in attempting to explain the relative positioning of the complex in my material. (1) the interchange complex is preferentially located at certain positions along the periphery of the three dimensional spindle; and (2) the plane of flattening is such as to cause the complex so located to lie in or near end positions of the flattened lateral equator.

(1) The complex may well be excluded in many cases from occupying the centre of the spindle, thus increasing its chance of eventually occupying end positions after squashing. But also, it must take up certain positions in the periphery of the spindle. There may be two such basic positions, opposite each other in the spindle; or alternatively only one of these may be preferentially occupied. It is not possible from the available data to decide this latter point since the two ends of the lateral metaphase equator cannot be distinguished.

Three interpretations of the apparent preference can be distinguished. Firstly, the complex may take up its selected position regardless of whether other positions are available (as yet unoccupied). This would be active preferential behaviour. Secondly, the complex might achieve positioning in the equator at a time, relative to the other bivalents, when only the "selected" positions are available. Or in other words, if the various positions in the spindle are filled in a reasonably sequential pattern and the complex comes to take up a position at a reason- ably specific time, relative to the bivalents, it might be forced to take

up only certain positions on the spindle.   This would be <u>passive</u>

preferential behaviour.   Thirdly, a preferential position on the equator

might conceivably be determined by preferential positioning at some stage

preceding prometaphase.   (These possibilities should be considered in

interpreting other available data on relative distribution of chromosomes

at metaphase.)   There is little or no evidence pointing to which of

these situations might pertain, and little indication of how the phenomenon

might be governed.   All present unsolved problems at present.

(2)   In order to <u>reveal</u> such preferential positioning of the complex

it is necessary that the plane of flattening, and thus lying, of the cells

must also be specific, relative to the spindle, so as to show the complex

most often at the ends of the two dimensional equator.   Random planes

of lying, and thus of flattening, would remove the indications of pre-

ferential placement.

It has already been pointed out that pollen mother cells in

<u>Allium triquetrum</u> preferentially lie in relation to the spindle so as to

present, lateral views of metaphase.   It is not surprising then to find

evidence that the relationship extends further.   Thus (a) either the

spindle has properties such that it always lies on its long axis and the

cell is flexible enough to accept any position, or the axis of the spindle

is always laid down relative to a constant morphological shape to the

pollen mother cell surface (which determines its constant plane of lying).

But also, (b) the position envisaged by rotating the spindle and its

oriented chromosomes on its axis must also be relatively fixed.

A further possibility can be conceived, by combining (1) and (2),

viz. that the position of the complex somehow determines, or influences,

the plane in which a cell tends to lie, so that whenever the complex is in a peripheral position there is a high probability that it occupies an end position after squashing. Why this should be so of course is impossible to say, but it is perhaps conceivable.

It will be desirable in future to establish whether or not the bivalents in normal and interchange cells show specific relative positioning in the metaphase equator. The analysis could be partially informative only since bivalents can be identified as groups I, II and III, not as individuals.

The differences in orientation frequencies associated with different locations of the complex are also very difficult to understand at this point. A possible explanation will be presented later in conjunction with evidence from anaphase developmental stages in interchange cells.

8.   UNEQUAL BIVALENTS AT METAPHASE/EARLY ANAPHASE I.

Approximately 5% of cells at diplotene - metaphase I showed unequal bivalents in place of the interchange chain of four chromosomes. These can be identified also at metaphase/early anaphase I, though some caution is necessary in their identification here. The distal/terminal chiasma in the la segments of the complex is probably lost very early in anaphase I, particularly from an alternate arrangement. Such separated complexes can be confused with true, unequal bivalents. One can often distinguish these true and false unequal bivalents by the relative positions of the two halves of the complex, and by the fact that the ends of the la arms often give an impression of whether or not a chiasma has been present, but lost. These difficulties, however, precluded any accurate assessment of frequencies and orientation types of unequal bivalents at this stage. But there is little doubt that their frequency will be much the same as at earlier stages; and that they are oriented, relative to each other, in two ways, corresponding to the alternate and adjacent-1 arrangements of the complete complex. These two orientations will presumably occur in about equal proportions; this expected equality will not be disturbed, as in the complex, by inter-relations of more than two centromeres.

Fig. 54 illustrates a probable case of unequal bivalents in adjacent orientation at early anaphase I. Fig. 55 is a certain case observed in prometaphase in an unsquashed cell. Fig. 53 is a false pair of unequal bivalents, recognized by the relative positions of the non-associated arm pair and the drawn out nature of the ends of these arms

9.   DISCORDANT ORIENTATIONS IN THE COMPLEX AND THEIR SEGREGATION COMPLEMENTS.

As well as the two concordant orientations described previously two distinct types of discordant orientations of the complex have been identified at metaphase/early anaphase I.   These are described below along with their anaphase I and II segregation complements.

(la)   3: 1 orientations at early anaphase I.   Two general types of 3 : 1 orientations might be expected in chain-of-four complexes, those paralleling types a and b centrophilic complexes at prometaphase (Fig. 45).   In these configurations three centromeres are oriented to one pole and only one to the opposite pole.   Only one of these types occurs at metaphase/early anaphase I.   It is similar to type a at prometaphase, in which one group I centromere (in reference to the present form of the chromosomes) and two groups III centromeres are oriented to the one pole, and the other group I centromere to the opposite pole.   What characterises this orientation at early anaphase I, however, is the fact that it occurs (persists from prometaphase) only in complexes in which one of the segments 2a or 2b has a chiasma very close to the relevant pair of centromeres (Fig. 45a' and 57). Thus the complex never appears distinctly centrophilic, i.e. with the non-cooriented centromere approaching its nearest pole (compare with Fig. 46). Rather the non-cooriented centromere, together with its adjacent, homologous one, to which it is very closely tied, is no nearer to the pole than centromeres of some cooriented bivalents.   Its distance from the pole is determined by the proximity of the nearest chiasma.   Also, in contrast to the truly centrophilic orientation type a at prometaphase, which is unstable and never seen in cells in anaphase I (they must be converted to co-oriented

arrangements) this 3 : 1 arrangement is a relatively stable one, since it occurs in cells in which anaphase has been initiated, and it gives rise to its expected non-disjunction segregations at mid-anaphase I.

In the 516 cells examined in connection with the frequency of alternate and adjacent orientations three 3 : 1 orientations of the type just described were observed.

The other possible 3 : 1 orientation, similar to type b centrophilic complex of prometaphase, does not occur at metaphase/anaphase I.   This is verified both from studies of pollen sacs at early anaphase I and from analyses of segregation complements at mid-anaphase I.

It is possible that 2 : 2 orientations, paralleling type c centro- philic complex, may rarely occur at anaphase I, though I have not identified these at present.   They would in fact represent adjacent-2 orientations if they occur.   Their segregation products would be indistinguishable at mid-anaphase I from those of alternate orientations.   Their frequency could only be very low.

Professor Hagberg (pers. comm.) has identified 3 : 1 orientations in rye interchanges.   Also, the cases of tertiary trisomic individuals found in the progeny of many interchange heterozygotes (Burnham, 1956 for ref- erences) suggest that the phenomenon probably occurs quite regularly, though at low frequencies, in interchange complexes in general.

(Ib) 10 : 8 segregations at mid-anaphase I.   Numerous cases have been observed in which one polar group in a cell contains 10 and the other only 8 chromosomes, instead of 9 each as in most cells.   These cells are of only one type.   The complement with 10 chromosomes has 4, 2 and 4

chromosomes of groups I, II and III respectively; and the eight chromosomes complement, 4, 2 and 2 (Fig. 68). Normal complements are either 4, 2 and 3 in each group (alternate) or 5, 2, 2 and 3, 2, 4 (adjacent). Hence the abnormality arises in the group III chromosomes in an otherwise normal segregation pattern from an alternate orientation. No case of numerical non-disjunction was observed in 300 pollen mother cells from normal plants examined for this purpose, so it is reasonable to conclude that the above cases arise through non-disjunction in the interchange complex. They must arise from the occasional 3 : 1 orientations described above as occurring in the complex at metaphase I (Fig. 57).

It is possible that there are two sub-types of 10 : 8 segregations, depending on which of chromosomes 6 and 4^6 of the complex was abnormally segregated, just as there are possibly two types of 3 : 1 orientations. The two, however, as at metaphase, are not distinguishable.

10 : 8 segregations have been identified at all stages of anaphase I development in a pollen sac. Their overall frequency is about 1%, and as this figure is based on many hundreds of cells examined at anaphase I this should be a reasonably accurate estimate of the frequency of 3 : 1 orientations of the complex at metaphase.

No case of 10 : 8 numerical non-disjunction has been observed in which group I rather than group III chromosomes of the complex are abnormally distributed. Nor have 11 : 7 segregations been identified. These facts parallel the absence of other than the one type of abnormal 3 : 1 orientation at metaphase I. Occasional cases of 9 : 9 segregations may occur from

adjacent-2 type orientations but these would be indistinguishable from normal 9 : 9 segregations.

(Ic) Anaphase II complements. Mid-anaphase II complements arising from 10 : 8 orientations and segregations in the complex have also been identified. Normal cells have nine chromosomes in each group (Fig. 75).   The abnormal cells show maldistribution of one group III chromosome.   Thus in these abnormal cells two sister chromosome sets each have 8 chromosomes (4,2, 2 composition), the other two have 10 chromosomes each (4, 2, 4 composition) - Fig. 77 and 78.   These abnormal cells have been observed in an overall frequency of about 0.85%, which is in satisfactory agreement with the 1% value obtained for corresponding complements at anaphase I.

(IIa) 2 : 1 and 1 : 1 orientations - passive centromeres.   Occasionally in prometaphase cells, some of the centromeres of the complex appear to be passive in respect of activity on the spindle and orientation (Fig. 51). The majority of these do not persist, but rather these passive centromeres must eventually become active and co-oriented.   However, occasionally they persist to early and late anaphase I.   Two types have been identified in early anaphase I cells (a) involving one group III centromere (Fig. 58), and (b) involving both group III centromeres (Fig. 59).   No case involving group I centromeres has been seen in early anaphase I cells (though seen at prometaphase), but mid-anaphase I studies have shown that such cases do in fact occur (data below).

Inactive centromeres are identified by the absence of attenuation at the position of the centromere and absence of associated spindle fibres, and by the fact that the chromosome arms and chiasmata involved lie indifferently in relation to the equator and other oriented chromosomes in the

complex.   The interpretation is verified by the lagging behaviour of
these chromosomes in mid-anaphase I.

Hagberg (1954) described and illustrated configurations in barley
interchanges in which two alternate centromeres in a ring of four were
co-oriented in reference to poles and equator, but the two other centro-
meres remained unoriented.   John and Lewis (1965 p.78/79) described
similar instances in rings of four chromosomes in inbred rye, and Bauer
et. al. (1961) described the phenomenon in interchange trivalents of
Tipula,   My own observations are somewhat similar, though in my material
it is most often the adjacent (group I) centromeres that are co-oriented
and the two other non-linked centromeres that are passive.

The frequency of 2 : 1 and 1 : 1 orientations is somewhat indefinite
in my material.   In the 516 cells analysed previously not one case was
observed, and other pollen sacs similarly appear to lack them.   Other
pollen sacs, however, showed a frequency of about 2%.   Even pollen sacs
from different inflorescences but of the same plant show these extreme
frequencies.   Similar variation, extending to different anthers of the
same flower, was suggested from mid-anaphase I studies.   The phenomenon
deserves a thorough analysis.

In barley, 1 : 1 orientations apparently occur in a regular frequency.

(IIb) 9 : 8 plus laggard segregations at anaphase I.   The above orien-
tations of the complex produce characteristic segregation products at
mid-anaphase I.   The appropriate cells show a normal 4, 2, 3 complement

in one polar group, but usually a 4, 2, 2 complement in the other, with one stray group III chromosome lying on or near the metaphase equator (Fig. 69a). Similar cases have not been identified in normal plants so it is again reasonable to conclude that they arise from abnormal orientations in the complex. They must arise from the 2 : 1 plus passive centromere orientations of the complex at metaphase I described above (Fig. 58). In these orientations the passive centromere must remain passive during anaphase movement of the other chromosomes. After loss of the chiasma binding the passive chromosome to others of the complex this chromosome is left behind, most frequently near the previous meta-phase equator. (General congression of chromosomes at the start of prometaphase and co-orientation of other complex centromeres can be expected to bring and thus leave passive centromered chromosomes on or near the equator.)

Just after loss of chiasmata in anaphase it is difficult to identify these abnormal segregations positively. At later stages of anaphase, however, they are very clear and quite distinct from 10 : 8 segregations. In the latter the abnormal chromosome is closely aggregated with the other chromosomes and clearly oriented to one pole. The lagging, unoriented behaviour of the abnormal chromosome in 9 : 8 segregations makes these cases particularly clear. However, the fact that laggards sometimes show delayed activity suggests that there may be occasional misclassification of the two numerical non-disjunction types.

In many cases the laggard chromosomes at late anaphase I show a secondary type of orientation - with their chromatid centromeres oriented

to opposite poles and the chromosome lying more precisely on the previous metaphase equator (Fig. 69b). No instance of ultimate separation of chromatids during late anaphase has been identified. In other cases at late anaphase I the laggards appear to have become positioned towards one of the two poles and give the appearance at the centromere regions of weak, belated attempts at polar movement (Fig. 69c). This phenomenon is similar to the late anaphase behaviour of univalent chromosomes at meiosis I that have become oriented along the equator but in which chromatids fail to separate (Östergren, 1951).

If this belated centromere activity were to begin relatively earlier, then these types might be confused with 10 : 8 segregations in some cases. This may be so, though the summary given later shows that this is very unlikely to be the case for all apparent 10 : 8 segregations, and that there are indeed two distinct types of numerical non-disjunction.

During telophase I the laggard chromosomes become transformed into an interphase state, paralleling the normal behaviour of intact nuclei (Fig. 70d).

The occurrence of one laggard group III chromosome at mid anaphase I is parallel to the observations of a group III passive centromere in the complex at metaphase I (cf. Figs. 58 and 69). Occasional cells with other laggard chromosomes, and in some cases multiple laggards, have been observed. Fig. 70b shows both group III chromosomes as laggards, paralleling Fig. 59 of early anaphase I. Fig. 70a shows two lagging group I chromosomes, and Fig. 70c one group I and one group III laggard.

Thus all the centromeres of the complex are apparently prone to inactivity on the spindle during normal orientation phases.

The conspicuous nature of the laggards at late anaphase I permits a reasonably accurate assessment to be made of the frequency of such behaviour. The phenomenon, as discussed previously from the point of view of metaphase I, appears to be very erratic in occurrence. Frequently some pollen sacs are quite devoid of laggards, while others show them in variable frequencies of usually 4 or 5 but up to 10 in a total of about 300 to 400 cells.

The following points characterize and distinguish 10 : 8 and 9 : 8 segregations at mid-anaphase I.

(a) 10 : 8. (1) Of regular occurrence.

(2) Definite orientation of the abnormally segregating chromosome, with no lagging.

(3) No cases other than those involving one group III chromosome.

(4) Actual 3 : 1 orientations observed at metaphase/early anaphase I.

(b) 9 : 8 (1) Erratic occurrence

(2) Absence of, or weak, belated orientation of the chromosome involved, with distinct lagging at anaphase I.

(3) All chromosomes in complex capable of showing the phenomenon.

(4) 2 : 1 orientations with passive centromeres observed at metaphase/early anaphase I.

The discordant orientations of Hagberg (1954) already referred to initially behave during anaphase as in my material, i.e. the passive chromosomes show distinct lagging in the metaphase equator. They also finally become oriented via their two chromatid centromeres in late anaphase. However, in barley these laggards eventually showed segregation in late anaphase of their two chromatids, a phenomenon that does not occur in my material.

(IIc) Anaphase II complements. A number of cells have been observed at interphase, prophase II and later stages containing one micronucleus in which the chromatin was in a non-dividing state. One cell with such a micronucleus has been observed with both chromosome complements in anaphase II, from which it was established that the persistently non-condensed micronucleus must have contained one complete, double (two chromatid) group III chromosome, there being one group III chromosome short in each of two sister complements that were in anaphase II (Fig.74). It is probable that this and other micronucleate cells arise from 9 : 8 plus laggard orientations and segregations, the micronucleus containing the double laggard chromosome 6 or 4^6 (the chromosomes most often involved in lagging) remaining in its previous telophase/interphase condition.

10. THE RELATIONSHIP BETWEEN THE RELATIVE FREQUENCY OF ALTERNATE AND
    ADJACENT TYPE CELLS AT MID ANAPHASE AND DEVELOPMENTAL STAGES OF
    ANAPHASE IN A POLLEN SAC.

Ia. Developmental studies in anaphase I pollen sacs.

The relative frequency of anaphase complements derived from alternate

and adjacent-1 orientations was expected to be approximately the same as

the relative orientation frequency at metaphase/early anaphase I; i.e.

approximately 3 alternate to 1 adjacent. This expectation was roughly

realized with the overall data obtained from many different anthers,

though it was noted occasionally that data obtained from a large sample

of cells in an anther showed rather deviant frequencies that approached

significance at the 5% level of probability. It was then thought possible

that the frequency might change through developmental stages of anaphase

in an anther; i.e. in an old anther, where most cells had passed anaphase,

the remaining cells might show an alternate : adjacent segregation

frequency different from that in cells first entering anaphase in a re-

latively younger anther. This possibility contrasts with the original

expectation (virtually an assumption) that the frequency would remain

constant at approximately 3 : 1 through development of anaphase in the

anther. Further consideration of the two metaphase orientations them-

selves gave rise to the following conditional hypothesis: (a) if alternate

arrangements with three pairs of adjacent, co-oriented centromeres took

longer to achieve full orientation than adjacent arrangements with only

two pairs of co-oriented centromeres, and (b) if in the former the cells

as a whole were consequently delayed in entry into anaphase I compared with the latter cells, then (c) the ratio of the frequency of cells from alternate to adjacent orientations should rise throughout the developmental stages of anaphase I in an anther. Discussion presented later shows that both (a) and (b) were not entirely improbable conditions on which to base the hypothesis (c), and hence it was decided to examine this possibility and, if it proved incorrect, obtain information on what changes, if any, occur in the developmental stages of anaphase I in an anther.

Preliminary observations showed that two main precautions were necessary in order to conduct the analysis: (1) individual pollen sacs rather than whole anthers had to be used, and (2) a reasonably constant method of slide preparation, in relation to spreading and squashing, and of scoring procedure should be adopted.

The phase differences in meiosis in different pollen sacs of an anther necessitated (1), since a mixture of mother cells at different stages of meiosis from different pollen sacs in some cases would preclude any accurate classification of developmental age. The procedure used for isolating the contents of individual pollen sacs has already been described.

Within a pollen sac at the more prolonged stages of meiosis the mother cells are very closely synchronised. At shorter stages involving chromosome movements the synchrony gets a little out of step in the first division. Within a pollen sac showing stages involving chromosome

movements the slightly different stages, as far as can be ascertained from general observations of sectioned material, are scattered at random throughout the sac with no isolated groups of cells at a particular stage (cf. Moens, 1964 in tomato). Rees and Naylor (1960) showed that slight but significant differences in chiasma frequency and mitotic timing occur in different parts of rye anthers according to proximity to the vascular supply. Such small differences may be present in my material. To reduce any possible error these differences, if present, might introduce (and also to aid in even squashing) the contents of a pollen sac were shaken with a needle to break up groups of cells and mix the contents, which were then distributed as evenly as possible over the approximate area of a coverslip.

The analyses were conducted over a relatively unbroken period of four months. Throughout the study as constant a degree of squashing as possible was maintained, to ensure that cells at approximately the same middle stage of anaphase were scored for segregation types in different pollen sacs. Neither cells in which all the chiasmata had not yet been lost (early anaphase) nor those where the chromosomes were noticeably aggregated around the poles (late anaphase I) were scored for segregation types, even though at least some of these probably could have been. Some differences in types of cells scored undoubtedly arose, though it is considered that the above procedure provided a reasonably objective frame of reference on which to make scoring decisions. Cells were scored only when both complementary groups of chromosomes in a cell

could be fully scored for numbers of group I, II and III chromosomes.

Slides were kept for a day or so, ringed with nail polish, in case any check proved desirable, but they were not made permanent. In all nearly 3,000 cells were scored from 41 pollen sacs from 10 different interchange plants. A number of pollen sacs were also examined from normal material. Each interchange pollen sac was scored from two points of view: (1) frequency of alternate and adjacent segregation in those cells in mid-anaphase, and (2) percentage of cells at each of early, mid and late-past anaphase stages. For (1) searching was carried out systematically using a moveable stage and 10 x 40 magnifications, and then scoring done under oil immersion. For (2) the slide was covered a second time under 10 x 40 magnifications. Usually the whole slide was covered for both scorings, though occasionally only in part when pollen sacs were in mid developmental stages.

The frequency of segregation types was expressed as a ratio; alternate/adjacent. The developmental age of each pollen sac was computed from the following formula, which is the same, basically, as that used to study metaphase I developmental stages (MI index) by Rees and Naylor (1960).

$$\text{Anaphase index} = \frac{100 - X + Y}{2}$$

where X = percentage of cells in early anaphase (= "pre" mid-anaphase)
and Y = percentage of cells in late or past anaphase I (="past"
mid-anaphase).

The application of this formula is discussed after presentation of results. For the purpose of discussion and preliminary statistical analysis the pollen sacs were divided into four groups according to their

index values, the groups being determined after examination of the data:

(1) early (E) developmental stage; index values 0 - 30.   At these stages
most of the pollen mother cells are still in early anaphase, with some in
mid stages (= "in" mid-anaphase) but few past;

(2) mid (M) developmental stages; index values 31 - 70.
Most cells here are in mid-anaphase I with moderate numbers in both
"pre" and "past" stages *;

(3) late (L) stages; index values 71 - 90; moderate number of cells
in mid anaphase, few "pre" and many "past";

(4) very late (VL); index values 91 - 100; few cells still in mid-
anaphase, most past.

The detailed data along with a partial summary are given in table
30, with pollen sacs arranged in sequence according to their index values.
The four developmental stages recognized are bracketed.

The data in the column on the right of this table, where alternate/
adjacent segregation ratios are shown, suggests a correlation between
developmental stage of anaphase and frequency of segregation types.   In
early developmental stages the ratio is somewhat above the expected 3 . 0;
it then falls to a little above half this original value in mid develop-
mental stages, and then rises gradually through late to very late
developmental stages to a value considerably above the original.   These
suggestions are more clearly manifest in the graphical presentation of the
data in Fig. 71 where index values are mapped along the Y and the segre-
gation ratios along the X axis.   Each point on the graph represents one
pollen sac.

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* i.e. pre- and past mid -anaphase, corresponding to X and Y above.

Table 30 Numbers of alternate and adjacent type mid-
anaphase I cells from 39 pollen sacs at successive
developmental stages of anaphase. Pre, in and past =
pre, in and past mid-anaphase I respectively. The
developmental index values were computed from the pre
and past values (formula in text). The pollen sacs and
their data are grouped into four developmental stages,
early (E), mid (M), late (L) and very late (VL).
Further explanations in text.

Group	P.s. no.	Index	% pre	% in	% past	Adj.	Alt.	Alt./Adj.
E.	1	2·5	95	5	–	1	5	5·0
	2	3	94	6	–	4	12	3·0
	3	5	90	10	–	5	20	4·0
	4	7	86	14	–	8	30	3·8
	5	11	78	22	–	12	59	5·0
	6	12	76	24	–	14	54	3·9
	7	13	74	26	–	12	53	4·4
	8	16	68	32	–	13	53	4·1
	9	22·5	58	39	3	11	43	3·9
	10	23·5	57	39	4	15	50	3·3
	11	26	54	40	6	17	64	3·8
	Totals					112	443	Mean 3·96
M.	12	32·5	40	55	5	31	67	2·2
	13	36	35	58	7	51	149	2·9
	14	38·5	32	59	9	33	102	3·1
	15	42·5	22	71	7	40	100	2·5
	16	44·5	21	69	10	31	78	2·5
	17	45	18	74	8	49	127	2·6
	18	55	10	70	20	23	60	2·6
	19	59	8	66	26	36	121	3·4
	20	64	2	68	30	34	104	3·1
	Totals					328	908	Mean 2·77
L.	21	71	4	50	46	16	66	4·1
	22	71	9	40	51	20	77	3·9
	23	77	4	38	58	18	82	4·6
	24	81·5	–	37	63	26	80	3·1
	25	83	2	30	68	21	60	2·9
	26	86	1	26	73	14	58	4·1
	Totals					115	423	Mean 3·68
VL.	27	91·5	1	15	84	7	41	5·9
	28	92·5	–	15	85	3	19	6·3
	29	93	–	14	86	6	35	6·0
	30	93	–	14	86	9	49	5·4
	31	93·5	–	13	87	7	33	4·7
	32	94·5	–	11	89	8	29	3·6
	33	95·5	–	9	91	4	18	4·5
	34	95·5	–	9	91	3	21	7·0
	35	96·5	–	7	93	3	19	6·3
	36	96·5	–	7	93	4	16	4·0
	37	97	–	6	94	3	15	5·0
	38	98·5	–	3	97	1	6	6·0
	39	99	–	2	98	1	3	3·0
	Totals					59	304	Mean 5·15

Table 30

Fig. 71 also indicates the somewhat variable ratios obtained, particularly in early and very late stages of development. The M stages show approximately the same ratio in different pollen sacs. The most variation is found in E and VL pollen sacs where many fewer cells in mid anaphase are available for scoring, compared to M stages.

The graphical presentation suggested the division of the data into the four developmental stages E, M, L and VL. The summed values and ratios so obtained from each of these groups are included in table 30. These mean ratios crystallize the suggested relationship between developmental age and segregation frequencies. This is further illustrated in a different way in Fig. 72.

The differences between the four developmental groups were tested statistically for significance at the 5% level of probability by means of a proportion test (Croxton, 1960, p.263.) Each group was found to be significantly different from its succeeding one (x/σ and p values as follows - for E and M groups; 2.886, p = 0.003: for M and L groups; 2.182, p = 0.029: for L and VL groups; 1.91, p = 0.056). The E and L values do not differ significantly from each other, though it is clear that they must be regarded as distinct since they are separated developmentally by the very distinct M group.

The data suggest that both the original hypotheses considered do not hold. Thus it appears that neither (1) a constant 3 : 1 ratio throughout developmental stages in a pollen sac, nor (2) an overall

positive correlation between alternate/adjacent ratio and developmental

stage is manifest.   Rather, the data suggest that initially, between

E and M developmental stages, a negative correlation holds, while between

M and VL stages a positive correlation holds. A single regression line

calculated for the total data is significant (p. $>$ 0.01 $<$ 0.05), but

the deviation from regression is very large.   This is not surprising

since the relationship suggested in Fig. 71 is clearly not a simple

linear one.   Linear regression lines were also computed for the data

to the right and left of the low value X 40, Y 2.5   Both these regressions

are very significant (p $<$ 0.01).   A curvilinear regression through the

data is very significant (p $<$ 0.01), and the curve gives a better overall

fit than the above, though again the deviation from regression is large.*

The regression analysis is confronted with one major problem,   If all

the alternate/adjacent points are treated as equal then they will carry

the same weight in an analysis, despite the large differences in sample

size of the various points.   Thus e.g. the value 3.0 of pollen sac number

39, which had been obtained from a sample of only four cells, will carry

the same weight as the same value (approximately) of pollen sac number 20,

which, however, had been obtained from a sample of 138 cells.   It would

be more appropriate if the latter value were to carry considerably more

weight than the former.   Numerous other similar situations occur in the

data.   In general values obtained from larger samples (e.g. those from

the M developmental stage) should carry more weight than those with small

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* Mr. S. Roberts kindly computed this curvilinear regression for me.

samples (e.g. those in E and L - VL developmental stages). These differences in sample size are of course inherent in this type of investigation. They are a main cause of the difficulty encountered in computing regression lines that have a reasonable goodness of fit. To overcome this problem it is necessary to weight appropriately each value, according to its sample size. This would permit a meaningful analysis of regression to be conducted. Professor B.I. Hayman and Mr. S. Roberts have kindly outlined to me the procedure for such a weighted regression.

(a) Instead of the ratio alt./adj. (table 30). alt./ (alt. + adj.) should be used. This enables binomial distribution theory to be applied.*

(b) The appropriate weight for each pollen sac is calculated from $w = 1/\text{var. } y$ where $y = \text{ratio alt.}/(\text{alt.} + \text{adj.})$.

(c) The regression equation $y = a + bi + ci^2$ is then calculated, where i = the anaphase index, by minimising $w(y - a - bi - ci^2)^2$. This provides an approximate regression equation, which is then used in an iterative process to gradually refine the equation. Thus y is predicted for each i from the calculated equation, these values of y are then sub-stituted in w above, the regression equation recalculated, and so on.

This iterative process clearly requires a computer program, and so far I have been unable to conduct this myself. Also, some further data is to be sought, particularly for the important M developmental stage. The simplified analyses conducted so far on the present data,

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* These have been calculated and used to construct a graph similar to that in Fig. 71. The two ratios give virtually identical pictures. These ratios were also used in calculating the regression lines mentioned above.

while serving to give a strong suggestion of a relationship between
alternate/adjacent ratio and developmental stage, and serving to give
some insight into the nature of this relationship, do not permit
rigorous conclusions to be drawn from the data.   In a following
discussion (p. 207 and Fig. 71a) a hypothesis concerning the detailed
nature of the relationship is proposed.   Further data with more detailed
analyses will be necessary to substantiate the hypothesis.

Ib   Composition of developmental stages.

The percentage of cells still in early anaphase ("pre" mid-
anaphase) in E pollen sacs must contain some cells that would determine
the alternate : adjacent ratio at the M stage, and virtually all those
that would be responsible for it at L and VL stages.   Also, at a stage
in M pollen sacs when the maximum percentage of cells in mid anaphase is
achieved (index 45), these cells must contain the bulk of those that would
have been "in" cells of E pollen sacs (since few of these have as yet
passed out of anaphase; see "past" percentages), plus those that would
contribute largely to the L stages.   And the bulk of the "pre" cells at
index 45 would comprise the "in" cells of VL pollen sacs.   What is most
interesting is the fact that those cells entering mid anaphase from E
stages that raise the percentage of "in" cells to the 74% of M stages,
must contain a frequency of alternate and adjacent types of cells such as
to lower the overall ratio from approximately 4 : 1 to 2.6 : 1.   Thus
for example, (a) the 40% "in" for pollen sac 11 can be considered to con-
tain about 32 alternate and 8 adjacent type cells (about 4 : 1), and
similarly (b) the 74% "in" of pollen sac 17 must contain about 54 alternate

and 21 adjacent type cells (about 2.6 : 1*).   Now since the "in" cells

at the developmental stage represented by pollen sac 17 contain almost

all the "in" cells at the developmental stage of pollen sac 11, then the

extra cells having entered mid anaphase from "pre" cells of 11 that raise

the "in" of 17 to 74% (an extra 34%) must have contained about 13 (21

minus 8) adjacent : 22 (54 minus 32) alternate type cells.   That is,

the 34% of cells entering mid anaphase between the developmental stages

represented by pollen sacs 11 and 17 must contain an alternate : adjacent

ratio of approximately 2 : 1 or a little less.   These values can only

be taken as approximate, though they do give a usefil further insight into

the changing alternate : adjacent type frequency during development of

anaphase within a pollen sac.

It will be of use in future studies along these lines to attempt

to score cells that are still in early anaphase as well as those in mid

anaphase.   Such an analysis would enable the above considerations on

group composition to be placed on a more firm basis.   This was attempted

towards the end of the study when its desirability became evident, but so

far the data are insufficient for any detailed considerations.   Expec-

tation, however, appears to be realized.   Thus e.g. in pollen sac 17, with

18% of cells still in early anaphase, a total of 28 satisfactory cells were

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* It is probable that the ratio 2.6 : 1 in this and similar developmental
  stages (pollen sacs 15 and 16 as well as 17) is a good estimate of the
  actual value since the sample of cells scored here is reasonably high.
  This value was used in preference to the near 2.8 : 1 value for the
  whole M stage since the latter appears to be affected by pollen sacs
  of slightly different developmental stages on either side of the low
  value reached.   It is difficult to assess pollen sac no. 11 in this
  respect.

scored (see p. 139 for precautions), which showed 23 alternate and 5 adjacent orientations, giving a ratio of 4.6 : 1. It was indicated above that most of the "pre" cells at such a developmental stage as re- presented by pollen sac 17 will comprise the "in" cells of very late pollen sacs, and are thus expected to show a parallel ratio of alternate : adjacent orientations of about 5.15 : 1, or a bit less since these "pre" cells might also include some of the "in" cells of L sacs. The observed value shows a good fit considering the sample size scored. Some difficulty with this type of analysis has been encountered at present since the method and degree of squashing desirable for mid anaphase studies is not fully satisfactory for study of early anaphase stages, and vice versa. Hence many early anaphase cells in the above pollen sac and the few others analysed could not be scored satisfactorily, an undesirable situation. This problem might be overcome in future by resquashing preparations for the studies at early anaphase.

Ic.   Synchrony at anaphase I in normal material.

In the interchange material a maximum of 74% of cells in a pollen sac in mid anaphase at once has been recorded (pollen sac 17 in table 30). This must be very close to the maximum generally achieved (see rapid rise of "past" percentages thereafter). In eight pollen sacs of normal material examined a maximum of 85% has been observed (2% in early anaphase and the rest in late anaphase/telophase). Thus the maximum in interchange material appears to be somewhat lower than normal; i.e. the pollen sacs at these stages are less closely synchronized in respect of entry into anaphase I.

Id.   Movement of cells into anaphase I.

From the percentages of cells at the three different anaphase
stages in table 30 there is a gradual increase in the "past" value and
a gradual decrease in the "pre" value.   This of course is to be expected.
However, the "past" value appears to be very slow in increasing to begin
with, even though the "pre" and "in" values show a sharp decrease and
increase respectively.   Thus between pollen sacs 8 and 17 the "pre"
value has decreased from 68 to 18 and the "in" value has increased from
32 to 74, while the "past" value has risen only from 0 to about 8 or 10.
But this is also to be expected theoretically, since the progression
through "in" to "past" theoretically represents a greater magnitude of
chromosome movement than through "pre" to "in".   On this basis one would
also expect, then, a more rapid increase of "past" cells at later
developmental stages;   and just this is seen (e.g. between pollen sacs
17 and 26).   Overall, then, between developmental stages where all three
phases of anaphase are present in a pollen sac (from nos. 8/9 to 25/26,
disregarding the apparently atypical 1% tardy cells still in "pre" of
pollen sac 27), the percentage "pre" has decreased from about 65 to 0
and the "past" increased from 0 to 73.   These are comparable figures
that are expected on the basis of the above explanation.

Through the developmental stages the percentage "in" increases to
its maximum and then gradually decreases, as expected.

As far as can be judged from these data then, the progression of
cells into, through and out of mid anaphase continues at a fairly steady

rate. If on the other hand, entry into anaphase were to occur to a marked degree in spasms, then this would have a considerable influence on the ratio of alternate : adjacent type cells at a particular developmental stage. For example, if further entry into anaphase I were to be delayed in pollen sac 11 (index 26; 54% pre, 40% in, 6% past), until a stage when say 20% of cells remain "in" and 30% are "past", then its developmental age would, at this stage, be comparable to that of pollen sac 18 (index 55). But it would probably show an alternate : adjacent ratio somewhat different from that otherwise expected. Thus if but few of the cells contributing to the low ratio of mid developmental stages had entered anaphase at an index 26 stage, then the "delayed entry" pollen sac at an index 55 stage would have an alternate : adjacent ratio higher than otherwise would pertain. Numerous other possibilities can be envisaged.

Data from two pollen sacs, given separately below, suggest that just such a phenomenon occurs.

Pollen sac No.	Index	%pre	%in	%past	Adj.	Alt.	Alt/Adj.
40	28	67	10	23	7	24	3.4
41	37	59	8	33	7	14	2.0

The percentage data from these two pollen sacs do not fit into the general pattern of table 30. Normally at index values of 28 and 37 there is a much higher percentage of cells in mid anaphase and a much lower percentage both pre and past this stage. It appears, therefore,

that in these two pollen sacs anaphase, in a proportion of cells, has been allowed to proceed with very little or no further entry of cells into mid anaphase (all "pre" cells had fully oriented bivalents and complex).

These two pollen sacs have been identified as distinct (and thus kept separate) because of their extreme differences from the norm. To what extent they represent the extreme in a gradation of similar types is not possible to say at present. Certainly these extreme or near extreme types are very rare, thus suggesting that the phenomenon is rather uncommon. But it may be that some similar but less extreme pollen sacs have been undetected as being such, and thus incorporated into the data of table 30. If so, such pollen sacs might be contributing to the variability in alternate : adjacent ratios seen in similarly aged pollen sacs in table 30. Statistically these variations can probably be accounted for on the basis of sampling errors alone. Detection of any other under-lying cause, such as that suggested above, will require further data and analysis.

The numbers of cells scored for alternate and adjacent type com-plements in pollen sacs 40 and 41 are too low to be clearly informative. It is very desirable to obtain further data from similar pollen sacs, since they could contribute much information to the heterogeneous make-up of developmental stages.

Ie.   Estimation of an overall alternate : adjacent ratio at mid-anaphase.

It is clear from the above observations that neither the overall frequency of alternate : adjacent type cells (3.4 : 1 in table 30), nor

individual developmental stage frequencies, can be used as an accurate indication of an overall frequency that should be comparable to the actual alternate : adjacent orientation ratio of metaphase I (approximately 3 : 1). Previously observations designed for this purpose would probably have been conducted from mid-stage pollen sacs (anthers?), because of the larger numbers of cells available for scoring, but this would likely produce too low an overall value; and from other developmental stages the value obtained would likely be too high. The overall 3 : 1 value lies between E/M and M/L stages. An estimate of the overall value can be obtained from the data of table 30. For example, in pollen sac 17 with an alternate : adjacent ratio of 2.6 : 1 (see footnote on p. 176), the 8% past at this stage must represent the first 8% of cells to have entered anaphase in this pollen sac, and thus would have showed an alternate : adjacent ratio of about 4 : 1. Similarly the 18% remaining in early anaphase I must largely represent those cells that would be in mid anaphase at the very late developmental stage, and therefore contain a frequency of about 5 alternate to 1 adjacent type cells. The desired calculations are shown below.

| | Composition | Cell types | |
		Alternate	Adjacent
	74% at 2.6 : 1	53.4	20.6
	8% at 4.0 : 1	6.4	1.6
	18% at 5.0 : 1	15.0	3.0
Totals	100%	74.8	25.2
		approx. 3 : 1	

The calculated expected frequency in the whole of pollen sac 17 is thus very close to the alternate : adjacent orientation ratio for metaphase I pollen sacs. This pollen sac was chosen for these calculations since it is possible to judge reasonably accurately what ratios of alternate and adjacent orientations would have occurred in "pre" and "past" cells, and the unknown quantities are minimal. Other pollen sacs of similar age give similar results.

If. <u>Scoring for alternate and adjacent orientations.</u>

It was mentioned on page 140 that scoring cells for relative frequency of alternate and adjacent orientations must be confined to pollen sacs with all cells at metaphase/early anaphase I. This is because the changes recorded during anaphase in the ratio of alternate : adjacent segregations in mid-anaphase cells imply contemporary changes of frequency in those cells still in early anaphase. Hence scoring pollen sacs in which all cells are not fully synchronized at metaphase/early anaphase I is bound to produce inaccurate results in this material.

Ig. <u>The anaphase index formula.</u>

The use of the changing proportions of cells in "pre", "in" and "past" mid-anaphase as a basis for calculating values indicative of development in a pollen sac hinges, for its validity, on whether or not a pollen sac is synchronized at prophase and metaphase stages. This has been shown to be so. Any asynchrony that might occur could not possibly be great, since if it were then it would be highly unlikely that a developmental sequence of the type revealed would have been detected in the first place.

The anaphase index formula used also depends for its accuracy

on a more or less steady flow of cells from metaphase to telophase.
Two examples where this appears not to be so have been detected, though
possible errors introduced here again cannot be large, for if so the
definite trends shown likewise would not have been detected.

II.   Developmental studies in anaphase II pollen sacs.

Essentially the same technique used for anaphase I studies was
employed to see how far the phenomenon previously revealed is carried over
or modified in the second division anaphase.

Detailed observations on two pollen sacs showed that in about 80%
of cases the two halves of a mother cell are closely synchronized.   In
the remaining 20% either one half is at metaphase II and the other at
mid-late anaphase II, or else one at mid anaphase and the other at telo-
phase.   (The former occur mainly in pollen sacs at mid developmental
                                                                    were
stages, the latter in late - very late stages.)   In both cases such cells/
scored according to the stage of the advanced half; i.e. the former were
classed as "in" mid anaphase II and were scored for complement types where
possible; and the latter were classed as "past" mid anaphase II and not
so scored.   Similar decisions had to be made occasionally in scoring cells
with less obvious asynchrony.   Some slight differences amongst pollen sacs
are likely to occur, but these will not be of great importance, particu-
larly since the frequency of cells with marked asynchrony is relatively
low.

Heating and sudden squashing readily burst the original mother

cell wall round the two halves, which then often become well displaced
from each other and thus impossible to pair in an analysis. Hence it is
necessary to flatten cells very slowly, without heating. With care the
mother cell wall is broken, though the halves only occasionally become
separated from each other. These occasional cells were not incorporated
into either of the two scorings.

The distinction between cells "in" and "past" mid anaphase II was
based on the same criterion as in anaphase I. On the other hand, the
distinction between "pre" and "in" of mid-anaphase II is a little more
subjective than for anaphase I. In order to be consistent and to allow
some comparison with the anaphase I data, anaphase II cells were classed
as "in" mid anaphase II when the chromosome groups were separated to a
degree adjudged to be comparable to that in anaphase I after complete
loss of chiasmata; i.e. when anaphase had reached a stage where a small
space separated the ends of separating, straightened chromosomes (e.g.
upper half of the cell in Fig. 74.)

Cell walls are laid down between the daughter nuclei of meiosis I
so that the halves could be expected to behave somewhat independently of
each other, with no co-operation between the two genomes. In contrast
to halves from the alternate arrangement and segregation of the complex,
where both members of a pair are chromosomally and genetically balanced,
those from the adjacent orientation and segregation are both deficient
and duplicated for certain chromosome segments, and thus genetically
unbalanced. These genome differences in separated cells might conceivably

cause differential behaviour of the two cells in respect of timing of meiosis II. Observations which had already been made on pollen grain mitosis showed that this was not altogether improbable. However, asynchronized cells appear in meiosis II of normal material, and in about the same frequency; and also, theoretically, it is probable that many conditions for meiosis II are determined from the original diploid pollen mother cell nucleus. Hence, it would not be surprising if the two halves were to behave similarly in entry to anaphase II, regardless of their balance or unbalance.

Two pollen sacs were analysed in the above respect, scoring closely synchronized and clearly asynchronized cells separately. In the first sac, which was in a mid developmental stage and showed asynchronized cells mainly with the advanced half in mid anaphase II, the ratio of alternate : adjacent type complements was 24 : 8 (3 : 1) in asynchronized cells; and 86 : 32 (2.7 : 1) in synchronized cells. In the second, later pollen sac, with most asynchronized cells with the retarded half in mid anaphase, the respective values were 16 : 6 (2.7 :1) and 63 : 19 (3.3 : 1). Thus there is at least no clear cut relationship between unbalanced complements and asynchrony within a cell.

In the analysis of alternate and adjacent type frequencies at different developmental stages, nearly 2,000 cells were scored from 28 pollen sacs. In analysing these cells for complement types a minimum of two of the four chromosome groups were classified before reaching a decision on the cell as a whole. The results are shown in detail in table 31 and graphically in Fig. 79.

Table 31   Numbers of alternate and adjacent type mid-
anaphase IIcells from 28 pollen sacs at successive stages
of development of anaphase.   Abbreviations etc. as in
table 30.

Table 31

	P.s. no.	Index	% pre	% in	% past		Adj.	Alt.	Alt./Adj.
E.	1	1	98	2	-		1	7	7.0
	2	2	96	4	-		1	3	3.0
	3	4	92	8	-		4	26	6.5
	4	4	92	8	-		4	19	4.8
	5	5	90	10	-		6	21	3.5
	6	10.5	79	21	-		12	46	3.8
	7	11.5	65	35	-		25	81	3.2
	8	23.5	54	45	1		30	99	3.3
					Totals		83	302	Mean 3.64
M.	9	35	40	50	10		29	94	3.2
	10	47.5	24	57	19		24	61	2.5
	11	50.5	22	55	23		40	110	2.8
	12	56.5	17	53	30		39	115	2.9
	13	58.5	9	65	26		44	122	2.8
	14	59	11	60	29		24	82	3.4
	15	61	8	62	30		33	111	3.4
	16	69.5	9	43	48		18	52	2.9
					Totals		251	747	Mean 2.98
L.	17	83	3	28	69		20	67	3.4
	18	85	3	24	73		10	29	2.9
	19	85	5	20	75		10	31	3.1
	20	88	1	22	77		8	40	5.0
	21	89.5	1	19	80		9	20	2.2
					Totals		57	187	Mean 3.28
VL.	22	92.5	-	15	85		7	28	4.0
	23	93	1	12	87		5	20	4.0
	24	94	-	12	88		7	27	3.9
	25	94.5	-	11	89		3	14	4.7
	26	95.5	0.5	8	91.5		5	13	2.6
	27	96	-	8	92		3	14	4.7
	28	97.5	-	3	97		1	5	5.0
					Totals		31	121	Mean 3.90

In table 31 a trend similar to that evident from anaphase I data
can be seen. However, the mean values of the four developmental stages
are not so sharply distinct here; and they are significantly different
at only round the 20% level of probability with these sample sizes.
Since the trend in anaphase II is basically the same as that of anaphase I
it is probably real.

A pattern of differences emerges when developmental stages of
tables 30 and 31 are compared. There is a general levelling out of
values, the higher ratios of E, L and VL anaphase I stages being lower
at anaphase II, the lower ratio of the M stage being higher. The levell-
ing out is of much the same magnitude in E, M and L stages, though it
appears to be somewhat greater at the VL stage. The smaller number of
pollen sacs and thus scorings in VL stages of anaphase II makes it im-
possible to conclude whether there is any significance in this latter
difference.

In attempting to explain this pattern of differences between the
anaphase I and anaphase II data it is necessary to enquire as to what
extent the anaphase I and II developmental stages are comparable. This
will depend on whether or not the "pre", "in" and "past" stages are
equivalent. The amount of centromere movement that occurs between the
initiation of anaphase and the stage where the cells are described as mid-
anaphase, will be substantially greater for anaphase II than for anaphase I *,
by virtue of the difference in proximity of chiasmata and linking substance
to the pairs of centromeres at the initiation of anaphase I and II
respectively. Thus the "pre" of anaphase II will tend to contain some

of the cells that if at anaphase I would be included in the "in"

category, so that particularly the "pre" and "in" categories will not

correspond very precisely.   If one makes the reasonable assumption that

the intercentromere-pole distances at metaphase I and II are the same,

then the alternate/adjacent composition of the "in" cells of the L stage

of anaphase II will tend to be lower than that for the "in" stages of

anaphase I, while the ratio of the "in" of M stages will tend to be higher.

The effect will be a levelling out of this part of the mid anaphase II

data compared with that of anaphase I, which is what is seen.

However, the levelling out appears to extend to all developmental

stages.   The factor pointed out above would not account for the lower

anaphase II values of E and VL stages.   Whereas an approximate maximum

of 75% of cells in mid-anaphase I is reached at one time, an approximate

10% lower value of synchrony occurs in anaphase II (pollen sac. no. 13).

(In normal material a maximum of 70% has been noted so far out of 10 M

pollen sacs examined; compare with 85% for anaphase I).   This apparent

'relaxation of synchrony' is, to some extent at least, an expression of

the same factor described above.   In addition, however, the sequence of

cells entering anaphase I may not be strictly maintained at anaphase II

because of more independent behaviour amongst the cells, unconnected with

prometaphase or other events of meiosis I.   The lower maximum of cells

scored as "in" at anaphase II, compared with anaphase I, may in part be

a manifestation of this also.   This factor would also bring about a

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*   Professor A.P. Wylie drew my attention to this important point.

levelling of group differences of alternate/adjacent ratios at anaphase I compared with anaphase II, even if these groups were strictly comparable. This might account for the lower ratios of anaphase II in E and VL developmental stages, as well as contribute in part to the differences in M and L stages.

11.   DISCUSSION ON PROMETAPHASE AND RELATED EVENTS.

I.   The Behaviour of chromosomes, bivalents and chromosome complexes
        during prometaphase.

Ia.   Introduction

        The process whereby chromosomes and bivalents achieve their

precise orientation along the metaphase equator, on which the whole

process of mitosis and meiosis hinges for its regularity, has long been

difficult to explain satisfactorily. The difficulty is increased by

the somewhat more diverse features that characterize prometaphase in-

volving chromosome complexes at meiosis. It is only in recent years

with the analyses in living endosperm and animal spermatocytes, and most

recently the beautiful experimental analyses by Nicklas, that a fuller

understanding of prometaphase mechanics is being made available, and a

closer approach to a general, unified hypothesis is being made. Before

discussing results of the prometaphase and related studies made on the

present interchange material, it will be necessary to summarize what

is known concerning prometaphase mechanics of chromosomes, bivalents,

and chromosome complexes.

Ib.   Prometaphase chromosomes and bivalents in mitosis and meiosis.

        A summary of the main features that characterize metaphase and

prometaphase in mitosis and meiosis has been published by myself (1965 -

see reprint here together with criticism and a reply, page 255).

The essential points are outlined below.

(a)   At complete metaphase chromosomes and bivalents exhibit co-orientation*

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*   See above mentioned reply.

of their centromere pairs, one centromere of a pair directed to one pole, the other directed to the opposite pole.

(b)   During prometaphase movements, chromosomes and bivalents may move first to one or other pole before becoming co-oriented along the metaphase equator.

(c)   Polar oriented chromosomes undergo reorientation, a process through which such chromosomes eventually become oriented along the equator.

(d)   The spindle apparatus is closely involved with the movements associated with the achievement and maintainance of metaphase.

(e)   The pairs of centromeres remain co-oriented in the equator, in an apparent equilibrial state, because of the presence of a spindle force operating between centromeres and poles.

A number of hypotheses have been advanced in an attempt to explain the equatorial positioning of bivalents and chromosomes at metaphase (see Rickards, 1965, Nicklas, 1967).   Most of these had been conceived prior to the identification of centrophilic bivalents and the phenomenon of reorientation as integral parts of normal prometaphase mechanics.   Consequently they have in most aspects proved untendable.   The most recent explanation that takes into account the above mentioned phenomena was originally suggested by Dietz (1958,) and similar considerations were arrived at independently by myself (1965).   The hypothesis considers that the basis of chromosome orientation is random reorientations where necessary, until, by chance, stable co-orientation in the equator is achieved.   Thus, if the two homologous centromeres of a bivalent become immediately oriented to

opposite poles at the initiation of prometaphase, then apparently stable

co-orientation is achieved at once.   Alternatively, both centromeres may

become oriented to the same pole, following which reorientation phases

occur until cooriantation is achieved, when the bivalent will become

stabilized in the metaphase equator.   If it is granted that disyntelic*,

co-oriented bivalents are stable and do not reorient, as the data of Bauer

et al. (1961) and Nicklas (1967) and other have established, and conversely,

that monosyntelic non-cooriented bivalents are unstable and are induced to

reorient, as is also evident from the same data, then it is possible in the

first analysis to give a reasonably satisfactory explanation of the events

and achievement of co-orientation.

