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ABSTRACT OF THESIS FOR Ph.D. DEGREE.

GAMETOGENESIS AND FERTILISATION IN CERTAIN MONOCOTYLEDONS.

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ABSTRACT OF THESIS.

Gametogenesis and Fertilisation in certain Monocotyledons.

At the outset of the investigation, it was hoped to elucidate details of meiosis and fertilisation in dioecious and monoecious monocotyledons, with especial reference to the possible presence of sex chromosomes. The chromosomes of the dioecious and monoecious forms examined however, have not lent themselves to such a study, whereas the chromosomes of the hermaphrodite Scilla nonscripta, have proved especially interesting. This plant has therefore latterly been studied almost to the exclusion of others.

Scilla nonscripta.

The chromosomes during mitosis and meiosis are conceived as always consisting of at least two regularly intertwined spiral chromonemata. An attempt has been made to harmonise this conception, already held by many writers for mitosis, with the appearances observed at meiosis.

At fertilisation, the egg nucleus is usually resting and the male nucleus is in a condition corresponding to late prophase. The membrane between the two nuclei breaks down and the reticulum from the male nucleus gradually spreads through the egg nucleus. The fusion of the second male nucleus with the two polar nuclei is similar.

In all the divisions of the endosperm nuclei studied, the chromosome complements from the male and polar nuclei

remain separate, but a mingling of the two chromosome complements in the nucleus of the embryo begins to occur after the first division.

Hydrocharis morsus-ranae.

The development of the flower buds has been traced, to find if the reputed lack of seeds in this country is due to any structural peculiarity. In the course of the investigation, however, seeds have been set and the significance of this is discussed. The problem of dioecism and monoecism in the plant has also been investigated.

Other Monocotyledons.

Five other monocotyledons have been examined, but have contributed little of special interest.

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INTRODUCTION.

At the present time, the study of the finer details of chromosome structure and behaviour has aroused the interest of a large number of cytologists and is proving of the greatest importance in the progress of plant breeding. The present investigation was undertaken in the hope of contributing a little to this subject. It was also felt that the details of fertilisation, especially the behaviour of the chromosomes at that time, would well repay further study. It seemed possible that the dioecious and monoecious forms would be more interesting, as they might have sex chromosomes, and a study of the details of their behaviour at meiosis and fertilisation, a subject which hitherto has been somewhat neglected, would be well worth while. Monocotyledons were known to afford more promising material than dicotyledons, as so many of them have been found to possess large and characteristic chromosomes. A few dicotyledons viz: Laurus nobilis, Bryonia dioica, Mercurialis perennis, Were however examined and later discarded on account of their small and numerous chromosomes.

In accordance with the above plans, fixations were made of flowers of a number of monocotyledons, amongst which were Typha latifolia, Sparganium erectum, Arum maculatum, Sagittaria sagittifolia, Hydrocharis morsus-ranae, Tamus

communis and Ruscus aculeatus. Of these, Typha latifolia, Sparganium erectum, and Tamus communis were discarded on account of their small and numerous chromosomes. Sagittaria sagittifolia is promising material, as it has large clear nuclei, but it was not available in abundance and was finally given up in favour of other forms. A considerable number of fixations of Ruscus aculeatus were made, but the material has not been thoroughly worked out, partly because much of the material unexpectedly proved to be hermaphrodite and therefore to offer small hope of distinctive sex chromosomes.

Much time was spent in fixing all stages in the flower buds of Arum maculatum, from the development of the archesporium before meiosis to the formation of young embryos, and in examining a great deal of this material. This study, however, has provided little of special interest, and the chromosomes are fairly small, numerous and without distinctive characteristics.

Hydrocharis morsus-ranae has been fixed extensively and has provided several points of interest in connection with seed production, but its nuclei are small.

In April 1932, after nearly a year spent in an endeavour to find suitable material among dioecious and monoecious plants, a re-examination of material of Scilla nonscripta, a hermaphrodite form previously fixed in 1931 for classwork, revealed chromosomes of such individuality and detailed

structure that it was considered worth while to undertake a further investigation of the plant. It was then just coming into flower, therefore careful fixations were made for a study of fertilisation. In the autumn and spring of the following year, a detailed study of the development of the flower bud was made, with special reference to the structure of the chromosomes at meiosis and mitosis. The plant has proved of such interest that the study of dioecious and monoecious forms has been practically abandoned in its favour.

A COMPARATIVE STUDY OF THE CHROMOSOMES OF SCILLA NON-SCRIPTA, HOFFM. AND LINK, DURING SOMATIC AND MEIOTIC MITOSES.

HISTORICAL REVIEW.

Chromosome structure. The last fifty years have seen a great increase in our knowledge of the processes involved in cell division, and a great many papers have been written on the subject. Interest has been further stimulated by the theory - first suggested by Sutton in 1902 and subsequently supported by a large and still accumulating mass of evidence - that the chromosomes are the bearers of hereditary characters. The mechanism of reduction division has not only been found to fit in with Mendel's Laws and the subsequent discoveries of genetical workers, but has frequently been able to throw new light upon the genetical facts thus discovered. A further relationship between chromosomes and hereditary characters was proposed by Janssens (1909, 1924), who by suggesting that chromosomes may exchange partners at the "chiasmata" formed during the diplotene of meiosis, supplied a cytological basis for genetical crossing over. This idea has been developed, modified and disputed, and is held in some form or another by most cytologists at the present time, its acceptance being mainly due to the work of Janssens (1909, 1924), Wenrich (1916), Morgan and his collaborators (1919, 1922, 1925 et seq)

Darlington and his co - workers (1928, 1929, 1930, 1931, 1932), Belling (1927, 1928, 1931), Belar (1929) and many others.

In this way, especially within the last fifteen years, a great mass of data has accumulated with regard to the various stages of meiosis, including the configurations of the chromosomes at meiosis and the conditions of chromosome pairing, in diploids, hybrids and polyploids of every conceivable form and complexity. Bound up with this has been the controversy which has raged round the parasynaptic or telosynaptic interpretations of chromosome pairing. At the same time, interest has gradually been more and more aroused in the study of the internal structure of the individual chromosome. Baranetsky (1880) was the first to find a chromatic spiral embedded in an achromatic matrix in the chromosomes of living pollen mother cells of Tradescantia. His interpretation was temporarily overshadowed, but was restated later by Bonnevie (1908, 1911), working on both mitosis and meiosis in fixed material of Allium and Ascaris and has subsequently been supported by a number of workers. Vejdowsky (1911, 1912) announced the presence of a spiral filament at certain stages of mitosis, to which he first gave the name "chromonema". Spiral filaments - either single or double - in somatic mitosis and meiosis have been described, to give further examples, by Van Herwerden (1910) Janssens (1924), Kuwada and Sugimoto (1926), Kuwada (1926, 1927), Newton (1924, 1927), Sakamura (1927), Kaufmann (1926, 1931),

Sharp (1929), Maeda (1928, 1930), Babcock and Clausen (1929), Telezynsky (1930, 1931), Shinke (1930), Sax (1930), Hedayetullah (1931), Taylor (1931), de Winiwarter (1931), Tuan (1931), Hsu-Siang (1932), Smith (1932), Darlington (1932) and Perry (1932). It is evident, therefore, that this conception has much support at the present time.

On the other hand, Grégoire (1903, 1906), suggested that the chromosomes were composed of a chromatic substance impregnating an achromatic core. From telophase to prophase of division, the chromatin was withdrawn more and more from certain parts of the achromatic core, thus giving the appearance of alveolation, until the result in the resting nucleus was a network of fine chromatic filaments on an achromatic groundwork. On this hypothesis the spirals observed by other workers were interpreted by stating that the chromatic network might sometimes (by chance) give the appearance of a spiral, but this had no structural importance or autonomy. Grégoire held that the alveolated chromosomes themselves fused in the resting nucleus, giving "un réseau de réseaux". The theory, or a variant of it, was supported by E. Strasburger (1905), Fraser and Snell (1911), Muller (1912), Overton (1922) and Sharp in his earlier work (1920). A modification has recently been put forward by Koerperich (1930), who says that the telophase nucleus forms a network of chromatin on an achromatic matrix; the network persists as such during interphase, but condenses into a spiral at prophase.

The idea of a reticulum in a matrix at telophase is therefore combined with that of a spiral filament at prophase. This is similar to the earlier descriptions of Overton and Sharp.

Bolles-Lee (1925) combined the conception of alveolisation with the presence of a spiral ridge running round the outside of the chromosome core. The chromosome was stated to consist of a chromatic cylinder, which is alveolated in plants and solid in animals. Around the alveolated chromophilous axis and forming part of it, is a peri-axial spiral, from the turns of which processes run outwards through the wide colourless sheath which he supposed to surround the axis itself.

Matens (1922, ~~1924~~, 1925) believed in an achromatic matrix, on which is a zigzag chromatic thread, neither spiral nor continuous. This involved a different conception of the method of chromosome division from that of most other workers, as it was not considered as a simple longitudinal division.

Sands (1923, 1925) in common with other workers, believed that the chromosome consists of an achromatic core, on which, the chromatic part is disposed as granules or chromomeres. These sometimes (by chance) assumed a spiral form. The idea of a row of granules in an achromatic groundwork was first put forward by Pfitzner (1882). Vejdowsky thought that the chromonemata became split up into chromomeres in late prophase, but joined together again to form a chromonema at teloplase. Bolles-Lee (1925) denied the existence of chromomeres,

stating that they were due to "illseen and faultily interpreted images and twists of the axes of the chromosomes". Kaufmann (1931), Smith (1932) and Maeda (1930) hold similar views, but Hedayetullah (1931) finds chromomeres in practically all stages of division when Merkel's fluid is used as a fixative. Chromomeres have been reported most often in meiosis, especially in prophase (Wenrich 1916, Gelei 1921; Reuter 1930 etc.), when each chromosome consists of a series of chromomeres of different sizes, connected by a less deeply staining thread. They have been most thoroughly investigated by Belling, who has been able to count them for Lilium and Aloe and has found that their number and linear arrangement is constant. At zygotene they pair specifically and identically, each with its homologous partner, but in later stages their individuality is hidden by extra chromatin which is produced around them. Microdissection of living chromosomes has supported this hypothesis (Chambers).

The Matrix of the Chromosome. Most recent workers on chromosome structure believe that the chromosome is composed of two parts - (1) chromatic spirals, filaments, reticula or chromomeres, (2) a less chromatic core in which these are embedded or on which they are supported. This duality has been confirmed, for example, by Baranetsky, Grégoire, Overton, Bonnevie, Martens, Sands, Sharp, Hedayetullah, Kaufmann, Taylor and Hsu-Siang, although these authors differ quite considerably amongst themselves on the interpretation they

give to the chromatic constituent. Most of them seem to regard the matrix as an autonomous unit of some importance, splitting at a regular stage in the chromosome cycle: usually from late prophase to early anaphase of the division in which the two halves will separate. Darlington, however, discounts the morphological evidence for the presence of chromatic and achromatic parts, but believes in the existence of a "pellicle" for reasons deduced from other data (Darlington 1932, p.269). Perry does not regard the matrix as a mould limiting the chromosomes, but as a fluid substance held between the turns of the spiral. The presence of an achromatic core with a chromatic periphery has been supported by the work of Sands (1925), Chambers and Sands (1923) and Telezynsky (1930) on living chromosomes, the former two using microdissection methods. The matrix apparently disappears into the resting nucleus, and it has therefore often been suggested that it is transformed either wholly or in part into the Karyolymph or a portion of the Karyolymph (Kaufmann, Siang; Taylor; Sharp). Where the new karyolymph comes into contact with the cytoplasm, the nuclear membrane is formed.

The resting nucleus. The structure of the resting nucleus has been the subject of some controversy in the past, certain investigators believing that the effects observed after fixation correspond to a real structure present in the living condition, whilst others believe that the reticulum observed is probably due to the precipitation by fixatives

of a structureless colloid. A reticulum was found in living cells by Martens (1927), Lundegardh (1912) and de Litardière (1921). Telezynsky (1930) finds that the living nucleus is filled with parallel rows of granules, which he interprets as optical sections through spiral filaments. Schaede (1925, ~~-31~~), Chambers (1924), Lewis and Lewis (1924) and Belar (1928), on the other hand, state that the living nucleus is homogeneous, with no visible or detectable internal structure and a reticulum only appears as the result of experimental injury or fixation. Direct methods are at present inconclusive for either view. The permancy of the chromosomes, however, has been indisputably established by indirect reasoning, and therefore the essential parts of the chromosomes must still be present, even though they may sometimes be invisible.

The split in the chromosome. One of the most controversial questions connected with chromosome structure centres round the time in the nuclear cycle at which the chromatic part divides, and its relation to the chromosome separation. The earliest conception was that of a split in the prophase immediately before the separation of the daughter chromosomes. Grégoire, Overton, Martens and others held this view, although Martens did not believe that the chromatic constituent divided longitudinally. Darlington (1932) states that in favourable preparations, the somatic chromosomes can be seen to be double from the earliest stages

of prophase. He therefore proposes a division in the interphase immediately preceding the anaphase in which the daughter chromosomes will separate. His most cogent reason for discounting an earlier division of the chromatic thread is that the leptotene threads appearing at the earlier prophases of meiosis are always single, and no trace of a split has ever been satisfactorily proved. His views will be discussed later.

The alveolation observed at telophase has been supposed in the past to be the cause of chromosome splitting: the alveoles always being median (Fraser and Snell 1911, Fraser 1914; Digby 1919; Carruthers 1921). The split might even be caused by the pull exerted by the anastomoses between the chromosomes (Fraser and Snell). This view was opposed by other workers (Grégoire, Sharp, Gates, Overton) on the grounds that the alveoles were not always median in position nor in a straight line.

Meanwhile, other investigators had suggested that the double nature of the chromatic thread was evident before this. Merriman (1904) Lundegardh (1912) and Sarbadhikari (1924) believed that the split revealed at telophase was initiated at anaphase. Granier and Boule (1911) found the double structure was evident at late metaphase, with some indications of it even earlier. The recent studies of Hedayetullah and Perry also suggest a split at metaphase. Other investigators have found that the division of the chromatic filament occurs even earlier, being first evident at late prophase (Kaufmann,

Telezynsky, Sharp, Smith, Hsu-Siang) but probably actually taking place at some still earlier stage. This means that for a short period in the nuclear cycle, namely from late prophase (at least) to metaphase, the chromatic complement is quadruple. Taylor (and also de Horne 1911) even finds evidences of the split in the telophase before this division (i.e. two divisions before the chromatic filaments actually separate to opposite poles).

A review of the most recent detailed work on mitosis in favourable material therefore reveals the fact that the majority of evidence supports the existence of two chromonemata in each daughter chromosome by at least early metaphase. An examination of the majority of papers on meiosis, however, reveals a widely divergent view. For example, the threads at leptotene are stated to be single by Newton (1927), Babcock and Clausen (1929), Belling (1928, 1931), Kuwada (1927), Shinke (1930), Darlington and his fellow workers (1929, 1930, 1931, 1932) and many others. In fact, so far there has been no satisfactory evidence that the chromosomes which pair in zygotene are ever double, although they have been carefully examined by hundreds of investigators. Kaufmann (1931) states that he finds evidence of a split in leptotene, but so far he has given no detailed descriptions or illustrations in support of his statement. The first indication of a split - giving the tetrad condition - has been reported in pachytene (Belling 1931, Darlington 1932) and has been observed in

diplotene by many investigators (Janssens ~~1924~~; Newton ~~1927~~; Babcock and Clausen; Shinke; Taylor etc.). From this stage onwards, evidence of the double nature of the two chromosomes becomes increasingly evident, until they separate at anaphase. In some material, a tetrad may never be seen, but modern work indicates that it is really present, although obscured from view.

Parasynapsis v. Telosynapsis. A great controversy has raged in past years around the telosynaptic and parasynaptic interpretations of chromosome pairing at meiosis, that is, whether the homologous chromosome pair end to end, or side by side. The telosynaptic view was first suggested by Haecker (1892), who assumed that the thick double threads seen at the prophase of meiosis (pachytene) are identical genetically with the double threads observed at the prophase of mitosis - i.e they are half chromosomes. The chromosomes at this time were supposed to be united at prophase into a "continuous spireme", and this "segmented" into the diploid number of chromosomes at mitosis in the sporophyte and into the haploid number of chromosomes at meiosis. This hypothesis was worked into a vigorous theory by Farmer and Moore (1903, 1905), Digby (1910, 1912, 1914, 1919), Mottier (1907, 1909, 1914), Fraser (1914), Gates (1911, 1921, 1924 et seq), Santos (1923, 1924), Latter (1926) and others. The theory was modified by the later workers as new facts were brought to light, and it was said that the paired chromosomes twisted

round each other at the "second contraction" or "brochonema" (Latter), so that they were brought side by side, and might not remain connected by the original point of union. This explained the observed presence of a "tetrad structure" - four chromatids - at diplotene.

The alternative theory of parasynapsis was put forward by de Winiwarter (1900). On this interpretation, the double threads seen at pachytene are whole homologous chromosomes which pair side by side along their length. Later they fall apart, both terminally and interstitially, and it is then evident that each chromosome has itself divided longitudinally, so that a tetrad structure is formed. This theory has been supported by Janssens, Grégoire, Wenrich, Robertson, Wilson, Gelei, Bouin, Strasburger, Yamanouchi, Babcock and Clausen, Belling, Bělar, Darlington and the majority of workers on meiosis at the present time.

The increased favour in which this last theory is held is mainly due to the following facts:-

1. The counting of the threads before and after zygotene pairing has shown that the single threads are really whole somatic chromosomes (Gelei 1921; Janssens 1924; Newton and Darlington 1929; Moffett 1932).

2. In some animals, the homologous pairs of chromosomes are heteromorphic, showing a constant difference in length, which would not be found in the halves of a split chromosome. These heteromorphic threads pair side by side in zygotene,

therefore here parasynapsis evidently occurs ([redacted] Gelei 1921; Wenrich 1916).

3. A continuous spireme can no longer be found in favourable material, and there is evidence that the absence of ends in the reticulum at prophase is at least partially due to their collapse against other threads, the nucleolus and the nuclear membrane, under the influence of the fixing fluids. (Martens 1929; Fikry 1930; Taylor 1931; Darlington 1932). With some fixatives, too, the paired chromosomes appear almost completely fused, and this has favoured the telosynaptic interpretation in the past (Gates 1931; Latter 1932).

4. Careful observations on the behaviour of hybrid polyploids show that the threads associate in threes and fours etc. at zygotene, any two of them pairing at random along the threads. This can only be interpreted from the parasynaptic standpoint. (Belling 1927, 1928; Newton and Darlington 1929; Darlington 1931; Catcheside 1931.)

5. Newton (1927 on Tulip) and others have traced the development of the bivalents from the stage where the two threads associate at zygotene, up to diakinesis, and have shown how the second split arises in the paired chromosomes themselves, and not by folding over. The splits as they appear have been carefully investigated by believers in the chiasmatype theory (Janssens 1924; Belling 1930; Darlington 1932) who have shown that the coiled threads of strepsitene are really

identical with the looped threads of diplotene.

The strongest case for the telosynaptic interpretation has been obtained from the study of Oenothera and its mutants. Here the chromosomes are attached end to end in diakinesis, and the obvious explanation is that this configuration arises from telosynaptic pairing. Recently, Darlington (1931) has put forward an alternative explanation of the phenomenon based upon the suggestion that the end of one chromosome is homologous with the end of another and therefore pairs with it. The ring formation is ultimately produced by terminalisation of the chiasmata. This explanation has been verified for Rhoeo, Pisum and a few other forms with chains of chromosomes at diakinesis. Further evidence for the essential correctness of the theory for Oenothera has recently been obtained by Catcheside (1931) and by Gates (1931), " one of the pioneer workers on Oenothera from the telosynaptic point of view. It is evident, therefore, that telosynapsis is becoming less and less adequate to fit the facts revealed by improving technique. Parasynapsis is also much more compatible with the theory of linear differentiation of the chromosome, as upheld by genetical workers.

The chiasmatypy theory. When the two homologous chromosomes fall apart at diplotene, they form rings and crosses with one another, and in favourable material it may actually be seen where the two chromosomes cross, a half chromosome (chromatid) from one chromosome has crossed over to take the

place of a chromatid belonging to the homologous partner, and vice versa. It has now been proved by genetical work (see Plough, Morgan, Sturtevant etc.) that crossing over of genetical characters does actually occur during meiosis. Since the chromosomes are now regarded as the main bearers of hereditary characters, it is evident that these genetical cross-overs must be correlated with actual crossing over between homologous chromosomes. The cross-overs observed at diplotene therefore provide the most obvious mechanism to corrobate genetical evidence. The idea was suggested by Ruckert (1892) that the chromosomes exchanged material at these points, - now known as chiasmata - but the hypothesis was at first overshadowed by the conception of chromosome permanency.

Meanwhile Janssens (1909; 1924) put forward his "chiasmatypy theory" as the result of outstanding careful work on the details of meiosis in certain insects. Janssens considered that a chiasma might arise in three ways:-

1. By crossing over occurring "before the division of the chromosomes into four chromatids", so that all the four chromatids of a tetrad cross over at a chiasma. This is "total chiasmatypy".

2. By only two of the four chromatids formed after division exchanging partners at the point of crossing. This is "partial chiasmatypy".

3. Two of the four chromatids might break and reunite in such a way as to leave no exchange of partner.

In all of these alternative conceptions, Janssens assumed that the sister chromatids are always held together, and the partner chromatids repelled at the diplotene loops. All the loops may therefore be said to be reductional. This view is in contrast to the alternative theory of Wenrich (1916) who thought that no crossing over occurs, but that a chiasma is due to the meeting of loops separating the chromatids equationally and reductionally. This is generally known as the "classical theory".

Chodat (1925) and Maeda (1930) suppose that chiasmata arise through the opening of reductional and equational loops, as Wenrich suggested, but as the chromosomes begin to separate at anaphase, they break and reunite in such a way that the chromatids in the arms distal to the chiasma need not pull apart.

Darlington (1929) originally thought it possible that the chiasmata arose by the meeting of reductional and equational loops without crossing over. They then broke and reunited at diplotene in such a way as to resolve the chiasmata. This theory has now been discarded by Darlington as untenable in the face of new facts.

Sax (1930) also believes in a similar resolution of the chiasmata at diplotene. The chiasmata are points of interchange of partners among paired chromatids (as in the classical theory). Strains on them, however, are liable to cause breaks at the points of contact. The strains are caused by

the internodes widening, and the chromonemata twisting and contracting unequally. Subsequently the broken ends of the chromatids reunite, with exchange of partners, so that the chiasmata are resolved. Some of them still remain unbroken, however, and they are then pulled apart at anaphase with no exchange of partners.

Darlington (1930-32) now regards the chiasmata at diplotene as indicating the points where exchange of partners between two chromatids has already occurred at pachytene. The loops at diplotene are always reductional and hence he has adopted and extended the original "partial chiasmatypy". theory of Janssens, discarding the other alternatives as inadequate. Belling (1927, 1928) has also adopted this point of view.

It is not within the scope of this paper to analyse the relative weight of the reasons given in favour of the various hypotheses enumerated above. These are mainly genetical and are reviewed by Catcheside (1931) and Darlington (1931, 1932). As Catcheside says "Most are agreed that the point of exchange of chromatid segments is actually at the chiasma". Genetical evidence derived from work on Drosophila destroys Janssen's theory of total chiasmatypy. The occurrence of terminalisation cannot be explained satisfactorily on Sax's hypothesis, and the figures of interlocking that should be expected from it in certain hybrids have never been obtained. (Darlington 1932, p. 258). Genetical and cytological data (Bridges and

Anderson 1925; Darlington 1930; Catcheside 1931) show that crossing over occurs in the pachytene stage. It is therefore probable that the "chiasmatypy hypothesis" as advanced by Darlington and Belling is substantially correct.

Correlated with the chiasmatypy hypothesis of Darlington is his conception of the terminalisation of chiasmata in certain plants. The points of crossing of the already interchanged partners of the chromatids slip towards the ends of the chromosomes, with the result that the number of chiasmata seen between diplotene and metaphase is progressively reduced. Terminalisation may be either absent, partial or complete in different species of plants and animals.

THE SCOPE OF THE PRESENT WORK.

On examining the mass of rather confused and conflicting data thus presented to our view, the fact emerges that few comparative studies of meiosis and mitosis have yet been made, and even these make little effort to discuss or correlate the modern point of view. On one hand, the investigators of somatic mitoses are moving towards unanimity concerning the presence of two chromonemata, probably surrounded by a common matrix, within each daughter chromosome, giving a quadruple structure by at least the beginning of metaphase. On the other hand, the mass of work done on large chromosomes at meiosis assumes the existence of

single threads at leptotene, pairing at zygotene, and each dividing for the first time at pachytene. Theories of crossing over have been founded on, or strongly supported by, this point of view (Belling, 1928, 1931; Darlington 1931, 1932). Such comparative accounts of meiosis and mitosis as have been encountered during the study of the literature (eg. Smith 1932) have ignored the point of view of a large body of investigators of meiosis. Darlington (1932) discusses mitosis in relation to meiosis, but dismisses as inadequate or faultily interpreted the point of view of the most careful students of mitosis, a dismissal which seems to the writer to be unwarranted in the face of the mass of detailed information thus acquired.

It seems, therefore, that a comparative account of somatic and meiotic mitoses, with special reference to chromosome structure is urgently needed at the present time. Even if the problems raised are not immediately solved, their discussion may stimulate an enquiry amongst other material, which may prove more decisive and may help to remove some of the inconsistencies with which cytologists are at present confronted.

MATERIAL AND METHODS.

In the autumn of 1930-31, young flower buds of Scilla non-scripta were cut and stained, for a class study in cytological technique. The material was fixed in Carnoy's fluid, or in a modification of Carnoy containing less absolute alcohol. In the course of examining this material

it was discovered that the somatic chromosomes showed very clear double spiral chromonemata in the metaphase and anaphase stages of division. The study of these interesting structures, however, was not resumed until the autumn of 1932.

The plants used were mainly those of Scilla nonscripta, the common bluebell, obtained either semi-wild in the grounds of Royal Holloway College or from the College botanical garden. The wild and garden plants were kept separate, but no difference was found in their nuclear constitution. They were also compared occasionally with flowers of Scilla campanulata (Hoffm. and Link.) also grown in the College garden, but the nuclei of the latter plants seemed identical in all respects with those of Scilla nonscripta.

Stages of meiosis in the anthers were mainly studied by means of pollen smears, but they were supplemented by material fixed by ordinary methods, and sectioned in paraffin. Suitable stages, both for smearing and fixing, were first identified with the aid of temporary iron aceto-carmine smears, as recommended by Belling (1921). Permanent pollen smears were made by a modification of Taylor's smear method, given by La Cour (1931). Pollen mother cells were teased out on to a glass slide, flattened along it by one smooth even stroke of a clean scalpel, and then inverted immediately, face downwards, in a dish of fixative. The dishes used had two parallel ribs across the bottom, which prevented the smeared surface of the slide from coming into contact with the

rest of the dish. The smears were left in the fixative from two to three hours, after which they were washed in running water for an hour, then bleached in an aqueous solution of hydrogen peroxide in water (one volume hydrogen peroxide ; two volumes of water) for about half an hour, the slides being illuminated the while with bright electric light to hasten the reaction. (Where non-osmic fixatives were used, it was still found advisable in general to bleach the slides for a brief period to rid them of other precipitates. Clearer preparations were obtained in this way. This statement also applies to paraffin sections.) From this the slides were rinsed in water, and thus easily transferred to an aqueous stain. A variety of fixatives were employed, including the La Cour modifications of Flemming's solution (2BE and 2BD), Flemming's solutions weak, medium and strong, Benda's fluid, Navaschin's solution (Langlet's modification), Allen's modification of Bouin's fluid (in this case the smears were fixed in a dish of the fluid on the top of a warm oven and washed according to schedule in alcohol before staining), Merkel's fluid, Bouin's solution etc. Of these, the osmic fixatives and Navaschin's fluid proved by far the best for meiosis in this material, the favourite osmic fixatives being (in order of merit) 2BE, Flemming's weak solution and 2BD. 2BE seemed slightly better for chromosome structure and gave very pretty preparations, but Flemming's weak solution was almost as good in most cases.

Navaschin's fluid was also found to be excellent for chromosome structure, especially in the later stages of meiosis, and sometimes gave a better effect than the 2BE, as the component parts of the chromosomes were more clearly differentiated. 2BE and Flemming's weak, however, were better for the prophase stages of the first division, and 2BE gave more critical preparations of the second division of meiosis. The 2BD fixative was not quite so good for smears, yet its merits were not greatly below the other three fixatives. Flemming's strong solution appeared to coagulate the reticulum and cannot be recommended for this plant, whilst Flemming's medium solution was not much better. Benda's fluid was not extensively used for smears, as again a ragged effect was produced in the nucleus., and chromosome/structure was obscured. Allen's modification of Bouin's fluid or Merkel's fluid gave clear preparations, but again chromosome structure was less evident, although indicated in favourable slides. Bouin's solution, medium chrom-acetic solution etc. gave very poor fixation.

