CHROMOSOMAL BEHAVIOUR DURING MEIOSIS IN MOSSES

by

FREDERICK JOHN DILL

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Department of Biology and Botany

The University of British Columbia,
Vancouver 8, Canada

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ABSTRACT

The primary purpose of the present work was to examine meiosis in mosses, concentrating on the detail of prophase I, a meiotic stage which has been described in detail only in Pleurozium schreberi. Secondarily, an investigation was undertaken to study the effect of heat stress on meiosis.

Hypnum circinale Hook. and Brachythecium frigidum (C.M.) Besch. were used to study meiosis. The procedure of preparing spore mother cell squashes was similar to that used by Steere et al. (1954), with modifications in handling the plants prior to fixation to ensure against production of heat induced anomalies.

Except for late prophase I, meiosis in both species conformed to that found in P. schreberi. In H. circinale and B. frigidum diplotene was followed by chromosome elongation and resulted in a diffuse stage. This stage is morphologically analogous to the dictyotene stage of the growing oocytes of many animals, and appears to have been described, in plants, only in Balsamina hortensis. It is probable that the stage occurs in many moss families; at present its functional significance is unknown.

In the heat stress experiments, plants of H. circinale were either maintained under laboratory temperatures while being studied or they were treated with a heat shock over a period of four or six hours with the maximum temperature in the general ranges of 25°C., 31°C., and 36°C. The maximum temperature was maintained for 4 hours in the 25°C. experiment and

1/2 hour in the remaining experiments. The heating and cooling gradients were almost equivalent (1°C./5 min.), and the starting and finishing temperature was 14°C. The temperature of the natural environment during the study ranged between 7-11°C.

Severe anomalies, including chromosome clumping and multiple association, precocious disjunction, chromosome contraction, spindle breakdown and inhibition, premature meiotic induction and meiotic abortion were observed to some extent in spore mother cells from all treatments except the ones from the 25°C. heat shock experiment.

Room temperature accelerates prophase I stages of H. circinale . The time available for these stages appears to be too brief for synthesis of necessary products leading to active stages, thus causing severe abnormalities which result in abortion of meiosis.

On the basis of these results, it is apparent that cytologists working with moss material should take care in handling the plants prior to fixation to ensure against heat induced meiotic anomalies.

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INTRODUCTION

The volume of literature pertaining to chromosomal and nuclear behaviour in mosses is small when compared to that concerning higher plants and animals. It is significant that many of the earliest works in the field of cytology and cytogenetics in mosses made valuable contributions to the study of plant cytology and cytogenetics in general. Outstanding among these contributions was the work of Allen (1917, 1919) who initiated the study of sex chromosomes in plants and that of the Marchals (1907 et seq., cited by Allen, 1935) who first produced artificial polyploids in mosses and supplemented their work with cytological evidence. Bryophyte material was used by Heitz (1928, cited by Steere et al., 1954) in his studies on nuclear behaviour in which he introduced the term "heterochromatin", and furthered the study of sex chromosomes in plants. Several other authors have also made notable cytological contributions utilizing bryophytes. This early work, which is mainly genetical, is adequately reviewed by Allen (1935, 1945) and more recently by Lewis (1960).

In the last twenty years, cytological study of wild populations of mosses has developed substantially and most publications have been concerned with establishing chromosome numbers and studying chromosomal behaviour. Mehra and Khanna (1961) have reviewed this period quite critically and have indicated that although the trend has been shifted to the study of chromosome number and in some cases behaviour, there are rare applications of these findings to clarify taxonomic problems.

It is noteworthy that although most of the chromosome

counts reported for mosses have been obtained from the study of meiotic metaphases, only rarely has the detail of the meiotic process been studied. One of the earliest studies of meiosis in a moss was that of Wilson (1909), who presented a fairly detailed account of meiosis in Mnium hornum. He was limited, however, by poor techniques and apparently did not accurately observe some of the early prophase stages. In 1932, Scheuber attempted to describe meiosis in Timmia cucullata but failed to observe many of the stages in prophase I. Not until the work of Vaarama (1954a,1954 b) were the details of meiosis in mosses (especially those of prophase I) well documented and in his description of meiosis in Pleurozium schreberi, Vaarama (1954b) has presented the most complete details of meiosis in mosses to date.

The purpose of the present study was primarily to provide a detailed account (especially of prophase I) of meiosis in one or more mosses. The need for such a study is obvious because many workers have conducted cytotaxonomical studies with a limited knowledge of meiosis in the plants they were studying. A secondary purpose grew from observations made during the preliminary investigation to this study and also as a consequence of several observations made during a previous study (Dill, unpublished data). These observations had suggested that bringing mosses into the laboratory, even for a short period, may cause anomalous chromosomal behaviour. There was also some indirect evidence from the literature which supported these observations. Since several authors (Steere et al., 1954; Bryan, 1956 et seq.) had suggested bringing mosses into the laboratory to speed maturation of the capsules, it was felt that an investigation of the effects of temperature stress on mosses was warranted. This matter is discussed in Chapter 2.

CHAPTER 1

Meiosis in *Hypnum circinale* Hook. and *Brachythecium frigidum* (C.M.) Besch.

In the pioneering studies concerning meiosis in mosses the workers were severely hampered by a limitation in techniques available. All of the work was performed by the embedding and sectioning technique; the fixation was poor and many artifacts were introduced. Heitz (1928, cited by Vaarama, 1949) developed the squash technique for mosses, a technique which has been used almost exclusively since that time.

Early in the present investigation, attempts were made to study meiosis in several moss taxa. It became apparent that not all would be suitable, as in many the prophase I chromosomes did not stain well or they were seldom observed. This was especially true of members of the Grimmiaceae and some other acrocarpous families. Vaarama (1949) found this to be true when he attempted to study meiosis in several members of the Grimmiaceae. It was soon discovered that the pleurocarpous mosses, especially the Hypnaceae (*sensu-lato*), had prophase I stages that could be easily stained. The chromosome number was also an important factor to be considered, as obviously a low number would permit much more accurate observations of the individual chromosomes during prophase I. For these reasons, *Hypnum circinale* Hook. and *Brachythecium frigidum* (C.M.) Besch. were chosen. Neither species had been cytologically studied.

METHODS

The Handling and Preparation of Material for Cytological Study:

Preparing slides of spore mother cells (SMC) for meiotic study was essentially the technique of Steere et al. (1954). However, careful consideration was given to the handling of plants prior to fixation to prevent heat induced abnormalities (see Chapter 2).

Plants were collected in plastic bags and were brought to the laboratory as quickly as possible, in an attempt to subject the plants to as little temperature change as possible. They were then put in flats in a shaded area of the roof on the biological science building. An attempt was made to simulate natural temperature and humidity conditions. Adequate water was supplied. As most of the plants for this study were collected during the months of October and November of 1963, the outside temperature was between 7-11°C., and the humidity was high as there was some precipitation almost every day.

When plants were to be studied, a portion of the collection was brought into the laboratory and immediately hung outside the window in a shaded basket. A small portion of this material was used when slides were to be made and any material brought in from the basket was discarded if unused after 10-15 minutes.

Aceto-orcein was the only stain used for the present study and was prepared according to the method described by Darlington and LaCour (1960). This is the only stain that has proven satisfactory for chromosome work with mosses.[1] Pre-fixation of SMC

[1] cf. footnote page 13

was usually done with 3:1 alcohol-acetic acid.

Squash preparations are made as follows: plants bearing bright green mature-sized capsules are selected from the collection. A sporophyte is severed from the gametophyte and transferred to a clean slide on the stage of a dissecting microscope. Holding the base of the capsule with forceps, both the calyptra and operculum are removed using a dissecting needle which is then placed at the base of the capsule and rolled toward the mouth of the capsule using slight downward pressure. In capsules possessing a differentiated archesporium this will cause the columella to be extruded through the mouth of the capsule and the SMC mass will follow. Both the columella and empty capsule are removed from the slide and the SMC are spread slightly to ensure even and rapid penetration of the fixative and stain. Immediately following this spreading procedure a drop of fixative is applied to the SMC. When the fixative has almost evaporated, the drop of stain is applied and the slide is set aside. Before the stain begins to dry (about 5 minutes after application) a clean coverslip is gently applied to the preparation. After about 5 minutes, pressure is applied to the coverslip, thus flattening the SMC and spreading the chromosomes. The coverslip is then sealed with a semi-permanent gum-mastic-paraffin (1:1) sealing medium. Stain intensity is generally satisfactory at this time.

Although heating the slide intensifies and speeds staining, there is evidence that heating the slide causes clumping of the chromosomes (Al-Aish and Anderson, 1961). The present author has found this to be true with some species, thus omitted this procedure to keep the chromosomes as close to their natural state as possible.

Permanent slides of some of the material of each population

were prepared according to the method of Darlington and LaCour (1960).

All bright field observations were made using an Olympus binocular compound microscope with N.A. = 1.40 Ach. Condenser, a 125 mm. oil immersion objective and X10 oculars. Photographs and phase contrast work were done on a Zeiss Photomicroscope.

Material:

Material of Hypnum circinale Hook. was collected from three populations: Alice Lake, B. C., Dill #1363; University of British Columbia Endowment Lands, Vancouver, B.C., Dill #2263; and Mt. Seymour, B.C. (2000 ft.), Dill #2463. Brachythecium frigidum (C.M.) Besch. was collected from one population: Mt. Seymour, B.C. (2000 ft.), Dill #2563.[2] Voucher specimens have been deposited in the University of British Columbia Herbarium.

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[2] This specimen was determined by Harold Robinson, Smithsonian Institution, Washington, D.C.

OBSERVATIONS

Meiosis in Hypnum circinale:

Observations of meiosis in each population of H. circinale were made at the same time the respective populations were used as the experimental material in the heat stress experiments (Chapter 2). It was found, that, with the exception of one sporangium (a member of population #2463, sporangium #6) all three populations were cytologically and morphologically indistinguishable. They differed only in the maturation time of the capsules. In general, members of population #2463 matured two to three weeks later than those of #1363 and #2262. This difference was attributed to the difference in the altitudes where the three populations occurred in nature. Number 2463 was collected at 2000 ft. while #1363 and #2263 were collected at sea level. For this reason the description of the meiotic process of these populations is combined and is considered to be representative for the species.

The archesporial layer of H. circinale has differentiated when the capsules reach mature size, but before there is any indication of browning of the annulus. When the spore mother cells are first differentiated they are quadrate and stain weakly with aceto-orcein. No internal structure is discernable. Meiosis appears to be initiated in all the SMC of a capsule simultaneously and, in most sporangia, proceeds in an almost synchronous manner until the initiation of diakinesis. Thus all SMC in each prepared slide appear to be at the same stage.

On initiation of meiosis SMC become spherical and are characterized by an acentrically placed, weakly staining chromatin mass (PL. 1, fig. 1-3). At this stage, which is probably

leptotene, the chromatin is a mass of very entangled threads. The threads are definitely single and in rare cases chromonemata can be discerned (PL. 1, fig. 3). It is questionable whether the entire nucleus or only the chromatin is acentrically placed, for in some cells threads of chromatin appear to traverse up to 2/3 the width of the cell (PL. 1, fig. 1). While the chromatin is acentrically placed synapsis occurs; often SMC can be observed in which the threads are double but cells are otherwise identical to those of the leptotene stage. Evidence for synapsis was obtained by phase contrast observation. One SMC was noted in which two single threads paired for a short portion of their length, while the unpaired sections were widely separated (PL. 1, fig. 2). Other cells of the same slide had mostly single-threaded chromatin.

When synapsis is complete the chromosomes become shorter and thicker. At the same time, the chromatin mass loosens and migrates to the center of the cell (PL. 1, fig. 4-9). Chromosomes during this time assume a typical pachytene morphology, but no nucleoli are evident. As pachytene proceeds, the chromosomes shorten and thicken, and the diplotene phase appears as the repulsion of homologs becomes evident (PL. 1, fig. 10,11; PL2, fig. 1,2). In rare SMC, all six bivalents are clearly discernible at diplotene (PL. 2, fig. 2) but usually only one or two are separated from the chromosomal mass (PL. 1, fig. 11). The smallest bivalent, which is heterochromatic, often becomes highly condensed during pachytene and diplotene (PL. 1, fig. 10; PL. 2, fig. 2). In addition, there are certain segments of other chromosomes which are heterochromatic and stain deeply during some period of diplotene, (PL. 1, fig. 11). However, it was not possible to identify the chromosomes

by these regions as they were seldom well enough separated from the mass to make accurate observations then. Occasionally nucleoli could be observed during diplotene (PL. 2, fig. 2), but generally they stained either too poorly to be seen or were obscured in the chromosomal mass. On the basis of the diplotene stage as shown in PL. 2, fig. 2, it is apparent that the nucleolar organizing chromosome is one of the larger bivalents, (probably the second largest). It is possible that the small heterochromatic bivalent is also associated with the nucleolus.

The degree of coiling of the chromosomes during diplotene is not as great as that found in most plants. The bivalent separated from the mass shown in PL. 1, fig. 11, represents the highest degree of coiling observed during diplotene. The bivalent shown in PL. 2, fig. 1 (highly squashed cell) is probably the large bivalent which has such a distinctive morphology at metaphase I.

Diplotene was the most commonly observed prophase stage during the time the populations were studied. It was difficult to estimate the period of time the diplotene persists under natural conditions, but from the large number of sporangia found in this stage during the period of study (about 2 weeks) it appears that this stage may persist as long as a week.

Near the end of diplotene the bivalents become less well defined (PL. 2, fig. 3), and chromosomes gradually lengthen and lose their discreteness, at which time the nuclear contents assume an interphase-like appearance (PL. 2, fig. 4,5). Under phase contrast, fine chromatin threads and what appear to be chiasmata were visible. During this phase some short sections of bivalent members were seen (PL. 2, fig. 4), suggesting that

the diplotene structure of the chromosomes had been maintained and that the chromosomes were in an uncoiled state. The cells at this stage usually contain one large deeply staining chromatin body. As in the earlier stages, no nuclear membrane was distinguishable during this phase, but since the chromatin was spread almost to the periphery of the cell the nucleus was probably only slightly smaller than the SMC itself.

Up to and including this chromosome elongation stage, the divisions of the SMC of one capsule are almost synchronous. Although most sporangia had some transition stages (eg. pachytene-diplotene or diplotene-elongation stage) the majority of the SMC appeared in the same stage. From this stage on, however, synchrony is lost. Each cell may remain in the elongation stage an estimated two days, then each may proceed to the next stage in a random order by a rapid condensation of the bivalents. In sporangia with some SMC possessing condensed bivalents (ie. diakinesis), all other cells were either in later stages of meiosis or were in the elongation stage. Only in extremely rare cases did earlier stages of prophase occur after condensation had begun. This observation clearly indicates that the elongation stage follows diplotene and therefore cannot be interpreted as a prediplotene stage.

Condensation or coiling of the bivalents could not be followed accurately since this appears to proceed very rapidly. In some cells, the chromatin was diffuse and in one large mass (PL. 2, fig. 6). This stage occurred between the elongation stage and diakinesis, but because of its irregular and infrequent occurrence it may represent an abnormal or poorly fixed elongation

stage. Without further study it cannot be accepted as a usual occurrence in the meiotic sequence of Hypnum circinale.

Diakinesis is characterized by a general clumping of the highly condensed bivalents (PL. 2, fig. 7,8) in the center of the cell. In conjunction with spindle formation the clumped bivalents separate and assume a metaphase I configuration. Metaphase I of Hypnum circinale is characterized by six bivalents including four which are medium in size, one which is usually distinguishable as smaller than the rest and one which is very large and morphologically distinct. In the majority of cells this large bivalent was located at one side of the metaphase I plate (PL. 2, fig. 9). Disjunction of the bivalents usually occurred quite synchronously (PL. 2, fig. 10) with the large bivalent often slightly preceding the others to the poles. On completion of the first division the chromosomes partially uncoil and assume a configuration similar to prophase (PL. 2, fig. 11) which usually contains one large heterochromatic region. Although cell wall formation is not complete until after telophase II, a constriction of the cytoplasm frequently appears after telophase I (PL. 2, fig. 11). The chromosomes at metaphase II are again condensed and form a clumped configuration. Only rarely can a chromosome count be made at this stage. At anaphase II the chromosomes appear to be sticky and temporary bridges often formed (PL. 2, fig. 12). However, these apparently do not cause abnormal separation of the chromatids as only seldom do fragments or extra nuclear chromatin appear in telophase II and the tetrad stage. During telophase II, wall formation begins (PL. 3, fig. 1). The cytoplasm appears at first to be constricted on the

plane of the first division and then on the plane of the second division. The meiotic sequence is concluded by production of a tetrad of spores (PL. 3, fig. 2).