Though it is possible on the above basis to explain formally

how and what reorientation achieves (as we can the spindle e.g.) it is

much more difficult to account for its underlying mechanism.   Most frequently

reorientation is induced when bivalents occupy polar positions (see Fig. 3

and 4 in my 1965 account), suggesting that polar orientation may be instru-

mental in the actual induction of reorientation.   This may be so. However

a polar position is not an absolute prerequisite as emphasized by Nicklas

(1967); occasionally a bivalent in monosyntelic orientation, but lying near

the equator along with cooriented bivalents, may be induced to reorient.

These observations suggest that something directly related to monosyntelic

orientation itself induces reorientation.   However, on this basis it is

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*   The terms disyntelic and monosyntelic are defined in the reply mentioned
    above (page 256).

difficult to explain the characteristic, repeated reorientation of univalents (which cannot show monosyntelic orientation). Some possible explanations are pointed out by Nicklas (1967), but as he suggested, information is grossly lacking along these lines and no satisfactory account of the origin and mechanism of reorientation is yet available. The data presented already and discussed in this chapter provide some additional information in this connection.[*]

In the spermatocytes studied by Bauer et al. (1961) about 10% of bivalents became centrophilic before achieving equatorial co-orientation, the other 90% attaining immediate co-orientation. A similar value was obtained in <u>Melanoplus</u> by Nicklas (1967) (no exact figures available). It is not possible to obtain a precise assessment of the frequency of centrophilic behaviour from the fixed preparations used in my present study. This is because one is unable to ascertain the earliest stage of prometaphase in a pollen sac, in which no reorientation of centrophilic bivalents has occurred, in order to compare relative frequencies of centrophilic and non-centrophilic bivalents in those cells in prometaphase. However, one pollen sac in an early prometaphase developmental stage (approx. 50% of cells at late diakinesis or very early prometaphase - i.e. no clear signs of orien-tation in these cells) had 20 cells with full co-orientation of bivalents and interchange complex and 118 cells with centrophilic bivalents and/or complex in 138 prometaphase/metaphase cells scored . Of the latter 118, 92 showed centrophilic bivalents with or without centrophilic complexes, the remaining 26 with only centrophilic complexes, the seven bivalents in these cells being fully co-oriented.

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[*] See very recent data in Addendum, p. 269

Thus 46 (20 + 26) or 33 - 34% of cells showed complete co-orientation
of all seven bivalents.   In the 92 cells showing centrophily there was a
total of 105 centrophilic bivalents (80 singles, 11 doubles, one triple)
and 539 co-oriented bivalents. Thus in all 138 cells there were 105 centro-
philic bivalents and 861 co-oriented ones, giving an overall frequency of
10.9% centrophily.   This value should be compared with the 10% value ob-
tained by Bauer et al. (1961) in the following light.

(a) If the pollen sac concerned had been at a very early developmental
stage and no reorientation of centrophilic bivalents had yet occurred, then
this value is strictly comparable with that of Bauer et al., suggesting,
then, that the degree of centrophily in the seven bivalents is about the
same as in Tipula spermatocytes.

(b) The value obtained will be too low as an overall figure if some
centrophilic bivalents had already become reoriented to equatorial co-
orientations.   Hence the data reveal a minimum degree of centrophilic
behaviour in these bivalents of about 10-11%.   This figure is only approx-
imate since some misidentification as well as sampling errors undoubtedly
occur.   Thus e.g. no distinction has been possible between the two basic
types of centrophilic bivalents, *(p. '23)* and some monosyntelic bivalents may have been
still positioned near the equator and thus confused with co-oriented bivalents.
It is interesting, however, that the frequency suggested from this analysis
is similar to that obtained in other materials (Tipula and Melanoplus).

Nicklas (1967 p.43) has pointed out that while it is necessary on
the one hand to explain the mechanism of reorientation and its relationship

to the achievement of co-orientation of centrophilic bivalents, "it is just as necessary to explain the flawless orientation of most bivalents" at the initiation of their prometaphase movements.   With completely independent initial orientation of the two homologous centromeres of a bivalent, a frequency of 50% centrophily should be shown.   Clearly then, there must be some bias associated with pairs of linked centromeres that causes them most frequently to become initially oriented towards opposite poles.

Ostergren (1951) considered from some available evidence (Nicklas,(1967) for references) that in a bivalent the two pairs of sister chromatid centromeres, each pair acting as a unit, are dispositioned back-to-back in the two associated homologous chromosomes, so that one unit faces one way, the other faces the opposite way.   When spindle fibres form, Ostergren suggested that they do so to the most direct pole.   Secondly, Ostergren postulated that the pull of one centromere to a pole during its orientation will cause the other centromere to become directed more precisely to the opposite pole, and hence it will interact with that pole to achieve co-orientation.

The micromanipulation studies of Nicklas (1967) have vividly shown that preferential directioning of a centromere towards a pole nearly always results in its orientation to that pole during initial prometaphase events.   Nicklas points out that since the bivalent chromosomes are not radically bent between the two homologous centromeres, the properties of (a) back-to-back centromere disposition and (b) preferential orientation through position, alone are sufficient to account for about 75% initial co-orientation

of bivalents randomly positioned at the start of prometaphase, without relying on Ostergren's second postulate. Pulling of one centromere on another would tend to enhance initial co-orientation if the centromeres of the two half bivalents do not become active at once, an event that at times certainly occurs. Hence as Nicklas (p. 45) has put it, "the presently available data confirm a restricted form of Ostergren's theory, which satisfactorily accounts for the co-orientation of the quantitatively most significant group of bivalents".

Nicklas (1967) observed in his micromanipulation induced re-orientation that anaphase was never initiated until detached, non-cooriented bivalents regained their equatorial position, and points out that the phenomenon suggests the presence of a "block" induced by the as yet non-cooriented bivalents, such that cells will/pass not through to anaphase until complete orientation has been achieved.

Considerable precedent exists for such a general "block" concept. Thus in mitosis Bajer and Mole-Bajer (1956) and Uretz et al. (1954) found that equatorially oriented chromosomes "waited" in metaphase until centrophilic members of the complement likewise became co-oriented. And these authors noted that anaphase was initiated immediately on the achievement of the correct orientation in all the chromosomes of the complement. The data from two meiotic sources, one published by Wagenaar (1961) and the other from my own analyses, point strongly towards the same conclusion (re-examination of data and discussion presented later).

Ic.   Prometaphase chromosome complexes in meiosis I.

Until recent years most studies on the orientation of chromosome complexes at meiosis I have been done at metaphase/early anaphase from fixed material.   Hughes-Schrader (1943) and Inamdar (1949) studied some details of prometaphase in XXY trivalents of mantids from fixed preparations.

The studies in prometaphase from living material by Bauer et al. (1961) on X-ray induced interchange trivalents are most informative, and point strongly to the need for further detailed studies along these lines. These studies have shown that, paralleling the behaviour of bivalents and chromosomes in mitosis and meiosis, chromosome complexes do not necessarily move directly onto the equator in a fully co-oriented, stable state. They may do so in some cells, while in others they may first take up polar, partially or fully centrophilic, relatively unstable positions, before undergoing phases of reorientation to achieve their more stable orientation states.   The static studies reported here are in full agreement with such conclusions.

As well as bivalents and univalents two types of trivalent, involving the same three chromosomes, were studied by Bauer et al. during prometaphase/metaphase; a Q-type trivalent with three chiasmata, and a chain type with two chiasmata (Figs. 88 and 89).   The latter approaches closely, in structure and behaviour, the chain of four chromosomes with three chiasmata studied in A. triquetrum, and hence emphasis will be placed in this summary on this type,

At the very earliest stages of prometaphase, chain forming trivalents with syntelic centromeres had either:

(a) two end centromeres oriented to one pole, the middle member to the opposite pole, giving V configurations (Fig. 88c), or

(b) one end centromere plus the middle centromere to one pole, the other end centromere to the opposite pole, giving rod configurations (Fig. 88 b, d & e). Two sub-types of rod configurations occurred, depending on particular centromere relationships (Fig. 88 d & e).

The third possible type of chain trivalent with synoriented centromeres (the monopolar type with all three centromeres oriented to the same pole; Fig. 88a) may also have arisen, but if so must have reoriented very quickly to other configurations, as did those few monopolar trivalents which were derived by reorientation from V types later in prometaphase.

In Q-type trivalents the two centromeres of the ring were usually oriented to opposite poles, the rod centromere to either pole (Fig. 89 c & d). In the very earliest stages of prometaphase the two other possible types of syntelic Q orientations may also have arisen and very quickly changed their orientation to other types, as with chain trivalents.[*]

All three basic types of orientations in chain trivalents are potentially able to undergo reorientation. Monopolar trivalents reorient to either V or rod configurations. Rod trivalents may reorient to V's and vice versa. In Fig. 90, initial orientation of the trivalent resulted in

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[*] The amphitelic orientations observed by Bauer et al. are not considered here.
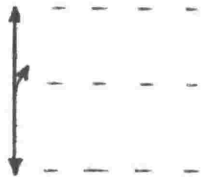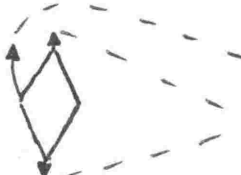
a V configuration which did not undergo reorientation. In Fig. 91. one end centromere from an initial V configuration reoriented to the opposite pole to give a rod configuration(Fig. 88 c to d), from which anaphase proceded. Conversely, in Fig. 92 an initial rod configuration was converted to a V configuration by reorientation of one end centromere (Fig. 88 e to c). Other more complex patterns of reerientation were recorded by Bauer et al.

By the completion of prometaphase, just as no monosyntelic bivalents persisted, no trivalents occurred with all three centromeres oriented to the same pole. V and rod configurations occurred in approximately equal frequencies.

Though all configurations are capable of reorientation during prometaphase differences in reorientation potential were recorded by Bauer et al. Monosyntelic trivalents, bivalents and equivalent orientations in univalents show increasing stabilities. Disyntelic bivalents on the other hand are completely stable, never undergoing reorientation. Different centromeres within the other trivalents show varying reorientation potentials, depending on the configuration occurring and the centromere relationships within the configuration. These different reorientation potentials are reviewed on the following page.

My present studies at prometaphase have identified monosyntelic bivalents and also all four basic types of centrophilic or partially

Relative reorientation potentials in

Tipula oleracea interchange cells

(from Bauer et al 1961).

UNIVALENTS   X 1

MONOSYNTELIC BIVALENTS   X 0.5 *

MONOSYNTELIC TRIVALENTS   X 0.1

DISYNTELIC BIVALENTS   X ∞   (Completely stable)

V-TRIVALENTS   (all centromeres) X 9

ROD TRIVALENTS   X 6

                        reorients but frequency
                        indeterminable

                        X 20

Q-TRIVALENTS   X 3

                        stable

---

*   i.e. monosyntelic bivalents wait before reorientation half as
     long as univalents do; or in other words, monosyntelic bivalents
     have twice as great reorientation potential as do univalents.

centrophilic complexes of four chromosomes (diagramatically illustrated in Fig. 45 a - d). Centrophilic bivalents are clearly unstable since they are not seen in early anaphase pollen sacs, and bivalent numerical non-disjunction has also not been identified. They must be reoriented to disyntelic co-orientations in the equator. Though it cannot be stated emphatically from these static studies, it can fairly safely be concluded, in view of the findings of Bauer et al. (1961) and Nicklas (1967) and others, that bivalents once co-oriented in a disyntelic fashion, remain so until anaphase.

The six possible orientations of the prometaphase chain of four (Fig. 45 a - f) parallel those observed in tipulids for a chain of three (Fig. 88 a - c); the extra number in my material arising because of the extra chromosome in the complex. The fully centrophilic, monosyntelic orientation (d) directly parallels that of the chain of three in tipulids; the others are not so clearly comparable.

Observations in synchronized pollen sacs at metaphase/early anaphase I have shown that two of these five orientations (the alternate and adjacent I types, e and f) comprise about 98% of cells; the remaining 2 % are special cases that do not fit precisely into any of a - f. These two orientations must therefore be the only stable, or at least the most stable ones; whereas a - d must be unstable and converted through re-orientations to e and f during the development of metaphase in these cells.

Frequency of unstable types. Only tentative conclusions have been made, which can now be reconsidered.

(1)   In both early and late stages of prometaphase development in a
pollen sac most unstable complexes are of types a and b, c and d being
much more infrequent.   This undoubtedly means that the latter two are
actually formed less frequently, and the former more frequently at the
initiation of prometaphase.   This is in reasonable agreement with the
data of Bauer et al., particularly when it is recalled that here four
rather than three centromeres are involved in the complex.   In types
a and b only one centromere is out of place in respect of a stable type,
while in c and d two are.   Thus taking into account that some cells
undoubtedly show stable orientation types e and f at the initiation of
prometaphase (further discussion later) there is a tendency for the complex
tо orient initially in either the stable or more nearly stable configurations.
This pattern of prometaphase initiation is similar to that of bivalents
which show mostly stable rather than unstable orientation initially.

(2)   Whereas in early developmental stages of prometaphase in a pollen
sac the ratio of the frequencies of a : b types is relatively low, it is
relatively high in late developmental stages (table 27 and p. 136   ).
Two interpretations can be reconsidered here;   (1) the a configurations
may be more stable than the b types and thus take a longer time to achieve
stable orientation.   In view of the evidence of differential stabilities
of configuration types noted in Tipulids, this explanation is not unlikely.
(ii) At least in part, types b - d may be converted to stable types via
type a.   It will be shown below that this explanation is also likely.
(i) and (ii) may work in concert.

Reorientation patterns.   Though configurations e and f are
regarded as the two stable types, it cannot be certain that once achieved

from these states

they do not ever reorient/.   This is probably so, though such reorientations
might appear possible in view of the fact that the somewhat similar V
configuration in <u>Tipula</u> trivalents does so.   However, at least in mid-
late developmental stages of prometaphase, reorientation of <u>e</u> and <u>f</u> do not
occur, since by complete metaphase in a pollen sac no unstable configura-
tions are found.   Type <u>f</u> might conceivably be converted directly to <u>e</u>,
or vice versa, though again there is no evidence that suggests this.[*]
Only observations on living material will ascertain these points, though
the absence of other configurations at anaphase (in contrast to the cells
of Bauer et al.) makes it very probable that <u>e</u> and <u>f</u> configurations are
either completely stable or only rarely undergo reorientation.

These apparently stable configurations are characterized by
having the two end pairs of adjacent centromeres co-oriented in the equator
as two bivalents.   It seems probable that, at least in part, it is this
fact that imparts stability to the two configurations.   Also, in types <u>e</u>
and <u>f</u> two centromeres are oriented to each pole in such a way that the
whole complex lies in the equatorial region along with the other bivalents.

In contrast to the above, unstable orientations do not have both
end pairs of adjacent centromeres co-oriented in the equator, and parts
or the whole of the complex lie outside (above and below) the equator.
In type <u>c</u>, two centromeres are oriented to each pole as in <u>e</u> and <u>f</u>, but
the end adjacent centromere pairs are not co-oriented, and thus lie above
and below the equator.   Centromeres are not evenly divided between the
two poles in the other unstable orientations.

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[*] See footnote in Addendum.

Considerations presented below and later suggest that adjacent
pairs of centromeres, when co-oriented and thus lying in or near the
equator, are either fully stable or considerably more stable than non-
cooriented centromeres. If this is so then in type a the three centro-
meres that are co-oriented as two adjacent pairs are either fully stable
as their equivalents in configuration e, or else undergo infrequent
reorientation compared with the remaining centromere. This remaining
centromere that lies above the equator towards one pole and not co-
oriented in relation to its adjacent one, is probably the one that most
frequently reorients. Its reorientation to the opposite pole will convert
the a configuration to a type e. Similar reasoning can be applied to
type c configuration. If both end centromeres reoriented at approximately
the same time, then the configuration would be reoriented to type e.
With non-simultaneous reorientation the same result would be achieved in-
directly, the configuration first being converted to type a.

In type b, the two non-cooriented centromeres are probably those
that undergo reorientation in the main. Reorientation of the terminal
centromere alone would convert type b into a stable type f. Reorien-
tation of the inner centromere alone would produce type e; while reorien-
tation of both non-cooriented centromeres would produce an e configuration
via type a. It is possible that the two non-cooriented centromeres of the
b configuration behave differentially in respect of reorientation frequency.

Type d configuration will probably produce all other types in
its reorientation (as with monopolar trivalents in tipulids), the unstable

ones of which would undergo further phases of reorientation.

If the above considerations are essentially correct, then b - d unstable configurations may frequently reorient to type a before undergoing further reorientation to a stable state. This, together with any differential stability, would account for the observation that the decrease in the frequency of b, c and d types during the development of prometaphase in a pollen sac is more rapid than that of type a.

The origin of reorientation. Bauer and colleagues pointed out from their studies that while certain patterns characterize prometaphase reorientation in Tipula oleracea, other tipulids, and more strikingly other organisms, show different patterns. The most striking difference in the behaviour of chain forming trivalents is found between the X-ray induced interchange ones of Tipula oleracea and the XXY sex trivalents of mantids (Hughes-Schrader, 1943; Inamder, 1949). The mantid studies from fixed preparations showed that while in prometaphase all possible orientation configurations occur (similar to tipulid ones), at full metaphase nearly all configurations were of the V-type, suggesting: (i) other configurations reorient to the V-type, and (ii) that the V-type is the only stable, or most stable orientation. The most striking feature noted by Bauer et al. is the very marked differences in stability (inverse of reorientation potential) of the V configuration; it being very strong in mantids but apparently weak in tipulids, the configurations in the latter being far more prone to reorientation. Superficially similar comparisons might be valid with the zig-zag alternate orientation in my

material, which resembles two V trivalents.   This orientation appears
to be very stable and is thus similar in behaviour to the V-type in
mantids.

If the considerations presented above on the patterns of
reorientation of the centrophilic interchange complex types in this
material are essentially correct, then it appears that instability (and
thus origin of reorientation) and polar location of a centromere are at
least to some extent associated with each other, perhaps functionally so.
From this point of view consideration can be given to one further orien-
tation type, that illustrated in Fig. 45a'.   It is apparently a stable
one.   It is characterized by having one end pair of adjacent centromeres
in monosyntelic orientation and three centromeres oriented to one pole,
only one to the other.   The configuration differs from the normal type a
in that the inner chiasma of the non-cooriented pair of centromeres is
very proximal in position.   Consequently, the abnormally oriented centro-
mere is not positioned markedly towards one pole as is usual for most type
a configurations (Figs. 46 and 57).   It has been suggested that such
configurations rather than the usual type a are responsible for the
10 : 8 segregations observed at anaphase I since they alone appear to
persist at early anaphase I.   Their presence and persistence might on
one hand be regarded as "accidents"; though the facts that only one type
of 3 : 1 orientation occurs in anaphase I and that there are peculiarities
associated with this one type, suggest rather that they are reasonably
stable.   Their frequency is low, probably not through frequent reorientation,

but because they <u>arise</u> in a low frequency.   The frequency of such proximal chiasmata at diakinesis and metaphase I is low and only a few of these complexes can be expected to become orienated in a type <u>a</u>' fashion.

The two non-cooriented, <u>end</u> centromeres of type <u>a</u>' show monosyntelic orientation, a feature shown by all unstable orientations, but not by the stable ones.   Monosyntelic orientation of adjacent centromeres therefore appears insufficient in itself in this material to induce reorientation.   On the other hand, the non-cooriented centromere and associated arms in <u>a</u>' are not located close to one pole as in type <u>a</u> and other unstable configurations.   This suggests that it is the near equatorial location of the whole complex that confers stability or relative stability on the type <u>a</u>' orientation.

Adjacent monosyntelic orientation is also found in stable adjacent orientations of interchange complexes (Fig. 45).   The equivalent orientations appear to be stable in the complex heterozygote <u>Rhoeo</u> <u>spathacea</u> also (Zimmermann, 1968).   In these cases perhaps the fact that each member of the monosyntelic pair of centromeres is cooriented with another adjacent centromere stabilizes the complex; that is, disyntelic coorientation suppresses the induction of reorientation.   On the other hand, it might be the equatorial position of adjacent complexes that suppresses reorientation of adjacent, monosyntelic orientation.

Considerations such as these suggest that factors which initiate or suppress stability and instability might interact with and counteract each other; and thus produce different reorientation potentials within a complex.

This discussion has emphasized monosyntelic orientation of adjacent centromeres, and polar or near polar location of reorienting centromeres, since the available evidence suggests these two features as being in some way directly associated with the induction of reorientations. It is clear that more detailed observations must be made, in fixed but more importantly in living material, in order to understand these phenomena in full.

II.   Developmental stages of anaphase I and their relationship with events of prometaphase.

Present studies have revealed a direct relationship between the frequency of alternate and adjacent complements at mid anaphase I and the developmental stage of anaphase in a pollen sac. It seems very improbable that the observed correlations arises through events associated with anaphase alone. Rather, the anaphase events appear to stem from certain characteristics of prometaphase. Two principles are considered: (1) The first cells that enter anaphase I in a pollen sac were the first cells to have achieved complete, stable orientation in bivalents and complex in the preceding prometaphase. The last cells that enter anaphase I were the last to have achieved full metaphase orientation. Similarly for the intermediate cells; (2) The first cells that achieve complete orientation and thus enter anaphase first show a frequency of about 4 alternate to one adjacent orientation. Cells that are somewhat later in achieving complete orientation and thus are later in entering anaphase, show a much lower frequency; while in still later cells this frequency increases, to about

5 : 1 in the last cells to reach full orientation.

There is some precedent for the hypothesis of a relationship between anaphase developmental patterns and events of the previous prometaphase. Wagenaar (1961) found in Triticum timopheevi X durum hybrids that metaphase I cells of anthers at late and very late developmental stages had significantly more univalents than those of early and mid developmental stages. All the results supported the interpretation that "the transition within an anther from metaphase I to anaphase I is a gradual process in which cells with few univalents proceed into anaphase I earliest, while the most irregular cells, containing the most univalents, pass into anaphase latest" (Wagenaar, 1961). The close synchrony observed in anthers at prophase stages indicated that the developmental sequence stemmed from events of orientation that led to metaphase I, rather than of anaphase I per se. The most regular cells (with few univalents and mostly bivalents) achieve a certain 'state of order', with full or nearly full equatorial orientation, relatively early in development, and were thus the first to enter anaphase. The more irregular cells were delayed in the attainment of this state of order and hence were delayed in their entry into anaphase. And the more irregular the cell the greater the delay in achieving the state of order.

This "state of order" concept was originally suggested by Carlson (1956) (see also Goulden and Carlson, 1951) from observations on grasshopper neuroblasts, in which anaphase was not initiated until certain centrophilic chromosomes reached the metaphase equator. Observations on

centrophilic bivalents and chromosomes in other materials agree with the concept (Bloom et al., 1955; Uretz et al., 1955; Bajer and Mole-Bajer, 1956; Nicklas, 1967) - cells do not progress through to anaphase I until full equatorial orientation has been achieved. It will become evident that my own data point strongly towards the same conclusion.

The developmental data of anaphase and prometaphase of the present interchange complement each other at least in part. As prometaphase in a pollen sac progresses the frequencies of type a and b centrophilic complexes change, that of b decreasing more rapidly than that of a (table 27). Whatever the underlying cause of this, if, as suggested previously, type a gives rise to alternate orientation and type b to adjacent orientation and the "state of order" concept holds, the alternate type segregations at anaphase should theoretically show an increase in frequency at least at later developmental stages of a pollen sac, as is seen (table 30). Fig. 71a is a duplicate of Fig. 71 with three regions A, B and C marked for reference in the following discussion.

Centrophilic complexes may not achieve full, stable orientation (alternate or adjacent) on passing through a single phase of reorientation. Evidence from the studies of Bauer et al. on tipulid spermatocytes showed indeed that one phase of reorientation does not necessarily bring about stable orientation (e.g. Fig. 3 in Rickards, 1965).

It was suggested previously from the basis of probable stable and unstable centromeres of the complex that during reorientation complex types b - d might be converted in part to type a before passing through

further phases of reorientation, and thus might be converted to alternate rather than adjacent stable orientations.   The greater the number of successive reorientations that a centrophilic complex has to undertake the greater the chance that it will become a type _a_ and then an alternate rather than adjacent orientation.

Originally it was thought that successive random reorientations of equally unstable complex types might provide a satisfactory explanation of the anaphase data.   Thus in Fig. 71a region A might represent those cells in which the complex was oriented in a stable fashion at the initiation of prometaphase, region B those in which the complex passes through but a single phase of reorientation, and region C those in which the complex passes through further successive reorientations.   However, a number of difficulties arise with this hypothesis.   Thus e.g. it is difficult to account precisely for the low frequency of alternate : adjacent orientation that is necessary to account for the sharp drop between E and M developmental stages of anaphase.   Also, it is not clear where cells fit into this scheme of successive random reorientation in which bivalents (but not the complex) have to pass through reorientation. They might be expected to show an alternate : adjacent frequency similar to region A (complex fully stabilized initially; 4 : 1) but enter anaphase in regions B and C.   This would probably be expected to cover up somewhat any general change in alternate : adjacent frequency during development.

These difficulties I feel make this explanation somewhat questionable and unsatisfactory.   Rather, a concept of differential instability, as

described below, gives a good explanation of the available data, and the basic tenets of such a concept have been adequately established in the work of Bauer et al. on tipulid interchange trivalents. Indeed, the present studies appear to provide a useful addition to these data on differential reorientation potentials.

It is possible that a large majority of centrophilic complexes, particularly the most frequent ones a and b, achieve stable orientation after only a single phase of reorientation. Indeed, Nicklas (1967) has suggested that there may be certain factors that directly stimulate disyntelic co-orientation during reorientation of monosyntelic bivalents. This might be so for chromosome complexes as well. Also observations on living material of tipulid spermatocytes indicate that different centrophilic complexes and bivalents may show marked differences in instability. Thus (i) monosyntelic bivalents are reoriented more quickly than unstable trivalents, (ii) different trivalents may show differential reorientation potentials and (iii) different unstable centromeres within a centrophilic complex may show differential instability. Similar cir-cumstances might occur in my material.

Regions A, B and C of Fig. 71a are described below from the point of view of differential instability. Basically these regions represent: (A) those cells in which the interchange complex was fully stabilized (alternate or adjacent orientation) at the initiation of prometaphase: (B) those cells in which type b centrophilic complex occurred and which reoriented to adjacent types in a high proportion of cases and

(C) those cells in which type a complex occurred and which reoriented to alternate types.

Region A.   Cells in which complete orientation in all bivalents and the complex is achieved at the initiation of prometaphase (require no reorientation) will be the first to enter anaphase (region A in part) and apparently do so in a frequency of 4 alternate : 1 adjacent (table 30 and Fig. 71).

Cells that pass through a phase of reorientation in one bivalent, the other bivalents and the complex having been fully oriented initially, would be expected to show alternate and adjacent orientation and segregation complements in a frequency of about 4 : 1 as well.   And it is probable that these cells are indeed included in region A, since reorientation in bivalents probably takes place some time before reorientation in a complex (Bauer et al, 1961 and Table 27 here).   Also, some or all of the centromeres of the complex are particularly prone to delays in spindle activity (the persistent activity that is sometimes observed is probably a pronounced expression of this phenomenon), which would cause delays in the achievement of complete orientation in the complex.

Similar argument probably applies to those cells with centrophilic bivalents and complex.   The bivalents tend to orient first leaving the centrophilic complex to determine the time at which prometaphase is completed in the cell.   In general it seems probable that the time a cell achieves full, stable orientation and hence the time a cell enters anaphase depends largely on the time at which the interchange complex

becomes fully oriented in a stable state.   Indeed, if this were not so,
but rather there were substantial compounding in anaphase of cells having
undergone reorientation of bivalents and complex at random times, then
it is difficult to see how a pronounced relationship between developmental
age and alternate : adjacent frequencies of orientation in the complex
would have been recognized.

If the above explanations are correct then region A as a whole
consists of cells in which the complex was initially oriented in a stable
state at the beginning of prometaphase.   No information is available at
present that would give a direct estimate of the number of cells in a pollen
sac in this category, though the impression gained from table 30 and Fig. 71
is that about 40% of cells are involved.

Regions B and C.   Of the cells passing through phases of
reorientation involving centrophilic complexes those with types a and b
(Fig. 45) are the most frequent and thus most important to consider.   It
is likely in view of available evidence that reorientations of these two
different complex types do not occur at equivalent times, or, in other
words, show differential reorientation potential.   It is suggested that
complex type b is more unstable than type a, cells involved in reorien-
tation of type b complexes to alternate or adjacent types being represented
in region B of Fig. 71a.   Reorientation of type a complexes might occur
relatively later in the development of prometaphase, the cells involved
being converted to alternate orientations in the complex and being
represented in region C.   Differential instability of this order is

considered since the prometaphase and anaphase data are most readily explained in this way.

Compared with other developmental stages a relatively high proportion of cells entering anaphase shortly following E stages are of the adjacent type. As suggested above this change is brought about by the entry into full metaphase and thus anaphase of type b complexes after reorientation. If it is assumed to begin with that the drop in alternate :adjacent frequency in M developmental stages is caused entirely through entry into division of those cells in which type b complexes were reoriented - no overlap occurring from region A or cells undergoing later reorientation - then one should examine how the apparent frequency of alternate : adjacent orientations (a little less than 2 : 1; see page 176) might be obtained in these cells. If only the end, unstable centromere of type b were to be reoriented, then only adjacent orientations would be produced. Similarly, reorientation of only the inner unstable centromere would produce only alternate orientations. If, however, the two unstable centromeres reorient with equal frequencies, then a 1 : 1 alternate : adjacent ratio would be produced.

It is probable, however, that the suggested differences in stability of centrophilic complex types a and b and unstable bivalents are not absolute. Also, all cells in a pollen sac do not enter prometaphase at precisely the same moment. For these two reasons there is probably some overlap in region B from both regions A and C, both of which are characterized by a relatively high frequency of alternate type

cells.   Further, table 27 suggests that centrophilic complex types c
and d are both reoriented very early in development since they are found
almost entirely in E pollen sacs.   On reorientation both these complex
types probably produce a high proportion of alternate type cells (see p.201).
Hence it is probable that reorientation of centrophilic complex type b
will be producing a higher frequency of adjacent orientations than super-
ficially evident from the data.

As indicated, region C probably represents a large proportion
of those cells in which centrophilic complex type a occurred and which
reoriented relatively late in development to alternate arrangements.
In the progression of anaphase in a pollen sac, as region A cells pass
out of mid anaphase (and therefore not scored any more) followed eventually
by region B cells, cells involving reorientations of type a complex become
increasingly more manifest, thus gradually raising the alternate : adjacent
ratio in the final stages of development.

Overall, four main classes of cells can thus be recognized as
passing through prometaphase to metaphase in sequence during development
in a pollen sac: (i)   those cells which achieve complete stable
orientation in all bivalents and the interchange complex at the initiation
of prometaphase.   No reorientation is necessary in these cells.
(ii) those cells in which the complex is initially oriented in a stable
alternate or adjacent arrangement, but in which centrophilic bivalents
occur and are reoriented (iii) those cells in which centrophilic complex
type b occurs (with or without centrophilic bivalents) and which are

reoriented to a high frequency of adjacent orientation. (iv) those cells in which centrophilic complex type a occurs (with or without centrophilic bivalents) and which are reoriented to alternate orientations.

Cells with complex type c probably complete prometaphase with or near those of (iii). Those with complex type d might complete prometaphase near those of (iii) though perhaps, at least in respect of the complex, before those of (ii) (completely monopolar trivalents in tipulids are more unstable than monosyntelic bivalents).

The above sequence is probably not absolute since not all cells enter prometaphase at precisely the same moment and differences in reorientation potential in bivalents and complex types are probably not absolute. The sequence, however, must be of sufficient order to be recognized at anaphase I.

III.   Chiasmata and orientation. Orientation frequencies in region A.

It has been argued that in a closed ring of four chromosomes a basic frequency of 2 alternate : 1 adjacent-1 : 1 adjacent-2 orientation should be expected at metaphase I (Rickards 1964; see also similar suggestions in Lewis and John, 1965[*], 1966), rather than a 1 : 1 : 1 ratio as sometimes considered (e.g. Jones, 1964). The reason for this is that orientation in a complex apparently has its basis in co-orientation of pairs of adjacent, linked centromeres, as in a bivalent, It has been considered further that the basic ratio of 2 : 1 : 1 is modified in chain forming complexes to a basic 3 alternate : 1 adjacent orientation, because one adjacent orientation is eliminated, these being converted to further alternate orientations.

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[*] See under John & Lewis, 1965.

This was considered to account for the ratio obtained in the present interchange complex.

It now appears more probable that these conclusions are not fully justified. While it is apparently true that the final stable orientation of a complex has its basis in co-orientation of pairs of linked centromeres, the actual process of orientation involves a tendency in many cases towards more random orientations of single centromeres, this apparently being the basis of the origin of centrophilic complexes. Thus general considerations on final overall orientation frequencies must take into account the types and frequencies of centrophilic complexes that arise during prometaphase, and then their behaviour during reorientation, if they are to explain adequately the achievement of a certain overall alternate : adjacent frequency.

The earlier general hypothesis may be more applicable to those complexes that achieve stable alternate and adjacent orientations at the initiation of prometaphase: i.e. do not pass through stages of centrophily and reorientation (region A of Fig. 71a). These complexes are being oriented initially on a strict bivalent basis. Present information shows that orientation of different pairs of linked centromeres in a complex may be modified by the genotype and by such features as the number and distribution of chiasmata (Rickards, 1964 e.g.). Some relevant publications will be summarised as a prelude to further discussion.

(a)   Selection and disjunction frequency.   The frequency of disjunctional segregation of interchange rings and chains in rye is at least partly under gene control (Thompson, 1956) and the frequency is, as expected, therefore also subject to selection (Lawrence, 1958). Thus

selection for high fertility, which accompanies and is determined by high disjunction frequency, will, over a number of generations, produce a relatively stable increase in disjunction frequency. Sun and Rees (1967) directly examined the effect of selection upon disjunction frequency in rye, showing that selection for high and low disjunction frequency in pollen mother cells at metaphase gives rise to distinct lines with relatively high and low disjunction respectively.

(b)   Chiasma frequency and disjunction. The work of Rees and collaborators on rye interchanges has also contributed evidence towards greater appreciation of Darlington's (1937) view that high chiasma frequency leads to a preference towards non-disjunction, and few, terminal chiasmata to disjunction. Rees and Sun (1967) established from comparisons of chiasma frequency in pollen mother cells within and between plants that variation in the disjunction frequency is dependent largely on the chiasma frequency of the interchange association. Thus high chiasma frequency in the interchange complex is associated with low probability of alternate disjunctional orientation.

(c)   The relationship between (a) and (b). Rees and Sun (1967) point outthat since chiasma frequency in rye is under gene control and subject to selection, as is disjunctional frequency in interchange complexes, then selective change in disjunction frequency may work through selective change in chiasma frequency, since chiasma frequency directly influences disjunction frequency. That is, the observation in rye summarised in (a) and (b) above are directly related to each other, being different manifestations of the same phenomenon. In part, similar considerations were presented by myself (1964) from theoretical grounds. Sun (1963 quoted by Rees and Sun, 1967) provided direct support for the suggestion, showing that selection

lines with high and low disjunction have low and high chiasma frequencies respectively.

(d)   A mechanistic view of the relationship between chaismata and disjunction.

The inference put on the view of Darlington (1937) concerning the relationship between chiasma frequency and disjunction is that under high frequency and non-terminalised chiasmata the mechanical forces of orientation are less able to manipulate a chromosome complex into an alternate disjunctional arrangement, which requires (apparently) a half twist being imposed upon the complex to achieve its disjunctional orientation and segregation (see comments in Rees and Sun, 1967; Rickards, 1964 e.g.). This inference, however, I feel is challengeable. While there can be little doubt that rigidity in a complex caused through many non-terminal chiasmata would affect mechanical manipulation of the complex into a zig-zag during orientation, it is not clear that a zig-zag form to the complex is necessary for disjunctional orientation and segregation. It is conceivable that such orientation and segregation from a ring could be achieved with the complex lying more in a flat plane on the equator without necessarily having to be manipulated into a zig-zag. Also, it may be that the chiasmata influence orientation in a more direct way, by influencing the actual orientation assumed in the first place by adjacent centromeres. The following comments summarise how this might occur.

The data and considerations of Nicklas (1967) strongly suggest that initial orientation to opposite poles of the two centromeres of a bivalent is determined by the apparent back-to-back disposition of homologous centromeres, in conjunction with their preferential orientation towards the pole

to which they most directly face (p. 194). If this is so, then a
greater degree of maintainance of the back-to-back disposition will
lead to a greater frequency of initial orientation to opposite poles.
Hence it can be suggested that the closer the chiasmata to the centro-
meres of a bivalent the greater will be the likelihood that a back-to-back
disposition of the centromeres will be maintained, and thus greater will
be their tendency to orient to opposite poles at the initiation of pro-
metaphase. Now in an interchange complex of four chromosomes for
example, the chiasmata may cause homologous pairs of centromeres to orient
to opposite poles in preference to non-homologous ones. Under conditions
where chiasmata occur and remain close to the centromeres in all four
pachytene parms of an interchange cross in which both interstitial seg-
ments are small, there would probably be but little disruption of the
prophase cross-like configuration before orientation. Homologous centro-
meres will therefore tend to retain a strict back-to-back disposition,
which will lead most frequently to their co-orientation, at the same time
leaving little chance for non-homologous centromeres to be positioned for
co-orientation to opposite poles. Complexes of this type might produce
a predominance of adjacent-1 type orientations in the complex as a whole.
The rigidity in the complex caused through chiasmata may perhaps interfere
with coorientation forces manipulating the complex into a zig-zag necessary

complex are remote from the centromeres, then the back-to-back disposition of homologous ones will tend to become relatively more disrupted than otherwise, thus leading to the potentiality of both non-homologous and homologous centromeres being oriented to opposite poles. This will produce a potential for disjunctional orientation. Further, any increase in chiasma frequency will tend to change this type of complex into one like the first mentioned, and thus cause a reduction in potential disjunctional orientation. Thus any increase in chiasma frequency in the complex will tend to produce a relative increase in non-disjunction, not because of increased rigidity in the complex per se, but because of the increased directing effect that proximal chiasmata have on homologous centromere orientation; and vice versa for a reduction in chiasma frequency.

This interpretation of the mechanical aspects of chiasma influence on orientation appears to provide better understanding to at least certain other problems of orientation of interchange complexes (Rickards, 1964). For example, it is well known that interstitial chiasmata reduce the frequency of adjacent-2 orientation in rings of four to almost nil (Burnham, 1956; Lewis and John, 1965; Rickards, 1964 p. 148).* An examination of Fig. 10 in my article, in the light of the above hypothesis, will suggest that with chiasmata at "a" and "b" the back-to-back disposition of the associated homologous centromeres is likely to be retained to a consider-ably greater degree than in a similar complex without the interstitial chiasma. Thus these two centromeres will orient to opposite poles at the initiation of prometaphase in a very large proportion of cases, leading to an absence of adjacent-2 orientation in the complex as a whole. It is perhaps significant in this respect that the one complex in the Chorthippus

* See John T Lewis, 1965.

interchange studied by Lewis and John (1963a) which had an interstitial chiasma and yet oriented in an adjacent-2 fashion, had half loops in place of closed loops i.e. no chiasma in the pairing segment "outside" the chiasmate interstitial segment. The open nature of the loop would tend to promote a greater disruption of back-to-back centromere disposition than with a closed loop, thus leading to a greater chance of these homologous centromeres becoming oriented to the same pole and thus the chance of adjacent-2 orientation in the complex as a whole.

Also relevant here are the general comments of Lewis and John (1966) on the coorientation of homologous centromeres. They consider that any preference of homologous centromeres to show orientation to opposite poles in interchange complexes does not rely on homology per se but on preferential position, i.e. on preferential centromere position in relation to neighbouring chiasmata. This I agree with entirely. It might be brought about at least in part by the chiasmata maintaining a back-to-back disposition of homologous centromeres, and thus causing their preferred orientation to opposite poles.

It is possible that relative centromere disposition is one of the principal factors determining initial centromere orientation in chromosome complexes (and bivalents), and thus one of the main factors determining the frequency of stable orientations that arise immediately at prometaphase initiation (require no reorientation). However, it is very difficult to conceive a likely disposition which would explain the alternate :adjacent frequency (4 : 1) in region A of Fig. 71a, which has been hypothesized as

comprising the cells in which the complex initially oriented in stable arrangements.   Some other guiding factor appears to be important here as well as centromere disposition.   Some evidence available at present suggests that this factor might be the relative position of the complex before orientation and its relation to the future metaphase poles. Evidence concerning relative placement of the interchange chromosomes at certain stages of meiosis has already been presented.   Relatively specific placement of the complex prior to metaphase is conceivable, and if present may be an important factor in regulating orientation patterns at the initiation of prometaphase.   A study of this problem is planned.

There are a number of ways by which the main hypothesis above can be tested.   These are being investigated and a fuller discussion will be prepared later for publication.

IV.   <u>Prefential position of the complex at MI and the relationship between position and orientation type.</u>

This topic has already been discussed in part (p. 149).   A clue towards a possible explanation of the relationship between position and orientation type, and thus perhaps a clue to the selective positioning phenomenon itself, comes from the fact that when the complex <u>does</u> occupy most central positions in the equatorial plane, it is mostly oriented in the alternate arrangement, which is the arrangement that arises in the <u>last</u> cells to pass through reorientation in the complex before completing prometaphase in the cell.

During initial prometaphase events the full complex, or initially co-oriented part of a centrophilic complex, probably takes up a selected

position in the metaphase equator such that it occupies end or near end positions in the lateral equator.   In those cells in which type b complex is reoriented, the delay in completing orientation might not affect ultimate positioning of reoriented complex parts and hence might not upset positioning of the whole complex.   In those cells in which type a complexes are reoriented (probably the last cells to complete prometaphase) the delay, however, might inhibit correct positioning, forcing at least the final co-oriented pair of centromeres (and possibly also the rest of the complex to some extent) into another position.   This latter position might be one or both of the two central positions in the three-dimensional spindle, which conceivably might be the only one(s) available.   The fact that the complexes involved here reorient to alternate arrangements would account for the relationship between position and orientation type.

The above comments deliberately have been kept superficial since the hypothesis is largely speculative/at present barely justified.   They point, however, to some of the difficulties encountered in explaining the perplexing phenomenon.   Clearly a lot more study is warranted.

V.   The "state of order" concept.   It was noted previously that the developmental data of Wagenaar, and my own, would be very difficult to understand but for recognition of the "state of order" concept.   The data can be adduced as evidence in support of the concept.   Some "block" at prometaphase/metaphase must be imposed upon a cell in division which inhibits its progression through to anaphase until orientation is complete. This overall block would be necessary of course since the initiation of

anaphase involves the complement of chromosomes as a whole and is not a
phenomenon associated with each chromosome at separate times. The block
is manifest in anaphase as developmental sequences of the type revealed
in this study.

The state of order in many instances is apparently not normally
achieved until every chromosome reaches equatorial orientation, as evident
e.g. in the experimental studies of Nicklas (1967). On the other hand,
removal of four or five bivalents from metaphase I cells of Melanoplus
does not block anaphase (Nicklas and Staehly, unpub., see Nicklas 1967,
p. 43; also Nicklas, pers. comm.). Thus the presence of a/non-cooriented
bivalent (or chromosome), or some feature associated with it, appears to
induce the block.

A state of order in hybrid Triticum cells was achieved when
approximately 70% of univalents reached the equatorial plate, making a
total of slightly more than 14 bodies in the equator, the number found
(as bivalents) in normal T. timopheevi and T. durum (Wagenaar, 1961).
This number appears to fulfil the cell's requirements in respect of the
state of order, univalents being able to substitute for bivalents.

In normal Allium triquetrum the state of order is probably reached
only when all nine bivalents have become co-oriented in the equator, as
evidenced by the absence of numerical non-disjunction in normal cells.
In fully oriented interchange cells there are seven bivalents and one
complex. In cells such as Fig. 47 with partially centrophilic complexes,
there are at least eight pairs of centromeres co-oriented in the equator
as bivalents, yet the state of order in these and similar cells is clearly

not yet reached (no corresponding anaphase segregations).   The presence
of centrophilic chromosomes in such cells may inhibit entry into anaphase,
rather than the presence of insufficient equatorially oriented bodies.   Thus
whereas cells with centrophilic complexes as Fig. 46 are blocked and do not
enter anaphase, those as Fig. 57 in which no truly centrophilic,polar
chromosomes or centromeres occur, are allowed to progress through into ana-
phase.   Thus the presence of centrophilic, non-cooriented centromeres in
some way may induce the anaphase block.   This is undoubtedly not the whole
answer, in some species at least, since in the studies of Wagenaar the
conditions for anaphase were satisfied when approximately 70%, not all, polar
univalents have moved to the equator.   Such a concept would, however, account
for the observation of Nicklas that removal of bivalents does not block
anaphase.

The presence of centromeres which apparently are and remain passive
during prometaphase (p. 161) does not appear to induce an anaphase block in
A. triquetrum, since cells containing such centromeres regularly pass into
anaphase.   Thus the presence of a block appears to centre round presence
of active, non-cooriented, centrophilic centromeres (rather than whole chromo-
somes).   Thus inactivity of a centromere in the complex of interchange cells
here is possibly a parallel to the removal of bivalents in Melanoplus.

The two pollen sacs specially recorded on p. 179 are characterized
by having a relatively high proportion of cells past and pre mid-anaphase I
compared with more normal pollen sacs of equivalent developmental indices.
It appears as if some factor has inhibited further entry of cells into anaphase
in these pollen sacs.   This is possibly related at least indirectly to achieve-

ment of a state of order in these cells.   However, it may be significant

that the joint percentages of cells "in" and "past" in each of these two

pollen sacs is about 40%; which suggests a relationship with region A of

Fig. 71a.   Thus the delay in entry of further cells into anaphase in these

pollen sacs might centre round reorientation in the interchange complex.

These comments again emphasize the desirability of obtaining further in-

formation along these lines.

1.   POLLEN   STUDIES

<u>İ.</u>   <u>Pollen abortion</u>

Spores produced from mother cells in which the interchange complex was oriented in an alternate fashion are chromosomally and genetically balanced.   Though half of these spores will contain the normal chromosomes 4 and 6 and the other half the interchange chromosomes 4^6 and 6^4, both can be expected to be fully functional.

Spores produced from mother cells in which the interchange complex was oriented in an adjacent fashion, on the other hand, are chromosomally and thus genetically unbalanced.

(a)   Half of these spores contain both chromosomes 4 and 6^4.   They will be duplicated, therefore, for most of the short arm of 4, and deficient for most of the short arm of 6 (only a small amount of chromatin).

(b)   The other half of these spores contains chromosomes 6 and 4^6.   They will be duplicated for most of the short arm of chromosome 6 and deficient for most of the short arm of 4.

Comparing these two types of spores - (a) and (b) - it will be seen that the duplication in (a) is considerably greater than that of (b), while conversely the deficiency in (b) is considerably greater than that of (a). Both types of spores might be expected to abort because of their genetic unbalance.

For some time after the completion of meiosis the microspores remain within the original mother cell wall.   There is no sign of impending abortion at this stage.   Some time before any sign of the initiation of pollen

grain mitosis the spores are liberated from the mother cell wall.   At this stage they have enlarged somewhat; they possess one or two nucleoli.

The first visible signs of abortion occur shortly before the onset of pollen grain mitosis I, the two phenomena undoubtedly being related.   The initiation of pollen grain mitosis is indicated by a movement of the nucleus to one side of the spore, where it becomes characteristically flattened and then transformed into prophase chromosomes.   Aborting nuclei show this movement, but there is no or very weak transformation into chromosomes.   Rather, the chromatin becomes broken down from within outwards giving, at intermediate stages, a very characteristic "ring" configuration to the nucleus (Fig. 81).   The breakdown continues to completeness, and during the same period of time the cytoplasm also breaks down, as evidenced by the gradual loss of staining capacity (aceto-carmine preparation). Finally the spores are completely void of contents.   Most abortive spores are empty by the time pollen grain mitosis in normal spores has been completed throughout a pollen sac,   Following pollen grain mitosis normal spores grow considerably so that at later stages of development there are considerable size differences between normal and abortive pollen (Fig. 80).

A second type of aborting spore is often encountered.   Instead of a "ring" stage being formed the nucleus clumps into one corner of the spore and becomes rather vacuolate (Fig. 81).   The nucleus eventually breaks down from outside inwards (cf. above).

There is unlikely to be any clear cut relationship between genome differences and type of abortion, since the "ring" type predominates very

strongly over the "clump" type.   However, neither of these two types of abortion have been seen in the small percentages of abortive pollen observed in normal material, so it appears as if they are characteristic of the interchange.   Indeed, at the equivalent stage of pollen sac development there is virtually no pollen abortion in normal plants.

As pollen grains approach maturity some additional aborting spores appear.   These are usually smaller grains with heavily darkened cell walls and weakly staining contents.   These can be seen in both normal and interchange material.

II.   Percentage pollen abortion

The technique employed and the precautions necessary have been described.   Preliminary observations on normal material revealed some important facts to be considered in attempting to assess the percentage of pollen abortion brought about by the interchange.

(a)   the percentage abortion shown in normal plants differs considerably between plants of different clones, even when these have been grown under very similar environmental conditions.   This is probably due to genetic differences between different clones.   For this reason only control 9 plants (see under material) were used to obtain abortion percentages for normal plants.   As discussed previously the interchange plants are closely related to these control plants and pollen abortion studies on normal plants obtained from selfing interchange plants have shown that these are very

similar to control 9 plants in respect of percentage abortion.

(b)   The percentage abortion varies between different anthers of the same flower, different flowers of the same plant, and different plants within a clone.   This is probably due largely to environmental differences during flower development.

(c)   Most abortion occurs round the time of pollen grain mitosis though it increases slightly during development of an anther from a pollen grain mitosis stage up to dehiscence.

In the present abortion studies anthers were selected for analysis when cells were at a stage shortly after pollen grain mitosis I.   General morphological conditions (pollen size, nuclear size, cytoplasm density etc.) enable one to judge reasonably accurately the developmental stage of the anther. Restricting studies to this one developmental stage enabled a reasonably accurate assessment to be made of abortion brought about by the interchange.

In the normal plants used the percentage pollen abortion at the desired developmental stage ranged from approximately 0.5% to 5.0% in ten anthers examined, with a mean in this sample of 2.1% (details in appendix table 4a).

In interchange plants visible abortion, as evidenced by the proportion of empty grains, ranged from approximately 10.5% to 15.2% in 25 anthers examined, with a mean in this sample of 12.2% (details in appendix table 4b).   This is clearly much higher than that found in normal plants.

Since the adjacent orientation of the complex at meiosis occurs at a frequency of about 25%, and since these orientations give rise to

unbalanced spores, about 25% of pollen abortion should be expected, if all duplicate/deficient spores abort at this developmental stage. The obtained percentage is considerably lower than this expected value, suggesting that some of the duplicate/deficient spores are passing through pollen grain mitosis, and thus not showing up at this developmental stage as abortive grains. An examination of anthers at a developmental stage just prior to dehiscence showed that here also the percentage abortion is only about half that expected.

The differences between the extremes in the range of abortion in normal and interchange plants are approximately the same, i.e. about 5%. It is reasonable to expect that interchange plants would show the same "natural" abortion (that not directly associated with the interchange) as occurs in normal plants. Thus if one subtracts the mean abortion of normal plants from the mean of interchange plants, a value of a little over 10% is obtained*. This value of a little over 10% must be that which is directly attributable to the interchange.