Experiments with varying proportions of the constituents of Flemming's and La Cour's fixatives, mixtures of these fluids etc. gave moderately good results, but none were so good as the standard solutions. Such experiments however, were not systematically carried out.

Permanent aceto carmine smears were made, using Steere's (1931) modification of Belling's temporary stain. Slides can

be made and mounted by this method in twenty to thirty minutes (or less with practice). For detailed structure, however, the stain proved disappointing, as differentiation was not sufficiently distinct between chromosomes and cytoplasm, and fixation was inferior to that obtained by ordinary smear methods. Its use was therefore discontinued, especially as the ordinary smear methods were completed in four hours if necessary, and this length of time did not prove inconveniently protracted.

Stages of meiosis and the development of the gametophyte in the ovary, also comparative stages of meiosis in the anthers were made by means of fixing whole buds or ovaries. In some cases, pieces of anthers and ovaries were put into the fixative and a pump used to sink them in the liquid. In general, however, the method first suggested by Kihara (1924) or a modification of it, was used. (see also Maeda 1930; Babcock and Clausen 1929; La Cour 1931 etc.) This consisted in dropping whole buds - or preferably ovaries for the study of meiosis in the megaspore mother cells - into Carnoy's fluid for from fifteen seconds to one minute, and then rapidly transferring them to another fixative for about twenty four hours. Fixation by this method seemed as good or better than when Carnoy's fluid was not used, and the laborious and possibly injurious use of the pump was avoided. They were then washed in water for three to four hours on top of a warm oven, changing the water about every half hour.

(La Cour)

~~(La Cour)~~. After this, they were taken up the alcohols and embedded in paraffin wax according to the schedule recommended by La Cour, using chloroform as the solvent for the paraffin. In general, about three to four days on and in the oven sufficed for adequate infiltration of the wax, but the rate of infiltration must necessarily depend on the type of material, its size and its stage of development. The fixatives used were very varied, and once more Navaschin's fluid, 2BE, 2BD and Flemming's weak solutions were the four best fluids, being all more or less equally suitable and equally used for stages in meiosis. For somatic structure, Navaschin's fluid was the clearest, but was closely rivalled in effect by 2BE. The effect of other fixatives was similar to that described for smear preparations, Merkel's fluid ranking as the next best fixative.

Sections were cut from 16 - 18μ. This thickness was chosen, after experimentation, as the one which gave whole nuclei fairly easily in favourable sections, and yet did not obscure the structure of individual nuclei by causing others to lie under them or on top of them to too great an extent.

Somatic structure was determined almost entirely from nuclear divisions in the nucleus or walls of the ovary and anthers. A few comparative studies were made from root tips fixed in various fixatives, but in general, chromosome structure was not so clearly shown in these, though it was obviously similar in type. Additional treatment consisted

of fixing root tips for a short time (2 - 5 minutes) in boiling water (Sakamura 1927). This, however, as Sharp remarks (1929) dissolves the chromosomes in later stages of division, so that only early prophase stages are shown and these are somewhat contorted. Experiments with shorter periods of treatment with boiling water were not carried out, owing to lack of time, but it would be worth while striving to imitate Sakamura's beautiful preparations.

Of the stains used, Newton's modification of Gentian Violet - iodine proved by far the most useful, and it was finally adopted almost exclusively. It gave beautifully transparent smear preparations, in which the chromosomes stood out clearly defined and well differentiated internally. Haematoxylin was found to be greatly inferior for smears, for not only were the cytoplasm and nuclei less clearly contrasted, but the non-transparent stain frequently obscured the nuclei by clinging to the mucilage and disorganised tapetal cells with which the pollen mother cells were necessarily surrounded, and by accentuating the overlying clumps in which the pollen mother cells themselves were often found. Safranin and light green were tried, but were found a very poor combination for details of nuclear structure. Similarly in sections of the thickness chosen, gentian violet was unequalled in every way for transparency, clearness and delicate contrast of parts. It was sometimes used with a counterstain of orange G, light green, or

erythrosin in clove oil (generally the first), but was usually preferred without a counterstain, as this often seemed to obscure rather than clarify detailed structure. Haematoxylin was tried, and was found to be successful in showing up structure, especially when used according to the short schedules recommended by Kaufmann (1926) and Hedayetullah (1930). Such slides were mainly used merely for comparison, as they were much less clear in detail than the gentian violet preparations.

LITERATURE CITED.

1929 Babcock,E.B. and Clausen,J. : Meiosis in two species and three hybrids of Crepis and its bearing on taxonomic relationship; Univ. Calif. Publ. Agric. Sci. 2, 401-425.

1880 Baranetsky,J. : Die Kernteilung in den Pollenmutterzellen einiger Tradescantien; Bot. Zeit, 38, 241-247, 265-274, 281-296.

1928 Belar,K. : Uber die Naturtreue des fixierten Präparats; V. Int. Kong. Vererb, 1, 402-407.

1929 Id. : Beiträge zur Kausalanalyse der Mitose. III Untersuchungen an den Staubfaden Haarzellen und Blattmeristemzellen von Tradescantia virginica; Zeits. Zellforsch. u. mikr. Anat., 10, 73-134.

1927 Belling,J. : Configurations of bivalents of Hyacinthus with regard to segmental interchange; Biol. Bull., 52, 480-487.

1928 Id. : A working hypothesis for segmental interchange between homologous chromosomes in flowering plants; Univ. Calif. Publ. Bot., 14, 283-291.

1928 Id. : Segmental interchange and crossing over; Nature, 121, 282-283.

1931 Id. : Chromomeres of Liliaceous plants; Univ. Calif. Publ. Bot., 16, 153-170.

1898 Blackman,V.H. : On the cytological features of fertilisation and related phenomena in Pinus sylvestris; Phil. Trans. Roy. Soc., 190, 395-426.

1913 Blackman,V.H. and Welsford,E.J. : Fertilisation in Lilium; Ann. Bot. 27, 111-113.

1908 Bonnevie,K. : Chromosomenstudien. I. Chromosomen von Ascaris, Allium und Amphinema. Ein Beitrag zur Lehre der Chromosomenindividualitat; Arch. f. Zellf, 1, 450-514.

1911 Id. : Chromosomenstudien III. Chromatinreifung in Allium cepa; Arch. f. Zellf, 6, 190-253.

1925 Bouin,P. : Les cinèses de maturation et la double spermatogenèse chez Scolopendra cingulata; La Cellule, 35, 371-423.

1925 Bridges,C.B. and Anderson,E.G. : Crossing over in the X-chromosomes of triploid females of Drosophila melanogaster; Genetics, 10, 418-441.

1921 Carruthers,D. : The somatic mitoses in Hyacinthus orientalis, var, albulus; Arch. f. Zellf., 15, 370-376.

1931 Catcheside,D.G. : Critical evidence of Parasynapsis in Oenothera; Proc. Roy. Soc., 109, B, 165-184.

1899 Chamberlain,C.J. : Oogenesis in Pinus laricio; Bot. Gaz., 27, 268-280.

1916 Id. : Stangeria paradoxa; Ibid, 61, 353-372.

1923 Chambers,R. and Sands,H.C. : A dissection of the chromosomes in the pollen mother cells of Tradescantia virginica; Jour. Gen. Physiol., 5, 815-819.

1924 Chambers,R. : The physical structure of the protoplasm as determined by microdissection and injection; Gen. Cytology, ed. E.Cowdry, Chicago, 235-309.

1925 Chodat,R. : La chiasmatypie et la cinèse de maturation dans l'Allium ursinum; Bull. Soc. Bot. Genève, 1-30.

1901 Conklin,J. : Karyokinesis and cytokinesis in the maturation, fertilisation and cleavage of Cripidula and other Gasteropoda; Jour. Acad. Nat. Sci. Phil., 12, 1-121.

1903 Coulter,J.M. and Chamberlain,C.J. : Morphology of Angiosperms, New York.

1926 Darlington,C.D. : Chromosome studies in the Scilleae; Jour. Genetics, 16, 237-249.

1928 Id. : Studies in Prunus, I and II; Ibid., 19, 213-256.

1929 Id. : Meiosis in Polyploids, II; Ibid, 21, 17-56.

1929 Id. : Chromosome behaviour and structural hybridity in the Tradescantiae; Ibid., 21, 207-286.

1930 Id. : Studies in Prunus, III.; Ibid, 22, 65-93.

1930 Id. : A cytological demonstration of "genetic" crossing over; Proc. Roy. Soc., B, 107, 50-59.

1931 Id. : Meiosis; Biol. Revs., 6, 221-264.

1931 Id. : The cytological theory of inheritance in Oenothera; Jour. Genetics, 24, 405-474.

1932 Id. : Recent advances in cytology; London, J. and A. Churchill.

1910 Digby,L. : The somatic, premeiotic and meiotic nuclear divisions of Galtonia candicans; Ann. Bot., 24, 727-757.

1912 Id. : The cytology of Primula Kewensis and of other related Primula hybrids; Ann. Bot. 26, 357-388.

1914 Id. : A critical study of the cytology of Crepis virens; Arch. f. Zellf., 12, 97-146.

1919 Id. : On the archesporial and meiotic mitoses of Osmunda; Ann. Bot., 33, 135-172.

1927 Earl,R.O. : The nature of chromosomes. I. Effects of reagents on root tip sections of Vicia faba; Bot. Gaz., 84, 58-74.

1902 Ernst,A. : Chromosome reduktion, entwickelung des Embryosackes und Befruchtung bei Paris quadrifolia und Trillium grandiflorum; Flora, 91, 1-36.

1903 Farmer,J.B. and Moore,J.E.S. : New investigations into the reduction phenomena of animals and plants; Proc. Roy. Soc., 72, 104-108.

1905 Id. : On the meiotic phase (reduction division) in animals and plants; Quar. Jour. Micr. Sci., 48, IV, 489-557.

1901 Ferguson,M.C. : The development of the egg, and fertilisation in Pinus strobus; Ann. Bot., 15, 435-479.

1904 Id. : Contributions to the knowledge of the life history of Pinus, with special reference to sporogenesis, the development of the gametophytes and fertilisation; Proc. Wash. Acad. Sci., 6, 1-202.

1930 Fikry,M.A. : Phenomena of heterotypic division in the pollen mother cells of a tetraploid form of Rumex scutatus, var. typicus; Jour. Roy. Micr. Soc., 50, 387-419.

1925 Finn,W.W. : Male cells in Angiosperms; Bot. Gaz., 80, 1-24.

1911 Fraser,H.C.I. and Snell,J. : The vegetative divisions of Vicia Faba; Ann. Bot., 25, 845-855.

1914 Fraser,H.C.I. : The behaviour of the chromatin in the meiotic divisions of Vicia Faba; Ann. Bot., 28, 633-642.

1911 Gates,R.R. : Pollen formation in Oenothera gigas; Ann. Bot., 25, 909-940.

1921 Gates,R.R. and Rees,E.M. : A cytological study of the pollen development in Lactuca; Ann. Bot., 35, 365-398.

1924 Gates,RR. : Meiosis and crossing over; Jour. Hered., 15, 237-240.

1931 Gates,R.R. and Goodwin,K.M. : Meiosis in Oenothera purpurata and Oe. blandina; Proc. Roy. Soc., 109, B, 149-164.

1921 Gelei,J. : Weitere studien über die oogenese des Dendrocoelum; Arch. f. Zellf., 16, 88-169.

1911 Granier,J. and Boule,L. : Sur les cinèses somatiques chez Endymion nutans; C. R. Acad. Sci. Paris, 152, 153-154.

1911 Id. : Sur le phénomène de conjugaison des chromosomes à la prophase de la première cinèse réductrice (microsporogenèse chez Endymion nutans); Ibid, 152, 393-396.

1903 Grégoire,V. and Wygaerts,A. : La reconstitution du noyau et la formation des chromosomes dans les cinèses somatiques; La Cellule, 21, 7-74.

1906 Grégoire,V. : La structure de l'élément chromosomique au repos et en division dans les cellules végétales; Ibid., 23, 311-357.

1882 Guignard,L. : Recherches sur le sac embryonnaire des Phanérogames Angiospermes; Ann. Sci. Nat. Bot., Série VI, 13, 136-199.

1891 Id. : Nouvelles etudes sur la fécondation; Ibid., Sér. VII, 14, 163-296.

1899 Id. : Les découvertes récentes sur la fécondation chez les végétaux Angiospermes; Soc. de Biologie, Volume Jubilaire, Paris, 189-197.

1899 Id. : Sur les anthérozoides et la copulation sexuelle chez les végétaux Angiospermes; C. R. Acad. Sci. Paris, 128, 864-871.

1900 Id. : Nouvelles recherches sur la double fécondation chez les végétaux Angiospermes; Ibid., 131, 153-160.

1893 Haeker,V. : Die heterotypische Kernteilung im Cyklus der generativen Zellen; Ber. Naturf. Ges. Freib., 6, 160-193.

1896 Id. : Ueber die Selbständigkeit der väterlichen und mütterlichen Keinbestandleile während der Embryonalentwicklung von 'Cyclops'; Arch. Mikr. Anat., 46, 579-618.

1931 Hedayetullah,S. : On the structure and division of the somatic chromosomes in Narcissus; Jour. Roy. Micr. Soc., 51, 347-386.

1911 Horne,A.de : Recherches sur la division de la cellule. I. Le duplicisme constant de chromosome somatique chez Salamandra maculosa et chez Allium cepa; Arch. f. Zellf., 6, 613-639.

1915 Hutchinson,A.H. : Fertilisation in Abies balsamea; Bot. Gaz. 60, 457-473.

1909 Janssens,F.A. : La théorie de la chiasmatypie. Nouvelle interprétation des cinèses de maturation; La Cellule, 25, 389-411.

1924 Id. : La chiasmatypie dans les insectes; Ibid., 34, 135-359.

1926 Kaufmann,B.P. : Chromosome structure and its relationship to the chromosome cycle; I. Somatic mitoses in Tradescantia pilosa; Amer. Jour. Bot., 13, 59-80. II. Podophyllum peltatum; Ibid., 13, 355-363.

1931 Id. : Chromonemata in somatic and meiotic mitoses; Amer. Nat., 65, 280-283.

1924 Kihara,H. : Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rüchsicht auf das verhalten der Chromosomen und die Sterilität in der Bastarden; Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, 1, 1-200.

1930 Koerperich,J. : Etude comparative du noyau des chromosomes et leurs relations avec le cytoplasme; La Cellule, 39, 309-398.

1891 Kruch,O. : Appuerte sullo sirluppe degli organi sessuali e sulla fecundazione della Riella clausonis; Malpighia, 4, 1-5.

1926 Kuwada,Y. and Sakamura,T. : A contribution to the colloid-chemical and morphological study of chromosomes; Protoplasma, 1, 239-254.

1926 Kuwada,Y. and Sugimoto,T. : On the structure of chromosomes in Tradescantia virginica; Bot. Mag. Tokyo, 40, 19-20.

1926 Kuwada,Y. : On the structure of the anaphasic chromosomes in the somatic mitoses of Vicia faba, with special reference to the so-called longitudinal split of the chromosomes at telophase; Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, 2, 1-13.

1927 Id. : On the spiral structure of the chromosomes; Bot. Mag. Tokyo, 41, 100-109.

1931 La Cour,L. : Improvements in everyday technique in plant cytology; Jour. Roy. Micr. Soc., 51, 119-126.

1926 Latter,J. : The pollen development of Lathyrus odoratus; Ann. Bot., 40, 277-313.

1932 Id. : The meiotic divisions in the pollen mother cells of Malva sylvestris; Ann. Bot., 46, 1-10.

1925 Lee,A.Bolles : The chromosomes of Paris quadrefolia and the mechanism of their division; Quart. Jour. Micr. Sci., 69, 1-25.

1924 Lewis,W.H. and Lewis,M.R. : Behaviour of cells in tissue cultures; Gen. Cytology, ed. E.Cowdry, Chicago, 235-309.

1921 Litardière,R.de : Recherches sur l'élément chromosomique dans la caryocinèse somatique des Filicinées; La Cellule, 31, 253-475.

1912 Lundegardh,H.: Die Kernteilung bei höheren Organismen nach Untersuchungen an lebendem Material; Jahb. f. Wiss. Bot., 51, 236-282.

1900 McClung,C.E. : The spermatocyte divisions of the Acrididae; Kansas Univ. Quart., 9A, 73-100.

1898 McKenney,R.E.B. : Observations on the development of some embryo-sacs; Contrib. Bot. Lab. Univ. Pennsylvania, 2, No. 1, 80-86.

1929 Madge,M.A.P. : Spermatogenesis and fertilisation in the cleistogamous flower of Viola odorata, var. praecox; Ann. Bot., 43, 545-577.

1928 Maeda,T. : The spiral structure of chromosomes in the sweet pea (Lathyrus odoratus,L.); Bot. Mag. Tokyo, 42, 191-195.

1930 Id. : The meiotic divisions in the pollen mother cells of the sweet pea (Lathyrus odoratus,L.) with special referenee to the cytological basis of crossing over; Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, 5, 89-123.

1922 Martens,P. : Le cycle du chromosome somatique dans les Phanérogames. I. Paris quadrifolia; La Cellule, 32, 331-428.

1925 Id. : II. Listera ovata; Ibid., 36, 125-214.

1927 Id. : III. Recherches expérimentales sur la cinèse dans la cellule vivante; Ibid., 38, 67-174.

1929 Id. : Nouvelles recherches expérimentales sur la cinèse dans la cellule vivante; Ibid., 39, 167-216.

1904 Merriman,M.L. : The vegetative divisions in Allium; Bot. Gaz., 37, 178-207.

1932 Moffett,A.A. : Chromosomes studies in Anemone. I. A new type of chiasma behaviour; Cytologia, 3.

1919 Morgan,T.H. : The physical basis of heredity; Philadelphia.

1922 Id. : On the mechanism of heredity; Proc. Roy. Soc., London, B, 94, 162-197.

1922 Morgan,T.H., Sturtevant,A.H., Muller,H.J. and Bridges,C.B. : The mechanism of Mendelian heredity; New York.

1925 Morgan,T.H. : The theory of the gene; New Haven.

1898 Mottier,D.M. : Ueber das Verhalten der Kerne bei der Entwickelung des Embryosacks und die Vorgänge bei der Befruchtung; Jahrb. Wiss. Bot., 31, 125-158.

1904 Id. : Fecondation in plants; Carnegie Inst. Publ., 15.

1907 Id. : The development of the heterotypic chromosomes in pollen mother cells; Ann. Bot., 21, 309-347.

1909 Id. On the prophases of the heterotypic mitosis in the embryo sac mother cell of Lilium; Ibid., 23, 343-352.

1914 Id. : Mitosis in the pollen mother cells of Acer negundo and Staphylea trifolia; Ibid., 28, 115-133.

1912 Muller,H.A.C. : Kernstudien an Pflanzen, I and II; Arch. f. Zellf., 8, 1-51.

1900 Murrill,W.A. : The development of the archegonium and fertilisation in the hemlock spruce (Tsuga canadensis); Ann. Bot., 14, 583-607.

1898 Navaschin,S. : Resultate einer Revision der Befruchtungsvorgänge bei Lilium Martagon und Fritillaria tenella; Bull. Acad. Imp. Sci. St. Petersbourg, 9, 377-382.

1899 Id. Neuen Beobachtungen über Befruchtung bei Fritillaria tenella und Lilium Martagon; Bot. Centralbl., 77, 62.

1910 Id. : Naheres über die Bildung der spermakerne bei Lilium Martagon; Ann. Jard. Bot. Buitenzorg, Ser. 2, Suppl. III, 871-901.

1927 Newton,W.C.F. : Chromosome studies in Tulipa and some related genera; Linn. Soc. Jour. Bot., London, 47, 339-354.

1929 Newton,W.C.F. and Darlington,C.D. : Meiosis in Polyploids Jour. Genetics, 21, 1-56.

1907 Noren,C.O. : Zur Entwickelungsgeschichte des Juniperus communis; Uppsala Universitets Arsskrift, 64,

1918 Nothnagel,M.: Fecundation and formation of the primary endosperm nucleus in certain Liliaceae; Bot. Gaz., 66, 143-161.

1893 Overton,J. : Ueber die Reduktion der Chromosomen in der Kernen der Pflanzen; Vierteljahrssch. Naturf. Gesell. Zurich, 38, 169-186.

1922 Id. : On the organisation of the nuclei in the root tips of Podophyllum peltatum; Trans. Wisconsin Acad. Sci., 20, 275-322.

1907 Pace,L. : Fertilisation in Cypripedium; Bot. Gaz., 44, 353-372.

1932 Perry,K. : Mitosis in Galanthus nivalis; Jour. Roy. Micr. Soc., 52, 344-356.

1882 Pfitzner,W. : Über den Feineren Bau der bei der Zelltheilung auftretenden fadenförmigen differenzirungen des Zellkerns; Morph. Jahrb.,7, 289-311.

1921 Plough,H.H. : Further studies on the effect of temperature on crossing over; Jour. Exp. Zool., 32, 197-202.

1930 Reuter,E. : Beiträge zur einer Einheitlichen, Auffaussung gewissen Chromosomenfragen; Acta Zool. Fennica, 9, 487.

1915 Robertson,W.R.B. : Chromosome studies, III. Inequalities and deficiencies in homologous chromosomes; their bearing upon synapsis and the loss of unit characters; Jour. Morphol., 26, 109-141.

1931 Id. : Chromosome studies II. Synapsis in the Tettigidae, with special reference to the presynapsis split; Ibid., 51, 119-139.

1895 Ruckert,J. : Ueber das selbständig bleiben der väterlichen und mütterlichen Kernsubstanz während der ersten Entwicklung des befruchteten Cyclopseies; Arch. Mikr. Anat., 45, 339-369.

1927 Sakamura,T. : Chromosomenforschung an frischem Material; Protoplasma, 1, 537-565.

1923 Sands,H.C. : The structure of the chromosomes in Tradescantia virginica; Amer. Jour. Bot., 10, 343-360.

1925 Id. : A microdissection of the pachytene threads of Tradescantis virginica, with observations on some aspects of mitosis; Jour. Gen. Physiol., 9, 2, 181-188.

1923 Santos,J.K. : Differentiation among chromosomes in Elodea; Bot. Gaz., 75, 42-59.

1924 Id. : Determination of sex in Elodea; Bot. Gaz., 77, 353-376.

1924 Sarbadhikari,P.C. : Cytology of Osmunda and Doodia. I. On the somatic and meiotic mitoses of Doodia; Ann. Bot., 38, 1-26.

1896 Sargent,E. : The formation of the sexual nuclei in Lilium Martagon; Ann. Bot., 10, 445-478.

1916 Sax,K. : Fertilisation in Fritillaria pudica; Bull. Torrey Bot. Club, 43, 505-522.

1918 Id. : The behaviour of the chromosomes in fertilisation; Genetics, 3, 309-327.

1930 Id. : Chromosome structure and the mechanism of crossing over; Jour. Arnold Arboretum, 11, 193-220.

1925 Schaede,R. : Untersuchungen über Zelle, Kern und ihre Teilung am lebenden objekt; Beitr. Biol. Pflanzen (Cohn), 14, 231-260.

1901 Schniewind-Thies,J. : Die Reduktion der chromosomenzahl und die ihr folgendenkernteilungen in den Embryosackmutterzellen der Angiospermen; Jena.

1920 Sharp,L.W. : Somatic mitosis in Tradescantia; Amer. Jour. Bot., 7, 341-354.

1926 Id. : An introduction to cytology; New York.

1929 Id. : The structure of large somatic chromosomes; Bot. Gaz., 88, 349-377.

1930 Shinke,N. : On the spiral structure of the chromosomes in some higher plants; Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, 5, 239-245.

1932 Siang-Hsu. : The structure of somatic chromosomes in Lilium tigrinum; La Cellule, 41, 165-178.

1932 Smith,F.H. : The structure of the somatic and meiotic chromosomes of Galtonia candicans; La Cellule, 41, 243-263.

1931 Steere,W.C. : Permanent aceto-carmine smears; Stain Technology.

1932 Stone,L.H. and Mather,K. : The origin and behaviour of chiasmata. IV. Diploid and triploid Hyacinthus; Cytologia, 4, 16-25.

1900 Strasburger,E. : Einiger Bemerkungen zur Frage nach der doppelten Befruchtung bei den Angiosperms; Bot. Zeit., 58, 293-316.

1905 Id. : Typische und allotypische Kernteilung Histologische Beiträge zur Vererburgsfrage; Jour. Wiss. Bot., 42, 1-71.

1921 Sturtevant,A.H. : Genetic studies on Drosophila simulans. III. Autosomal genes; Genetics, 6, 179-207.

Id. : Linkage variation and chromosome maps; Proc. Nat. Acad. Sci., 7, 181-183.

1902 Sutton,W.S. : The chromosomes in heredity; Biol. Bull., 4, 231-251.

1924 Taylor,W.R. :The smear method for plant cytology; Bot. Gaz., 78, 236-238.

1931 Id. : Chromosome studies on Gasteria; Amer. Jour. Bot., 18, 367-386.

1930 Telezynsky,H. : Cycle évolutif du chromosome somatique. I. Observations vitales sur les poils staminaux de Tradescantia virginica; Acta Soc. Bot. Poloniae, 7, 381-433

1880 Treub,M. and Mellink,J.F.A. : Notice sur le développement du sac embryonnaire; Arch. Néerl., 15, 452-457.

1931 Tuan, Hsu-Chuan : Unuasual aspects of meiotic and post meiotic chromosomes of Gasteria; Bot. Gaz., 92, 45-64.

1910 Van Herwerden,M.A. : Uber die Kernstruktur in den Speicheldrüsen der Chironomus latve; Anat. Anz., 36, 193-207.

1911 Vejdovsky,F. : Zum Problem der Vererbungsträgen; Koïnigl. Böhemische Gesels d. Wiss., Prag.

1927 Id. Structure and development of living matter; Pub. Roy. Boh. Soc. Sci., Prague.

1878 Vesque,J.: Nouvelles recherches sur le développement du sac embryonnaire; Ann. Sci. Nat. Bot., 6, Ser. 6, 261-392.

1914 Welsford,E.J. : The genesis of the male nuclei in Lilium; Ann.Bot., 28, 265-270.

1918 Weniger,W. : Fertilisation in Lilium; Bot. Gaz., 66, 259-268.

1916 Wenrich,D.H. : The spermatogenesis of Phrynotettix Magnus, with special reference to synapsis and the individuality of the chromosomes; Bull, Mus. Comp. Zoöl. Harvard Coll., 60, 55-136.

1929 Whyte,R.O. : Studies in Ranunculus. II. The cytological basis of sex in R. acris L.; Jour. Genetics, 21, 183-191.

1912 Wilson,E.B. : Studies on chromosomes. VIII. Observations on the maturation phenomena in certain Hemiptera and other forms, with considerations on synapsis and reduction; Jour. Exp. Zool., 13, 345-431.

1900 Winiwarter,H.de : Recherches sur l'ovogenèse et l'organogenèse de l'ovaire des Mammifères (Lapin et Homme); Arch. de Biol., 17, 33-199.

1931 Id. : Evolution de l'Héterochromosome chez Tettigonia (Decticus) albifrons; Ibid., 42, 201-288.

1904 Wylie,R.B. : The morphology of Elodea canadensis; Bot. Gaz., 37, 1-20.

1923 Id. : Sperms of Vallisneria spiralis; Ibid, 75, 191-201.

1908 Yamanouchi,S. : Spermatogenesis in Nephrodium; Bot. Gaz., 45, 1-30.

1928. Zirkle,C. : The nucleolus in root tips mitosis in Zea Mays; Bot. Gaz., 86, 402-418.

PRESENT INVESTIGATION

A COMPARATIVE STUDY OF THE CHROMOSOMES OF SCILLA NONSCRIPTA DURING SOMATIC AND MEIOTIC MITOSIS.

See accompanying separate La Cellule, January 1934 from page 10.

A COMPARATIVE STUDY OF THE CHROMOSOMES

OF

SCILLA NONSCRIPTA

DURING SOMATIC AND MEIOTIC MITOSIS

BY

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Sommaire du fascicule 1 :

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INTRODUCTION.

Scilla nonscripta, although one of the commonest flowers of the English woodland in Spring, seems to have been curiously neglected by cytologists. This is especially surprising, as it is a member of the Liliaceae family, members of which have yielded such a rich harvest of information with regard to details of chromosome structure and the mechanism of division. Mc KENNEY (1898) mentioned that in *Scilla hyacinthoides* and *S. campanula* the sixteen chromosomes are reduced to eight in meiosis, but he did not describe any details of the division. SCHNIEWIND-THIES (1901) recorded a similar observation, and mentioned that the chromosomes at the metaphase of the first division of meiosis may be shaped like loops or crosses.

In 1911, a brief note appeared by GRANIER & BOULE on the somatic divisions in the root tips of *Endymion nutans* (= *Scilla nonscripta*). They stated that the chromosomes were already double by the end of the metaphase preceding that at which the halves would separate, and that this doubleness remained evident until the end of the telophase. This was followed in the same year by a note on meiosis by these two authors. The threads seen at prophase were described as quadri-partite, due to the pairing of the two double chromosomes. The double nature was seen occasionally, where the gemini were further apart. Eight paired chromosomes were counted. Neither of these notes were accompanied by drawings.