Meiosis in Sporangium #6, Population #2463:

In only one sporangium of the three populations of H. circinale examined did meiosis differ distinctly from what was considered normal. In this sporangium SMC were present in all stages later than diplotene of prophase I with the majority in metaphase I and diakinesis. In about 40% of those in metaphase I and diakinesis the smallest bivalent had disjoined precociously. Usually only a single member of the pair was separated from the main chromosomal mass (PL. 3, fig. 3-6). However, there were some metaphases in which both the univalents were evident and others in which the bivalent had not divided (PL. 3, fig. 7). The remainder of the bivalents appeared to act as they did in 'normal' sporangia. Later stages of division in this sporangium had a noticeably high frequency of abnormalities. About 15% of the SMC in first and second telophase contained a chromatin bridge and/or laggards (PL. 3, fig. 8,9). These anomalies occurred with a frequency of 2-3% in the cells of the normal sporangia. Often more than one bivalent participated in bridge formation and usually the small bivalent was not involved (PL. 3, fig. 8). Furthermore, in many of those with only one bridge or laggard the amount of participating chromatin was greater than the size of the small bivalent. On the other hand, there were some cases (PL. 3, fig. 9) in which the small bivalent was completely omitted from the spindle apparatus. Evidently something was causing an upset in meiosis in this plant but this could not be demonstrated on morphological grounds. A structural aberration

might possibly have been detected at diplotene or pachytene but unfortunately neither of these stages was present in the slide. Heat shock might also have caused such anomalies but this sporangia was treated in the same method as the others, making this possibility slight. The consequences of heat shock are discussed in detail later.

Meiosis in Brachythecium frigidum:

Observations of meiosis of B. frigidum were made for only a single population. The main purpose of this study was to determine if the elongation stage observed after diplotene in H. circinale was unique to that species. Thus, on the basis of the results, a study of one population was sufficient.

Essentially the pattern of meiosis in B. frigidum was the same as that of H. circinale. However there were certain facets of the sequence which, although seemingly different in the two species, were probably more clearly demonstratable in B. frigidum.

The first of these is the presence of the nucleolus during early prophase. Early prophase of B. frigidum is characterized by the acentric location of a nucleus containing single-stranded chromosomes. Phase contrast observations of this stage revealed the presence of a large nucleolus (PL. 3, fig. 10, 11). This nucleolus[3] was not evident when the slides were initially prepared

[3] The author has since observed large nucleoli in leptotene through to early diplotene in a number of species viz. Rhacomitrium microcarpon, Claopodium crispifolium, Isothecium stoloniferum, Plagiothecium undulatum as well as B. frigidum using proprionic-carmine with iron stain. The stain was made according to the method of Darlington and La Cour (1960) for aceto-carmine. However, proprionic acid replaced the acetic acid and the iron acetate was not added. Iron was added by suspending forceps

but became evident only after standing a few months.[3] Cells in migrating pachytene and diplotene phases (PL. 3, fig. 12; PL. 4, fig, 1,2) appeared to have no visible nucleolus using phase contrast or bright field illumination. During pachytene and diplotene of B. frigidum all the bivalents have the same stain intensity except for a heteropycnotic section of one of the chromosomes (PL. 4, fig. 2). This chromosome could not be identified specifically. Following diplotene, the chromosomes uncoil as in H. circinale and the chromatin mass assumes an interphase appearance (PL. 4, fig. 3,4). In B. frigidum the threads appear more coarse than those of H. circinale during the same stage. One remarkable difference noted between the two species was that the nuclear membrane in B. frigidum was clearly defined in many of the SMC (PL. 4, fig. 3). Condensation of the chromosomes follows the elongation phase and the bivalents assume a clumped configuration (PL. 4, fig. 5). This resembles the diakinesis in H.circinale, but in B. frigidum the clumped configuration is followed by a more normal diakinesis in which the highly condensed bivalents are not clumped together and are distributed quite evenly in the SMC (PL. 4, fig. 6). Six bivalents of similar size are present at metaphase I. One of

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[3](continued from page 13)

in a drop of the stain just before the stain was used.

It was found that fresh material as well as material fixed in either 1:3 acetic alcohol or Newcomer's solution (Newcomer, 1953) stained well. Glacial acetic acid, followed by a washing in 1:3 acetic alcohol fix could be employed after normal fixation to remove oil droplets. This treatment did not impair the stainability of the nucleoli or chromatin.

these bivalents is rod-shaped and is usually located at one side of the metaphase plate (PL. 4, fig. 7,8). This bivalent is usually very long at metaphase I and often dissociates precociously. Its behaviour is analogous to that of the large bivalent of H. circinale. Anaphase I (PL. 4, fig. 9) was remarkably regular in this species and the remainder of the meiotic sequence was similar to that in H. circinale. Abnormalities at second division were extremely rare and tetrad formation appeared normal.

DISCUSSION

The study of the meiotic prophase of mosses has been severely neglected. For the most part, students of moss cytology have been concerned only with acquiring chromosome counts and determining, rather superficially, the behaviour and morphology of the bivalents at metaphase I. The chromosome numbers of at least 700 of the 20,000 described species of mosses have now been established (Mehra and Khanna, 1961, estimated 650 species). Over 90% of the established numbers have been presented since 1948. Although the accumulated work has contributed limited knowledge to the taxonomy and evolution of mosses, the contribution to basic cytogenetics and genetics has been small.

The most outstanding of the work that has been published since 1948 is that of Vaarama. In an attempt to study the meiotic prophase of Hedwigia ciliata Br. & Sch. and some members of the Grimmiaceae, Vaarama (1949, 1954a) found that poor stainability of the SMC at prophase I rendered detailed examination impossible. He stated (1949) that prophase stages later than pachytene had been rarely observed, which suggested that these stages were of short duration. However, during his study of H. ciliata, the same author described the behaviour of a 'special bivalent' during the early prophase. This bivalent appeared as a spherical opaque nucleolus-like vesicle bounded by four heteropycnotic bodies. Although the rest of the chromatin in prophase I was almost impossible to observe in detail, the vesicle was quite conspicuous and persisted until diakinesis, whereupon it became negatively heteropycnotic and underwent unusual segregation behaviour. At the time, Vaarama stated that the relationship

between the nucleolus-like vesicle and the ordinary nucleolus was not known. It is interesting to note that during a pilot study using propionic-carmine as a stain for nucleoli in the prophase I of several mosses (cf. footnote page 13) the present author observed a similar special bivalent in prophase I of Plagiothecium undulatum (Hedw.) Bry. Eur. In addition to the nucleolus-like vesicle there was a normal nucleolus present which appeared entirely separate from the special bivalent. Also, the vesicle of the special bivalent stained in a manner similar to that of the nucleolus, indicating that the vesicle is possibly nucleolar in structure.

The first and only detailed study of the general behaviour of the chromosomes at meiotic prophase in mosses was reported by Vaarama (1954b). He found that the chromosomes of Pleurozium schreberi (Brid.) Mitt. stained well during prophase I and that, because they were few in number (5), observations of individual chromosomes during the early stages were possible. He presented the only published figure of true diplotene in mosses known to the present author. The early prophase of Pleurozium schreberi compares quite closely with the early prophase of H. circinale and B. frigidum. However, Vaarama did not describe or depict the elongation stage that followed diplotene in the two species presented here and he indicated that a clumped diakinesis directly followed diplotene. Further mention of this unusual stage will be made in the discussion of each prophase stage.

Leptotene and Zygotene:

The acentrically localized nucleus at the earliest prophase I

stage is of wide occurrence (Vaarama, 1954b). The present author has observed the phenomenon in all of the approximately thirty species of mosses examined during the past three years (unpublished data). The chromosomes can be observed either single stranded, partly double stranded (as in H. circinale, PL. 1, fig. 2), or completely double stranded, indicating that synapsis takes place during this period of chromatin polarization. Since the nuclear membrane has not been observed at this stage, it is difficult to say whether the chromatin is bound closely on all sides by it or whether the nucleus is actually quite large (ie. extending to or past the middle of the SMC) with the chromatin localized at one side. As noted earlier, on rare occasions in H. circinale long, presumably single, threads of chromatin extended well past the center of the cell while the remainder of the chromatin was acentrically localized. Furthermore, the nucleolus of some of the species studied with propionic-carmine (cf. footnote page 13) was often quite separate from the chromatin mass and was located closer to the center of the cell than the mass. In addition, when the chromosomes migrate to the center of the cell in early pachytene, initiation of this movement is by one or a few of the chromosomes which become loosened from the mass. Most of the chromatin remains localized for some time after this migration begins, indicating that the nucleus at this time must extend past the middle of the cell. Further studies, possibly using fluorescent microscopy with unfixed material may help to elucidate this matter.

Pachytene-Diplotene:

The migration of the paired chromosomes to the center of

the SMC, as observed in both H. circinale and B. frigidum is similar to that described by Vaarama (1954b) in Pleurozium schreberi.

The transition between pachytene and diplotene stage is not precise. In fact, Vaarama preferred to lump the two stages in many cases and referred to pachytene-diplotene stages. Soon after the chromosomes have migrated, sections of the bivalents begin to repel each other but only seldom can this be followed in one chromosome as usually some part of every chromosome is entangled with the others. However, when the bivalents are short enough that one or two are easily separated from the rest, repulsion is completed along the whole chromosome except at the contact points. These would usually be designated as chiasmata. Vaarama (1954b) has presented evidence in his study on P. schreberi that the contact points observed during diplotene are not true chiasmata, but merely areas of sticky heterochromatin. No special study of the method of chiasma formation was made in this investigation so the question must remain open for the present.

The Elongation Stage:

The most remarkable aspect of the meiotic sequence observed in H. circinale and B. frigidum was the occurrence of the chromosome elongation stage which followed diplotene. Vaarama (1954b) did not describe or depict this stage for Pleurozium schreberi. However it remains quite possible that this stage is present in Pleurozium schreberi as well as in many other mosses. The present author has, since the initial studies on H. circinale and B. frigidum , examined meiosis in Claopodium

crispifolium (Hook.) R. & C. and found that the elongation stage occurred in that species as well. Furthermore, an examination of permanent slides of twelve moss taxa studied at an earlier date (Dill, unpublished data), suggests that all have this elongation stage. Unfortunately this cannot be established conclusively until the complete sequence of meiosis is examined for each of the species. The fact that H. circinale, B. frigidum, and C. crispifolium represent three moss families indicates also that the stage is probably widespread in the mosses.

A post-diplotene elongation stage has been reported on rare occasions in the plant kingdom. Violante (1929) described a stage, termed streptsitene, in the dicot Balsamina hortensis (Impatiens balsamina) similar to that observed by the present author in Hypnum and Brachythecium. The elongation of the chromosomes was also accompanied by nuclear growth. He indicated that Lewitsky (1927) and Modilevsky (1918) had observed similar stages in other plants and that Lewitsky had apparently considered the stage artifact. Violante also mentioned that Szakien (1927) had observed this stage in the fern Osmunda regalis, but examination of Szakien's paper indicates that this is not the case. There is also a post-synaptic elongation stage in the developing asci of many ascomycetes, but this phase occurs before diplotene and is a typical pachytene stage (Carr and Olive, 1958). Wilson (1952) has reported that the mature resistant sporangia of Allomyces is in a state of suspended prophase I of meiosis, but he was unable to determine which stage of prophase I. The photographs do not resemble those of the elongation stage reported here.

According to Pratt and Long (1917), von Winiwarter (1900) described an elongation stage in prophase I which followed diplotene in the maturing oocytes of rabbit and man. Von Winiwarter gave the term 'Noyoux dictyé' (sic) to this phase. More recently Ohno, Makino, Kaplan and Kinosita (1961), and Ohno, Klinger and Atkin (1962), have reaffirmed von Winiwarter's original observations on oogenesis in man and have indicated that the dictyé stage (termed dictyotene or dictyate stage in recent papers)[4] is a 'resting' stage in which the chromosomes are highly extended and maintain their diplotene structure. The oocytes remain in this phase until ovulation when the meiotic sequence is resumed. During the intervening period the cells grow and accumulate nutrient utilized by the future embryo.

In many amphibians and some insects, the lampbrush stage is homologous with the dictyotene stage (Seshachar and Bagga, 1963). Furthermore, according to Callan (1963) probably all animals have a stage which corresponds to the dictyotene or lampbrush stage in their developing oocytes. He also indicates that the lateral projections of the diplotene chromosome of many primary spermatocytes may also represent a lampbrush-like behaviour, but that the chromosomes are not greatly extended in length as they are in the oocytes. Although the dictyotene chromosomes vary in gross morphology among the different animal groups it is apparent that the chromosomes are basically diplotene in structure (Ris, 1945).

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[4] Wilson (1925) refers to this stage as the dictyotic stage.

Aside from the few instances mentioned in the fungi, which probably do not represent the dictyotene stage, the only satisfactory evidence for the occurrence of such a stage in plants to my knowledge has been reported in Balsamina hortensis (Impatiens Balsamina). In this case the sporocyte nuclei apparently increased in size during this phase, in a manner similar to the growth found in oocytes. However, the growth was not so marked. At the present time, it is impossible to say if the relationship between the animal dictyotene and that found in mosses and in Balsamina is superficial or is based on similar structure. The SMC of mosses do not enlarge during dictyotene and the duration of the stage is not long in relation to the other meiotic events, as it is in oocytes. The metabolism of the SMC during meiosis is virtually unknown and it is quite possible that a high rate of metabolic activity may take place without appreciable growth in size of the cells.

It appears probable that the dictyotene stage has some functional significance in the meiotic sequence of mosses, but until details of the metabolic events taking place during this stage are discovered little can be said about this matter.

Diakinesis and the Remainder of Meiosis:

The clumped diakinesis observed in both species studied here is commonly found in mosses. Vaarama (1954b) indicated that a similar situation occurred in P. schreberi and many other mosses. Usually bivalents at diakinesis in higher plants are evenly distributed about the nucleus or are near the nuclear membrane. Steere, et al. (1954) have reported making many counts from

diakinesis stages in which the bivalents were widespread. Possibly the prometaphase stage observed in B. frigidum corresponds to this same stage. In this case, H. circinale and Pleurozium schreberi would not have a classical type of diakinesis and the clumped stage cannot be referred to as diakinesis. However, B. frigidum and many other species have the clumped stage following dictyotene and before prometaphase; it may be another distinctive feature of the meiotic sequence in mosses.

Metaphase I conforms well with that of the higher plants in the two species studied here. The large rod-shaped bivalent which is usually located at the periphery of the metaphase I plate has been described in many moss species (Mehra and Khanna, 1961). They are usually referred to as heterochromatic, but no evidence for this was displayed during prophase I in either of the two species discussed here. Studies on somatic tissue would help to elucidate this matter. Possibly these bivalents correspond to the large 'H' chromosomes of mitotic cells described by Yano (1957a,b,c). The small heterochromatic bivalent found in Hypnum circinale perhaps falls into the class of "morphologically indistinguishable minute bivalents" of Mehra and Khanna (1961), a category that these authors say corresponds to Yano's (1957 a,b,c) heterochromatic 'h' chromosomes of mitosis. Its behaviour, (ie. precocious disjunction) in sporangium #6 of the population # 2464 was reminiscent of the minute bivalent's behaviour.

The remainder of the meiotic sequence in both the species studied here is unremarkable. Both have normal second divisions and normal tetrad formation, indicating the presence of a well-balanced genetic system. The time and method of wall formation

during meiosis in mosses has not been studied closely. Until this is done one can only say that it appears that the wall begins to form toward the end of the first division. This is indicated by a light staining and apparently constricted area of cytoplasm which traverses the cells following telophase I. Quite likely this mechanism varies in different mosses as it does in different higher plants.

The chromosome counts (n=6) for H. circinale and B. frigidum have not been previously reported. Both Hypnum and Brachythecium have a great variety of chromosome numbers reported, a variation that lies not only between species but also within the same species. Much of this variation may be attributable to inaccurate observations and until the counts are clearly established discussions of their taxonomic significance are relatively menaingless.

In conclusion, it could be said that, with the exception of the dictyotene stage, the meiotic sequence in these mosses conforms closely to that found in most higher plants. The dictyotene stage, although an unusual feature of plant meiosis, may be of common occurrence in many moss taxa.

CHAPTER 2

The Effect of Heat Stress on Meiosis in *Hypnum circinale* Hook.

It is well-known that meiotic stages in most mosses are stained most effectively if the capsules are immersed in acetic alcohol for a very brief period. With most mosses a 24 hour fixation period will reduce the chromosome stainability to such an extent that detailed observations are impaired. For this reason, many authors (Steere *et al.*, 1954; Bryan, 1956 *et seq.*; Anderson and Crum , 1959) have brought the plants directly to the laboratory, and have made the preparations in the laboratory from living material. It has been suggested that plants which are not mature enough for meiosis at the time of collection can be grown in the laboratory until they mature. Usually the plants are kept in covered plastic dishes. The above mentioned authors also state that maturing plants can be refrigerated in order to slow down meiosis. Although there are presumably no abnormalities induced by use of either of these techniques, no controlled experiments have been conducted to substantiate this claim.