A careful study of apparently normal pollen grains (i.e. normal as far as stainability in acetocarmine is concerned) in interchange anthers showed that at the developmental stages examined occasional pollen grains occur that appear "good", except that they have not passed through pollen grain mitosis. These spores probably abort or remain non-functional at later stages of development. An estimation of the percentage of these

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* Strictly, a small correction should be employed here since some abortion of interchange anthers will have been brought about jointly by "natural" and interchange causes. This correction, however, is so small as to be negligible as far as general conclusions are concerned.

grains is difficult to obtain since they can be confused with normal grains in which the generative and vegetative nuclei are lying atop each other and thus virtually indistinguishable. However, their frequency is in the order of 1 or 2%. These probably also represent pollen that is at least non functional because of effects brought about by the interchange.

The percentage abortion obtained in interchange plants itself suggests that one of the two types of unbalanced pollen grains is not aborting at this developmental stage, but rather is passing through pollen grain mitosis to give apparently normal spores. On this basis, an abortion of about 12.5% would be expected, a proportion that is closely approached if those spores which do not pass through pollen grain mitosis but otherwise appear good, are taken into account.

It is not altogether surprising to find such a low percentage of abortion compared to expected in this interchange material, since one of the two types of unbalanced spores is deficient for only a very small piece of chromatin. It is generally recognized that deficiencies cause abortion rather than duplications, and that smaller deficiencies are more tolerable than larger ones. Burnham (1956) found in maize that small interchange deficiencies may not cause abortion of spores on the male as well as the female side.

Studies of cells in pollen grain mitosis reported below reveal that the above suggestions are correct.

III. Pollen grain mitosis I.

Those chromosomes which can be identified in root-tip mitoses

(section 1) can often be distinguished at metaphase of pollen grain mitosis I, though sometimes not so clearly. The chromosomes are somewhat smaller than in even moderately contracted, colchicine, root-tip preparations. Fig. 82 shows the complement from a normal plant. The satellites on chromosomes 7 and 9 are usually visible, that of the former, as before, being most variable in morphology. When both satellites are clear as in Fig. 82 all the group III chromosomes can be distinguished with certainty.

Two types of pollen grains are produced from the balanced alternate orientation and segregation of the complex at meiosis. One of these contains the normal chromosomes 4 and 6 and is thus indistinguishable from the complement of normal material. The other type contains both interchange chromosomes 4^6 and 6^4, and can be distinguished from the former type most readily by the relative lengths of the group III chromosomes. In normal pollen, as in root-tip cells, chromosome 6 is larger than 7 (Fig. 82) while in interchange pollen 4^6 is considerably shorter than 7 (Fig. 83), hence distinguishing this type of pollen grain. Identification of other group II and III chromosomes (e.g. 4 or 6^4) is somewhat hazardous because they do not always lie in one plane and thus can appear fore shortened.

Of the two types of pollen grains produced from the adjacent orientation and segregation of the complex, one type passes through pollen grain mitosis I. This/type (a) of p.228 with both group I chromosomes 4 and 6^4, and the one containing the smallest deficiency (Fig. 84). These pollen grains are readily identified by the presence of only two group III chromosomes (7 and 9) and five group I (1 - 4 plus 6^4).

No case of the other type of duplicate/deficient pollen grain (type (b) of p. 228 ;3I, 2II, 4III) has been seen in the many cells in

mitosis that have been examined.   These must be the ones that abort.

One other type of pollen grain passes through mitosis I, the duplicated product from 3 : 1 numerical non-disjunctional in meiosis. These grains have a total of 10 chromosomes, with 4 instead of 3 group III types (Fig.85).   The complementary, deficient spore has not been observed and probably does not pass through pollen mitosis I.

Initial studies attempting to observe unbalanced pollen grains passing through pollen grain mitosis I were completely unsuccessful, even though considerable numbers of grains were examined at metaphase.   Later, one anther was obtained in which a very high and unexpected percentage of pollen grains examined were of the unbalanced type, and this, together with the developmental stage of the anther involved, suggested that a relation-ship exists between time of entry of these unbalanced pollen grains into mitosis and the developmental age of the anther.   This was subsequently confirmed on examination of a further 9 pollen sacs (rather than anthers; for the same reason as before) at a variety of developmental stages.

Pollen sacs showing mitotic stages can be divided roughly into three developmental stages: (a) those showing most grains still in inter-phase or very early prophase, with only a few (about 25% or so) in various stages of mitosis;   (b) those in which a high percentage or grains are in mitotic stages (up to 65% has been noted so far), the rest about evenly past or pre division;   and (c) those in which most grains have passed division, but a few (about 20% or so) remain at various stages.   There is still quite a considerable degree of synchrony within a pollen sac at these

stages, a carry over from the early meiotic condition.   In these three

broad developmental stages, designated early (E), mid (M) and late (L)

below, noticeable differences in the percentage of aborting and aborted

pollen grains can be observed, with the frequency approaching 10% or so

at the later stages examined.

Of the nine pollen sacs scored in this analysis, two were judged

to be at E, four at M and three at L developmental stages.   Table 32

presents the data obtained.

In both E pollen sacs all 73 grains scored were of either the

normal or interchange balanced complements.   In the M sacs only 5 of the

215 pollen grains observed at metaphase were of the unbalanced type (approx.

2.5%).   However, in the three L sacs, 33 out of 61 (approx. 54%) were of

the unbalanced type.

The above data contrast vividly with expectation.   If all of this one

type of unbalanced spore are viable at this stage, then about 17% of pollen

in mitosis should be of such a type*.   The data, along with the evidence

obtained before the detailed analysis was conducted, show that the unbalanced

grains are delayed in their entry into mitosis compared with the normal

balanced grains.   This fact becomes even more striking when it is recalled

that late pollen sacs in anaphase I and II show a higher than average

frequency of alternate type complements, i.e. ones that contribute to

balanced pollen grains.

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* Anthers consist of approximately 75% balanced and 12.5% unbalanced grains
  that enter mitosis, plus 12.5% that abort.   Of the grains entering
  mitosis, 12.5/75 x 100/1, or about 17% are therefore of the unbalanced
  type.

Table 32   Numbers of normal and unbalanced pollen grains

in mitosis I from nine pollen sacs at early (E), mid (M)

and late (L) developmental stages (see text for criteria

of classification).   The whole pollen sac was not scored

when at a mid stage of development.

Table 33   Data on mean seed set per flower from four

types of pollination using normal (N) and interchange (I)

plants.   Data in brackets from normal plants of clone A

control 9.

* Not significantly different.

** Very significantly different (p. $<$ 0.001).

Pollen sac no.	D/ment stage	No. of pollen scored	Normal	Unbalanced 9 c/some	Unbalanced 10 c/some
1	E	33	33	0	0
2	E	40	40	0	0
3	M	60	60	0	0
4	M	59	57	2	0
5	M	41	40	1	0
6	M	55	53	2	0
7	L	34	16	17	1
8	L	16	8	8	0
9	L	11	2	8	1

Table 32

Nature of pollinat-ion.	No. of flowers	Total no. of seeds.	Mean no. of seeds per flower
N x N	151 (58)	620 (246)	4·11 (4·24)
N x I	235 (47)	953 (190)	4·06 (4·04)
I x I	245	761	3·11
I x N	236 (25)	756 (77)	3·20 (3·08)

Table 33

In pollen sacs 3, 4 and 7 the frequencies of the two types of pollen from the balanced, alternate orientation and segregation of the complex were scored. In pollen sacs 3 and 4, 35 cases were able to be scored with certainty, 20 of which had the normal complement(chromosomes 4 and 6) and 15 had the interchange (chromosomes 4^6 and 6^4) complement. In pollen sac 7, nine cases were scored with values 4 and 5 respectively. The figures give no/suggestion, therefore, that these two types of pollen grain show differential behaviour in respect of entry into mitosis. This was expected since both types of pollen are balanced, though it is interesting that the rearrangement of the chromosomes in the interchange has not caused any noticeable deviation from expected in respect of viability and entry into mitosis.

Two cases of pollen grains with 10 chromosomes as in Fig. 85 were observed in the 9 pollen sacs scored. Both occurred in pollen sacs at late developmental stages (table 32). Perhaps these unbalanced grains are also relatively delayed in their entry into mitosis, though the data are insufficient to be clear on this point.

In view of the above phenomenon it is impossible to evaluate directly the percentage of unbalanced pollen passing through mitosis I. Some points can be considered, however, in respect of pollen sac no. 7, in which some further details of developmental stage were obtained. The pollen sac showed the following, approximate composition: 4% pre mitosis, 17% in mitosis and 69% past, with the remaining 10% abortive or nearly so.

Of the 17% in division about 50% of cells at metaphase stages showed unbalanced pollen complements, and at least this frequency probably

extends to those cells in prophase and pre division.   Thus about 50% of

21% (4% plus 17%) or 10.5% of the pollen grains in the sac are of the

unbalanced type.   This is close to but a little less than the expected

12.5% if all such spores are entering mitosis.   A few unbalanced pollen

grains in this sac had probably already passed division (as evidenced by

their rare occurrence in sacs 4 - 6) and this would contribute to the

above discrepancy.   But also, the last pollen grains to enter division

probably have a higher than 50% frequency of unbalanced grains.   This is

evidenced by pollen sac 9, the only other from which the desired details

were obtained*.   The composition of this pollen sac was 2% pre **, 3% in

and 84% past and the remaining 11% abortive.   Of the 11 pollen grains

scored nearly 73% were of the unbalanced type.

Thus it appears that virtually all unbalanced pollen grains of the

potentially viable type pass through mitosis I, are roughly equally delayed

in entering division, and are thus nearly all present in or pre division in

a pollen sac at a developmental stage as that of no. 7.

Several authers have published reports on differential behaviour

of pollen in respect of entry into mitosis.   Östergren and Östergren (1964)

found that pollen grains with X-ray induced undivided chromosomes enter

division later than normal grains.   Gülcan and Sybenga (1967) reported that

in auto tetraploids of Tradescantia virginiana , Lycopersicon esculentum and

Secale cereale the first pollen grain mitosis took place considerably later

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* Initially developmental age was assessed roughly as indicated previously.
Preparations from pollen sacs 7 and 9 had been kept for photography etc.
in a semi permanent state and were thus able to be scored more precisely
when the desirability of this became evident.

** Some of these probably never pass through mitosis I, but eventually
-- abort; see comment on p. 232 .

in hypo and hyper aneuploid cells than in euploid ones. But of most interest here is the report of such differential behaviour at pollen grain mitosis in _Allium_ _paniculatum_ by Ved Brat (1967). Here unbalanced haploid and subhaploid pollen grains, produced through meiotic irregularities in a clone of structural hybrids, are delayed in entry into mitosis I, such pollen grains being more frequent in anthers in late developmental stages compared to early ones. This is very similar to the situation described above for A. triquetrum .

Preliminary investigations have been made on pollen germination in normal and interchange material to test whether the above unbalanced pollen grains germinate or not. These studies are largely imcomplete at present and do not warrant reporting. Indications are that in interchange material there is indeed a proportion of pollen grains that does not germinate, or is retarded in germination, this proportion probably representing the un-balanced pollen. Further investigations are to be carried out to test these suggestions.

2.   OVULE AND EMBRYOSAC STUDIES

In normal plants two ovules develop in each of the three locules
of the ovary, giving a total of six ovules per flower.   At maturity (as
judged from style conditions) the ovules are large and very fleshy.

Interchange flowers also have six ovules per ovary (no exception
in 25 flowers examined), and at maturity the ovules are indistinguishable
from those of normal flowers in size and general morphological features.
From this point of view, then, there is no ovule abortion in these interchange
plants.

Ovule abortion, however, is not the precise female equivalent of
pollen abortion;   hence some embryo sac studies were conducted.   Three
mature ovaries were serially sectioned longitudinally, and the embryo sac
of each ovule was reconstructed from these sections.   One ovary from a
normal flower was also sectioned to act as a control.

A thorough investigation of the nature and development of the
ambryo-sac in normal material has yet to be carried out, but general obser-
vations show that the sac is eight nucleate at maturity, Fig. 86a illustrates
a normal ovule (from an interchange flower) showing general features;   Fig. 86b
shows the embryo sac from the same ovule in greater detail;   the section shows
only one large nucleus (probably an endoploid synergid) of the eight nucleate
embryo sac.   This picture is obtained from all ovules of normal plants and most
ovules of interchange plants.

Ovules with abortive embryo sacs have been identified in interchange
material.   Fig. 87a shows one of these from the same flower as Fig. 86 (the two
ovules are thus of the same developmental age).   No embryo sac is present in

this ovule. The space within the inner integuments, which is normally occupied by the mature embryo sac at this developmental age, is virtually empty (Fig. 87b). A tongue-like structure (in sectional view) projects from the base into the cavity. This was identified for me by Dr F.B. Sampson as being indicative of a complete failure to form an embryo sac. No signs of megaspore or other relevant cells could be identified within this structure. Apart from the absence of an embryo sac, the ovule is apparently normal, thus accounting for the inability to recognize these abortive ovules when unsectioned.

Of the 18 ovules examined from the three interchange ovaries sectioned, three (17% approximately) were of the abortive type, all others being apparently normal. These values are too low at present to be clearly indicative of trends, though it appears likely that all unbalanced spores on the female side (assumed 25%) fail to give rise to functional embryo sacs. Further studies are desirable to determine the precise nature and development of the normal embryo sac, to establish the developmental stage at which breakdown occurs in interchange material, whether any fine differences occur in the method and timing of breakdown between the two types of unbalanced spores, and whether these abortive ovules occur in the expected frequency of approximately 25%. That the latter is so is strongly suggested from fecundity studies described in the next section.

3.   SEED SET IN NORMAL AND INTERCHANGE PLANTS

I.   The Data Obtained

Details of seed set were obtained from crosses between normal
and interchange plants (both ways), and from selfed and crossed interchange
and normal plants. Some details were also obtained from apparently normal
populations growing in the wild. The data are summarized in table 33(p. 236).

(a) Crosses between normal plants (N X N). Most of these crosses were
made between and within cultivated clones obtained for diverse reasons from
a number of different localities. Crosses made between plants of only one
clone (clone A of control 9) showed no significant differences. The mean
number of seed set per flower was 4.11 (range from 0 - 6; flowers with 0
not scored).

(b) Normal (female) X Interchange (male) (N X I). The normal plants used
initially here were from three different clones (indistinguishable from each
other cytologically, though with minor floral differences). Data obtained
from crosses with clone A showed no significant differences. Mean seed set
4.06; not significantly different from (a) above.

(c) Interchange X interchange (I X I). Data from these crosses showed no
significant differences between crossings and selfings (interchange plants are
probably all of one clone, so the distinction is probably meaningless). The
data in table 33 include both these types of pollination. Mean seed set 3.11;
very significantly different from (a) and (b) above (p. $<$ 0.001).

(d) Interchange (female) X normal (male) (I X N). Normal plants initially
as (b) above; later data only from clone A (no significant differences). Mean
seed set 3.20; not significantly different from (c); very significantly diff-
erent from (a) and (b).

    The range in seed set (0 - 6) is the same for all types of crosses.

    One fact emerges clearly from these results. The mean seed set of
approximately 4 per flower when normal plants are the female parents
((a) and (b)) is reduced to approximately 3 per flower when interchange
plants are the female parents ((c) and (d)). The reduction of one quarter
is not affected by the male parent (interchange or normal).

Flowers with no seed set. As indicated above the number of seeds set per
flower ranges from 0 - 6 (the latter rare) in all types of pollinations.
The later flowers on an inflorescence generally form fewer seeds than the
first formed ones; and in the more heavily flowered inflorescences the bottom,
youngest flowers are the ones that commonly set no seed at all. This same
tendency occurs in natural pollinations of plants in culture, and also appears
to be a general phenomenon of wild populations, though some of the latter
appear to show lower frequencies of flowers with no seed set. There appeared
to be no significant differences in the number of flowers failing to set
seed between hand and naturally pollinated cultured plants used in these
studies.

    Since the failure of some flowers to set seed appears to be a
variable yet general phenomenon, not related specifically to the interchange
or normal plants used in these studies, they were not recorded in the analyses
reported above. Scorings were made only when flowers set at least one seed.
It is probable that the chief factor, discussed below, that causes the

reduction in seed set when interchange plants are the female parents, would cause some flowers to set no seed when otherwise they would have set one or a few. These failures should technically have been incorporated into the data. Hence the difference between mean values obtained above when normal and interchange plants are the female parents, a measure of the effect such interchange plants have on fecundity, is probably a little smaller than actually obtains. However, the frequency of such types of total seed set failure is likely to be very small and hence of little significance to the general results and conclusions (further comments below).

II.   Post pollination development of ovules.

A day or so following pollination the fertilized ovules and ovary as a whole begin to swell. As the ovules develop their testas darken, eventually to a uniform black. Slight differences in seed size are usually observed in all crosses, but not until long after seeds have been shed and have become dried and wrinkled.

The ovules that fail to develop into seeds show none of the usual post pollination signs of development. They remain apparently unchanged for some time as the fertilized ovules enlarge and darken, but eventually they wither and shrink to a small white ghost, still attached to the placenta. Normally their testas show no darkening, though occasionally one or both sides may develop patches of grey or black colouring when they lie adjacent to one, or between two, normally developing (darkening) ovules. This appears to be a direct influence of fertilized ovules on the appearance of adjacent, non-developing ones. These undeveloping ovules with small dark patches wither

and shrink eventually as do unaffected ones.

No differences can be detected in the course of events followed by non-developing ovules in the different pollinations conducted. Also, these events, in crosses involving interchange plants as the female parent, are indistinguishable from those that follow in ovules of emasculated normal flowers that are bagged to prevent pollination.

III.   The nature of failure to set seed

(a)   A Major factors causing the failure of some ovules to develop into seeds in crosses within wild and cultivated normal populations are probably hand pollination techniques and environmental influences. Unripe pollen or immature stigmas may have been used in some pollinations, or environmental conditions may have been adverse, all of which would be expected to have reduced seed set. Ved Brat (1965) states that in Allium triquetrum the later flowers of an inflorescence set only 1 - 2 seeds per capsule because they are self rather than cross pollinated (reduced protandry in later developed flowers). Though there is a reduction in seed set between first and later flowers on an inflorescence in plants I have examined, it appears unlikely that selfing in place of cross pollination is a principal cause. I have noticed no reduced protandry in later developed flowers of an inflorescence; there is no clear difference in seed set between selfed and crossed plants of different clones used here; and first formed flowers of an inflorescence, when selfed, frequently set 4 - 6 seeds per flower. The later developed flowers on an

inflorescence and their pedicels never grow as large as the first formed ones; indeed the whole vigor of these flowers is somewhat less than first formed ones, and it appears that reduced seed set is a further manifestation of this phenomenon (though this of course is not an explanation).

(b)   Variations in pollination technique and environmental conditions can be assumed to be random in occurrence, so the reduction in seed set between normal and interchange plants, when the latter are the female parents, must be of a different nature than in (a) above. The reduction achieved is approximately 25%, roughly the same as the percentage of unbalanced spores produced in male meiosis. Since it is probable that female meiosis produces the same percentage of unbalanced megaspores, the observations suggest that these unbalanced female spores are the cause of the reduction in seed set. This would account for both the percentage reduction achieved and the fact that it is shown only when interchange plants are the female parents*.

The data from these seed set studies and those previously reported on embryo sac abortion clearly complement each other. Indeed, the 25% reduction in seed set can be adduced as evidence in support of the suggestion made previously that about one in four megaspores on the female side is of the unbalanced type, indicating 25% adjacent orientation in the complex during female meiosis, and that these unbalanced spores fail to develop into functional embryo sacs.

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*   A particular ovule has 1 chance in 4 of being an unbalanced type. Hence the chance of all ovules in a flower being of the unbalanced type is $1/4^6$ (see relevant comment on p. 244).

Thus the data on orientation frequencies and abortion percentages on the male and female sides parallel each other.

In interchanges in general, ovule "abortion" is usually only revealed as undeveloped seeds (Burnham, 1956). In maize, plants with a 1 : 1 ratio of alternate : adjacent type orientations at metaphase I show about 50% pollen abortion and only 50% of the normal seed set (Brink, 1927; Burnham, 1962).

SUMMARY

A study was made of normal and interchange heterozygous individuals of the onion weed, _Allium triquetrum_. Somatic and meiotic chromosomes were studied, an investigation made of meiosis, particularly in the interchange material, and pollen, ovule and seed set studies have also been conducted. In these studies particular emphasis was given to phenomena associated with the achievement of metaphase I in meiosis, aspects of which have been discussed in the light of the data obtained and other published works.

1. The somatic chromosomes of normal _Allium triquetrum_ $(2n = 18)$ can be classified into three distinct groups (I, II, and III) according to sizes and centromere positions. One of the four pairs of the group I chromosomes (4) can be recognised consistently and thus characterised well because of its smaller size and asymmetry. The two pairs of chromosomes in group II (5 and 8) can be recognised with reasonable consistency. In group III all three pairs of chromosomes (6, 7 and 9) can be recognised consistently and thus characterised well because of size differences and different satellite characteristics.

2. All six of the nine pairs of chromosomes that can be recognised consistently show a tendency to associate in homologous pairs in root tip cells.

3. The arms of chromosomes 4 and 6 (the two examined in some detail) show essentially the same degree of interhomologous variation over the 16 cells examined in detail.

4. The recognisably distinct chromosomes of <u>Allium triquetrum</u> do not show differential patterns of contraction in long and short chromosomes or chromosome arms over the range of lengths studied.

5. The somatic chromosome complement of the interchange <u>Allium triquetrum</u> studied here is different from the normal complement in respect of chromosomes 4 and 6. It is concluded that the interchange involved one of each of these chromosomes. The interchanged members are referred to as 4^6 and 6^4. Measurements of normal and interchange members of pairs 4 and 6 showed that the interchange involved the short arm of both these chromosomes. The interchange break points must have been close to the centromeres of both chromosomes.

6. As in normal material the chromosomes of interchange material show a tendency towards association of homologues. Members of 4 and 4^6 show association but not 4^6 and 6. This evidence confirms the identification of the chromosomes and chromosome arms involved in the interchange.

7. A general survey and comparison of meiosis in normal and interchange material show that the interchange appears as a chain of four chromosomes, or rarely as a pair of unequal bivalents, in prophase - metaphase I stages. The chain is oriented in two basic ways at metaphase I (alternate and adjacent types), though occasional abnormal orientations arise. The alternate and adjacent arrangements occur in a frequency of 3 : 1. Segregation from the basic orientations gives rise to distinct chromosome complements at anaphase I and anaphase II.

8. Late pachytene/early diplotene stages of meiosis in interchange material revealed certain unusual features that are characteristic of this interchange complex : (a) consistent asynapsis in localised segments of the complex, (b) wide separation of the complex 'halves', and (c) stretching of unpaired segments. The latter two features are also found in later diplotene stages.

Limited measurements of the chromosomes at pachytene/diplotene stages are compatible with the conclusion that the interchange involved the short arms of chromosomes 4 and 6, and confirm also the conclusion that the interchange break points must have been close to the centromeres in both chromosomes.

9. Chiasma frequency is proportional to chromosome length in this species .

Chiasma studies in normal and interchange cells revealed (a) a significant reduction in the expected chiasma frequency in the inter-change complex, brought about by asynapsis at pachytene; (b) a reduction in the overall mean chiasma frequency per interchange cell compared to that of normal cells, brought about by the reduction in the complex; and (c) no evidence of negative correlation between groups or within groups of bivalents in normal and interchange cells.

10. Prometaphase stages in normal material reveal polar oriented (centrophilic) bivalents which later, however, must be reoriented to stable orientations along the equator. Equivalent polar or partly polar orientations involving the complex are found in interchange material. There are four of these centrophilic orientations in the complex (Fig. 45) and these show evidence of changing relative frequency during the development of prometaphase in a pollen sac. Thus types

c and d occur frequently in young pollen sacs but not in old ones; and in early pollen sacs type b predominates while in late sacs type a does. Overall these centrophilic orientations in the complex are unstable and are reoriented to alternate or adjacent orientations during prometaphase.

11. Though the relative frequency of the alternate and adjacent orientations at metaphase I is approximately 3 : 1, that of their anaphase I segregation types is shown to vary with the developmental age of the pollen sac. The relative frequency is about 4 : 1 in young pollen sacs, 2.5 : 1 in mid aged pollen sacs, and about 5 : 1 in old pollen sacs. A similar though less obvious relationship is shown to occur for anaphase II segregation types. It is thus essential that when scoring for alternate and adjacent orientation frequencies in this material to score pollen sacs in which all the cells in the sac are synchronised at metaphase I.

12. The developmental data of prometaphase (10 above) and anaphase (11 above) complement each other, and can be explained on a basis of (a) a 'state of order' concept whereby cells do not proceed into anaphase until all chromosomes are fully oriented along the metaphase equator, and (b) a concept of differential behaviour of unstable centromeres of centrophilic complexes during prometaphase reorientation. The available data are regarded as providing new evidence in support of both the above concepts.

13. The interchange complex is shown to be positioned preferentially in the three dimensional spindle such that it most often occupies outside or near outside positions in the flattened lateral metaphase plate. Also the type of orientation in the complex is related in part to position in the equatorial plate. At present these phenomena are largely

unexplainable, though they mean that it is essential when scoring for alternate and adjacent orientation frequencies at metaphase I to examine only those preparations in which a very large proportion of cells can be scored.

14. Persistent discordant orientations in the interchange complex occur in low frequencies at metaphase I, these being of two main types : (a) those in which three centromeres in the complex are oriented to one pole, only one to the other pole; and (b) those in which one centromere in the complex is inactive during prometaphase and following stages. The former orientations produce segregation complements with 10 chromosomes in one group and only eight in the other, instead of the normal nine per group. The latter orientations produce laggard chromosomes during anaphase I and micronuclei at prophase II - telophase II. The persistent 3 : 1 orientations are considered to arise through certain unusual centromere/chiasma relationships in the complex such that the expected instability of the 3 : 1 orientation is suppressed. The origin of passive centromeres during prometaphase is not fully explicable with the available data.

15. The interchange produces characteristic aborting pollen grains at the pollen grain mitosis developmental stage in unbalanced pollen produced from adjacent orientation in the complex. The frequency of abortion brought about by the interchange is about 12.5% rather than the expected 25% (the frequency of adjacent orientation) because one type of unbalanced spore passes through pollen grain mitosis and so does not abort. These spores, however, are retarded in their entry into pollen grain mitosis relative to normal ones, being found almost entirely

in pollen sacs at late developmental stages of pollen grain mitosis.

Pollen grains with 10 chromosomes from 3 : 1 orientations in the complex also pass through pollen grain mitosis.

16. Ovule abortion from adjacent orientation is revealed as undeveloped embryosacs.

17. Average seedset in normal plants is reduced by 25% in interchange plants, because of 25% abortive ovules produced from adjacent orientation in the complex.

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general discussion.

A REPLY

Nicklas (1966) has published a criticism of my 1965 analysis of co-orientation, to which a number of points can be made in reply.

There appear to be genuine differences of opinion regarding the use of the terms auto- and co-orientation between Nicklas and myself. To the comments published by both writers the following can be added.

When describing the orientation phenomenon we must ask whether there are indeed natural differences between mitosis and meiosis. In the first we observe two chromatid centromeres linked on either side and oriented axially in the spindle in relation to each other. In meiosis we observe two chromosome centromeres linked together by chiasmata and oriented axially in the spindle. And both these orientations appear to arise through the same basic mechanism. One difference lies in the orientation of chromatid or chromosome centromeres. But this difference is on the same level of magnitude as that between linking substance and chiasmata. These are not basic differences but represent necessarily different means of achieving equivalent ends. Thus both phenomena appear to fit logically into Darlington's concept of co-orientation (Lima-De-Faria, 1958; Virkki, 1967). It anything the situation in mitosis is more strictly co-orientation of a pair of centromeres than that in meiosis for chromatid centromeres in meiotic metaphase I can be distinguished structurally and functionally. But they behave as a unit in the final state and so can be referred to as a unit. Also it should at least be considered that sub-chromatid centromeres might at times be transiently functional in mitosis (see the similar behaviour of undivided chromosomes in mitosis and univalent chromosomes in meiosis; Rickards, 1965 section 4).

I suggested recognition of a broad concept of co-orientation for all orientations along the equator in both mitosis and meiosis. A differential terminology is clearly desirable (see below) but to retain the term auto-orientation (e.g. in reference to the fact that in mitosis chromatid centromeres are oriented while in meiosis chromosome centromeres are) would, in my opinion, warrant a redefinition of the term other than that given it by Darlington (I suggested that the term was without meaning as coined by Darlington).

Two points in Nicklas' criticism appear to have arisen through misinterpretation (Nicklas pers. comm.) and need clarification. Firstly, the comments on p.149 on the origin of polar or centrophilic bivalents or chromosomes do not describe my interpretations, which were (see p. 345) that such chromosomes arise through orientation and thus movement of both centromeres, simultaneously, to the one pole. Secondly, Nicklas states that I conclude that the terms syntelic and amphitelic are "unnecessary" (p. 150). The term I used was 'cover', or include under (p.348); or in other words, these terms are included under a general concept of co-orientation. Rather than being unnecessary these terms are most useful for describing in detail the differential behaviour seen during prometaphase and metaphase. Thus in mitosis, chromatid centromeres are the basic units of orientation at metaphase, whereas in meiosis the two chromatid centromeres of a chromosome act as a unit in the final state, thus providing a bivalent with essentially a pair of centromeres. Chromatid centromeres at meiosis do, however, transiently react with the spindle as individual units during late prometaphase (Bauer et al, 1961; Nicklas, 1967); such behaviour has occasionally been seen by myself in fixed preparations of <u>Allium triquetrum</u> . Thus/sister
centromeres of
chromatids may be directed to the same pole (<u>syntelic</u> orientation) or

directed to opposite poles (amphitelic orientation)(Bauer et al. 1961).
These terms are widely used in recent literature on prometaphase
phenomena. In meiosis the stable metaphase orientation of a bivalent
can thus be described as di-syntelic co-orientation with the two pairs
of associated chromatid centromeres oriented to opposite poles, and the
corresponding orientation of a chromosome at mitosis as amphitelic
co-orientation (Lewis and John, 1963;'co' my own).

However, the main point arising from the criticism of Nicklas
concerns the interpretation of the overall purpose of my publication.
This was to present an analysis of orientation as envisaged by myself,
applying it to both mitosis and meiosis. As pointed out already (p.190)
I had been unaware at that time that similar ideas applied to meiosis
(only) had been proposed by Dietz (1958). Nicklas has criticised my
analysis, suggesting that it presents nothing new. This is correct
in part, from the point of view of basic ideas applied to meiosis, since
these had already been proposed by Dietz. It appears that the fact that
Nicklas was aware of Dietz's contribution, whereas I was not at that
time, was the main reason for the differences of opinion between Nicklas
and myself (Nicklas pers. comm.). My analysis in no way attempted to pre-
sent a theory for the underlying mechanism of reorientation, but rather
to present this phenomenon as an integral part of normal prometaphase events
and so give a general theory of how metaphase orientation is achieved,
in a similar manner in both mitosis and meiosis.

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ADDENDUM

Nicklas and Koch (1969) have very recently published important data concerning the origin and control of reorientation from micromanipulation experiments in living grasshopper spermatocytes. Their experiments tested the possibility that the normal spindle tension developed between centromere and pole provides for the stability of bivalents cooriented in the equator, while the absence of such tension apparent in a centrophilic (unipolar oriented) bivalent induces instability and thus reorientation. They found, as predicted on this hypothesis, that tension artificially applied to a unipolar oriented bivalent by micromanipulation, without exception inhibited its normal reorientation, thus conferring on it an unnatural stability. These experiments clearly identified spindle tension as a source of orientation stability, and the absence of such tension as a source of instability and thus reorientation.

In their discussion Nicklas and Koch point out that spindle tension may provide a general explanation for the initiation and control of reorientation, in meiosis and mitosis. In respect of interchange and other multivalent associations they suggest that when two or more adjacent centromeres are oriented to the same pole the situation might be similar to unipolar orientation in a bivalent, in that spindle tension on (certain) centromeres so oriented is reduced. This promotes reorientation of these arrangements to stable types in which tension is fully developed on all centromeres (e.g. the alternate orientation). This suggestion would account for the apparent instability and thus absence at metaphase I of many theoretically possible orientations in multivalents in general, and, in particular, the centrophilic 'adjacent' orientations in the interchange complex studied in this thesis (Fig. 45 a - d). On the other hand,

Nicklas and Koch point out that the adjacent orientation as Fig. 45f here would, on the tension hypothesis, be predictably stable, since uniform bipolar forces are produced on each/centromeres. *pair of cooriented* Whether indeed such orientations in certain materials do form and then reorient to alternate types, as sometimes claimed (Sybenga, 1968)[*]is not clear at present. Certainly in the present interchange this adjacent orientation appears to be stable and thus follows prediction.

The tension hypothesis may provide adequate explanation for the apparent differential instability of the centrophilic complexes studied in the present interchange material. The degree of instability/stability may be related to the degree of tension developed between the unstable centromeres and poles, less tension promoting greater instability and vice versa. This would account for the apparent greater instability of monosyntelic bivalents and complexes compared with partially centrophilic complexes (p.212), since the unstable centromeres of the latter are likely to be under some tension to their pole because of orientation of one centromere in the complex to the opposite pole, whereas this tension is not present in monosyntelic bivalents and complexes. Also, this application of the tension hypothesis would probably account satisfactorily for the differential instability suggested for complex types a and b (pp. 137 & 213). Thus the end, non-cooriented centromere of the type b complex might well be subjected to less tension than the non-cooriented centromere of the type a complex, thus promoting its relatively greater instability. Also, on this basis one would expect the type a' complex (Fig. 45) to be stable, as the evidence suggests it is (p. 205).

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[*] See on following page.

On the other hand, it is difficult to adequately explain on this basis certain aspects of the differential behaviour of the rod and V trivalents of tipulids studied by Bauer et al. (1961) (see data on p. 199 here). Thus, for example, it is difficult to account for the reorientation of the middle centromere of the V configuration, which, on the tension hypothesis, should be stable.

The artificial tension applied to unipolar oriented bivalents by Nicklas and Koch was regulated to duplicate as nearly as possible the natural tension developed in cooriented bivalents, and hence no experimental data are available at present that would provide substance for the application of the tension hypothesis to differential instability in centrophilic interchange complexes.

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*

With the available information it is not possible to decide whether the conclusion made by Sybenga (1968), that between early and late metaphase I adjacent orientations reorient frequently to alternate ones, is valid or not. The criteria used for distinguishing early and late metaphase I anthers (not pollen sacs) are not given by Sybenga, and without this and other data on which to make a decision, the possibility exists that the apparent change in relative frequency of alternate and adjacent orientations arises through developmental variation similar to that revealed in this thesis, and thus is open to a very different interpretation.

Such differences in interpretation apply also to the metaphase I/ anaphase - telophase data of Zimmermann (1968), who claimed that unequal orientations in the multiple interchange ring of Rhoeo spathacea must reorient to equal types during metaphase I.

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Fig. 1   a - c. Reproductions of Levan's (1932) figures of the
Allium triquetrum complement studied by him. a. Photomicrograph
of a pollen grain in first mitosis. b. Diagram of Levan's S_4
chromosome, showing the complex nature of its satellite. d. Drawings
of two haploid sets.

         d. Reproduction of the diploid root tip complement
of Allium triquetrum studied by Kurita (1958).

Fig. 1
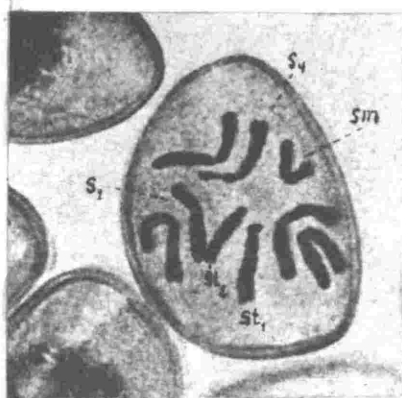
a.

b.
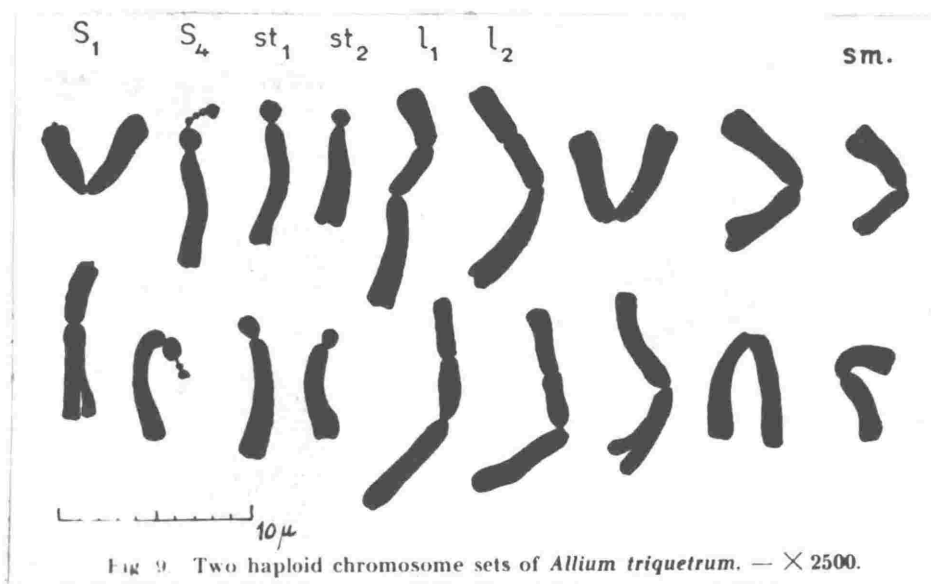
Fig. 9 Two haploid chromosome sets of *Allium triquetrum.* — × 2500.

c.

d.

Figs. 2 - 5   Somatic chromosome complements from root tips of
normal _Allium triquetrum_ studied by myself. Colchicine/2BD/
Feulgen preparations. The morphologically distinct homologous
pairs are labelled 4/4, 6/6, 7/7, and 9/9. Note the satellites
on pairs 7 and 9. The remaining chromosomes can be paired
according to relative sizes and centromere positions only
roughly (Fig. 6), not with certainty because of arm reversals
and reversals of order (see comments in text).

Fig. 2 and Fig. 3 moderately contracted; Fig. 4 weakly contracted.
Fig. 5 highly contracted complement showing retention of asymmetry
in chromosomes 4, group II (5 and 8) and 6, 7 and 9. Two chromosomes
in this figure were broken at their centromeres during extra
squashing prior to photographing the complement.

Fig. 2
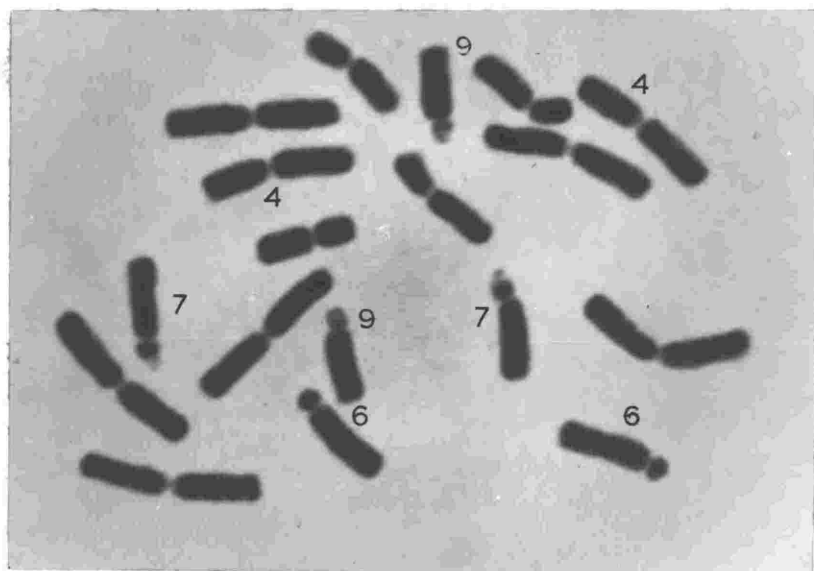
Fig. 3
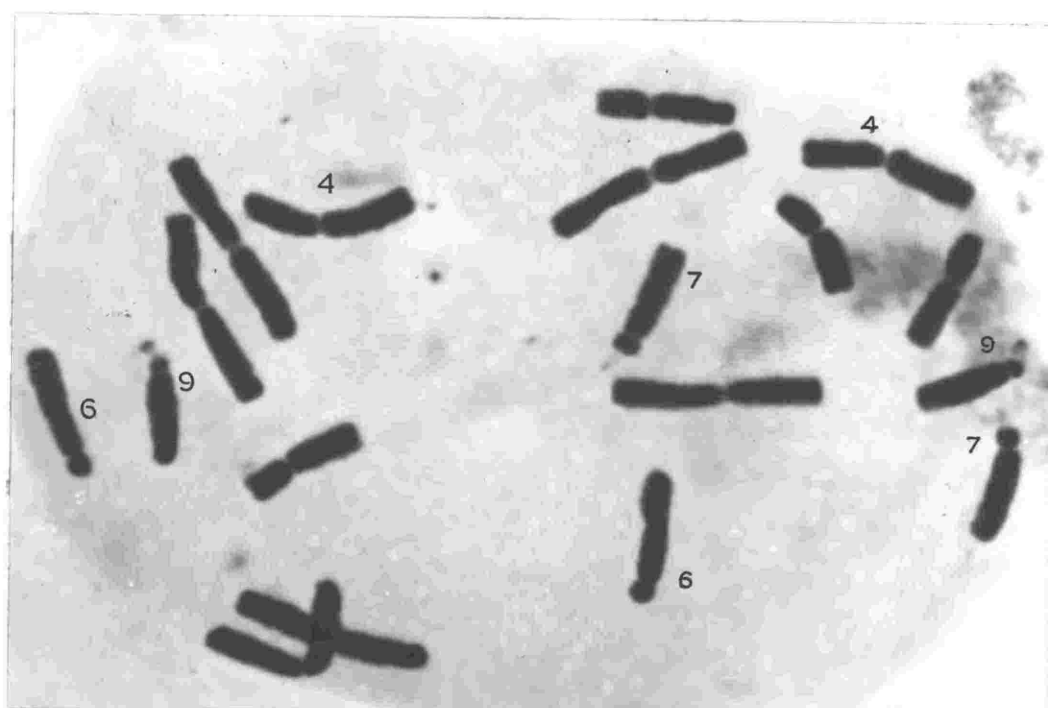
Fig. 4
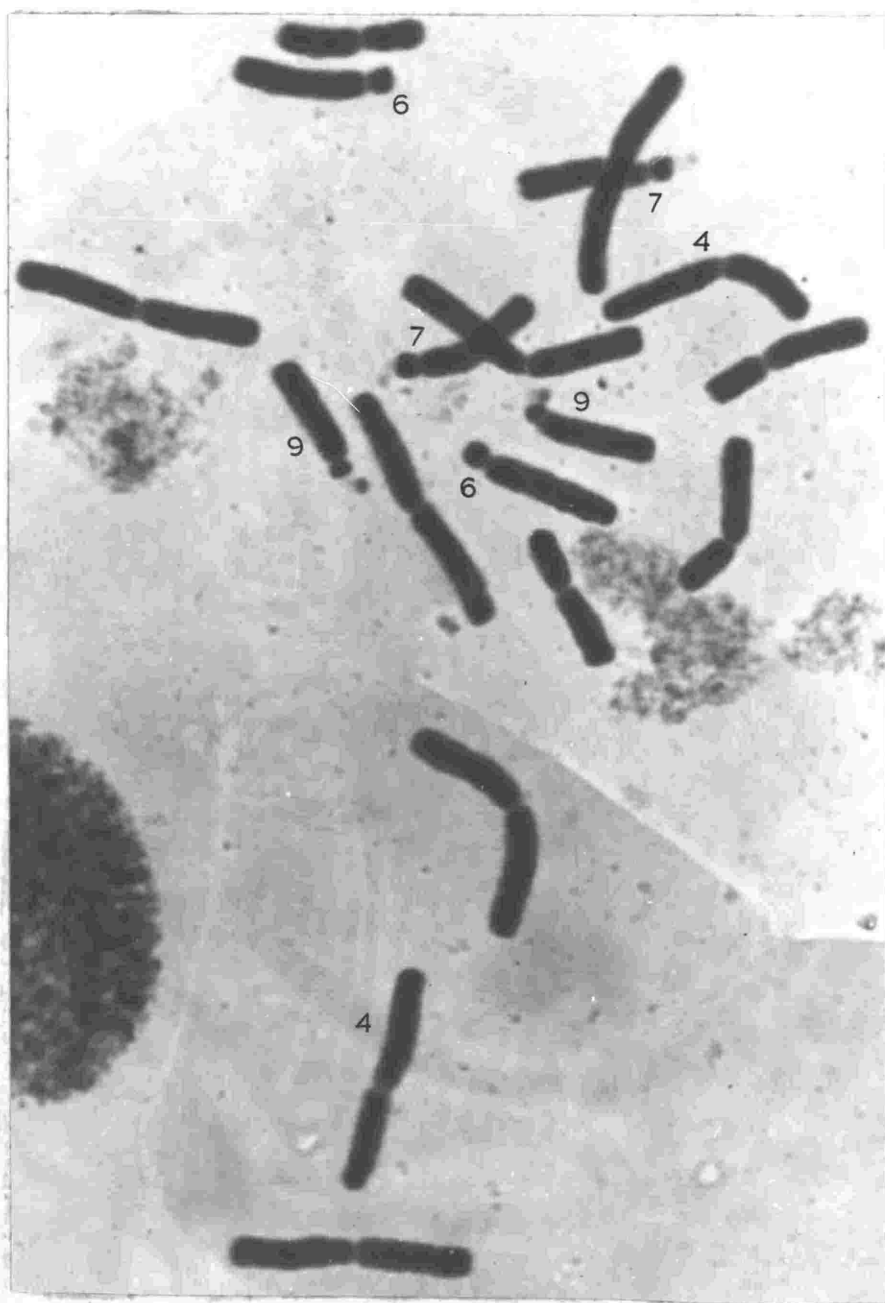
Fig. 5
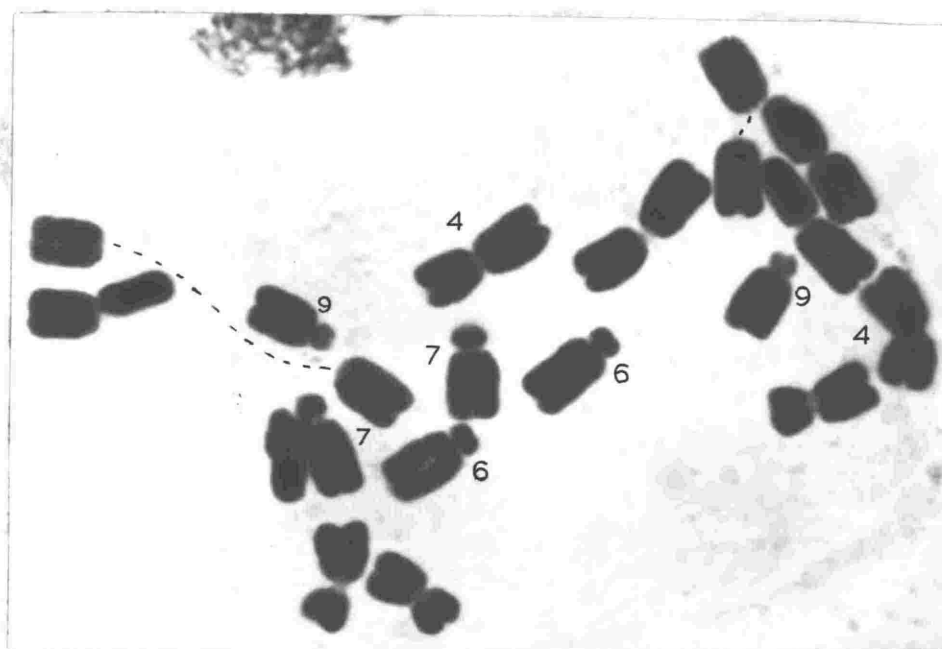
Fig. 6   Apparent [*] diagramatic and photographic idiograms from

normal <u>Allium triquetrum</u>, illustrating chromosome numbering and

group classification. The diagrams were drawn up from mean

lengths and arm ratios of the 10 cells of Appendix table 1.

For chromosomes 4, 6, 7 and 9 the idiograms will be accurate

since these pairs can be recognised unequivocally. For chromosomes

1 - 3 the idiograms are probably not as accurate since instances

of incorrect pairing of chromosomes and arms cannot be eliminated.

This is probably also true for pairs 5 and 8 though to a lesser

extent (further comments in text).

[*]   The adjective 'apparent' is used here as in Simak (1966) to

distinguish the idiogram from a <u>true</u> one, which can be drawn up

only if all the chromosomes in the complement can be identified

unequivocally.