DARLINGTON (1926) investigated the chromosome types of *Scilla nutans* (= *S. nonscripta*) by means of sections through root tips, and smears showing stages of pollen grain division. He distinguished eight types of chromosome, which are more or less the same in both diploid and haploid mitosis. Some variations in the occurence of chromosome constrictions were noted and the occasional appearance of satellites was described.

SCOPE OF THE PRESENT WORK.

On reviewing the number of papers which have been published in recent years on the structure of the chromosome during somatic and meiotic mitosis, the fact emerges that few comparative studies of meiosis and mitosis have yet been made, and even these make little effort to discuss or correlate the modern point of view. On one hand, the investigators of somatic mitosis are moving towards unanimity

concerning the presence of two chromonemata, probably within a common matrix, in each daughter chromosome, giving a quadruple structure by at least the beginning of metaphase : KAUFMANN (1926, 1931), SHARP (1929), TELEZYNSKY (1930), HEDAYETULLAH (1931), PERRY (1932), SMITH (1932), HSU-SIANG (1932). On the other hand, the mass of work done on large chromosomes at meiosis assumes the existence of single threads at leptotene, pairing at zygotene, and division for the first time at pachytene : NEWTON (1927), KUWADA (1926, 1927), BELLING (1928, 1931), BABCOCK & CLAUSEN (1929), SHINKE (1930), DARLINGTON and his fellow workers (1928, 1929, 1930, 1931, 1932). Theories of crossing over have been founded on, or strongly supported by, this point of view : BELLING (1928, 1931), DARLINGTON (1931, 1932). Such comparative accounts of meiosis and mitosis as have been encountered during a study of the literature (eg. SMITH, 1932) have ignored the point of view of a large body of investigators of meiosis. DARLINGTON (1932) discusses mitosis in relation to meiosis, but dismisses as inadequate or faultily interpreted the point of view of many students of mitosis, a dismissal which seems to the writer to be unwarranted in the face of the mass of detailed information thus acquired.

It seems, therefore, that a comparative account of somatic and meiotic mitosis, with special reference to chromosome structure is urgently needed at the present time. Even if the problems raised are not immediately solved, their discussion may stimulate an enquiry amongst other material, which may prove more decisive and may help to remove some of the inconsistencies, with which cytologists are at present confronted.

MATERIAL AND METHODS.

In the autumn of 1930-31, young flower buds of *Scilla nonscripta* were cut and stained for a class study in cytological technique. In the course of examining this material, it was discovered that the somatic chromosomes showed very clear double spiral chromonemata in the metaphase and anaphase stages of division. The study of these interesting structures, however, was not resumed until the autumn of 1932.

The plants used were mainly those of *Scilla nonscripta*, the common bluebell, obtained either semi-wild in the grounds of Royal Holloway College or from the College botanical garden. The wild and garden plants were kept separate, but no difference was found in their nuclear constitution.

Stages of meiosis in the anthers were mainly studied by means of pollen smears, but they were supplemented by material fixed by ordinary methods, and sectioned in paraffin. Suitable stages, both for smearing and fixing, were first identified with the aid of temporary iron aceto-carmine smears, as recommended by BELLING. Permanent pollen smears were made by a modification of TAYLOR's (1924) smear method, given by LA COUR (1931). A variety of fixatives were employed, including the LA COUR modifications of FLEMMING's solution (2BE and 2BD); FLEMMING's solutions weak, medium and strong; BENDA's fluid; NAVASCHIN's solution (LANGLET's modi-

fication); MERKEL's fluid; BOUIN's solution; ALLEN's modification of BOUIN's fluid. In this last case the smears were fixed in a dish of the fluid on the top of a warm oven and washed according to schedule in alcohol before staining. Of these, the osmic fixatives and NAVASCHIN's fluid proved by far the best for meiosis in this material, the favourite osmic fixatives being (in order of merit) 2BE, FLEMMING's weak solution and 2BD.

Stages of meiosis and the development of the gametophyte in the ovary, also comparative stages of meiosis in the anthers, were obtained by means of fixing whole buds or ovaries. In some cases, pieces of anthers and ovaries were put into the fixative and a pump used to sink them in the liquid. In general, however, the method first suggested by KIHARA (1924) or a modification of it, was used (see also MAEDA, 1930; BABCOCK & CLAUSEN, 1929; LA COUR, 1931, etc.). This consisted of dropping whole buds — or preferably ovaries — to be used for the study of meiosis in the megaspore mother cells, into CARNOY's fluid for from fifteen seconds to one minute, and then rapidly transferring them to another fixative for about twenty four hours. Fixation by this method seemed as good or better than when CARNOY's fluid was not used, and the laborious and possibly injurious use of the pump was avoided. They were then washed, taken up through the alcohols and embedded in paraffin wax according to the schedule recommended by LA COUR, using chloroform as the solvent for the paraffin. The fixatives used were very varied, and once more NAVASCHIN's fluid, 2BE, 2BD and FLEMMING's weak solutions were the best.

Sections were cut from 16-18 μ. This thickness was chosen, after some experimentation, as the one which gave whole nuclei fairly easily in favourable sections, and yet did not obscure the structure of individual nuclei by including others, to too great an extent, either below or above them.

Somatic structure was determined almost entirely from nuclear divisions in the nucellus or walls of the ovary and anthers. A few comparative studies were made from root tips fixed in various fixatives, but in general, chromosome structure was not so clearly shown in these, though it is obviously similar in type.

Of the stains used, NEWTON's modification of the gentian violet-iodine method proved by far the most useful, and it was finally adopted almost exclusively. It gave beautifully transparent smear preparations, in which the chromosomes stood out clearly defined and well differentiated internally.

It was sometimes used with a counterstain of orange G, light green, or erythrosin in clove oil (generally the first), but was usually preferred without a counterstain, as this often seemed to obscure rather than clarify detailed structure. Haematoxylin was tried, and was found to be successful in showing up structure, especially when used according to the short schedules recommended by KAUFMANN (1926) and HEDAYETULLAH (1930). Such slides were mainly used merely for comparison, as they were much less clear in detail than the gentian violet preparations.

OBSERVATIONS.

I. SOMATIC MITOSIS.

§ 1. Anaphase, telophase and resting stage.

The usual custom of describing the anaphase first will be followed in this paper, thus avoiding the difficulties of interpretation encountered by beginning with the early prophase stages.

Sixteen chromosomes pass to each pole from the metaphase plate. They fall into eight types, which are represented in the accompanying diagram, lettered in order of size (TEXT FIG. 1). They have been described for the species by DARLINGTON (1926). Their shapes, which are constant for each chromosome, vary with the position of the attachment constriction. A has a subterminal attachment constriction. B and C are roughly equal in size and also have a subterminal constriction, their arms being still more unequal in length than those of A; C may frequently be distinguished from B by the presence of a pronounced secondary constriction in the longer arm. The attachment constriction of D seems to vary in position, apparently being either terminal or slightly subterminal, or the constriction may be drawn out to give

TEXT FIGURE 1. Diagram of the eight characteristic chromosome types of *Scilla nonscripta*, lettered in order of size.

a satellite at the end. E has a median attachment constriction, whilst that of F is slightly submedian in position. G shows the same variation as has been described for D. H has a submedian attachment constriction. All may be characterised in prophase by the presence of marked secondary constrictions at intervals along their length, but these become obscured at metaphase. The chromosome types may be most clearly discerned during the second division of meiosis and the division of the gametophytic nuclei, when each chromosome type is represented only once in the dividing nucleus.

During anaphase, the internal structure of each chromosome is revealed with great distinctness (FIG. **10**). Each chromosome appears to be made up of two spiral chromonemata, twisted round a lightly staining region which may represent a less chromatic matrix. It is not possible to be certain whether these spirals are invariably intertwined round one another, as the points where they cross are not usually in a sufficiently different focus for the upper thread of each cross to be determined with certainty. Occasionally an overlying thread stands out with great clearness. Assuming that the appearance is due to the presence of two spiral threads, two alternatives

present themselves to account for the configuration at this stage. Either the spiral chromonemata turn in the same direction, and therefore intertwine at every turn, or they turn in opposite directions but again with the same degree of twist so that they interlock rather than intertwine. KUWADA's (1927) suggestion that the two threads spiral together ar random, and therefore sometimes intertwine and sometimes do not, would not produce the regular curves and crossings found invariably in this material. The twining is very regular, often giving the superficial appearance of a chromatic matrix with a median row of alveoles, but in favourable material the turns of the two spiral threads seem to be clearly defined. The number of turns of the spiral, as counted by the number of spaces between the crossing threads, varies from about four in the smallest chromo- some to nine in the largest one, and these numbers remain remarkably constant when corresponding chromosomes from different nuclei are compared. The ends of the spirals are often rather indistinct, probably owing to the fact that the end of one spiral may curl round and run beside the end of the other, but it is quite common to find a horned tip to the chromosome, the horns being presumably the ends of the two spirals.

Sometimes as the chromosomes pass towards the poles, they turn their ends at right angles to the rest of their length. Looking down on to the end, as it were through the length of the chromosomes, it can be seen that each chromosome is roughly circular in end view, with one or two projections standing out from the circumference, indicating the position of the ends of the spirals. If these ends are carefully focussed, and the thread continuing downards from them is followed by manipulating the micrometer screw, the turns of the spirals can be traced, curling around below. Figure **9**, which represents half chromosomes in early metaphase, shows a very similar structure.

The position of the attachment constriction is frequently marked by the chromatin spirals being drawn out into a finer thread at this point. At other times it is distinguished by a darker staining piece of chromatin. According to DARLINGTON (1932) the spiralling of the chromosome probably starts with the attachment constriction as a pivot, and therefore the spirals on either side should be reversed. It is impossible to ascertain this for *Scilla nonscripta* however, since — as described above — the direction of turn is difficult to determine.

The chromosomes pass to the poles without apparent shortening in length or alteration of the angle between the spirals. The latter still seem to be very clearly shown (1).

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(1) At the poles, the chromosomes often seem to clump together to give the « tassement polaire ». This feature of mitosis was first described by GRÉGOIRE, and has since been found to a greater or less extent by a number of workers (TELEZYNSKI, KAUFMANN, MARTENS, SHARP, HEDAYETULLAH, HSU-SIANG). OVERTON, however, regards it as a fixation artefact, and finds no clumping in material fixed with MERKEL's fluid. The consensus of opinion seems to be that a certain drawing together of the chromosomes at this stage is actually present but is exaggerated by fixation. The boundaries of the chromosomes are therefore not lost, as certain poorer preparations would suggest; but their individuality is kept, although they are often pressed closely together. Structure is generally obscured on the whole, but individuals showing spirals may sometimes still be distinguished by means of their

After a period of close polar clumping, the chromosomes loosen slightly, but still remain very close together and deeply chromatic. They seem to be shorter and thinner, but their proportions and the number of spiral turns of the chromonemata still remain more or less constant (FIG. **11**). A number of delicate anastomoses are seen between them for the first time, but they grow more pronounced as the nucleus passes more and more into resting stage (1).

When the chromosomes first loosen from the tassement polaire, the spiral chromonemata seem to be exceptionally clear and frequently appear to be really intertwined, a fact which could not be ascertained with certainty in earlier stages. Sometimes the two ends of the spirals are very evident, being more pulled out from the rest of the chromosome than they have been hitherto. The « achromatic matrix » is sometimes still distinguishable, but there is very little difference between the colourableness of the spaces between the chromonemata and the spaces between the chromosomes at this stage. The nuclear membrane is well marked by now, having appeared towards the end of the tassement polaire.

In certain chromosomes at this stage, the two spirals are no longer regularly intertwined, but give the appearance of single spirals at certain points (FIG. **11**). If the chromonemata are really intertwined, this can be explained as due to the rotation of the spirals towards each other (or of one of the spirals through an angle of ninety degrees) until they lie parallel. There is no need for them to untwist to

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projecting ends, which may show entwined chromonemata persisting unchanged (KAUFMANN, SIANG, etc.). This point of view is supported by the present investigation, for varying degrees of « clumping » have frequently been found at telophase, even in material fixed with MERKEL's fluid (cf. OVERTON). All stages have been found from those in which structure was obscured to those in which the chromosome boundaries can still be distinguished and entwined chromonemata are visible at their projecting ends. The observed presence of a slight tassement polaire in all fixatives and, in an incomplete form, in living nuclei (MARTENS, TELEZYNSKI) would seem to indicate its occurrence in nature, although doubtless in a less exaggerated form than is usually found in fixed material.

(1) These anastomoses have been variously described as parts pulled from the surface of the chromosomes by their mutual contact during tassement polaire (GRÉGOIRE) or as active outgrowths from the chromosome threads, which ultimately join together (SARBADHIKARI, KAUFMANN, SMITH). PERRY further states that anastomoses appear between chromosomes which could not have touched each other during clumping. MARTENS and TELEZYNSKI have described anastomoses between the chromosomes of living material. Passing reference may be made to the view of FRASER and SNELL that these anastomoses actually pull the chromosomes apart and cause their split for the next division. It seems to the writer that since certain of the anastomoses appear immediately after the tassement polaire, it is probable that they have been caused by the close approximation of sticky chromatin, as GRÉGOIRE suggests. It seems improbable, however, that any essential parts of the chromosomes are pulled out in this way. Furthermore, in certain diplotene and diakinesis stages of meiosis, when the chromatids are fine and delicate, exactly the same phenomena of anastomosis and outgrowths from the surfaces of the chromosomes have been recorded in less adequately fixed material of *Scilla nonscripta*. In this case, the outgrowths are plainly artefacts. It therefore seems probable that some at least of the outgrowths in late telophase may be regarded as artefacts of a similar nature — perhaps due to currents of stainable material passing between the chromosomes, or perhaps merely due to the contortion of the threads during fixation, owing to their highly sensitive and delicate state at this stage. This suggestion is supported by their comparative absence in MERKEL's fluid.

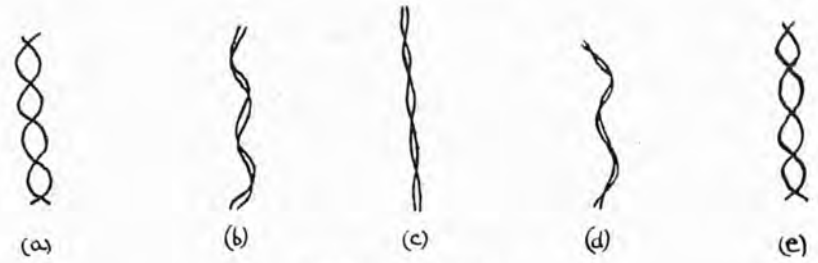
become parallel, as HEDAYETULLAH would suggest at this stage. Chromosomes are frequently found in which parts show an apparently single spiral whilst other parts show the two spirals still distinct (FIG. **12**). The closer association of the chromonemata may be due to a greater attraction between them at this stage, and it is of special interest in view of the appearance of the nucleus in prophase. The approximation of the two spirals in this way has also been commented on by SHARP (1929). This behaviour, however, is complicated by the increasing evidence and distinctnes of the anastomoses and by threads running across the chromosomes themselves, and the curves of the spirals tend to appear more angular (FIG. **13**). Finally, individual chromosomes can only be distinguished with difficulty, and the nucleus enters into the resting condition. It then appears to be made up of a mass of fine threads, whose curves give the nucleus a granular appearance. Sometimes the resting nucleus seems to be made up of very fine, tightly curled spirals. Whilst the structures observed in the resting nucleus correspond to the chromonemata and make up the permanent parts of the chromosome, it is probable that the chromatin threads are in a highly sensitive state at this time and are therefore much more liable to injury from the action of fixatives than many observers have supposed. Therefore too much emphasis should not be placed upon all the structures observed at this time.

§ 2. Prophase and metaphase.

The first indication of the beginning of prophase is given by the condensation of the reticulum into thin, apparently single, spiral threads (FIG. **1**). These, in well fixed material, give no indication of anastomoses between them. The spirals thicken and pull out slightly (FIGS. **2**, **3**), and then splits are seen at intervals along them (FIG. **4**). These may be considered at first as due to one of two causes : either the two half chromosomes have separated during resting stage and come together during early prophase ; or the split evident in the preceding telophase has been hidden in early prophase and is now reappearing. A study of the prophase stages supports the latter view. There is never any sign of an intermediate stage showing the two halves coming together, and a split does not become evident throughout their length until later in the prophase, as shown by the relative thickness at this stage of the two component threads. There are all grades between threads which are apparently single ; slightly thicker threads which show faint indications of duality ; and still thicker threads with the split well marked. The apparently single loops seen here and there in telophase also support the view that the spirals at least partially approximate in interphase.

The two threads thus revealed at early prophase are entwined round each other, at first lying closely upon each other but later becoming more and more widely separated between the points where they cross (FIG. **5**). Parallel with this increasing evidence of the split is the gradual thickening and straightening of the tight little spirals seen at earlier prophase (FIG. **6**). The tightness of the spirals seems to vary within certain limits with the fixative employed, although regular spirals are

always found in early prophase, even when MERKEL's fluid is used. Hence, such spirals are probably produced naturally along the thread at this stage, although their closeness of coiling may be exaggerated by the use of a fixative. As the threads thicken, they straighten until no trace of their spirals remains.



TEXT FIGURE 2.

Two pieces of modelling wax can be made into two spiral threads, twisting in the same direction and intertwining at every turn, to resemble the configuration seen in the telophase chromosomes [TEXT FIGURE 2, (*a*)]. If they are then twisted towards each other, the two spirals will run parallel, and can be approximated so that evidence of duality is more or less lost, and the effect a single spiral is produced [TEXT FIGURE 2, (*b*)]. If this common spiral is then stretched out from either end, the threads will be seen still twisted around each other, once for every half turn of the original spiral [TEXT FIGURE 2, (*c*)]. This rope-like double thread can be made into a new spiral, when the distinctness of the two halves composing the thread will be still further obscured [TEXT FIGURE 2, (*d*)]. Upon pulling this spiral out again, the original twists will reappear in the straightened double thread, and they can be loosened so that the duality of the double thread will become more evident [TEXT FIGURE 2, (*e*)]. The number of turns about each other will still be twice the number of whole turns of the original spirals at the telophase configuration. This is apparently what is happening from telophase to prophase of mitosis, although at prophase, the closeness of approximation of the two halves and the degree of tightness of coiling in the new secondary spiral may both be exaggerated by fixation. The configurations of the spirals are also influenced by a lengthwise stretching of each half chromosome thread itself during interphase (such as would be given by a piece of elastic) and its gradual contraction during prophase. The stretching would help to obscure the duality of the double thread, and this contraction would reveal it. An increase or decrease in the number of original twists about each other of the telophase spirals may be expected to occur during resting stage and early prophase. The number seen at early prophase, however, seems to approximate more or less to the number of turns expected from the frequency of intertwining in the telophase chromosomes.

The succeeding stages of the prophase consist mainly in a gradual thickening, intertwining and contraction of the double threads. Colourless portions in them, representing the positions of constrictions (both primary and secondary) can sometimes be seen along the threads. At later stages, however, these generally become more and

more obscured. Favourable preparations during early middle prophase show a markedly beaded appearance but this is obscured again as the threads thicken still further.

Chromomeres are never very evident in this material, even when fixed in MERKEL's fluid : cf. HEDAYETULLAH on *Narcissus ;* but irregular thickenings have frequently been seen along the threads at prophase, and may be explained as due to contorted aggregations of chromomeric swellings. The increased beadiness of the middle prophase chromosomes has been noted by several observers eg. KAUFMANN, PERRY. They are supposed by the former to be the centres of internal differentiation of two new chromonemata. In view of the sudden appearance of these chromonemata at a slightly later stage, the writer also believes that they may be connected with them in some way, but of this there is no proof. The subject will be discussed later.

Towards the end of prophase, the chromosomes begin to arrange themselves in a more definite manner, so that they gradually become more or less parallel with one another, and their ends point towards one or the other of two opposite poles. (FIG. **1**). The two halves of each chromosome are now twisted around each other only from two to four times, and sixteen pairs of half chromosomes can easily be counted. The nuclear membrane gradually disappears and the ends of the chromosomes are drawn towards the equator. During all this time, the thickening daughter chromosomes remain chromatic and uniformly stained, and all endeavours to discern any convincing internal structure have failed. Now, however, as the chromosomes move towards the equator each daughter chromosome suddenly reveals that it is itself apparently double, and seems to be composed of two spiral intertwined chromonemata with lighter spaces between them (FIG. **8**.) The construction of the two daughter chromosomes is in fact identical in every way with that which they will have when they pass to the poles at anaphase. The chromonemata are regularly intertwined, with about the same number of median spaces between the twists as have been described for anaphase chromosomes. When the daughter chromosomes are seen in end view they appear more or less circular, with the spirals curling behind them from the two projecting ends, just as they appear at anaphase. (FIG. **9**.) After their sudden appearance in early metaphase the two new chromonemata in each chromosome remain apparently unchanged throughout metaphase and anaphase until late telophase. The metaphase changes consist in the completion of the untwisting of the now loosely intertwined daughter chromosomes, and their close approximation at full metaphase, followed by their separation from each other — beginning at the attachment constriction — and their progress towards opposite poles. This brings us to the anaphase of the nuclear cycle with which we commenced our description.

II. THE LAST ARCHESPORIAL MITOSIS.

The archesporial mitoses in the anthers of *Scilla nonscripta* were carefully investigated from sections of young flower buds. It was extremely difficult, however, to be certain that the divisions found were actually the *last* divisions before meiosis. Divisions occurring in cells scattered amongst obvious pollen mother cells were pro-

bably tapetal, and in younger buds the size of the anther or of the archesporium was not a sufficient criterion for determining the exact stage reached. The archesporial tissue of a large number of buds was examined, especially from buds which although in the same inflorescence as buds which contained young pollen mother cells were situated immediately above them on the flower stalk. In no case was there the slightest indication that the mitoses in this tissue differed in any way from normal somatic mitoses. DIGBY (1919) and others have noted this absence of any peculiarity in the last division before meiosis, although she also confesses her inability to be certain whether she could identify the last division in all cases. There is no evidence, however, that the last archesporial mitosis is in any way different from that occurring in ordinary somatic cells. Therefore in the telophase, each chromosome probably consists of two spirally intertwined chromonemata as described above.

III. MEIOTIC MITOSIS.

§ 1. First division.

After the last archesporial mitosis, the nuclei of the pollen mother cells rest for a short time, but this stage passes almost imperceptibly into early prophase, or leptotene. The nuclei have meanwhile enlarged quite considerably, and each is filled with a number of very fine threads, in the meshes of which one to six nucleoli are embedded. These threads presumably correspond to the apparently single spirals seen in the early prophase of mitosis and therefore are probably really double in nature, being composed of two closely approximated chromonemata, although all signs of duality are hidden. In this way, they agree with other descriptions of leptotene given by previous writers. In *Scilla nonscripta* however, the relationship of the leptotene threads to those of the early prophase of somatic mitosis does not seem difficult of interpretation. The nuclei of the pollen mother cells have increased in volume, and therefore the leptotene threads are much thinner and more drawn out than are the corresponding threads of a somatic mitosis. Hence, if the duality of the prophase thread is temporarily hidden in somatic mitosis, it will necessarily be much more so in these thinner threads. It is also quite possible that the temporary attraction between the two component halves may be greater at this stage than at any other. The question will be discussed in a later section.

At leptotene, then, the nucleus is filled with these apparently single but probably double threads. They are very finely beaded along their length, the beads no doubt representing chromomeres. Leptotene is of comparatively short duration, and soon it is seen that some of the threads are beginning to run parallel to one another along certain portions of their length, pairing closely with each other here and there. (FIGS. **14**, **15**.) This is zygotene, in which homologous chromosomes are pairing side by side. In some cases, the threads seem to twist round each other as they pair. It could not be ascertained in this material whether the chromomeres pair specifically, as they are so small and fine in *Scilla nonscripta*, but it is probable

from BELLING's and DARLINGTON's work that they actually do so. Pairing takes place very rapidly when once it is initiated, and soon it is complete along all the threads in the nucleus. (FIGS **16**, **17**.) This is pachytene.

Contact is very close between the two pairing homologues, so that they often together give the appearance of a single beaded structure. Careful examination, however, reveals the presence of tiny spaces between the two homologous chromosomes, and occasionally free ends are found with the two chromosomes plainly separate. Especially clear cases of this have been seen during pachytene in megaspore mother cells. (FIG. **17**.) The paired chromosomes frequently give the appearance of a tightly twisted double spiral. They are arranged at random through the nucleus, no trace of a « hollow spireme » ever being found in smear preparations. The chromosomes have now thickened and contracted and free ends are distinguished with ease, although it is not yet possible to distinguish the limits of the chromosomes. Pachytene is of considerable duration, being in fact more prolonged than any other stage of meiosis in this material (1).

After some weeks spent in this condition, the spaces between the chromosomes become more evident, and the threads fall apart from each other at certain places. (FIGS. **18**, **19**, **20**.) As this happens, it can be seen very clearly that each of the apparently single threads which paired at zygotene is now double in itself, so that a tetrad of half chromosomes can be distinguished for the first time. The half chromosomes will be called chromatids, adopting the term first given to them by MC CLUNG (1900) and now widely used. The splits therefore separate pairs of chromatids. Where these pairs meet, it can often be seen that they have exchanged partners, thus forming a chiasma. That the exchange has really occurred, however, becomes more evident in *Scilla nonscripta* at late diakinesis and metaphase. Each chromatid appears to consist of a single spiral, which is either intertwined with, or fitted into, the spiral of its partner.

Further contraction and the widened curves of the diplotene loops have now allowed eight chromosome pairs to be distinguished. These are graded in size and may be roughly classified as three large pairs, three medium sized ones, and two much smaller ones. A description of the eight types of chromosome has already been given. These bivalent chromosomes shorten and thicken still more, until the diakinesis stage is reached. (FIGS. **21**, **22**.) The chromosomes are still distributed at random through the nucleus, never being found exclusively at the periphery as some accounts of meiosis in other plants suggest. The spiral structure of the chromosomes

(1) *Note on Pollen Smears.* It is a fact of some significance that there is no « contraction » or « synizesis » stage found in pollen smears of *Scilla nonscripta* although a slight contraction of the chromosomes towards one side of the nucleus is sometimes found during early diplotene in material which has been sectioned from paraffin. This seems to support the contention put forward by DARLINGTON (1932) that synizesis or contraction during meiosis is not a natural phenomenon, but is produced by the action of a fixative upon the chromatin threads of the nucleus, which are peculiarly sensitive at this stage. There is no doubt, however, that the collapse of the nuclear threads shows much more readily in the nuclei of some species than it does in others under the same conditions, and the nucleus of *Scilla nonscripta* does not seem to be as susceptible as many others in this respect.

now becomes very evident, and increasingly so as the metaphase stage approaches. The nuclear membrane then breaks down, and the chromosome pairs are drawn on to the equator of the spindle. Each chromatid is an unmistakable spiral, but it is uncertain whether it is intertwined with or fitted into the spiral of its partner. (Figs. **23-27**.)

At this stage, too, the fact that an actual exchange of partner has occurred at a chiasma can be clearly seen. Especially clear cases of this have been found in a metaphase plate of a megaspore mother cell. (Fig. **24**.) Here, in each of the three chromosomes marked C, E and F, a thread from one pair of chromatids can be seen distinctly as passing over into the other pair of chromatids at the chiasma. Figure **24** is also particularily interesting as it illustrates the first clear case of crossing over of chromatids at a chiasma formed during meiosis in the megaspore mother cell of any plant. Hitherto, such crossing over has only been demonstrated in pollen mother cells. It also represents the first record of a definite spiral structure of the chromosomes in the megaspore mother cell or female gametophyte of any plant. It is satisfactory to have a clear demonstration of the fact that the details of meiosis and chromosome structure are the same in the megaspore mother cell as in the pollen mother cell of a plant, a correspondence which could hitherto only be inferred from the similarity of their less delicate nuclear structures.

The chromosomes now begin to separate to opposite poles, beginning at the attachment constriction, the position of which determines the shape which each chromosome will assume. (Figs. **28-32**.) The relationship of the attachment constriction to the chiasmata seems to be such that the half chromosomes with terminal and subterminal attachment constrictions which pass together to the same poles are mainly composed of homologous chromatids, and show single spirals. They are shaped like two inverted J's placed shoulder to shoulder. The half chromosomes with median attachment constrictions, however, pass to the poles mainly as sister chromatids, with the spirals still unseparated except at the ends. The position and behaviour of the chiasmata will be described later.

As the chromosomes separate at anaphase, the spirals are somewhat pulled out, but they still remain very evident, even at telophase. (Fig. **34**.) The chromosomes collect together as a bunch at the poles, corresponding to the « tassement polaire ». They then loosen, and the threads gradually become pulled out, until finally the spirals can only be traced here and there. (Figs. **35, 36**.) A membrane has meanwhile appeared around the nucleus. The threads twine among each other, giving almost the appearance of a network, but there are never anastomoses between them at this stage. The daughter nuclei never enter into a true resting stage, but remain for a brief time in an early interphase condition. (Fig. **36**.)