These same authors note one particular striking feature of meiosis in wild populations of mosses: many chromosomal aberrations occur and often some bivalents tend to disjoin precociously at metaphase I. To quote Steere *et al.*, (1954):

> "In the present investigation, it is observed that the sporocytes of mosses brought in from the field early in the day were often in late prophase or tetrad stages. Exposure to light and warmth, however, induced the young sporocytes to resume meiosis. In view of the many chromosomal aberrations noted at the first and second meiotic divisions, a careful

> study of prophase behaviour is urgently needed, especially of pachytene stages."

It is puzzling that the authors did not attempt to connect the two facts and suggest that the abnormalities may have arisen as a result of the treatment.

Bryan (1956b) suggested that clumping of chromatin in Phascum SMC seemed to be related to the low water content of the cells. The material she studied that particularly showed this phenomenon had been mailed to her and the capsules were flaccid on arrival. She mentioned that if the plants were watered and stored at reduced (unspecified) temperatures, they had normal meiosis. She (1956a) observed similar clumping and unusual metaphases in Bruchia but no mention was made concerning the treatment given the plants.

Khanna (1960), in a chromosomal study of some Himalayan mosses, has indicated that all his cytological studies were carried out in the field because stored material did not yield satisfactory results, but he did not say why they were unsatisfactory. On the other hand, he indicated that abnormal weather as well as the fixative used could cause clumping of meiotic chromosomes. This was especially apparent in Barbula constricta Mitt. which had chromosomal clumping and other abnormalities in hot dry weather, while it had normal meiosis in cool wet weather. This appears to be the only observation concerning bryophytes in which meiotic abnormalities were induced by environmental conditions.

In an earlier study (Dill, unpublished data), there was a suggestion that bringing mosses indoors to ripen might cause abnormal meiosis. Mnium venustum, Mitt. , when examined directly from the field, had 12 closely packed bivalents at metaphase I.

However, material that had been kept indoors in a humid container for 2-3 days had metaphases in which the chromosomes were loosely spread throughout the cell. In addition, many of the bivalents disjoined precociously. A collection of Heterocladium heteropteroides Best was also brought into the laboratory before the capsules had matured. After 2 weeks the SMC underwent severely abnormal meiosis (PL. 7, fig.3). No good tetrads were observed. Since the capsules were never flaccid it seemed unlikely that humidity was related to this anomalous condition.

Studies using higher plants and various animals clearly demonstrate that heat stress can severely affect meiosis. The bulk of this work, both cytological and genetical, has dealt with chiasma frequency at various temperatures (Yanney Wilson, 1959), but some has dealt with temperature effects on chromosome coiling, spindle formation, centromere behaviour, and nucleoli behaviour (Swanson, 1942, 1943; Dorwick, 1957; Jain, 1957 et seq.; and Henderson, 1962).

In view of these facts, an experiment seemed justified to ascertain whether or not room temperatures were sufficiently high to cause such anomalies. Three major questions arose concerning heat and its effect on meiosis in mosses. First, if the room temperature caused meiotic abnormalities, how sensitive are moss SMC to slight heat stress? Second, if the sensitivity is great, might not natural heat stress in the environment be a mechanism inducing mutations or structural/numerical alterations of the chromosomes? Third, what is the mode of response of the SMC to heat stress?

Several experiments were designed to answer some or all of these questions. Although complete answers were not supplied for all the questions, certain trends and some definite conclusions were obtained.

METHODS

Methods of Treating the Plants:

In the following experiments, several different types of heat treatment were applied. In some, special equipment was needed in order to attain a reproducible experimental procedure.

The first experiments involved treating the plants to short term heat stress (ie. a period of about 4 hours). A longer period did not seem realistic since a longer period of abnormal heat stress would not be expected under natural conditions. Furthermore, pilot experiments showed that the shorter period was sufficient to cause severe anomalies in plants subjected to the higher ranges of temperature used (30-37°C.). To ensure replication it was necessary to control the rate of temperature change when heating as well as when cooling the plants, thus a chamber possessing a heating plus a cooling unit, both of which would give approximately similar rates of temperature change, was required.

These requirements were partially met by the use of the Warburg respirometer. The apparatus was used as a water bath and was fitted with an aluminium cooling coil which was cooled with tap water. The top of the bath was insulated with blocks of styrifoam.

The plants were put into covered 8" - 5" plastic cake dishes which were then partially submerged (to within 1/2 " from the upper rim) with weights. A hole was drilled in the cover of one of the dishes to insert a thermometer. The humidity of the dishes was not controlled but the plants were well watered and the inside

of the containers were kept damp so that moisture was never lacking. Since the humidity outdoors was close to or at 100% during most of the study, the humidity of the plastic dishes probably approximated the control humidity quite closely.

The heating gradient remained constant (1 °C. / 5 min.). Cooling was not as easily controlled since the volume of cold water passing through the cooling coils had to be increased as the temperature approached room temperature. Furthermore the system could not cool fast enough at temperatures below that of the room. It was felt that this was not critical since the error was introduced at temperatures which were not considered abnormal to the plants.

At the beginning of each experiment the water bath was at 12-14 °C. It was felt that any temperature under 15°C. would not be deleterious to the plants. Plants in plastic containers were placed into the water bath. The temperatures in the containers and the water bath were allowed to equalize before the heating cycle was begun. In most of the experiments performed using this apparatus, the plants were kept at the desired maximum temperature for 30 minutes. The water bath could be held at any desired temperature by using the thermo-regulator. Difficulties were encountered in keeping precise control of the temperature within the plastic containers. The temperature of the plastic containers tended to lag behind that of the water bath at a constant rate until the desired maximum temperature was reached in the water bath. At this time the thermostat maintained the water bath temperature. The rate of increase in temperature within the containers dropped considerably at this time, so that their temperature never reached

that of the water bath. For this reason the maximum temperature within the plastic container fluctuated approximately 1°C. while the water bath remained at a constant temperature. Thus, maximum temperature for all experiments are given with an error of 1°C.

The cooling cycle was begun by turning off the heating coil and starting a flow of tap water through the cooling coil. This was continually regulated so that the rate of temperature change was similar for cooling and for heating. When the bath was cooled to 14°C., the plants were removed and immediately taken back to the roof where they had been previously stored.

Methods of Analyzing Treated Plants:

The methods for cytological study of these plants are described in Chapter 1.

Sampling--In most of the experiments involving the use of the Warburg apparatus some treated sporangia were examined immediately after their removal at the end of the heat shock period. An attempt was made to obtain representatives of all stages of meiosis at each sampling time. This was extremely difficult because only a limited number of slides could be prepared at one time. Many of the sporophytes of any selected clump of plants were either too young or too old for study, so that only about one-half the slides made at one time were suitable for analysis. It was essential to leave enough young capsules for further study as well. Because more than one experiment was being conducted at the same time, progressive sampling of each treatment was performed approximately every second day. However, day to day

sampling was performed when time was available.

Each slide was examined as soon as it was made and any meiotic irregularities were noted. If certain stages of meiosis were not represented in the group of slides made at one sampling time then further attempts were made to choose capsules which might be at the desired stage.

Analysis--Later the slides were examined systematically and careful observations were made. An attempt was made to procure some quantitative data on the treatments. This was done by tabulating the abnormalities found in cells which had completed or had begun to complete meiosis after the treatment period. This tabulation was made concerning slides in which the majority of the SMC were in late anaphase II or the tetrad stage of development. Five hundred cells from each satisfactory slide were analysed and tabulated as follows, using a laboratory counter:

(0) normal tetrads with four cells, all of which have nuclei of comparable size and which possess no micronuclei, extra nuclear chromosomes or fragments of chromosomes (PL. 3, fig. 2).

(1) tetrads with four cells each with nuclei but possessing one extra nuclear chromosome or fragment. The size of the fragment was not particularly considered but some were obviously larger than any of the bivalents of normal metaphase (PL. 5, fig. 3).

(2) same as (1) but possessing two extra nuclear chromosomes (PL. 5, fig. 4).

(3) same as (1) but possessing 3 or more extra nuclear chromosomes (PL. 5, fig. 5).

(4) degenerate tetrads of SMC showing aborted meiosis. This included tetrads which had either more than, or less than, four cells with nuclei. Almost all of these had extra nuclear chromosomes, (PL. 5, fig. 6-8).

Control plants were examined and analysed from the same population used in the experiments, and at the same time the experiments were conducted. Observations presented earlier (Chapter 1) concerning Hypnum circinale were derived from the controls. The tetrad analysis of the controls, however, is included with the results of the experiments.

Materials:

Pilot experiments were performed in the spring of 1963 with plants of Hypnum subimponens Lesq. from populations collected in the University of British Columbia Endowment Lands, Vancouver, B.C. (Dill #5163).

Experiments conducted in the fall of 1963 were performed using the same populations of H. circinale that were used in the preparations described in Chapter 1.

Since the material was from wild populations it cannot be said that the sporangia from any one population were genetically the same. Certainly sporangia from different populations would not be genetically identical. However, since thedense populations of gametophytes of H. circinale probably have been produced, to a large extent, by asexual means, it is suspected that the gametophyte members are quite uniform genetically. In addition, as the species is monoecious and the gametophytes probably self-fertile, the sporophytes of one clump, or small population, also

would be genetically quite uniform.

RESULTS

The results of pilot experiments on Hypnum subimponens in the spring of 1963 indicated that the species was, during its meiotic cycle, extremely sensitive to short term heat stress (4 hour period with maximum temperature range at 32, 36, & 37° C.). Many unusual meiotic aberrations were observed, including complete clumping of metaphase I chromosomes, precocious disjunction of all or some of the bivalents, abnormal tetrad formation and complete abortion of meiosis. However, insufficient material was available to make a satisfactory analysis. In the fall of 1963 Hypnum circinale produced abundant sporophytes and was therefore selected as the material with which to continue the work. Unfortunately, due to the low yield of pertinent slides during sampling even this material was insufficient to indicate more than trends in abnormal behaviour.

Experiment #1:

Material--Hypnum circinale, population #1363.

Treatment--The plants were maintained in the water bath for a period of 4 1/2 hours while the temperature was raised to 36.5 ± 0.5°C. and held at that temperature for 30 minutes; then returned to 14°C. Following the heat shock period the plants were stored outside (7-11°C.).

Observations--The first 20 slides were prepared 2 1/2 hours after completion of the heat shock period. Each slide contained all the SMC of a sporangium. The majority of the sporangia had completed meiosis and only young spores were seen. These were

discarded. The five usable slides fell into the following categories: one had old tetrads; two were mostly tetrads; one had leptotene, zygotene and pachytene; and one had leptotene and zygotene.

A few metaphase I cells were observed in the slides with tetrads and all of these were severely clumped (similar to those shown in PL.7, fig. 7). Some of the tetrads contained extra nuclear chromatin, indicating that the last part of meiosis was completed during the heat shock period. Only two of the slides with tetrads were suitable for analysis; these are sporangia 1 and 3 in Table I.

Table I: Tetrad analysis of sporangia of experiment #1.

Sporangium No.	Time of sampling after the end of the heat shock period.	% cells of each analysis category of 500 examined cells per sporangium. 0	1	2	3	4	Comments
1	2.5 hrs.	88.4	8.4	1.6	0.6	1.0	slide had mostly tetrads
3	2.5 hrs.	91.0	6.0	1.4	0.4	1.2	"
6	26 hrs.	65.8	11.2	1.2	1.0	19.8	"
23	4 days	26.8	13.0	7.8	8.6	43.8	"

The next 12 slides were made 26 hours after termination of the heat shock period. These fell into the following categories: four contained young spores; two had old tetrads with no active stages[5]; two had tetrads with some active stages; two had mostly

[5]the term "active stages" is used to denote all the stages from diakinesis to telophase II.

Table II: Tetrad analysis of control plants of population #1363 which were used in experiments 1 and 2.

Sporangium No.	% cells of each analysis category of 500 examined cells per sporangium. 0	1	2	3	4	Comments
1	94.0	4.0	0.0	1.0	1.0	mostly tetrads
5	93.6	5.0	0.0	0.8	0.6	"
7	89.0	5.6	4.6	0.4	0.4	"
11	96.8	2.6	0.0	0.3	0.3	"
23	95.6	3.0	0.4	0.6	0.4	"
Average	93.8	4.0	1.0	0.6^{+}	0.5^{+}	

dictyotene stages (one of these slides had some metaphase I cells); and two had leptotene and zygotene. The active stages in all of these slides were abnormal. Some of the metaphase I were clumped, but in those that were not clumped, over 70% had bivalents that were not oriented on a proper spindle (PL. 4, fig. 10). Anaphase I and II contained many laggards and bridges. Again prophase I stages could not be distinguished from prophase I stages observed in the controls. One slide (#6) was suitable for tetrad analysis and is included in Table I (Page 35).

Seven slides were made 48 hours after the termination of the heat shock period. They fell into the following categories: two had young spores; two had predominantly active stages; one had primarily migrating pachytene; and two had leptotene, zygotene and some pachytene. In one of the slides containing young spores, all the spores were spherical and appeared normal. However in

a second slide there were some (approximately 10%) large cells that were hyaline, in this respect resembling young spores. These cells contained pycnotic chromosomes in various stages of meiosis; such cells may be termed restitution cells. It is apparent that these cells were SMC in which meiosis had aborted.

All active stages were abnormal (PL. 4, fig. 11, 12; PL. 5, fig. 1). Metaphase I stages were sometimes partially, but never completely clumped. Unclumped metaphases were not organized on a proper spindle and univalents and fragments were scattered around the cell. Anaphases were severely disrupted and in many of the cells it was impossible to determine what stage of meiosis the chromosomes were in. Telophase I nuclei were clumped into globular masses. Some of these cells, although abnormal, had completed anaphase I without leaving laggards and fragments outside the telophase I nuclei, (PL. 5, fig. 2). No slides were suitable for tetrad analysis.

After four days five additional slides were made. By this time only a few suitable capsules remained in the treated lot of material. These slides fell into the following categories: two contained mostly restitution SMC and some broken tetrads; one possessed only tetrads; one had mostly dictyotene, but had some diplotene, migrating pachytene and metaphase I cells; and one had all diplotene and pachytene. About 70% of the restitution SMC were in dictyotene and diplotene stages (PL. 5, fig. 9), the remainder were in active stages that appeared abnormal. One slide was suitable for tetrad analysis and is included in Table I (see page 35).

All metaphase I stages were disrupted and appeared unoriented

as the chromosomes were spread all over the cell. Generally many univalents were present.

Two observations can be made at this point. First, the synchrony of the prophase stages appeared to be completely disrupted; never were pachytene, diplotene, dictyotene and metaphase I stages observed in one plant in the control material. Second, the first diplotene phases were seen at the fourth day of sampling. Although this may be due to sampling error it suggests that the duration of diplotene was possibly shortened immediately following the heat shock period.

Experiment #2:

Material--Hypnum circinale, population #1363

Treatment--The plants were maintained in the water bath for a period of 3 1/2 hours while the temperature was raised to $30.0 \pm 0.5^{\circ}$ C., held at that temperature for 30 minutes and then returned to 14°C. Following the heat shock period the plants were stored outside ($7\text{-}11^{\circ}$C.).

Observations--The first 8 slides were prepared immediately after the end of the heat shock period and fell into the following classes: one had mostly tetrads with some active stages; one had active stages and dictyotene stages; two had mostly dictyotene with some diplotene; two had migrating pachytene and diplotene; and two were in leptotene and zygotene.

Many metaphase I stages were observed in this material. About 20% were clumped (PL. 5, fig. 10) while the remainder varied from partially clumped (PL. 5, fig. 11,12) to those

arranged on an apparently normal spindle (PL. 6, fig. 1-3). Rare metaphase I stages were observed with unoriented and divided bivalents (PL. 6, fig. 4). The chromosomes of many of the metaphase I cells with oriented bivalents appeared more highly condensed and more globular than those of the controls. However, in many of the cells the chromosomes were indistinguishable from those in the controls. Unfortunately only one slide was suitable for tetrad analysis, and is sporangium 4 in Table III.

Table III: Tetrad analysis of sporangia of experiment #2.

Sporangium No.	Time of sampling after the end of the heat shock period.	% cells of each analysis category of 500 examined cells per sporangium.					Comments
		0	1	2	3	4	
4	0 hrs.	85.6	6.8	0.6	0.6	6.4	mostly tetrads
13	72 hrs.	57.8	22.0	6.6	5.6	8.0	"

Of seven slides made 24 hours after the termination of the heat shock period, four were discarded because the plants were much too young for meiotic stages. The remainder fell into the following classes: two were in pachytene, and diplotene; and one had young spores.

The prophase I stages and the young spores appeared normal. No material was available for tetrad analysis.

Three slides were made 72 hours after termination of the heat shock period. These fell into the following classes: one

had old tetrads with about 1% active stages and two were in leptotene and pachytene.