Fig. 6
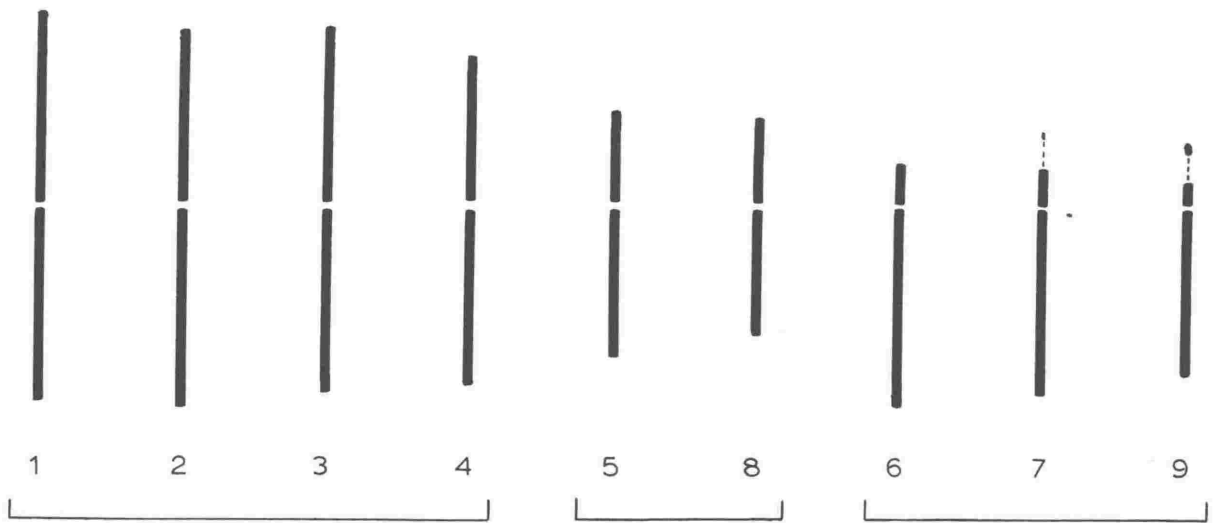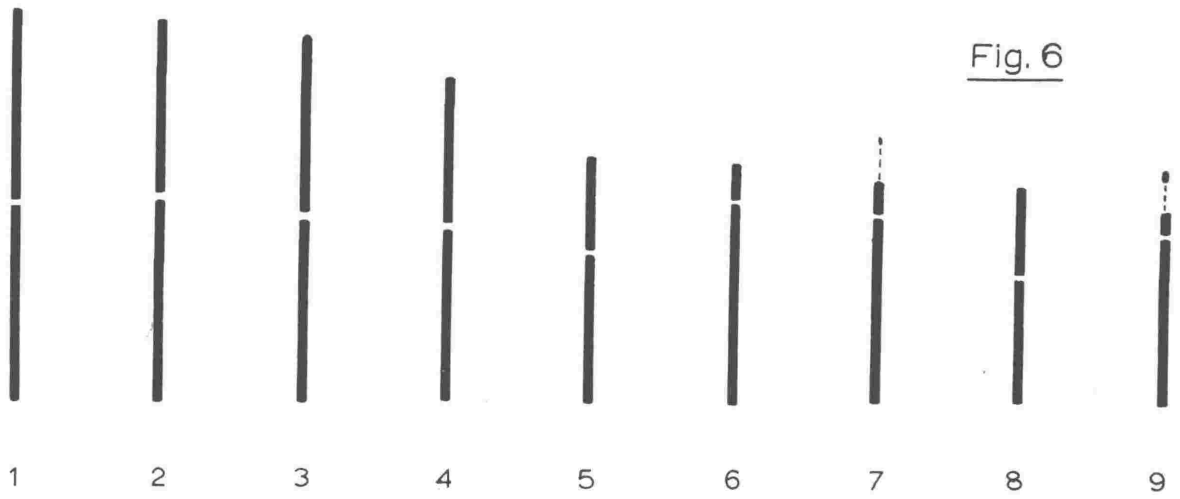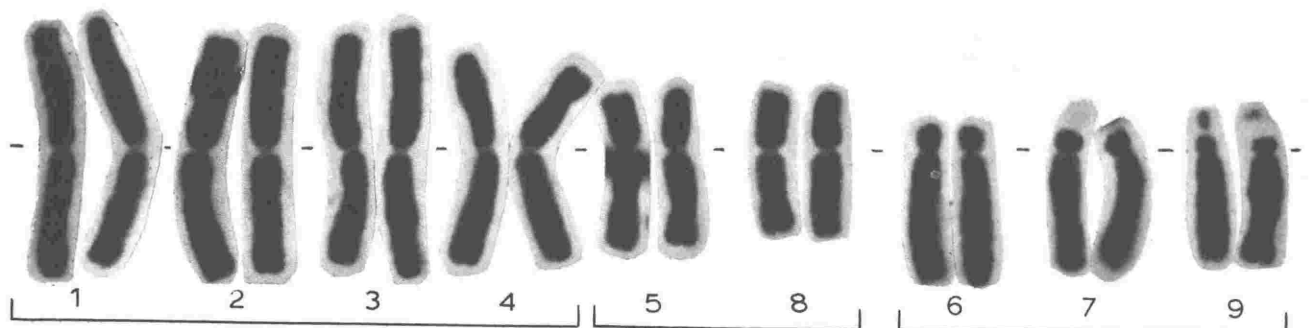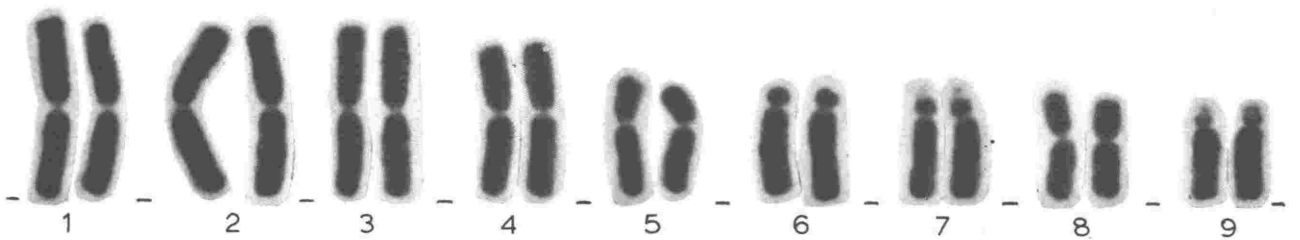
Fig. 7 Satellited chromosomes of <u>Allium triquetrum</u>.

a - e. Chromosome 7 showing the extreme variation found in this
satellite. c from pollen grain mitosis, alcoholic carmine;
others from root tip mitoses, colchicine/Feulgen's. Compare
c and d with Levan's S_4 satellite (Fig. 1b here).

f and g. Chromosome 9, showing the two basic satellite types,
f with a distinct attachment thread, g with a very short or no
apparent attachment thread. Root tip/colchicine/Feulgen's.

Fig. 7

Figs. 8 - 11   Somatic chromosome complements of interchange

Allium triquetrum. Colchicine/2BD/Feulgen preparations from root

tips.

Figs. 8 and 9. Weakly contracted complements. Note satellites

on chromosomes 7 and 9 as in normal cells. Note also the length

differences in both long and short arms of chromosomes 6 and 4^6

and the presence of only one normal chromosome 4 member.

Figs. 10 and 11. Moderate - strongly contracted complements in

which the above differences are less clear.

The identification of chromosome 6^4 in each cell cannot be taken

as unequivocal since it may be confused at times with a symmetrical

members of normal pairs 1 - 3.

Fig. 8

Fig. 9
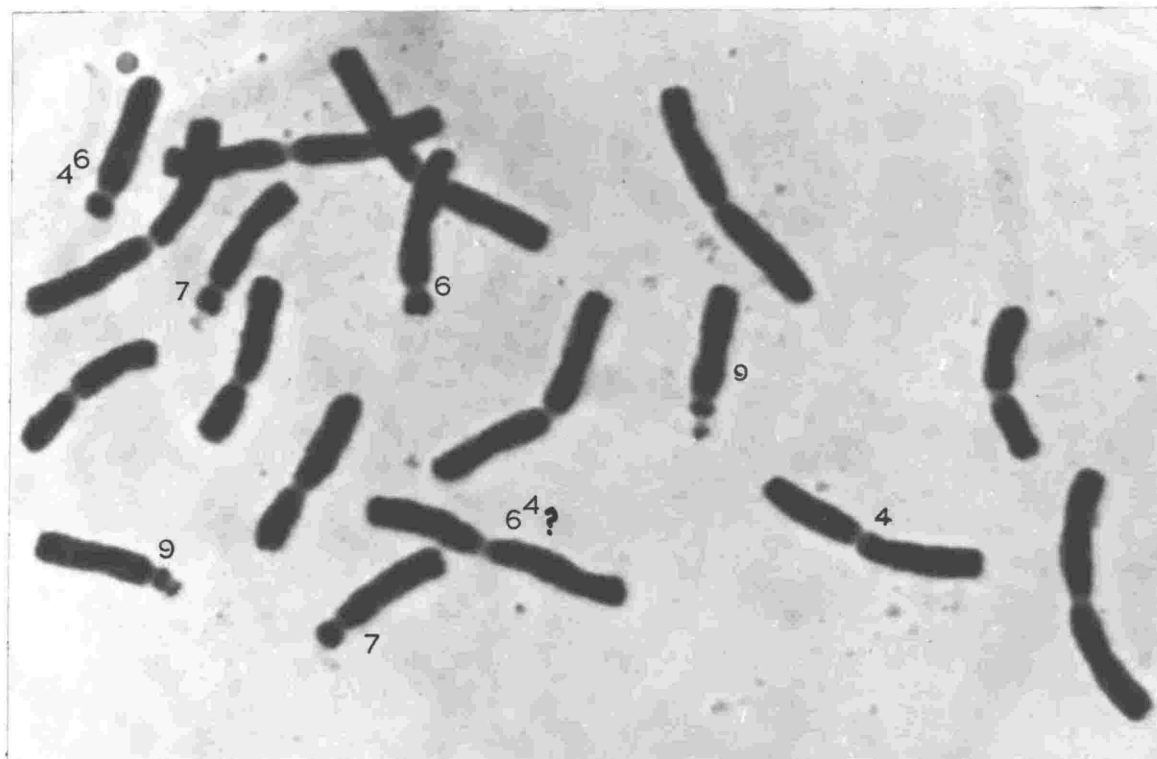
Fig.10
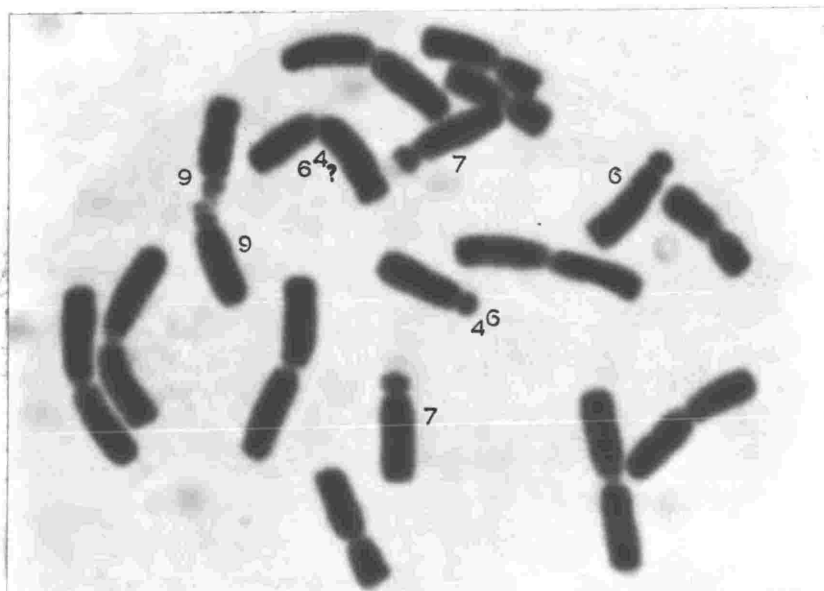
Fig.11
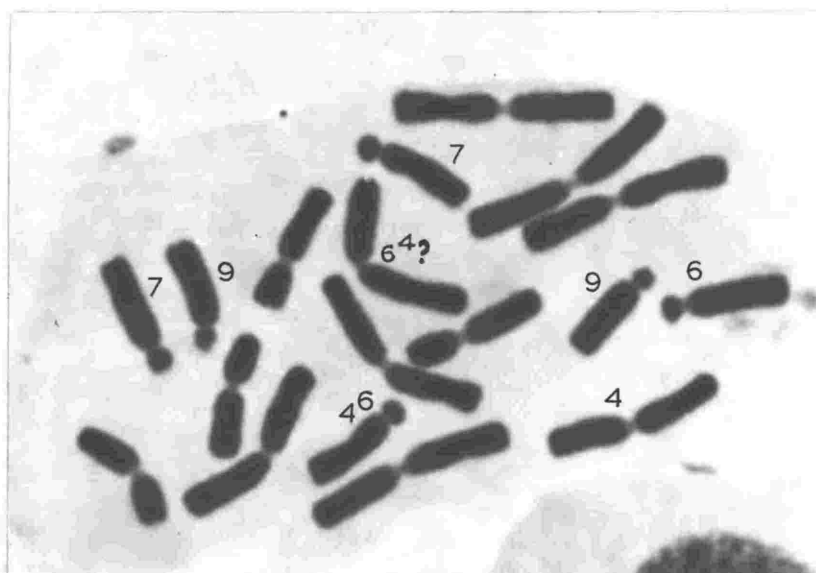
Fig. 12   Apparent diagramatic and photographic idiograms from
interchange _Allium triquetrum_, drawn up from mean lengths and
arm ratios of the 8 cells of Appendix table 2. In the diagram
only the interchange chromosomes are illustrated. The idiograms
for chromosomes 6, 4^6 and 4 (as well as pairs 7 and 9) will be
reasonably accurate; those for chromosome 6^4 and other pairs
are probably not so accurate (see relevant comments in caption
to fig. 6 and in text). Compare with fig. 6. The chromosomes
have been arranged to facilitate desired comparisons. The
photographic idiograms are from two complements (figs. 11
and 8 respectively).

Fig. 12
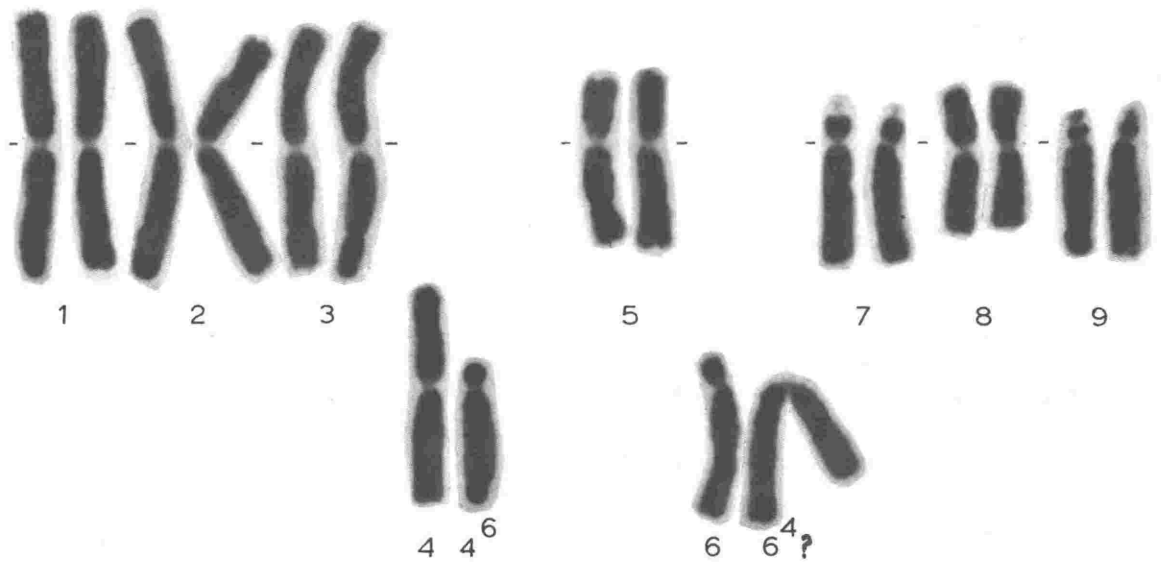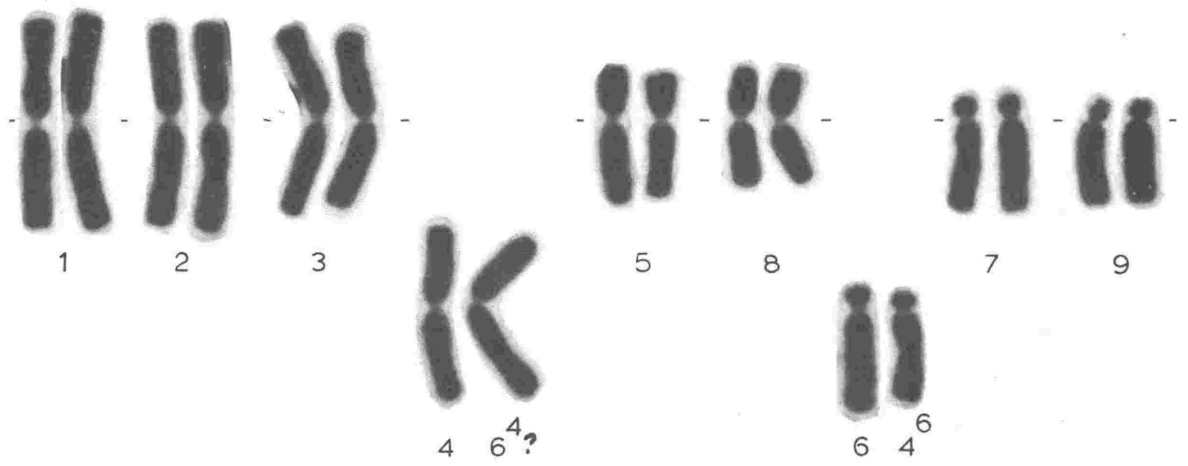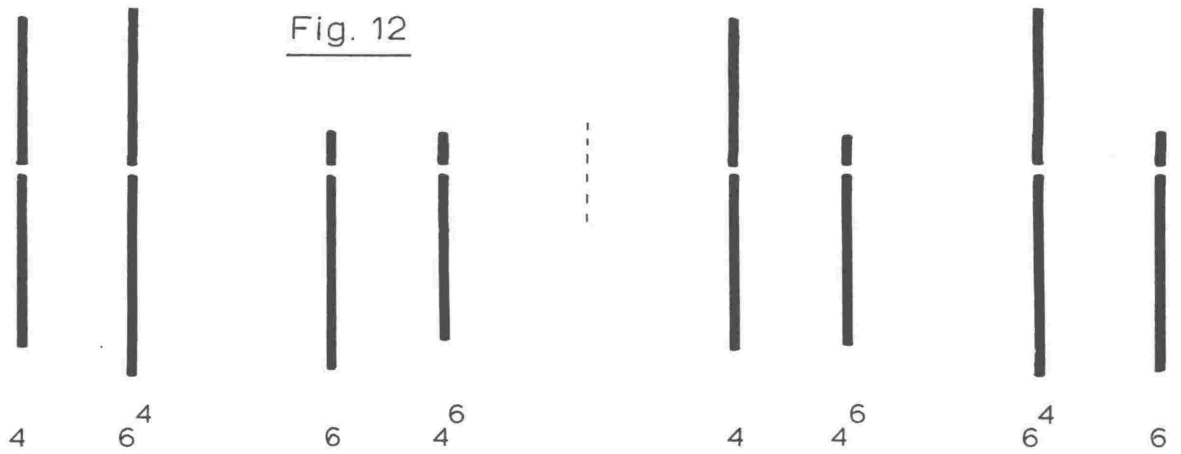
Fig 13   Diagrams of the two general types of interchange involving

a group I and group III chromosome initially considered possible

for the present material. Both types produce chains of four

chromosomes through failure of chiasmata in the short pachytene

arm; and give alternate and adjacent orientations at metaphase I

of the types seen. In (1) the interchange involves the long arm

of the group III chromosome (A), the centromeres at pachytene are

on the vertical axis (B), and the adjacent orientation at metaphase

I is adjacent-2 (C). In (2) the interchange involves the short

arm of the group III chromosome (a), the centromeres at pachytene

are on the horizontal axis (b), and the adjacent orientation is

adjacent-1 (c). Previous studies assumed the interchange was

of type (1); detailed studies here have shown it to have been of

type (2).

Fig. 13
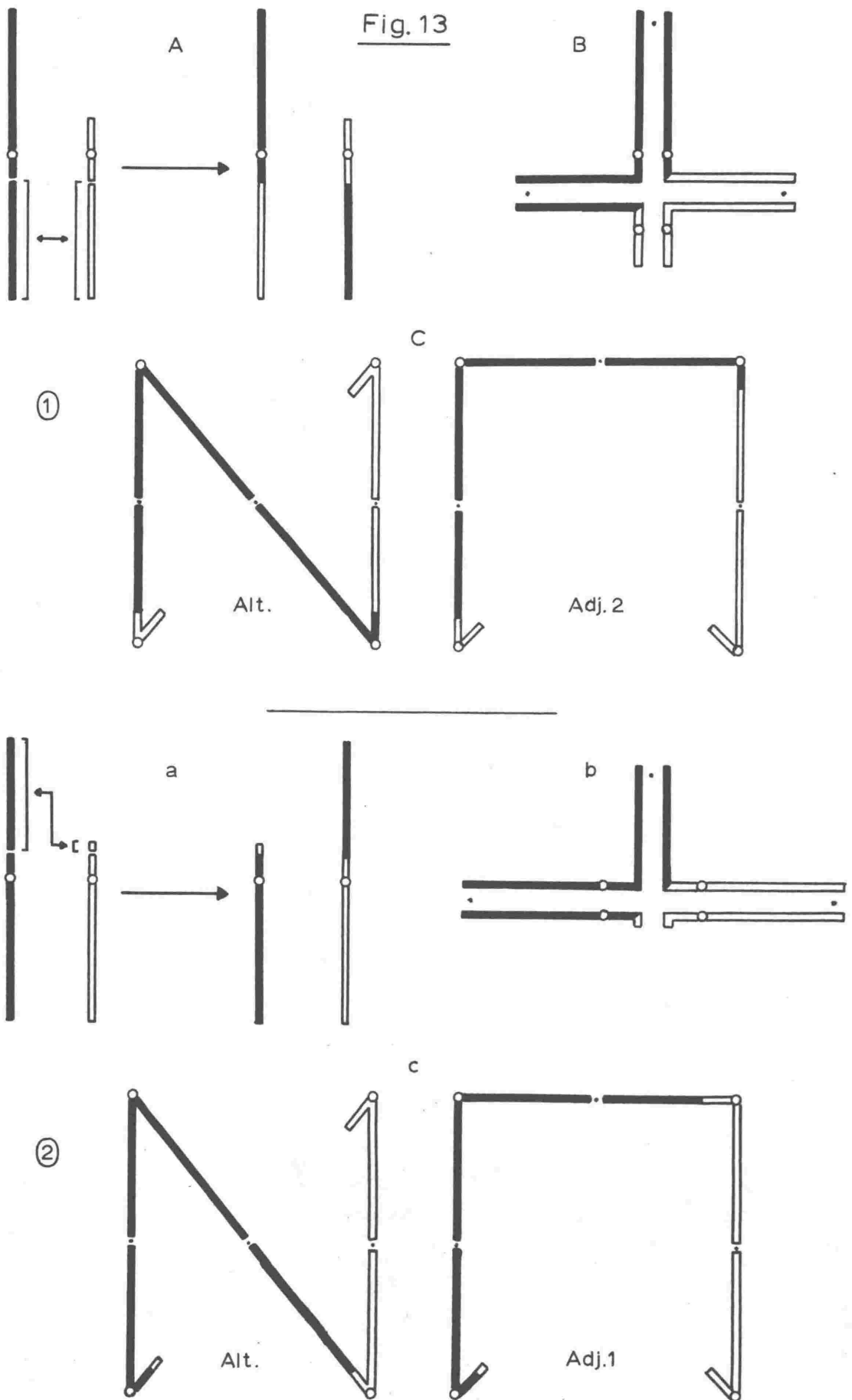
Fig. 14   Illustration of the proximal (upper) and distal break
point limits for the present interchange between chromosomes
4 (left) and 6, based on measurements of the interchange
somatic chromosomes .

Fig.14

at proximal
end of arm.

distal to c/m.
by approx.1/6 of arm .

distal to c/m.
by 1/4-1/5 of arm.

at distal
end of arm.
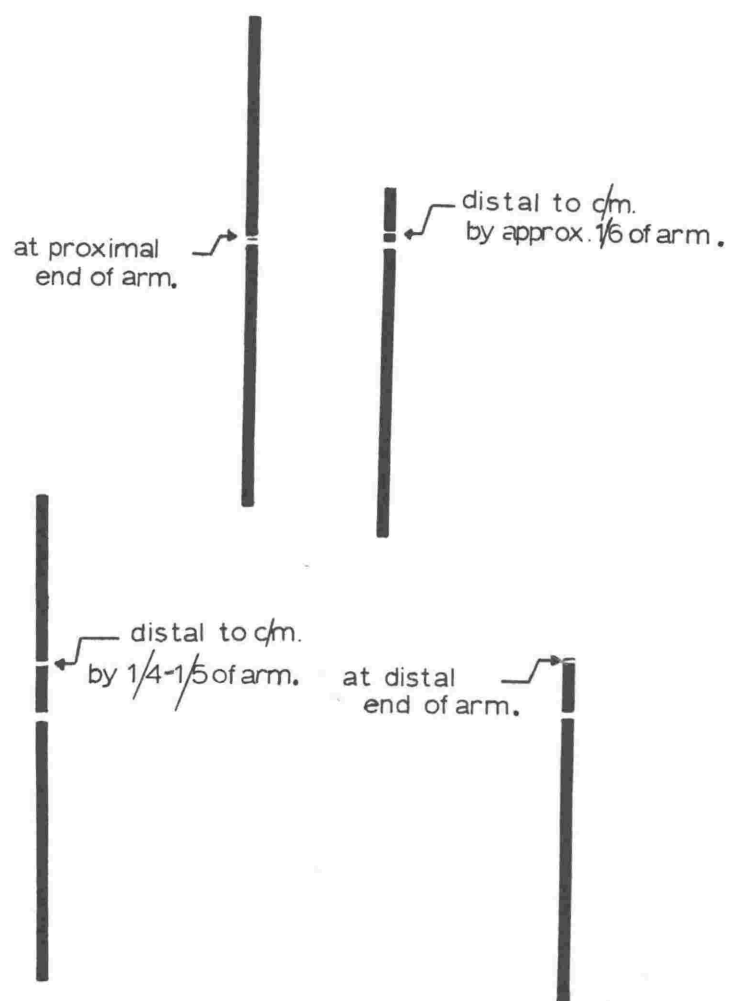
Fig. 15   Somatic chromosome complement of a normal plant derived
from a selfed interchange plant. Note the approximate equal lengths
of members of pairs 6 and 4 and their relative lengths compared to
other chromosomes (see table 14 and compare with figs. 2 - 4 on
one hand and 8 - 11 on the other).

Fig. 16   Apparent photographic idiogram of a second normal
complement. Comments as above. See also relevant comments in
caption to fig. 6.

Both figures from colchicine/2BD/Feulgen preparations of root
tips.

Fig. 15

1   2   3   4   5   6   7   8   9

Fig. 16

Figs. 17 and 18   Somatic chromosome complement and its apparent
idiogram from an interchange homozygote plant derived from a
selfed interchange heterozygote. Note arm and total relative
lengths of pair 4^6 in particular, and also the relatively large
asymmetrical pair labelled 6^4 which possibly represents the other
interchange members. ·From colchicine/2BD/Feulgen preparations
of root tips.
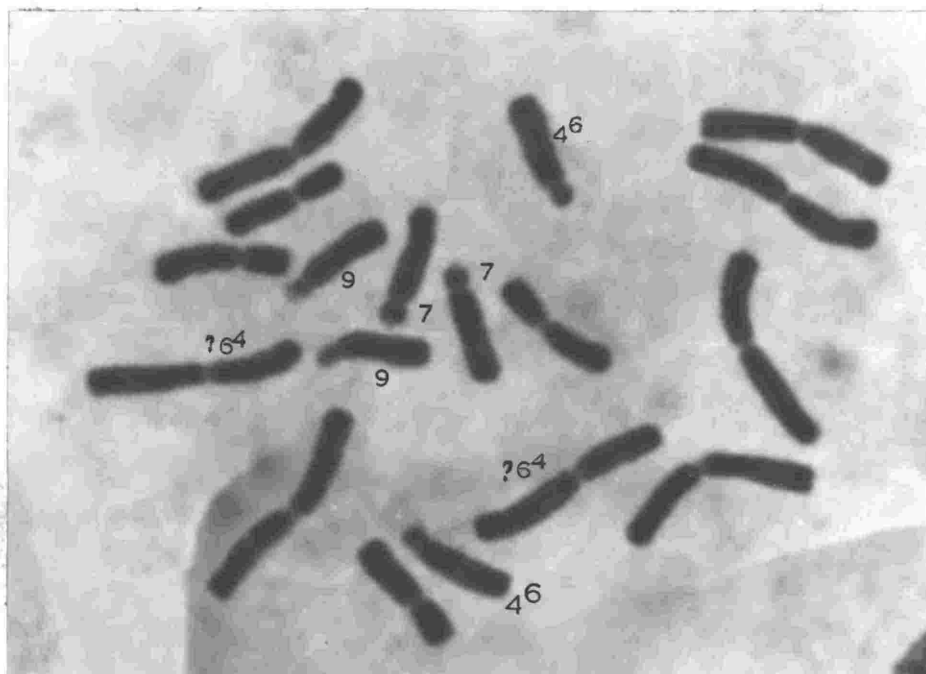
Fig. 17
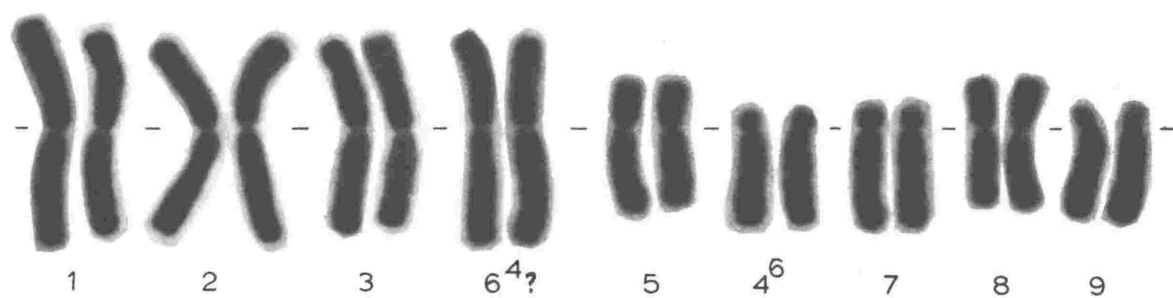
Fig.18

Fig. 19 A precise illustration of the interchange

taking into account the relative total and arm lengths

of the chromosomes involved. The most proximal break

points compatible with somatic chromosome measurements

(see text) have been used to construct the figure. The

configuration expected at pachytene is also illustrated.

Fig. 19

at c/m.
in short arm.

distal to c/m.
by 13% of
arm length.

4

6

4^6

6^4

4

6^4

4^6

6
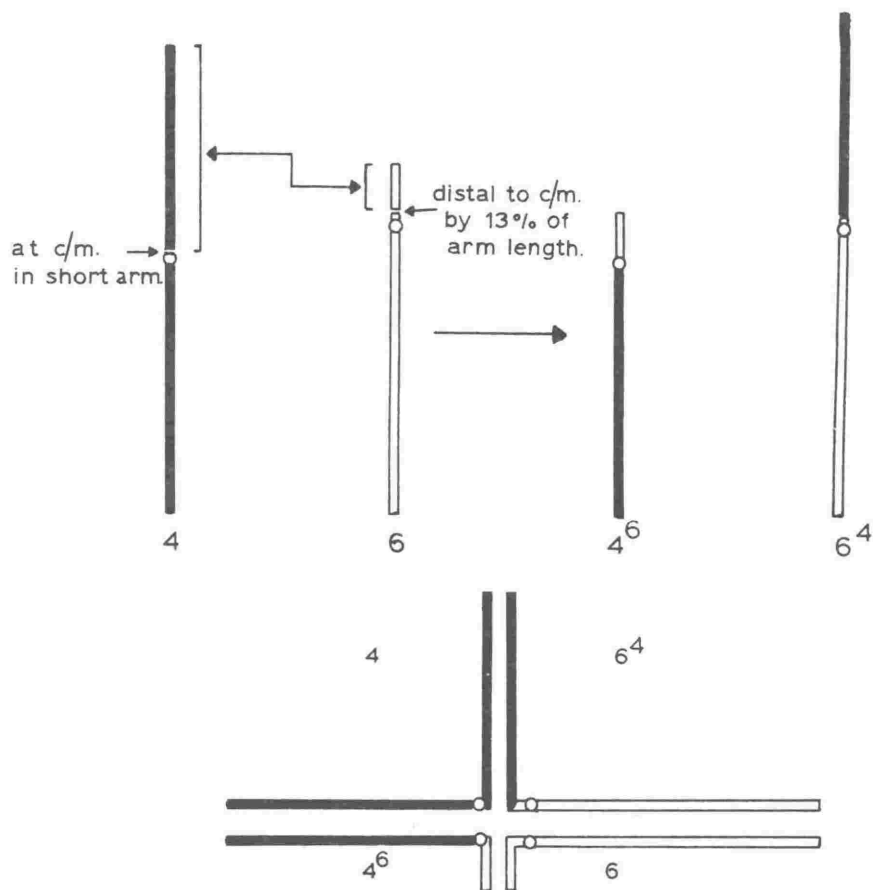
Fig. 20   Late pachytene/early diplotene of the interchange
complex.   b. is a sketch of the complex from the photograph
a.   Arrows in b. point out attenuated portions in the
complex.   An interpretive figure is given in c, showing
homologous unpaired segments $(x,x'$ and $y,y')$, the
chromosomes in the complex and the terminology used in
reference to the axes.   Feulgen preparation.

The interchange proves to be difficult to illustrate
at this stage, largely because the complex invariably
has one or other of its axes stretched across the
nucleus in characteristic fashion (see discussion in
text).

Fig. 20

a.

b.

c.

Axis 1a.

6^4

4

6

]y

x'

x

Axis 2a.

Axis 1b. (yy').

]y'

4^6

Axis 2b.
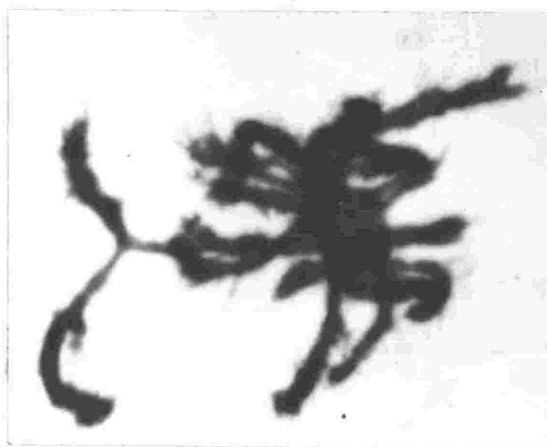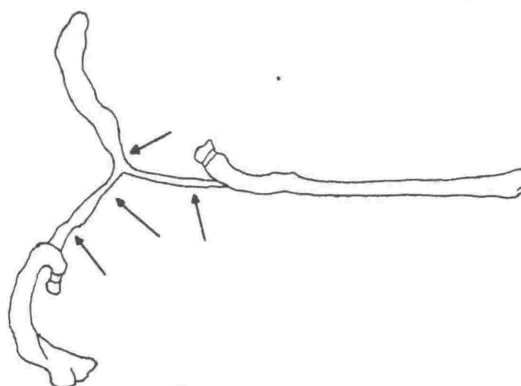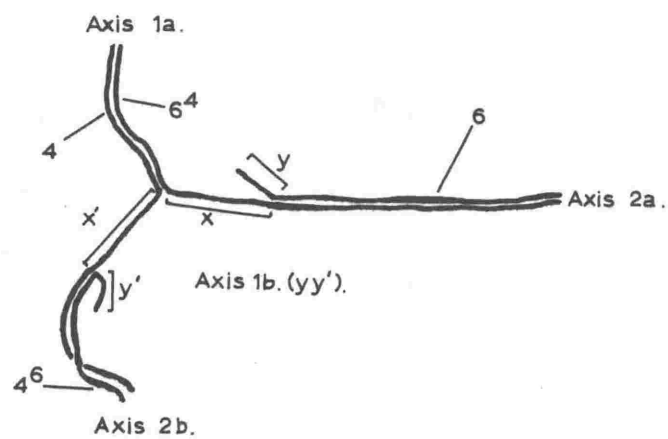
Fig. 21 Early diplotene with first clear signs of desynapsis in the complex (line drawing). Note the spatial separation of the complex halves as in fig. 20. The arrow points to apparent stretching in a bivalent. Alcoholic carmine.

Fig. 22 Diplotene/diakinesis showing the interchange complex (c) and the other seven bivalents, labelled according to their group classification. Chromosome 9 is distinguished because of its association with the nucleolus (stipple); the arrow points to the chromosome 9 satellite. Chiasma frequency is given for each bivalent, for the complex, and for the cell as a whole. Alcoholic carmine.

Fig. 21
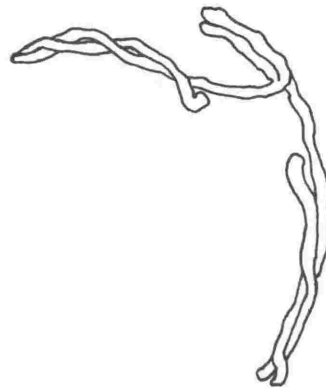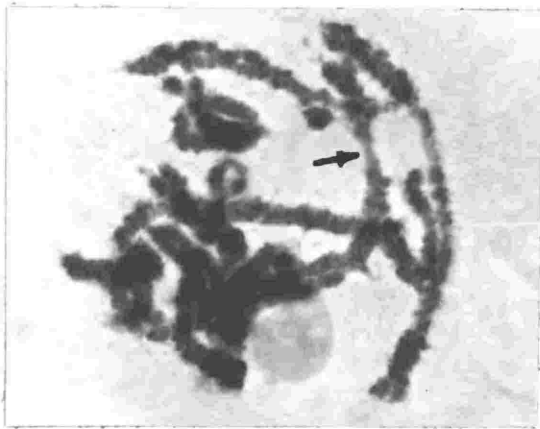
Fig. 22
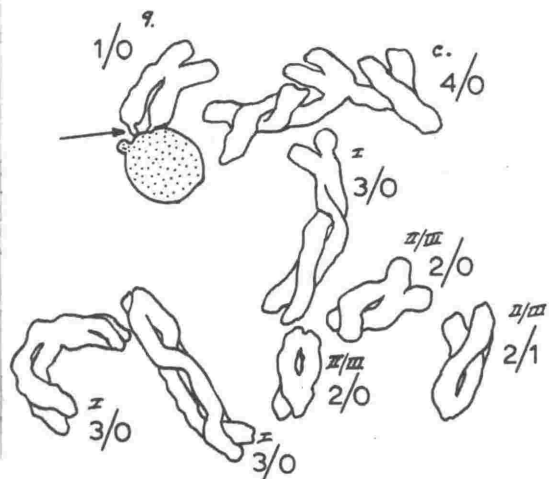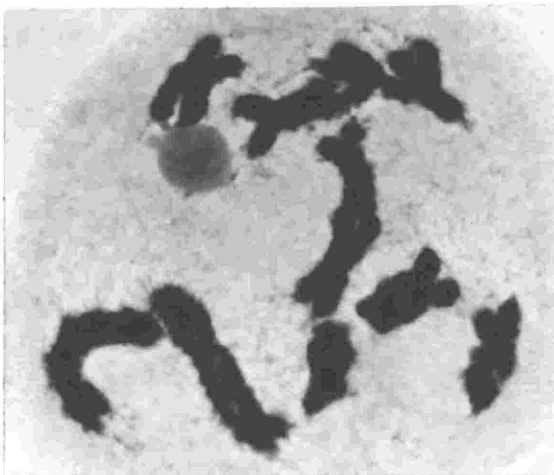
Fig. 23a and b Two diplotene cells showing the characteristic

wide separation of the complex halves and attenuation in

some segments of the complex (arrow in line drawing).

a. Feulgens, b. alcoholic carmine.

Fig. 23

a.
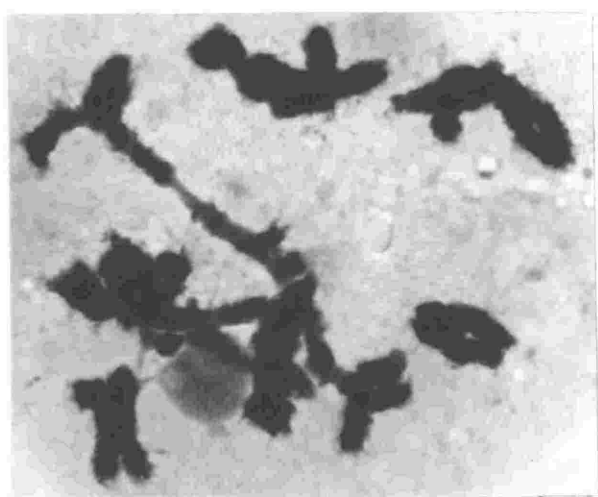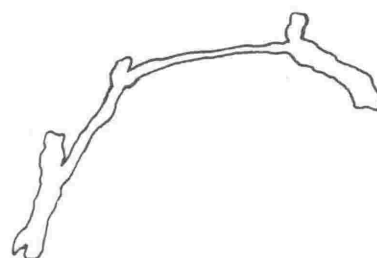
b.

Fig. 24 Diplotene cell from an unsquashed preparation showing wide separation of the complex parts and attenuation in localised segments (arrows in line drawing). a and b are two levels of focus of the same cell; c the same cell after hard squashing to show retention of the general placement of the complex parts and chromosome breakage rather than stretching (arrow). Alcoholic carmine.

Fig. 24
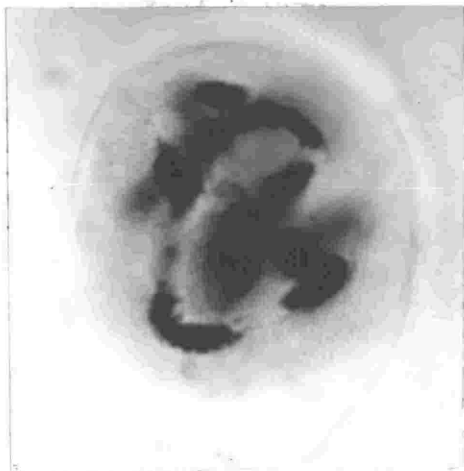
a.
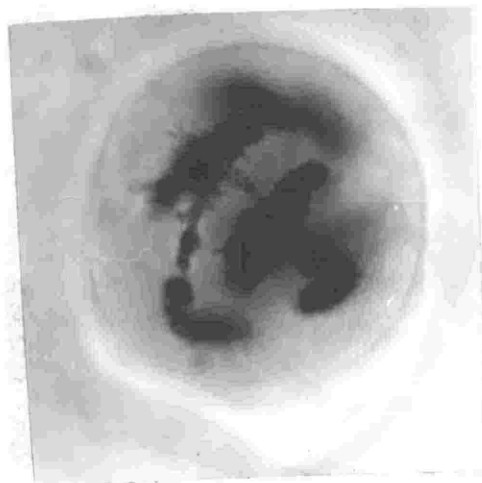
b.
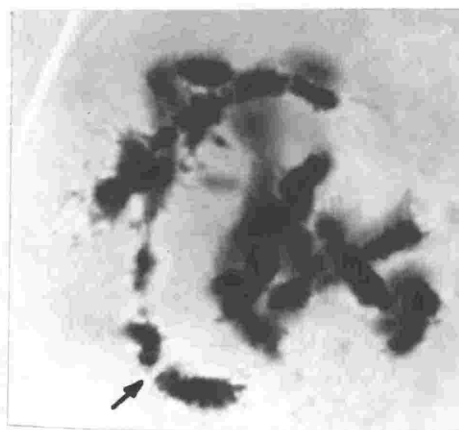
c.

Fig. 25 The interchange complex at diplotene/diakinesis.

a. with three chiasmata; b. with four chiasmata, two in

axis 2a (or 2b); c. with four chiasmata, two in axis 1a.

In all figures the arrows point to chiasmata. Notice also

the suggestion still of separation of the complex halves

and attenuation.

Fig. 25
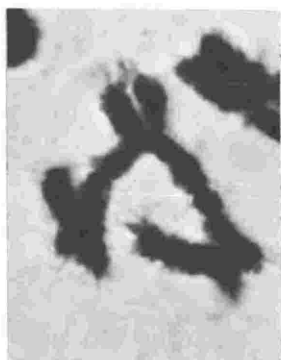
a.
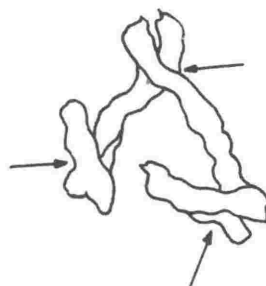
b.
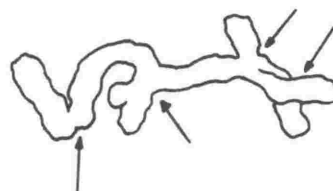
c.
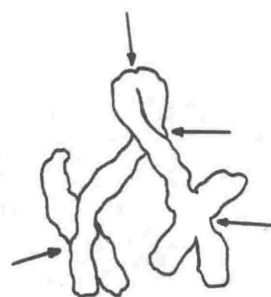
Fig. 26 Diplotene/diakinesis from normal material

showing bivalent types and their chiasmata. Nucleolus

not present. Alcoholic carmine.

Fig.27 Group I bivalent with a single chiasma.

Alcoholic carmine.

Figs. 28 & 29 Chromosome 9 bivalents each showing

satellites (arrow) attached to the nucleolus.

Alcoholic carmine.

Fig. 26

II/III
2/1

II/III
1/0

II/III
1/0

I
2/0

II/III
1/0

I
3/1

I
2/0

I
3/1

II/III
2/1
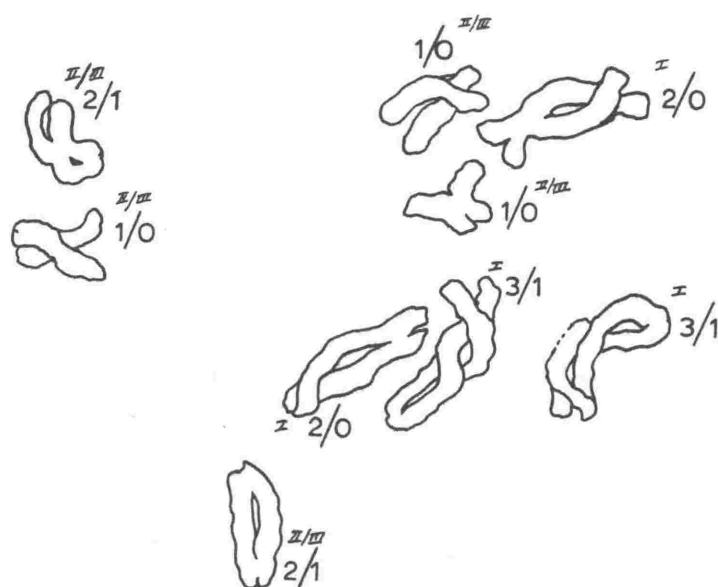
Total = 17/4
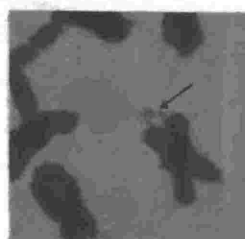
Figs. 27   28   29

Figs. 30 - 32 Unequal bivalents representing the
interchange complex at diplotene/diakinesis (Figs.
30 & 31) and diakinesis/metaphase I (Fig. 32). The
dotted arrows indicate the region where chiasmata
failed to arise; straight arrows indicate chiasmata
that have formed. The tailed arrow in Fig. 32
indicates an oriented centromere. All Feulgens.

Fig. 30
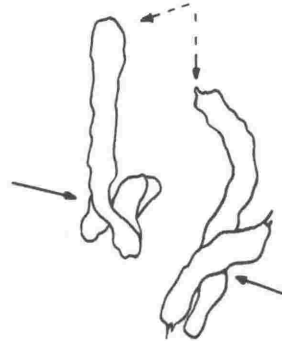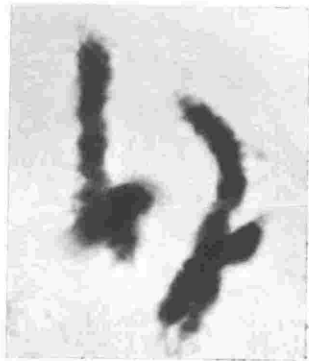
Fig. 31
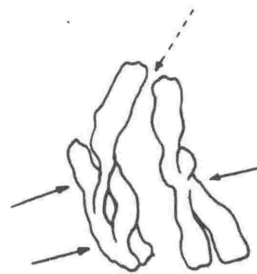
Fig. 32
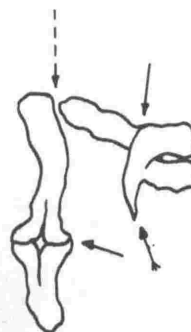
Fig. 33 Interchange metaphase I showing bivalent
types, the complex (c), and their chiasmata, and the
total chiasmata in the cell. Alcoholic carmine.

Fig. 34 Normal metaphase I showing the nine bivalents
and their chiasmata. Alcoholic carmine.

Figs. 35 - 37 Bivalents from normal cells. Fig. 35,
a group I bivalent with a single chiasma; Figs. 36 &
37, group III bivalents with a chiasma in the short arm
pair of each. Feulgens.

Fig. 33

I 2/1
I 2/0
III 1/0
I 2/1
I 2/0
c. 3/1
III 1/1
II 2/1

Total = 15/5

Fig. 34

III 1/0
I 2/1
I 2/1
III 2/1
I 2/1
II 2/1
I 2/0
I 3/1
III 1/0
I 2/2

Total = 17/7
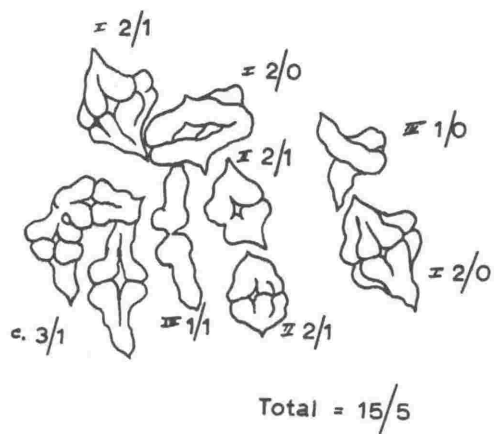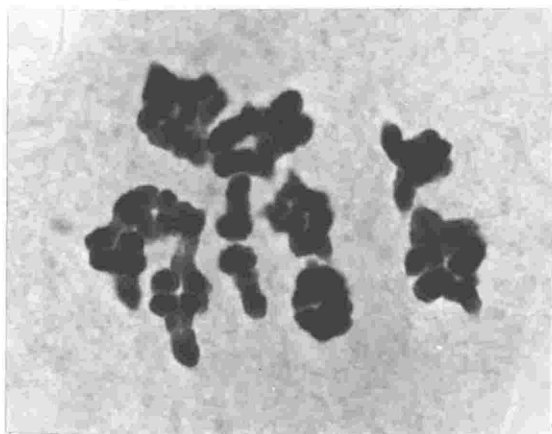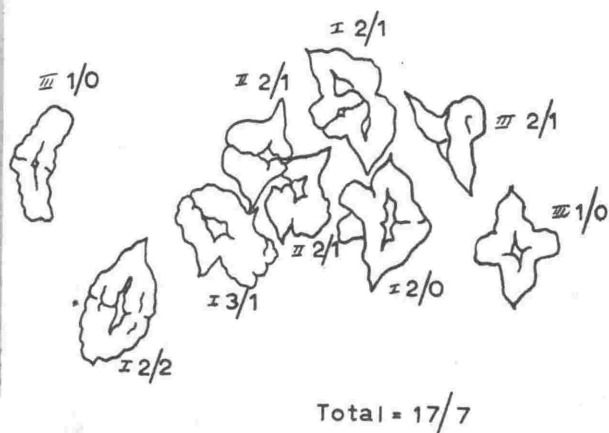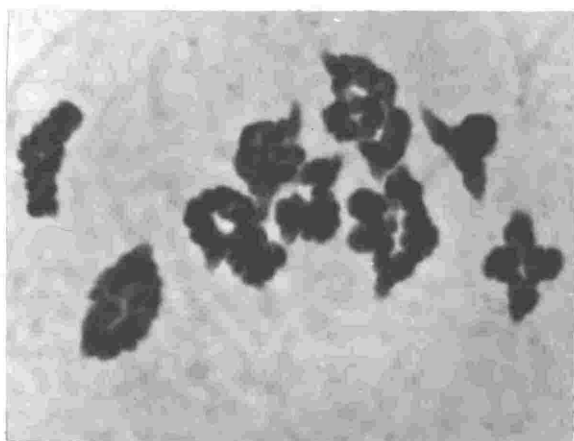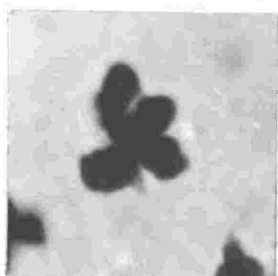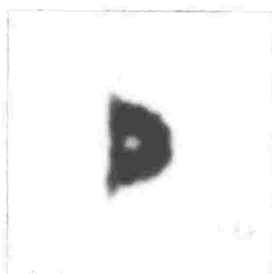
Figs. 35   36   37
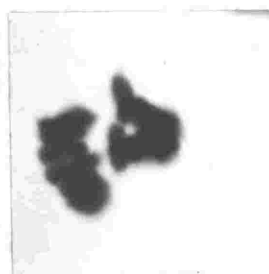
Figs. 38 and 39 Interchange complex types at diakinesis/

metaphase I : Fig. 38 with three chiasmata, one in each

of the long pairs of arms; Fig. 39 with four chiasmata,

two in axis 2a (perhaps 2b), one very near to the

centromeres. The tailed arrows indicate centromeres;

non-tailed arrows indicate chiasmata. Fig. 38 Feulgens,

Fig. 39 alcoholic carmine.