§ 2. Second division.

The first evidence of the prophase of the next division is a shortening and thickening of the chromosome threads. In favourable preparations, it can be seen that

the paired chromatids are still widely separated, but are attached at the constriction, so that they more or less retain the configuration with which they went into the preceding telophase. In early prophase, very slight evidences of a split may be seen in each chromatid thread in suitable preparations (FIG. **37**), but in later prophases, the chromatids once more appear as homologous chromatic rods. In early metaphase, there are eight pairs of chromatids in the nucleus, which are side by side and apparently no longer attached together at the constriction. Each of the chromatids is now revealed as apparently double in itself, consisting of two spiral chromonemata entwined together, and showing less deeply stained spaces between them. (FIG. **38**.) The configuration and approximate number of the spirals and their time of appearance are very similar to the behaviour of the spirals in the chromosomes of a somatic mitosis. The short arms of the chromosomes with subterminal constrictions often stain homogeneously as dark heads at the tops of the douhle spirals of te long arms. Crescent shaped tips to the half chromosomes, and free ends may be readily distinguished in certain nuclei. The characteristic chromosome types A-H are also easily identifiable at this stage (FIGS. **39-41**.) The chromatids separate to opposite poles, but the spirals remain more or less unchanged from the moment of their appearance until early telophase. (FIGS. **42-44**.) The chromosomes then clump at the poles and occasionally, as they loosen, a few fine anastomoses may be seen between them (FIG. **45**.) The spirals remain evident for some time, but gradually pass into the resting stage. All these stages of this division are very like the corresponding ones of a somatic mitosis.

IV. BEHAVIOUR OF CHIASMATA.

The chromatids fall apart in pairs at diplotene, but the two pairs on either side of the loops meet at intervals along the thread and exchange partners at these chiasmata. The chiasmata in each of the eight chromosomes of the nuclear complement of *Scilla nonscripta* have been counted for a number of nuclei at diplotene, diakinesis and metaphase. From these data have been computed (*a*) the average number of chiasmata for each chromosome at these three stages, (*b*) the number of chiasmata which are terminalised. The results are given in the following table, the chromosomes being lettered in order of size :

	No. of chiasmata per bivalent.								*No. of chiasmata terminalised.*							
	A.	B.	C.	D.	E.	F.	G.	H.	A.	B.	C.	D.	E.	F.	G.	H.
Diplotene	4	3.	2.5	2.3	2.2	2.0	1.8	1.5	0.8	1.0	0.8.	0.7	1.3	1.0	0,5	0,5
Diakinesis	2.5	2.3	2.1	1.9	2.1	1.7	1.5	1.3	0.8	1.0	1.0	1.0	1.3	0,8	0,8	0.4
Metaphase	2.5	2.2	2.2	2.0	2.1	1.5	1.6	1.1	0.8	1.0	1.0	.8	1.1	0.5	0.8	0.1

§ 1. Diplotene.

This table shows that the number of chiasmata decreases more or less proportionally with the size of the chromosome, the relationship being especially clear in diplotene. This agrees with the quantitative records made by STONE and MATHER (1932). It illustrates the fact that the occurrence of one chiasma tends to prevent the occurrence of another in the same pair of chromosomes, a phenomenon known as cytological interference. (DARLINGTON, 1932.) The number of terminal chiasmata which occur in each chromosome pair (averaging one or less), bears no relation to the size of the chromosome, except that where only one chiasma is found in a bivalent it is invariably interstitial. The inference is, that when the chiasmata are first formed, they are not at random to one another, but are at random in respect of the exact part of the chromosome at which they may appear. The very small number of terminal chiasmata at diplotene, however, and the interstitial position assumed by a chiasma when it is the only one in a bivalent, indicate that chiasmata rarely (and probably never in the first instance) arise at the ends of the chromosomes, although this position may be adopted at a later stage. Chiasmata arising strictly terminally at pachytene would be incompatible with the hypothesis of partial chiasmatypy. (DARLINGTON, 1932, p. 313.)

§ 2. Diakinesis.

From diplotene to diakinesis, a slight amount of terminalisation occurs, so that here the average number of chiasmata for all the chromosomes in a nucleus falls to between three and one. At the same time the number of chiasmata terminalised rises very slightly. It is noticable, however, that the average number of terminal chiasmata in a chromosome is still one or less, so that there is seldom a terminal chiasma at each end of a chromosome. Chromosomes A and B, which show the greatest decrease in the number of chiasmata, show no perceptible increase whatsoever in the number terminalised. This means that terminalisation occurs much more readily towards one end, and it can be seen by a study of the chromosomes at metaphase that this is always the end away from the attachment constriction. This is readily understandable on the assumption that chiasmata will be less likely to form in a short arm than in a long arm of a chromosome, the chance of their formation being proportional to the respective lengths of the arms. Hence, in a chromosome with a subterminal or almost terminal attachment constriction, there is usually no chiasma formed between the point of attachment and the end of the short arm, and since in any case during terminalisation movement is away from the attachment constriction (DARLINGTON), the short arm of the chromosome will show no terminal chiasma. The chiasmata of the chromosomes with median or submedian attachment (E and F) may be localised in origin nearer one end than the other for some reason, but it is significant that the average number terminalised for chromosome E is higher than that of any chromosome in the complement.

§ 3. Metaphase.

The configurations seen at diakinesis remain more or less unchanged until metaphase. Since most of the chromosomes have a subterminal or almost terminal attachment, and the chiasmata are mainly localised between the attachment constriction and the end of the long arm of each, it follows that the two chromatids which pass to the poles at anaphase will be widely separated from each other, and each will show a single spiral through most of its length. But the three chromosomes that pass to the poles at anaphase with median or submedian attachment constrictions, will each remain with their spirals intertwined for a short part of their length on either side of the constriction, until their separation at a chiasma pulls them apart. In general these chromosomes at anaphase appear with a central portion showing entwined spirals and two free ends either side of it. The behaviour of the chromosomes during meiosis in *Scilla nonscripta* is therefore compatible with the chiasmatypy hypothesis. A slight terminalisation occurs, the movement being away from the attachment constriction, to give the configurations observed at diakinesis and metaphase.

V. COMPARISON OF MEIOSIS IN THE POLLEN MOTHER CELL AND MEGASPORE MOTHER CELL.

A comparison of the corresponding stages of meiosis in the megaspore mother cell and pollen mother cell is best made by means of drawings, when the close similarity in the two mother cells becomes immediately evident. (See Figs. **14-24** for a comparison of the prophase stages and metaphase of the first division.) The early prophases correspond in every way, but diakinesis obtained in the megaspore mother cell does not show the spiral chromatids very clearly, although there are indications in many of the chromosomes that they are really present. At metaphase beatifully clear spirals have been seen in the megaspore mother cell, which are obviously of the same type as those which are found in the pollen mother cell at this phase. (Figs. **23**, **24**.) The demonstration in this plant, apparently for the first time in plants, of spiral chromatids and chiasmata in the megaspore mother cell during the first division of meiosis, has already been noted in an earlier section. The number of chiasmata present in each chromosome at metaphase of the megaspore mother cell has been estimated in the same way as has been described above for the pollen mother cells. The average number of chiasmata and amount of terminalisation has been found to be approximately the same in both the pollen mother cell and megaspore mother cell nuclei at this phase. The average number of chiasmata and the number of these which are terminalised at diplotene and diakinesis also apparently correspond, although too few counts have been made during these stages in the megaspore mother cell to permit a really representative average to be calculated.

The chromosomes of the pollen mother cells show clear spirals during anaphase and early telophase, but they have not been demonstrated so far in the megaspore

mother cells at these phases. Late telophases, however, show them very clearly once more in both pollen mother cells and megaspore mother cells. (FIGS. **34-36**.)

The account given of chromosome structure during the second division of meiosis has been obtained exclusively from the pollen mother cells, as only uniformly stained chromosomes have been found in the megaspore mother cells. In late telophase, however, two apparently intertwined spirals in each chromosome are seen clearly in the tetrad of megaspores which have been produced, and these structures correspond to the double spirals in the telophase chromosomes in the tetrad of newly formed pollen grains. It is therefore probable that the details of chromosome structure in this division are essentially the same in both megaspore mother cells and pollen mother cells. Chromosome structure in the megaspore mother cell, however, must necessarily be more difficult of demonstration, for its deep-seated position in the nucellus makes good fixation difficult, and its study must be undertaken by means of paraffin sections, which usually show the finer details of chromosome structure less clearly than pollen smears.

VI. DIVISION IN THE POLLEN GRAIN.

After a certain period of rest, the nucleus of each pollen grain enters into the prophase of division. The prophase threads unlike the chromosomes in the early prophase of somatic mitosis reveal their duality from their earliest appearance, and at later stages they separate very widely from each other, except at the points where they are still intertwined. They are markedly beaded throughout early and middle prophases, but become more uniformly cylindrical at late prophase. No spiral chromonemata have been observed in the chromosomes during this division, although there are occasional indications of lighter areas in the centre of certain chromosomes which suggest that chromonemata may be really present, though obscured. The absence of visible structure in the chromosomes is probably due to the presence of the thick pollen grain wall, which would make it difficult for fixatives to penetrate rapidly.

Eight chromosomes pass to each pole at anaphase. They correspond with the types already described. Secondary constrictions are often marked along their length, and chromosomes D and G frequently show a satellite on the end nearest the attachment constriction.

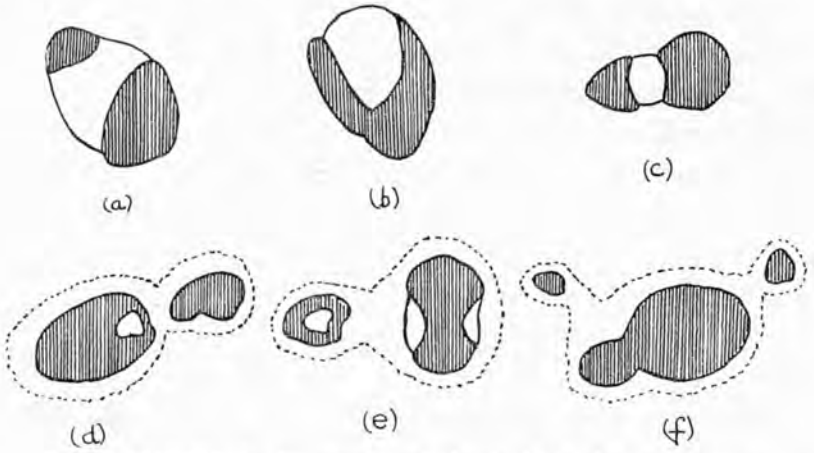
VII. ENDOSPERM DIVISION.

Some very clear cases of chromosomes showing an apparent by spiral structure were observed in certain endosperm nuclei of *Scilla nonscripta*. In these, two intertwined spirals were seen in each chromosome from late prophase to telophase, and the division stages resembled those of a normal mitosis. The demonstration of spiral chromonemata in endosperm tissue has not hitherto been recorded for any plant.

VIII. THE NUCLEOLUS.

The behaviour of the nucleolus during meiosis has presented several points of interest. It buds vigorously throughout early prophase and resting stages, and it does not entirely disappear during metaphase and anaphase, as the nucleoli of most plants seem to do. Time has not permitted the various stages to be studied in detail nor can their significance be adequately computed. A brief description of the nucleolar behaviour, however, will be given.

The nucleoli are first observed in meiosis of the pollen mother cell during leptotene and zygotene, when they are apparently budding vigorously. Their behaviour can be seen most clearly when they are stained with haematoxylin.



TEXT FIGURE 3. Stages of nucleolar budding during zygotene.

The large nucleoli are irregularly spherical with two darkly stained portions on either hand and with a lighter patch in the centre. (TEXT FIG. 3*a* et *b*). Other nucleoli are elongated and narrower, with the lighter patch rather more drawn out. A series can be arranged to show an apparent sequence from the elongation of a nucleolus, the drawing out of the lighter patch, and the final separation of the nucleolus into two parts along the centre of the lighter stained bridge. (TEXT FIG. 3*a-d*). One part thus newly separated off from the original nucleolus is usually much larger than the other part, which forms a kind of « bud » (TEXT FIG. 3*d-f*). A portion of the lighter bridge usually remains either in one or both of the daughter nucleoli thus formed (TEXT FIG. 4*e*). There are usually three to eight nucleoli of varying sizes in the zygotene nucleus. In the cytoplasm around the nucleus are numerous faintly staining bodies, which greatly resemble the nucleolar buds. They have been found just against the nuclear membrane both in the nucleus and immediately outside it, but have never been observed actually to penetrate the membrane. It seems probable, however, that the buds extruded from the larger nucleoli pass into the cytoplasm, where they gradually grow smaller and stain more faintly until they can no longer be distinguished. Other small nucleoli seem to fade into the karyolymph, for tiny faint fragments, similar to those in the cytoplasm, may be seen amongst

the chromatin threads. A similar nucleolar budding has been described for these phases by others workers. It seems difficult to interpret the buds as the result of fixation (ZIRKLE, 1928; FIKRY, 1930) for they have been found both in osmic fixatives and in NAVASCHIN's fluid.

As prophase advances, the nucleoli bud less actively, and the number of nucleoli in each nucleus grows progressively smaller. Fragments may still be seen in the cytoplasm at metaphase. The large nucleoli become smaller and fainter in late prophase, until at metaphase only one or two small pale ones remain. These pass to the side of the metaphase plate, or into the cytoplasm near it (FIG. **27**.) As the chromosomes move to the poles, the nucleoli move to the centre or to the side at the equator (FIGS. **29**, **30-33**), and by this time there is usually only one nucleolus (sometimes two) in each dividing nucleus. Where there is one, it moves towards one of the daughter nuclei now formed (FIG. **34**), but where there are two they usually move to opposite poles. There is a little evidence in some nuclei that the single nucleolus may divide and the halves pass to opposite poles at this stage, but this does not often occur. The daughter nuclei form nuclear membranes but remain either without nucleoli or with only small ones. The persistent nucleoli move very close to the nuclear membranes, but no conclusive evidence has been obtained to prove that they actually pass through, although their appearance is sometimes suggestive of this. (FIG. **36**.) The next stage, however, shows the daughter nuclei with one to three large nucleoli — usually one of these nuclei has a much larger nucleolus than the other, whilst the cytoplasm shows only small or faint nucleolar bodies dying away in the cytoplasm. There is thus the possibility that the persistent nucleolus from the metaphase of meiosis may pass into one of the daughter nuclei, but there is also evidence that at least some of the nucleolar material is formed *de novo* in the newly formed nuclei.

The nucleoli now bud vigorously once more through interphase and into the prophase of the second division, the buds passing as a rule into the cytoplasm. One or two persistent nucleoli remain at the equator of the spindle as in the previous division, and migrate towards the poles at telophase. One of the nuclei of the tetrad frequently shows a much larger nucleolus than the other three, which is again suggestive of the fact that it may have received the original persistent nucleolus of the first meiotic metaphase, which has remained fundamentally unchanged even though it has budded many pieces from its surface.

The nucleoli in the nuclei of the tetrads of pollen grains now proceed to bud extremely vigorously and material is apparently exuded with great activity into the cytoplasm. The cytoplasm around these nuclei is therefore filled with these chromatic bodies, some of them being quite large and deeply coloured. (FIG. **45**.) Similar bodies are still found during the division of the pollen grain, but the behaviour of the nucleolus during mitosis has not been followed. Occasionally, a persistent nucleolus at a metaphase plate has been noted, so that apparently a similar persistence and behaviour may also be seen in diploid and haploid mitosis as is found in meiosis. The behaviour of the nucleolus has been observed during meiosis in the megaspore

mother cell, and is found to be similar in all essentials to that in the pollen mother cell.

Persistent nucleoli are rare in higher plants, except in certain hybrids, when they are probably abnormal. A few animals are reported to have nucleoli which persist and may even divide and re-enter the daughter nuclei. There seem to be no other records of a similar behaviour in higher plants. It is difficult to estimate its importance in *Scilla nonscripta*, since the observations are unsupported by microchemical tests or comparative staining reactions. It seems probable that the nucleolus is a store of ergastic material (as FIKRY suggests) or that it is the centre from which such material is differentiated. It is unlikely that it represents any special store of chromatin as some workers suggest, for ZIRKLE has found by microchemical tests that it is not composed of chromatin, and a direct contact between it and the chromosomes is no longer considered likely, in the absence of a continuous spireme. The persistence of the nucleolus through metaphase and anaphase in *Scilla nonscripta* is probably due merely to the relative conditions of viscosity of the cytoplasm and the nucleolus or to some similar physical cause. A further study of such persistent nucleoli, however, may throw some light on the behaviour of the nucleoli of other plants, in which they become invisible or used up in some way during the metaphase, anaphase and telophase of division.

DISCUSSION OF RESULTS.

I. THE CHROMOSOME THROUGHOUT THE PHASES OF MITOSIS AND MEIOSIS.

The chromosomes of *Scilla nonscripta* have been traced through all the sucessive phases of mitosis and meiosis, and spiral chromonemata or chromatids have been described as constantly recurring features. At metaphase and anaphase of a somatic mitosis, each chromosome appears to consist of two spiral filaments coiled round a lighter coloured core which may or may not exist as such. These are conceived to be true spirals because of their appearance both in side and in end view, and they have been demonstrated with varying degrees of clarity in a great variety of fixatives. They persist more or less unchanged until late telophase. After this they apparently approximate closely to one another, so that in early prophase they appear as single threads but they gradually separate at a slightly later stage.

In fact, the split which earlier investigators have observed at prophase is probably not a new division of the formerly single chromosome, but an old split which dates from at least the preceding early metaphase, and is now reappearing after a short period of obscurity. The closing together of the two chromonemata, so that they appear as a single spiral in early prophase, has been reported by other investigators (SHARP, SMITH). It is difficult to understand why they should suddenly approximate as they do in *Scilla nonscripta*, after remaining at such a constant distance apart from one another through so many phases of division. Throughout prophase,

the wide double spiral of metaphase and anaphase is never regained, although the two sister chromonemata, are still twisted round each other. The two chromonemata may repel each other from metaphase to telophase, and may therefore spiral apart from each other as widely as possible. If in this case, they are prevented from indefinite expansion, as though held in a cylindrical membrane of constant diameter, and are intertwined about each other, they will take up a position giving the configuration seen in a somatic chromosome, and will never touch each other at any point along their length. If this repulsion is gradually replaced by an attraction towards each other, the two spirals will twist until they lie parallel, and will give the effect of a single spiral such as is seen during early prophase. This approximation will be further assisted if a lengthwise stretching of the two spirals is presumed to occur at the same time, as seems probable. The attraction between the two chromonemata may now be gradually overcome, when the original split will appear. Their shortening and thickening, an increasing repulsion between them, and a diminution of the tension which has kept them twisted until now, will all tend to the gradual untwisting of the two chromonemata (now known as daughter chromosomes). Finally, under conditions of maximum repulsion, they will separate and pass to the poles. It is possible that some such alternation of attraction and repulsion may cause the movements of the chromonemata during nuclear division.

The new pair of chromonemata seen to make their first appearance in the daughter chromosomes at very late prophase, and this time of appearance coincides with that noted by most recent observers. It is true that workers appear to differ in opinion as to where the split first appears : KAUFMANN, TELEZYNSKY, SHARP, TAYLOR, TUAN, SMITH and SIANG describe it as occurring in late prophase; HEDAYETULLAH and PERRY in early metaphase : but is not this difference apparent rather than real, since these phases grade into one another? In *Scilla nonscripta* the nuclear membrane has disappeared before the new chromonemata are visible in the daughter chromosomes, but the chromosomes are not yet completely arranged on the equatorial plate, and the daughter chromosomes are still twisted round each other. Whether this stage is called late prophase or early metaphase depends upon the limits placed upon the respective stages. The newly visible spirals, however, are so regularly arranged that it is difficult to believe that they have only just formed, unless the repulsion between them develops very suddenly. The actual division may have been initiated a little earlier in prophase, in which case the markedly beaded appearance noted by many observers (KAUFMANN, PERRY) at middle prophase may be connected with the gradual separation of the two spirals (KAUFMANN) or it may be the stage in which the longitudinal split is actually occurring. As yet, however, there is no direct evidence that the split occurs earlier than late prophase.

Assuming that the chromonemata are composed of chromomeres, arranged like beads on a string and bound together by accessory chromatin, three ways of splitting the thread are possible.

1° The beads may split longitudinally and in the same plane, thus producing two straight chromonemata. These must then twist round each other, possibly to relieve

the tension set up by their division, to form the intertwined spirals observed at a later stage.

2° The beads may split longitudinally, but not in the same plane, so that two irregular zigzag threads are produced (EARL, 1927). These must become regularly intertwined spirals by further twisting.

3° The beads may split longitudinally and in different planes, and the split itself, together with the adjacent splits, may actually form a spiral. In this case, the new chromonemata have formed regularly intertwined spirals from their first inception, and need only to separate from each other to give the appearances seen at late prophase in *Scilla nonscripta*.

The alternatives 1° and 3° seem to provide the most adequate explanations of the appearances seen in *Scilla nonscripta*, but it is impossible to decide between them; although the lack of an intermediate stage between the presence of a single chromonema and its division into two regularly intertwined daughter chromonemata may indicate that the third suggestion is the more probable one.

It is evident, however, from the foregoing account, that each telophase chromosome may be conceived as consisting of two chromonemata. The last archesporial mitosis is apparently similar in all respects to a normal mitosis, therefore each chromosome is probably made up of two chromonemata when it enters the resting stage. The apparently single threads appearing at leptotene are in consequence also double. The threads, however, have never been observed to show their double nature, except by KAUFMANN (1931), who reports double threads at leptotene in his material, but has so far given no figures in support of his brief statement. Most workers describe them as single. In *Scilla nonscripta* however, the somatic chromosomes are also apparently single in early prophase, although they are really double; hence, it is not difficult to conclude that the leptotene threads are double, but that the doubleness is hidden by the extreme thinness and stretched condition of the strands at this phase and perhaps also by a great attraction between the two component halves.

It is therefore probable that the two chromosomes which pair at zygotene are double themselves, so that the tetrad condition of four half chromosomes is produced at this phase. It is not revealed, however, until the following late pachytene or diplotene.

DARLINGTON in his « precocity theory » has recently denied this interpretation. According to this theory the chromosome divides during the interphase of mitosis, so that it is double from the earliest prophase. The prophase of meiosis, however, is precocious, therefore the chromosome is still single when it appears at leptotene. Chromosome threads, are constructed so that they feel an attraction for each other in pairs, and hence the leptotene threads pair with their homologues. « The attraction of the single threads is unsatisfied. They pair with homologues as far as they can ». The theory offers a superficial explanation of why meiosis occurs. Similarly, DARLINGTON explains the repulsion of the pairs of chromatids at diplotene by assuming that the paired chromosomes divide at pachytene, « the temporary equilibrium is upset and they fall apart again into pairs of threads, being held together only by exchanges

of partner amongst 'the pairs ». This and other corollaries of the assumed attraction in pairs amongst the chromosome threads do not seem to the writer to provide an adequate explanation of all the phenomena observed, especially during somatic mitosis.

It will be supposed therefore, that the chromosomes pair at zygotene owing to some unknown attraction between them, and remain closely paired until diplotene. At diplotene, the chromosomes repel each other for some reason, and fall apart in pairs of half chromosomes, being held together only by the chiasmata where they have exchanged partners. In the description of meiosis the half chromosomes have been referred to as chromatids, following the general terminology employed, but in *Scilla nonscripta*, they correspond in appearance to the chromonemata of the somatic mitosis, except that they actually originated one division earlier. From diplotene onwards, the spiral crhomatids become more obvious and at metaphase are very clear. They differ from the chromonemata of a somatic mitosis only in being less regularly intertwined with wider spiral turns, and the body of the chromatid is thicker. Such differences however, are not fundamental, and are correlated with the greater degree of lengthwise contraction which occurs in the meiotic chromosomes. During the anaphase of meiosis, the presence of chiasmata causes the two chromatids passing to each pole to be widely separated for at least part of their length, and they are pulled out of their metaphase configurations. In mitosis, they remain unchanged at anaphase. Hence, in interphase they are usually wide apart after the first meiotic division, and are close together in mitosis. The closeness of the spiral threads is associated in some way with the presence of anastomoses in the interphase nucleus, for they are absent in the interphase of meiosis, and present in all other interphases.

The second division of meiosis resembles a normal mitosis except that the half chromosomes in prophase are wide apart, as they are in the preceding telophase, instead of being intertwined. At the end of prophase, as in mitosis, two new chromonemata are observed suddenly in each half chromosome at the end of prophase giving a quadruple condition for a brief period. The two intertwined chromonemata seen in each chromosome then pass unchanged to the poles, and the resting nucleus is constituted as in a normal mitosis.

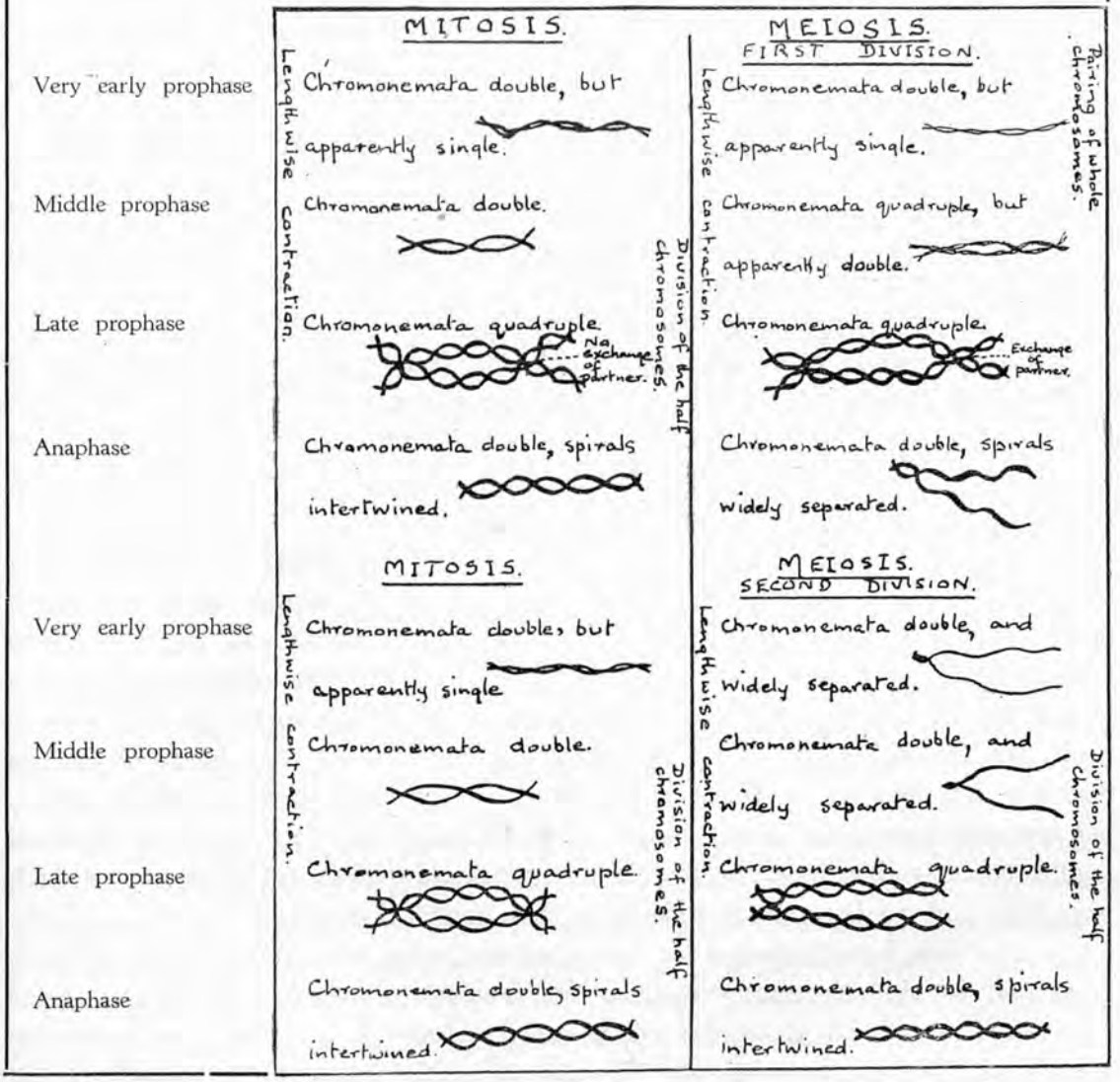
The main differences between the two divisions, which are suggested in the text figure 4, are as follows :

1. The chromosomes at mitosis probably divide during prophase to give four chromonemata. This split is suppressed in meiosis, being replaced by an earlier pairing of whole homologous chromosomes, which are double in themselves. Therefore a quadruple condition is obtained at meiosis, but by the pairing of two sets of half chromosomes (chromatids) rather than by the presence of four quarter chromosomes (chromonemata).