The slide with tetrads was analysed and is sporangium 13 in Table III (page 39). The tetrads contained many abnormalities (PL. 6, fig. 7-9). The active stages, which were all anaphases, contained laggards or exhibited abnormal segregation. Prophase I stages appeared normal.

Experiment #3:

Material--Hypnum circinale, population #2267

Treatment--The plants were maintained in the water bath for 3 3/4 hours, while the temperature was raised to 31.5 ± 0.5° C., left at that temperature for 30 minutes, and then lowered to 14° C. Following the heat shock period the plants were stored outside (7-11°C.)

Observations--Five slides were prepared immediately after the heat shock period. These fell into the following classes: one had mostly tetrads with some active stages; one had dictyotene and diplotene; and three had earlier stages of prophase I.

All metaphase I stages observed had some degree of clumping, although it was not as severe as that in experiment #1. All observed anaphases had laggards and prophase I cells appeared normal. One slide was suitable for tetrad analysis and is sporangium 3 in Table IV (page 41).

Eight slides were made five hours after the end of the heat shock period, and fell into the following classes: one had tetrads; one had dictyotene; one had transitional stages between

Table IV: Tetrad analysis of sporangia from experiment #3.

Sporangium No.	Time of sampling after the end of the heat shock period.	% cells of each analysis category of 500 examined cells per sporangium. 0	1	2	3	4	Comments
3	0 hrs.	93.0	1.8	0.8	0.8	3.6	mostly tetrads
7	5 hrs.	73.0	11.6	3.0	3.0	8.4	"
17	20 hrs.	87.0	3.0	0.0	0.0	10.0	young tetrads many active stages
18	20 hrs.	63.0	7.8	1.2	0.2	27.2	old tetrads
19	20 hrs.	45.6	13.0	3.2	1.2	27.0	mostly tetrads
21	5 days	56.6	14.2	8.4	9.0	16.0	tetrads with many active stages
22	5 days	54.8	17.8	9.8	8.4	9.2	mostly tetrads
26	5 days	68.0	12.0	2.2	0.8	17.0	old tetrads
31	7 days	77.0	13.6	1.4	0.8	7.2	active stages with young tetrads
32	7 days	65.2	18.6	5.8	2.4	8.0	old tetrads

diplotene and dictyotene ; four had leptotene to pachytene; and one had diplotene and migrating pachytene.

No active stages were observed in the sampling. The prophase I stages appeared normal, except that synchrony of division within each sporangium was lost. In contrast to the findings in experiment 1 and 2, some diplotene figures were observed. One

slide was suitable for tetrad analysis and is sporangium 7 in Table IV (page 41).

Table V: Tetrad analysis of control sporangia of population # 2263 which were used in experiment #3.

Sporangium No.	% cells of each analysis category of 500 cells per sporangium					Comments
	0	1	2	3	4	
4	95.0	3.6	1.0	0.0	0.4	old tetrads; staining would not allow accurate observations of micronuclei
8	99.2	-	-	-	0.8	
10	94.0	4.4	0.8	0.2	0.6	

Six slides were made 20 hours after the end of the heat shock period. They fell into the following classes: two had old tetrads with some active stages; one had active stages, young tetrads; one had mostly dictyotene with diakinesis and rare metaphase I stages; and two had mostly dictyotene with early prophase I stages.

Most of the active stages in the most active sporangium appeared normal; twelve of one hundred metaphases counted appeared to have somewhat abnormal orientation on the spindle. The frequency of laggards during anaphases was quite low. However, in the sporangia with older tetrads all anaphases observed were abnormal. This discrepancy may be explained by the fact that the latter sporangia were at the active stages during the heat shock while the former ones were in late prophase. This superficially suggests that the heat shock affected less severely

the late stages of prophase I than it did the metaphase I and later stages.

The prophase stages again showed lack of synchrony but otherwise appeared normal. Three sporangia were satisfactory for tetrad analysis and are sporangia 17, 18, and 19 in Table IV (page 41).

Five days after the end of the heat shock period 9 slides were prepared. They fell into the following classes: one had old tetrads and young spores; one had old tetrads; two had tetrads and active stages (one of these had only rare active stages); one had dictyotene and many active stages; and four had various unsynchronized prophase I stages.

In the sporangia with tetrads and some active stages, most of the active stages were abnormal. Very few metaphases were observed and most of these were oriented on a spindle, but often the chromosomes were partially clumped or there were disjoined bivalents. The bivalents appeared highly condensed. Anaphases had many laggards and bridges, or appeared unoriented (PL. 6, fig. 10). In the sporangium with predominantly dictyotene and active stages, there were many metaphase I stages. These, for the most part (71 of 100 cells counted) had normal arrangement on the spindle. In the metaphase cells with abnormal bivalent arrangement, the bivalents were usually highly condensed.

Three sporangia were suitable for tetrad analysis and are sporangia 21, 22, and 26 in Table IV (page 41).

Seven days following the heat shock 6 more slides were prepared and fell into the following classes: one had spores; one had old tetrads; one had active stages and young tetrads;

two were in dictyotene and diplotene with rare pachytene stages; and one was in leptotene.

Again the metaphase I stages ranged from what appeared to be normal to those that were abnormal. Fifty-one of the metaphase I stages counted had apparently normal orientation on the spindle. Also there were many cases where it was difficult to establish whether the orientation was normal or abnormal. The chromosomes often were condensed slightly more than those in the controls (PL. 6, fig. 11), but there were also those that could not be distinguished from those of the controls (PL. 7, fig. 1). Of the stages observed, anaphases were the most abnormal. The chromosomes were not oriented and there were many laggards. Also, nondisjunction of one or more chromosomes was most commonly observed (PL. 7, fig. 2). Two slides were suitable for tetrad analysis and are sporangia 31 and 32 in Table IV (page 41).

Experiment #4:

Material--Hypnum circinale, population #2463

Treatment--The plants were maintained in the water bath for a period of 6 1/2 hours during which the temperature was raised to 25.0 $\pm$ 0.5°C., kept at that temperature for 4 hours and lowered to 14°C. The plants were placed outside following the treatment (7-11°C.).

Observations--No slides were prepared immediately after the treatment. After 24 hours 6 slides were made and these fell into the following classes: one had active stages and young tetrads; two had dictyotene and active stages; and three had

mostly dictyotene with some transitional stages between diplotene and dictyotene, and also rare pachytene.

The active stages observed during this sampling appeared predominantly normal. There was an occasional metaphase in which the bivalents appeared unoriented and were over-condensed, but in general behaviour was not significantly different from the control. Prophase I stages exhibited some asynchrony and diplotene stages were rare. One plant was suitable for tetrad analysis and is sporangium 6 in Table VI (page 46).

Thirteen slides were prepared 72 days after the end of the treatment and these fell into the following classes: four had old tetrads with few or no active stages; one had mostly tetrads with some active stages; two had mostly active stages; two had mostly dictyotene with rare diplotene, diakinesis and metaphase I figures; one had diplotene-dictyotene transitional stage; two had diplotene and pachytene; and one had leptotene, zygotene and rare pachytene stages.

Again the active stages appeared predominantly normal. Only a few (probably less than 5%) of the metaphases appeared slightly unoriented with highly-condensed bivalents. The prophase stage exhibited some asynchrony since stages from diplotene to metaphase I were observed in two slides. Four slides were suitable for tetrad analysis and are sporangia 17, 12, 14 and 19 in Table VI (page 46).

Five days after the end of the heat shock period 11 more slides were prepared and these fell into the following classes: eight had spores; one had active stages and young tetrads; one

Table VI: Tetrad analysis of sporangia of experiment #4,

Sporangium No.	Time of sampling after the end of the heat shock period.	% cells of each analysis category of 500 examined cells per sporangium					Comments
		0	1	2	3	4	
6	24 hrs.	83.0	7.6	1.4	1.0	7.0	active stages and young tetrads
17	72 hrs.	90.2	3.4	0.2	0.4	5.8	old tetrads
12	72 hrs.	73.8	6.6	1.2	1.0	17.4	mostly tetrads
14	72 hrs.	87.4	7.2	1.8	0.2	3.4	"
19	72 hrs.	84.2	5.8	1.8	0.2	8.0	"
21	5 days	80.6	10.4	1.6	0.6	6.8	active stages and young tetrads

Table VII: Tetrad analysis of sporangia from population #2463 which were used in experiments 4-7.

Sporangium No.	% cells in each analysis category of 500 examined cells per sporangium					Comments
	0	1	2	3	4	
8	88.6	3.2	0.6	0.2	7.4	mostly tetrads
9	95.8	3.4	0.4	0.0	0.4	"
10	94.6	3.8	0.4	0.0	1.2	"
12	94.0	2.6	0.2	0.2	3.0	"
13	93.6	2.8	0.4	0.0	3.2	"
Average	93.3^{+}	3.2^{-}	0.4	0.1^{-}	3.0^{+}	

had dictyotene with some metaphase I and rare diplotene stages; and one had mostly dictyotene.

The spores observed appeared normal, as did the active stages, with the exception of a few questionable metaphase I stages. Prophase I stages appeared slightly asynchronous. One slide was suitable for tetrad analysis and is sporangium 21 in Table VI (page 46).

Experiment #5:

Material--Hypnum circinale, population #2463

Treatment--In an attempt to determine the immediate effects of raising the temperature to a level that would induce severe anomalies, the plants were put into the water bath and the temperature was raised in the normal manner to 37 $\pm$ 0.2°C. The material was removed at that temperature and slides were made as soon as possible.

Observations--Of the twenty slides made immediately after the treatment period 9 were discarded because the sporangia were too old. The remaining 11 fell into the following classes: two had old tetrads; four had mostly tetrads and some active stages; two had mostly dictyotene and some metaphase I stages; three had diplotene and late pachytene stages; and one had leptotene.

All metaphase I stages observed were severely clumped; the bivalents had fused into a globular spheres of chromatin (PL. 7, fig. 7). Only a few of the anaphases and telophase stages had laggards, indicating perhaps that these stages were completed

before the temperature was critically high. Many of the dictyotene stages had been affected by the heat shock, for often the chromatin appeared reduced in area. Chromosomes were apparently partially condensed around the chromocentre. The earlier prophase stages appeared normal (PL. 7, fig. 5,6). There were occasional instances of abnormal pairing (PL. 7, fig. 4) but as this anomaly was rare it could not be determined whether it was caused by the heat shock or whether it was due to natural abnormality. Five slides were suitable for tetrad analysis and are sporangia 4, 10, 2, 7, and 11 in Table VIII (page 49).

Eleven slides were made 4 hours after the end of the treatment and these fell into the following classes: two had mostly old tetrads with a few active stages; two had many active and some tetrads; one had dictyotene and metaphase I stages; and six had early prophase stages in which diplotene was rare.

Again the metaphase I stages were severely clumped. In cells at telophase I, the chromosomes of the two nuclei were clumped into globular spheres. Anaphase stages were not observed, indicating that active movement of chromosomes had been temporarily arrested. The dictyotene stages had more heterochromatic areas than those of controls and the chromatin threads appeared thicker and stuck together in certain areas. In addition, the size of the chromatin mass at dictyotene had been reduced. Early prophase I stages appeared normal. Four slides were suitable for tetrad analysis and are sporangia 12, 17, 20 and 22 in Table VIII (page 49).

Twelve slides were made 72 hours following the end of the

Table VIII: Tetrad analysis of sporangia of experiment #5

Sporangium No.	Time of sampling after the end of the heat shock period.	% cells in each analysis category of 500 examined cells per sporangium					Comments
		0	1	2	3	4	
4	0 hrs.	91.4	5.2	1.0	0.6	1.8	old tetrads
10	0 hrs.	90.2	3.6	1.4	0.2	4.6	"
2	0 hrs.	85.0	6.8	3.0	1.4	3.8	active stages and young tetrads
7	0 hrs.	73.8	15.0	3.6	2.8	4.8	"
11	0 hrs.	67.0	14.0	4.6	5.0	9.4	"
12	4 hrs.	90.8	5.6	0.8	0.0	2.8	old tetrads
17	4 hrs.	84.6	8.4	1.2	1.2	4.6	mostly tetrads
20	4 hrs.	65.0	14.0	4.0	4.0	13.0	active stages and tetrads
22	4 hrs.	70.6	14.4	7.0	2.0	6.0	active stages and young tetrads
25	72 hrs.	80.0	6.8	1.6	0.0	11.6	active stages and old tetrads
39	5 days	71.8	12.8	3.8	3.0	7.6	old tetrads
42	5 days	46.4	13.8	1.6	1.4	36.8	active stages and tetrads

treatment. These fell into the following classes: two had young spores; one had active stages and tetrads, one had mostly active stages; two had mostly dictyotene; five had unsynchronized

early prophase stages including dictyotene; and one had leptotene and zygotene.

In the slides with young spores, restitution SMC were common, indicating that meiosis had been completely arrested in some cases. No clumping was observed at metaphase I, but generally spindle formation was abnormal. Fifty-two of 200 metaphases, examined appeared to be arranged on a spindle (PL. 7, fig. 8; PL. 8, fig. 12), but in all cases the chromosomes were highly condensed and disjunction was not synchronous. Anaphases had many laggards but 88 of 100 examined telophase I stages exhibited no abnormalities. There are at least two possible explanations for this discrepancy. One is that the laggards at anaphase were included in the telophase nuclei despite their apparent lack of movement; the second is that meiosis had been arrested soon after the treatment and that these cells would eventually become restitution SMC . Neither explanation is entirely satisfactory. The first explanation is contrary to the findings elsewhere in these studies and the second is puzzling because if meiosis had been arrested for three days the SMC had not gone into restitution, while restitution SMC were observed in plants with young spores. Only one slide was suitable for tetrad analysis and is sporangium 25 in Table VIII (page 49).

Eight slides were made 5 days after the treatment and these fell into the following classes: two had young spores; one had old tetrads; one had tetrads and active stages; one had dictyotene and diplotene, and three had various early prophase I stages.

The slides with young spores had many restitution SMC and

abnormally shaped spores (PL. 8, fig. 3-5). Often spores were spherical but were joined together by threads of cell wall material (PL. 8, fig. 4). In many cases the spores and restitution SMC were clumped into large aggregated (PL. 8, fig. 5). The active stages were markedly abnormal and probably had been arrested. Prophase stages appeared normal. Two slides were suitable for tetrad analysis and are sporangia 39 and 42 in Table VIII (page 49).

Experiment #6:

Material--Hypnum circinale, population #2463

Treatment--In this experiment the plants were brought into the laboratory and put into covered plastic dishes. The laboratory temperature was 22$\pm$ 2°C. (normal indoor conditions). The purpose of the treatment was to determine the behaviour of meiotic chromosomes under laboratory conditions.

Observations--The first slides, made 24 hours after the plants had been brought indoors, were markedly abnormal. In 50-60% of the metaphase I stages there was no spindle orientation, and in almost all the cells the metaphase I bivalents were highly condensed. Moreover, there were many cells with partially clumped bivalents as well as those with precociously disjoining bivalents. There were also those containing a mixture of the various abnormalities. Anaphases had many laggards and appeared disorganized. Prophase stages were apparently normal. No satisfactory diplotene was observed; only transitional stages between diplotene and dictyotene were present. Two slides were suitable

for tetrad analysis and are sporangia 1 and 2 in Table IX.

Table IX: Tetrad analysis of sporangia of experiment 6.

Sporangium No.	Time of sampling after plants were brought indoors.	% cells of each analysis category of 500 examined cells per sporangium.					Comments
		0	1	2	3	4	
1	24 hrs.	14.8	5.2	1.2	0.0	78.8	mostly tetrads
2	24 hrs.	27.2	2.0	0.4	0.2	70.2	"
6	30 hrs.	18.0	10.0	0.0	0.0	72.0	active stages and tetrads
10	30 hrs.	39.6	9.2	2.2	0.4	48.6	old tetrads
12*	72 hrs.	1.2	0.0	0.0	0.0	98.8	active stages and tetrads
17*	72 hrs.	0.0	1.0	0.0	0.0	99.0	"
18*	72 hrs.	0.8	0.0	0.0	0.0	99.2	"

* in these three sporangia no normal active stages were observed and in most of the SMC meiosis had aborted before second division began.

Thirty hours after plants were brought indoors 6 more slides were prepared. These fell into the following classes: two had old tetrads; one had active stages and tetrads; one had dictyotene and active stages; and two had pachytene stages.

Again the active stages were abnormal; over 60% of the metaphases had abnormal spindle formation (PL. 8, fig. 6-9). Even cells with some metaphase orientation had highly condensed bivalents, one or more of which usually displayed precocious disjunction. All anaphases had laggards and in many cases the

chromosomes were spread all through the cell. The pachytene stages appeared normal. Two slides were suitable for tetrad analysis and are sporangia 6 and 10 in Table IX (page 52).