Fig. 38
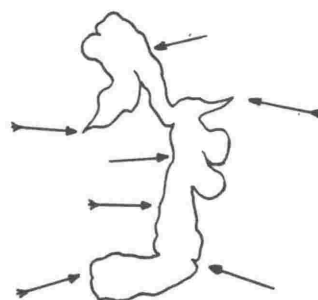
Fig. 39
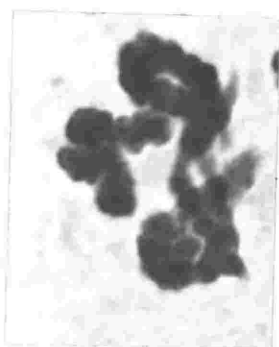
Fig. 40 Unsquashed prometaphase cell from normal
material showing two centrophilic bivalents (one group
I - 'above' - and one group III). In the group III
bivalent both centromeres were clearly identified and
were oriented to the same pole. a and b are two focal
levels of the same cell to show both centromeres of
the group III bivalent. c is a composite diagram.
Alcoholic carmine.

In this and subsequent prometaphase/metaphase
diagrams the ordinary arrows indicate chiasmata while
the tailed arrows indicate centromeres. The approximate
limits of the spindle and the equator are indicated
by dotted lines.

Fig. 41 Centrophilic group II bivalent in a mildly
squashed preparation. Alcoholic carmine.

Fig. 40
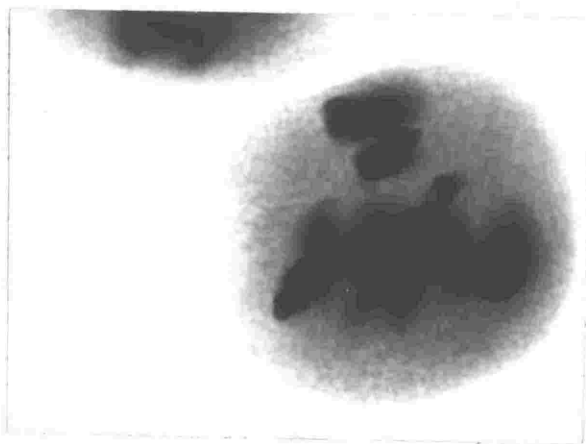
a.
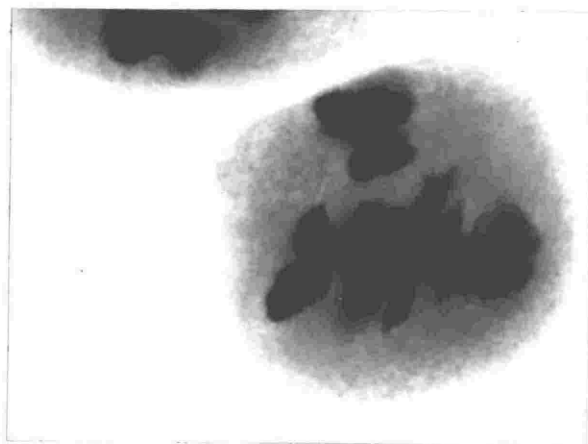
b.
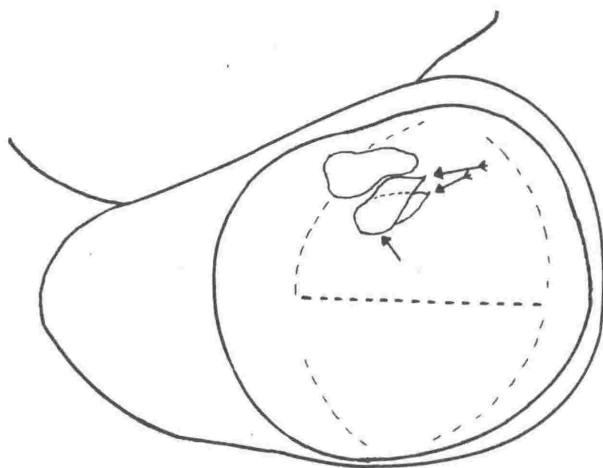
c.

Fig. 41
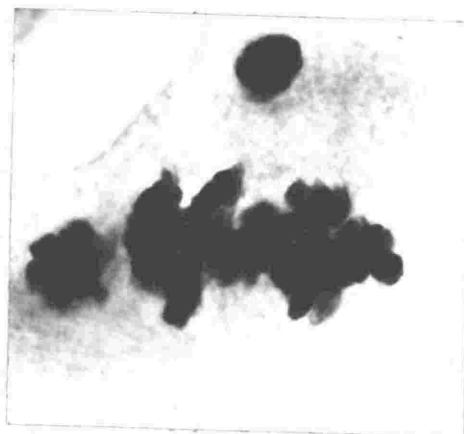
Figs. 42 - 44 Interchange cells at metaphase/early

anaphase, showing seven bivalents and the interchange

complex (outline diagram). Fig. 42 shows the

interchange complex in alternate orientation; Fig.43

in adjacent-1 orientation with three distal chiasmata;

Fig. 44 in adjacent-1 orientation with one chiasma well

in towards the centromeres. Feulgens.

Fig. 42
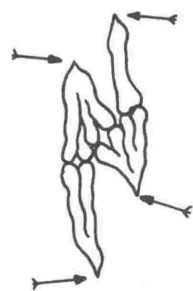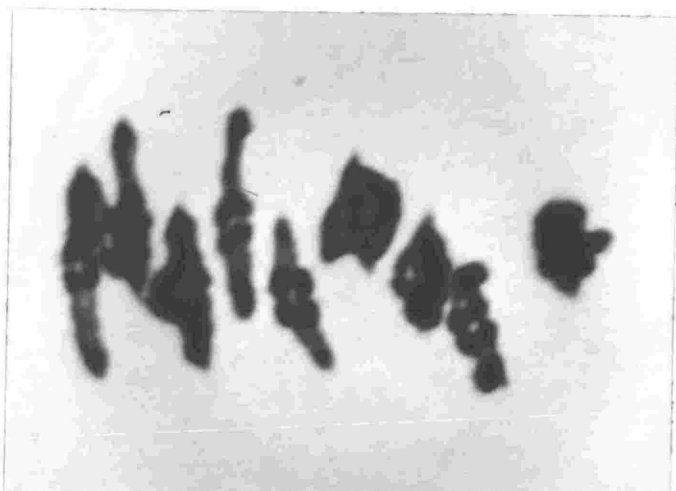
Fig. 43

Fig. 44
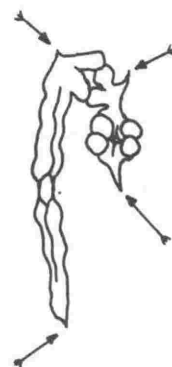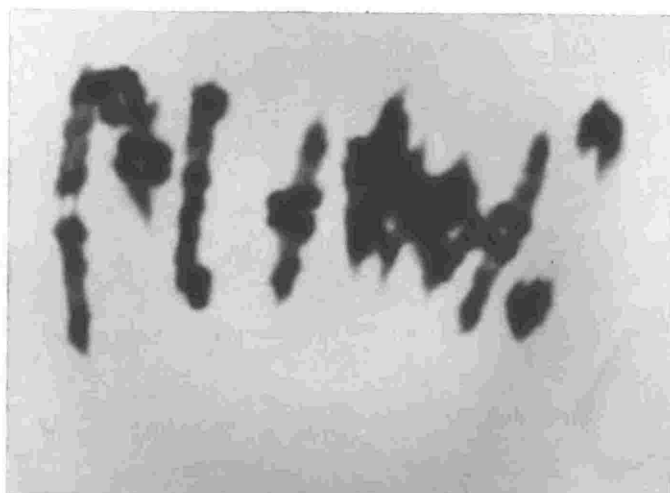
Fig. 45 a - d and a'； diagrammatic illustrations of
centrophilic and partially centrophilic complexes
expected in a chain of four chromosomes. e and f;
stable, fully cooriented complexes (alternate and
adjacent orientations).

The horizontal lines indicate the approximate
position of the metaphase equator. Arrows indicate
centromeres and their direction of orientation.

Fig. 45

a

b

c

d

e

f

a′
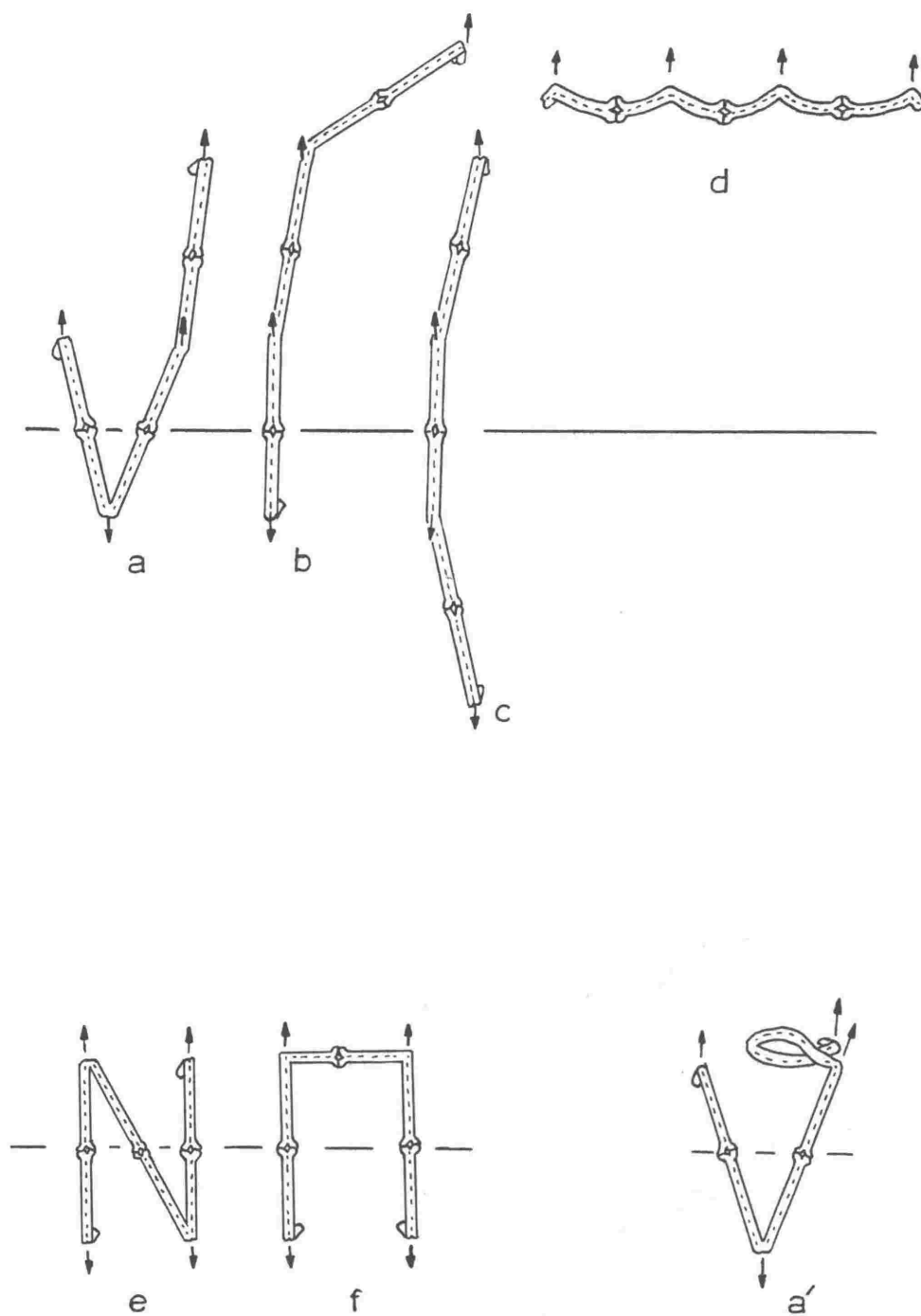
Fig. 46 Unsquashed prometaphase cell from interchange

material showing type a (Fig. 45) centrophilic complex

and all bivalents cooriented. a and b are two focal levels

of the same cell; c is a diagrammatic illustration.

Alcoholic carmine.

Fig. 46

a.

b.

c.
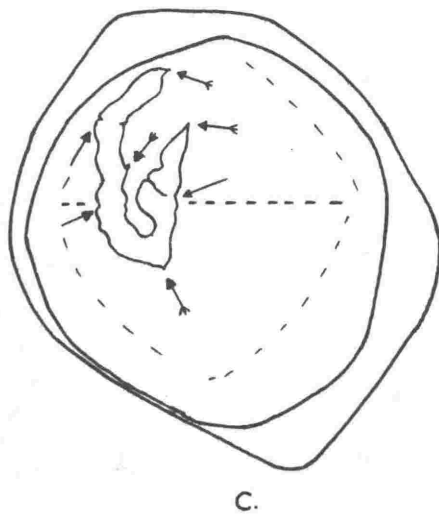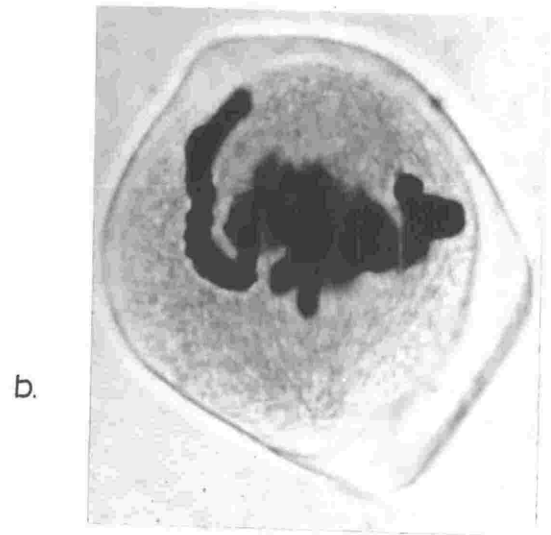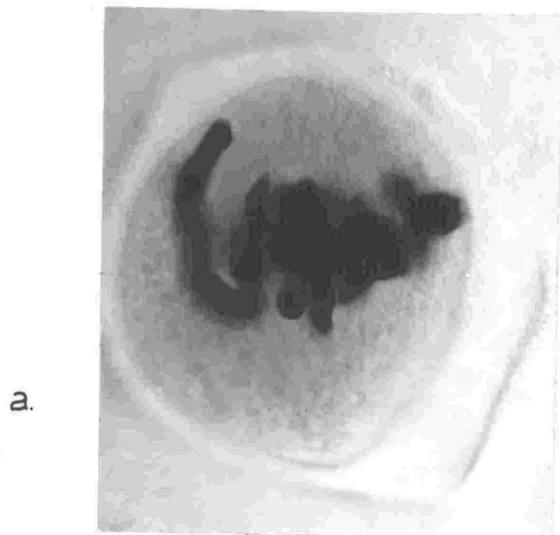
Fig. 47 Unsquashed prometaphase cell showing type b
centrophilic complex; all bivalents cooriented.
a and b; two focal levels of the same cell. c; diagr-
ammatic illustration. Lower photograph; same cell after
squashing. Alcoholic carmine.

Fig. 47

a.

b.

c.
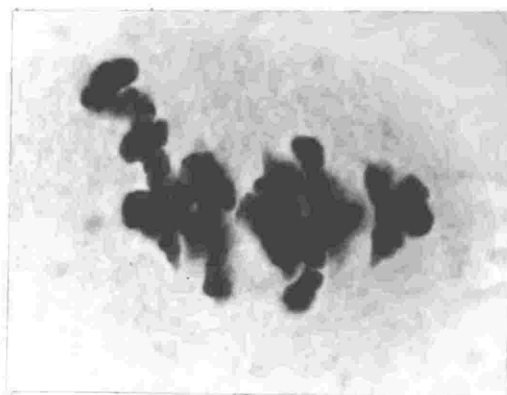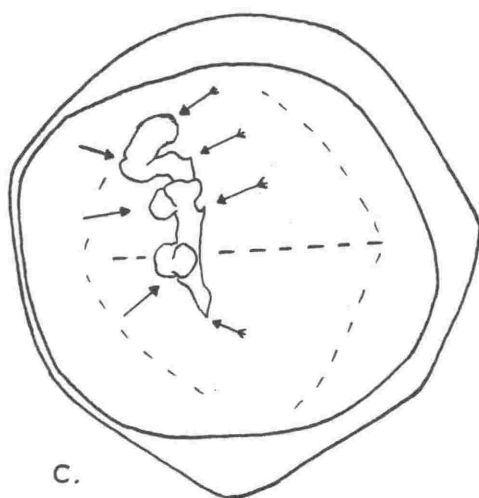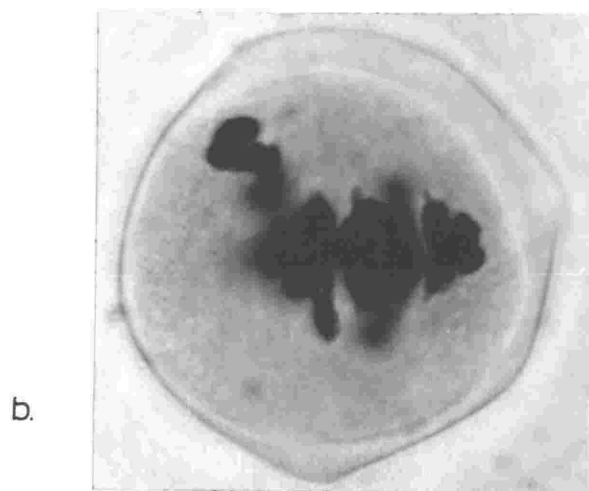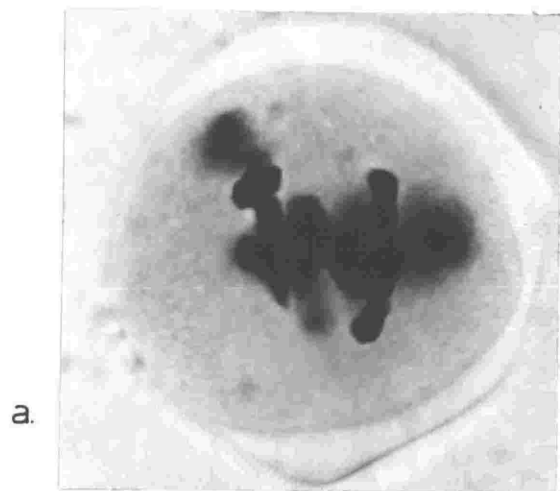
Fig. 48   Unsquashed prometaphase cell showing a type b

centrophilic complex (see also Fig. 47). b; from the same

cell as a but after light flattening. Alcoholic

carmine.

Fig. 48

a.
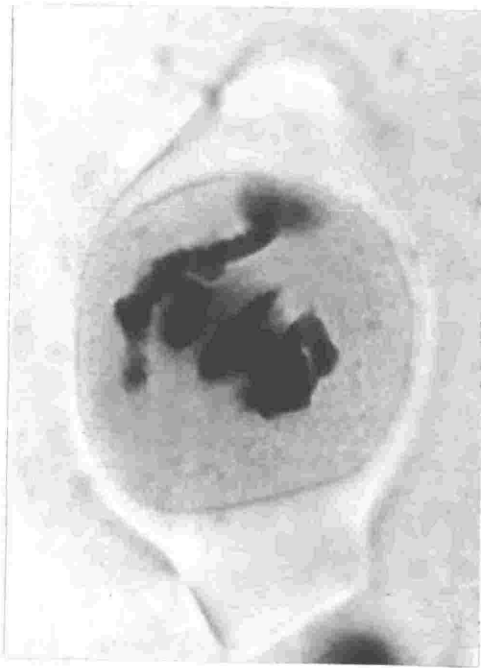
b.
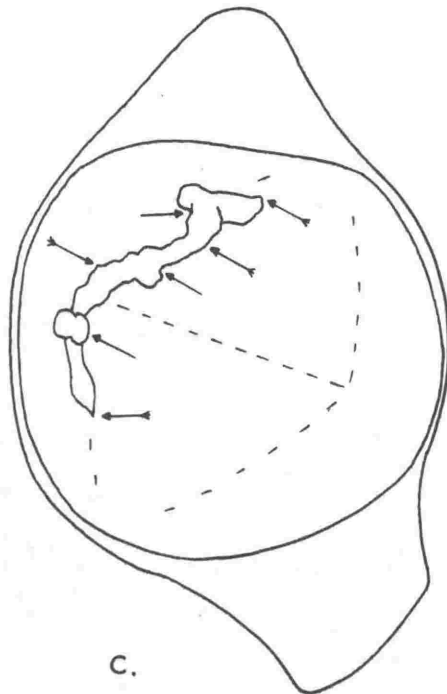
c.
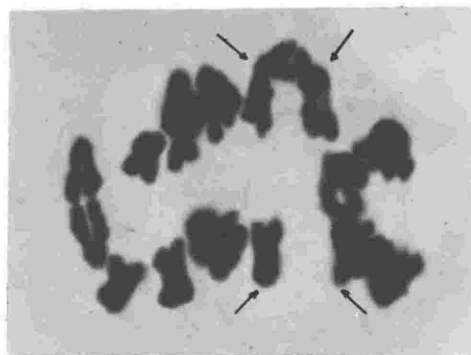
Fig. 49 Unsquashed prometaphase cell showing type c (Fig. 45)
centrophilic complex; all bivalents cooriented. a and b;
two focal levels of the same cell. c; diagrammatic
illustration. The lowermost centromere was difficult to
locate precisely. Alcoholic carmine.

Fig. 49
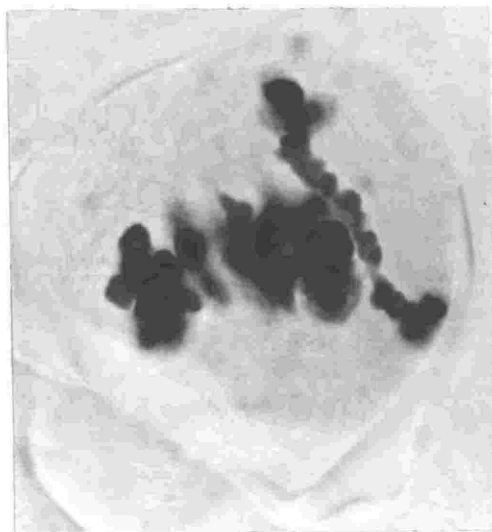
a.
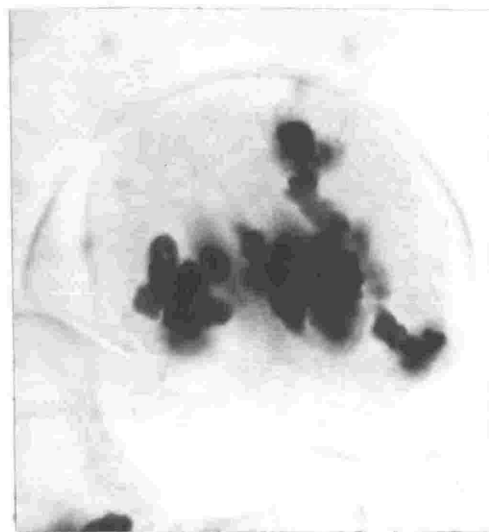
b.
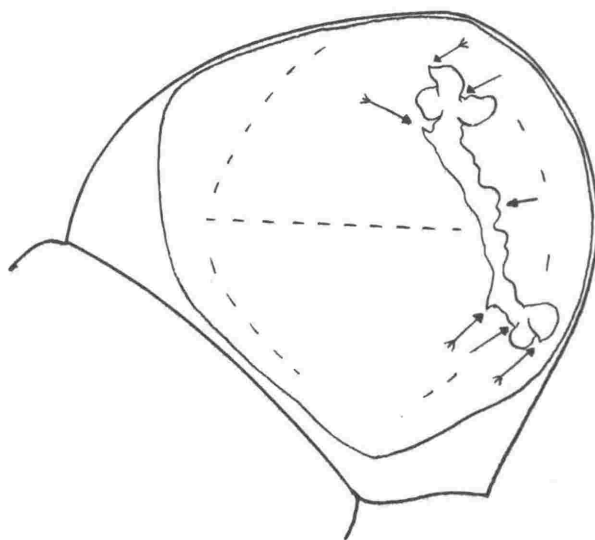
c.

Fig. 50 Unsquashed prometaphase cell showing type d
(Fig. 45) centrophilic complex; all bivalents
cooriented. b; after light flattening. c; line
interpretation. d; diagrammatic illustration.
Only two of the four centromeres could be clearly
identified. Alcoholic carmine.

Fig. 50

a.

b.

c.

d.
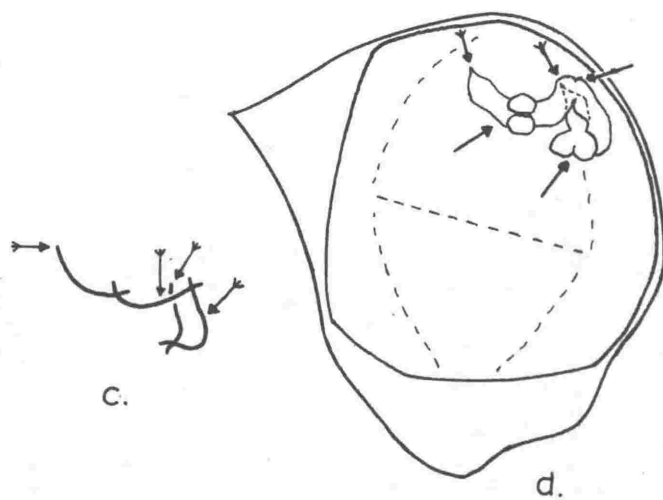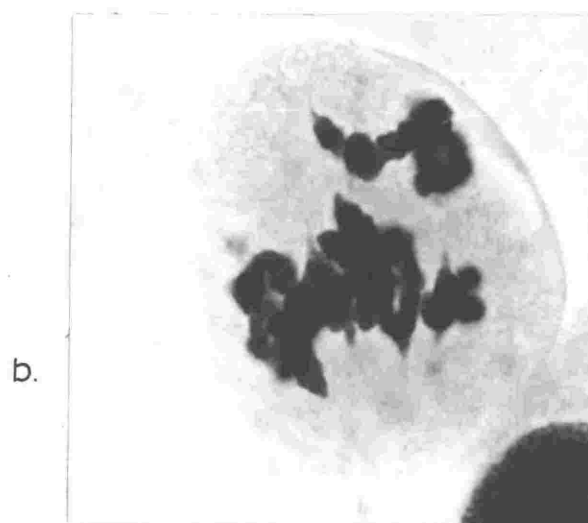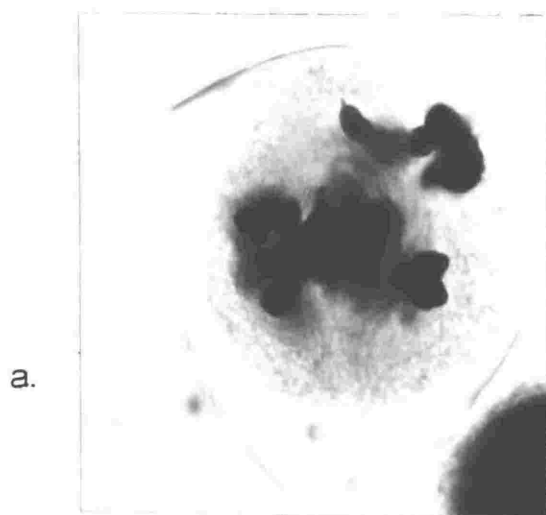
Fig. 51 Unsquashed prometaphase cell showing the complex
in a centrophilic state. The centromere indicated by the
sigmoid arrow appeared to have just been (re)oriented
to the 'lower' pole. Diagrammatic illustration below.
Alcoholic carmine.

Fig.51
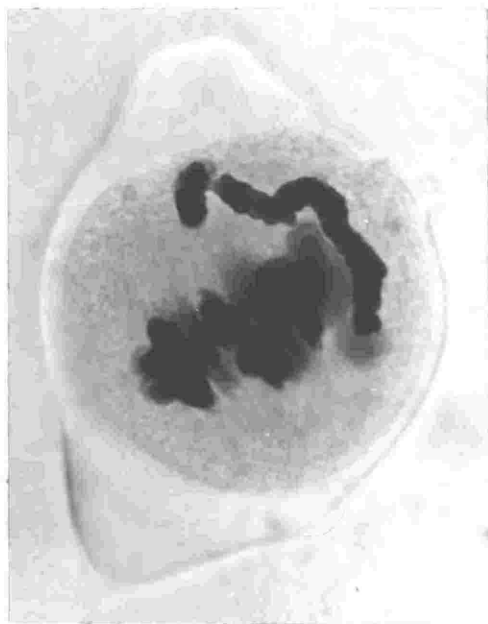
Fig. 51a   Unsquashed prometaphase cell showing centrophilic

complex type b along with a centrophilic bivalent (at left).

Two focal levels above and a diagrammatic illustration

below.   Alcoholic carmine.
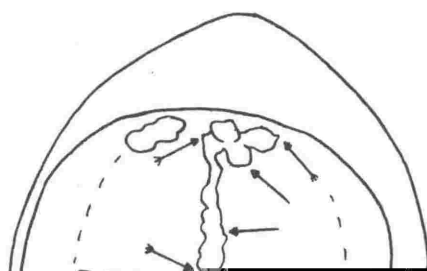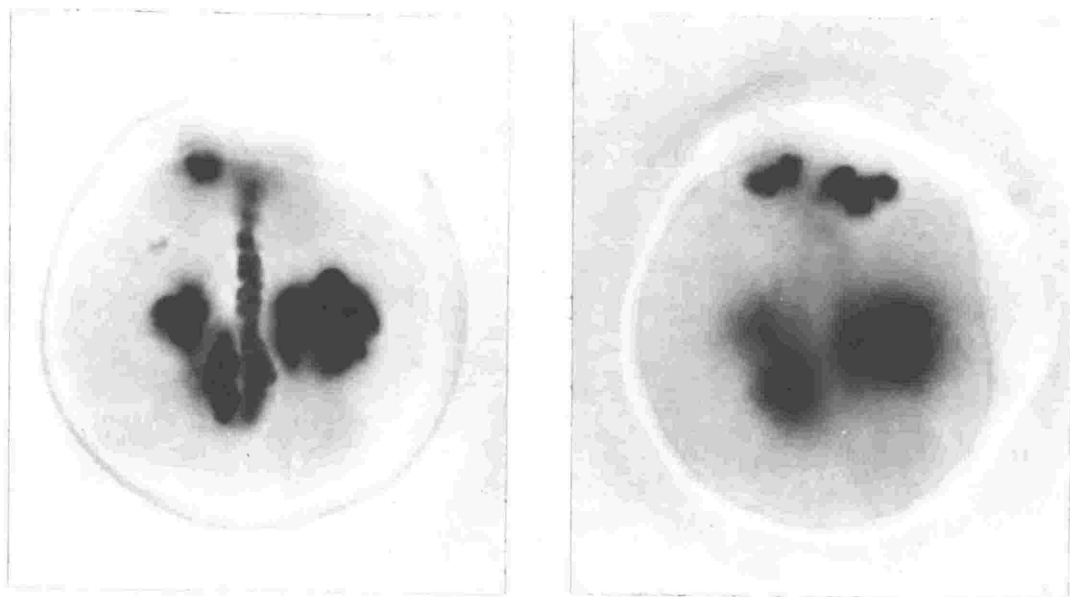
Fig. 52 Unsquashed prometaphase cell showing a
centrophilic complex in which one end centromere
appears to have just been (re)oriented to the lower
pole. The cell also shows a centrophilic bivalent
(at right). Alcoholic carmine.

Fig. 52

a.

b.
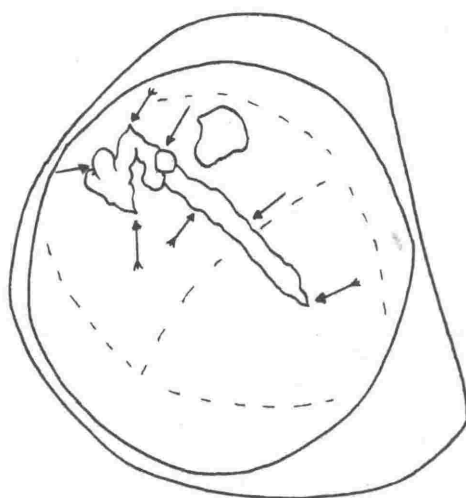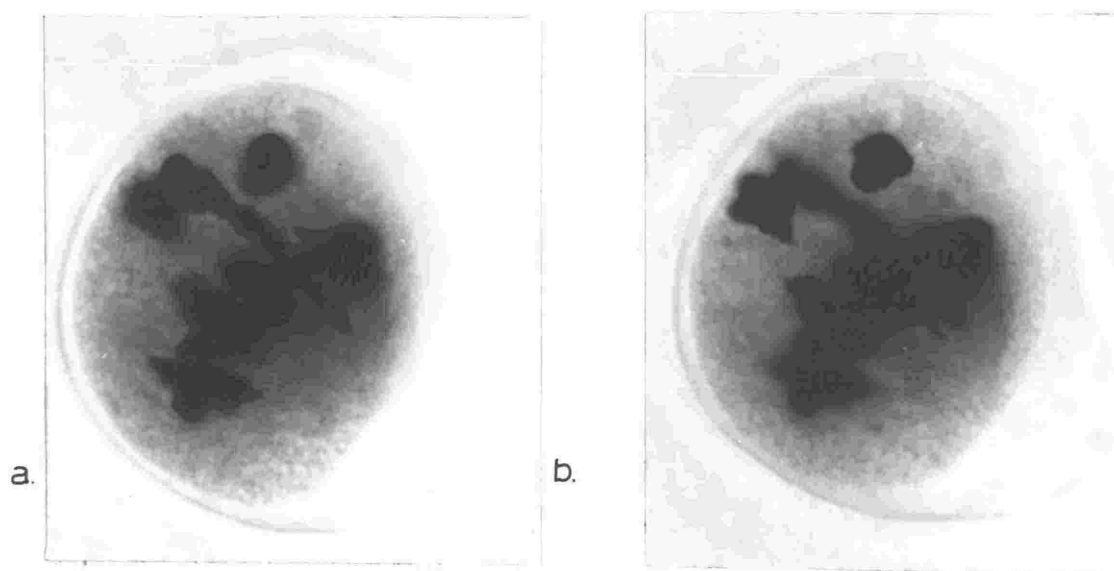
c.

Figs. 53 and 54 Unequal bivalents representing the four
interchange chromosomes at metaphase I. In Fig. 53 the
unequal bivalents are probably 'false' in that the middle
chiasma (arrows) has been lost through anaphase separation
or squashing during preparation. The chromatin at the
chromosome ends where the chiasma was lost is noticeably
drawn out. In Fig. 54 the unequal bivalents are
probably true ones i.e. brought about through a failure
of chiasma prior to metaphase/anaphase I. The chromosome
ends where no chiasma is present showed no attenuation
of chromatin as in Fig. 53.

Both Feulgen preparations.

Fig. 53
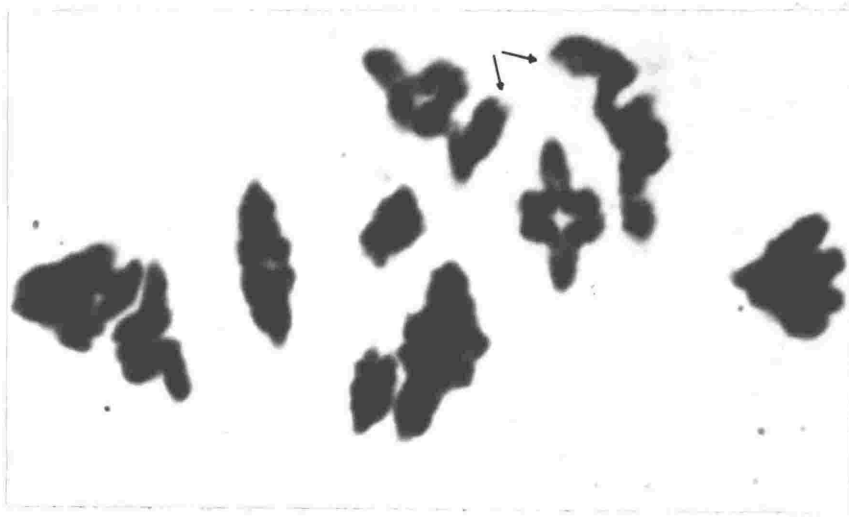
Fig. 54
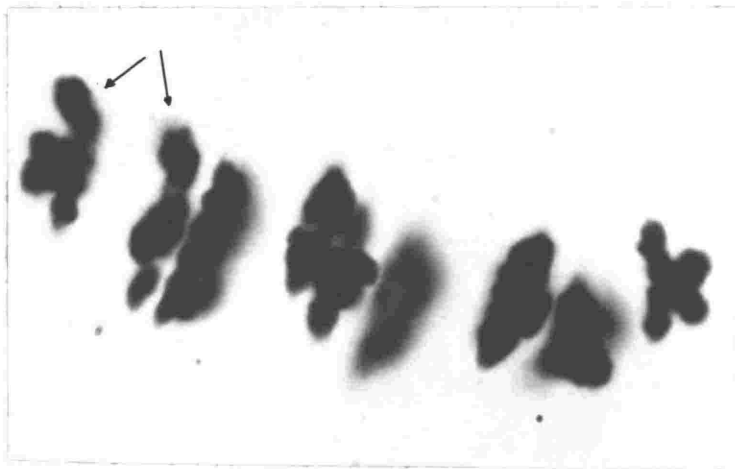
Fig. 55 Unequal bivalents representing the interchange

chromosomes in an unsquashed cell at prometaphase I. A

centrophilic bivalent lies near the 'upper' pole.

Alcoholic carmine.

Fig. 56 Early - mid anaphase I showing separation of

the complex (centromeres arrowed) from adjacent

orientation. The middle chiasma is still present

and is presumably lost 'passively' during later

anaphase. Feulgens.

Fig. 55
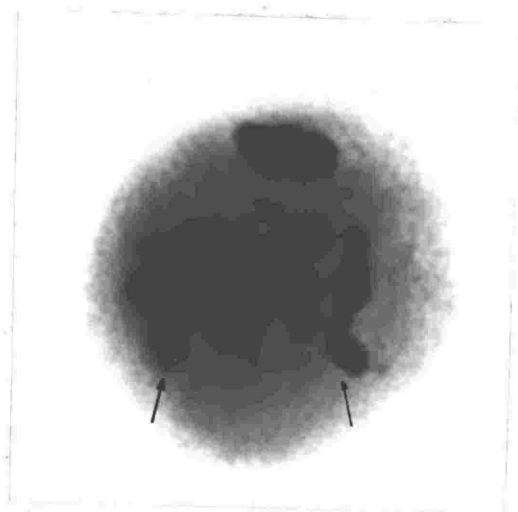
Fig. 56

Fig. 57 Unsquashed prometaphase I cell showing type a'
(Fig. 45) centrophilic complex. The upper portion of the
complex has two chiasmata in the arm pair, one of which
is very close to the centromeres. Note the position of
the non-cooriented centromere in relation to proximity
to its pole; compare in this respect with Fig. 46.
a; unsquashed. b; diagrammatic illustration. c; after
light flattening. Alcoholic carmine.

Fig.57
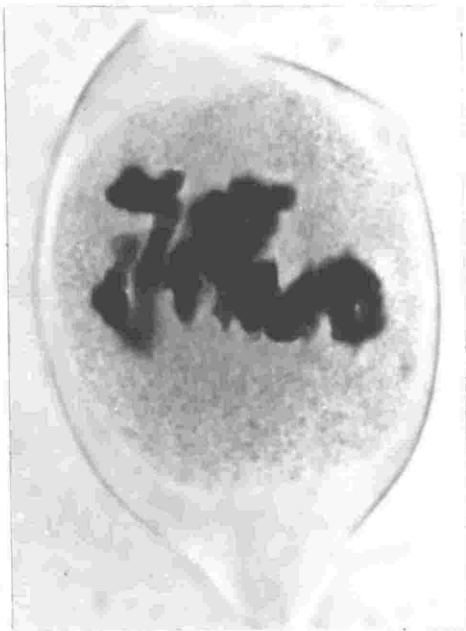
a.
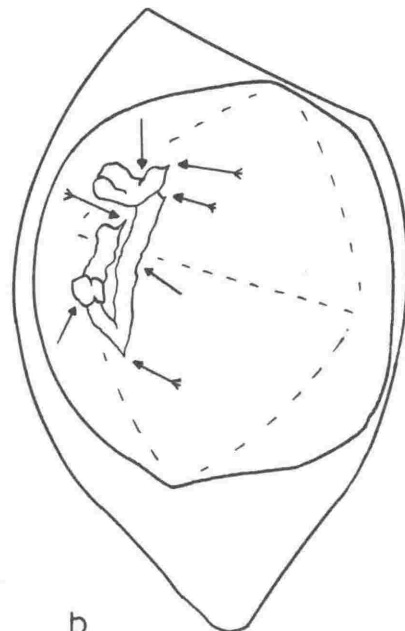
b.
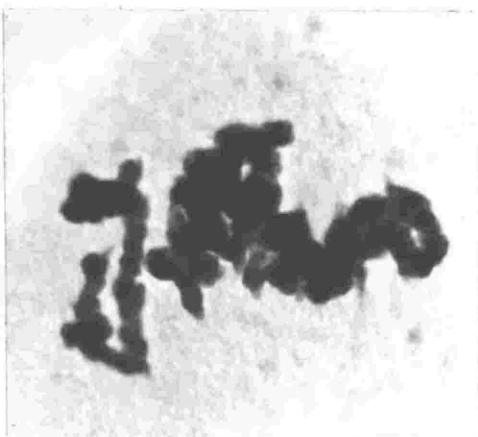
c.

Figs. 58 and 59 Passive centromeres (sigmoid arrows) in the interchange complex in early anaphase I cells. In Fig. 58 one group III centromere is passive. In Fig. 59 two group III centromeres are passive. In the latter the middle chiasma has been lost through anaphase movement (bisected arrow). From lightly squashed, alcoholic carmine preparations.

Fig. 58
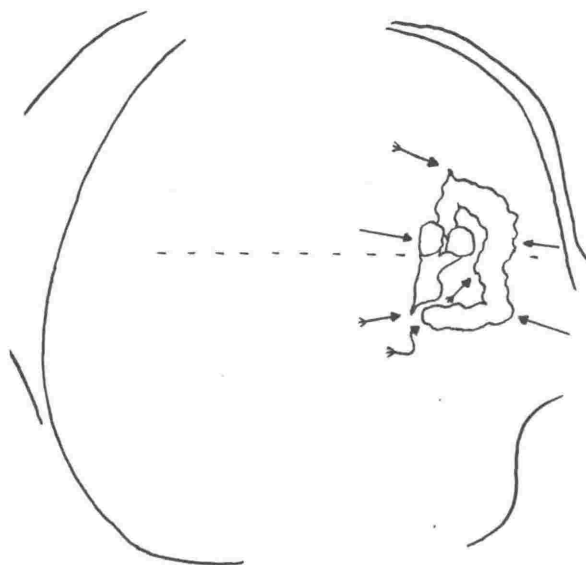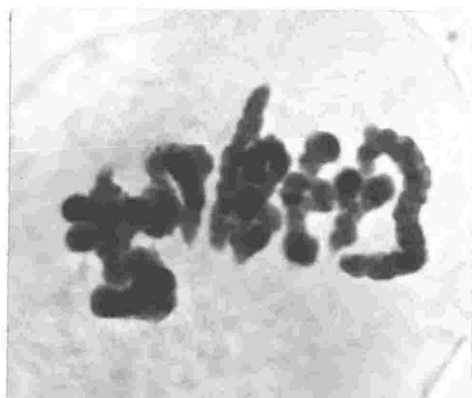
Fig. 59
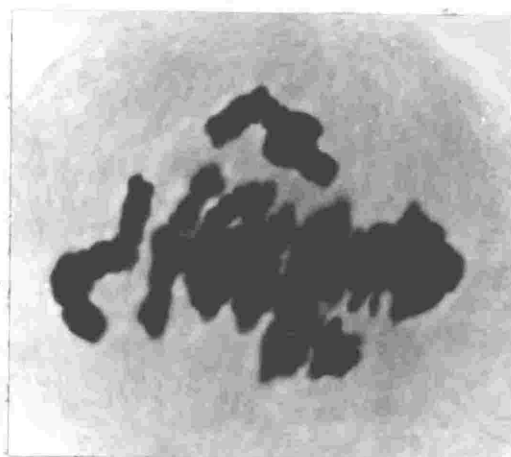
Fig. 60 Early anaphase I from an unsquashed interchange
preparation showing wide separation of the complex halves
in adjacent orientation . Diagrammatic illustration of
the complex on the right. Alcoholic carmine.

Fig. 61   Early anaphase I from an unsquashed interchange
preparation showing extreme attenuation at the middle
chiasma of the complex, accompanied by wide separation
of the complex halves. a; unsquashed. b; diagrammatic
illustration of the complex (centromeres arrowed).
c; after light flattening. Alcoholic carmine.

Fig. 60

Fig. 61

b.

a.
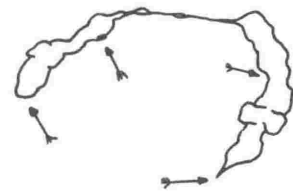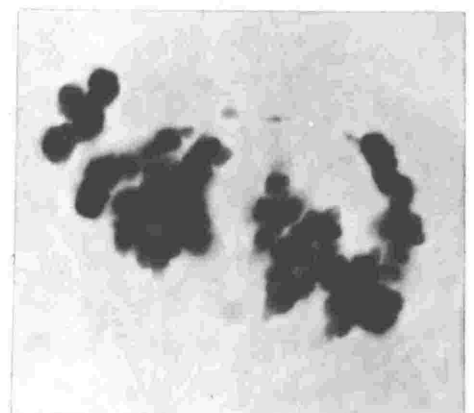
c.

Figs. 62 and 63   Polar aspects of metaphase I.   Fig.62
from normal material showing two central and seven
peripheral bivalents (right hand cell of the two
shown.) Fig. 63 from interchhange material showing
two central and seven peripheral 'bodies'; seven of the
total bodies are bivalents, the other two represent the
two halves of the interchange complex (indistinguishable
because of difficulties with depth of focus).   Both
cells from alcoholic carmine preparations.

Fig. 62
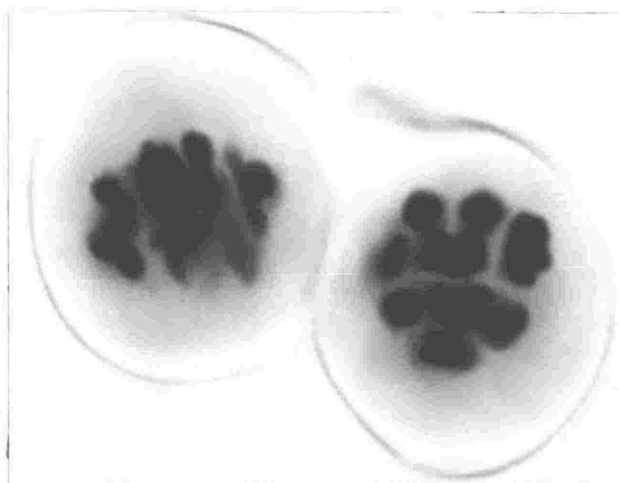
Fig. 63
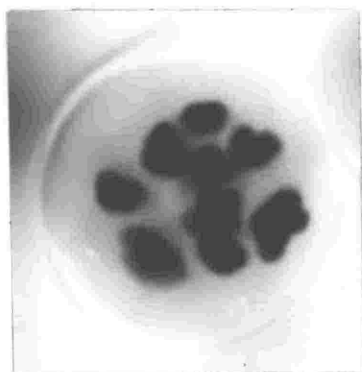
Fig. 64   Illustration of the method used in classifying
the position of the interchange complex in lateral
metaphase plates, according to the position of its two
halves in relation to the other seven bivalents in a
total of nine available positions. Further details in
text.

Fig. 65 Diagrammatic illustration of metaphase I in
polar aspect with nine numbered bivalents. Below is
depicted the relative order these bivalents are likely
to take up in lateral aspects of metaphase following
different planes of flattening (aa', bb', cc').
Further details in text.

Fig. 64

N | | | | | | 1/2

⊓ | | | | | | 1/3

| | N | | | | 3/5

| | ⊓ | | | 3/6

Fig. 65
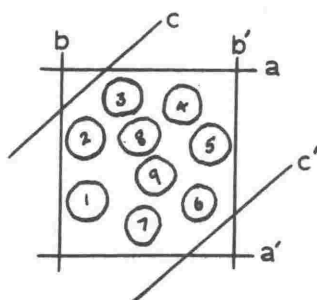
For aa' plane of flattening ⟶ 213879465

   "   bb'   "   "   " ⟶ 342859167

   "   cc'   "   "   " ⟶ 172986354

Fig. 66   Mid-anaphase I from alternate orientation in
the interchange complex.   Each polar group has four
group I, two group II and three group III type chromosomes.

Fig. 67   Mid-anaphase I from adjacent orientation in
the interchange complex.   The upper polar group has
a 5, 2, 2 composition (groups I - III respectively),
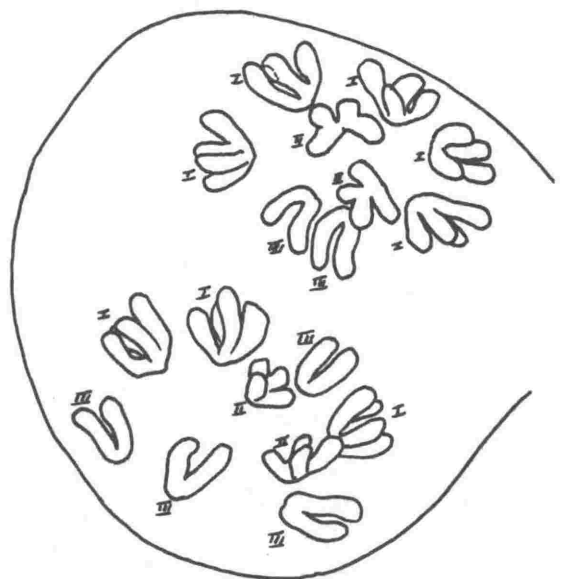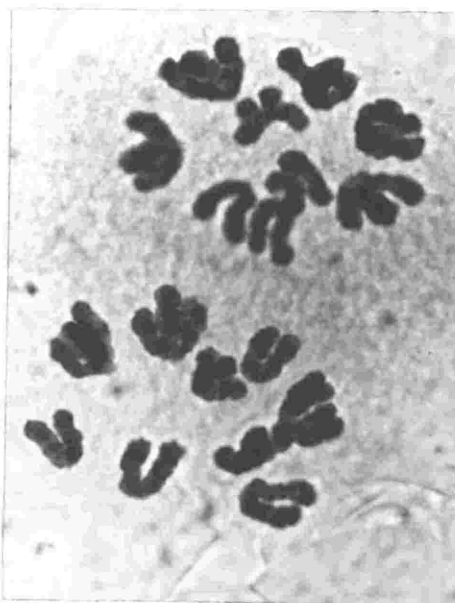the lower group 3, 2, 4.