2. The quadruple condition is not observed until very late prophase of mitosis, but is visible at middle prophase (late pachytene to diplotene) of meiosis. It is probable that the pairing of whole chromosomes at zygotene gives a precocious quadruple condition, and this may suppress the occurrence of the normal division of the daughter chromosomes. In this case, the split giving four chromatids cannot have been initiated before zygotene, and therefore the corresponding split cannot have occurred in mitosis

before early prophase (unless it is suppressed fore some reason in meiosis). It may therefore be supposed that the split giving the four chromonemata in mitosis probably occurs at some time between early and late prophase.

SUMMARY OF THE SUGGESTED COMPARISON BETWEEN MITOSIS AND MEIOSIS.



TEXT FIGURE 4

3. The spiral chromatids passing to the poles at the first division of meiosis are widely separated, whereas the chromonemata at the anaphase of mitosis are seen to be closely intertwined. This difference is probably due to the presence of chiasmata at earlier stages of meiosis. In the second division of meiosis therefore, the two half chromosomes never approximate and appear single in early prophase as they do at mitosis, but remain widely separated. The gradual untwining of the two halves during prophase of mitosis is also generally absent in the prophase of the second division of meiosis. Later stages of the division, however, show identical spiral structures, the only difference being that the chromosome complement of the second division of meiosis has been halved.

Meiosis may therefore be derived in theory from mitosis by the supposition of a sudden attraction between the homologous chromosomes, which causes them to pair. This pairing may then suppress the next division of the chromo- nemata.

II. THE SPIRAL STRUCTURE OF THE CHROMOSOME.

The details of spiral structure during meiosis and mitosis have been a little difficult to interpret in *Scilla nonscripta*. Two alternative hypothesis present themselves to account for the appearances observed :

1. The pairs of spiral chromonemata associated together may be twining in opposite directions, with the same number of turns, so that the two spirals are mirror images. Such spirals would fit into one another to give the configurations seen during diakinesis and metaphase of meiosis and from early metaphase to telophase of mitosis. In face view they would give regularly crossing spirals like those which are so frequently seen, and in side view they would give the apparently single spirals which are often encountered during diakinesis and metaphase of meiosis, if it is supposed that the thread underneath is hidden or does not stain in these cases. At anaphase the chromatids would easily separate unchanged, for they would not be intertwined, and would therefore come apart without difficulty.

2. The pairs of chromonemata associated together may twine in the same direction, and therefore intertwine at every turn. These spirals would also give the configurations observed from early metaphase to telophase of mitosis and during diakinesis and metaphase of meiosis. The apparently single threads often observed during diakinesis and metaphase of meiosis could be explained as due to the parallel approximation of the two spiral chromonemata at these points. (It may be noted that the double spirals discussed by KUWADA, which twine in the same direction as one another but are only *inter*-twined for a certain random number of twists would not give the regular spaces and crosses observed in an optical view of the chromosomes of *Scilla nonscripta*, and need not be considered further in this discussion.)

The first hypothesis was at first considered the more probable. It accounted quite well for the structures seen from late prophase to telophase of meiosis, and it was hoped to extend it to the spirals seen at mitosis. In attempting to correlate mitosis with meiosis, however, the following difficulties arose :

1. The threads which enter the prophase of meiosis are those which went into the telophase of the last somatic mitosis. Each of the telophase chromosomes had apparently provided two of these threads, which were first observed as spiral chromonemata in the preceding late prophase. It is difficult to imagine, however, how two chromonemata with oppositely twined and *interlocked* spirals could be derived from the single chromonema of earlier prophase, unless the thread first split, the halves separated, spiralled apart from each other, and them came together again and fitted into each other's spirals. There is no evidence that such a process occurs. *Intertwined* spirals, on the other hand, can easily be produced from a single thread. Even if it

is supposed that the chromonemata are intertwined during mitosis, but straighten out in the interphase before meiosis and then fit into each other during meiosis, the two spiral threads twining in opposite directions would still need to be pulled apart and spiralled separately at some stage between zygotene and pachytene and there is never the slightest evidence for such a separation.

2. If the chromonemata are *interlocked* at mitosis, why does the characteristic side view never appear during metaphase, anaphase and early telophase? Where a structure shows at all, the spirals always appear to be very regularly twisted during these stages. This agrees with the theory that they are *intertwined* in which case the spirals would give the same appearance in all side views of the chromosome.

It therefore seems that the second hypothesis viz : that the two chromonemata are intertwined, is the more adequate one to fit the facts observed. Some workers have found it difficult to imagine how the intertwined spirals can separate (eg. BABCOCK & CLAUSEN). They can do so quite easily, however, as long as they can slip past each other witnout sticking, and are not pulled out sideways at more than one point at a time. The attachment constriction is situated at one point only in each daughter chromosome, and the ends of these daughter chromosomes are pulled from each other in a lengthwise direction, towards opposite poles. The chromonemata are probably not very sticky at this stage, otherwise the half chromosomes would be likely to pull pieces from each other's surface when they press closely against each other at full metaphase. Hence, wherever the position of the attachment constriction, intertwined chromonemata can pull apart from each other without difficulty at anaphase.

The chromosomes which pass to the poles at the anaphase of the first division of meiosis show clear spirals, the chromatids usually being widely separated. The spirals are almost more evident at this stage than they are at any other period of the nuclear cycle. They sometimes give the appearance of being slightly pulled out, but this can easily be accounted for by the resistance to separation offered at the chiasmata. Such comparatively undisturbed spirals are expected if the two spirals at metaphase are fitted into one another, but if they are intertwined, their separation presents a little difficulty. Intertwined spirals, if they are pulled apart by two forces each moving in a straight line, will untwist in order to separate, therefore the chromatids at anaphase should be straight. The only way in which such spirals would be left undisturbed is by the forces which pull the chromosomes to the poles also rotating in a spiral path, with the same width of twist and direction of turn as the intertwined spirals have at metaphase. In this case, the two spiral chromonemata will slip past each other and emerge at anaphase each with the same spiral formation as they had at metaphase. It is difficult to state, however, whether such a rotation can actually occur in nature.

The formation of chiasmata in pachytene has been regarded hitherto as facilitated by the division of the previously unsplit paired chromosomes at this stage. BELLING (1931) suggests that the paired chromosomes twist half round each other at certain random points along their length. The chromomeres, which constitute the

essential parts of the chromosomes, then divide, and new « fibres » grow between the half chromosomes thus formed, so that four chromatids are produced in the place of two chromosomes. Where a twist occurs, however, the new « fibres » will take the shortest distance between the chromomeres, and in this way crossing over will occur between two of the four chromatids at a twist. It will be a matter of chance which two of the four chromatids cross over, and this agrees with genetical observations. The theory as it stands, however, does not seem to take account of the phenomenon of interference. DARLINGTON also supports the view that the division of two relatively thick chromosomes into four thin strands, coupled with a torsion set up between the four chromatids is responsible for breaks occurring and giving chiasmata. « If breakage amongst the four threads associated at the end of pachytene is caused by torsion, reunion amongst the broken ends is most likely to occur in such a way as to relieve the strain. The occurrence of one break will therefore reduce the chance of another break in the neighbourhood. The interference observed is therefore expected on the theory of torsion ».

It is probable that torsion of this kind is responsible for the breaks in the threads at pachytene, but it does not seem necessary to suppose that these breaks are dependent on a new division occurring in the chromosomes at this stage. If each chromosome consists of two intertwined chromatids, and is at the same time twisted round its homologous partner (it may be recalled that the paired pachytene threads often give the appearance of a two-stranded rope) it is easy to conceive of breaks occurring in the doubly twisted threads, and these would probably be mended in such a way as to relieve the strain of torsion. One break will reduce the chance of another break occurring near it, therefore « interference » will be expected. Four parallel threads all twisted equally together (as the chromatids would presumably be on DARLINGTON's hypothesis) would probably all break at once, as they are subjected to equal strain; whereas four chromatids entwined first in pairs and then the pairs round each other would be more likely to feel the strain of twisting unequally and give the crossing over in pairs which occurs. Whether this is the mechanism which produces chiasmata or not, it remains evident that the division of the chromosomes at pachytene need not be a necessary part of an explanation of how chiasmata are formed.

III. THE MATRIX.

The chromonemata appearing in the fully formed chromosomes at mitosis and meiosis have been described in the foregoing account as probably forming intertwining spirals between which is a lighter coloured core. This shows between the spirals on their inner surface, but never extends beyond them, therefore the spirals would seem to be arranged on its periphery. It appears to be coloured by the same stain as the chromonemata, but more faintly. This tint, however, may be due to the reflection through it of the coloured spirals rather than to any stained material in the « matrix » itself. A « matrix », or lighter coloured centre, appears in the daughter chro-

mosomes at late prophase, at the same time as the new spiral chromonemata become visible. It persists until the « tassement polaire », when it disappears until the following late prophase, at which stage two new « matrices » appear in the half chromosomes. This description of the behaviour of the « matrix » at mitosis agrees with KAUFMANN's observations. At meiosis, two spiral chromatids are associated together in a common « matrix » from diplotene to metaphase, so that there are as many « matrices » as there are whole chromosomes. At anaphase, if the chromatic spirals separate, the single spirals observed are each accompanied by a « matrix », so that presumably the common « matrix » has divided at this stage. Where double intertwined spirals still persist at anaphase, however, the « matrix » does not divide. It disappears into early telophase and does not reappear again until the two spiral chromonemata become visible in the half chromosomes at late prophase, when a « matrix » is found between the two spirals.

A matrix has been described in the chromosome by a number of investigators. A survey of the behaviour of the « matrix » in *Scilla nonscripta*, however, does not suggest an autonomous unit, and it disappears completely between late telophase and middle to late prophase. It is suggested that the « matrix » is part of the karyolymph, which is perhaps altered in some way by its close contact with the spiral chromonemata, and is held inside them by surface tension. When the spirals loosen out, the tension is overcome, and the « matrix » passes back again into the karyolymph — which is itself only the product of an interaction between the chromosomes and cytoplasm. At anaphase of meiosis, the « matrix » divides into two parts if the chromonemata are separated, but not otherwise, therefore it is entirely dependent upon the behaviour of the chromonemata, which form the essential parts of the chromosome. PERRY's statement is supported, that the « matrix » is a fluid substance held within the turns of the chromonemata, but not binding them in any way.

DARLINGTON has given several reasons which indirectly support the existence of a « pellicle » around the outside of the chromosome. It may be this which limits the size of the spiral turns assumed by the chromonemata and therefore determines the width of the chromosome. No visible external pellicle has been observed round the chromosomes of *Scilla nonscripta*, however, and the reasons for its existence can only be deduced indirectly from other data.

CONCLUSION.

Whatever the exact nature of the matrix may be, it has been shown that each chromosome of *Scilla nonscripta* probably always consists of at least two chromatic threads, and may even be quadruple for a brief period; each chromosome, therefore may be considered as inherently dual in structure. The two threads usually appear as spirals, each presumably being intertwined with the spiral of its partner. One or more spiral filaments have been found in the chromosome by the great majority of recent cytological workers, and in this investigation they are reported for

yet another plant. The suggestion may therefore be put forward with renewed confidence that spiral chromatic threads are probably universally present in dividing nuclei and form the permanent parts of the chromosome. The observations of GRÉGOIRE, OVERTON, BOLLES LEE and other workers who obtained alveoles, discontinuous filaments, or any structure other than spiral chromosomes or chromonemata are therefore probably faulty interpretations of the true nature of the chromosome. The division which gives the two chromonemata in a somatic mitosis has been placed earlier and earlier in the chromosome cycle as technique has improved, and many recent observers now suggest that it occurs in the late prophase or metaphase of the division which precedes their separation. Division at this stage has been reported by KAUFMANN, SHARP, TAYLOR, HEDAYETULLAH, PERRY, SMITH and SIANG. It may occur universally at this time, and may possibly be found wherever structure can be clearly distinguished in chromosomes.

It has been necessary to correlate these conclusions with the conflicting ones current among students of meiosis. It has been shown that the absence of a visible duality at leptotene is no proof of its non-existence (on the analogy of the behaviour at prophase of mitosis) and meiosis has been described on the hypothesis that two already double chromosomes pair side by side, to give a tetrad structure at zygotene. This may suppress a further split in the half chromosomes at late prophase. The appearances seen at meiosis have been found on this interpretation to harmonise with those which are so frequently described at mitosis. The conception of the permanent duality of the chromatic constituents of a chromosome at mitosis has been extended to meiosis, where there are always at least two chromatic threads associated together, and four are present during the greater part of prophase and metaphase of the first division.

SUMMARY.

1. The chromosomes of *Scilla nonscripta* during meiosis and mitosis are conceived as always consisting of at least two regularly intertwined spiral chromonemata, which may be coiled within a lightly staining matrix.

2. *Mitosis.* The chromonemata are first observed when late prophase is passing into early metaphase, the new chromonemata formed being those which will separate as chromosomes at anaphase of the next division.

3. The two chromonemata of each chromosome pass to the poles as intertwined spirals. During late telophase and interphase they apparently approximate to one another, so that at early prophase they appear as a single thread. Their duality reappears in later prophase, after which they gradually untwist from one another, in readiness for their separation at anaphase.

4. The last archesporial mitosis resembles a normal mitosis in all respects.

5. *Meiosis.* The chromosomes pairing at zygotene are therefore probably each made up of two chromonemata (chromatids), although this duality is obscured until diplotene. The pairing of the homologous chromosomes at zygotene seems to

suppress the split which becomes visible at early metaphase in *mitosis*. Spiral chromatids are first distinguished at diplotene.

6. The second division of meiosis resembles a normal mitosis except that the number of chromosomes is halved, and the chromatids are generally more widely separated from each other during prophase. Chromonemata appear in these chromatids for the first time at early metaphase.

7. The number and behaviour of the chiasmata are described for the eight chromosome bivalents at meiosis, and are found to agree with the chiasmatypy hypothesis. A torsion of the pachytene threads is suggested as a possible cause of chiasma formation.

8. The eight chromosome types are described, with special reference to the position of their attachment constrictions.

9. Meiosis in the pollen mother cell is compared with meiosis in the megaspore mother cell, and is found to be identical, even to the finer details of chromosome structure. This is the first time for any angiosperm that (*a*) intertwined spiral chromatids and (*b*) chiasmata have been described in the megaspore mother cell.

10. The spiral nature and the behaviour of the chromonemata in mitosis are compared with the corresponding phenomena in meiosis, and are interpreted as similar in many essentials. The apparent differences are enumerated and discussed.

11. Spiral chromonemata are described in the nuclei of the endosperm tissue for the first time for any angiosperm.

12. Stages in the budding of the nucleoli during meiosis in *Scilla nonscripta* are described and discussed. The nucleoli persist in the cytoplasm during metaphase, anaphase and telophase of division, and may possibly re-enter the two new daughter nuclei which are formed.

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LITERATURE CITED.

1929 *Babcock, E. B. & Clausen, J.* : Meiosis in two species and three hybrids of *Crepis* and its bearing on taxonomic relationship ; Univ. Calif. Publ. Agric. Sci., **2**, 401-425.

1928 *Belling, J.* : A working hypothesis for segmental interchange between homologous chromosomes in flowering plants ; Univ. Calif. Publ. Bot., **14**, 283-291.

1928 *Id.* : Segmental interchange and crossing over ; Nature, **121**, 282-283.

1931 *Id.* : Chromomeres of Liliaceous plants ; Univ. Calif. Publ. Bot., **16**, 153-170.

1926 *Darlington, C. D.* : Chromosome studies in the *Scilleae ;* Journ. Gen., **16**, 237-251.

1928 *Id.* : Studies in *Prunus*, I and II ; Ibid., **19**, 213-256.

1929 *Id.* : Meiosis in Polyploids, II ; Ibid., **21**, 17-56.

1929 *Id.* : Chromosome behaviour and structural hybridity in the *Tradescantiae ;* Ibid., **21**, 207-286.

1930 *Id.* : Studies in *Prunus*, III ; Ibid., **22**, 65-93.

1930 *Id.* : A cytological demonstration of « genetic » crossing over ; Proc. Roy. Soc. B., **107**, 50-59.

1931 *Id.* : Meiosis ; Biol. Revs., **6**, 221-264.

1932 *Id.* : Recent advances in cytology ; London, J. & A. Churchill.

1919 *Digby, L.* : On the archesporial and meiotic mitosis of *Osmunda ;* Ann. Bot., **33**, 135-172.

1927 *Earl, R. O.* : The nature of chromosomes. I. Effects of reagents on root tips sections of *Vicia faba ;* Bot. Gaz., **84**, 58-74.

1930 *Fikry, M. A.* : Phenomena of heterotypic division in the pollen mother cells of a tetraploid form of *Rumex scutatus var. typicus ;* Journ. Roy. Micr. Soc., **50**, 387-419.

1911 *Fraser, H. C. I. & Snell, J.* : The vegetative divisions of *Vicia Faba ;* Ann. Bot., **25**, 845-855.

1911 *Granier, J. & Boule, L.* : Sur les cinèses somatiques chez *Endymion nutans ;* C. R. Ac. Sc. Paris, **152**, 153-154.

1911 *Id.* : Sur le phénomène de conjugaison des chromosomes à la prophase de la première cinèse réductrice (microsporogénèse chez *Endymion nutans*) ; Ibid., **152**, 393-396.

1904 *Grégoire, V. & Wygaerts, A.* : La reconstitution du noyau et la formation des chromosomes dans les cinèses somatiques ; La Cellule, **21**, 5-76.

1906 *Grégoire, V.* : La structure de l'élément chromosomique au repos et en division dans les cellules végétales ; Ibid., **23**, 311-357.

1931 *Hedayetullah, S.* : On the structure and division of the somatic chromosomes in *Narcissus* ; Journ. Roy. Micr. Soc., **51**, 347-386.

1926 *Kaufmann, B. P.* : Chromosome structure and its relationship to the chromosome cycle. I. Somatic mitosis in *Tradescantia pilosa* ; Amer. Journ. Bot., **13**, 59-80. II. *Podophyllum peltatum* ; Ibid., **13**, 355-363.

1931 *Id.* :Chromonemata in somatic and meiotic mitoses ; Amer. Nat., **65**, 280-283.

1924 *Kihara, H.* : Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rüchsicht auf das verhalten der Chromosomen und die Sterilität in der Bastarden ; Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, **1**, 1-200.

1926 *Kuwada, Y. & Sakamura, T.* : A contribution to the colloidchemical and morphological study of chromosomes ; Protoplasma, **1**, 239-254.

1926 *Kuwada, Y.* : On the structure of the anaphasic chromosomes in the somatic mitoses of *Vicia faba*, with special reference to the so-called longitudinal split of the chromosomes at telophase ; Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, **2**, 1-13.

1927 *Id.* : On the spiral structure of chromosomes ; Bot. Mag. Tokio, **41**, 100-109.

1931 *La Cour, L.* : Improvements in everyday technique in plant cytology ; Journ. Roy. Micr. Soc., **51**, 119-126.

1925 *Lee, A. Bolles* : The chromosomes of *Paris quadrifolia* and the mechanism of their division ; Quart. Journ. Micr. Sci., **69**, 1-25.

1900 *Mc Clung, C. E.* : The spermatocyte divisions of the *Acrididae* ; Kansas Univ. Quart. **9**A, 73-100.

1898 *Mc Kenney, R. E. B.* : Observations on the development of some embryo-sacs ; Contrib. Bot. Lab. Univ. Pennsylvania, **2**, N° 1, 80-86.

1928 *Maeda, T.* : The spiral structure of chromosomes in the sweet pea (*Lathyrus odoratus*, L.) ; Bot. Mag. Tokyo, **42**, 191-195.

1930 *Id.* : The meiotic divisions in the pollen mother cells of the sweet pea (*Lathyrus odoratus*, L.) with special reference to the cytological basis of crossing over ; Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B, **5**, 89-123.

1922 *Martens, P.* : Le cycle du chromosome somatique dans les Phanérogames. I. *Paris quadrifolia* ; La Cellule, **32**, 331-428.

1925 *Id.* : Id. II. *Listera ovata* ; Ibid., **36**, 125-214.

1927 *Id.* : Id. III. Recherches expérimentales sur la cinèse dans la cellule vivante ; Ibid., **38**, 67-174.

1929 *Id.* : Nouvelles recherches expérimentales sur la cinèse dans la cellule vivante ; Ibid., **39**, 167-216.

1927 *Newton, W. C. F.* : Chromosome studies in *Tulipa* and some related genera ; Linn. Soc. Journ. Bot. (London), **47**, 339-354.

1922 *Overton, J. B.* : On the organisation of the nuclei in the root tips of *Podophyllum peltatum ;* Trans. Wisconsin Acad. Sci., **20**, 275-322.

1932 *Perry, K.* : Mitosis in *Galanthus nivalis ;* Journ. Roy. Micr. Soc., **52**, 344-356.

1924 *Sarbadhikari, P. C.* : Cytology of *Osmunda* and *Doodia*. I. On the somatic and meiotic mitosis of *Doodia ;* Ann. Bot., **38**, 1-26.

1901 *Schniewind-Thies, J.* : Die Reduktion der Chromosomenzahl und die ihr folgenden Kernteilungen in den Embryosackmutterzellen der Angiospermen ; Jena, 1901.

1929 *Sharp, L.* : The structure of large somatic chromosomes ; Bot. Gaz., **88**, 349-377.

1930 *Shinke, N.* : On the spiral structure of chromosomes in some higher plants ; Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, **5**, 239-245.

1932 *Siang, Hsu* : The structure of somatic chromosomes in *Lilium tigrinum ;* La Cellule, **41**, 165-178.

1932 *Smith, F. H.* : The structure of the somatic and meiotic chromosomes of *Galtonia candicans ;* La Cellule, **41**, 243-263.

1932 *Stone, L. H. & Mather, K.* : The origin and behaviour of chiasmata. IV. Diploid and triploid *Hyacinthus ;* Cytologia, **4**, 16-25.

1924 *Taylor, W. R.* : The smear method for plant cytology ; Bot. Gaz., **78**, 236-238.

1931 *Id.* : Chromosome studies on *Gasteria ;* Amer. Journ. Bot., **18**, 367-386.

1930 *Telezynsky, H.* : Cycle évolutif du chromosome somatique. I. Observations vitales sur les poils staminaux de *Tradescantia virginiana ;* Acta Soc. Bot. Poloniae, **7**, 381-433.

1931 *Tuan, Hsu-Chuan* : Unusual aspects of meiotic and postmeiotic chromosomes of *Gasteria ;* Bot. Gaz., **92**, 45-64.

1928 *Zirkle, C.* : The nucleolus in root tip mitosis in *Zea Mays ;* Bot. Gaz., **86**, 402-418.

EXPLANATION OF PLATES.

All drawings were made with the aid of a camera lucida, using a ZEISS *2 mm. apochromatic objective N. A. 1.4 and compensating oculars K 12 (× 15), or (× 30) Magnification : × 1800 or × 3600.*

PLATE I.

FIGS. **1-13**. Portions of nuclei in somatic mitosis : 1-7 in prophase, 8 and 9 in early metaphase, 10-13 in telophase.

FIG. **1**. Very early prophase. × 1800.

FIG. **2**. A later stage : chromosome spirals apparently single, but really double. × 3600.

FIG. **3**. A later stage : spirals very evident, still apparently single. × 3600.

FIG. **4**. A later stage : duality of spirals apparent in certain parts of the chromosomes. × 3600.

FIG. **5**. Mid prophase : each chromosome showing two intertwined spiral chromonemata. × 3600.

FIG. **6**. A later stage : chromosomes clearly double throughout their length. × 3600.

FIG. **7**. Late prophase : chromonemata have gradually untwisted from each other and have shortened and thickened. × 3600.

FIG. **8**. Late prophase to early metaphase : five pairs of half chromosomes incompletely shown. Two intertwined chromonemata have now formed in each half chromosome. × 3600.

FIG. **9**. The same : polar view of one pair of half chromosomes, showing spiral structure. × 3600.

FIG. **10**. Late anaphase : chromosomes passing to the poles (seven only shown), each one with two intertwined chromonemata. × 3600.

FIG. **11**. Mid telophase : chromosomes now connected by a few anastomoses. Spiral chromonemata are still visible, the two spirals appearing as one in the chromosome on the left. × 3600.

FIG. **12**. The same : part of one chromosome, in which two spirals are apparent at one end, but only one at the other end. × 3600.

FIG. **13**. Late telophase : double spirals still evident in most places. Chromonemata are thinner and less easily distinguishable from the increasingly evident anastomoses. × 3600.

FIGS. **14-36**. Nuclei in first division of meiosis.

FIGS. **14-24**. A comparison of the nucleus in the pollen mother cell (p. m. c.) and the megaspore mother cell (m. m. c.).

FIGS. **14**. (p. m. c.) and **15** (m. m. c.). Early zygotene : chromosomes beginning to pair. × 1800.

FIGS. **16** (p. m. c.) and **17** (m. m. c.). Pachytene : chromosomes closely paired. The individual members of each pair are more evident in m. m. c. × 1800.

FIGS. **18** and **19** (p. m. c.) and **20** (m. m. c.). Diplotene : the members of each pair now very distinct and clearly double in themselves. In FIG. **20** one bivalent has been displaced by the microtome knife. In FIG. **19** a selected bivalent from a p. m. c. shows clearly the spiral form assumed by each chromatid. × 1800.

FIGS. **21** (p. m. c.) and **22** (m. m. c.). Diakinesis : the eight bivalents show the spiral structure of the chromatids in the p. m. c. but not in the m. m. c. × 1800.

FIGS. **23** (p. m. c.) and **24** (m. m. c.). Metaphase : eight bivalents, A-H, each chromosome of the pair with two spirally intertwined chromatids. Chiasmata are very evident in the bivalents C, E and F of the m. m. c. × 3600.

FIGS. **25** and **26**. Metaphase in p. m. c. : three bivalents from one nucleus and two from another, selected for their very evident spiral structure. × 3600.

PLATE II.

FIG. **27**. The same : the eight bivalents slightly spread out in smearing. The four spiral chromatids in each bivalent are clearly shown. A nucleolus persists on the left of the drawing. × 1800.

FIG. **28**. Early anaphase in p. m. c. : seven bivalents drawn spread out for clearness sake, and the eighth omitted. × 1800.

FIG. **29**. The same : six of the eight bivalents shown in position. Two persistent nucleoli lie on either hand. × 1800.

FIG. **30**. The same : seven bivalents shown, and two persistent nucleoli. × 1800.

FIG. **31**. Late anaphase in p. m. c. : spiral chromatids still clearly distinct. Note the influence of the attachment constriction on the shape of the chromosome. Nucleolus shown on right. × 1800.

FIG. **32**. The same : spiral chromatids not shown. The characteristic shapes of the individual chromosomes are very distinct. Nucleolus shown on left. × 1800.

FIG. **33**. Early telophase in p. m. c. : spiral chromatids not shown One nucleolus. × 1800.

FIG. **34**. Mid telophase in p. m. c. : spiral chromatids clearly shown. One nucleolus. × 1800.

FIG. **35**. Late telophase in m. m. c. : spiral chromatids shown. A wall has been formed between the two daughter nuclei. × 1800.

FIG. **36**. Interphase in m. m. c. : spiral chromatids still distinct, though less evident. The nucleolus on the left is apparently just piercing the nuclear membrane. The nucleolus on the right is still outside the nucleus. × 1800.

FIGS. **37-45.** Nuclei in second division of meiosis.

FIG. **37.** Early prophase in p. m. c. : spiral nature of chromatids more or less obscured. Along some of the threads there is slight evidence of the new split. A wall has been formed between the two daughter nuclei. × 1800.

FIG. **38.** Late prophase to early metaphase in p. m. c. : six half chromosomes (chromatids) selected from one nucleus to show that two intertwined spiral chromonemata have now been formed in each. × 3600.

FIGS. **39** (p. m. c.) and **40** (m. m. c.). Metaphase : spiral chromonemata obscured. The eight chromosome types A-H are more clearly shown in p. m. c. × 1800.

FIG. **41.** Anaphase in m. m. c. : spiral chromonemata obscured. The chromosome types are clearly shown. × 1800.