Seventy-two hours after the plants were brought into the laboratory 9 slides were prepared and these fell into the following classes: one had young spores and old tetrads; four had active stages and tetrads; one had active stages; two had dictyotene and active stages (there were also some early prophase I stages in one slide); and one had dictyotene.

It was noted that the plants were maturing extremely rapidly by this time. Many of the capsules that were not full size had begun meiosis prematurely.

The sporangium with young spores had about 80% restitution SMC and many degenerate tetrads. The active stages of meiosis were completely aborted (PL. 9, fig. 1) and it appeared that in many cases the complete tetrad stage would never be revealed. Prophase I stages were asynchronous and no diplotenes were observed. The dictyotene chromosomes were often condensed around the chromocentre (similar to that in PL. 9, fig. 4) and many cells were observed in which the chromosomes were clumped into a tightly packed ball (similar to that in Pll 9, fig. 5). This stage could not be accurately interpreted, but was possibly an aborted pachytene or diplotene. Three slides were suitable for tetrad analysis and are sporangia 12, 17 and 18 in Table IX (page 52). These tetrad stages very seldom had four cells since meiosis had aborted at or before second division (PL. 9, fig. 2, 3). In addition, it was often noted that individual chromosomes had formed micronuclei.

Observations were made again at 4 and 6 days after the

plants were brought into the laboratory. Meiosis had completely aborted; by 4 days many of the SMC had undergone restitution. Those that had not undergone restitution possessed either aborted active stages or prophase stages which were closely clumped (PL. 9, fig. 5), as previously mentioned. At 6 days the capsules with any sign of meiotic activity were extremely small (about 1/2 full size) and these had only developed a few archesporial cells. Meiosis had aborted.

Experiment #7:

Material--Hypnum circinale, population #2463

Treatment--The plants were brought into the laboratory and put into open trays. One of the trays was put into a cupboard during the evenings and at night in order to simulate the normal outdoor photoperiod. The other was left out on a bench. The purpose of this experiment was twofold: (a) to determine whether the humidity in the plastic dishes was contributing to or counterbalancing the effect of the indoor temperature, (b) to demonstrate whether a photoperiod response was contributing to the abnormalities.

Observations--Slides were made from both trays at certain times after the plants were brought indoors, much as in experiment 6. The abnormalities observed were similar to those described in experiment 6, and meiosis had aborted 72 hours after the plants had been brought indoors.

Tetrad analysis of sporangia from both trays are included in Tables X and XI. The analyses indicated that the severity

of the anomalies was comparable to those observed in experiment 6.

Table X: Tetrad analysis of sporangia of experiment 7, plants under laboratory conditions.

Sporangium No.	Time of samples after the plants were brought indoors.	% cells of each analysis category of 500 examined cells per sporangium					Comments
		0	1	2	3	4	
7	36 hrs.	71.2	5.0	0.0	0.0	23.8	old tetrads
12	36 hrs.	68.8	4.2	0.0	0.0	27.0	"
9	36 hrs.	56.0	8.6	1.2	0.0	34.4	mostly tetrads
11	36 hrs.	49.6	4.6	0.4	0.0	45.4	"
13	36 hrs.	47.6	8.6	1.4	0.6	41.8	"
14	36 hrs.	42.2	9.6	0.2	0.0	48.0	"
16	72 hrs.	2.6	1.2	0.4	0.2	95.0	arrested active stages and tetrads

Table XI: Tetrad analysis of sporangia of experiment 7, plants under normal photoperiod.

Sporangium No.	Time of sampling after plants were brought indoors.	% cells in each analysis category of 500 examined cells per sporangium. 0	1	2	3	4	Comments
1	24 hrs.	78.4	0.6	0.0	0.0	21.0	very old tetrads
4	24 hrs.	86.0	8.2	0.0	0.0	5.8	"
2	24 hrs.	30.2	9.6	1.2	0.0	58.8	"
18	72 hrs.	4.2	0.0	0.0	0.0	95.8	old tetrads
19	72 hrs.	2.2	0.4	0.0	0.0	97.4	"
20	72 hrs.	0.0	0.8	0.0	0.0	99.2	active stages and tetrads
22	72 hrs.	3.0	0.0	0.0	0.0	97.0	mostly tetrads
23	72 hrs.	0.8	0.2	0.0	0.0	99.0	active stages and tetrads

DISCUSSION

It is realized that the results of these experiments suggest little more than trends of abnormal behaviour and that much more meaningful information could have been derived if the sample sizes had been larger. This was especially evident in those experiments in which the heat shock was relatively mild and the possibility of recovery after such shock was superficially suggested from the tetrad analysis (see experiment 3, page 40). In addition it is obvious that the experiments could be refined by using controlled temperature, humidity, and photoperiod rooms which would give more accurate and probably more meaningful results. Although the interpretation of the experiments possess these limitations, some informative conclusions can be drawn.

It was stated earlier that the purpose of conducting the heat shock experiments was fourfold:

(1) to determine whether errors in interpretation of chromosomal behaviour would be introduced if mosses were brought indoors to mature the sporangia,

(2) to determine the sensitivity of moss SMC to slight heat stress,

(3) to indicate the likelihood that natural heat stress in the environment might be a mechanism of inducing mutations or structural/numerical alterations of the chromosomes, and

(4) to determine the mode of response of the SMC to heat stress

These four categories are discussed under separate headings.

(1) Chromosomal Behaviour of Mosses under Laboratory Conditions.

Experiment 6 (page 51) and 7 (page 54) were primarily undertaken to investigate the effects on chromosomal behaviour of mosses under laboratory conditions. The results clearly indicate that severe abnormalities may be introduced by bringing mosses indoors to ripen. Furthermore, plants kept in the very humid containers had abnormalities similar to those in the open and relatively arid tray. Although the sporangia of those in the open tray did not become flaccid, they were in much drier conditions than those in the plastic dishes. In fact, a comparison of the analysis of experiment 6 and the first part of experiment 7 suggests that possibly the plants under the lower relative humidity responded with severely abnormal meiotic behaviour more slowly than did those in the humid plastic dishes. This evidence is not conclusive, however, since normal variation in day-to-day laboratory temperature may account for these differences. In any event, after 72 hours, both of these environments had induced complete abortion of meiosis.

The second part of experiment 7, in which the plants were subjected to normal photoperiods in open trays, was not expected to indicate any influence of meiotic response to day length. This supposition was borne out by observations of meiosis and by the results of the tetrad analysis (Table XI, page 56).

Although these results are valid only for the species tested (H. circinale) , it certainly emphasizes the necessity of great care when handling mosses prior to fixation. It is quite likely that mosses maturing in warmer climatic areas of the

continent or in late spring or summer in the cooler climatic regions are not as sensitive to slight heat stress as are those maturing during cooler temperatures. Steere et al. (1954) stated that most of the Californian mosses studied matured in winter and early spring. Many of these species might well be heat sensitive. However, in their study they did not indicate how individual plants were handled so it is impossible to evaluate any abnormalities they reported. It is logical, considering the way many of the mosses must have been handled by many authors (Steere et al., 1954; Bryan, 1956a et seq.; Anderson and Crum, 1959) that at least some of the unusual features described for meiosis in mosses (ie. clumping, multiple association and precocious disjunction of certain bivalents), can be attributed to the influence of heat shock prior to fixation.

(2) The Sensitivity of Moss SMC to Slight Heat Stress

The present results indicate that H. circinale is extremely sensitive to heat stress. However, the temperature level at which no abnormalities were produced (if there is an absolute level) was not determined. It is significant that even the treatment applied in experiment 4 (heat stress with a maximum temperature of 25°C. for 4 hours) was sufficient to cause some reduction in normal tetrad formation. In experiments 6 and 7, the sustained room temperature caused complete cessation of meiosis after three days in such an environment. This suggests that long periods at temperatures between 15-20°C. may produce abnormalities.

It is difficult to compare the sensitivity of H. circinale to other organisms and to higher plants in particular because

a wide variety of methods have been used in applying heat stress. Heat shock experiments such as those performed by Barber (1941, 1942), Emsweller and Brierley (1943) and Pao and Li (1940) are uncommon in the literature compared to the sustained temperature experiments of Elliot (1955), Jain (1957 et seq.), Yanney Wilson (1959) and Henderson (1962). Even those experiments using sustained temperatures exhibit conflicting results because the length of time needed to complete the meiotic sequence at a specific temperature was seldom taken into account (Yanney Wilson, 1959). A constant criterion on which to base sensitivity is also difficult to establish. Most writers deal with chiasma frequency at metaphase; only rarely is mention made of the final outcome of meiosis. Chiasma frequency is very difficult to study in moss bivalents bedause the chromosomes are small and seldom have a sufficiently distinct morphology for accurate chiasma counts. Jain (1957 et seq.) has studied heat effects on meiosis of a variety of Lolium perenne L. Although he was not interested in the final product of meiosis he observed that pollen mother cells aborted at metaphase, the chromosomes formed restitution nuclei and no viable pollen grains were produced after treatments of (1) $35 \pm 2^{\circ}C.$ for 58 hours, and (2) $34 \pm 3^{\circ}C.$ for 72 hours, and (3) $33 \pm 2^{\circ}C.$ for 86 hours. Henderson (1962) showed that in the desert locust, Schistocerca gregaria, maintained at $40^{\circ}C.$ for the duration of meiosis, many of the spermatocytes failed to undergo cleavage and became pycnotic. Both of these treatments are much more severe and prolonged than those necessary to cause abortion of meiosis in H. circinale. It is not surprising to find that H. circinale

and probably many other mosses are particularly sensitive to temperature increase because the optimum meiotic temperature is much lower than it is for higher plants which flower in the summer. It is interesting to note that Pao and Li (1940) observed a high degree of univalence in pollen mother cells of Vicia cracca L. immediately after a heat shock as mild as 25°C. for 30 minutes. These plants were studied in January when the temperature was 7°C. Fall and winter-maturing mosses are presumably adapted to these lower temperature ranges. In Lolium the optimum meiotic temperature may be between 15-20 °C. The relative difference between this temperature and room temperature could be considered insufficient to produce anomalous meiosis. It seems obvious that comparison of heat sensitivity between various late spring-maturing mosses and fall/winter-maturing mosses would probably aid in determining the adaptive significance of heat stress.

(3) Natural Occuring Heat Stress as a Mechanism of Inducing Mutation or Structural/Numerical Alterations of the Chromosomes.

This question is only a matter for speculation at this time. The present experiments were neither sufficiently refined nor specifically directed to answer this question.

Abnormalities induced by natural heat stress similar to those induced experimentally have been observed in wild populations in both higher plants and mosses. Beamish (1961a, 1961b) has observed severe clumping of metaphase I stages and highly abnormal anaphases in pollen mother cells of Saxifraga species under field conditions. It is suspected (Beamish, 1961b)

that these abnormalities resulted from high temperatures. Khanna (1960) has observed a similar effect in the moss Barbula constricta Mitt. One might speculate from these observations that less severe heat stress may induce less severe and possibly isolated occurrence of anomalous meiosis. However, the types of anomalies (ie. mutations, chromosomal aberrations) that could be produced is presently unknown. For instance, it is currently impossibly to say whether point mutations are possibly produced by this mechanism. On the other hand, it is reasonable to consider that chromosomal aberrations of either the structural or numerical type might be produced. From the high frequency of univalency at metaphase observed in these experiments and others (see Henderson 1962), nondisjunction, leading to aneuploidy, might be the most common anomaly produced by slight heat stress. To be significant in the population, of course, the aneuploid types must of necessity be viable and also fertile. Nondisjunction was observed occasionaly in the treated moss material used in the present experiments. However, only in rare anaphases could all chromosomes be counted accurately, so no reliable estimate could be made of the frequency of this anomaly.

(4) The Mode of Response of the SMC to Heat Stress.

The response of the SMC to increased temperatures was manifest in several interrelated ways. The different categories can be classified generally as: metaphase I clumping and multiple association of bivalents; extreme condensation or coiling of the bivalents; precocious disjunction; spindle destruction or inhibition; and prophase asynchrony and premature meiotic induction. In no experiment performed here, however, were all of these

categories of response observed. This simply reflects the diversity of time and intensity of heat stress used. Table XII (page 64) indicates in general the mode of action of different categories of heat stress used on the SMC of H. circinale.

Clumping and Multiple Association of the Bivalents:

Severe clumping was observed only under conditions of very high temperature (ie. above approximately 33°C.). In experiments 2(page 38) and 3 (page 40) in which the maximum temperature was between 30° and 32°C., some of the metaphase I stages were clumped immediately after the treatment, but this clumping was not considered severe. With temperatures above 33°C. the areas of clumped chromatin were globular and shiny. Severe clumping is a primary response to high temperatures and does not occur in cells entering metaphase I after the plants have been returned to normal temperatures (see experiments 1 (page 34) and 5 (page 47) . It is expected, although not confirmed that severely clumped cells do not continue meiosis to completion. Relaxation of the clumped chromatin mass may occur however, but the cells probably undergo restitution.

Multiple association of the bivalents was observed consistently in all experiments except experiment 4 (page 44) and is related to the clumping effect observed at high temperatures. In experiment 2 and 3, mentioned above, association ranged from clumping to almost no multiple bivalent association immediately after treatment. Experiments 6 and 7 indicated that multiple association occurs at room temperature after a day indoors.

Table XII: A general summary indicating the mode of action of different categories of heat stress on the SMC of H. circinale.

Heat Stress	Primary Effect	Secondary Effect
36 ± 1°C. Shock	-metaphase I clumping -arrest of active stages -condensation of dictyotene -premature meiotic induction (not manifest) -prophase asynchrony	-spindle breakdown, or inhibition -condensation of metaphase I bivalents -asynchrony of anaphase I, and laggard and bridge formation -restitution -prophase asynchrony -severe reduction in good tetrad formation
31 ± 1°C. Shock	-metaphase I partial clumping -laggard and bridge formation at anaphase -normal appearing prophase -premature meiotic induction (not manifest) -prophase asynchrony	-metaphase I bivalent association, precocious disjunction and condensation -partial spindle breakdown or inhibition -prophase asynchrony -significant reduction in good tetrad production
25 ± 1°C. Shock	-not determined, but premature meiotic induction is presumed to occur	-metaphase I with slightly abnormal orientation on spindle -some anomalous anaphases -slight reduction in good tetrad formation
22 ± 2°C Maintained	-premature meiotic induction -prophase asynchrony	-spindle inhibition and breakdown -disorientation at metaphase I -abortion of meiosis

Multiple association, a primary effect of moderate temperature stress, (ie. heat shock to 31°C.) is also a secondary effect of temperature stress. In experiment 3, multiple association was observed in cells at metaphase I 5 days after the treatment period. It is expected that these cells were probably in early prophase during the treatment. Although the prophase stages appeared normal after the treatment, they must have been affected in some way. This may be related to the prophase asynchrony which is mentioned later.

Extreme Condensation or Coiling of the Bivalents:

This feature was one of the most consistent anomalies observed in all experiments except #4. Abnormal coiling phenomenon associated with heat stress has been noted often in other organisms (Pao and Li, 1940; Swanson, 1942, 1942; Jain, 1957; and Henderson, 1962). Pao and Li (1940) observed severe contraction of the bivalents similar to that noted in H. circinale after heat shock in several species. However, in the sustained temperature experiments performed by the other authors mentioned above, the coiling was asynchronous and usually delayed at metaphase I. The discrepancy may be explained by taking into account the different types of treatments utilized. In the heat shock experiments there is insufficient time between the treatment and the examination for the sporangia to exhibit, at metaphase I, the results of delayed prophase coiling and asynchrony during the treatment period. The high condensation of bivalents at metaphase I observed in moderately heat-treated H. circinale is possibly caused by a failure of the metaphase I bivalents to uncoil to the extent they do following the extreme condensation

at the clumped diakinesis. In the control material the metaphase chromosomes do not appear as condensed as those in diakinesis. This failure of the bivalents to relax at metaphase I may be related to the anomalies in the spindle apparatus under similar conditions.

Precocious Disjunction:

The precocious disjunction of bivalents at metaphase I is closely related to the coiling anomalies found in treated material. In all treatments except experiment 4, precocious disjunction of one or more bivalents was associated with condensation, multiple association, and abnormal spindle formation. This disjunction of bivalents is probably caused by precocious terminalization of the chiasmata at diakinesis so that the bivalent members lose all contact points and drift apart. Precocious chiasma terminalization may be a direct effect of the heat (ie. speeding up the process) or it may be caused by a delay in spindle formation. Swanson (1942) has indicated that in heat-treated material of Tradescantia coiling was asynchronous and delayed. The terminal chiasma frequency was much higher in the more condensed metaphase I bivalents of the controls of Tradescantia than in the relatively uncoiled metaphase I bivalents of the heat treated material. It is possible that the high contraction noted in heat treated H. circinale bivalents caused chiasm terminalization earlier than in the controls, thus contributing to the early disjunction.