Fig. 66
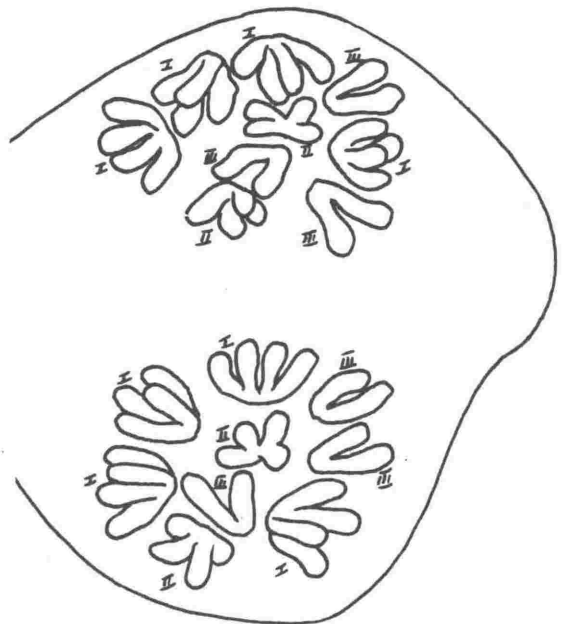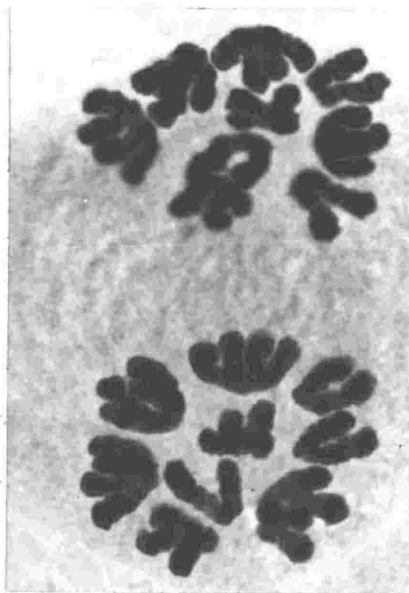
Fig. 67

Fig. 68   Mid-anaphase I cell showing 10 : 8 numerical

non-disjunction arising from 3 : 1 segregation from the

interchange complex.   The upper polar group has a

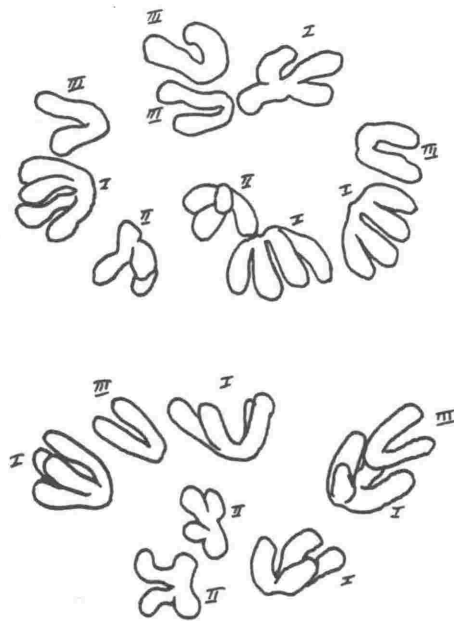4, 2, 4 composition (groups I - III respectively),
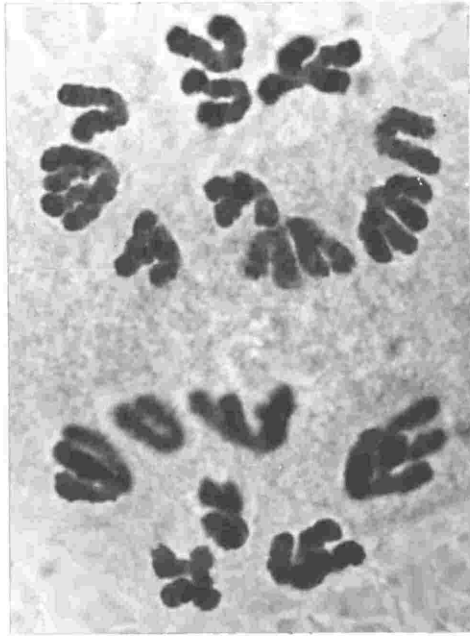
the lower group 4, 2, 2.

Fig. 68

Fig. 69 Lagging group III type chromosomes at late
anaphase I arising from passive centromeres in the
interchange complex. In a and b the chromatid centromeres
of the laggards have become oriented to opposite poles;
in the latter both chromatid centromeres and associated
spindle fibres were very clear (see accompanying illust-
ration on right). In c the laggard is positioned near
the upper pole, apparently showing belated centromere
activity. All figures alcoholic carmine.

Fig. 69.
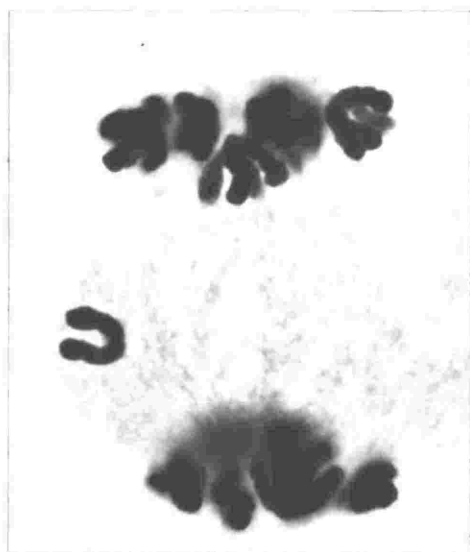
a.
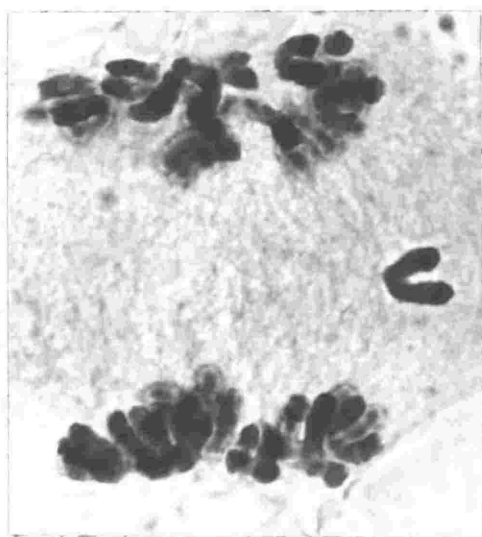
b.
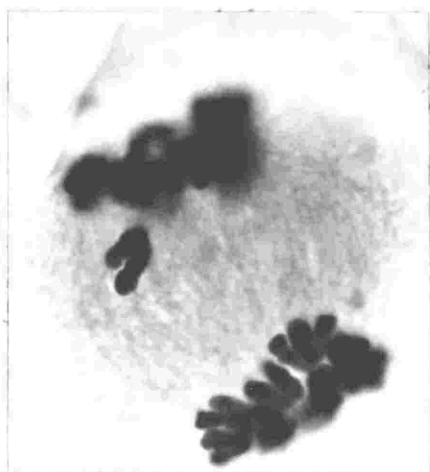
c.

Fig. 70 Laggard chromosomes arising from passive
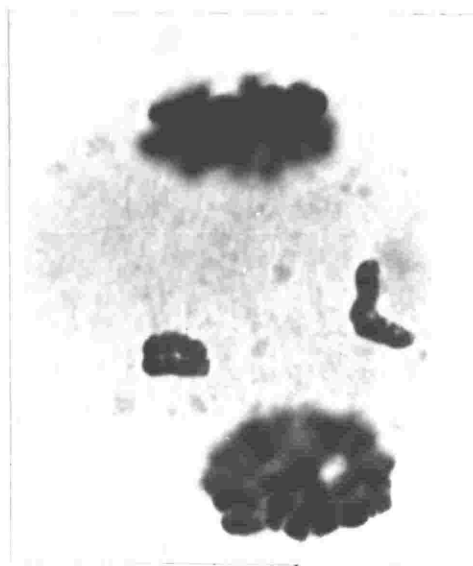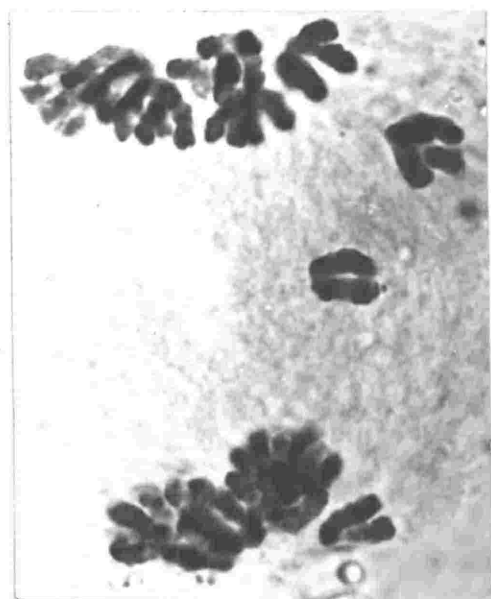centromeres in the interchange complex at late
anaphase (a and c) and telophase (b and d). In
a, two group I chromosomes are lagging; in b, two of
group III; and in c, one group I and one group III
chromosome. In d the one group III laggard is becoming
transformed into an interphase state along with the
rest of the chromosomes. Alcoholic carmine.

Fig. 70

a.

b.

c.
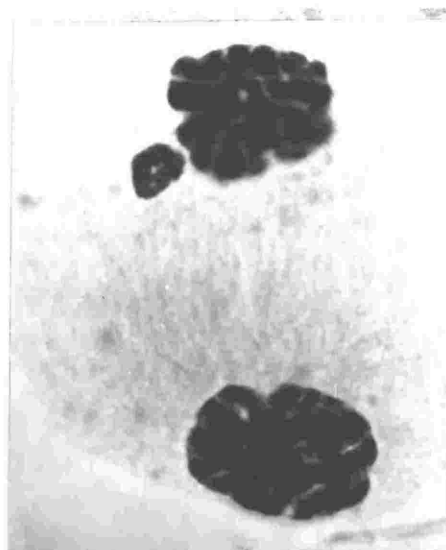
d.

Fig. 71   Graphical presentation of the data in table 30
showing the relationship between developmental age
(anaphase I index values) and alternate/adjacent type
complements. Each point in the graph represents the
data from one pollen sac.

Fig. 71a Diagrammatic interpretation of the relationship
shown in Fig. 71. Regions A, B and C are described in
detail in the text.

Anaphase I Index values.

Ratio of alt./adj. type complements.
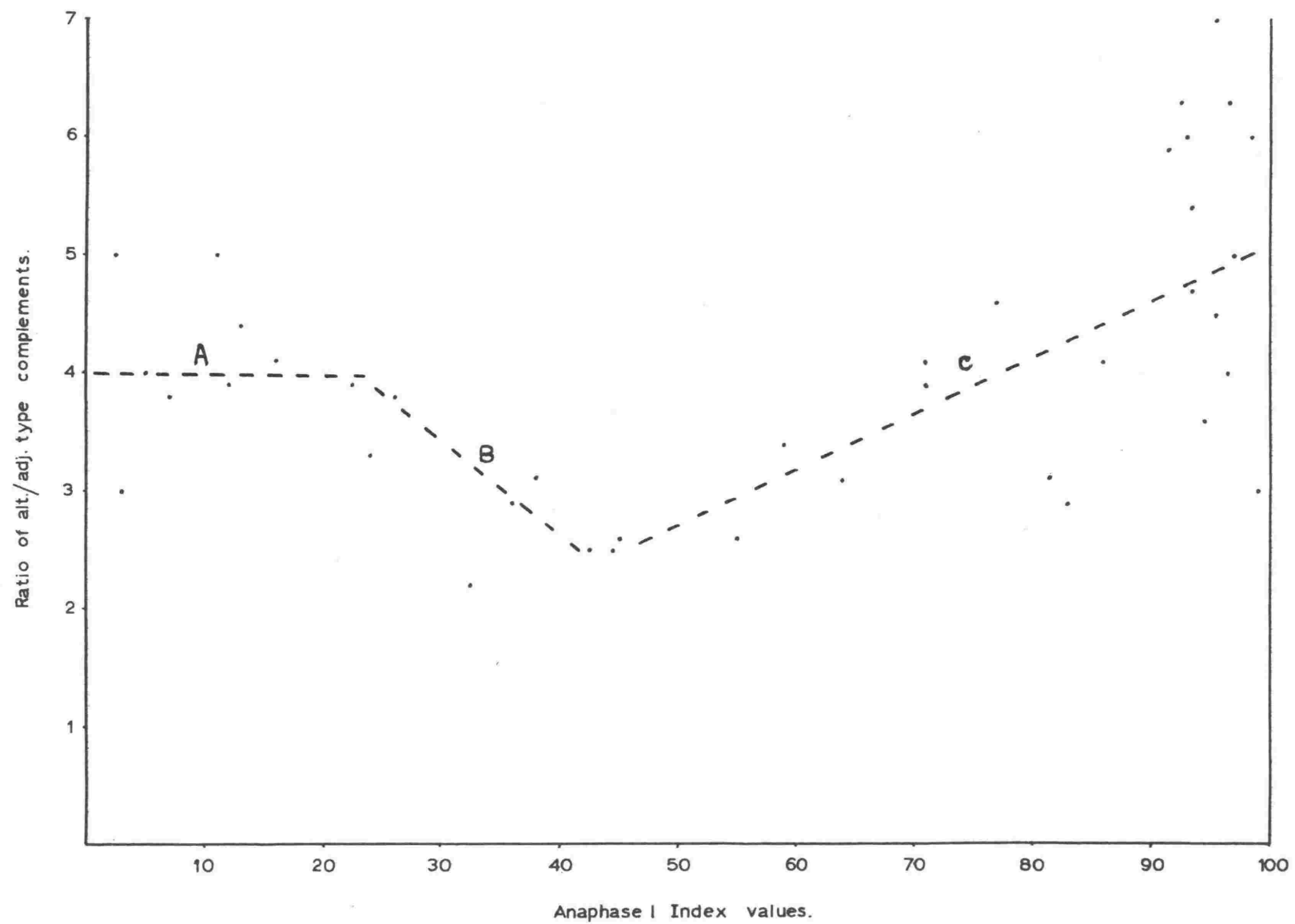
Fig. 72 Graphical presentation of the data in table 30.
_____

Here the alternate and adjacent complement frequencies

in each pollen sac are plotted against each other at

E, M, L, and VL developmental stages.

Fig. 72

Alternate type complements

Adjacent type complements

Very late

Late

Early

Mid
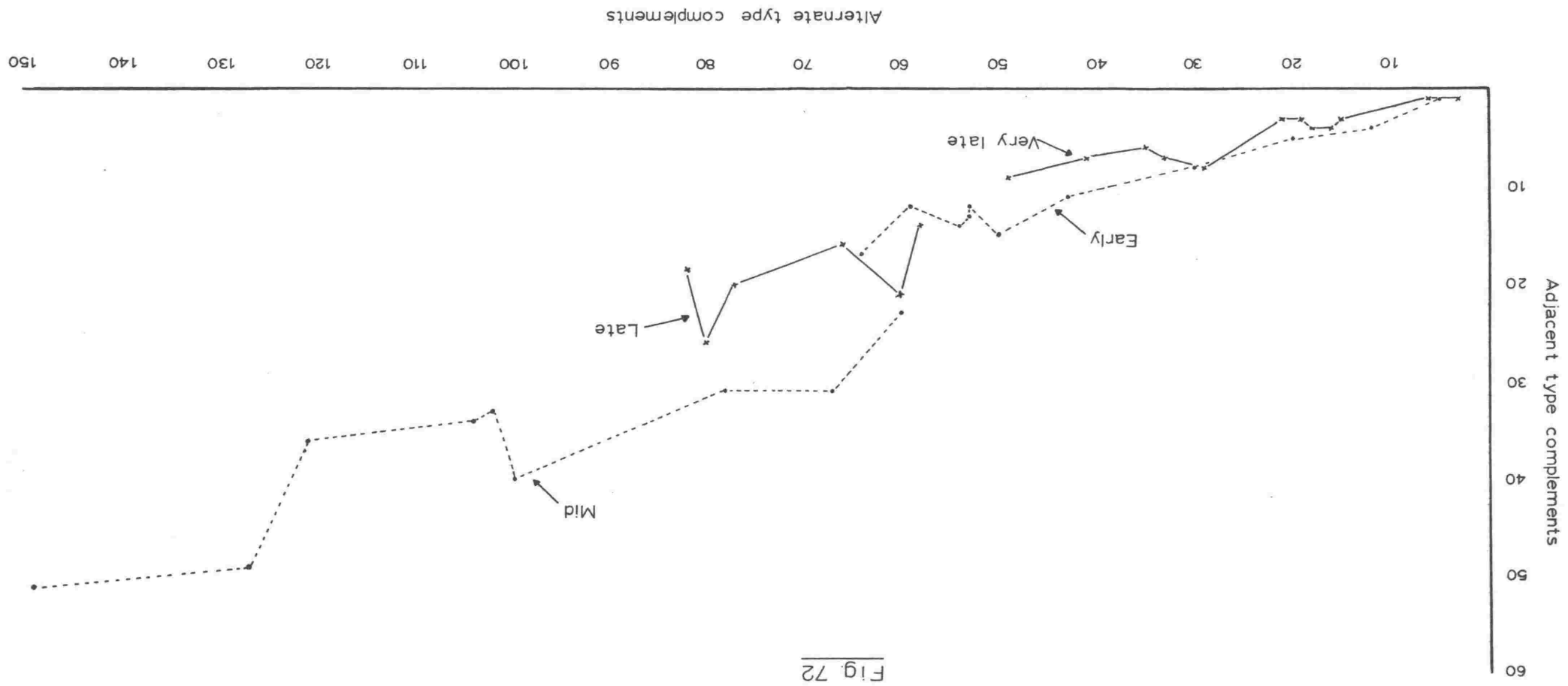
Fig. 73   Mid-late anaphase II cell from normal material

showing the three groups of chromosomes, I, II, and III

(diagrammatic illustration of the top polar group of

chromosomes on right of photograph). Note, however, that

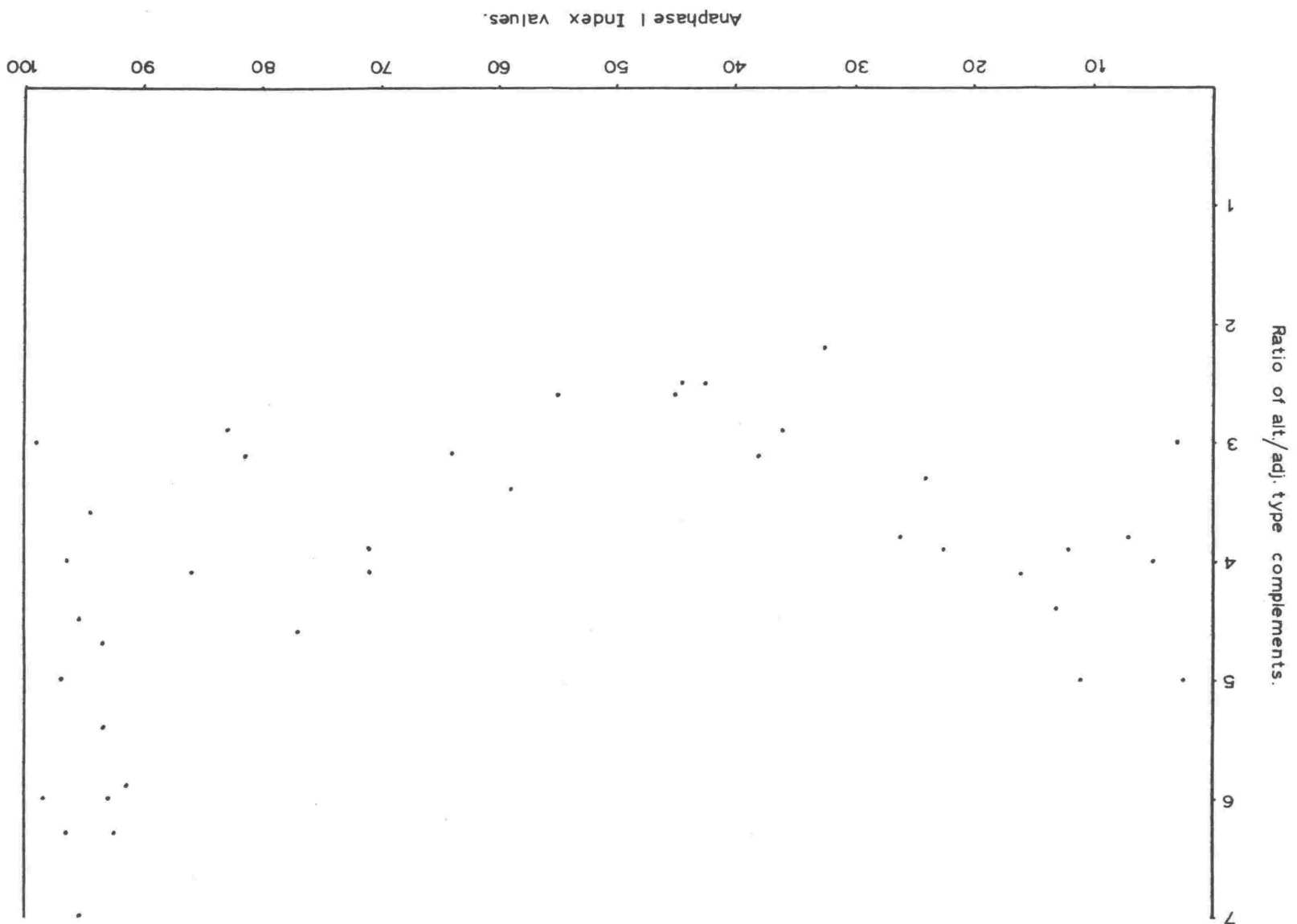no satellites appear on chromosomes 7 and 9 of group III

so that the individual chromosomes within this group

cannot be identified with complete certainty (cf. in

root tip cells). The smaller size and asymmetry of the

group I chromosome arrowed indicates that this is

probably chromosome 4. Alcoholic carmine.

Fig. 74   Mid-anaphase II cell produced from 2 : 1 plus

laggard segregation of the interchange complex at

meiosis I. The two upper sister complements each have

only two group III chromosomes (arrows) and thus a total

of only eight. The lower two sister complements are

normal. The micronucleus (blackened in in the diagram),

which did not transform into chromosomes during prophase

II, contains the remaining two group III chromosomes

(unseparated pair of chromatids) and represents the

laggard of anaphase I. Alcoholic carmin.

Fig. 73

Fig. 74

Fig. 75   Mid-anaphase II complements derived from
alternate segregation of the interchange complex.
Each complement has three group III chromosomes (arrows
in diagram). The two pairs of complements cannot be
distinguished into normal and interchange types.
Alcoholic carmine.

Fig. 76   Mid-anaphase II complements from adjacent
segregation of the interchange complex. The upper pair
of complements has four group III chromosomes in each,
the lower pair only two. Alcoholic carmine.

Fig. 75
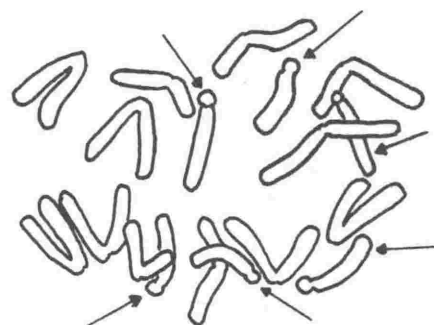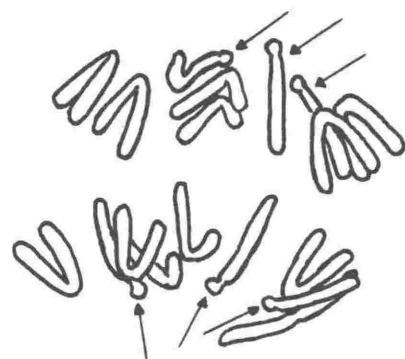
Fig. 76
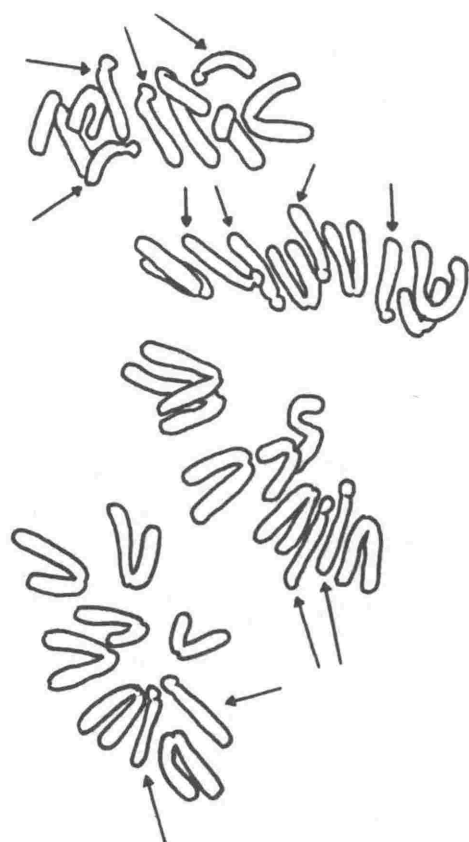
Figs 77 and 78   Mid-anaphase II complements produced

from 3 : 1 segregation of the interchange complex. In

Fig. 78 the upper pair of complements each has two

group III (arrows in diagram) and a total of only eight

chromosomes; while the lower pair each has four group III

and a total of ten chromosomes. Fig. 78 is similar

except that the complement pairs are inverted compared

with Fig. 77. Alcoholic carmine.

Fig. 77

Fig. 78
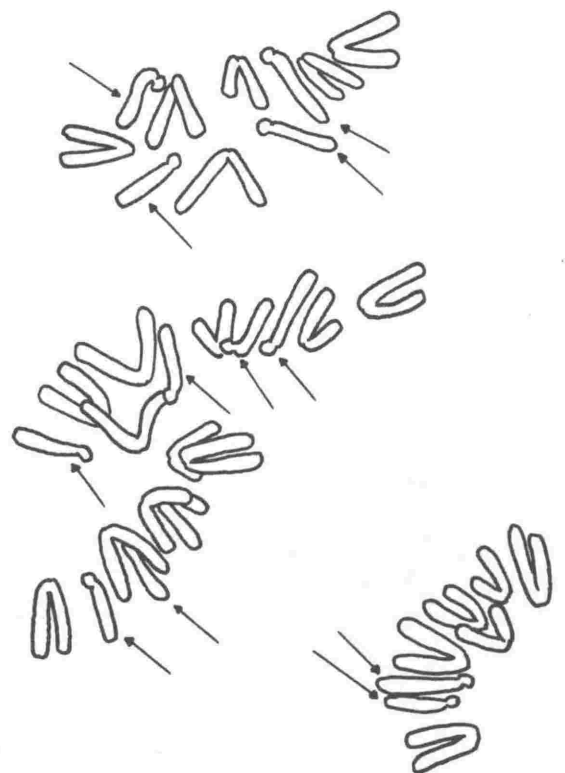
Fig. 79   Graphical presentation of the data in table 31

showing remnants of a relationship between developmental

age (anaphase II index values) and alternate/adjacent

type complements.   Each point in the graph represents

the data from one pollen sac.   Compare with Fig. 71.

Fig. 79.

Anaphase II Index values.

Ratio of alt./adj. type complements.
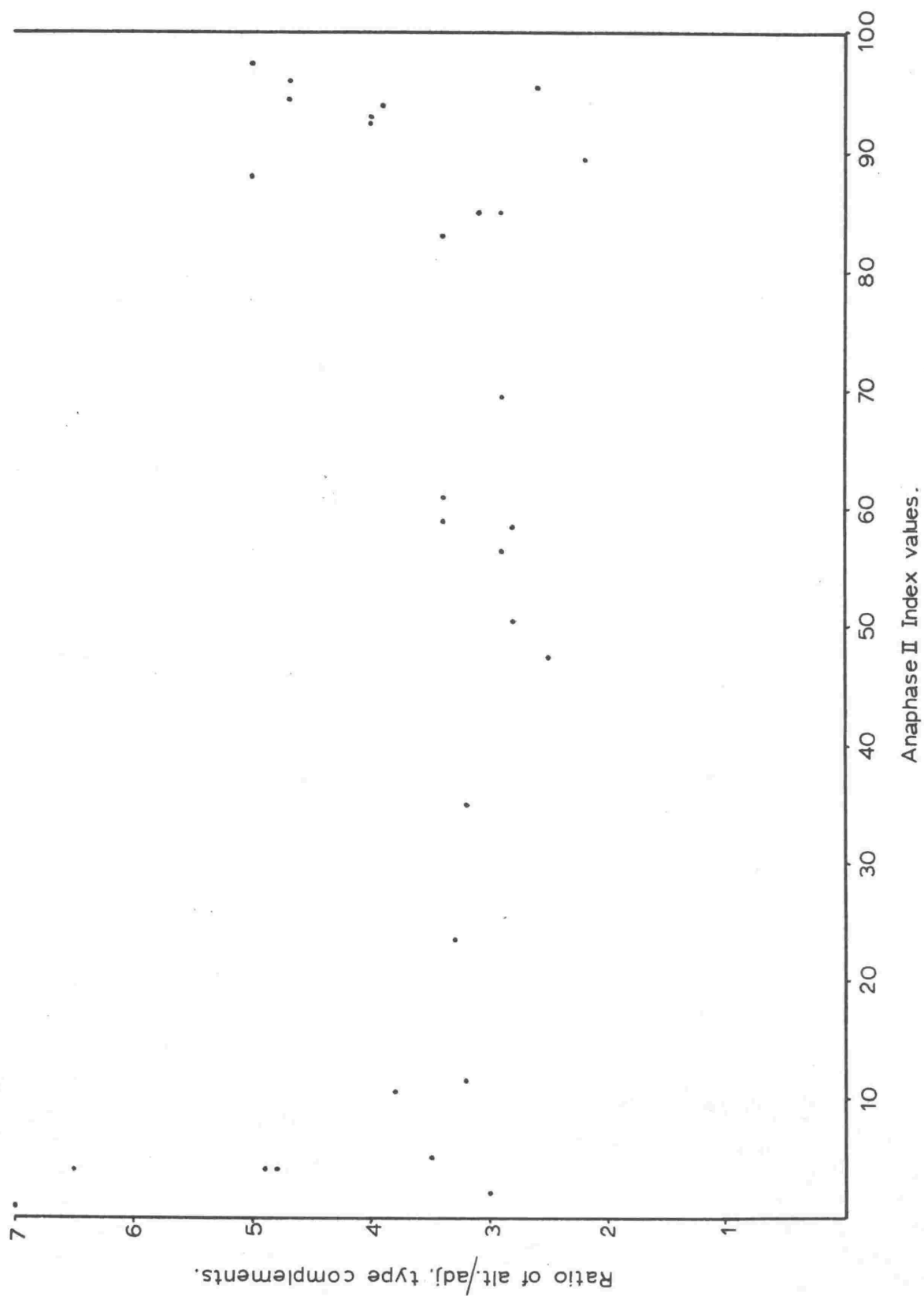
Fig. 80 Pollen from a mature anther of an interchange

plant showing abortive (small, empty shells) and

normal grains. Aceto-carmine. Approx. × 600.

Fig. 81 Immature pollen from an interchange anther

shortly before the pollen grain mitosis stage of

development. Two types of aborting grains are shown:

the 'clump' type (upper two at left) and the 'ring'

type (lower two at right). The remaining pollen grain

is normal and undergoing preparations for mitosis.

Aceto-carmine.   Approx. × 1,200.

Fig. 80
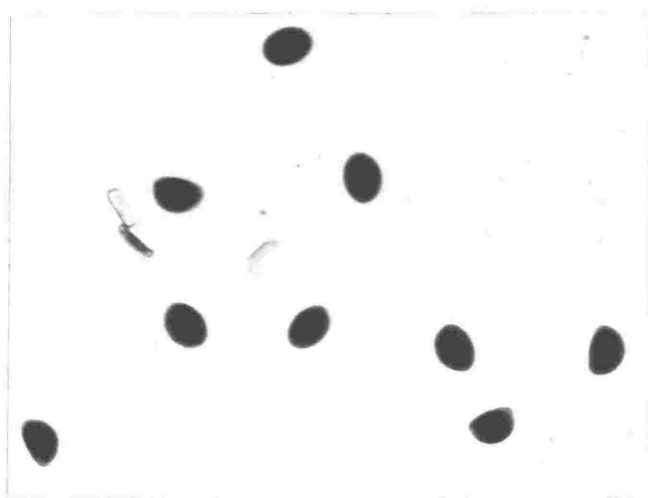
Fig. 81
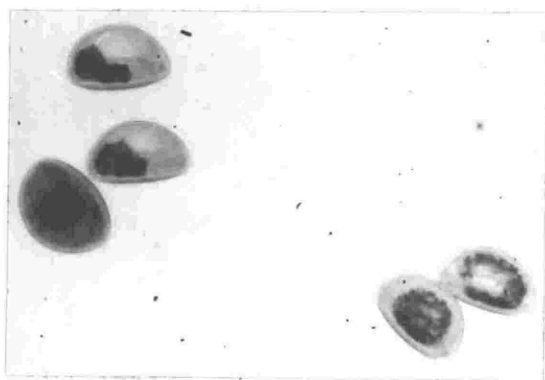
Fig. 82   Metaphase of pollen grain mitosis 1 from

normal material showing the three groups of chromosomes,

I, II and III, and chromosomes 6, 7, and 9 of the

haploid set (refer to diagrammatic illustration on

right). Note the satellites on chromosomes 7 and 9.

A larger and a smaller group II chromosome can be

distinguished, these probably representing chromosomes

5 and 8 respectively (cf. Fig. 6). The smaller asymmetr-

ical group I chromosome on the right is probably chromosome

4. Alcoholic carmine.

Fig. 83   Metaphase of pollen grain mitosis 1 from

interchange material showing the interchange complement

derived from alternate segregation of the complex during

meiosis. Note the relative lengths of the group III

chromosomes compared with Fig. 82: the relatively smaller

non-satellited member is the interchange chromosome 4^6.

The other interchange chromosome 6^4 cannot be distinguished

from the other group I members. Alcoholic carmine.

Fig. 82
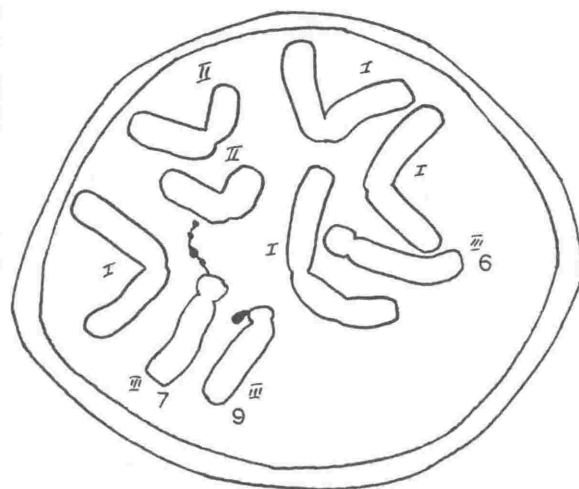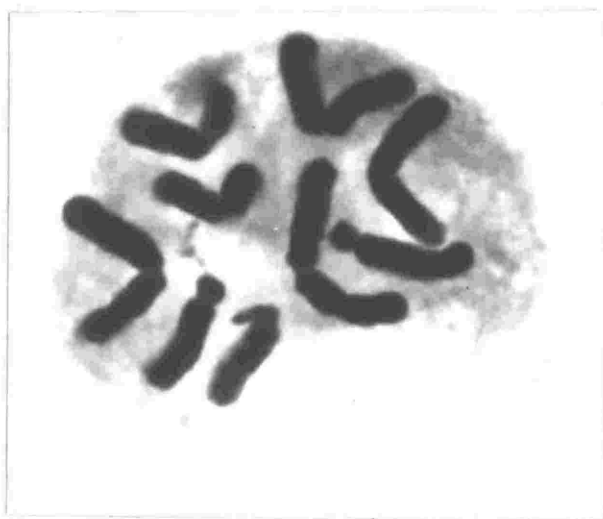
Fig. 83
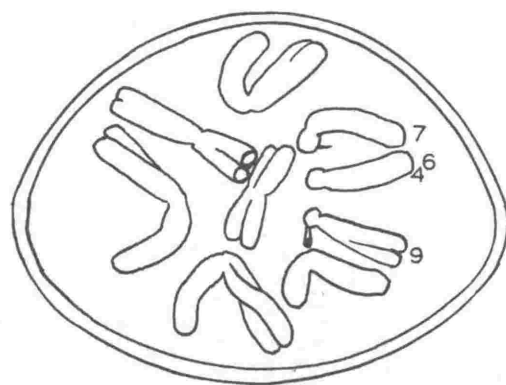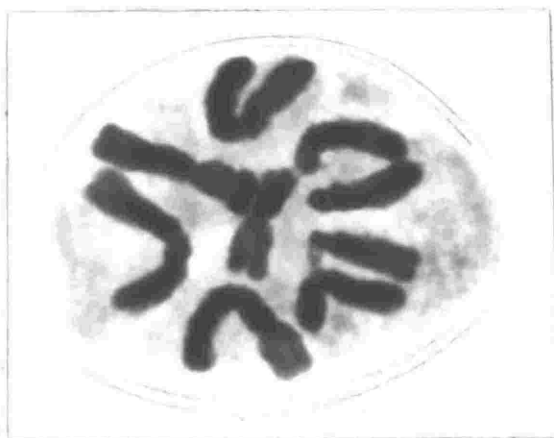
Fig. 84   Metaphase of pollen grain mitosis 1 from
interchange material showing the one viable complement
derived from adjacent-1 segregation of the complex
during meiosis. The complement has five group I
chromosomes, two group II and two group III chromosomes
(compare with Figs. 82 and 83). Alcoholic carmine.

Fig. 85 Metaphase of pollen grain mitosis 1 from
interchange material showing a numerically unbalanced
spore derived from 3 : 1 segregation of the complex
during meiosis. The complement has four group I,
two group II and four group III and thus a total of ten
chromosomes. Alcoholic carmine.

Fig. 84
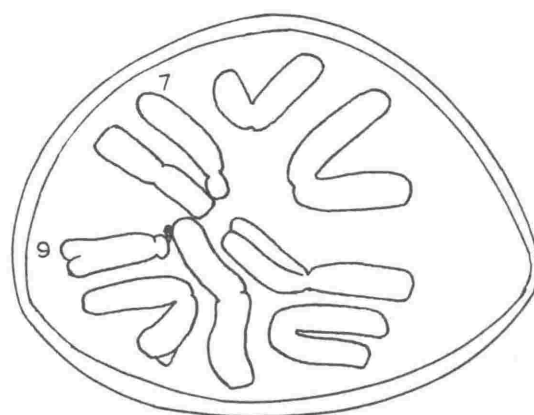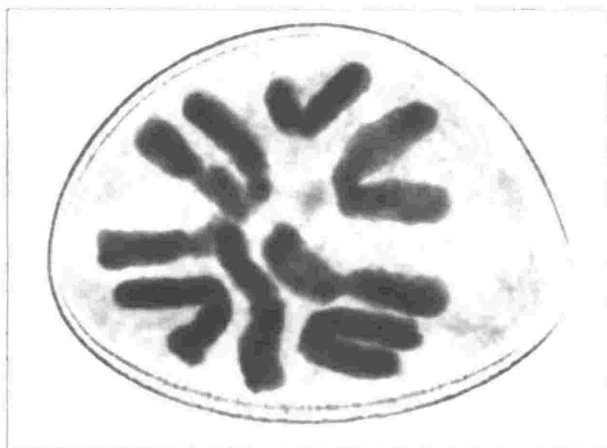
Fig. 85
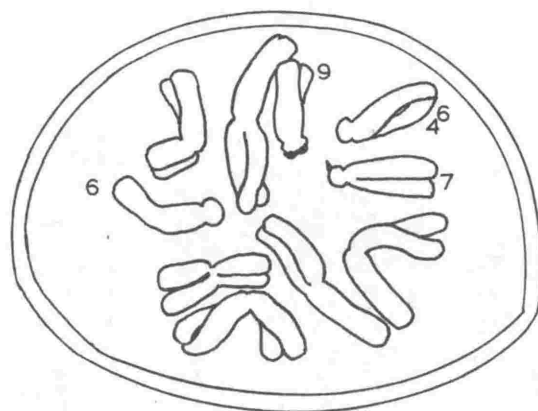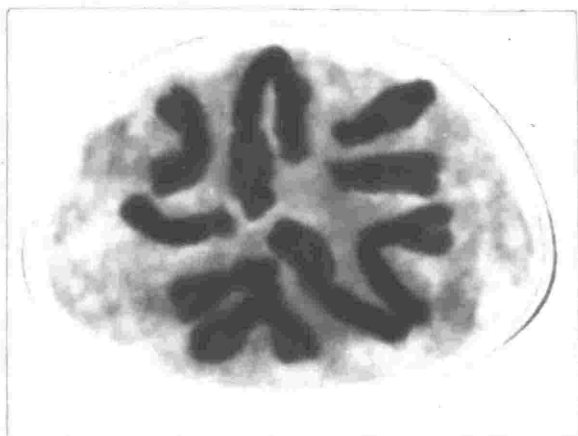
Figs. 86 and 87 Longitudinal sections of two equivalent

aged ovules from an ovary of interchange material. a;

general view of the ovule. b; enlarged view of the

embryo sac and associated structures from the same ovule.

In Fig. 86 the embryo sac is normal, the section showing

one of the eight nuclei, probably an enlarged, endopleid

synergid.   In Fig. 87 the embryo sac has completely

failed to develop, leaving an empty space between the

two inner integument sections. The abortive embryo sac

probably results from unbalanced adjacent segregation

of the interchange complex during female meiosis.

Heidenhain's haematoxylin.

es = embryo sac; n = nucellus; oi = outer integument;

ii = inner integument; s = synergid; x = tongue-like

tissue associated with the undeveloped embryo sac.

Fig. 86

a.

b.

Fig. 87

a.

b.
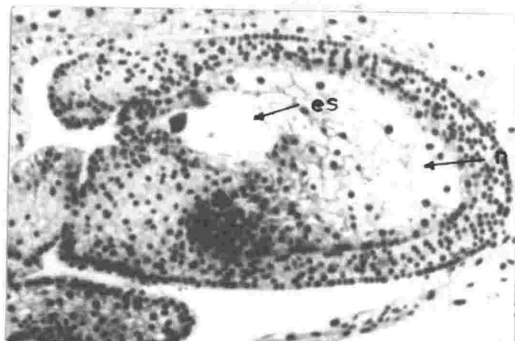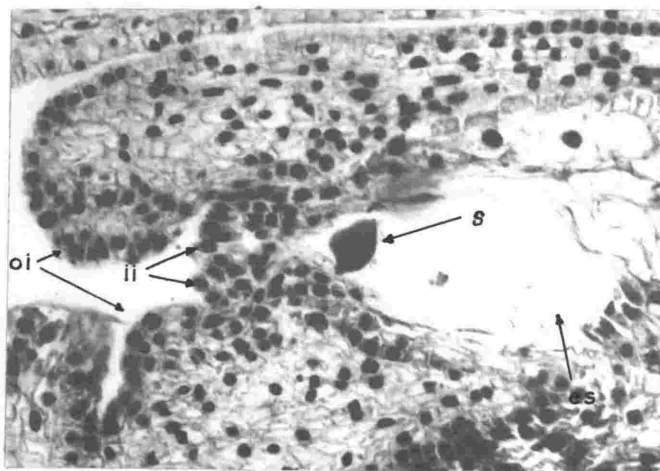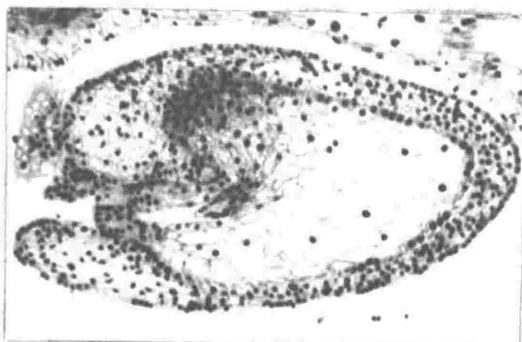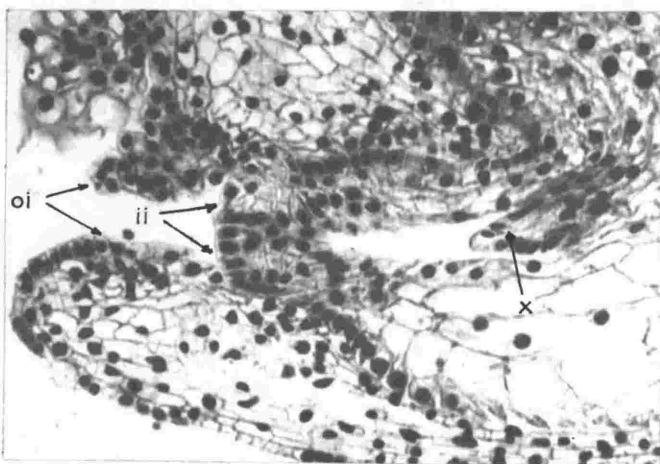
0.25mm.

0.25mm.

Fig. 88   Prometaphase I orientations of chain forming interchange trivalents in _Tipula oleracea_. The three basic orientations are shown in a, b and c. In a, the whole complex is located at one pole. d and e are two sub-types of b. Reproduced from Bauer et al. (1961), Fig. 26.

Fig. 89   Prometaphase I orientations of Q forming interchange trivalents in _Tipula oleracea_. a, b and c are the three basic orientations. In a the whole complex is located at one pole. d is a sub-type of c. From Bauer et al. (1961), Fig. 25.

Fig. 88
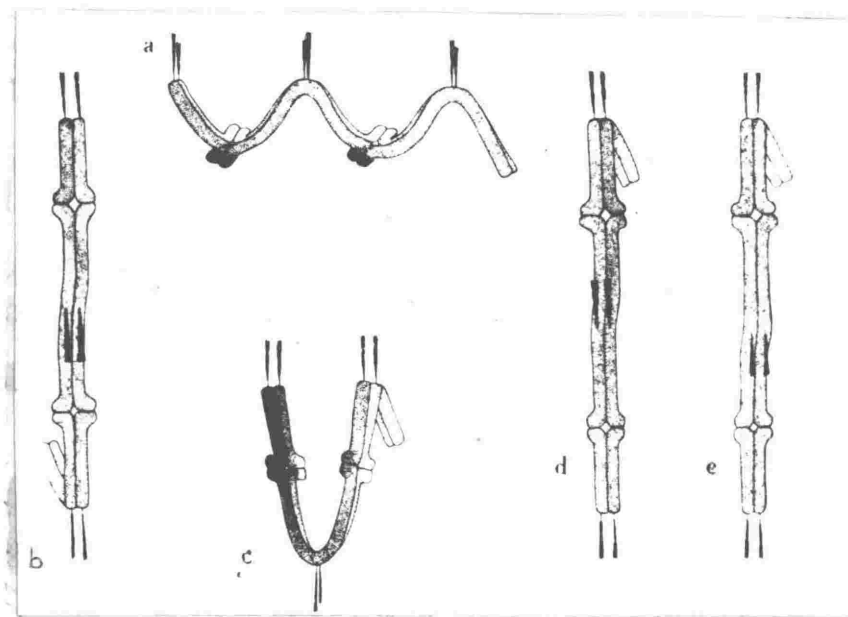
Fig. 89
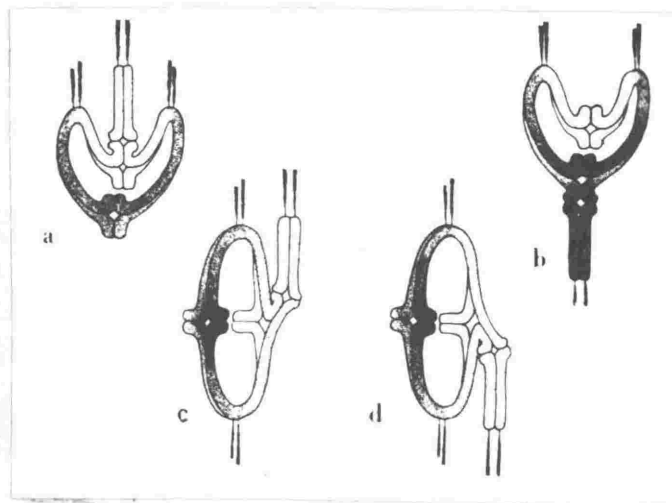
Figs. 90 and 91 Prometaphase orientation and reorientation

movements in interchange chain trivalents in Tipula

oleracea depicted in graphical and diagrammatic fashion.

O marks the position of the metaphase equator. The

upper and lower lines plotted on the graphs represent

the positions of the poles. The more central lines

plot the paths of the centromeres according to distances

from the equator and successive times at which recordings

were made. In Fig/ 90 the trivalent oriented initially

in a V configuration (Fig. 88c) and remained so until

anaphase separation. In Fig. 91 the trivalent oriented

initially in a V configuration but at approximately the

52nd minute one end centromere reoriented to the 'lower'

pole to form a rod configuration (Fig. 88b), from which

anaphase proceeded.

The figures are reproduced from Bauer et al. (1961),

figs. 6 and 7 respectively.

Fig.90
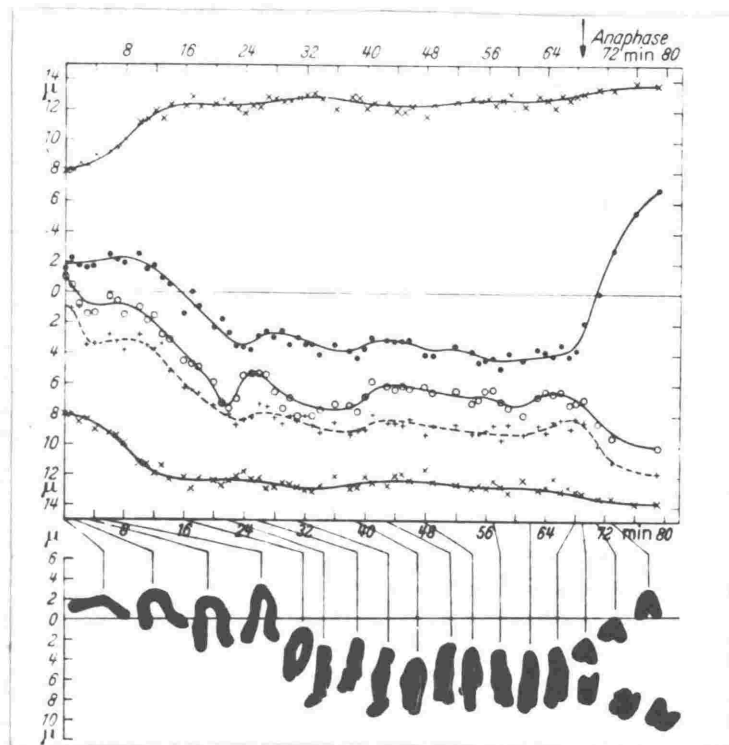
Fig.91
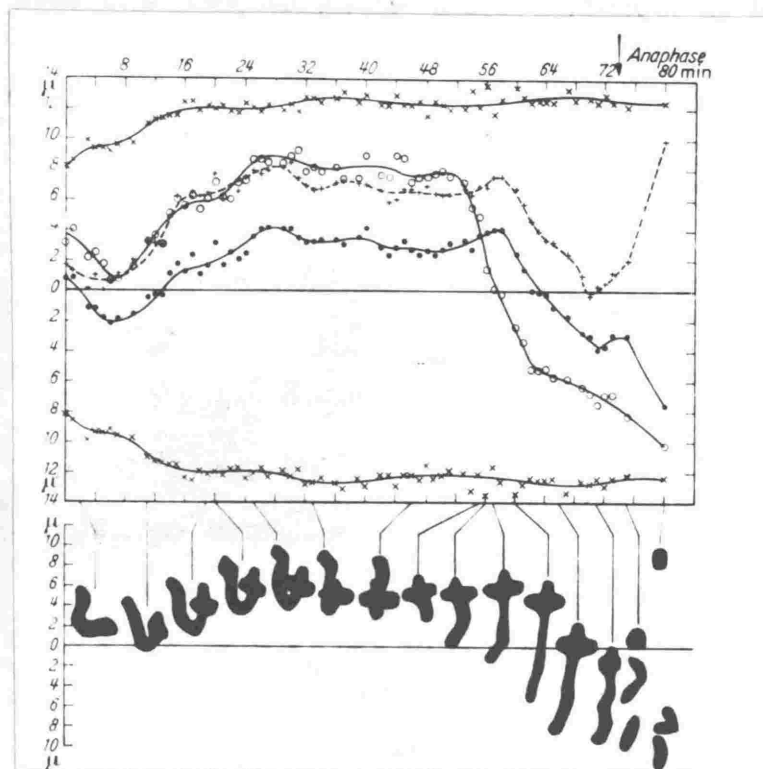
Fig. 92   As in Figs. 90 and 91, though at the initiation
of prometaphase the trivalent oriented in a rod config-
uration (Fig. 88e), and at approximately the 68th
minute the 'lower' end centromere reoriented to the
opposite pole to give a V configuration (Fig. 88c).
From Bauer et al. (1961), fig. 8.