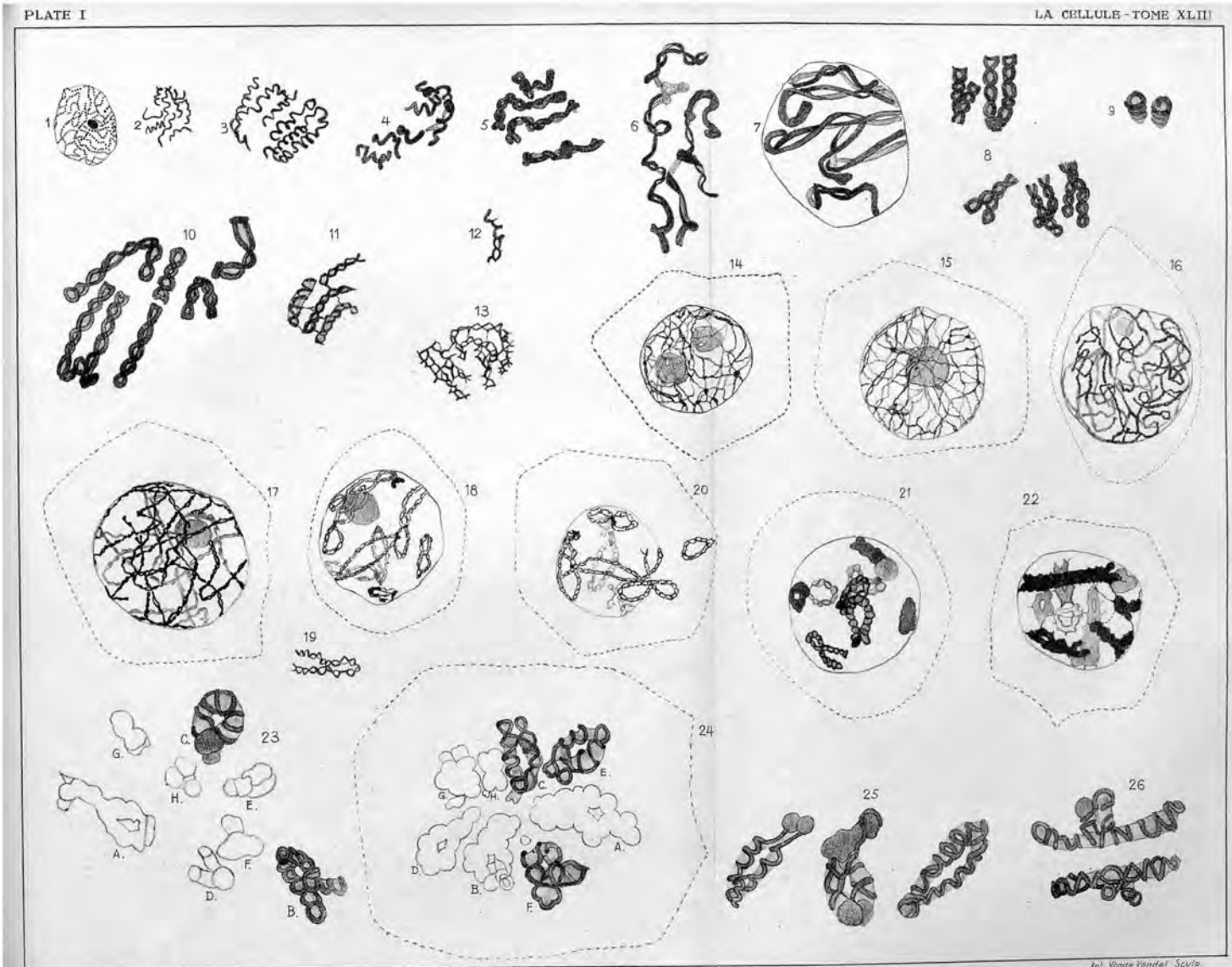
FIG. **42.** Early telophase in p. m. c. Some of the chromosomes have been selected, to show that each has two intertwined spiral chromonemata. × 3600.

FIG. **43.** Telophase in p. m. c. : four chromosomes drawn to show spiral chromonemata still distinct in each. × 1800.

FIG. **44.** Telophase in p. m. c., showing the four nuclei. All the chromosomes are not drawn. They are still distinct from one another, and each has two spiral chromonemata. × 1800.

FIG. **45.** Tetrad of young pollen grains : nuclei in late telophase. The chromosomes are beginning to show anastomoses, but the two spiral chromonemata are still fairly distinct in each. The nucleoli are apparently budding with great vigour, and pieces of nucleolar material are seen in the cytoplasm. × 1800.

CONTENTS.



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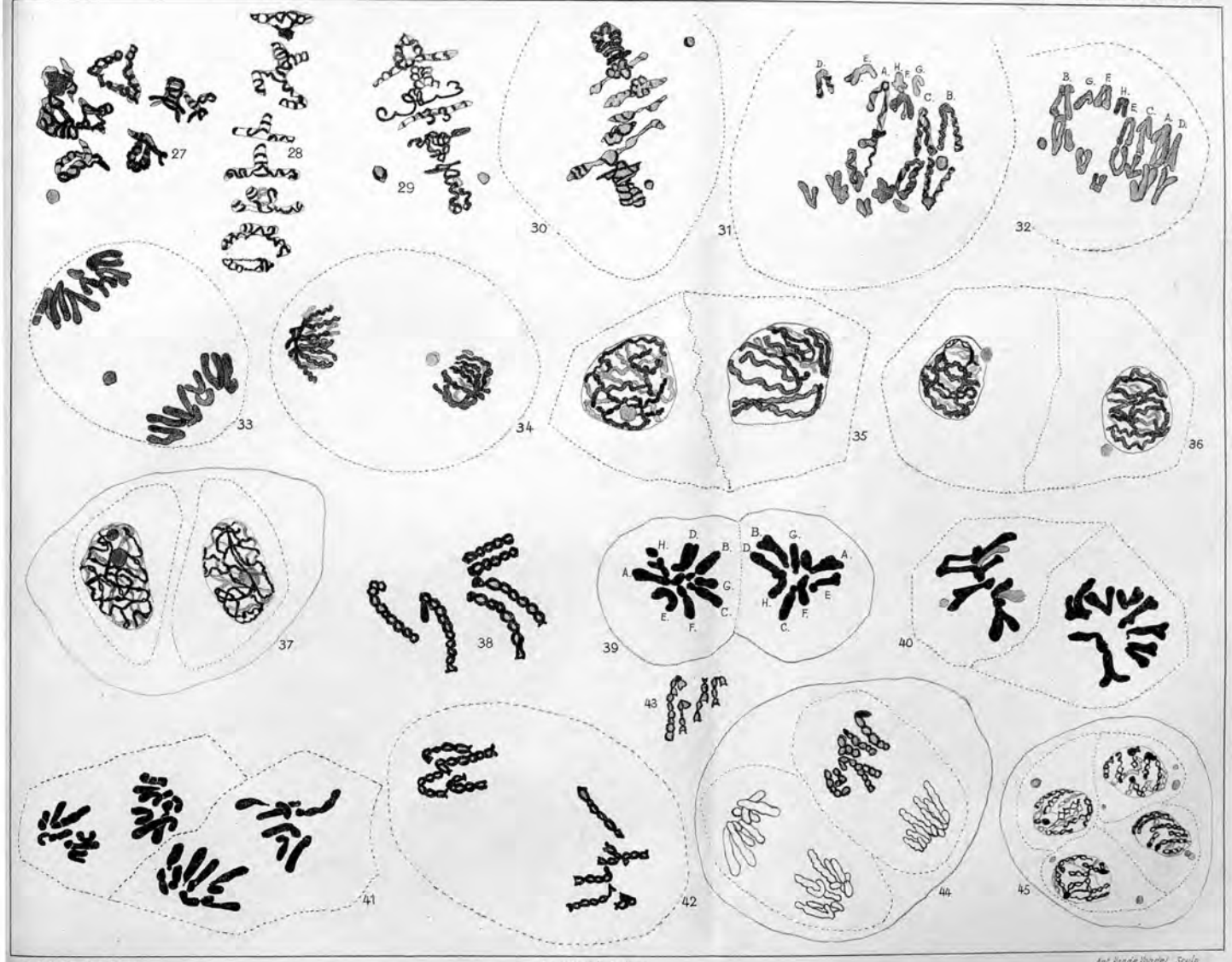

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GAMETOGENESIS AND FERTILISATION IN SCILLA NONSCRIPTA.

See accompanying separate, La Cellule, December 1933.

GAMETOGENESIS AND FERTILISATION

IN

SCILLA NONSCRIPTA

BY

Gladys V. HOARE, B. Sc.

DEPARTMENT OF BOTANY, ROYAL HOLLOWAY COLLEGE, UNIVERSITY OF LONDON

(Extrait de « La Cellule », tome XLII, fascicule 3, 1934)

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Sommaire du fascicule 3 :

GAMETOGENESIS AND FERTILISATION

IN

SCILLA NONSCRIPTA

BY

Gladys V. HOARE, B. Sc.

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GAMETOGENESIS AND FERTILISATION

IN

SCILLA NONSCRIPTA HOFFM. & LINK.

SUMMARY OF PREVIOUS WORK AND SCOPE OF THE PRESENT INVESTIGATION.

There are surprisingly few recorded investigations of embryo sac development and fertilisation in *Scilla* species, considering that the genus offers peculiarly convenient and promising material.

The earliest record of embryo sac development was made by J. VESQUE (1878), which account however, has been corrected by later workers. The first accurate account of embryo sac development in *Scilla* was given by TREUB & MELLINK (1880) for *Agraphis patula* (= *Scilla hispanica*). The production of two binucleate cells from the « embryo sac mother cell » was described. These cells are at first of equal size. The upper cell then gradually enlarges, and its nuclei divide twice, producing eight nuclei, of which four are at the base of the sac and four at the top. The upper nuclei form the synergids and egg, the lower nuclei sometimes form antipodal *cells*, but usually stay free. Meanwhile the lower cell of the original binucleate pair remains approximately the same size, but is later found constantly with four nuclei.

This account was briefly confirmed by GUIGNARD (1882) for *Agraphis* (= *Scilla*) *campanulata*, and his work is cited by OVERTON (1893). The next description was by Mc KENNEY (1898) for *Scilla hyacinthoides* and *Scilla campanulata*. The hypodermal cell of a young ovule was said to divide into two, of which the upper one is tapetal and divides again into two, whilst the lower one also divides into two, the upper cell of which degenerates later on ; hence, a row of four cells is obtained, of which the lowest one becomes the megaspore mother cell. The reduction of sixteen chromosomes to eight is briefly noted in anaphase and telophase of meiosis. The upper cell from this heterotype division becomes the embryo sac. The succeeding stages of development in the female gametophyte were outlined and were found to be as described by TREUB & MELLINK. Mc KENNEY stated that the lower quadrinucleate cell gradually degenerates, the degeneration being complete at about the period of fertilisation.

In 1901, SCHNIEWIND-THIES described the development of the embryo sac in *Scilla sibirica*. The origin and development of the embryo sac were outlined, the description agreeing with the foregoing accounts. The chromosomes of the nucleus were found to vary from eight to sixteen. Their behaviour at meiosis was briefly described, it being stated that they may form hooplike figures or crosses and are frequently bent at the ends. Two nuclear divisions were stated to occur in the pollen grain, but this statement cannot be confirmed.

A brief account of fertilisation in *Scilla* was given by GUIGNARD (1891 and 1899) for *Agraphis cernua* and *Endymion mutans* (= *Scilla nonscripta*). The two polar nuclei unite before the penetration of the pollen tube, but they do not fuse. Fertilisation occurs very rapidly, the male nucleus which unites with the egg being smaller than the one which fuses with the polar nuclei. The male nucleus often remains distinct within the paired polar nuclei for some time, but the fusion of the egg and « antherozoid » is quite rapid. The male nuclei are always smaller and more chromatic than those of the egg and polars, and usually possess one to three nucleoli.

An examination of these accounts reveals several gaps in our knowledge of the plant. The development of the male gametophyte and the germination of the pollen tubes in the style have not been traced. Fertilisation stages are scanty, details of fusion and the subsequent nuclear divisions being entirely absent. The accounts of development and fertilisation which do exist are made without a knowledge of modern technique, descriptions of division stages are few and are obscured by obsolete terminology. It therefore seemed worth while to work on gametogenesis and fertilisation in *Scilla nonscripta*, especially as the large nuclei are such favourable material for detailed study of nuclear structure.

METHODS ADOPTED IN THE PRESENT INVESTIGATION.

The development of the pollen grain until the time of its germination was studied by means of pollen smears. Stages in the development of the female gametophyte, the growth of the pollen tube, fertilisation and the development of the embryo and endosperm were obtained from paraffin sections. The principle fixatives used were FLEMMING's fluid weak, LA COUR's 2BD and 2BE (LA COUR, 1931) and NAVASCHIN's fluid. Gentian violet was the stain usually employed and with it a counterstain (Orange G or erythrosin) was often used, especially for the fertilisation stages. Methods adopted in smearing, fixing and embedding were similar to those recommended by LA COUR. Sections were cut from 18 μ to 20 μ, in thickness.

Note. The superiority of the smear method is revealed by the fact that the contraction formerly known as « synizesis » does not appear in smear preparations. The nuclear membrane is much less distinct, so that its limits are frequently difficult to discern, and there is never any sign of spindle fibres in the cell during metaphase and anaphase. All these structures are emphasised by inferior fixation, and their lack of definition in smears, points to the greater efficiency of fixation obtained by this method.

OBSERVATIONS.

§ 1. Formation and development of the microsporangium.

Buds cut during early October show that next year's stamens are already formed, but are extremely small, the whole of each being composed of a uniform tissue of active, dividing cells, with no archesporium as yet discernible. Later in

the month, a definite archesporium is differentiated, and it is distinguished from the surrounding tissue by its larger cells, each with a large nucleus and dense cytoplasm. The archesporial cells divide actively during November and December, but pollen mother cells have begun to form in some buds by the beginning of December, their nuclei passing fairly rapidly into pachytene. In anthers examined at the beginning of January the nuclei of the pollen mother cells are almost invariably in pachytene, and this condition persists until the end of January. Diplotene and later stages then begin to be fairly common, the subsequent stages of meiosis following one another in rapid succession, so that all stages frequently may be found together in the same bud. Such stages of meiosis can be procured from suitable buds until about the end of February. This time schedule has proved remarkably constant during the three years of observation, 1930-33.

After the first division of meiosis a wall is invariably laid down between the two daughter nuclei. The spindles of the second division may be at any angle to this wall, but are usually more or less parallel with it, and therefore parallel with one another. About 5-10 % of the dividing pollen has the two spindles at right angles to one another. Further walls are formed delimiting the four pollen grains. The shape of the tetrad varies with the angle previously assumed by the spindles, and is therefore usually cruciform, with all four nuclei more or less in one plane, but all grades may be found between this and a roughly tetrahedral shape.

The pollen grains gradually loosen from their tetrad condition, until they are quite separate from one another. Each is then roughly pyriform, with a single resting nucleus. The grains remain in the anther for some weeks in this condition.

§ 2. Nuclear division in the pollen grain.

After a time the nucleus of each grain enters into prophase and divides (Figs. **6-10**). The two daughter nuclei are at first very much alike (Fig. **10**), but soon the one nearest the pointed end of the pyriform grain seems to be slightly smaller and more chromatic than the other. This difference becomes more and more marked, as the nucleus in the centre of the grain (the tube nucleus) enlarges and passes into the resting stage, whilst the smaller one (the generative nucleus) develops a thick chromatic reticulum. The generative nucleus remains in the pointed tip of the grain, and is surrounded by a small quantity of dense cytoplasm. This is separated from the cytoplasm of the tube nucleus by a clear concave space; that is, two separate cells have been differentiated. The nucleus of the generative cell gradually elongates until it has assumed a characteristic vermiform shape, often coiling round somewhat in the cytoplasm, and the thick bands of chromatin give it a striped appearance. The tube nucleus, by contrast, is large and spherical, with very diffuse and scarcely stainable chromatin (Fig. **11**). In this condition the pollen grain remains until it is shed.

§ 3. Development of the megasporangium and the embryo sac.

The early development of the megaspore mother cell is parallel in time with that of the pollen mother cell and both nuclei reach leptotene by about the end of

December. The nucleus of the megaspore mother cell remains in this phase however, until the pollen mother cells have completed meiosis and formed tetrads of pollen grains. It then passes into diplotene and ultimately divides, a wall being formed between the two daughter nuclei. The position taken up by this wall varies considerably, and is not always strictly at right angles to a plane through the micropyle. The second division of meiosis now occurs, the angle between the two spindles again showing considerable variation. As a consequence the tetrad of nuclei formed is rarely of the regular linear type so characteristic of megaspores in most angiosperms. A wall is never laid down between the daughter nuclei formed by this second division, so that two binucleate cells are produced (FIG. **1**).

The nuclei now rest until after the first nuclear division in the pollen grain. The two cells are at first of equal size, but the upper one gradually increases and elongates towards the micropyle, whilst the lower one (the « antigone » of TREUB & MELLINK and of GUIGNARD) remains constant. The upper cell always becomes the embryo sac in *Scilla nonscripta*. Its two nuclei pass to opposite ends and a vacuole appears between them. They then divide, and at the same time the two nuclei of the « antigone » also divide, so that two 4-nucleate cells are produced (FIGS. **2** AND **3**). The nuclei of the « antigone », however, never divide again; those of the embryo sac enter into a second division (FIG. **4**). The embryo sac then contains eight nuclei (four at each end) whilst the « antigone » has only four, which show no polarisation (FIG. **5**). A nucleus from each end of the embryo sac migrates towards the centre, and the two nuclei unite without fusing, to form the « paired polar nuclei ». The common membrane between them always remains intact so that the outlines of the two component nuclei are never lost, and each retains its own nucleolus and chromatic reticulum. The three nuclei at the micropylar end are now organised into cells, — the two synergids having vacuoles at their base and a fairly well defined filiform apparatus, whilst the egg is between them with a large vacuole in the upper part of the cytoplasm. The three nuclei at the antipodal end are not organised into cells as a general rule. They usually appear very active, but occasionally may degenerate. Below the embryo sac is the relatively small « antigone », containing four apparently active looking nuclei, which usually persist until after fertilisation. Mc KENNEY found that they gradually degenerate, and some evidence of a tendency towards degeneration of the antigone has been found in the present investigation. In one preparation the embryo sac contains two nuclei which are in the metaphase of division and the « antigone » contains thirty-two chromosomes in a common mass, probably the result of a fusion of two nuclei in metaphase. Another slide shows four nuclei in metaphase in the embryo sac, and a mass of thirty-two chromosomes in the « antigone ».

In one preparation a vertical wall shows at the base of the antigone, partially dividing it into two binucleate portions, but it grows fainter and fades away nearer the embryo sac end. The production of a vertical wall as a rare occurrence in the « antigone » was noted by GUIGNARD.

It is interesting to note the regular alternation in time of nuclear divisions in *Scilla nonscripta*. They are summarised in the following table; the periods of active division being *in italics*.

	Development of microsporangium, pollen grains and pollen tubes	*Development of megasporangium and embryo sac.*
December-January	Meiosis. Leptotene — pachytene	Meiosis. Leptotene — pachytene.
February	*Meiosis. Diplotene — tetrads of pollen grains.*	Meiosis. Leptotene — pachytene.
February-March	Tetrads and pollen grains formed.	*Meiosis. Diplotene — tetrads of megaspores.*
March-April	*Division of pollen grain nucleus.*	Enlargement of potential embryo sac.
April	Binucleate pollen grains.	*Division in embryo sac, producing eight nuclei.*
May	Shedding of pollen grains. Germination of pollen tubes. *Division of generative nucleus in the pollen tube.*	8-nucleate sac organised.

SARGANT (1896) recorded that meiosis in the megaspore mother cell lags behind that of the pollen mother cell in *Lilium Martagon*, and is not completed in this plant until after the division of the pollen grain nucleus.

WYTE (1929) claims a similar alternation of a « male » and « female » stage in *Ranunculus acris*. Here meiosis does not occur in the megaspore mother cells until it has been completed in the pollen mother cells, and pollen grains have been formed. He finds that unless this lagging occurs, normal hermaphrodite flowers cannot be produced.

§ 4. Germination of the pollen grain. Fertilisation.

Stages of pollen tube growth and fertilisation were obtained by pollinating flowers by hand. Anthers were removed from buds which were on the point of opening, and the buds were then enveloped in muslin bags, to protect them from stray pollen. At intervals after pollination, the ovaries were removed from a few flowers, and were fixed, embedded in paraffin and cut.

Pollen grains were also germinated artificially in sugar, and were then either embedded, or stained with iron aceto-carmine, but the best stages of pollen tube growth were obtained from styles which had been embedded and cut.

The embryo sac is organised and ready for fertilisation some weeks before the flower opens. The pollen grains germinate on the stigma, and a tube from each grows down through the tissues of the style. The tube and generative nuclei pass into it, the tube nucleus either preceding or following the generative nucleus. The tube nucleus is still only slightly chromatic, but the generative nucleus is already in fairly late prophase of division, with thick chromatic strands running through it (FIG. **12**). As it passes downwards through the style, it elongates considerably, and the prophase threads begin to show their double nature. The amount of stylar tissue traversed by the generative nucleus before it divides varies considerably, but is usually from a half to a third of the total length. The division differs somewhat from a normal mitosis, for the chromosomes remain long and closely pressed together in the confinement

of the pollen tube; they may double back on themselves or twist among each other (FIG. **13**). The chromosomes are never all in one plane at the equator at metaphase, again because of the restriction placed upon them by the width of the tube, but their ends approximate to the centre of the nucleus before the daughter halves finally separate to opposite poles (FIG. **14**). Spindle fibres have not been seen, but they are frequently not clearly defined even in a normal mitosis in this material, the distinctness of the spindle varying with the type of fixative employed and inversely with the rapidity of its penetration.

The two male nuclei are now organised. They separate rather widely from each other at telophase, since they lie freely in the pollen tube. When they are first formed, they elongate slightly lengthwise and the chromosomes become drawn out into finer threads (FIG. **15**). They never pass into resting stage, however, and soon contract again somewhat and the threads thicken once more (FIG. **16**). In favourable cases, it can be seen that they are surrounded by a small amount of their own cytoplasm, so that two male cells have been produced. Each nucleus is still slightly elongated, and of the vermiform shape so characteristic of male nuclei. It is made up of thick chromatic threads, which have the appearance of chromosomes in mid prophase. Usually both nuclei of a pair are about the same size and are pointed a little, either at one or at both ends. When a slight difference in size can be seen, the larger one seems to be in front, as found for *Fritillaria pudica* (SAX, 1916), but not for *Lilium* (WELSFORD, 1914). They follow closely behind each other down the pollen tubes in the tissues of the style. It is uncertain at late stages whether they are still surrounded by their own cytoplasm, but it is probable that they remain as organised cells until they are discharged into the embryo sac. At this stage, they are always revealed as naked nuclei. The tube nucleus meanwhile shows increasing signs of degeneration, stains deeply and uniformly and sometimes appears to have broken up into two or three pieces. After the three nuclei have passed through, callose plugs are formed in the pollen tubes behind them, but no encysted nuclei have been observed within the plugs, as in *Elodea canadensis* (WYLIE, 1904).

Having reached the base of the style, the pollen tube grows along the surface of the axile placenta, until it reaches an ovule. It then grows along the surface of the funiculus and enters the micropyle of the ovule. It penetrates the embryo sac, almost invariably disorganising one synergid as it passes inwards, although a few fertilised sacs have been found with both synergids intact. The two nuclei are then discharged into the embryo sac. The disorganised synergid finally contains very dense cytoplasm, and one to three dark, degenerating bodies of varying size. These are probably the tube nucleus, the nucleus of the synergid, and perhaps the darkened remains of some of the cytoplasm which ensheathed the male nuclei. The second synergid remains intact with an active nucleus for a considerable time.

The two male nuclei are evidently ejected with some considerable force, so that they are thrown into the cytoplasm of the embryo sac a little distance below the egg (FIG. **17**). In the example figured the lower one is very slightly larger than the upper one, and will probably be the one which will fuse with the polar nuclei.

Hence, when a difference in size exists between the two nuclei, the larger one is the leading one in the pollen tube, and its weight will probably cause it to be ejected further into the embryo sac. It will therefore probably be the one which will fuse with the polar nuclei. This agrees with GUIGNARD's findings for *Lilium*, but WELSFORD states that in her material the first nucleus to be ejected fertilises the egg. A comparision of figures illustrating the male nuclei fusing with the egg and polar nuclei respectively shows that the one concerned with triple fusion is slightly the larger, but a sufficient number of triple fusions have not been obtained to allow of any generalisation with regard to a constant difference in size at this stage. GUIGNARD (1899), however, states definitely that in *Scilla* the male nucleus that fuses with the egg is always smaller than the one that fuses with the polars.

It seems probable, as GUIGNARD supposes, that the passage of the first male nucleus through the cytoplasm, and its fusion with the polar nuclei occur very rapidly, for only a few rare instances have been obtained among the hundreds of embryo sacs examined. The fusion of the egg and second male nucleus is also probably fairly rapid, but is slower than the triple fusion. Instances have been found where the membranes between the egg and male nucleus are still intact, yet in the same embryo sac triple fusion is already completed.

The vermiform shape assumed by the male nuclei is suggestive of independent movement through the cytoplasm, but this shape would also be the one which would offer the least resistance to its carriage by cytoplasmic currents. As the sperm applies itself to the egg nucleus, it coils closely round it, and thus again suggests some independent motion. It might equally well be explained, however, as the result of chromatic attraction along all its length. The two pieces of evidence considered together do, however, indicate a possible active movement of the male nucleus, though they are by no means conclusive.

The male nucleus penetrates the cytoplasm of the egg and becomes closely pressed against the nuclear membrane (FIG. **18**). The nucleus of the egg is either in a resting state, or in very early prophase, and a finely chromatic and slightly granular reticulum can be seen in it, within which is the single nucleolus. The male nucleus, on the other hand, is very much smaller, often less than one quarter the size of the egg nucleus, but is deeply chromatic, the thickness of its chromatic threads corresponding to those in middle prophase of mitosis. A nucleolus is either absent or hidden within the chromatic reticulum, more probably the latter, for one becomes evident at a later stage. After a short period in contact with each other, the membranes of the two nuclei begin to break down at the point of contact (FIGS. **19** AND **20**). The chromosome threads of the male nucleus are then seen massed upon the side of the egg nucleus, and perfectly distinct from it. They gradually spread out among the reticulum of the egg nucleus, and the fusion nucleus becomes more or less spherical, although a slight bulge at the point of entry of the male nucleus can frequently be distinguished, after all trace of the distinction between the two nuclei has been lost. The male chromatic threads can at first be readily distinguished, owing to their greater thickness and more abundant chromatin (FIG. **21**); but they

gradually become longer, thinner and less chromatic until they can be distinguished only with difficulty from the reticulum of the egg (FIG. **22**). Finally all distinction is lost and the fusion nucleus rests for some time. The time taken for complete fusion apparently varies from 35 to 74 hours after pollination.

Soon after the entry of the male nucleus, a large and a small nucleolus can be clearly distinguished in the fertilised egg, the small one having presumably been contributed by the male nucleus. These two nucleoli sometimes fuse, but they often remain separate, keeping their characteristic difference in size. The presence of a large and a small nucleolus, however, as ERNST (1902) points out, is no absolute criterion that fertilisation has occurred, for unfertilised eggs have been found to possess them in some cases and fertilised eggs may frequently have only one large nucleolus, the product of fusion between the original two.

Meanwhile the second male nucleus has penetrated the cytoplasm around the paired polars, and has applied itself to one of the polar nuclei, coiling round it in the same manner as has been described for the male nucleus fusing with the egg (FIG. **23**). GUIGNARD states that the male nucleus applies itself to the common membrane between the two polar nuclei, but this has not been found in the present investigation. An even greater difference in size exists between the male nucleus and the polars than between the male nucleus and that of the egg, and the polars resemble the egg nucleus in being in resting stage, each with a faintly chromatic and granular reticulum, and a single large nucleolus. The male nucleus is filled with thick chromatic bands corresponding to middle prophase of division, and no nucleolus is visible. The membranes between the male nucleus and one polar nucleus break down, and the chromatic threads of the male enter the polar nucleus until practically all trace of their point of entry is lost (FIG. **24**). The thick threads of the male nucleus, however, remain distinct from the thin threads of the polar nucleus, in exactly the same way as has been described for the fertilised egg (FIG. **25**), until they elongate, become thinner and paler, and finally can no longer be distinguished. The fertilised polars are now in a resting condition.

After the entry of the male nucleus, a large and a small nucleolus can be distinguished in the polar nucleus which has been fertilised. The small nucleolus probably belongs to the male nucleus, and sometimes fuses with the nucleolus of the polar nucleus but often remains distinct. In this case, the unfertilised polar has a single large nucleolus and the fertilised polar has one large nucleolus and one small one. As in the case of the fertilised egg, however, the number of nucleoli cannot be taken as an absolute criterion that the entrance of the male nucleus has occurred.

§ 5. Free nuclear division of the endosperm.

The fertilised polars rest for a brief period, but within a few hours they begin to pass into prophase of division. Both of the polar nuclei then form distinct chromatic threads. At the side of the polar nucleus which has fused with the male nucleus a group of threads now appears, these being thicker and more deeply stained than the threads in the rest of the nucleus. This distinction still remains at late prophase,

the darker group probably representing the chromosomes of the male nucleus, which are still separate from those of the polar (FIG. **26**). Individual chromosomes begin to be distinguishable, the presence of secondary constrictions along them being very evident at this stage. The wall between the two polar nuclei now at last breaks down, and the chromosomes from both are drawn on to a common spindle. Unfortunately, no uncut examples of this first metaphase have been obtained. There is no visible evidence of grouping in the cut nuclei which have been examined, but a study of the chromosome types suggests that the eight chromosomes from each nucleus remain in separate groups even at this stage. Uncut nuclei of later stages of endosperm division have been obtained and confirm this idea. Figure **27** represents a nucleus from a sac which contained about eight endosperm nuclei. Sixteen of the chromosomes are pointing towards one pole and eight towards the other. The sixteen chromosomes on one side of the equator can be clearly distinguished as two sets of eight.