Spindle Destruction or Inhibition, Prophase Asynchrony and Premature Meiotic Induction:

Spindle destruction or inhibition by heat stress can only be inferred from these data as the actual spindle fibers cannot be seen using present techniques. The degree of bivalent orientation at metaphase I and the effectiveness of anaphase movement can be used as criterion denoting the state of the spindle. With temperatures above 36°C., it is apparent that the spindle apparatus is destroyed, probably by a denaturation of its functional structure. However, at the moderate heat shock (31 ± 1°C.) the spindle was never completely destroyed as usually the metaphase I stages varied from almost normal orientation to severely abnormal orientation.

Complete inhibition of the production of an effective spindle apparatus was inferred from observations of the material maintained at room temperature. As was mentioned earlier the prophase asynchrony and premature meiotic induction (ie. stimulation of premeiotic SMC to begin meiosis when the capsule is about 1/2 size) was evident in the plants kept at room temperature. At the same time prophase I was accelerated so that the process of visible chromosomal participation was completed in a period of 1-2 days instead of 1-2 weeks. It would seem unlikely that all the synthetic mechanisms leading to the active stages of meiosis could take place satisfactorily in such a short time; thus it is logical to suspect that spindle production is actually inhibited at room temperature. In addition, the spindle inhibition may not be due directly to the heat stress but due to a reduction in time available for completion of necessary syntehtic mechanisms.

Asynchrony of development of SMC in prophase within one

sporangium following various heat treatments is probably directly related to the mechanism of premature meiotic induction and again reflects an acceleration of the meiotic processes. Reducing the time available for normal completion of specific stages (especially diplotene) is apparently contributory to the abnormal spindle effectiveness observed several days following the treatment.

Heat induced spindle breakdown or inhibition has been noted many times in previous studies (Jain, 1957 et seq.; Swanson, 1942; and Henderson, 1962). These were continuous temperature experiments at relatively high temperatures. Failure of the spindle to develop was based on observations of cleavage failure.

The biochemical mechanisms of heat induced spindle failure have not been investigated. A study in variations of synthetic activity during normal and heat shocked prophase may prove to be a useful tool in acquiring a better knowledge of the production and function of the meiotic spindle.

SUMMARY

1. An account of meiosis emphasizing the prophase I stages has been presented for two moss species; Hypnum circinale Hook. and Brachythecium frigidum (C.M.) Besch. In both species, meiosis followed the general pattern of events found in most plants except for a chromosome elongation stage which followed diplotene. Evidence was presented that the stage may be found in many moss species. This stage, which is morphologically similar to the dictyotene stage of the developing oocytes of most animals, has been described in Balsamina hortensis (Impatiens balsamina). The functional significance of the dictyotene stage in moss meiosis is presently unknown.

2. The effects of heat stress of meiotic spore mother cells of H. circinale has been studied. Both heat shock and sustained temperature experiments were performed. Severe anomalies such as metaphase I clumping, bivalent association and contraction, precocious disjunction, spindle destruction or inhibition, prophase asynchrony, premature meiotic induction, and meiotic abortion were observed in most of the experiments. The results indicate that H. circinale is extremely sensitive to heat stress and that cytologists working with mosses should be careful in handling the plants prior to fixation to insure against heat induced meiotic anomalies.

The effects of heat stress under natural conditions, and the mode of response of the SMC to heat stress are discussed.

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(to follow page 73)

APPENDIX

PLATES 1-9

(to face Plate 1)

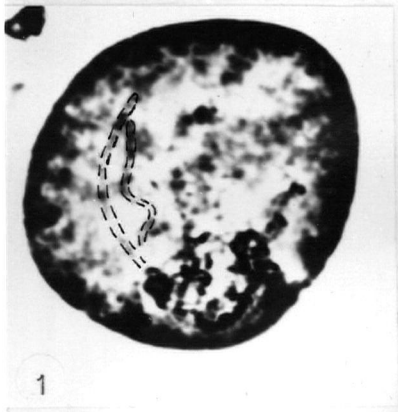
DESCRIPTION OF FIGURES OF PLATE 1

Figures 1-3, H. circinale; showing the acentrically placed chromatin at leptotene and zygotene; fig. 1; leptotene with chromosome strands extending past the middle of the SMC (strands appear thick because the slide was old at the time the photograph was taken and the chromosomes have swelled); fig. 2; transition between leptotene and zygotene, arrow indicates two strands which appear to be in the process of pairing (phase contrast); fig. 3; strands with chromomeres, all X2400.

Figures 4-9, H. circinale; showing various stages of pachytene; fig. 4,5; beginning of migration of the chromatin to the center of the SMC; fig. 7,8; mid-pachytene; fig. 9; late pachytene with chromatin centrally located, all X2400.

Figures 10&11, H. circinale; diplotene; fig. 10; diplotene showing the condensed heterochromatic small bivalent; fig. 11; showing one of the larger bivalents with a heterochromatic segment (phase contrast), X2400.

PLATE I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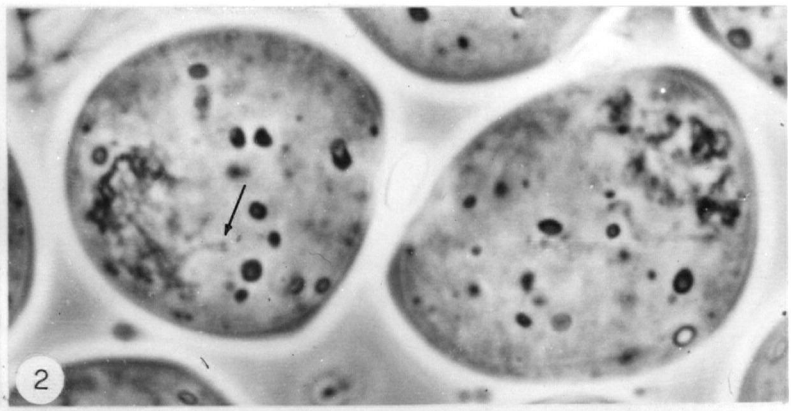
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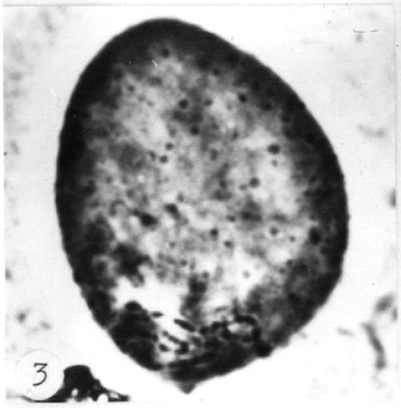
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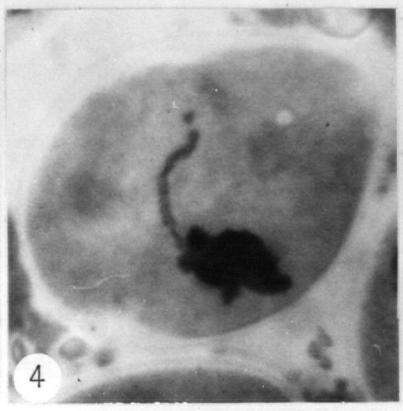
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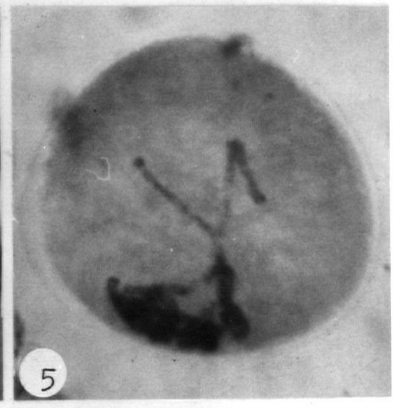
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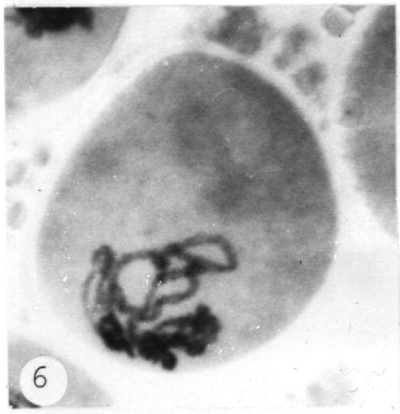
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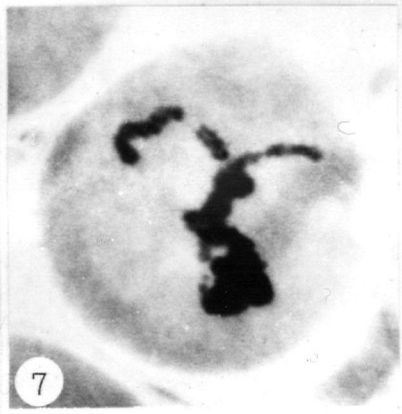
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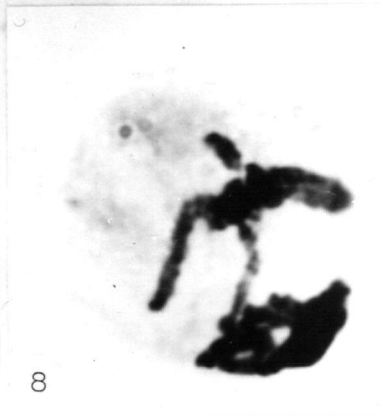
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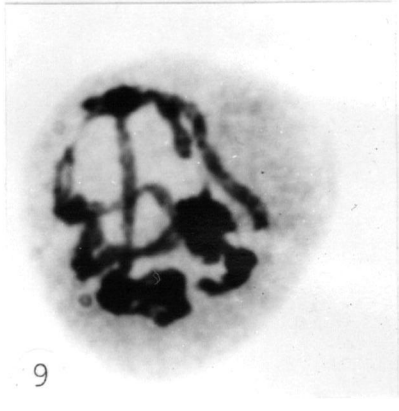
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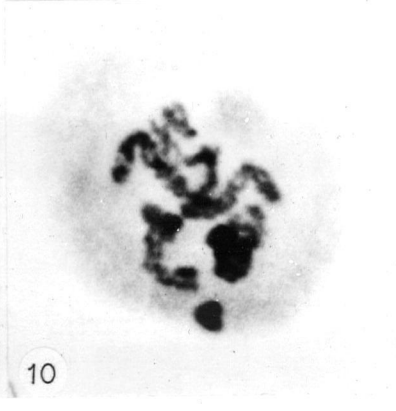
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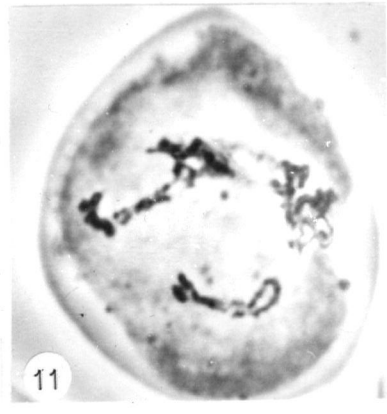

11

(to face Plate 2)

DESCRIPTION OF FIGURES OF PLATE 2

Figures 1&2, H. circinale; diplotene; fig. 1; a highly squashed SMC showing the detailed structure of the large bivalent, X 4500; fig. 2; late diplotene showing all six bivalents, including the heterochromatic small bivalent and the nucleolus (both phase contrast), X2400.

Figure 3, H. circinale; transitional stage between diplotene and the elongation stage (phase contrast) X2400.

Figures 4 &5, H. circinale; elongation stage; fig. 4; elongation stage (arrow indicates a section where the chromosomes are still closely paired); fig. 5; elongation stage showing the interphase-like appearance of the cell (both phase contrast), X2400.

Figure 6, H. circinale; a SMC with diffuse appearance of chromatin. This stage is probably artifact, X2400.

Figure 7&8, H. circinale; diakinesis; fig. 7; densely clumped bivalents at diakinesis (phase contrast); fig. 8; late diakinesis, X2400.

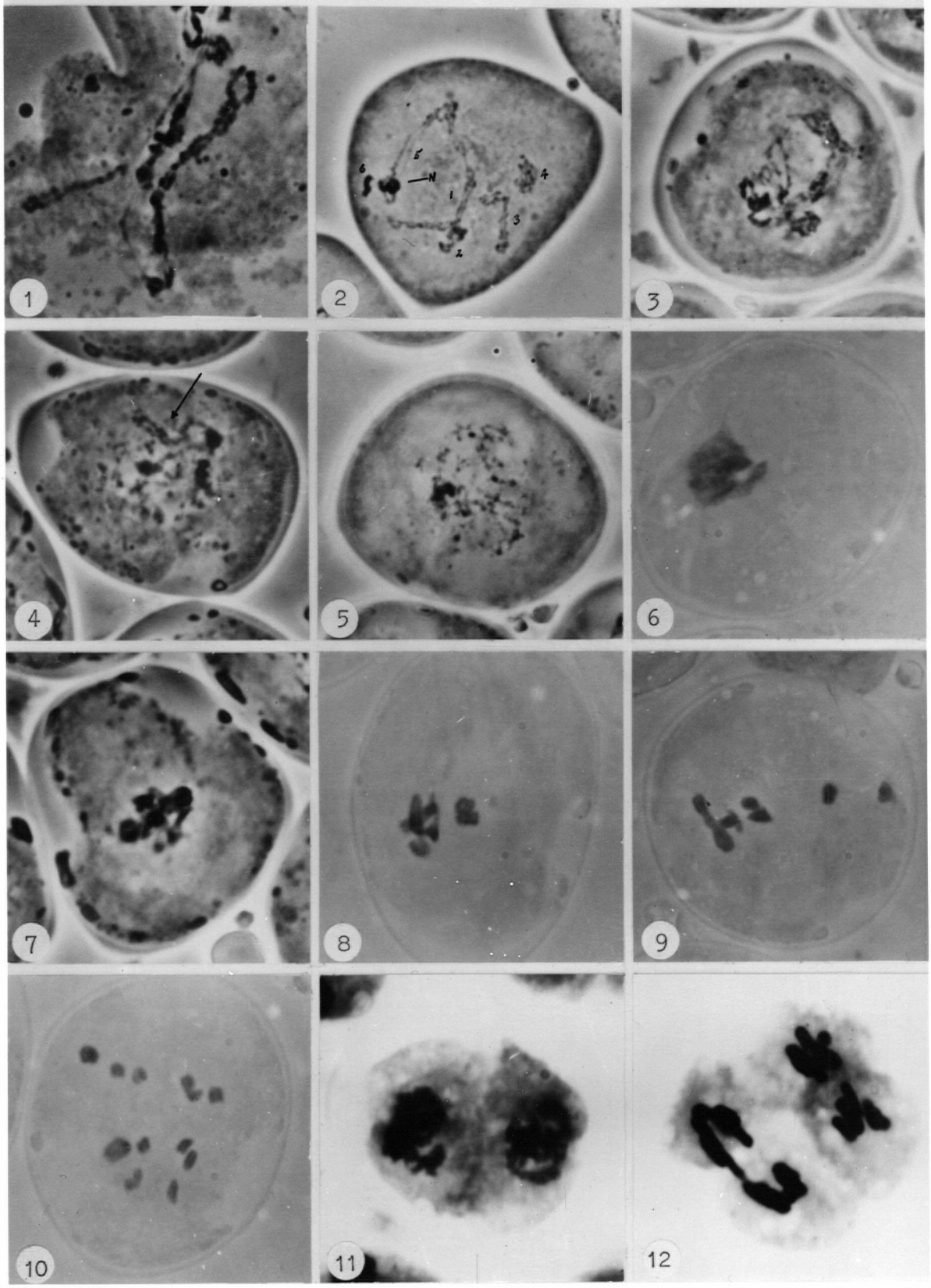
Figure 9, H. circinale; metaphase I showing six bivalents with the large bivalent in a peripheral location. The smallest bivalent is difficult to identify in this case, X2400.

Figure 10, H. circinale; anaphase I, X2400.

Figure 11, H. circinale; interphase or prophase II showing a suggestion of wall formation between the two nuclei, X2400.

Figure 12, H. circinale; anaphase II showing closely packed chromosomes and a temporary bridge formation, X2400.

PLATE 2



(to face Plate 3)

DESCRIPTION OF FIGURES OF PLATE 3

Figure 1, H. circinale; telophase II showing the beginning of wall formation, X2400.

Figure 2, H. circinale; a normal tetrad, X2400.