Fig. 92
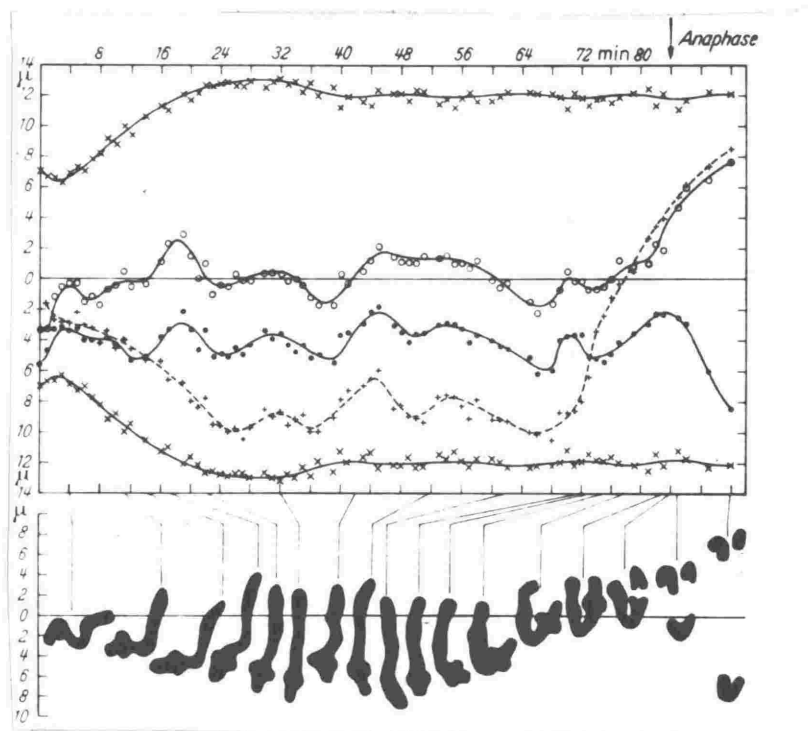
Appendix table 1   Details of measurements of the chromosomes from
10 normal cells of <u>Allium triquetrum</u>.

T = total, L.a. = long arm, Cm. = centromere, S.a. = short arm,
R = ratio (S.a./L.a.).

For pairs 4, 6, 7 and 9 the pairing of homologous members and of
their arms is accurate and probably nearly as accurate for pairs
5 and 8. For pairs 1 - 3 instances of incorrect pairing of homologues
and homologous arms cannot be ruled out, so that the apparent
pairing carried out here and arm ratio determinations for these
chromosomes are probably somewhat unreal (see further comments in
text).

Appendix Table 1.

Details of chromosome measurements from 10 normal cells.

23.75 units of table = approx. 10μ.

Chromosome 4					Chromosome 5					Chromosome 6					Chromosome 7					Chromosome 8					Chromosome 9				
T.	La.	Cm.	S.a.	R.	T.	La.	Cm.	S.a.	R.	T.	La.	Cm.	S.a.	R.	T.	La.	Cm.	S.a.	R.	T.	La.	Cm.	S.a.	R.	T.	La	Cm.	S.a.	R.
38.00	20.75	1.00	16.25	0.783	30.00	17.75	1.25	11.00	0.620	27.50	22.75	1.00	3.75	0.165	25.75	21.00	1.25	3.50	0.167	26.00	14.00	1.25	10.75	0.768	21.50	18.75	0.75	2.00	0.107
40.75	22.00	1.50	17.25	0.784	30.50	18.00	1.50	11.00	0.611	28.50	23.50	1.00	4.00	0.170	24.50	20.00	1.00	3.50	0.175	27.75	15.00	1.50	11.25	0.750	22.00	19.00	0.75	2.25	0.118
38.50	20.25	1.00	17.25	0.852	29.25	17.25	1.25	10.75	0.623	27.25	22.75	0.75	3.75	0.165	26.00	21.50	1.00	3.50	0.163	25.25	14.50	1.00	9.75	0.672	22.00	19.25	0.75	2.00	0.104
39.50	20.75	1.00	17.75	0.855	31.75	18.50	2.00	11.25	0.608	28.50	23.50	1.00	4.00	0.170	26.00	21.50	0.75	3.75	0.165	27.00	15.00	1.00	11.00	0.733	21.75	19.00	0.75	2.00	0.105
36.00	19.00	1.25	15.75	0.829	30.50	18.25	1.25	11.00	0.603	26.50	21.75	1.00	3.75	0.172	24.75	20.50	0.75	3.50	0.171	24.75	14.25	1.00	9.50	0.667	21.00	18.00	1.00	2.00	0.111
37.50	19.75	1.25	16.50	0.835	28.25	16.25	1.00	11.00	0.677	27.00	22.25	1.00	3.75	0.170	24.50	20.00	1.00	3.50	0.175	26.75	14.50	1.50	10.75	0.741	21.75	18.50	1.00	2.25	0.122
38.25	20.00	1.25	17.00	0.850	28.25	17.25	1.00	10.00	0.580	27.50	22.75	0.75	4.00	0.176	26.25	21.50	1.00	3.75	0.174	25.00	13.75	1.00	10.25	0.745	21.00	18.00	0.75	2.25	0.125
38.25	20.50	1.50	16.25	0.793	28.25	17.00	1.00	10.25	0.603	27.00	22.00	1.00	4.00	0.182	25.75	21.25	0.75	3.75	0.176	24.50	13.50	0.75	10.25	0.759	22.50	19.00	1.00	2.50	0.132
34.25	18.00	1.25	15.00	0.947	26.50	15.50	1.00	10.00	0.645	26.75	21.75	1.00	4.00	0.184	23.75	19.25	0.75	3.75	0.195	23.75	13.00	1.00	9.75	0.750	21.25	18.00	0.75	2.50	0.139
35.00	18.25	1.50	15.25	0.836	26.50	15.50	1.00	10.00	0.645	26.50	21.50	1.00	4.00	0.186	23.75	19.25	1.00	3.50	0.182	24.50	13.50	1.25	9.75	0.722	21.25	18.25	0.75	2.25	0.123
34.75	18.25	1.00	15.50	0.849	26.50	14.50	1.25	10.75	0.741	27.00	22.00	1.00	4.00	0.182	24.75	20.25	1.00	3.50	0.173	22.50	11.75	1.00	9.75	0.829	20.75	17.75	1.00	2.00	0.113
36.25	19.25	1.00	16.00	0.831	27.25	16.00	1.00	10.25	0.641	26.75	21.75	1.00	4.00	0.184	24.50	20.25	1.00	3.25	0.160	23.00	12.25	1.00	9.75	0.796	20.25	17.00	1.00	2.25	0.132
32.75	17.25	1.25	14.25	0.812	25.00	14.25	1.00	9.75	0.684	23.25	18.75	1.00	3.50	0.187	22.50	18.00	1.00	3.50	0.194	21.50	12.00	1.00	8.50	0.709	19.50	16.50	1.00	2.00	0.121
31.50	16.25	1.25	14.00	0.862	23.50	14.25	1.00	8.25	0.579	24.50	19.75	1.25	3.50	0.178	21.50	17.25	1.00	3.25	0.188	21.50	12.25	1.00	8.25	0.673	19.75	16.75	1.00	2.00	0.119
31.50	16.00	1.25	14.25	0.890	23.75	13.75	1.00	9.00	0.655	24.00	19.25	1.00	3.75	0.195	21.50	17.25	0.75	3.50	0.203	20.50	11.50	1.00	8.00	0.696	19.50	16.75	0.75	2.00	0.119
31.25	16.50	1.00	13.75	0.833	25.00	14.50	1.25	9.25	0.638	23.25	18.50	1.00	3.75	0.208	22.50	18.00	1.00	3.50	0.194	20.50	11.50	1.00	8.00	0.696	18.50	15.75	0.75	2.00	0.127
30.00	16.00	1.00	13.00	0.828	23.00	14.00	0.75	8.25	0.589	22.00	18.00	0.75	3.25	0.180	20.25	16.50	1.00	2.75	0.166	21.50	11.50	1.50	8.50	0.737	18.25	15.75	0.75	1.75	0.111
30.00	15.25	1.50	13.25	0.869	21.00	12.75	1.00	7.25	0.569	22.50	18.25	1.00	3.25	0.178	20.00	16.25	1.00	2.75	0.169	19.75	11.00	1.00	7.75	0.705	18.25	15.75	0.50	2.00	0.127
24.75	13.00	1.00	10.75	0.827	20.00	11.75	1.00	7.25	0.617	18.50	14.75	0.75	3.00	0.203	16.50	13.25	0.75	2.50	0.189	16.50	9.00	1.00	6.50	0.722	14.25	12.00	0.75	1.50	0.126
25.25	13.25	1.00	11.00	0.830	18.75	11.00	1.00	6.75	0.614	18.25	14.50	0.75	3.00	0.207	16.25	13.00	0.75	2.50	0.192	18.00	9.75	1.00	7.25	0.744	14.25	12.00	0.75	1.50	0.126

	Chromosome 1					Chromosome 2					Chromosome 3				
Cell no.	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.
1	46.50	22.75	1.75	22.00	0.967	43.25	22.75	1.00	19.50	0.857	42.25	21.00	1.25	20.00	0.952
	48.00	24.00	1.00	23.00	0.958	42.75	22.25	1.25	19.25	0.865	44.50	22.00	1.25	21.25	0.966
2	45.25	22.00	2.00	21.25	0.966	42.50	22.25	1.00	19.25	0.865	42.75	21.25	1.00	20.50	0.965
	45.75	22.00	1.75	22.00	1.000	43.00	22.75	1.00	19.25	0.857	42.50	20.50	1.50	20.50	1.000
3	44.00	21.75	1.50	20.75	0.954	45.00	24.00	1.50	19.50	0.813	42.50	21.00	1.50	20.00	0.952
	44.00	21.50	1.50	21.00	0.977	44.50	23.00	1.50	20.00	0.870	42.25	20.75	1.50	20.00	0.964
4	43.50	21.75	1.00	20.75	0.954	43.75	23.50	1.00	19.25	0.819	40.00	20.00	1.25	18.75	0.938
	42.50	21.25	1.25	20.00	0.941	44.25	21.00	1.25	19.00	0.905	41.50	20.25	1.25	20.00	0.988
5	42.00	20.50	1.50	20.00	0.976	41.00	21.25	1.25	18.50	0.870	39.00	19.00	2.00	18.00	0.947
	41.50	20.50	1.50	19.50	0.951	42.50	21.75	1.50	19.25	0.885	39.25	19.00	1.50	18.75	0.987
6	40.00	19.75	1.00	19.25	0.975	39.75	21.25	1.00	17.50	0.824	38.00	18.75	1.25	18.00	0.960
	39.50	19.75	1.00	18.75	0.949	42.25	22.25	1.00	19.00	0.854	38.50	19.00	1.00	18.50	0.974
7	38.50	19.00	1.50	18.00	0.947	37.25	19.25	1.00	17.00	0.883	36.50	17.75	1.50	17.25	0.971
	38.75	18.75	1.50	18.50	0.987	37.25	19.00	1.25	17.00	0.895	35.00	17.50	1.25	16.25	0.929
8	37.75	18.50	1.50	17.75	0.959	39.75	20.50	1.75	17.50	0.854	34.75	17.00	1.00	16.75	0.985
	38.50	18.25	2.00	18.25	1.000	37.50	19.25	1.50	16.75	0.870	35.50	17.50	1.00	17.00	1.000
9	35.25	17.25	1.25	16.75	0.971	35.25	18.50	1.00	15.75	0.851	33.50	16.50	1.00	16.00	0.970
	36.25	17.50	1.50	17.25	0.986	36.00	18.25	1.50	16.25	0.890	34.50	17.00	1.25	16.25	0.956
10	29.50	14.25	1.25	14.00	0.982	29.00	14.50	1.00	13.50	0.931	27.75	13.50	1.00	13.25	0.981
	28.50	13.50	1.50	13.50	1.000	28.00	14.00	1.00	13.00	0.912	28.25	13.75	1.00	13.50	0.982

Cell No.	% difference.	
1	24.32	24.20
2	16.00	15.58
3	18.71	17.93
4	16.22	23.13
5	18.18	17.91
6	16.30	19.55
7	19.67	14.88
8	11.57	18.18
9	20.69	14.04
10	18.95	18.56

Overall Mean   18.23.

Appendix table 1a Percentage differences between short and long arms of chromosome 4 members in the 10 measured cells of Appendix table 1. Values were calculated as $\frac{\text{long arm} - \text{short arm}}{\text{mean length of both arms}}$ X $\frac{100}{1}$.

Appendix table 2   Details of chromosome measurements from 8

cells from interchange heterozygous plants. Abbreviations as

in Appendix table 1. Comments concerning instances of unreal

pairing noted for Appendix table 1 apply here to chromosomes

1 - 3 and 6^4.

Appendix Table 2.

Details of chromosome measurements from 8 interchange cells.

23.75 units of table = approx. 10μ.

Chromosome 4/6[4]					Chromosome 5					Chromosome 6/4[6]					Chromosome 7					Chromosome 8					Chromosome 9			
L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.
19.50	1.25	16.25	0.833	28.50	17.25	1.00	10.25	0.594	24.00	19.75	0.75	3.50	0.177	24.75	19.75	1.25	3.75	0.190	25.50	14.00	1.00	10.50	0.750	21.75	18.75	1.00	2.00	0.107
23.00	1.25	18.25	0.804	28.50	17.00	0.75	10.75	0.632	28.00	23.00	1.25	3.75	0.163	23.75	19.00	1.00	3.75	0.197	26.75	14.50	1.50	10.75	0.741	21.50	18.50	0.75	2.25	0.122
20.25	1.50	17.50	0.864	28.00	16.50	0.75	10.75	0.652	23.50	19.50	0.75	3.25	0.167	25.25	21.00	0.50	3.75	0.178	25.25	14.00	1.00	10.25	0.732	23.50	20.00	1.00	2.50	0.125
22.50	1.50	18.50	0.822	28.00	17.00	1.00	10.00	0.588	27.00	22.00	1.00	4.00	0.182	24.75	20.50	0.50	3.75	0.183	26.25	15.00	1.00	10.25	0.683	22.50	19.75	0.50	2.25	0.114
20.25	1.75	17.00	0.840	29.25	17.00	1.25	11.25	0.661	27.25	22.50	1.00	3.75	0.166	24.75	20.00	1.00	3.75	0.188	25.50	14.25	1.00	10.25	0.719	20.75	18.00	0.75	2.00	0.111
22.50	1.50	18.25	0.811	28.00	17.00	1.00	10.00	0.588	23.00	19.00	0.50	3.50	0.184	24.50	20.00	1.00	3.50	0.175	24.75	14.00	0.50	10.25	0.732	20.75	17.75	1.00	2.00	0.113
20.00	1.50	16.50	0.825	29.25	17.75	1.25	10.25	0.577	25.50	20.75	1.00	3.75	0.181	22.75	19.00	0.75	3.00	0.158	24.00	13.00	1.25	9.75	0.750	20.00	17.00	1.00	2.00	0.118
22.50	2.00	17.75	0.789	28.50	17.25	1.00	10.25	0.594	22.00	17.75	1.00	3.25	0.183	23.00	19.25	0.75	3.00	0.156	23.25	13.00	1.25	9.00	0.692	20.25	17.25	1.00	2.00	0.116
18.00	1.50	15.25	0.847	26.25	15.25	1.75	9.25	0.607	26.00	21.00	1.25	3.75	0.178	24.00	19.00	1.25	3.75	0.197	22.50	12.00	1.75	8.75	0.729	21.50	18.25	1.00	2.25	0.123
22.25	2.00	17.00	0.764	28.00	16.00	1.50	10.50	0.644	22.50	18.25	1.00	3.25	0.179	24.00	19.75	0.75	3.50	0.177	22.75	12.25	1.50	9.00	0.735	21.00	18.00	0.75	2.25	0.125
17.50	1.50	14.00	0.800	24.25	14.00	1.25	9.00	0.643	23.25	18.75	1.25	3.25	0.173	23.00	18.75	1.25	3.00	0.160	21.75	12.25	1.00	8.50	0.694	20.00	17.00	1.00	2.00	0.118
19.75	1.75	15.50	0.785	25.25	14.25	1.50	9.50	0.667	19.25	15.50	0.75	3.00	0.194	23.25	19.00	1.25	3.00	0.158	20.00	11.25	1.00	7.75	0.689	20.25	17.25	1.00	2.00	0.116
15.00	1.50	12.75	0.833	21.00	12.00	1.50	7.50	0.625	21.25	16.50	1.25	3.50	0.212	19.25	15.00	1.25	3.00	0.200	19.75	10.75	1.25	7.75	0.721	16.00	13.00	1.00	2.00	0.154
17.50	1.25	13.25	0.757	22.75	13.00	1.75	8.00	0.615	18.50	14.50	1.00	3.00	0.207	19.25	15.25	1.00	3.00	0.197	19.75	10.50	1.75	7.50	0.714	16.00	13.25	1.00	1.75	0.132
13.25	1.00	11.50	0.868	20.25	12.00	1.00	7.25	0.604	18.75	15.00	0.50	3.25	0.217	17.75	14.50	0.50	2.75	0.190	18.00	9.75	1.25	7.00	0.718	16.00	13.75	0.50	1.75	0.127
15.50	0.75	12.00	0.774	20.25	11.75	1.00	7.50	0.639	17.25	13.75	1.00	2.50	0.182	18.25	14.75	0.75	2.75	0.186	18.25	10.00	1.25	7.00	0.700	15.75	13.50	0.50	1.75	0.130

Cell no.	Chromosome 1					Chromosome 2					Chromosome 3					T.
	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.	T.	L.a.	Cm.	S.a.	R.	
1	44.75	21.75	1.50	21.50	0.989	45.25	24.00	1.50	19.75	0.823	42.00	20.25	1.50	20.25	1.000	37.00
	43.00	21.00	1.25	20.75	0.988	46.50	24.00	1.75	20.75	0.865	42.00	20.50	1.25	20.25	0.988	42.75
2	44.25	22.00	0.75	21.50	0.977	43.00	22.25	1.25	19.50	0.876	42.25	21.00	0.75	20.50	0.976	39.25
	42.75	20.75	1.50	20.50	0.988	44.00	22.50	1.50	20.00	0.889	41.25	20.25	1.00	20.00	0.988	42.50
3	45.00	22.25	1.50	21.25	0.955	44.50	22.75	1.50	20.25	0.890	42.25	21.00	1.25	20.00	0.952	39.00
	44.00	21.75	1.25	21.00	0.966	43.50	22.75	1.25	19.50	0.857	44.75	20.25	1.50	20.00	0.987	42.25
4	44.50	21.50	2.00	21.00	0.977	43.25	22.50	1.75	19.00	0.844	40.00	19.00	2.00	19.00	1.000	38.00
	43.50	21.75	1.75	20.00	0.920	43.50	22.00	2.00	19.50	0.886	41.75	20.00	1.75	20.00	1.000	42.25
5	41.00	20.00	1.50	19.50	0.975	43.00	22.00	1.50	19.50	0.886	39.25	18.75	1.75	18.75	1.000	34.75
	42.25	20.50	1.25	20.50	1.000	41.75	22.00	1.25	18.50	0.841	40.75	20.00	1.50	19.25	0.963	41.25
6	39.50	19.00	1.50	19.00	1.000	39.25	20.25	2.00	17.00	0.840	34.25	16.25	0.985	16.25	0.485	33.00
	37.25	18.00	1.50	17.75	0.986	36.50	18.50	1.75	16.50	0.904	36.75	17.50	1.00	17.50	1.000	37.00
7	34.50	16.50	1.50	16.50	1.000	34.50	18.00	1.50	15.00	0.833	30.75	15.00	1.25	14.50	0.967	29.25
	35.75	17.00	1.75	17.00	1.000	33.25	17.25	1.00	15.00	0.870	32.25	15.50	1.50	15.25	0.984	32.00
8	31.00	15.00	1.00	15.00	1.000	30.50	16.00	0.50	14.00	0.875	30.25	15.00	0.75	14.50	0.967	25.75
	31.50	15.25	1.50	14.75	0.967	31.00	15.75	1.25	14.00	0.889	30.50	14.75	1.00	14.75	1.000	28.25

Appendix table 2a.

Cell No.	Chromosome 4			Chromosomes 6/4[6]	
	L.a.	S.a.		L.a.	S.a.
9	19.75	16.75		23.25	4.00
				20.25	3.50
10	20.00	16.50		21.25	4.00
				18.50	3.25
11	18.25	15.25		21.25	3.75
				18.50	3.50
12	16.50	13.75		19.25	3.75
				16.50	3.25
13	17.00	14.00		19.00	3.75
				16.50	3.25
14	15.25	13.00		16.75	3.25
				14.50	3.00
15	15.00	12.75		16.25	3.00
				14.50	2.75
16	15.25	12.50		17.25	3.50
				15.25	3.00

Appendix table 2a   Details of measurements from eight interchange cells of chromosomes 4, 6 and 4[6]. Abbreviations as in Appendix table 2.

Appendix table 3   Chiasma frequencies in normal and
interchange cells at diplotene/diakinesis (E) and
metaphase I (L).

Appendix table 3

	13	14	15	16	17	18	19	20	21	22	23
E — From 25 normal cells	0	0	1	2	1	3	3	5	6	3	1
E — From 27 interch. cells	0	1	1	1	1	6	9	4	1	2	1
L — From 34 normal cells	1	4	6	7	6	5	3	0	2	0	0
L — From 30 interch. cells	1	3	12	9	1	1	2	1	0	0	0

Number of cells

Anther	Total	Abortive	% abortive
1	1782	9	0.51
2	3657	21	0.57
3	2700	68	2.52
4	2660	56	2.11
5	2010	20	1.00
6	1800	9	0.50
7	2710	140	5.12
8	2001	35	1.75
9	1880	41	2.17
10	1999	95	4.75
		Mean	2.10

Appendix table 4a   Pollen abortion details from 10 anthers of normal plants.

Anther	Total	Abortive	% abortive
1	2652	329	12.41
2	2488	260	10.45
3	3199	432	13.50
4	1982	230	11.60
5	2446	295	12.06
6	3637	383	10.53
7	2319	319	13.76
8	2792	359	12.86
9	2613	349	13.36
10	3311	505	15.25
11	2875	305	10.61
12	2601	291	11.19
13	2601	307	11.80
14	2425	323	13.32
15	2413	264	10.94
16	2229	311	13.95
17	2666	325	12.19
18	1819	256	14.07
19	2067	257	12.43
20	2799	365	13.04
21	3286	404	12.29
22	2330	252	10.82
23	2768	292	10.55
24	2709	292	10.78
25	2580	290	11.24
		Mean	12.20

Appendix table 4b   Pollen abortion details from 25 anthers of interchange plants.

A Note on Orientation in Mitosis and Meiosis

Kinetochore (= centromere) orientation during prometaphase determines the pattern of chromosome disjunction in anaphase and this in turn determines the chromosome complements of the daughter cells. Consequently, kinetochore orientation has been recognized as a central problem by all students of mitotic mechanisms and currently interests many other researchers because of recent studies on abnormal chromosome complements in man. Rickards (1965) has advanced a theory of orientation which to many might seem new and important. Before the theory gains wider currency its originality and power should be scrutinized.

Rickards' first point is that " . . . orientations along the spindle equator in mitosis and meiosis in general conform to the above principle of 'co-orientation in linked centromere pairs'." (p. 338.) Consider, for example, a mitotic chromosome. Rickards is suggesting that if two conditions are met, the chromosome will attain an equatorial position: condition 1: a force acting toward one pole at the kinetochore of one sister chromatid and a force acting toward the opposite pole at the kinetochore of the other sister chromatid; condition 2: a linkage between the chromatids which prevents their separation in spite of the bipolar forces (see Fig. 1(b)). This does not differ from the concept advanced by numerous earlier workers (see, e.g., Schrader, 1947; Östergren, 1950) except that Rickards cites recent morphological evidence (reviewed by Lima de Faria, 1958) for the doubleness of the kinetochore of mitotic chromosomes and concludes that the mitotic chromosome is co-oriented in the same sense that a meiotic bivalent is. The usage of "co-orientation" in this connection is considered below; here the significance of Rickards' modification is assessed. First, only Darlington and his school considered the mitotic kinetochore to be single until anaphase; other cytologists from Schrader (1936) on have regarded it as at least partly doubled by prometaphase. From the available evidence, Schrader, and later, Östergren (1951; see his figures 1 to 5 and associated discussion) argued that the portion of the kinetochore where the force is applied (the "spindle spherule") is double but the remainder of the kinetochore region is not (see Fig. 1(a)). So there is a genuine difference in views concerning the structure of the kinetochore region. But does this lead to different views of kinetochore orientation? No, for only the *position* of the linkage differs:

(1) undivided material between the spindle spherules versus (2) undivided material between the chromatids and immediately adjacent to the kinetochore (see Fig. 1). Thus that the second view is correct in certain cases, has in itself no bearing on the concept of orientation as developed by Schrader and Östergren (among others). Moreover, even if Rickards' argument were

FIG. 1. Diagrams showing those features of the mitotic prometaphase chromosome relevant to this discussion of orientation. (a) After Östergren (1951; based largely on data of Schrader, 1936). (b) After Rickards (1965; based largely on data of Lima de Faria, 1958 and earlier). Similar conventions are used in both drawings to facilitate comparison and therefore some structural details are incorrectly rendered, but the crucial points should be clear: the position of the interchromatid linkage (*l*) differs, but the position and number of the spindle fiber attachment sites (*s*) does not. The vertical arrows indicate the forces acting toward opposite poles on sister chromatids.

tenable, it lacks priority, for Lima de Faria (1958, p. 147) had drawn attention to the implications of his observations on kinetochore structure and concluded that mitotic chromosomes and meiotic bivalents are co-oriented.

All the above merely establishes the conditions underlying a stable position on the equator and as Rickards says, the real task is to account for orientation in the first place. Rickards reviews the impressive studies of Bauer, Dietz & Röbbelen (1961); he concludes "The author considers that the major orientation and reorientation movements outlined above are directly responsible for bringing about the necessary co-orientation of bivalents or chromosome centromeres during prometaphase." (p. 345.) Exactly what Rickards intends to suggest is unclear. Thus it is obvious that the movements to which he refers *reflect* the pattern of kinetochore orientation at any given time, and it is equally obvious that the final metaphase orientation is initiated by kinetochore orientation early in prometaphase; this initial orientation may be followed by reorientations later in prometaphase. On the other hand, if Rickards is going beyond this to suggest that the movements themselves induce the reorientation which co-orients initially mal-oriented chromosomes or bivalents, then his suggestion differs only by its vagueness from Östergrens' (1951) "co-orientation by pulling" hypothesis. The charitable assumption is that Rickards is suggesting neither "co-orientation by pulling" nor any

ovel view of orientation, but is only summing up the well attested facts of
ientation phenomena for the reader's convenience. Rickards then introduces
psi-orientation", and suggests that a given kinetochore orients independ-
atly of any other kinetochore; if the associated kinetochore does not orient
multaneously, movement to one pole follows. If the second kinetochore
ients to the opposite pole, co-orientation has been achieved and the
iromosome or bivalent will shortly move to the equator and will be divided
ormally in anaphase. However, in 10% of the bivalents studied by Bauer
_al_. (1961) all kinetochores are initially oriented to the same pole; re-
ientation always follows and eventually the bivalent is co-oriented. But
... does reorientation occur? This is the real question, as has been recognized
ice reorientation was first studied (Hughes-Schrader, 1943). Rickards'
iswer to the question is not very helpful: he proposes that proximity to a
ole is a pre-requisite to reorientation and the equatorial position is associated
... able orientation. Certainly reorientation occurs more frequently in
ir... ...mes lying near a pole, but this is by no means invariable. Indeed
imple ...f reorientation in other parts of the spindle are documented in a
per ... d by Rickards (Bauer, Dietz & Röbbelen, 1961, Figs 4 and 7).
Rickards also overlooks worrisome phenomena that should be kept in
ind. For example, in some forms co-orientation can be achieved even in the
osence of physical linkage of kinetochores ("distance conjugation", see, e.g.
orber, 1934). This does not necessarily mean that linkage is without
gnificance where it does occur, but the existence of distance conjugation
ises doubts that should stimulate further research.
Darlington (1937) distinguished between the orientation of a chromosome
mitosis (auto-orientation) and the orientation of bivalents in meiosis
o-orientation). Rickards (1965) suggests (p. 337) discarding the term
iuto-orientation" since in mitosis as in meiosis the final orientation gives a
polar arrangement of kinetochores. But this is the extent of the similarity.
ius as Schrader (1939) and Lima de Faria (1958) have observed, the two
iromosomes of a meiotic bivalent each have kinetochores as double
ructurally as those of a mitotic chromosome. Yet by meiotic metaphase in
ost organisms these doubled kinetochores behave as a single unit: both
netochores of one chromosome (i.e. one kinetochore for each chromatid)
e oriented to one pole, while those of the other chromosome are both
iented to the opposite pole. Contrast this with a mitotic chromosome, in
hich the chromatid kinetochores orient independently to opposite poles.
iis manifest difference in orientation was aptly designated by Darlington
937) (even though his causal analysis was incorrect) and his terms have
en widely used. More than linguistic conservation is at stake, however, for
iless our terminology clearly identifies natural differences we may remain in

ignorance of their origin. Additionally, distinct sequences of co- and auto-orientation in the two meiotic divisions can be recognized: while co-orientation at division one followed by auto-orientation at division two is the pattern in many organisms, the inverted sequence also occurs. This important difference in meiotic pattern is best described using "auto-" and "co-orientation" (Hughes-Schrader, 1955; her suggestion has been widely followed).

Rickards also concludes (p. 348) that the terms "syntelic" and "amphitelic" (Bauer *et al.*, 1961) are unnecessary. These terms succinctly describe the orientations of individual pairs of kinetochores. To see the necessity for differential terminology consider again the initially mal-oriented bivalents studied by Bauer *et al.* (1961). Their initial orientations cannot be described by the term co-orientation and yet the reorientation of such bivalents is just what, *inter alia*, a satisfactory theory of orientation must explain. Here again those interested in precise description and constructive thought may elect to ignore Rickards' terminological advice.

Finally, the agreement on important fundamentals should be stressed; indeed the major charge made above is the lack of useful novelty in Rickards' presentation. First, all of us are agreed that bipolar orientation of a chromosome or bivalent is necessary for attainment of the equatorial position and also for equipartition at anaphase. Second, since the initial orientation often differs from the bipolar orientations seen in metaphase, we agree that reorientation is the phenomenon of critical importance. Third, we must all admit that no satisfactory theory of reorientation is at hand.

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An Analysis of Co-orientation in Mitosis and Meiosis

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In this paper the type and the process of orientation of chromosomes during prometaphase of mitosis and meiosis are discussed. A theory is proposed suggesting that two principal factors govern the orientation of both mitotic chromosomes (as associated pairs of sister chromatids) and of meiotic bivalents (associated pairs of homologous chromosomes). The first of these factors is that orientation is achieved principally by the presence in either chromosomes or bivalents of two independently acting centromeres linked one to another, the linking being achieved through the chiasmata in bivalents, and the at present little understood substance or structure maintaining the association of chromatids of a mitotic chromosome. Orientation takes place in linked centromere pairs. The second factor is considered to stem from the capacity of one centromere to become influenced and oriented to one of the two spindle poles. Through these two factors stable orientation along the metaphase equator is achieved, with one of the pair of associated centromeres being influenced and directed to one pole, the other to the opposite pole, with the chromosome or bivalent remaining in this position because of the linking device shared between the two members of each. The movements involved in bringing about this orientation are also discussed.

1. Introduction

In a previous paper (Rickards, 1964) a hypothesis was put forward that chromosome complexes, that have arisen during meiosis as a result of a previous interchange of segments between non-homologous chromosomes, are oriented along the equator at metaphase I in a characteristic variety of ways (e.g. Plate I; see also Burnham, 1956 for a review) according to the combinations of two directly linked centromeres that the complex permits. The potential types of orientation of a particular complex are determined by the number of pairs of linked centromeres within the complex (three in each complex in Plate I), while its actual orientation depends not only on these pairs of linked centromeres, but also on the positions of the chiasmata relative to the centromeres and to each other. Thus emphasis was placed on the importance of the chiasma to the attainment of orientation—any two centromeres, homologous or not, that are directly linked by a chiasma are capable of orientation in
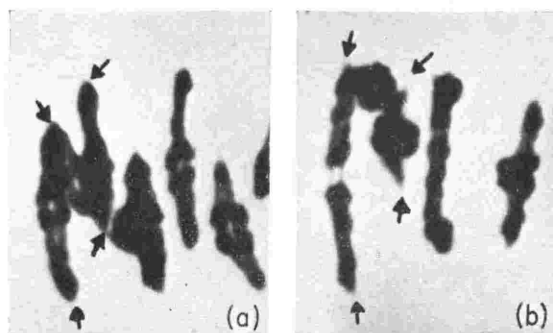
PLATE I. Chromosomes at anaphase I from two pollen mother cells of *Allium triquetrum*, heterozygous for an interchange. The meiotic complex of four chromosomes is seen co-oriented in each of the two possible ways at the left of each photograph (a) and (b). In (a) the four centromeres (arrows) are co-oriented in an "N" as three adjacent pairs. The chiasmata linking the centromeres show across the approximate position of the equator. In (b) the complex is co-oriented in an "□" as two adjacent pairs of centromeres; one chiasma (left along line of equator) is almost lost. (See Rickards, 1964.)
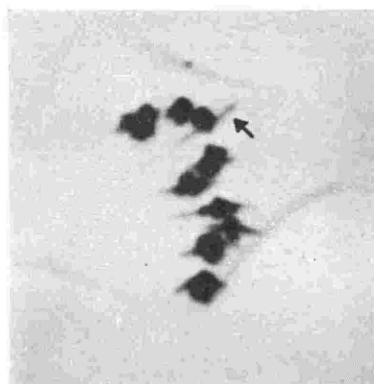

PLATE II. Nine bivalents of an *Allium triquetrum* pollen mother cell, co-oriented at metaphase I. The arrow points to the chromatin between centromere and chiasma that has been drawn out towards one pole.

linked pairs (co-orientation), but with centromeres that are not linked this is not possible.

From these considerations it was suggested that the actual feat of co-orientation in interchange complexes depends largely on the linking chiasma; the mechanism works upon the chiasma and thus accords to it a function above that of merely maintaining association of homologous centromeres.

Data from chain forming complexes in an interchange heterozygote of *Allium triquetrum* as in Plate I were given in support of this thesis. Also, support was provided by data (reviewed by Burnham, 1956) on the different interchange complex types known to occur and their methods of orientation. In presenting the hypothesis it was indicated that the suggested mechanism orienting interchange complexes is considered to be essentially the same as that responsible for orienting meiotic bivalents, i.e. adjacent pairs of centromeres in a complex such as in Plate I behave in their orientation essentially as does the pair of a simple bivalent. The present paper is designed to cover more fully the process of orientation of meiotic bivalents in terms of the author's hypothesis and to extend this hypothesis to cover the orientation of chromosomes at mitosis and also the behaviour of univalent and undivided chromosomes.

2. Co-orientation of Bivalents at Prometaphase of Meiosis

Under apparently normal conditions the two homologous centromeres of a bivalent separate and move to opposite poles during anaphase I of meiosis; both centromeres do not pass to the same pole and so a regular meiotic division is achieved. This regularity can be regarded as a consequence of an exact orientation of the bivalent at prometaphase I† and up to metaphase I with the two homologous centromeres directed to opposite poles (Plate II). At anaphase interactions between centromeres and spindle, which are considered to be the basis of chromosome movements at anaphase, ensure that homologous centromeres so oriented pass to opposite poles.

Darlington (1937) coined the term *co-orientation* to describe the arrangement of the centromeres of a bivalent at metaphase I, with the two oriented in positions relative to each other, one "above" the spindle equator directed to the "upper" pole, the other "below" the equator directed to the "lower" pole. The term has since been used chiefly in reference to the product rather than the process of orientation; but what has remained obscure is what cytological features are responsible for bringing this co-orientation about. The problem is simply expressed as one concerning Boveri's "exclusion

† Prometaphase refers to the stage following prophase and leading to metaphase in which chromosome movements of orientation take place.

principle" whereby once a centromere is oriented to one pole, that pole cannot apparently orient its homologue (Boveri, 1907).

Two significant hypotheses have been put forward to account for the co-orientation phenomenon. Darlington (1937) developed a scheme of repulsion forces between the two homologous centromeres, and between centromeres and poles. These repulsions were considered to move the chromosomes into the equator and co-orient the centromeres in equilibrium with the poles.

On the other hand, Östergren (1951) formulated a theory of "co-orientation by pulling" which envisaged (a) an orientation of an active side of a centromere to a pole, (b) an interaction between homologous centromeres to ensure they become influenced by opposite poles and (c) a pulling of one centromere upon the other, through the chiasma, to effect co-orientation.

In attempting to analyse some of the forces involved in cell division Östergren (1950) pointed out that there is but little evidence indicating that repulsion forces are principally active in attaining co-orientation. Swanson (1957) likewise considered from Schrader's (1947) data of metaphase mechanics in animal cells with clear centrioles marking the poles and conspicuous spindle elements, that repulsion forces do not seem to be operative. Also, it is difficult to see how actual co-orientation could be achieved by general repulsion (or attraction) forces of centromeres and poles.

While certain aspects of Östergren's hypothesis are based on observational evidence, it seems clear that his theory as set out is also not an adequate explanation of the co-orientation phenomenon. The crux of the theory rests on an interaction (of unknown quality) between homologous centromeres to ensure they become oriented to opposite poles, yet it is clear (see Rickards, 1964) from a consideration of orientation in interchange complexes that such an interaction is very unlikely. In interchange complexes that form closed rings of four chromosomes at diakinesis, homologous centromeres orient and segregate to the same pole in a very significant percentage of cells. This is so even where the complex is held together by chiasmata near the chromosome ends, a situation expected to best aid manoeuvring of the chromosomes if homologous centromeres interact to ensure they pass to opposite poles. Also, the observations in living material of *Tipula* by Bauer, Dietz & Robbelen (1961), where bivalents sometimes move from pole to pole as a result of centromere activity during prometaphase, clearly indicate that the pull of one centromere upon the other, through the chiasma, does not necessarily result in their co-orientation.

At this point it is necessary to consider in more detail what is involved in the co-orientation of bivalents (Plate II). The two centromeres lie in the long axis of the spindle at right angles to the equator and directed to opposite poles. At metaphase an apparent equilibrium is observed with the two centro-

meres approximately equidistant from the equator. The chromosome segments between the centromeres and their nearest chiasma are drawn out along the spindle axis (arrow in Plate II) indicating considerable resistance by the chiasma to an apparent attraction of the centromeres to the poles. Thus, in a co-oriented bivalent, one must consider the presence of two centromeres linked together by a shared chiasma(ta), with each centromere under an attraction to the pole to which it is oriented. And with a number of conditions that are discussed later it may be considered that a bivalent that has been actively stabilized in the equator at metaphase I *must* have its centromeres in a co-oriented position, one directed to and influenced by one pole, the other by the opposite pole; and that the bivalent remains in the equator, co-oriented, because of its linking chiasma. If the chiasma did not exist the attraction between a centromere and a pole would have its logical effect; and if both centromeres were oriented to one pole (i.e. not co-oriented) the bivalent would likewise not remain in the equator.

3. Co-orientation of Chromosomes at Prometaphase of Mitosis

Sister (perhaps mother/daughter) chromatids that have been formed from a previous chromosome duplication pass to opposite poles during anaphase of somatic mitosis due to interactions between their centromeres and the spindle. Up till metaphase, however, the centromere of a mitotic chromosome has, until recently, been considered not to be divided along with the rest of the chromosome; chromatids are fully formed but not their individual centromeres. Chromatid centromeres were considered to be formed at the initiation of anaphase. Thus the phenomenon of sister chromatids always passing to opposite poles during anaphase of mitosis has generally been considered to be the result of a "predivision polarization" of the as yet undivided chromosome centromere at metaphase (Darlington, 1937, p. 539), such that when the centromere divided at the initiation of anaphase the resulting two (chromatid) centromeres are engaged by opposite poles and thus pass to opposite poles.

Darlington (1937) coined the term *auto-orientation* to describe the arrangement of the chromosome centromeres at metaphase, i.e. oriented in no direct relation to each other (not regarding relative positions in the horizontal plane†) and lying directly on the equator. In this position each centromere undergoes a change to show a polarization prior to its anaphase division.

Darlington (1937) considered that repulsion forces between two poles and a centromere bring about the movement of a mitotic chromosome and its auto-orientation on the spindle equator. Somehow polarization of the centro-

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† Certain chromosomes of a complement may show preference for particular positions along the equator. This is not fully understood (see Schrader, 1953).

mere followed in a way to ensure a proper anaphase. This idea is not clearly compatible with the often complex and indirect paths that chromosomes follow during prometaphase orientation (see later) and it gives no concrete explanation of the engagement of sister centromeres to opposite poles.

Östergren (1951) extended Darlington's principle of polarization to stages before metaphase. The polarized centromere prior to prometaphase was considered to be in a state such that, wherever the chromosome lies, one polarized portion (future chromatid centromere) will face one pole, the other portion the other pole, and that there is no time when one is oriented to both poles or both to one pole. When attractions between the poles and a polarized centromere come into play the chromosome will move to an equilibrium position on the spindle equator such that the still inseparable half centromeres are engaged by opposite poles. This concept, however, does not clearly indicate how Boveri's exclusion principle is achieved. It was considered by Östergren that the nature of the chromatid coils creates so-called active faces to the centromere which always face opposite ways, but it is not clear how once an active face comes under the influence of a pole, that pole cannot engage the other active face. This difficulty is essentially the same as that in an interaction between homologous centromeres in a bivalent which was considered by Östergren to ensure that they orient to opposite poles.

The recent studies by a number of workers (see Lima-de-Faria, 1958 for a review) have now considerably advanced our knowledge of the structure of the centromere. Where detailed analysis of mitotic metaphase chromsomes has been made (in a variety of animal and plant species where chromosome size has permitted this) the centromere has been found to be a dual rather than a single structure. It comprises two, separate, structurally complete centromeres, each being the centromere unit of one chromatid.

Each chromatid centromere at metaphase generally appears as a break or lightly stained region in the chromatid arms though in certain cases a small, chromatic, centric granule connected on each side to the chromatid arms, or a pair of these, may visibly mark the centromere position. The two chromatids of the divided chromosome are lightly associated along their lengths apparently by some generalized forces of attraction, but also are held together at regions adjacent to their centromeres by a substance or structure formed in a well defined area (Fig. 1; Lima-de-Faria, 1958). This structure can be seen in the birefringent ciné-micrographs of living *Haemanthus* endosperm cells (Inoué & Bajer, 1961) and in a number of recent and not so recent publications, e.g. Gimenez-Martin, Lopez-Saez & Gonzalez-Fernandez (1963), Prakken & Levan (1946), and it seems more appropriate in the light of this evidence to regard the structural (and functional) duplicity of the mitotic chromosome centromere as having a sound basis.
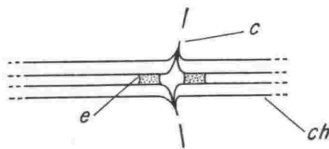
FIG. 1. Diagram of a chromosome at mitotic metaphase constructed from data in Lima-de-Faria (1958), Lewis & John (1964) and from the author's preparations of colchicine metaphase II chromosomes in *Allium triquetrum*. (In the latter preparations the centromeres are marked by a break in the chromatids; the linking structure is very pronounced.) The centromeres c and adjacent chromatin are drawn out to opposite poles. ch, chromatid, e, shared linking structure between the chromatids.

In the light of present evidence concerning the structure of metaphase centromeres it is necessary here to reconsider orientation of chromosomes in mitosis. At metaphase the sister chromatids lie in the axis of the spindle as do their centromeres. One centromere is "above" the equator directed to the "upper" pole, the other "below" the equator directed to the "lower" pole; and the chromatids remain linked at regions adjacent to the centromeres. Also, the centric granules (when visible) and the chromatin immediately adjacent to the centromeres are drawn out towards the pole to which the centromere is oriented (Fig. 1; Lima-de-Faria, 1958; Lewis & John, 1964). This drawing out of the centromeric chromatin must indicate some resistance by the linking structure to an apparent attraction of the centromeres to the poles.

The conclusion must be that chromosomes are *co-oriented* at metaphase of mitosis as are bivalents in meiosis, and the term auto-orientation as coined by Darlington appears without meaning. The relatively small distance between the chromatid centromeres (compared with that between the homologous centromeres of a bivalent) gives the impression of there being a single centromere oriented directly on the equator, but that the centromeres are indeed co-oriented can be clearly seen from the birefringent micrographs of Inoué & Bajer (1961) in living endosperm mitoses.

In considering the orientation of chromosomes in mitosis one must consider, as with bivalents, the presence of two centromeres linked together (in chromosomes by a structure shared between the two chromatids), with one centromere at metaphase under an apparent attraction to one pole, the other to the opposite pole. The chromosome centromeres remain in the equator, co-oriented, because of their shared linking device.

In the above considerations of co-orientation of bivalents in meiosis and of chromosomes in mitosis one main feature has been emphasized: co-orientation involves two independently acting centromeres linked together (by a chiasma in a bivalent; by the little understood structure shared between

the chromatids in a divided chromosome). The presence of the two centromeres and the linking device is essential to co-orientation. This was the same conclusion the author drew from observations on the orientation of chromosomes complexes (Rickards, 1964): centromeres in a chromosome complex co-orient in adjacent linked pairs: whether homologous or not two centromeres directly linked by a chiasma are capable of co-orientation and non-linked centromeres are not so capable.

In the author's opinion orientations along the spindle equator in mitosis and meiosis in general conform to the above principle of "co-orientation in linked centromere pairs". Further, the actual *process* of co-orientation is considered to hinge on this principle as well: the result of co-orientation is a direct reflection of the process of co-orientation. This is considered further below.

4. Links: Univalent and Undivided Chromosomes

In the light of what has been emphasized above on the importance of the chiasmata in co-orientation of meiotic bivalents, Darlington's theory of metaphase pairing is of significance. Darlington's theory (1937) states that homologous chromosomes are prevented from complete desynapsis and the formation of univalent chromosomes by the presence of chiasmata between them. Support for this theory comes from a number of observations as (a) the not infrequent presence of univalent chromosomes in the meiosis of auto-polyploid species due to the failure of chiasmata in some paired segments (Darlington, 1937), (b) the reduction of metaphase pairing paralleling the reduction in chiasma frequency (Rees, 1961), (c) the increase in the frequency of univalent chromosomes paralleling a reduction in chromosome size (and, therefore, of chiasma number) (Philp & Huskins, 1931), (d) the fact that diplotene bivalents generally desynapse except at points where chiasmata are present.

However, it is clear that chiasmata are not universally necessary for the maintenance of prophase pairing. It has long been recognized that chiasmata do not arise during *Drosophila* spermatogenesis and similar situations are found in a number a insect species (e.g. mantids, Hughes-Schrader, 1943a,b), but in these examples conjunction of chromosomes at metaphase still occurs by means of some unknown forces of generalized "attraction". Also, in female *Drosophila* meiosis where chiasmata normally arise, it has been demonstrated that suppression of these chiasmata does not induce desynapsis (Cooper, 1945). This situation may be expected from the one in male *Drosophila* though it clearly shows that something other than chiasmata may maintain association up to anaphase in certain species.

Homologous chromosomes of *Drosophila* and other organisms that maintain association at prometaphase I by some mechanism other than through the formation of chiasmata, regularly disjoin at anaphase I. The author considers that the mechanism maintaining chromosome association in such examples is also taking the place of the chiasmata of normal bivalents in effecting co-orientation and hence regular disjunction.

Regarding the importance of the substance or structure shared between the two chromatids of a mitotic chromosome in maintaining the association of these sister chromatids there is little experimental evidence available at present. The fact that the two chromatids of each meiotic chromosome at mid-anaphase I and prophase/metaphase II are separated except at their shared regions adjacent to the centromeres (Lima-de-Faria, 1958; Prakken & Levan, 1946; author's observations in *Allium triquetrum*) is an important indication.

The importance of the linking device to the maintenance of co-orientation is further emphasized here by a consideration of the apparent equilibrium that chromosomes or bivalents maintain during metaphase. The equilibrium position is with the co-oriented centromeres approximately equidistant from the spindle poles, though the position is not absolutely stable for the chromosomes oscillate in an irregular way above and below the equator (Bajer & Molè-Bajer, 1956; Bauer *et al.*, 1961).

It was considered earlier that co-oriented bivalent or chromosome centromeres are under the influence of the pole to which each is oriented at metaphase (Plate II and Fig. 1). The magnitude of the force or pull between centromeres and poles must vary directly with the centromere-to-pole distances if chromosomes and bivalents are to be held in the equator in an equilibrial position (Östergren, 1950, 1951). There is much support for this equilibrial concept and its importance, including (a) the observations of the "one-sided" position assumed by trivalents† in meiosis where the trivalent is oriented closer to the pole to which two of the three centromeres are oriented (Östergren, 1945; Bauer *et al.*, 1961), and (b) the experiments of Izutsu (1959) of bivalents being shifted towards one pole after radiation damage in the spindle or centromere of the other pole. The equilibrium concept is considered by Rashevsky (1941), Schrader (1953), Nicklas (1961) and Mazia (1961).

The importance of the linking device between co-oriented centromeres is clear from the above comments on metaphase equilibrium. Without the chiasma in a bivalent, for instance, the chromosome centromeres can be expected to be drawn towards the pole to which each is oriented; likewise in a mitotic chromosome.

† In a trivalent three homologous chromosomes are associated by chiasmata.