Figure **28** represents a nucleus from a sac containing about 128 nuclei; and has been selected from a number of examples at this stage. The metaphase plate is seen in polar view, and the three groups of eight chromosomes can be clearly identified, each set of eight being made up of the chromosomes (A-H) characteristic of *Scilla nonscripta* (DARLINGTON, 1926). Therefore even at the seventh division, the three groups have not yet mingled.

Nuclei in prophase have been seen in which the separation of the three groups is evident in that the nucleus has a three-lobed configuration, but later on, the groups can be recognised only by means of their chromosome types. Resting nuclei too are often three-lobed, and as a general rule possess either two large nucleoli and one small one, or one much larger nucleolus and one small one, as if two nucleoli had fused together. These configurations obtained from different stages of nuclear division, and from endosperm containing from two to two hundred nuclei, suggest that the three chromosome groups contributed by the nuclei which fuse in triple fusion may never mingle together at any stage in the production of endosperm nuclei. The fact that the groups are still distinct at the seventh division has not hitherto been ascertained by previous investigators.

§ 6. Early divisions in the embryo.

As the endosperm nucleus divides for the first time, that of the fertilised egg enters into early prophase of the first division, but it does not reach metaphase until eight or sixteen endosperm nuclei have been formed. During early prophase, a group of more deeply staining chromatin threads can be distinguished at one side of the nucleus, corresponding to the group seen in the division of the endosperm nucleus. At the last prophase, this can still be made out, the chromosomes being slightly shorter and thicker than those in the rest of the nucleus. An uncut nucleus stained at this stage has allowed the chromosome types to be indentified and counted, showing that eight chromosomes can be distinguished as belonging to the female nucleus, and eight thicker ones, more closely arranged, as belonging to the male nucleus (FIG. **29**). In this nucleus at least, the male and female chromosomes had not mingled.

The nuclear membrane now breaks down and the chromosomes are arranged on a spindle. Once more, however, no uncut metaphase plate has been obtained, in spite of the fact that sections of 18-20 μ were cut. A cut nucleus in metaphase, however, had two sets of eight chromosomes in slightly different planes, and the razor, by a lucky chance, passed between them. A composite drawing has therefore been made with comparative ease from the two sections, and the chances are that the relative positions of the chromosomes have not been greatly disturbed by the knife (FIG. 30). Once more it is evident that the two groups of chromosomes have not yet mingled. Uncut telophase nuclei have been obtained however, and in these the distinction is no longer maintained. The two groups are still more or less separate, but during their progress to the poles some of the chromosomes of one group have mingled with the chromosomes of the other group, so that it is no longer possible to draw a straight line of demarcation between them (FIG. 31).

Later stages in the development of the embryo have not been extensively obtained in this material. Late prophases and telophases of the second division have been examined, however, and no evidence of chromosome groups has been found, the chromosomes apparently being arranged more or less at random.

Thus there is evidence in *Scilla nonscripta* that although the two sets of chromosomes contributed by the male and female nuclei in fertilisation remain separate until the metaphase of the first division, yet the grouping is gradually lost after this, until little or no trace of it remains in the second division of the embryo. The chromosomes are then arranged more or less at random on the metaphase plate.

The early segmentation of the embryo follows a regular sequence. It has not been described before and is sufficiently distinct from the type cited by COULTER (1912, p. 193) as probably characteristic of the *Liliaceae* family, to merit a brief description. The first division of the egg is transverse, resulting in two equal cells. The upper one of these divides again transversely, so that a row of three cells is formed. The apical cell, that is the one furthest away from the micropyle, now divides longitudinally, after which the two other cells of the original row of three divide longitudinally. Subsequent divisions have not been followed.

DISCUSSION OF RESULTS AND HISTORICAL REVIEW OF FORMER WORK.

§ 1. Development of the embryo sac.

The account of the development of the embryo sac given above has been found to agree in all essential details with that given by TREUB & MELLINK, GUIGNARD, Mc KENNEY and SCHNIEWIND-THIES. The first fact of importance is that only one wall is formed during the two divisions of meiosis, so that two potential megaspores take part in the development of the embryo sac. The sac is therefore « bisporic ». An additional peculiarity of *Scilla nonscripta* is the development of a quadrinucleate « antigone », as a kind of abortive embryo sac below the functional eight-nucleate sac. It is probable that the « antigone » assists in passing nutriment to the embryo sac

itself, for its nuclei have been found still in an active condition when the dividing endosperm of the functioning embryo sac has produced as many as thirty two or more nuclei, and its four nuclei are often arranged in a line with the antipodals, as though they were associated together for some purpose.

§ 2. Formation of the generative and male cells.

The formation of the tube and generative cells, and the division of the latter into two male cells in the pollen tube agrees more or less with the detailed accounts given by WELSFORD (1913) and others, for *Lilium* and other plants. As in *Lilium*, the generative cell is always in middle to late prophase when it enters the pollen tube. It never divides in the pollen grain. The male nuclei formed as the result of this division never pass into a complete resting stage, but retain a thick chromatic reticulum characteristic of middle prophase (WELSFORD) or of telophase (NAVASCHIN, 1910). The male nuclei are apparently organised into cells in the pollen tubes, as in *Lilium* (WELSFORD) but are discharged into the embryo sac as naked nuclei. The presence of male nuclei, and not cells, in the embryo sac appears to be characteristic of angiosperms (GUIGNARD, 1891, 1899 et seq.; MOTTIER, 1898, 1904; NAVASCHIN, 1899, 1910; STRASBURGER, 1900; SAX, 1916; NOTHNAGEL, 1918, etc.) but male *cells* have been found in the embryo sac in *Vallisneria* (WYLIE, 1923), *Pinus* (FERGUSON, 1901), *Cypripedium* (PACE, 1907), *Asclepias* (FINN, 1925) and a few other forms. No male cytoplasm has been seen definitely entering the egg cytoplasm, although WYLIE considers it possible that some or all of it does so in *Vallisneria*. In *Scilla nonscripta* the male nuclei are apparently completely naked at the time of fusion.

§ 3. Fertilisation and triple fusion.

There have been very few detailed accounts of fertilisation since the burst of enthusiastic research which followed NAVASCHIN's (1898) and GUIGNARD's (1899) reports on the occurrence of double fertilisation in angiosperms, and which appeared to end abruptly in 1918. Later papers published by WYLIE (1923) and FINN (1925) are concerned mainly with the organisation of male cells, and either merely outline or omit the problems of fertilisation. In spite of this cessation of interest, there still remain several gaps in our knowledge of fertilisation and the subsequent divisions. As SHARP (1926 p. 321) points out, meiosis and syngamy are the two crises in the life of a plant. Meiosis has deservedly attracted a great deal of detailed investigation, and it is probable that fertilisation would also repay a little close study with modern technique.

Two main problems confront the student of the details of fertilisation :

1° the exact processes involved in the nuclear fusion : 2° the stages passed through in the nuclei during nuclear division in the embryo and endosperm. These two problems necessarily overlap if a true resting stage does not occur between fusion and division, for in this case the chromosomes entering into the fusion nucleus prepare immediately for the following division.

The first accounts of fertilisation in angiosperms stated that the male and female nuclei both entered into fusion in the resting condition. The membrane

between them broke down, and their contents mingled together in a common mass (GUIGNARD, 1891; NAVASCHIN, 1898, 1899; ERNST, 1902; MOTTIER, 1904, etc.). In 1899, GUIGNARD figured the reticulum of the male nucleus of *Scilla* as a deeper staining mass just inside the membrane of the egg nucleus, but he does not stress its importance. The first paper to arouse interest in the finer details of fusion and subsequent division was on *Pinus strobus* by FERGUSON (1901, 1904). This paper remained as a classic, referred to by most subsequent workers on fertilisation. The male nucleus applies itself to the female nucleus and loses its dense structure, passing into resting stage. The egg is also in resting stage. The two nuclei do not fuse, however, but each passes into early prophase, and it is not until late prophase that the membranes between them break down. Fusion and division therefore overlap in this plant.

PACE (1907) stated that although in *Cypripedium* fusion in the resting stage is usual, it is not uncommon to find separate « spiremes » already well advanced before the membrane between the fusing nuclei breaks down. She finds that this is true in this plant not only of the fusion between the egg and sperm, but also of triple fusion. In one preparation she found that the « spiremes » of the male and female nuclei had almost formed distinct chromosomes before they came into contact with one another. She presumed that they then pass immediately into metaphase. SAX (1916, 1918) finds similar stages to these in *Fritillaria*.

BLACKMAN and WELSFORD (1914), in describing the fertilisation of *Lilium* stated that the sperm is in a condition corresponding to prophase and the egg nucleus is usually resting, but that occasionally the latter may pass into a very early prophase stage before fusion. NOTHNAGEL (1918) and WENIGER (1918) confirm this for *Lilium*. During triple fusion in *Trillium* NOTHNAGEL finds that the three nuclei are resting when they come into contact, but usually form separate spiremes before the nuclear membranes between them break down.

In *Triticum*, SAX (1918) finds that the male nucleus is in prophase of division when it fuses with the egg, appearing as a dark homogeneous body against the membrane of the egg, which is in a resting condition. After fusion, the male nucleus increases in size, and forms a distinct compact « spireme » inside the nuclear membrane (cf. *Scilla*). Meanwhile the egg nucleus enters into early prophase, and the two nuclear « spiremes » ultimately divide. The sperm therefore never rests after its fusion with the egg. Triple fusion is essentially similar in its stages.

In all these cases cited above, the male nucleus either fuses with the egg in resting stage, or one or both of them are in prophase of division. In the latter case they pass directly into the succeeding division stages, with no intervening resting period. MADGE (1929), however, in a paper on fertilisation in the cleistogamous flower of *Viola*, reports several interesting divergences from the normal procedure. The male nucleus is in « spireme » when it comes into contact with the resting egg nucleus. As it enters the egg the spireme gradually loosens and spreads through the egg nucleus, at the same time staining more and more faintly. Finally, all distinction be-

tween the male and female chromatin is lost, and the fertilised egg rests for a time. A similar sequence is found during triple fusion. Most previous investigators have reported a rapid fusion of all three nuclei into a common endosperm nucleus (either the sperm nucleus and one polar fusing first, and then fusing with the second polar — e.g. in *Lilium* as reported by WELSFORD — or else all three nuclei fusing together — as SAX described for *Fritillaria*). In *Viola*, on the other hand, the male nucleus and one polar fuse and rest, whilst the second polar nucleus may still remain quite distinct, pressed against the other two but not fused with them. A definite membrane, however, is not found between the two polar nuclei at this stage, and their fusion occurs very early in the prophase of division.

This report agrees in most essentials with that recorded for *Scilla nonscripta*. In this plant at the time of fertilisation, the male nucleus is in a stage corresponding to middle prophase in the thickness of its chromatin threads, whilst the egg is in resting condition. The male reticulum remains perfectly distinct inside the membrane of the egg nucleus, but gradually loosens and spreads out. At the same time its threads become thinner until they finally approximate in appearance to those of the female nucleus, and distinction between the two is lost. The fertilised egg then rests before entering into the prophase of division. Since no true resting stage was formed after the division of the generative cell in the pollen tube, the male nuclei may be said to correspond to telophase nuclei, their resting stage having been postponed until after fertilisation has occurred.

The second male nucleus and one polar pass through similar stages of fusion as do the male and egg nuclei, and finally rest. The second polar, however, although united with the fertilised polar nucleus, remains perfectly distinct from it until the late prophase of the ensuing division, a definite nuclear membrane being readily distinguishable between them. This is very similar to MADGE's findings for *Viola*, but the line of demarcation between the two polars after fertilisation is even greater, and this common membrane between them does not break down until a considerably later stage of division. It is interesting, however, that such a similarity in essentials should be found in two such totally unrelated plants, and indicates that other plants will probably reveal similar stages. The postponement of actual fusion between the three nuclei involved in the production of endosperm, until late in their prophase of division, is of significance in any theoretical discussion on the nature of triple fusion.

§ 4. Early nuclear divisions of the embryo and endosperm.

The changes involved during the early division of the fertilised egg and polars are also of considerable interest. In 1891, GUIGNARD described the early prophase of a fertilised egg as showing distinct male and female « spiremes », which later « segmented » into two groups, each containing twelve chromosomes. Twelve were on the upper side of the equator of the spindle, and twelve on the lower side, and GUIGNARD supposed from a study of earlier stages, that the upper group represented the maternal chromosomes whilst the lower ones were contributed by the male. This early account, however, was unaccompanied by illustrations, and was overshadowed by the investi-

gations of later workers. NAVASCHIN (1898, 1899), ERNST and MOTTIER, all supposed that the male and female chromatin mingled and fused completely, so that no parental groups could be distinguished in the following prophase.

Meanwhile, workers on animals, and on groups of plants other than angiosperms, were reporting the separate entity of the male and female chromosome sets during prophase of the first division. Such reports were made by KRUCH (1891) for *Riella*; RUCKERT (1895) for *Cyclops*; HAECKER (1896) for *Cyclops*; CONKLIN (1901) for *Crepidula*; BLACKMAN (1898) for *Pinus*; CHAMBERLAIN (1899) for *Pinus*; MURRILL (1900) for *Tsuga*; NOREN (1907) for *Juniperus*, and many other investigators supported them. The most stimulating publication was by FERGUSON on *Pinus*, already referred to in the preceding section. The male nucleus in *Pinus* remains in contact with the female nucleus without fusing, and later they each form their separate « spiremes ». The membrane between them does not break down until late prophase, and the two groups of chromosomes remain separate until they are drawn on to the metaphase spindle. After this, the male and female chromosomes can no longer be distinguished, but the two groups reappear once more in the following prophase. It is therefore probable that they remain separate although they are not visibly arranged into two groups between metaphase and telophase. (cf. *Scilla*).

In 1915 HUTCHINSON put forward a very different conception of the division following fertilisation. According to his account of fertilisation in *Abies balsamea*, the homologous chromosomes from the male and female nuclei paired at the metaphase of the first division, twisted round each other, and segmented transversely at anaphase, so that paired halves passed to each pole. In 1916, CHAMBERLAIN reported a similar pairing in *Stangeria paradoxa*, but he does not explicitly state that there is a transverse segmentation during the first division. WENIGER (1918) put forward the same idea for *Lilium*. It may safely be stated at the present time, however, that the conception of transverse splitting of the chromosomes is contrary to all cytological and genetical work, and may be discarded. Single chromosomes possessing median constrictions were probably confused with pairs of whole ones. It is still uncertain, however, whether the pairing of homologous chromosomes may not sometimes occur at this stage, and the subject is worthy of fresh investigation. No trace of such pairing has ever been found in *Scilla*, and longitudinal splitting of individual chromosomes most certainly occurs.

SAX (1918) investigated *Fritillaria* and *Triticum* with special reference to the behaviour of the chromosomes after fertilisation. In *Fritillaria* the chromosomes show no indication of grouping during prophase or subsequent stages, either in the early nuclear divisions of the embryo or of the endosperm. In *Triticum*, however, the male nucleus forms a compact spireme inside the egg nucleus, and the latter subsequently also enters into prophase. The two separate groups are finally drawn on to the metaphase plate, where all distinction between them is lost. During triple fusion, three separate spiremes are produced and the three groups (in contrast with the chromosomes in the division of the embryo) remain distinct even at metaphase. Later division stages of the embryo and endosperm were unfortunately not investigated.

NOTHNAGEL (1918) gave a careful account of chromosome behaviour after fertilisation in *Trillium* and *Lilium*. In the first division of the endosperm three separate spiremes can be clearly distinguished, and have frequently begun to appear even before the breaking down of the nuclear membranes between the three fusing nuclei. A tripolar spindle is formed, which later becomes bipolar, and as this occurs, the chromosomes from the third arm are gradually pulled into line with the other two groups. The three groups of chromosomes are no longer visibly separate at metaphase, and their arrangement on the spindle is uncertain. The same grouping reappears in the prophase of the next division, however, and again at the third division of the endosperm of *Trillium*. No later stages were obtained.

Similarly, in the first division of the embryo, two separate thick spiremes are formed, giving an appearance very like that found in *Scilla*. The two sets of chromosomes are still separate after the disappearance of the nuclear membrane. A spindle is apparently formed about one group and the other one is pulled on to it, but the arrangement of the chromosomes at metaphase was not determined, nor were later stages in the division of the embryo investigated.

MADGE (1929) finds in *Viola* that the first division of the endosperm shows two separate « spiremes », and not three as other investigators have described. The chromosomes then collect on the spindle in two groups, which are side by side and at right angles to each other. The diploid number of chromosomes is present in one group, and the haploid number in the other, and the two sets remain distinct throughout telophase. Suggestions of two groups of chromosomes are seen for the first few divisions of the endosperm and as a correlated phenomenon, the resting nuclei show either one large nucleolus and one small one, or three spherical nucleoli of equal size. This is the first description of two groups of chromosomes in the early nuclear divisions of endosperm formation, and suggests that the fusion between the male nucleus and the first polar nucleus must be an extremely close one. The relation of the number of nucleoli to the separate chromosome groups is similar to that found in *Scilla*, where either three single nucleoli are formed in one endosperm nucleus, or one large nucleolus and one small one. It suggests a direct relationship between the nucleoli and the chromosomes, but the nature of this is difficult to decide, and is probably merely nutritive (FIKRY, 1930).

It is clear therefore that most of the investigated cases show a grouping for at least the first nuclear division of the endosperm and fertilised egg, but the total mass of our knowledge remains extremely scanty. SAX, NOTHNAGEL, WENIGER and MADGE all find that in the first division of the endosperm nucleus separate spiremes are formed, SAX, NOTHNAGEL and WENIGER finding three haploid groups and MADGE finding one diploid and one haploid group. At metaphase, the grouping may remain visibly distinct (SAX, MADGE) or may apparently be lost (NOTHNAGEL, WENIGER). A lack of visible distinction, however, need not necessarily mean a complete mingling of the chromosome sets, for NOTHNAGEL finds that the three groups reappear once more in the prophase of the second and third endosperm divisions of *Trillium*. Therefore the three sets are still distinct in the third division here, and there are indica-

tions that the grouping still remains for the first few divisions of *Viola*. SAX and WENIGER do not follow the problem in their material beyond the first metaphase. There are indications, however, from these incomplete accounts that the groups of chromosomes combining at triple fusion may frequently not intermingle until at least several divisions have elapsed. This surmise has been confirmed for *Scilla*. The three sets can still be identified on the metaphase plate (although at first sight each group as such cannot be distinguished from the mass) even when the dividing endosperm has produced 128 nuclei or more. It therefore seems probable that in this plant the chromosomes of the three component groups never intermingle in the endosperm tissue. It would be interesting to obtain comparable data from other plants.

Two distinct chromosome groups at the prophase of the first division of the embryo have been identified by GUIGNARD, PACE, SAX, NOTHNAGEL and WENIGER, and are presumably derived from the maternal and paternal nuclei after their fusion. None of these investigators, however, pursue the subject beyond the metaphase of the first division, by which time all obvious grouping has been lost. It may be recalled, however, that FERGUSON lost all evidence of separate male and female sets of chromosomes at the metaphase of the first division in the embryo of *Pinus*, yet in the second division of the embryo they reappeared. A lack of grouping which is visible to the casual glance is therefore no criterion that the chromosome sets have intermingled. This can only be determined by a study of the relative positions of the chromosome types which make up the haploid complement. Adopting this method for *Scilla nonscripta*, it has been found that the chromosomes remain in two distinct groups on the metaphase plate of the first division, but that at telophase, an intermingling has begun to occur and the chromosomes contributed by one nucleus at fertilisation cannot readily be separated by a single bounding line, from those contributed by the other nucleus. At the second division of the embryo, no evidence of grouping has been found, but a sufficient number of convincing examples have not been procured to permit of a dogmatic generalisation on this subject. It is probable that in *Scilla* intermingling begins to occur after the first metaphase, but it is unlikely that it will begin at exactly the same time, or continue at the same rate in all young *Scilla* embryos. The number of chromosomes crossing over into another group will probably depend on a chance arrangement of the spindle fibres. FERGUSON believes that the chromosome sets probably remain permanently separate in *Pinus*. It would be very interesting to discover what is the usual rate of intermingling of chromosomes in plants, and whether the two haploid sets are ever separate throughout the somatic divisions. In *Scilla* they are apparently associated at random in all divisions after the first.

It is curious that although intermingling occurs among the chromosomes of the embryo, the three haploid sets always apparently remain separate in the division of the endosperm. The fact that the three groups are not visibly distinct as such at metaphase indicates that there is probably no actual repulsion between them. Perhaps the spindle fibres belonging to the individual sets can never mingle for some reason — but this cannot be decided by ordinary cytological methods. The

fact that wherever the divisions of the endosperm have been studied in detail (*Triticum, Viola, Scilla*) separate grouping has been found, makes it seem probable that it often occurs.

Further investigations are much needed on this subject. At the moment generalisations are difficult and theorising a little premature, with such a limited number of facts at our disposal.

SUMMARY.

1. The development of the microsporangium and megasporangium is described, also the nuclear division in, and the germination of, the pollen grain, the development of the embryo sac, fertilisation and the early divisions of the embryo and endosperm tissue.

2. New facts, and facts of especial interest gleaned during the investigation are the following :

a) The megaspore mother cell divides into two cells, the upper of which becomes the embryo sac whilst the lower one becomes an « antigone ». The upper cell ultimately contains eight nuclei and the lower one four, these latter remaining active in appearance until after fertilisation.

b) The nuclear divisions in the male and female gametophytes show a regular alternation in time, the female generally lagging behind the male.

c) At fertilisation, the egg nucleus is spherical and either resting or in early prophase ; the male nucleus is vermiform, much smaller than the egg nucleus and in a condition corresponding to late prophase, but which is in reality the previous early telophase. The membrane between the two nuclei breaks down and the reticulum from the male nucleus gradually spreads through the egg nucleus. The chromosome threads from the male nucleus are at first quite distinct, but they gradually become thinner and fainter until all distinction between the male and female nuclei is lost, and the fusion nucleus rests for a time.

d) The two polar nuclei unite but do not fuse. The second male nucleus fuses with one polar nucleus, the stages of fusion resembling those which occur during the fertilisation of the egg.

e) After a short period of rest, the fertilised polars pass into the prophase of division, each polar nucleus forming distinct chromatic threads. A thicker and more deeply stained group of threads appears towards the side of one polar nucleus, and represents the chromosomes of the male nucleus. The wall between the two polar nuclei breaks down for the first time in late prophase, but the three chromosome groups remain separate throughout this and subsequent nuclear divisions, and are still distinct from one another even at the seventh division. It is therefore presumed that they never mingle at any time during the nuclear divisions of the endosperm. There is no evidence of grouping, as such, on the metaphase plates, but the three chromosome groups are identifiable through the eight types of chromosome which make up the haploid complement.

f) During the division of the fertilised egg, the chromosomes of the male nucleus are at first distinct from those of the egg, but during the first telophase the two groups begin to mingle together. No evidence of grouping has been found in the second nuclear division of the young embryo or at later stages of development. It is therefore presumed that the chromosomes contributed by the female and male nuclei at fertilisation mingle at random after the first division of the embryo.

3. The relation of this work to that of other investigators is discussed.

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LITERATURE CITED.

1898 *Blackman, V. H.* : On the cytological features of fertilisation and related phenomena in *Pinus sylvestris;* Phil. Trans. Roy. Soc., **190**, 395-426.

1913 *Blackman, V. H. & Welsford, E. J.* : Fertilisation in *Lilium;* Ann. Bot., **27**, 111-113.

1899 *Chamberlain, C. J.* : Oogenesis in *Pinus laricio;* Bot. Gaz., **27**, 268-280.

1916 *Id.* : *Stangeria paradoxa;* Ibid., **61**, 353-372.

1901 *Conklin, E. G.* : Karyokinesis and cytokinesis in the maturation, fertilisation and cleavage of *Cripidula* and other Gasteropoda; Journ. Acad. Nat. Sci. Phil., **12**, 1-121.

1903 *Coulter, J. M. & Chamberlain, C. J.* : Morphology of Angiosperms. New York.

1926 *Darlington, C. D.* : Chromosome studies in the Scilleae; Journ. Gen., **16**, 237-252.

1902 *Ernst, A.* : Chromosomenreduktion, Entwickelung des Embryosackes und Befruchtung bei *Paris quadrifolia* und *Trillium grandiflorum;* Flora, **91**, 1-46.

1901 *Ferguson, M. C.* : The development of the egg, and fertilisation in *Pinus strobus;* Ann. Bot., **15**, 435-479.

1904 *Id.* : Contributions to the knowledge of the life history of *Pinus*, with special reference to sporogenesis, the development of the gametophytes and fertilisation; Proc. Wash. Acad. Sci., **6**, 1-202.

1930 *Fikry, M. A.* : Phenomena of heterotypic division in the pollen mother cells of a tetraploid form of *Rumex scutatus* var. *typicus;* Journ. Roy. Micr. Soc., **50**, 387-419.

1925 *Finn, W. W.* : Male cells in Angiosperms; Bot. Gaz., **80**, 1-25.

1882 *Guignard, L.* : Recherches sur le sac embryonnaire des Phanérogames Angiospermes; Ann. Sci. Nat. Bot., Série VI, **13**, 136-199.

1891 *Id.* : Nouvelles études sur la fécondation; Ibid., Série VII, **14**, 163-296.

1899[a] *Id.* : Les découvertes récentes sur la fécondation chez les végétaux Angiospermes; Société de Biologie, Volume Jubilaire, Paris, p. 189-197.

1899[b] *Id.* : Sur les anthérozoides et la copulation sexuelle chez les végétaux Angiospermes; C. R. Acad. Sc. Paris, **128**, 864-871.

1900 *Id.* : Nouvelles recherches sur la double fécondation chez les végétaux Angiospermes; Ibid., **131**, 153-160.

1896 *Haecker, V.* : Ueber die Selbständigkeit der väterlichen und mütterlichen Kernbestandteile während der Embryonalentwicklung von *Cyclops*; Arch. Mikr. Anat., **46**, 579-618.

1915 *Hutchinson, A. H.* : Fertilisation in *Abies balsamea*; Bot. Gaz., **60**, 457-473.

1891 *Kruch, O.* : Appunti sullo sviluppo degli organi sessuali e sulla fecundazione della *Riella clausonis*; Malpighia, **4**, 1-5.

1931 *La Cour, L.* : Improvements in everyday technique in plant cytology; Journ. Roy. Micr. Soc., Serie III, **51**, 119-126.

1898 *Mc Kenney, R. E. B.* : Observations on the development of some embryo-sacs; Contrib. Bot. Lab. Univ. Pennsylvania, **2**, No. 1, 80-86.

1929 *Madge, M. A. P.* : Spermatogenesis and fertilisation in the cleistogamous flower of *Viola odorata*, var. *praecox*; Ann. Bot., **43**, 545-577.

1898 *Mottier, D. M.* : Ueber das Verhalten der Kerne bei der Entwickelung des Embryosacks und die Vorgänge bei der Befruchtung; Jahrb. Wiss. Bot., **31**, 125-158.

1904 *Id.* : Fecondation in Plants; Carnegie Inst. Publ., **15**.

1900 *Murrill, W. A.* : The development of the archegonium and fertilisation in the hemlock spruce (*Tsuga canadensis*); Ann. Bot., **14**, 583-607.

1898 *Navaschin, S.* : Resultate einer Revision der Befruchtungsvorgänge bei *Lilium Martagon* und *Fritillaria tenella*; Bull. Acad. Imp. Sci. St. Petersbourg, **9**, 377-382.

1899 *Id.* : Neuen Beobachtungen über Befruchtung bei *Fritillaria tenella* und *Lilium Martagon*; Bot. Centralbl., **77**, 62.

1910 *Id.* : Näheres über die Bildung der Spermakerne bei *Lilium Martagon*; Ann. Jard. Bot. Buitenzorg, 2e Série, Suppl. III, 871-904.

1907 *Noren, C. O.* : Zur Entwicklungsgeschichte des *Juniperus communis*; Uppsala Universitets Arsskrift, **64**.

1918 *Nothnagel, M.* : Fecundation and formation of the primary endosperm nucleus in certain Liliaceae; Bot. Gaz., **66**, 143-161.

1893 *Overton, J.* : Ueber die Reduktion der Chromosomen in den Kernen der Pflanzen; Vierteljahrssch. Naturf. Gesell. Zürich, **38**, 169-186.

1907 *Pace, L.* : Fertilisation in Cypripedium; Bot. Gaz., **44**, 353-372.

1895 *Ruckert, J.* : Ueber das Selbständigbleiben der väterlichen und mütterlichen Kernsubstanz während der ersten Entwicklung des befruchteten Cyclopseies; Arch. Mikr. Anat., **45**, 339-369.