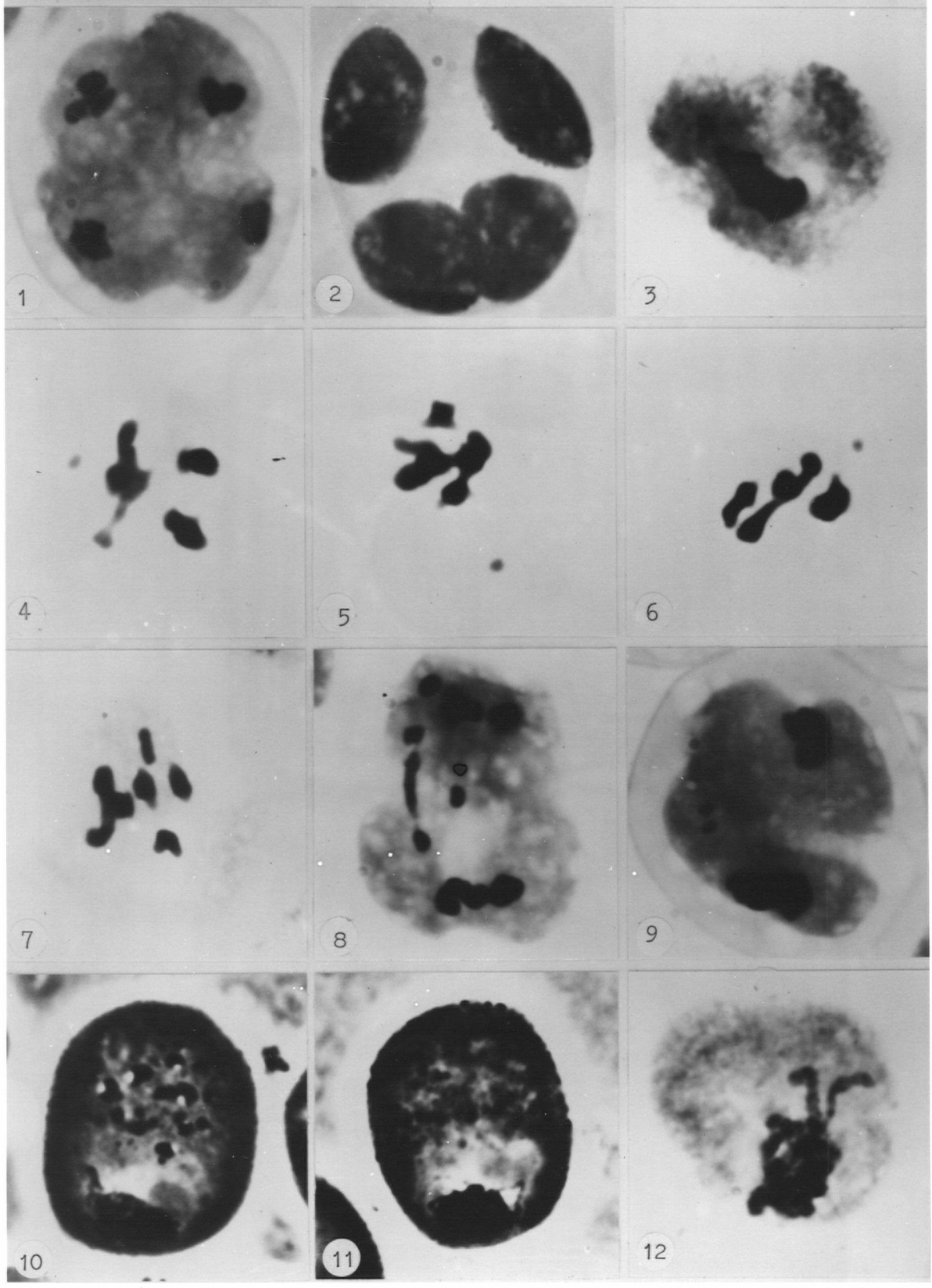
Figures 3-7, H. circinale; sporangium #6; diakinesis to metaphase I, showing the unusual behaviour of the smallest bivalent; fig. 3; early diakinesis with a univalent separate from the chromosomal mass; fig. 4-6; late diakinesis or early metaphase I, showing a univalent of the smallest bivalent separate from the mass. The other univalent of the smallest bivalent is closely associated with the remainder of the chromosomes and is not distinguishable; fig. 7; a metaphase I in which the smallest bivalent has not disjoined precociously, all X2400.

Figures 8&9, H. circinale; showing bridge and laggard formations at telophase I; fig. 8; a bridge and two univalents involving the larger chromosomes; fig. 9; showing the two univalents of the smallest bivalent left off the metaphase plate, both X2400.

Figures 10&11, B. frigidum; showing the acentrically placed chromatin at leptotene-zygotene. The nucleolus is in a more central location in each cell than is the chromatin and is of lighter density than the chromatin, both X2400 (phase contrast).

Figures 12, B. frigidum; early pachytene showing the beginning of chromosome migration, X2400.

PLATE 3



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DESCRIPTION OF FIGURES OF PLATE 4

Figure 1, B. frigidum; mid-pachytene, X2400.

Figure 2, B. frigidum; diplotene. The heterochromatic segment of one of the chromosomes is shown in the lower right hand corner of the cell; the other dark section is caused by overlap of chromosomes, X2400.

Figure 3&4, B. frigidum; SMC in the elongation stage. In fig. 3 the nuclear membrane is clearly evident; in fig. 4 it is less well defined, both X2400.

Figure 5, B. frigidum; showing a clumped diakinesis, X2400.

Figure 6, B. frigidum; prometaphase showing six bivalents, X2400.

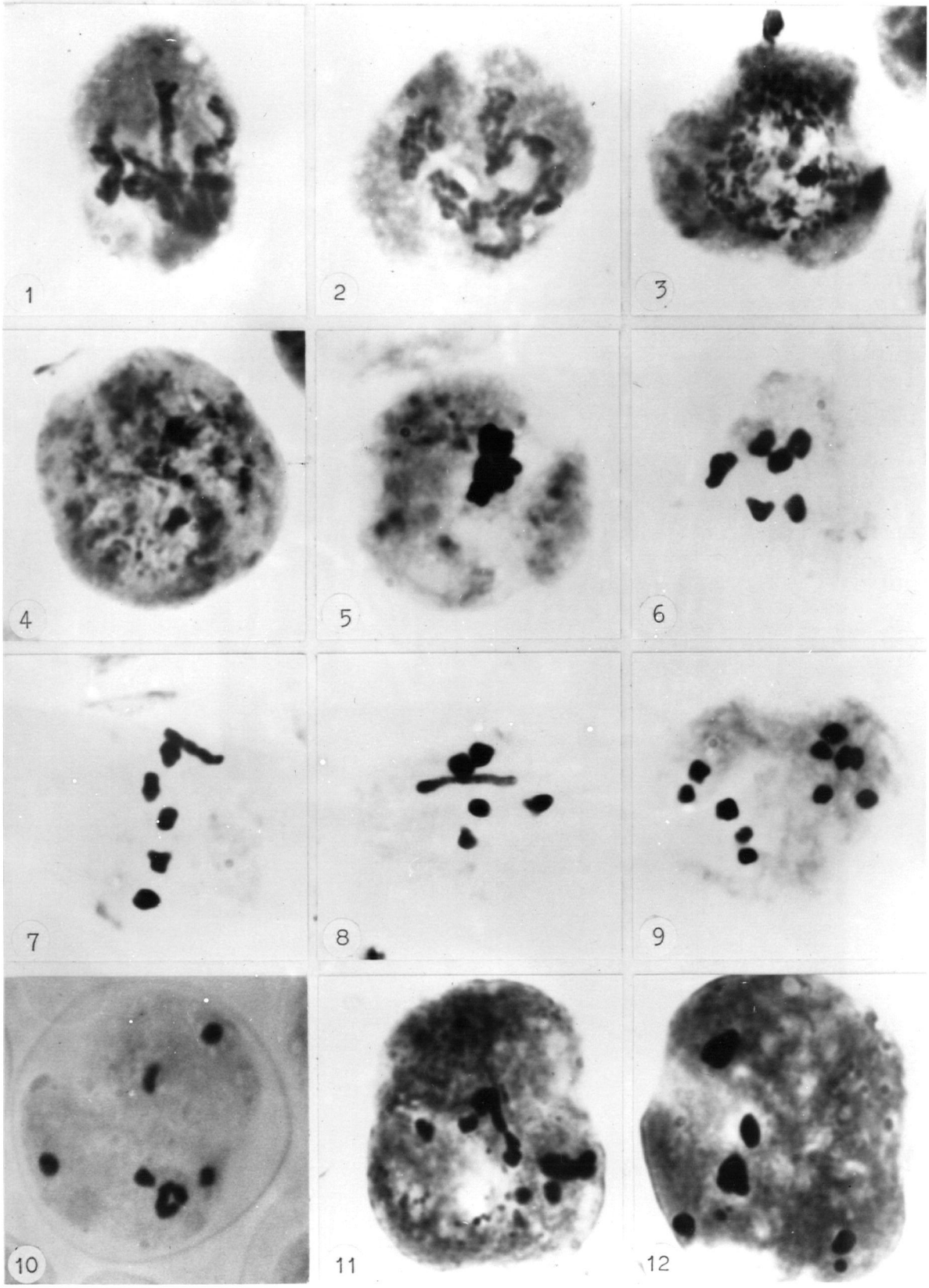
Figures 7&8, B. frigidum; metaphase I. In fig. 7 the long bivalent is located at the periphery. This is the most common situation, X2400.

Figure 9, B. frigidum; late anaphase I, X2400.

Figure 10, exp. 1, page 34; 26 hours after treatment; an unoriented metaphase I, X2400.

Figures 10&11, exp. 1, page 34; 48 hours after treatment; fig. 10; a partially clumped and poorly oriented metaphase I; fig. 11; diakinesis or unoriented metaphase I, both X2400.

PLATE 4



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DESCRIPTION OF FIGURES OF PLATE 5

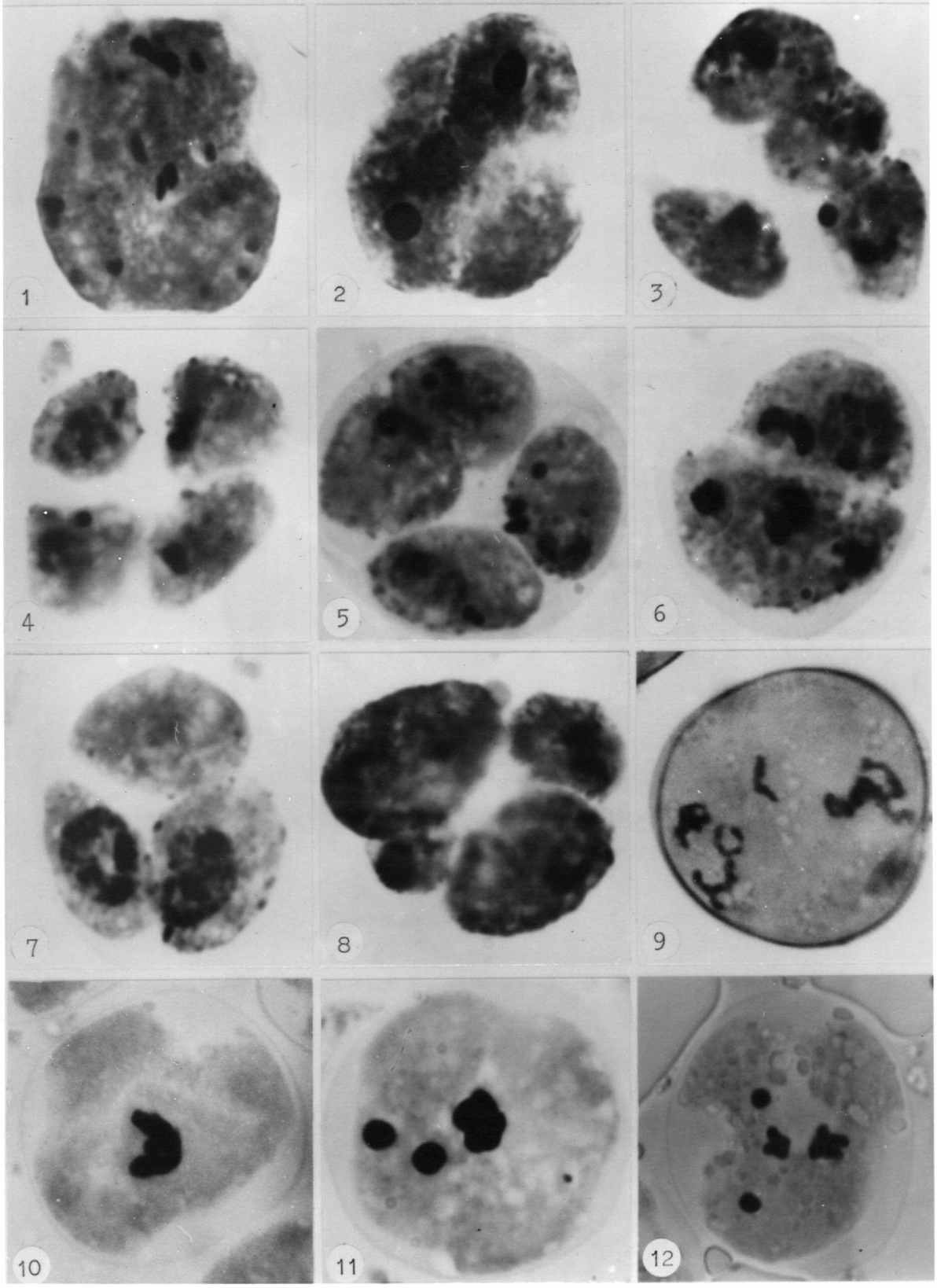
Figures 1&2, exp. 1, page 34; 48 hours after treatment; fig. 1; anaphase I or metaphase I with univalents; fig. 2; telophase I showing globular nuclei, but no laggards, both X2400.

Figures 3-8, exp. 1, page 34; 4 days after treatment; showing examples of the 4 abnormal analysis categories; fig. 3; category 1; fig. 4; category 2; fig. 5; category 3; fig. 6,7,8; category 4, all X2400.

Fig. 9, exp. 1, page 34, 4 days after treatment; showing a restitution SMC that was arrested at diplotene, X2400.

Figure 10-12, exp. 2, page 38; immediately after treatment; fig. 10; heat induced clumping at metaphase I; fig. 11; a partially clumped metaphase I with highly condensed bivalents; fig. 12; normal spindle formation with multiple association of bivalents, all X2400.

PLATE 5



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DESCRIPTION OF FIGURES OF PLATE 6

Figures 1-3, exp. 2, page 38; immediately after treatment; fig. 1; metaphase I with normal spindle formation; fig. 2; metaphase I with normal spindle formation but with highly condensed bivalents; fig. 3; normal bivalents at metaphase I, orientation is slightly abnormal, all X2400.

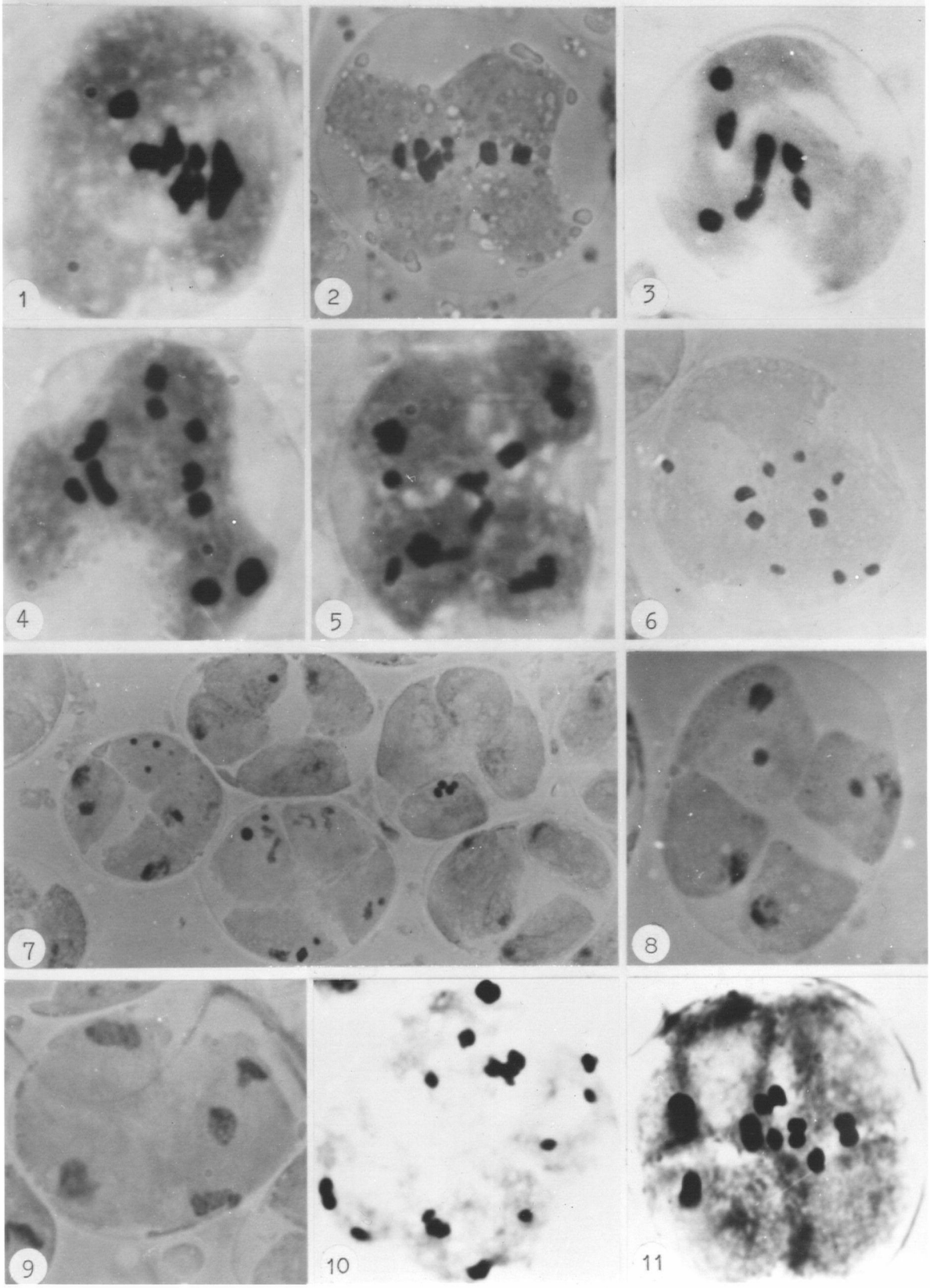
Figures 4&5, exp. 2, page 38; immediately after treatment; fig. 4; metaphase I with dissociated and unoriented bivalents; fig. 5; anaphase with lagging chromosomes, both X2400.