This comment is interesting in the light of the initiation of anaphase. In mitosis anaphase is visibly initiated by a lapse of attraction between sister chromatids and a "breaking" of the linking structure adjacent to the centromeres. This causes the chromatids to jump apart a short distance from each other (Mazia, 1961), a phenomenon vividly shown on the recent ciné-micrographs of living material. The same lapse of attraction between sister chromatids (except at their centromeric regions) also initiates anaphase I of meiosis by permitting the chiasmata to slip along the chromosomes as the homologous centromeres move to opposite poles (see comment by Lewis & John, 1963).†
Thus the same forces that are apparent at prometaphase and metaphase also account for anaphase movement.

The behaviour of univalents‡ on a meiotic spindle and of undivided chromosomes§ on a mitotic spindle is clearly of importance to this discussion on linking structures between pairs of centromeres.

Univalent chromosomes, whether present in meiosis of haploids, abnormal diploids, or triploids etc., that remain in the spindle during prometaphase I (they are sometimes lost from the spindle) show two distinct patterns of orientation, polar and equatorial; i.e. they are found either close to one or other pole, or oriented in an apparently stable position along the spindle equator (Darlington, 1937). As meiosis proceeds into anaphase an increasing number of univalents that are in a polar position undergo an apparent change in their orientation and move to a stable position along the equator (Bauer et al., 1961). Equatorial oriented univalents may remain in the equator as meiosis proceeds or a "univalent anaphase" may take place in which the chromosomes either move directly to a pole, or undergo separation into two univalent chromatids one of which passes to each pole. During these anaphase movements those univalents that do not change their orientation remain at the pole with which they originally became associated.

In considering these movements in the light of the principles of co-orientation outlined above, three explanations will be discussed. First, in those univalents that become positioned at one or other of the spindle poles, it is suggested that the single univalent centromere comes under the influence of a pole at prometaphase (as do those of a bivalent), but being without a second, linked centromere it moves to that pole being unable to become

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† This lapse of attraction between chromatids explains why these are widely separated from each other except at their centromeric regions at mid-anaphase I and until their complete segregation at anaphase II. However, it is possible that the lapse of chromatid attraction is not so complete as in mitosis for the chiasmata still afford some resistance to chromosome separation as shown by the more drawn out nature of the chromosome segments between centromeres and chiasmata that is commonly observed at early anaphase I.

‡ Chromosomes without homologous pairing partners.

§ Chromosomes in which reproduction to form chromatids has not occurred.

co-oriented. Single centromeres are unable to become oriented along the spindle equator. They undergo what may be described as a precocious anaphase.

But second, in further consideration it must be recalled that meiotic chromosomes of meiosis I are duplicated, though these duplicates do not separate until meiosis II (disregarding here the effect of crossing-over). Co-orientation of a bivalent normally involves the chromosome rather than the chromatid centromeres. Whether or not we regard the chromosome centromere as being double at meiosis I along with the rest of the chromosome (see Lima-de-Faria, 1958; Stahl, 1964) it is clear that the chromosome centromeres are the functional units in co-orientation at meiosis I. Co-orientation of sister chromatid centromeres has been observed in studies of living material of *Tipula* (Bauer *et al.*, 1961), but this condition is very unstable.

If in a univalent at meiosis I co-orientation is inhibited and the univalent becomes oriented at one of the spindle poles, its centromere may become functionally double during later phases of division. Univalent chromatid centromeres will then be capable of a change from their polar position to one of co-orientation along the equator, at late prometaphase I (as observed in *Tipula*; Bauer *et al.*, 1961) or later depending on the division cycle of the centromere and the duration of a potential orientation environment.

Thirdly, a univalent centromere may be in a sufficiently divided functional state at the start of prometaphase I to permit independent functioning of the chromatid centromeres and thus of their immediate co-orientation, as a pair of linked centromeres, along the equator in a perhaps relatively stable state. Thus we have an explanation of the characteristic behaviour of univalents that was outlined above.

The point suggested in the above discussion is that orientation of univalents along the equator at meiosis I conforms to the principle of co-orientation in linked centromere pairs as in bivalents and chromosome complexes in meiosis, and mitotic chromosomes.

The observations of a number of workers on stained and living materials of mitoses with undivided chromosomes (Beadle, 1933; Geitler, 1943; Johnson, 1944; Östergren & Bajer, 1961) should also be mentioned here. Undivided chromosomes, each consisting of a single chromatid, behave in a manner very similar to univalent chromosomes at meiosis I, and at prometaphase they exhibit great difficulties in forming a metaphase plate. At metaphase the chromatids that remain within the spindle may all be found aggregated round the poles or forming a relatively regular equatorial plate. Intermediate cases occur as in the living material of *Haemanthus* endosperm cells observed by Östergren & Bajer (1961) in which some chromosomes orient at the poles, others along the equator.

Undivided chromosomes that do not orient on the equator during mitosis must have undivided centromeres, and they cannot, therefore, co-orient on the equator. The one centromere of each comes under the influence of one pole at prometaphase and thus moves to that pole. Those undivided chromosomes that do orient on the equator can be regarded as having centromeres in a sufficiently divided state to allow them independent activity, and, therefore, co-orientation. If this is so the degree of undividedness of chromosomes and centromeres (in different species and in different chromosomes of a species) will determine the extent of formation of a metaphase plate; and it will determine the time after initiation of division (as compared with normal mitoses) at which co-orientation is possible.

The behaviour at meiosis II of daughter univalents from univalent chromosomes that have divided at meiosis I is essentially the same as that of undivided chromosomes (Darlington, 1937; Östergren, 1961). It is suggested that the reason as outlined for univalents is the same too.

The behaviour of chromosomes at meiosis II is essentially the same as divided chromosomes at somatic mitosis. The centromere is double at prometaphase II (Lima-de-Faria, 1958; author's observations in *Allium triquetrum*) and the two chromatids, maintaining association at regions proximal to the centromeres, co-orient on the equator during prometaphase II.

The above discussion of univalents and undivided chromosomes emphasizes the importance of the linking device between centromeres in effecting co-orientation. It also emphasizes that orientation along the spindle equator of chromosomes, bivalents and other chromosome arrangements is a phenomenon concerning *pairs* of centromeres. It is a phenomenon of co-orientation of pairs of centromeres that are linked to each other. The activity of the centromeres in bringing about co-orientation is thus discussed below.

5. Mechanics of Co-orientation

Mazia (1961) briefly covers present indications of the initiation of prometaphase and its relationship with the breakdown of the nuclear membrane. Evidence in the works of Bajer & Molè-Bajer (1956) & Bajer, 1958*b*) suggests that chromosome movements of prometaphase are initiated at the time of orientation of spindle substances into their "fibrous" state extending from centromeres to poles.

The ciné-micrographs and minute-by-minute sketches of mitosis in living materials (Bajer & Molè-Bajer, 1956; Bauer *et al.*, 1961), and the centromere irradiation experiments causing the disruption of movements by Bajer & Molè-Bajer (1961) have clearly shown that the centromere is the focal point

of prometaphase (and anaphase) chromosome movements. In these movements the centromeres lead the way, the chromosome arms following the centromeres in an irregular manner. Further, there is very strong evidence indicating that the centromere movements depend on interactions with the poles mediated through the spindle fibres. Evidence comes from direct observations of centromere-to-pole spindle connections (Schrader, 1947; Nicklas, 1961), and experimental disruption of movements with spindle poisons and irradiation (e.g. Mazia & Zimmerman, 1958; Izutsu, 1959). Also, the experiments of Mazia & Zimmerman (1958) inducing a "reversal" of metaphase by spindle poisons (after which removal of the "block" allows metaphase orientation to reoccur) indicate that the maintenance of metaphase is dependent on the oriented structure of the spindle.

We now have a clear picture of the movements of chromosomes during prometaphase of mitosis and meiosis in a variety of both plant and animal cells (Bajer & Molè-Bajer, 1956; Bauer et al., 1961). Of importance here, these observations indicate that during prometaphase the centromeres of a chromosome or bivalent need not move directly onto the equator in a co-oriented position. They may do so (Fig. 2) or they may move to within close proximity of one pole (the centromere often being referred to as "centrophilic" when in
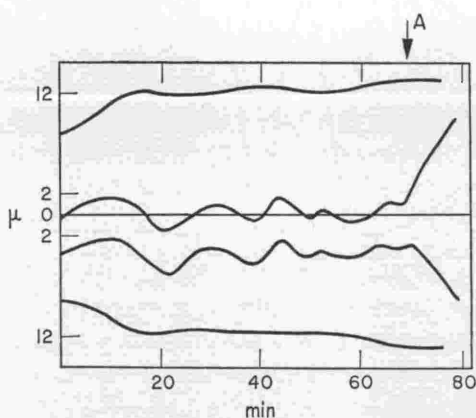
FIGS 2 to 4. Graphical representation of chromosome movements through prometaphase and metaphase to anaphase, constructed from minute-by-minute sketches in *Tipula oleracea* spermatocytes. The abscissa marks the time in minutes; the ordinate the distances in μ of the poles (upper and lower lines) and centromeres from the equator; A = anaphase. (Condensed from *Chromosoma* 12, 116–189 (1961), Springer-Verlag, Berlin, Göttingen, Heidelberg. Courtesy Dr. H. Bauer.)

FIG. 2. Bivalent orientation. The two centromeres co-oriented immediately at prometaphase, and remained so until anaphase.
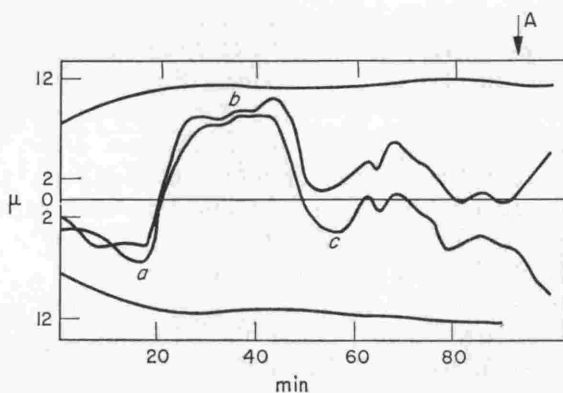
FIG. 3. Bivalent orientation. The two centromeres initially oriented to the same pole, *a*, and then both reoriented to the opposite pole, *b*, then one centromere again reoriented and brought the bivalent into an equatorial, co-oriented position, *c*.
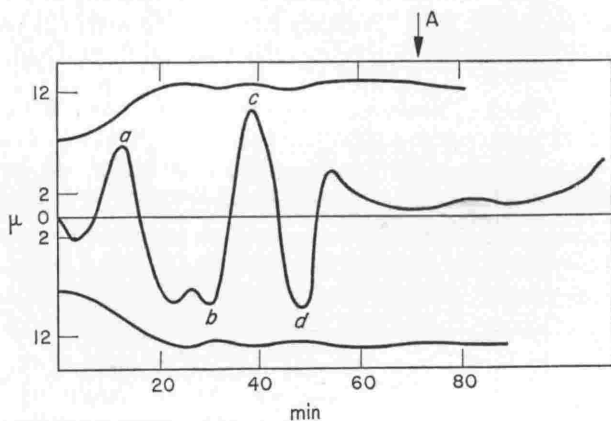

FIG. 4. Repeated reorientation (*a*, *b*, *c* and *d*) of a univalent centromere prior to its apparent co-orientation on the equator at late prometaphase.

this position), then perhaps to the other pole and back before finally co-orienting in the equator (Fig. 3). Also, once co-oriented in the equator the major (orientation) pole-to-pole movements cease and the bivalent or chromosome becomes stabilized except for the characteristic oscillations above and below the equator (Bauer *et al.*, 1961).

(The Figs 2 to 4 redrawn here are from meiotic prometaphase I. At meiosis the movements of individual (homologous) centromeres can readily

be traced because of their wider separation from each other as compared with the (chromatid) centromeres at mitosis. The basic facts above, however, apply equally well to the chromosome movements recorded by Bajer (1958) in endosperm mitoses.)

A second important phenomenon of prometaphase movements was referred to as *reorientation* by Bauer *et al.* (1961). A bivalent when in close proximity to a pole (centrophilic or showing unipolar orientation) will eventually re-orient and move either to the opposite pole or to a co-oriented position along the equator (Fig. 3). The term is also applicable to the similar orientation change of centrophilic chromosomes observed in endosperm mitoses by Bajer (1958). These reorientation movements are centred on the centromeres, and during reorientation the interactions between centromere and pole are abolished and then new interactions are established (Bauer *et al.*, 1961; see also Dietz, 1958; Nicklas, 1961).

The author considers that the major orientation and reorientation move-ments outlined above are directly responsible for bringing about the necessary co-orientation of bivalents or chromosome centromeres during prometaphase.

Prometaphase mechanics are considered to hinge on a concept referred to here as *ipsi-orientation—one centromere orients to and*, if unchecked, *moves to one pole*.

Consider first the prometaphase movements of univalents and undivided chromosomes with their single centromeres. It has already been suggested (p. 342) that these single chromosomes are incapable of co-orientation along the equator unless their centromeres have become functionally double, but they are capable of ipsi-orientation and reorientation. The single centromere comes under the influence of one pole and moves to that pole, and there it may undergo reorientation (Fig. 4). These polar movements have already been discussed as being characteristic of univalents and undivided chromo-somes.

Consider second a bivalent with its two linked centromeres. If each centromere acts independently of the other, the two may come under the influence of opposite poles, and the bivalent will then become co-oriented directly on the equator, one centromere being under the influence of and having potential movement to one pole (ipsi-orientation), the other to the opposite pole (Fig. 2); and the bivalent will remain stabilized on the equator through the action of the shared chiasma (Plate II).

However, the two centromeres of a bivalent may initially come under the influence of the same pole. The bivalent will then move to that pole (Fig. 3). The polar movement of a bivalent has been mentioned previously as being characteristic of prometaphase I and is considered here to be the result

of an independent ipsi-orientation of the two homologous centromeres such that they move to the same pole.†

In order to attain co-orientation of these centrophilic or unipolar oriented bivalents, one of the two centromeres must reorientate to the opposite pole. On reorientation of one centromere the bivalent will be drawn into the equator in a (bipolar) co-oriented position. These movements are clearly illustrated in the graphs of Bauer *et al.* (1961, see Fig. 3).

Thus co-orientation of a bivalent is considered to depend on the independent ipsi-orientation of the two homologous centromeres and, where necessary, reorientation of one centromere. And the same mechanism is considered to act upon the chromatid centromeres of a mitotic chromosome. Unipolar orientation, if arising, will give place to bipolar co-orientation as one centromere reorientates to the opposite pole. Otherwise the chromosome will move directly to the equator, its two chromatid centromeres in a co-oriented position. This is here considered to be the basis of the principal chromosome movements observed by Bajer (1958) and mentioned on p. 345.

Certain aspects of this concept of prometaphase mechanics should be discussed more fully here. First, further mention should be made of the equilibrium hypothesis discussed earlier (p. 339). It is necessary that the force acting between a centromere and the pole to which it is oriented should vary directly with centromere-to-pole distance not only to maintain co-orientation along the equator (as discussed earlier) but also to bring polar oriented chromosomes onto the equator after reorientation. The rapid movements of reorienting chromosomes noted by Bauer *et al.* (1961) and Nicklas (1961) present further support for this equilibrium theory.

Second, one must consider why equatorial co-oriented centromeres do not reorientate (Bauer *et al.*, 1961), but remain co-oriented. The answer may perhaps be in the large distance between the centromere and the poles, compared with that between a reorienting centromere and the pole with which it is associated. Proximity to a pole may be a prerequisite for reorientation. This distance principle may be the reason too why the chromatid centromeres of co-oriented bivalents do not themselves undergo co-orientation at late

† The present author considers that the apparently minor movements "above" and "below" the equator (e.g. Fig. 2) found in both mitosis and meiosis are not the same as the pole-to-pole movements brought about by the orientation and reorientation of centromeres. The bivalent centromeres as in Fig. 2 behave virtually as a unit during these movements, a situation that would not be expected with independent centromere reorientation. Also, under these movements the chromosomes never pass wholly to one pole as is often the case during reorientation movements (compare the equatorial movements in Fig. 2 with the reorientation movements in Fig. 3). Both types of movement are undoubtedly of centromere/spindle origin but the equatorial movements appear to result from variations in the strength of the "force" between a centromere and the pole to which it is oriented. Polar oriented univalents also show these "equatorial" movements, though at the poles, not at the equator (Fig. 4).

prometaphase or later, while those of univalent chromosomes that have become sufficiently divided at this stage may do so.

There may also be a relation between this phenomenon (reorientation ability) and changes in intensity of the prometaphase to anaphase "pulling forces" acting between poles and centromeres. These forces are strong at early prometaphase, grow weak towards metaphase and very weak at metaphase. They grow strong again at anaphase (Hughes-Schrader, 1943; Dietz, 1958; Taylor, 1959; Nicklas, 1961; Bauer et al., 1961).

Concerning the basis of a centromere's reorientation ability when it lies close to a pole, little is known at present. This reorientation property may be a feature of the pole or centromere alone or of a centromere/polar interaction. In this respect the transient reversal of prometaphase by spindle poisons (Mazia & Zimmerman, 1958), and the experiments by Bajer & Molè-Bajer (1961) in which irradiation of the centromere during prometaphase often brought about a kind of reorientation of movements are of interest as these may be related phenomena.

From the discussion on the importance of reorientation to prometaphase mechanics (p. 345) it may be inferred that reorientation is a property associated with individual centromeres. However, it may be considered that when two centromeres of a bivalent or a chromosome are oriented at a pole reorientation causes all interactions of the pair of centromeres to cease and the centromeres then start off afresh as it were and orient individually to either the same or opposite pole.

Thirdly, it is evident that the force causing ipsi-orientation at prometaphase (and the related force at metaphase and anaphase) is potentially able to act in the *whole* spindle; and it acts from one centromere to *one pole*. An alternate idea, that the force might act towards both poles during prometaphase and towards only one at anaphase (Östergren, 1950; Östergren et al., 1960; Bajer, 1958a), is perhaps suggested from a (here superseded) concept involving a pre-anaphase singleness of a chromosome centromere at somatic mitosis. But with a concept involving a double centromere at prometaphase and metaphase it is clear that an apparent two-way force is, in fact, two one-way forces involving two separate units. The force acts between one centromere and one pole at prometaphase, metaphase and anaphase, i.e. a centromere ipsi-orientates.†

The appearance of a force acting in the half spindle at metaphase (Bajer, 1958a) is merely the result of co-orientation imposed upon ipsi-orientation. Prior to co-orientation a centromere's orientation force potentially acts in the whole spindle, while its actual direction and range is determined by the posi-

† See also spindle fibre orientation during pole-to-pole movements of sex chromosomes in Nicklas (1961).

tion of the centromere relative to the pole from which it is influenced. This is of importance to any interpretation of the nature of the force between centromere and pole: that is the mechanics of the spindle. The "one-way" behaviour of the centromere may be caused through an active as apposed to a passive face of the centromere (Boveri, 1907; Östergren, 1951, 1961), but it may be a peculiar property of the centromere and its activity. This will perhaps be understood when we can explain more fully the structure and, in particular, the functioning of the centromere and the exact mechanics of centromere. spindle and polar interactions.

6. Conclusions

It seems probable that there are no essential differences in centromere structure, centromere orientation and the orientation mechanism between meiosis (bivalents) and somatic mitosis (divided chromosomes). There is no problem in prometaphase of an "exclusion principle" as stated by Boveri; both centromeres *can* orient to one pole simultaneously. Bipolar orientation leads immediately to co-orientation; unipolar orientation is transformed to bipolar co-orientation on reorientation of one of the bivalent or chromatid centromeres.

In the author's opinion the term co-orientation is applicable to all types of orientation of centromeres that have become positioned along the equator and covers the terms "syntelic" and "amphitelic" coined by Bauer *et al.* (1961) to describe the orientations of univalents and transient orientations of chromatid centromeres of bivalents, and also covers the extended terminology of Henderson (1962).

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SOME THEORETICAL ASPECTS OF SELECTIVE SEGREGATION IN INTERCHANGE COMPLEXES

By

G. K. RICKARDS

With 11 Figures in the Text

Introduction

In the course of cytological work on *Allium triquetrum* L., a very common introduced species in New Zealand, a number of plants were discovered that showed a complement anomaly at meiosis of pollen mother cells. Closer attention revealed that these plants were heterozygous for an interchange (reciprocal translocation). The meiotic complex was an open chain of four chromosomes.
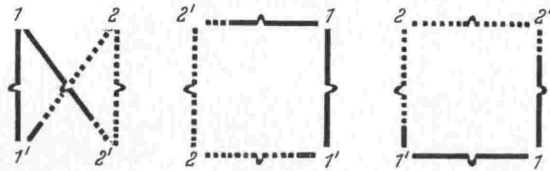

Fig. 1. Diagram showing the possible orientation types of a simple interchange complex at metaphase I. From left, alternate, adjacent, adjacent *1*, adjacent *2*. Centromeres indicated in homologous pairs *1,1'* and *2,2'*. Brackets indicate chiasmata

Initial observations on the types of orientation of this complex at metaphase I showed that there was an apparent selection in these plants for arrangements that produce viable products, to the extent of about 75%. Further observations at diakinesis and anaphase I led to some consideration of the possible reason(s) for this selection and also of already published theories related to this phenomenon.

Paralleling the phenomenon in bivalents where, under (apparently) normal conditions, the two homologous centromeres rarely pass to one pole, the centromeres of an interchange complex generally behave in twos, i.e. each pole receives, at anaphase, two of the four centromeres. However, in higher plants, of the three possible arrangements whereby each pole will receive two centromeres (Fig. 1) only one, the alternate arrangement, produces normal viable products (disregarding modifications caused through the occurrence of interstitial[1] chiasmata — see

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[1] Refers to chiasmata between the points of interchange and the centromeres.

BURNHAM, 1956). The adjacent arrangements give rise to products that are either deficient or duplicated for the interchanged segments and are generally inviable.

If each of the arrangements in Fig. 1 were to occur at random, about two thirds of the total meiotic products should be inviable. However, many interchange heterozygotes show 50% or more viability and in some it reaches as high as 90%. The ocurring percentage is related to the modes of orientation of the complexes at metaphase I (and to chiasmata when these are interstitial). A number of theories have been put forward in an effort to explain this apparent selection in interchange heterozygotes for those orientations that produce viable products. As is evident from BURNHAM's review (1956), the factors controlling segregation of interchange complexes are still imperfectly known.

The object of this paper is to present some new ideas on the mechanism of orientation of interchange complexes.

The Interchange[1]

Allium triquetrum has $2n = 18$ (LEVAN, 1932). The normal haploid set of nine includes four long chromosomes with median centromeres (here referred to as *m* chromosomes), two smaller ones with submedian centromeres (*sm*), and three which are about half as long as the *m*'s and have subterminal centromeres (*st*). The *m* and *st* groups can be further subdivided in mitotic divisions though these differences cannot be observed through the first division of meiosis.

The interchange complex involves one *st* and one *m* pair of chromosomes as shown from a number of metaphase I cells in which three *m*, two *sm* and two *st* bivalents as well as the complex could be identified (Fig. 2). Early anaphase I cells in which the complex had just separated showed an *m* and an *st* chromosome directed to each pole (Fig. 3). Both *m* chromosomes had arms of about the normal length (as far as can be judged from the contracted first division chromosomes). From these data alone it was not possible to ascertain whether the interchange involved portions of about equal length from each of the chromosomes involved or almost the whole of the *m* chromosome arm and a small portion of the short *st* chromosome arm. However, to judge from the regular occurrence of chains rather than unequal bivalents or univalents that would arise if more than the one chiasma in the complex failed, the points of interchange were probably close to the centromeres. This is also suggested from the fact that interstitial chiasmata did not form in the complexes.

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[1] Observations on the interchange material are continuing and will be reported later.
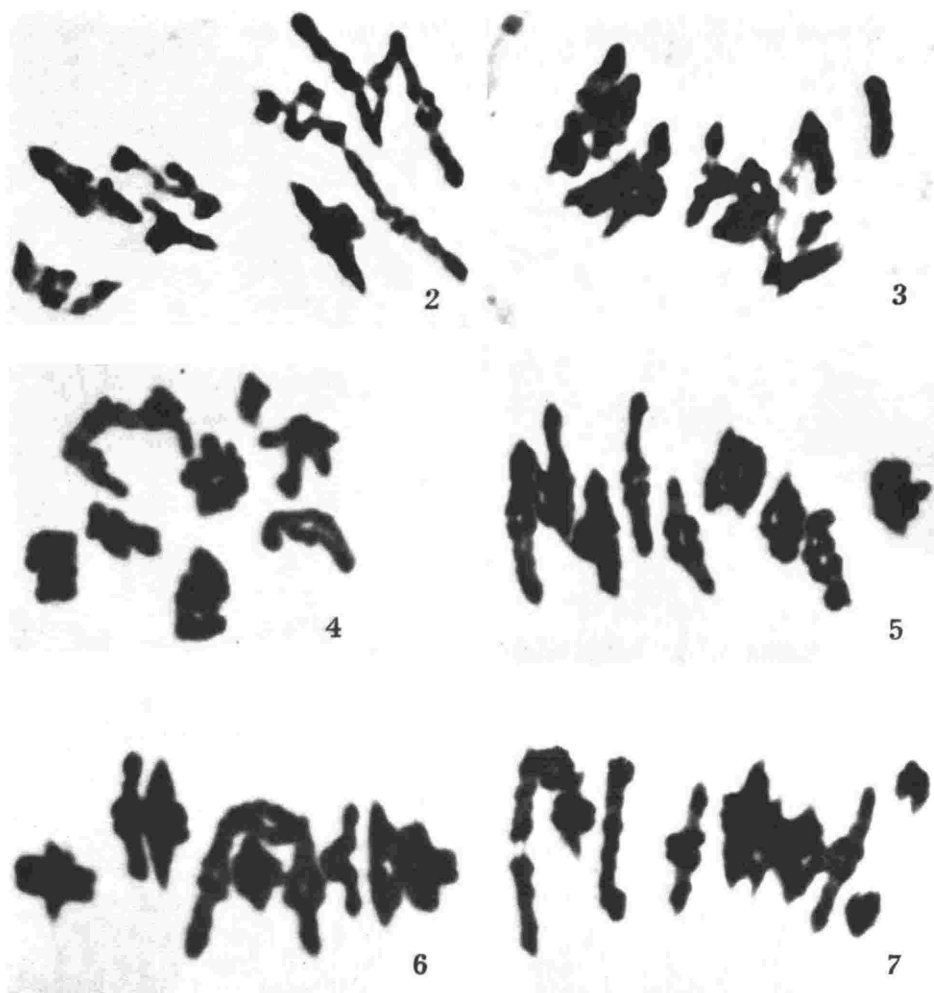
Figs. 2—7. × approx. 2700. Fig. 2. Metaphase 1 showing interchange complex plus seven bivalents. From left to right along approximate line of equator — *m, m, st, sm, m, sm, st,* complex

Fig. 3. Precocious separation of complex at anaphase 1. Complex separating into two *m* and two *st* chromosomes. Fig. 4. Diakinesis. Complex at top left. Fig. 5. Alternate orientation of complex (left). Fig. 6. Adjacent orientation of complex (middle); chiasmata near ends of chromosomes. Fig. 7. Adjacent orientation of complex (left); chiasma in right hand *st* 'bivalent' well in from chromosome ends

Chiasma frequency in the association of four was lower than it would have been in the unaltered bivalents. Most frequently the *m* bivalents have two chiasmata in each arm and the *st* bivalents, two in their long arms. The chain of four at diakinesis (Figs. 4 and 4a), however, only occasionally showed more than three chiasmata there being most commonly one chiasma, near the ends, in each of the three long pairs

of arms. The reduction in chiasma frequency is probably the consequence of reduced chromosome pairing at the break regions during pachytene.

Absence of chiasmata in the short arms of the *st* chromosomes was no doubt the cause of chains rather than rings being formed. The normal *st* bivalents are rods with chiasmata only in the long arms (Fig. 2).

The chief point of interest is that the complex has little suggestion of being a rigid association (as would be expected with a greater number

4a

5a

6a

7a

|————————————————|
70μ
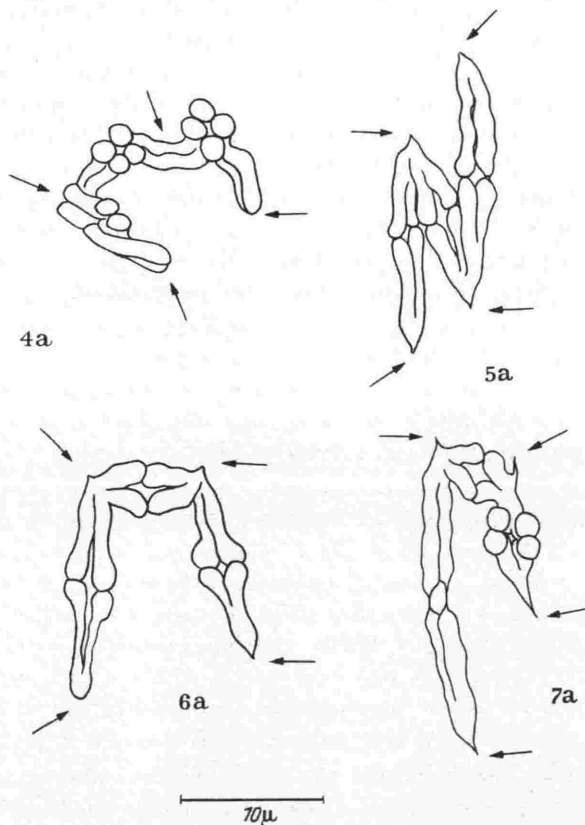
Figs. 4a—7a. Camera lucida drawings of complexes shown in Figs. 4—7. Fig. 4a. Diakinesis. Arrows indicate approximate positions of centromeres. Figs. 5a—7a. Metaphase. Arrows indicate centromeres. Fig. 5a. Alternate orientation. Fig. 6a. Adjacent orientation. Fig. 7a. Adjacent orientation; chiasma of right hand arm well in from chromosome ends

of, and less terminalized, chiasmata) and can, therefore, be expected to respond relatively freely to orientation forces of prometaphase.

Three other features of the complex should be noted for reference later, 1. the approximate equidistant positions of the centromeres, 2. the remoteness and approximate equidistance of the chiasmata from the

centromeres and 3. the shortness of the interstitial segment and the consequent absence of interstitial chiasmata.

The complex of four was oriented in an alternate fashion (Figs. 2, 3, 5 and 5a) in about 75% of cells. The remaining 25% of cases were all of one adjacent type (Figs. 6 and 7).

The mechanism of co-orientation

In maize, where orientation types in many different interchanges have received extensive study, alternate segregation in ring complexes involving four chromosomes occurs at a frequency of about 50% (ANDERSON et al., 1955a, b; BURNHAM, 1956 for a review). This frequency is approximated in a number of other cases e.g. in *Pisum* (HÅKANSSON, 1931), *Allium cernuum* (LEVAN, 1939), *Neurospora crassa* (McCLINTOCK, 1945), *Sorghum versicolor* (GARBER, 1948). Pollen and ovule abortion in these cases is about 50% due to approximately 25% of each of the non-viable adjacent 1 and adjacent 2 orientations. The examples are described as being semi-sterile or as having non-directed segregation.

In other species as rye and barley and including the multiple interchange complexes of such plants as *Oenothera*, the frequency of alternate segregation is in excess of 50% and in some it may be as high as 90%. These examples generally have low sterility and are described as having directed segregation (BURNHAM, 1956, p. 425).

A number of writers on the subject (e.g. DARLINGTON, 1937, SWANSON, 1957) have considered selection for viable products in the light of the fact that if the three ways that a complex can become oriented were to occur at random, alternate segregations should only be about one third of the total. On the other hand, BURNHAM considers from observations (alone) in maize, that a ring may orient itself as an open configuration in two ways (adjacent 1 and adjacent 2) and "for each type there is a corresponding alternate arrangement (all of one type) resulting in 50% alternate segregation" (BURNHAM, 1956, p. 425).

GLASS (1935) has attributed the skew towards viable combinations to the homologous centromeres possessing a single predetermined axis of segregation, while DOBZHANSKY (1933) related the phenomenon to forces of synapsis of the homologous segments. DARLINGTON (1937) considered that forces of repulsion associated with the centromere pairs effect regular segregation for viable products and that the presence of chiasmata, causing rigidity in the complex, results in non-disjunction[1].

ÖSTERGREN (1951) has postulated a pulling towards the poles by the spindle after an initial orientation of an active side of the centromeres

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[1] Non-disjunction here implies incomplete disjunction of all homologous chromosome parts.

towards the poles and an interaction between homologous[1] centromeres to ensure they pass to opposite poles. He believes that inflexibility in the ring is the principal cause of non-disjunction.

REES (1961) has suggested, from selection experiments in rye, a direct role of the genotype in controlling segregation.

Each of these theories provides an explanation for the occurrence of a high frequency of disjunction but does not, the author feels, satisfactorily explain why non-disjunction should occur at all. The explanation that is generally put forward is that chiasmata cause rigidity within the complex and with this rigidity it is unable to become oriented as it should (GAIRDNER and DARLINGTON, 1931; DARLINGTON, 1937, 1939). The orientation mechanism is ineffective in these 'special' cases. If this is so, the percentage of non-disjunction that may occur in a particular plant must be regarded as related merely to the position and frequency of chiasmata within the complex. Also, since the occurrence, terminalization, and localization of chiasmata are at least partly under genetic control and not determined simply by chance one is led to expect a very high frequency of non-disjunction; a regularity of chiasma position (in particular, a position which would be causing improper orientation) would result in just such a high frequency. Again, as BURNHAM (1956) has noted, this explanation is difficult to accept in those complexes with interstitial chiasmata. In these cases only adjacent 1 or alternate segregations occur (see later). If interstitial chiasmata affect 'correct' orientation, alternate rather than adjacent 2 segregations should be eliminated.

There need be little doubt that chiasmata may impart rigidity to a complex and so influence orientation at prometaphase but it is the author's opinion that such chiasmata are not the principal cause of adjacent orientations (see later).

The author is of the opinion that the orientation of a quadrivalent is governed by the same forces as that of a bivalent, that a 50% frequency of adjacent segregation is what basically should be expected in closed complexes, and that adjacent arrangements occur because the centromeres behave as *adjacent* pairs and not necessarily as homologous pairs.

If the two centromeres of a bivalent behaved independently of each other at prometaphase, a regular occurrence of bivalent non-disjunction should be expected.

Thus, from the regular behaviour of bivalents that is observed at anaphase and also the generally indifferent behaviour of univalents on a meiotic spindle (DARLINGTON 1937, p. 410), the author considers that

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[1] Used in connection with centromeres the term 'homologous' refers to the centromere pair of homologous chromosome segments. This applies throughout this paper.

the prometaphase orientation mechanism hinges on *the presence of two centromeres linked together through chiasmata*[1].

The actual mechanism that orientates bivalents is not of concern here though the role of 'homologies' should be mentioned. ÖSTERGREN (1951) states that the observed 100% disjunction of bivalents is "...proof enough of an interaction between homologous kinetochores". This could, however, be indicative of an interaction of some sort between the centromeres and the poles. And even in those complexes with high terminalization (Figs. 4a, 6a, e.g.) of four chromosomes the not infrequent occurrence of adjacent orientations throws much doubt on this contention, unless there are other strongly influential factors that cause 'incorrect' orientation.
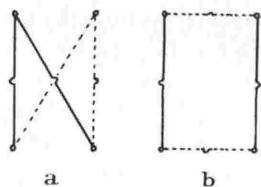

Fig. 8. a Showing three adjacent centromeres (circles joined by solid lines) co-oriented as two bivalents and b, four centromeres, in two separate adjacent pairs, cooriented as two bivalents

An interchange complex opens out during diplotene/diakinesis as does a bivalent, owing to the repulsion of its homologous parts. At prometaphase it is not only subjected to the same forces that are responsible for bivalent orientation but it is clear also that the centromeres *behave in twos* and not independently. Segregations giving odd numbers of centromeres at each pole are rare and if they occur, are the equivalent of non-disjunction in bivalents.

If disjunctional orientation of bivalents does not rely on the homologous nature of the two centromeres or other homologous parts, then adjacent (not necessarily homologous) centromeres of a complex will be expected to be co-oriented likewise. However, each centromere of a closed complex as in Fig. 1 has two adjacents. Three such centromeres may then act independently of the fourth centromere as in two bivalents (Fig. 8a). The alternate arrangement must result. But also, all four centromeres, in two separate pairs, may act as in two bivalents (Fig. 8b).

The adjacent arrangement will be formed (see p. 150 for a further consideration).

Thus each centromere always has one of its adjacents oriented towards the pole opposite to its own; the other adjacent is oriented to either pole. There are two arrangements possible (taking each centromere in turn), the alternate and the adjacent. But in half of the latter total, homologous centromeres will be oriented to opposite poles (adjacent 1), and in the other half to the same pole (adjacent 2). The ratio alternate : adjacent 1 : adjacent 2 would then be about 2:1:1 or as BURNHAM (1956) recognized in maize, an alternate arrangement for each adjacent arrangement.

The mechanism simply relies on the presence of two centromeres linked together by chiasmata. No interaction between homologous chromosome parts to ensure they pass to opposite poles is involved. Note Fig. 7, 7a, the two centromere pairs clearly behaving as if they were of two independent *st* bivalents.

Factors that affect orientation of complexes

The thesis outlined above emphasizes the role that chiasmata play in the mechanism responsible for orientation of bivalents at metaphase. The centromeres of a bivalent are oriented to opposite poles because they are linked through (one or more) chiasmata. Chromosome complexes are oriented in a number of ways according to the combinations of two linked centromeres that the complex permits. Basically then, 50% alternate, 50% adjacent orientations should occur in a complex with four chromosomes. However, it will be seen from the following that a number of factors may alter this basic frequency.

Rigidity

As a result of different positional relationships between centromeres and chiasmata, certain centromere pairs of a complex may become ininfluenced by orientation forces ahead of others. Complexes with centromere/chiasmata positions as in Fig. 9a are oriented with a tendency towards an apparent excess of alternate arrangements; those as in Fig. 9b are oriented with a tendency towards an apparent excess of adjacent 1 or alternate orientations (almost indifferently) with little or no adjacent 2.

It has been appreciated by a number of authors that rigidity imposed on a complex by chiasmata affects its orientation at prometaphase. This effect will be particularly marked if the rigidity is all round the complex. A complex rigidly bound together has little chance of orienting in an alternate manner. In *Allium ammophilum* (LEVAN, 1935) terminalization of chiasmata was low in a simple interchange complex and segregation was adjacent, seldom alternate. On the other hand, in a

complex in *Allium cernuum* (LEVAN, 1939) with high terminalization, alternate and adjacent segregations occurred at about equal frequencies. A more detailed relationship has been reported by SAX and ANDERSON (1933) in *Tradescantia edwardsiana*.

The regular cytological features of the described complex (p. 142, 143) had, as expected, eliminated possible modification of orientation frequencies in the above manner.
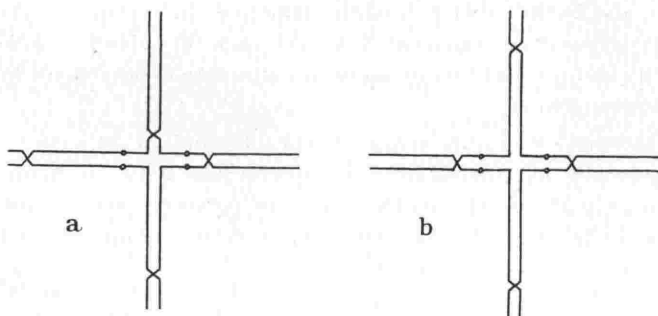

Fig. 9 a and b. Diagramatic pachytene configurations with chiasma positions that, if localized as such, favour orientation at metaphase in a, an alternate manner and b, an adjacent manner

Complexes with interstitial chiasmata

Chromosomes that have crossed over in the interstitial segment pass to opposite poles, i.e. adjacent 1 and alternate orientations occur but not adj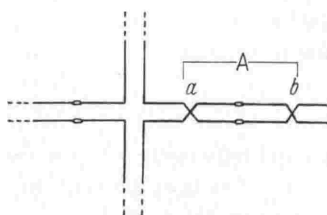acent 2 (McCLINTOCK 1945, BURNHAM 1949, 1950 b, 1956). BURNHAM (1956, p. 448) concluded from a variety of interchanges in maize that "...the length of the interstitial segment is one factor affecting segregation". If the interstitial segment is long enough and interstitial chiasmata form, then adjacent 2 segregations that occur in ring configurations without interstitial chiasmata are eliminated. Both alternate and adjacent 1 segregations occur in about equal frequencies. BURNHAM (1956, p. 449) remarked "This describes the behaviour in maize but does not explain the mechanism". The phenomenon is readily explainable: the interstitial chiasma (*a* in Fig. 10) forces the adjacent centromeres so linked to co-orientate invariably and so eliminates adjacent 2 orientations. For adjacent 2 orientations to occur non-homologous centromeres would have to react together, a feat virtually impossible with the presence of the interstitial chiasmata.

Fig. 10. Diagrammatic pachytene configuration with an interstitial chiasma at *a*

If interstitial chiasmata have formed then the number and positions of chiasmata in the other segments are not important to segregation. BURNHAM (1950b, p. 473) concluded just this from observations on interchanges involving chromosome 6 in maize.

Chain forming complexes

Fig. 11 (a) and (b) at left show the types of chain forming interchanges to be considered, (a) the centromeres on the same pachytene
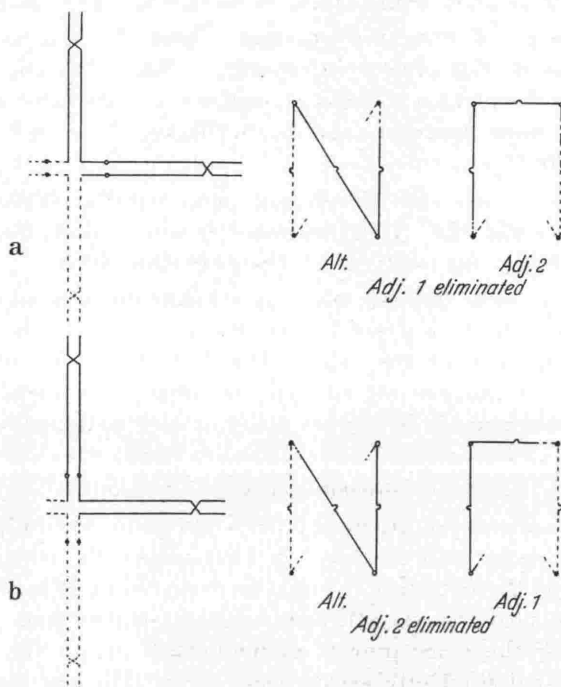

Fig. 11a and b. Diagrams of the two principal types of chain forming configurations at pachytene and their types of orientation at metaphase. a, with the centromeres on the pachytene axis in which the chain forming chiasma has failed and b, with the centromeres no the other axis

axis as that bearing the homologous segments in which chiasmata fail, (b) the centromeres on the other axis.

In considering orientation types the interchange break regions (and hence centromere positions at pachytene) must be known.

In chain forming interchanges involving one break in the satellite of chromosome 6 in maize (the centromere position then as in Fig. 11b) BURNHAM (1950a, b) noted the relationship — in interchanges with short interstitial segments adjacent 2 orientations are virtually nil.

Burnham concluded from his observations (though he could not under-
stand the reason) that chain forming interchanges without interstitial
chiasmata behave differently from rings of similar type in that adjacent 2
segregations are eliminated.

The reason is clear. Non-linked centromeres as those in Fig. 11 b
do not co-orientate each other though they always pass to opposite
poles when the other two centromeres do so because each co-orientates
with its adjacent linked centromere. Adjacent 2 segregations are
eliminated, their place being taken by further alternate arrangements.

The presence of interstitial chiasmata must alter this relationship.
This is as Burnham (1950a, b) reported. "The other chain forming
translocation has a long interstitial segment and its behaviour agrees
with ring forming translocations of similar type" i.e. 50% adjacent,
50% alternate.

The author is unaware of sufficient published data concerning con-
figurations as Fig. 11 a.* It is suggested however, that, for the same
reasons as above, adjacent 1 orientations are eliminated.

In general, chain forming interchanges eliminate one adjacent type
of orientation, the type depending on whether the centromeres are on
one pachytene axis or the other. The 75% frequency of alternate
orientation and absence of one type of adjacent orientation in the
described interchange is clearly the result of such modification.

Alternate orientations in ring complexes

The basic orientation frequency (50% alternate, 50% adjacent) can
be expected to be little modified in cases where the complex is not
displaced from the flat plane it would be expected to lie in as it opened
out at diplotene/diakinesis. If, however, the complex were to become
displaced from this plane prior to prometaphase orientation, one of the
two pairs of independently acting centromeres (Fig. 8 b) might be ex-
pected to become oriented 'up-side-down' as it were to the other. In
these cases alternate orientations would take the place of adjacent ones.
In extreme cases, half of the expected adjacent orientations would be
alternate ones. This would produce an approximate frequency of 75%
of the alternate arrangement.

The average frequency of the alternate orientation in barley is about
75% (Burnham et al. 1954).

The time between diakinesis and prometaphase may be an important
factor governing 'displacement' of a complex. It is interesting to note
that two related species as *Allium triquetrum* and *A. cernuum* appear
related in this respect and have adjacent orientations unmodified in
this manner.

* See, however, Lewis & John (1963): Chromosoma 14: 618-637 in
which the relevant interchange is described and where
similar considerations had been published shortly
before my own.

It would be of interest to know the difference in time lags between diakinesis and prometaphase in possible extreme cases as in barley and maize.

Of course there may be other factors effecting 'displacement' of a complex prior to orientation.

Relative lengths of pachytene axes

BURNHAM (1956, p. 448) concluded from studies on chain configurations in maize that the relative lengths of the two axes at pachytene does not appear to be a factor determining the type of segregation. In explanation, no matter the lengths of the axes, if the positions of the innermost chiasmata (assuming there are no interstitial ones) relative to the centromeres remain the same, no modification in orientation frequencies occurs. Only if chiasma positions as well were radically changed would segregation be affected. With chiasmata position under genetic control this would not be generally expected.

Multiple interchange heterozygotes

On the basis of the theory of co-orientation outlined in this paper, an appreciable frequency of other than complete alternate segregation should be expected in large ring complexes. However, adjacent and semi-adjacent orientations occur in very low frequencies in natural, true breeding populations of interchange heterozygotes as Oenothera. To explain the strongly directed segregation in Oenothera ÖSTERGREN (1951) put forward his 'co-orientation by pulling' theory. As already indicated, however, this theory does not satisfactorily explain the not infrequent occurrence of adjacent orientations in simple ring complexes.

The high frequency of alternate segregation in Oenothera is apparent not only in populations with complexes involving the complete chromosome complement ($2n = 14$), but also in populations with smaller rings of 4, 6, and 8 chromosomes (STEBBINS 1950). It is also apparent in multiple complexes of Rhoeo discolor and in a few other species. It appears then that there is in these species a fixed evolutionary entity in the form of a special cytogenetic feature that governs or modifies the orientation of their meiotic complexes. The present author does not consider it likely that an entirely unique process of orientation has been evolved in interchange heterozygotes such as Oenothera but, rather, that a secondary feature has been established which, together with the orientation mechanism outlined in this paper, enables a very high frequency of alternate segregation to be attained.

A number of distinct cytological features characterize the ring complexes in Oenothera, namely (a) the chromosomes are all of about the same size, (b) the centromeres all about median, (c) the interchanged

segments all of about equal lengths and (d) crossing over and chiasmata are localized near the ends of the homologous segments and interstitial chiasmata are rarely formed.

In wild populations, natural selection seems to have been against any cytological features not conforming to the above. Also, selection for the above features is probably related to the process by which the meiotic complex is oriented; Östergren considered this in relation to his theory of co-orientation by pulling.

As Darlington (1937) and Burnham (1956, p. 478) pointed out, the mechanism of orientation appears to rely upon the precocious passage of one chromosome onto the metaphase plate followed in order by the remaining chromosomes. If this is so, the author considers that alternate orientation will follow regularly throughout the large ring as a result of co-orientation of the successive adjacent centromeres that are linked together by chiasmata.

The precocious behaviour of one chromosome during orientation appears to be characteristic only of certain species and is probably the special orientation feature that these same species possess.

Genotypic control of segregation

Differences in the frequencies of orientation types between closely related species have been suggested as indicative of a role played by the genome in controlling segregation of complexes. Recent inbreeding experiments (Thompson 1956, Rees 1961) are suggestive that such genetic control is present in rye interchange heterozygotes.

The important place and modifying effects that chiasmata hold in the mechanism of orientation have been amply demonstrated and stressed and a discussion was included above on the absence of relationship between relative lenghts of the pachytene axes of a complex and its orientation frequencies if the chiasma positions are constant. But, if chiasma position varied as the genome controlling it varied, a certain amount of frequency modification might be expected. Variation in chiasma position caused through genetic variations may be the basis of an apparent genetic control of segregation.

It has been found in rye that chiasma frequency is subjected to genotypic control, that the control is polygenic and that the chiasmata-controlling genotypes of normally outbreeding populations are partly heterozygous (Rees, 1955). Inbred populations show marked reduction in the number of chiasmata in bivalents and the crossing of different inbred lines induces heterosis for chiasma frequency in the progeny (Rees et al. 1956). There is then in rye, genetic variability expressible in chiasma formation which might result in potential heterogeneity in segregation types in different lines with identical interchange complexes.

Conclusions

The co-orientation of a bivalent depends on its two centromeres being linked together by at least one chiasma; the homology of its centromeres is not an essential feature governing orientation. In a complex the possible combinations of two adjacent, linked centromeres govern its basic orientation frequency i.e. any two linked centromeres, homologous or not, have the potential of being co-oriented as in a bivalent. The positions of the linking chiasmata relative to the centromeres and to each other govern their actual orientation.

The suggestions put forward present a simple answer to the numerous 'exceptions' that present themselves to any of the previous explanations for 'selective' segregation.

Summary

1. A population of *Allium triquetrum* heterozygous for an interchange has been discovered. The meiotic complex of a chain of four is oriented in an alternate fashion in about 75% of cells.

2. The suggestion is put forward that adjacent centromeres of a complex, if linked by at least one chiasma, behave as in a bivalent. Because there are four centromeres in a complex of four chromosomes, the basic orientation frequency is 50% alternate, 50% adjacent. Adjacent orientations are not basically the result of rigidity caused by chiasmata, and an interaction between homologous centromeres or other chromosome parts is not involved in the orientation mechanism.

3. Chiasmata may impose rigidity on the complex and so affect orientation frequencies.

4. Two (necessarily homologous) centromeres linked by an interstitial chiasma will be co-oriented as in a bivalent and adjacent 2 orientations are eliminated.

5. In chain forming configurations without interstitial chiasmata, one type of adjacent orientation is eliminated, depending on whether the centromeres are situated on one or the other pachytene axis; the configuration eliminated is replaced by alternate orientations, the basic frequency of which is thus increased from 50% to 75%. Interstitial chiasmata in a chain configuration alter the orientation frequencies.

6. Ring complexes may orientate in an alternate fashion in 75% of cells.

7. Relative lenghts of pachytene axes have no effect on orientation frequencies.

8. Multiple interchange heterozygotes such as *Oenothera* have probably evolved a special feature that, together with the typical orientation mechanism found in most organisms, enables a very high frequency of alternate segregation to be attained.

9. Apparent genotypic control of segregation may be the result of genotypic control of chiasma position.

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