Figures6, exp. 2, page 38; 72 hours after treatment; showing an anaphase I displaying abnormal segregation, X2400.

Figures 7-9, exp. 2, page 38; 72 hours after treatment; fig. 7; a field of abnormal tetrads, X1650; fig. 8; a tetrad of category 2; fig. 9; a tetrad with five nuclei, both X2400.

Figure 10, exp. 3, page 40; 5 days after treatment; showing a metaphase I with good spindle arrangement and slightly over-condensed bivalents, X2400.

PLATE 6



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DESCRIPTION OF FIGURES OF PLATE 7

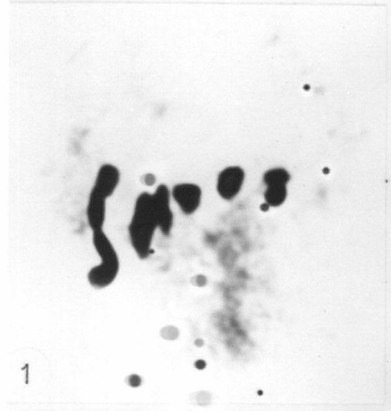
Figures 1&2, exp. 3, page 40; 7 days after treatment; fig. 1; a normal appearing metaphase I; fig. 2; anaphase I exhibiting nondisjunction of the large bivalent, both X2400.

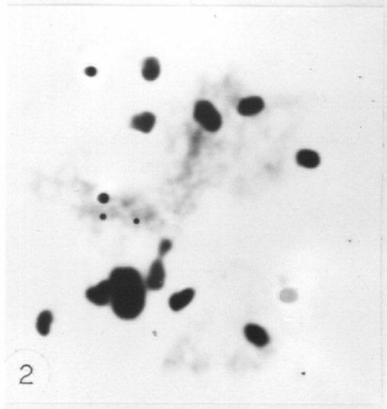
Figure 3, Heterocladium heteropteroides; abnormal active stages and tetrads after 2 weeks indoors, X2500.

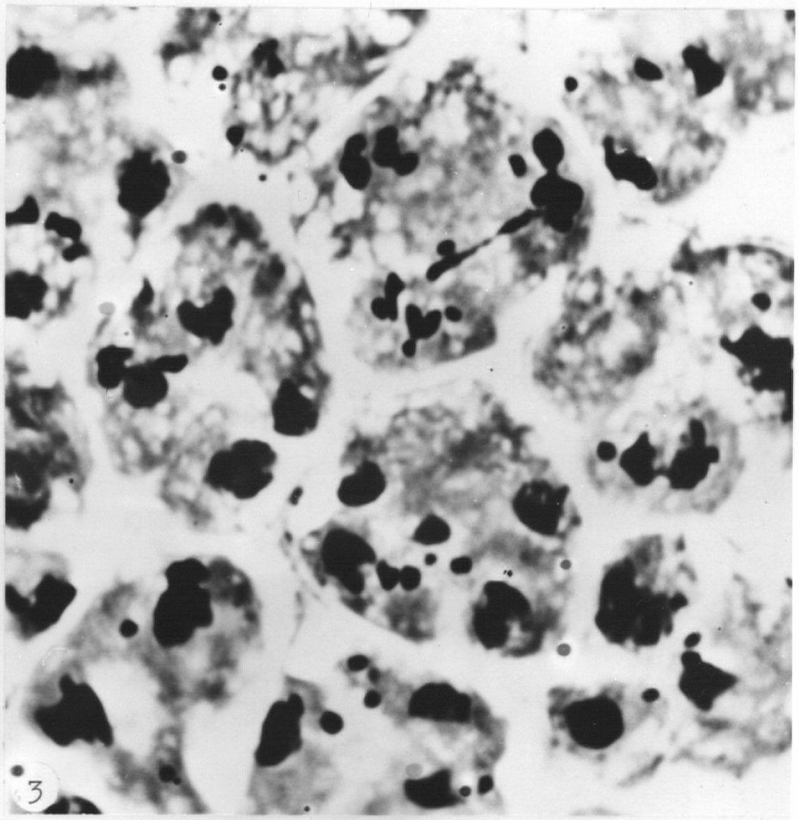
Figures 4-7, exp. 5, page 47; immediately after treatment; fig. 4; diplotene showing what appears to be abnormal pairing; fig. 5,6; a normal appearing late diplotene, all X2400; fig. 7; a field of SMC with severely clumped metaphase I, X1300.

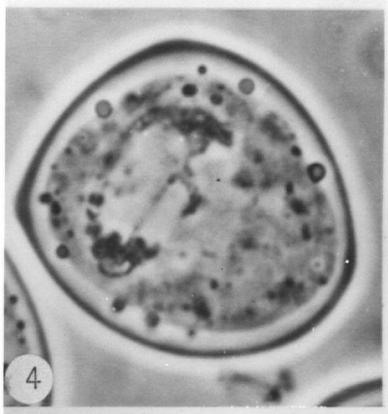
Figure 8, exp. 5, page 47; 72 hours after treatment; showing a metaphase I with some spindle orientation, but with not all the bivalents oriented, X2400.

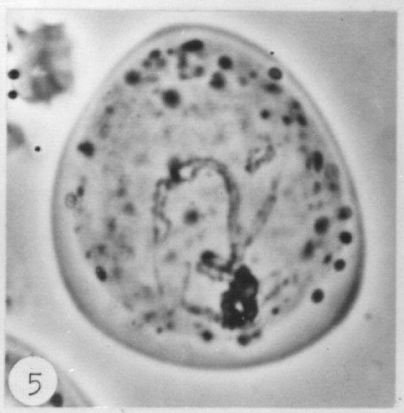
PLATE 7

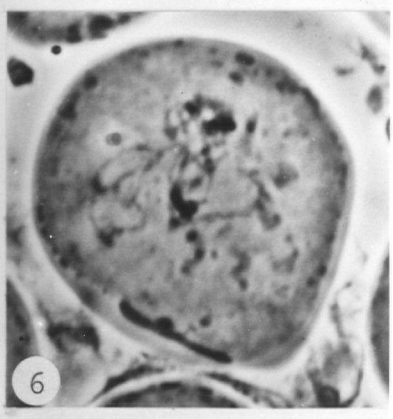


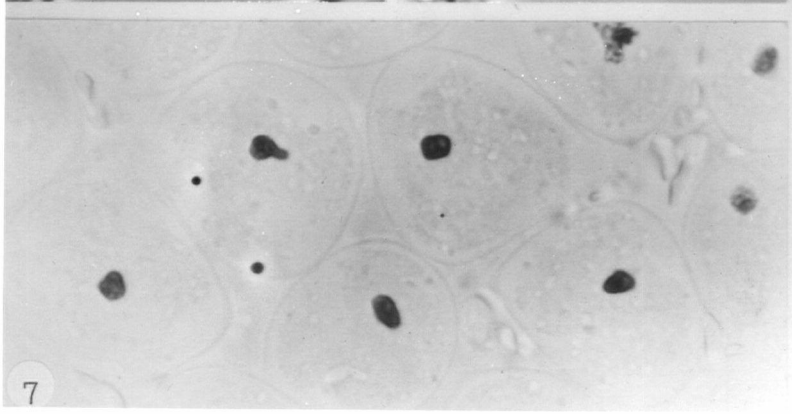


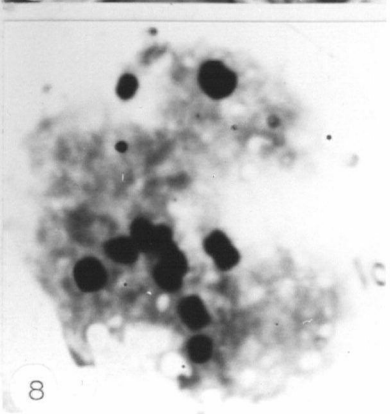












(to face Plate 8)

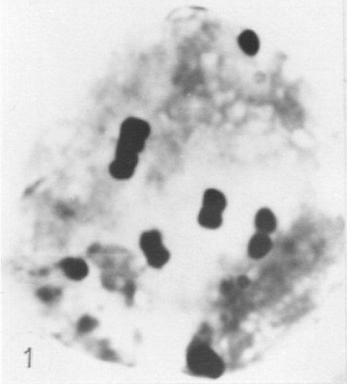
DESCRIPTION OF FIGURES OF PLATE 8

Figures 1&2, exp. 5, page 47; 72 hours after treatment; showing SMC with spindle arrangement, but with highly condensed bivalents exhibiting asynchronous disjunction or faulty orientation, both X2400.

Figures 3-5, exp. 5, page 47; 5 days after treatment; fig. 3; a restitution SMC which has partially divided; fig. 4; pycnotic spores and a restitution SMC (dotted lines indicate the fine threads of wall material which join two spores), both X2400; fig. 5; a low power field of spores and restitution SMC which have clustered into a large aggregate, X450.

Figures 6-9, exp. 6, page 51; 30 hours after the plants were brought indoors; showing various anomalies (ie. no bivalent orientation, partial clumping and precocious dissociation) at metaphase I, all X2400.

PLATE 8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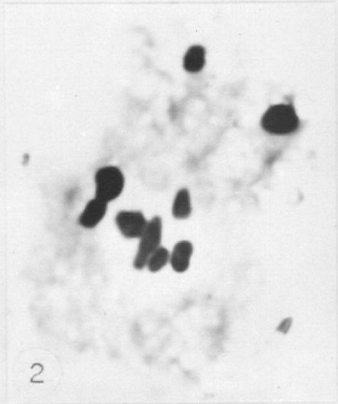
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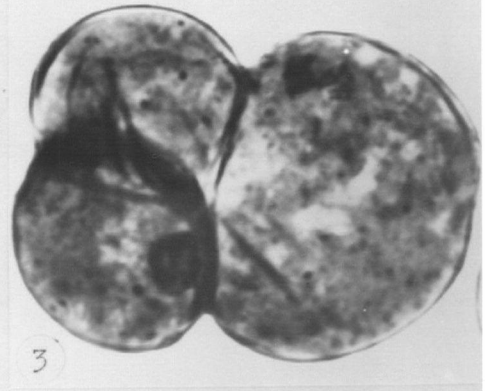
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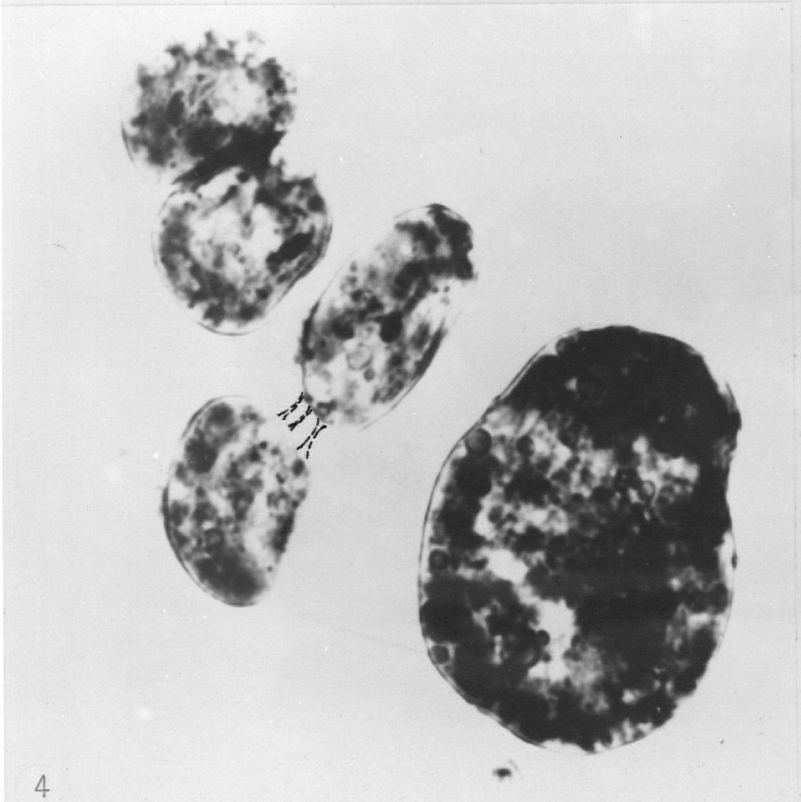
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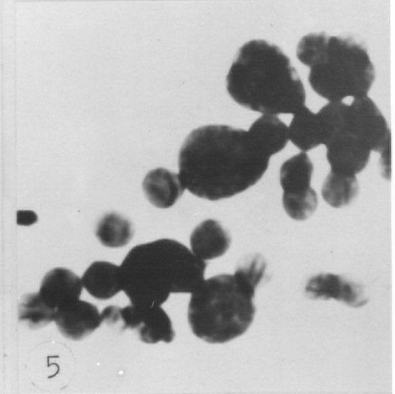
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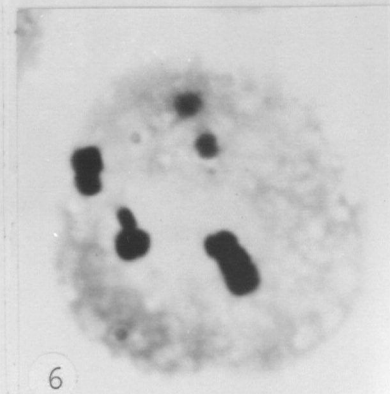
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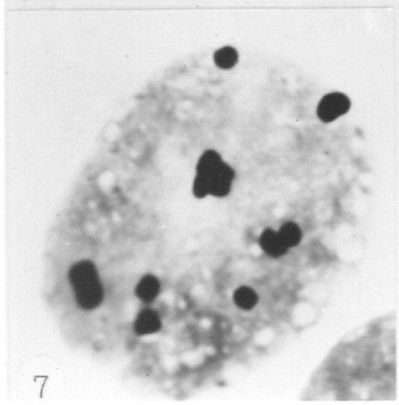
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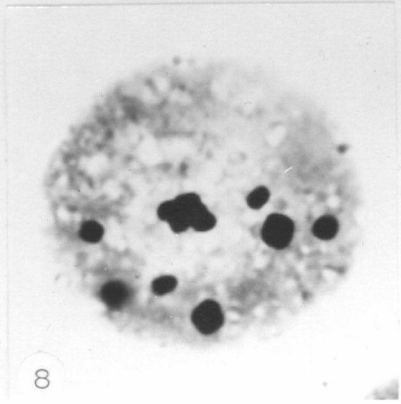
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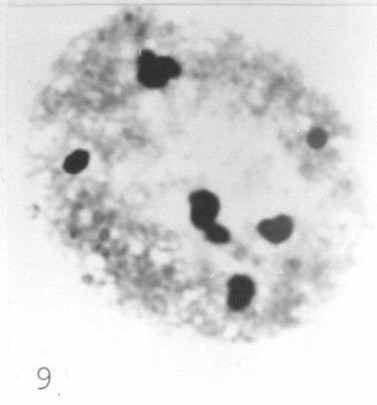
9

(to face Plate 9)

DESCRIPTION OF FIGURES OF PLATE 9

Figures 1-3, exp. 6, page 51; 72 hours after plants were brought indoors; fig. 1; aborted meiosis at first division; fig. 2; aborted meiosis during micronuclei formation, both X1300; fig. 3; a tetrad formed from a SMC after abortion of meiosis, X2400.

Figures 4&5, exp. 6, page 51; 4 days after plants were brought indoors; fig. 4; dictyotene with the chromatin pycnotic and clumped around the chromocentre; fig. 5; early prophase in which the chromatin has clumped into a ball, both X2400.

PLATE 9