1896 *Sargant, E.* : The formation of the sexual nuclei in *Lilium Martagon*; Ann. Bot., **10**, 445-478.

1916 *Sax, K.* : Fertilisation in *Fritillaria pudica*; Bull. Torrey Bot. Club, **43**, 505-522.

1918 *Id.* : The behaviour of the chromosomes in fertilisation; Genetics, **3**, 309-327.

1901 *Schniewind-Thies, J.* : Die Reduktion der Chromosomenzahl und die ihr folgenden Kernteilungen in den Embryosackmutterzellen der Angiospermen. Jena.

1926 *Sharp, L.* : An introduction to Cytology. New-York.

1900 *Strasburger, E.* : Einige Bemerkungen zur Frage nach der doppelten Befruchtung bei den Angiospermen ; Bot. Zeit., **58**, 293-316.

1880 *Treub, M. & Mellink, J. F. A.* : Notice sur le développement du sac embryonnaire ; Arch. Neerl., **15**, 452-457.

1879 *Vesque, J.* : Nouvelles recherches sur le développement du sac embryonnaire ; Ann. Sci. Nat. Bot., Série VI, **8**, 261-392.

1914 *Welsford, E. J.* : The genesis of the male nuclei in *Lilium ;* Ann. Bot., **28**, 265-270.

1918 *Weniger, W.* : Fertilisation in *Lilium ;* Bot. Gaz., **66**, 259-368.

1929 *Whyte, R. O.* : Studies in Ranunculus. II. The cytological basis of sex in *R. acris* L. ; Journ. Gen., **21**, 183-191.

1904 *Wylie, R. B.* : The morphology of *Elodea canadensis ;* Bot. Gaz., **37**, 1-20.

1923 *Id.* : Sperms of *Vallisneria spiralis ;* Ibid., **75**, 191-202.

EXPLANATION OF PLATES.

All drawings were made with the aid of a camera lucida, using a Zeiss *2 mm. apochromatic objective N. A. 1.4 and compensating oculars K4 (× 5), K8 (× 10), or K12 (× 15). Magnification : × 1800, except fig. 12 (× 1200), figs. 3 and 5 (× 600).*

PLATE I.

FIGS. **1-5.** Development of embryo sac.

FIG. **1.** Longitudinal section of 2-nucleate embryo sac with smaller 2-nucleate antigone at chalazal end, showing telophase of second meiotic division of megaspore mother cell. The cell wall between embryo sac and antigone was formed after the first meiotic division.

FIG. **2.** The same, showing metaphase of first division after meiosis.

FIG. **3.** Embryo sac and antigone each 4-nucleate. Embryo sac much enlarged. The fourth nucleus of the antigone is in the next section. The nuclei of the embryo sac have been cut through by the microtome knife.

FIG. **4.** Cross section through chalazal pair of nuclei in 4-nucleate embryo sac, showing telophase of the next division.

FIG. **5.** 8-nucleate sac with polar nuclei approaching one another : egg apparatus not yet organised. The 4-nucleate antigone develops no further.

FIGS. **6-16.** Nuclear division in pollen grain and pollen tube.

FIG. **6.** Young pollen grain with single nucleus resting and showing nucleolar budding.

FIG. **7.** The same, with nucleus in early prophase of first division after meiosis.

FIG. **8.** The same in metaphase, showing the eight characteristic chromosomes A-H.

FIG. **9.** The same in telophase.

FIG. **10.** Pollen grain with tube nucleus and smaller generative nucleus.

FIG. **11.** The same, with tube nucleus spherical and lightly stained, and generative nucleus elongated and deeply stained.

FIG. **12.** Germination of pollen grain : generative nucleus in early prophase, tube nucleus not shown.

FIG. **13.** Pollen tube in the style : generative nucleus in late prophase.

FIG. **14.** The same : generative nucleus in early anaphase.

FIG. **15.** The same : generative nucleus in telophase.

FIG. **16.** The same : with two male nuclei formed.

PLATE II.

FIGS. **17-22.** Fertilisation.

FIG. **17.** Egg nucleus and two male nuclei in embryo sac.

FIG. **18.** Male nucleus coiled about egg nucleus : egg nucleus in resting stage ; male nucleus in stage which is either late telophase or early prophase (there is no resting stage).

FIG. **19**. Micropylar end of embryo sac showing one synergid, and the egg at the beginning of fusion with a sperm : membrane between egg and sperm breaking down.

FIG. **20**. Fusion : membrane broken down.

FIG. **21**. Fusion : male reticulum mingling with female reticulum but still clearly distinct.

FIG. **22**. Fusion : male reticulum less distinct from that of female.

FIGS. **23-25**. Triple fusion.

FIG. **23**. Male nucleus coiled about one of the polar nuclei. (Other polar not shown.)

FIG. **24**. A later stage : male reticulum distinct.

FIG. **25**. A later stage : male reticulum less distinct. (Second polar in next section.)

FIGS. **26-28**. Development of endosperm.

FIG. **26**. Late prophase of first division of the triple fusion nucleus : membrane between the two polars just breaking down (shown at m.). The chromosomes shown crowded at the centre of the figure are the male chromosomes.

FIG. **27**. An early metaphase of the fourth division, showing three distinctly recognisable groups of chromosomes A-H, A′-H′, A″-H″.

FIG. **28**. A metaphase plate of the seventh division drawn from an embryo sac containing about 128 endosperm nuclei : the three groups of chromosomes still distinct.

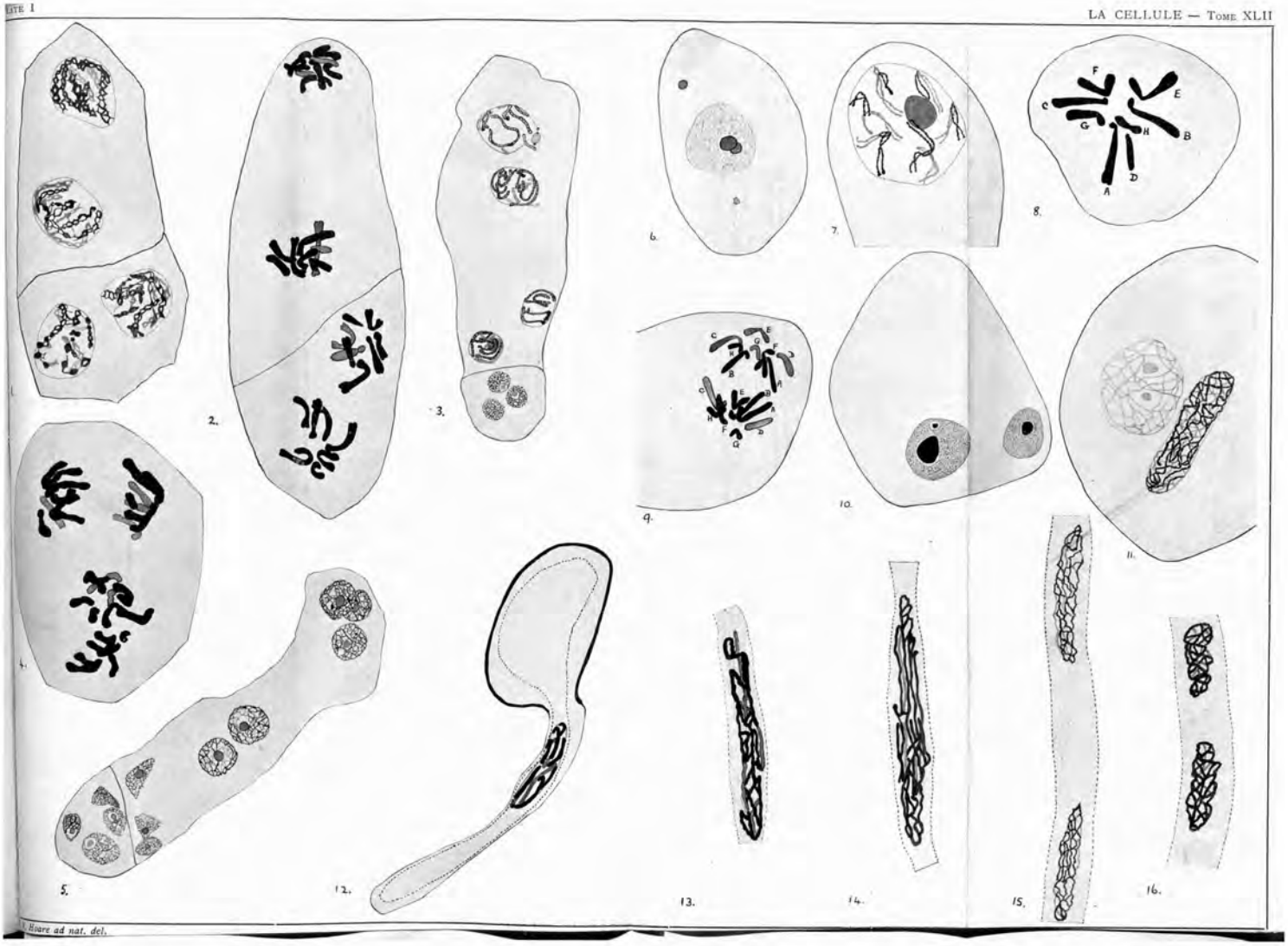
FIGS. **29-31**. Development of embryo.

FIG. **29**. Early prophase of first division of fertilised egg : showing two distinctly recognisable groups of chromosomes A-H, A′-H′.

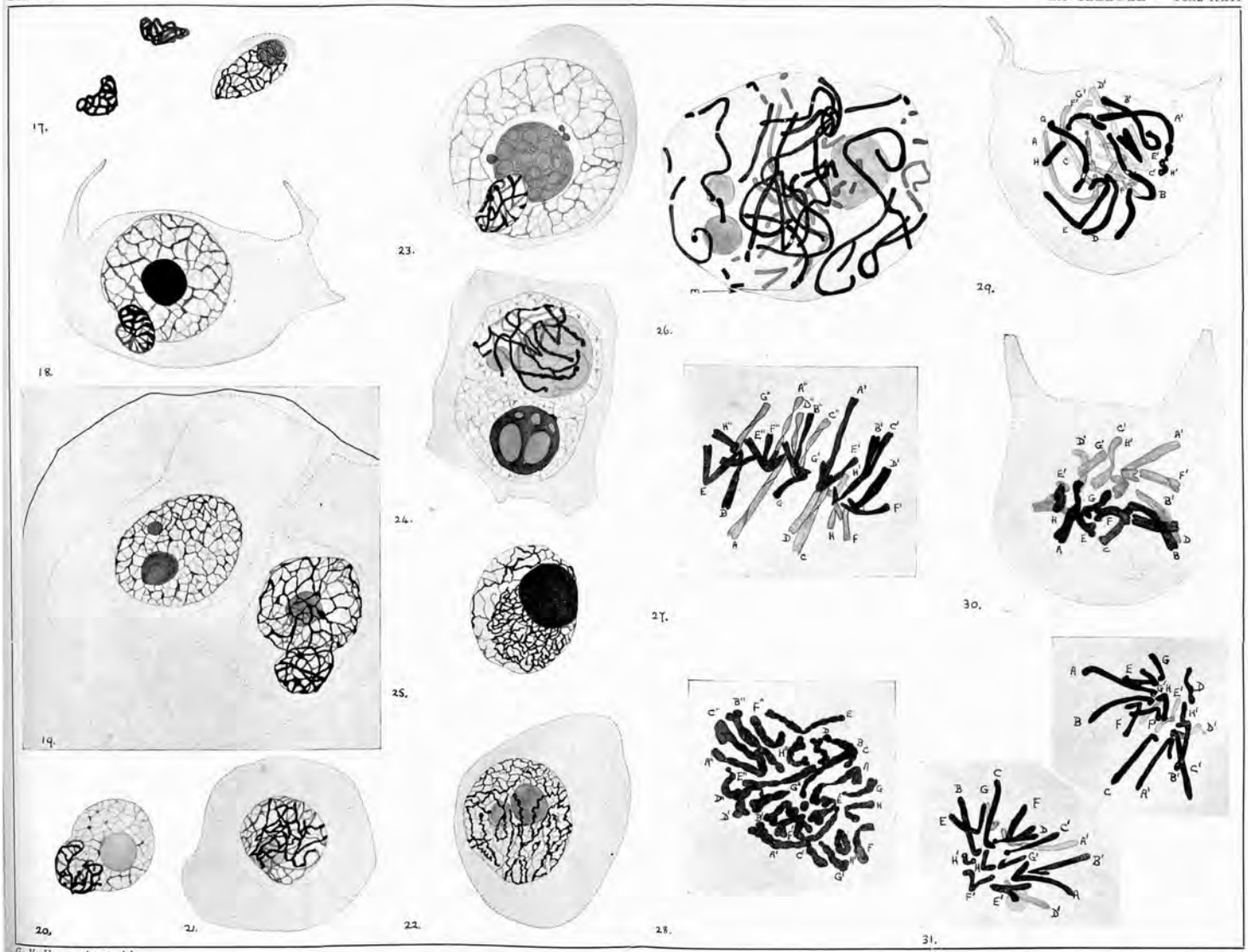
FIG. **30**. Metaphase of same division : the two groups of chromosomes still distinct.

FIG. **31**. Telophase of same division : the two groups of chromosomes beginning to mingle.

CONTENTS.



J. Hoare ad nat. del.



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SEED PRODUCTION IN HYDROCHARIS MORSUS-RANAE.

INTRODUCTION.

Although Hydrocharis morsus-ranae is not uncommon in the ponds and ditches of Britain, there have been few extensive investigations into its life history, and two problems in particular stand out as worthy of further study: (1) the reputed rarity of seed production, (2) the dioecious or monoecious habit.

It is commonly stated that Hydrocharis morsus-ranae does not set seed in England. Hooker (1884 p.382) writes: "Fruits I have not seen". Mrs.Arber (1920, p.46) states that "though the flowers of Frogbit are not uncommon, seed is hardly ever set in this country. The ripened seed ~~and~~ vessels are to be found, however, in Continental stations". She herself has never found fruits in England. The British Museum, however, had in 1931 four seeds in its possession, sent from Glastonbury in 1926, so that it is apparent that seeds are sometimes set under certain given conditions.

It is still uncertain whether the plant is monoecious or dioecious. It appears superficially to be dioecious and has been described as such in many of the standard floras and textbooks (Hooker, 1884; Engler and Prantl 1889; Warming 1895; Willis 1919.) One of the first investigators to throw doubt upon its dioecism was Lindberg (1873) who suggested that in reality the plant is either monoecious or

monoico - female, and found that if he removed the male and female"plants" from the water without breaking the stolons connecting them, they proved to be shoots belonging to the same branching vegetative system and were not separate individuals. Similarly Tuschnjakova (1929) states that the plants she studied were monoecious, but she also notes that dioecious forms do exist in the Moscow Botanic Gardens. Navaschin has found separate patches of male and female plants in South Russia, from which it may be inferred that they are probably dioecious there. It is interesting in this connection to note that there is in the British Museum a specimen of Hydrocharis Chevalieri, An African species, with two "plants", bearing a male flower and a fruit respectively, connected together by a runner.* This species is obviously monoecious and it is said that certain American species are also monoecious. Such facts lend additional weight to the reputed monoecism of Hydrocharis morsus-ranae. It is evident therefore from this review that the dioecism or monoecism of H. morsus-ranae is by no means conclusively established, and may possibly vary from one locality to another.

THE PRESENT STUDY.

Plants of Hydrocharis morsus-ranae have been examined both in the hope of throwing some light on the problem of

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* In the Herbarium of the British Museum: Hydrocharis Chevalieri (De Wild) Dandy, Tropical Africa, Cameroons. Bates 960.

their dioecism, and of ascertaining whether the lack of seed can be correlated with any abnormalities in the development of the pollen grain or embryo sac. An unexpected and interesting feature of the research, however, has been the production of ripe fruits and seeds in both years in which systematic investigation has been made.

MATERIAL AND METHODS.

Plants of Hydrocharis morsus-ranae grow in a semi-wild condition in the Botanic Garden of Royal Holloway College, and flower plentifully each year. The first material obtained was collected and fixed roughly in August 1930. Further collections were made in the summers of 1931, 1932 and 1933 and fixed in a variety of ways.

The methods of fixing and staining employed were similar to those already described for Scilla nonscripta. Stages of meiosis in the pollen mother cells were studied partly by means of pollen smears. It has been found, however, that the small size of the archesporium, the small size and delicate nature of the pollen mother cells and the tenacity with which these cling together, do not lend themselves very readily to the smear method, which was therefore supplemented by fixing whole buds, and then embedding and cutting them in the usual way. Sections were cut from 12-16 μ, this being the minimum thickness which permits the preservation of whole nuclei. Nuclear divisions in the pollen grains were studied mainly by the smear method,

which proved practicable at this stage.

OBSERVATIONS.

Somatic mitosis.

Root tips were cut and the metaphase plates examined. The chromosome types apparently agree with the account of them given by Tuschnjakova, There are fourteen pairs of chromosomes in the diploid somatic complement. Of these, one pair is large with subterminal attachment constrictions, and are therefore J - shaped as they pass to the poles; those of another pair are large with median attachment constrictions, so that they are U - shaped at anaphase; whilst the other chromosomes are smaller, and assume approximately U or J shapes, according to the position of the attachment constriction.

Development of the pollen mother cells and pollen grains.

The archesporium is already differentiated when the flower buds are still extremely small. Leptotene and zygotene are passed through fairly rapidly, but pachytene is of considerable duration, and is frequently accompanied by a well - marked "contraction". From zygotene to pachytene the nucleus appears to "bud" vigorously into the cytoplasm, agreeing in this with the similar phenomenon found in early prophases of Scilla nonscripta. It is possible that nucleolar budding at prophase is more widespread than has been commonly supposed, but the cause of such budding remains obscure. The

nucleoli do not persist beyond diakinesis (cf. again Scilla nonscripta.) From diplotene to metaphase the stages are passed through without any feature of special interest. Sex chromosomes have been sought for, but have not been identified. The chromosomes are too small and condensed at these stages for the types of chiasmata formation to be clearly distinguished, but the number of chiasmata formed varies as a rule between one and three and is rarely four in the two large pairs. Where there is one chiasma it is interstitial and the bivalents are cross - shaped at metaphase. Where there are two or more chiasmata formed, they usually seem to be terminalised at metaphase, so that ring shaped bivalents are produced. Anaphase and telophase follow the normal sequence and a wall is laid down between the two daughter nuclei formed. The second division of meiosis, which gives four pollen grains, then occurs, the angle adopted by the spindles showing great variation as in Scilla nonscripta.

The young pollen grains round off from one another and their nuclei rest for a comparatively short period. They then enter into a fairly lengthy prophase of division. The nuclei are very clear during this and the ensuing division, and the chromosome types may be more easily distinguished. A tube and a generative nucleus are produced and their cells are separated by a thin membrane. The division of the generative nucleus then follows rapidly, so that anthers have

been found which included pollen grains at both division stages. The two male nuclei produced are small and deeply stainable. They remain more or less spherical or slightly oval and are apparently not organised into cells. The tube nucleus becomes spherical, stains lightly, and rests completely. The pollen grain is shed in this three-nucleate condition.

The majority of the pollen grains follow the above sequence of division, but it is worth recording that about 10% of the pollen seems to be defective, and stains deeply and uniformly. Such defectiveness is usually confined to one whole anther or to all the anthers of a single flower, other flowers being perfectly healthy, and it may show itself either at the pollen mother cell stage or not until the pollen grains have been formed. Even the apparently healthy pollen grains show a most unusual variation in size, some being about twice their normal size and oval in shape, as if meiosis had failed and left diploid nuclei. These large grains have not been found containing chromosomes, however, therefore the hypothesis cannot be verified.

Development of the megasporangium and embryo sac.

The megaspore mother cell is usually two or three layers deep in the nucellus. It is an interesting peculiarity of the plant that in a large number of ovules - about 30 - 40% of the ovules studied - more than one megaspore mother cell is differentiated in the nucellus and enters into the

prophase of meiosis. Usually two megaspore mother cells are thus produced in the same ovule, but rarely there may be three or even more. Their nuclei develop as far as pachytene after which the chalazal one (but occasionally the micropylar one instead) completes the meiotic divisions and the others remain in pachytene. The dormant megaspore mother cells may persist unchanged until the developing one has completed meiosis and three megaspores have degenerated, or they may degenerate earlier. In any case they finally degenerate in their turn. Less commonly, more than one megaspore mother cell in an ovule may develop to a certain extent. Where meiosis occurs in both cells of a "twin", one is usually slightly ahead of the other in development; for example, one ovule examined showed the megaspore mother cell nearest the micropyle in the anaphase of the first division, while the chalazal one was in metaphase. "Twin" embryo sacs have also been found, each with a row of degenerating megaspores above them, and themselves in various stages of development. A further example of this interesting tendency towards duplication in this plant is shown in the double ovules which have occasionally been discovered.

Each megaspore mother cell passes rapidly through leptotene and zygotene, but the pachytene stage is of considerable duration and here too nucleolar budding is very marked. Further phases from diplotene to the telophase of

the first division follow the normal sequence. Chiasma formation is again difficult to interpret, but is similar in type to that observed in the pollen mother cell. The wall laid down between the two daughter cells is not always at right angles to the plane through the micropyle and the angle adopted by the spindles during the second division of meiosis also shows great variation. Hence, a linear row of megaspores is the exception rather than the rule. Frequent degeneration, or a tendency towards degeneration has been noted in the micropylar daughter nucleus of the first division. It is often smaller and deeply stainable and in some cases may never divide again so that a row of four megaspores is not then produced. In other cases it divides, but appears to be degenerating even as it does so. The chalazal nucleus does divide again, after which all except the megaspore at the chalazal end rapidly degenerate, often forming a deeply stainable cap at the top of the young embryo sac. To the cap above the embryo sac the degenerating "twin" megaspore mother cell, if present in the ovule, usually contributes. The cap may persists until the embryo sac has produced two or even more nuclei.

The embryo sac now enlarges, and its nucleus rests for a short time. It then prepares to divide, and three nuclear divisions in the embryo sac follow each other rapidly. Eight nuclei are thus produced after which in some sacs the two polar nuclei come together and fuse, so that the usual

Flowers have been pollinated artificially and subsequently fixed and examined. Withered and withering flowers from the ponds have also been fixed and examined. Pollen tubes have been observed entering the embryo sac and discharging the male nuclei, but actual stages of fertilisation have not been seen, although they have been extensively sought for. It is presumed that the process of fertilisation is passed through very rapidly, but there is little doubt that normal fertilisation does occur and apparently takes place about 20 - 30 hours after pollination. One synergid is usually broken down by the pollen tube as it enters.

Development of the young embryo and endosperm.

The fertilised egg cell rests for a time and then divides by means of a transverse wall to form a two celled pro-embryo. The cell nearest the micropyle enlarges a great deal and becomes the basal cell, and the other cell again divides transversely. A three celled pro-embryo is therefore formed. Further divisions are rather irregular, but agree more or less with the stages figured for Sagittaria (Coulter and Chamberlain, p.188). The resulting young pro-embryo has a very large basal cell, a short suspensor of two or three cells and a balloon shaped embryo of many cells.

The embryo sac is characterised meanwhile by a remarkable scarcity of endosperm. The fertilised egg frequently divides before the fertilised polars, although occasionally there are 2-4 endosperm nuclei formed at this stage. When

the embryo has formed three cells, there are usually about 4-8 endosperm nuclei in the sac, and sacs with quite large embryos show only about 16-24 endosperm nuclei clustered around the embryo at the top of the sac. Other sacs apparently form little or no endosperm The antipodals persist at the base of the sac for a time and then gradually degenerate, by which time the uninjured synergid has already degenerated.

It is interesting to note that only one or two ovules out of the many formed in the ovary produce embryos. The others do not seem to be fertilised, and gradually degenerate as the fertilised ones grow. Some of these unfertilised ovules may have contained the abnormal embryo sacs already noted.

The formation and dehiscence of ripe fruits.

In September 1932, ripe fruits were found in the ponds of Royal Holloway College. These fruits are about $\frac{3}{8}$" x $\frac{1}{4}$", with a smooth shining green surface and a somewhat woody texture. They are on long stalks which curve slightly to carry the fruits under the surface of the water. The capsules were brought into the laboratory and kept in glass vessels, in which they burst. Dehiscence occurs irregularly lengthwise down one side of each capsule and the seeds are liberated in a mass of mucilage. From two to six seeds and innumerable unfertilised ovules are set free from each capsule. Each seed is small, light brown and surrounded by a layer of enlarged cells, the walls of which are thickened spirally. According to Goebel (1891), it is from these enlarged cells

that the abundant mucilage is produced in which the seeds are embedded. Six seeds, chosen at random from different capsules, were dried. Of these six five shrivelled and were obviously non-viable and only one remained in good condition. Hence, it seems that of the seeds set, only a small percentage is viable.

These observations were published in brief form after the seeds were obtained in 1932, and in the following year a further note was published when more seeds were obtained.*

The remaining seeds from the capsules were sown in mud from the pond in which the fruits were produced and covered by pond water. They were left in this during the following year, the water being kept up to its original level by the regular addition of distilled water, and the vessel containing them being left out of doors in the sun, under as natural conditions as possible. It was hoped by these means to induce the seeds to germinate, but all efforts failed.

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* 1"Nature", November 1932, in "News and Views"
2"North Western Naturalist", December 1932, p.315.
3"Nature", October 1933, in "News and Views".

Ripe fruits and seeds were again formed in the autumn of 1933 and were again collected. The seeds were put into a dialyser in the pond in which they were produced, whilst other seeds were sent to Kew to see if they could be germinated there. The results of these experiments, however, will not be known until at least the summer of 1934. Even if germination then fails, it may be because the seeds require more than one year's rest before they can be induced to grow. Therefore it has not yet been possible to show what percentage of seeds are viable, although from the number which shrivelled on drying it is possible that quite a number of them may be incapable of germination.

DISCUSSION ON THE CAUSES OF SEED PRODUCTION.

The stages in the development of the gametes have been investigated with a view to discover whether any abnormalities occurred in their production which would hinder the development of seeds. The work was begun on the assumption that seeds are rarely or never set in England, but has led to the discovery that seeds have been set in both years in which they have been sought for. Embryos were found in fertilised ovules even in the year before, when actual fruits were not looked for in the autumn, so that it is probable that some fruits were formed even in that year. It appears therefore that the production of seeds by Hydrocharis morsus-ranae is not as rare as is commonly assumed. On the other hand, the summers of 1932 and 1933 were both extremely hot ones, and

the young embryos found in 1931 were collected and fixed during a heat wave in July. Similarly, it was during the hot summer of 1926 that seeds were sent from Glastonbury to the British Museum, these being the only seeds of Hydrocharis morsus-ranae then in the possession of the Museum. It would be interesting to discover if fruits and seeds were still produced in a cold summer. Several peculiarities have emerged from this study, which may throw some light on the alleged lack of seed production. The pollen contains a fair percentage of abortive grains, which are confined to the anthers of certain flowers. It is conceivable that this percentage may be increased during colder summers. Again, there are a considerable number of abnormal embryo sacs, which presumably would be incapable of normal fertilisation and the production of embryos. The percentage of these was higher in 1931 (a cooler summer) than in 1932 and 1933. It is therefore probable that the formation of normal embryo sacs may be affected adversely in some way by the cold. If cold is responsible for an increase in these abnormalities, it would explain why fruits and seeds have readily been gathered in warmer European stations, but are reputed to be rare in England.

Since embryos have been found in so many seeds, and pollen tubes have been seen discharging the male nuclei into the embryo sac, it is evident that fertilisation can occur in properly formed embryo sacs. It is possible,

however, that a colder summer may cause a dearth of the small flies which appear to pollinate the flowers and this may be an additional cause of the lack of seeds in such seasons.

There is an alternative explanation that the fruits and seeds may be formed every year but have been overlooked as they bend under water to dehisce. It is difficult to believe, however, that so eminent a naturalist as Hooker, in correspondence with other naturalists all over England, could have missed them in this way. The theory that heat is needed for their production is far more probable, but can only be tested in the next cold English summer.

STUDIES ON THE DIOECISM OF HYDROCHARIS MORSUS-RANAE.

The plants have been removed from the water as carefully as possible, so that the runners have not been broken, but all the plants connected together have proved to be either all male or all female. This seems to indicate that the variety under observation may be dioecious. In the autumn of 1932, plants were picked out which bore fruits, and the turions formed on them were removed. Turions on plants bearing male flowers were removed in a similar way. The two sets of turions were put in separate buckets of pond water, which were sunk in the ground near the ponds. In the summer of 1933, the turions rose to the surface and began to grow. They seemed quite healthy for some time, but later on the leaves turned brown and the plants looked unhealthy. They

were removed to wooden tubs, and as these failed to restore their healthy appearance, they were put into neighbouring ponds, but all efforts proved useless and finally they died. Before they died, however, the plants formed from the turions of the"male" plants had produced flowers, and they all proved to be male. Those from the "female" plants had not formed flowers at all. (The female flowers always seem to come slightly later than the earliest male flowers.) These incomplete experiments seem therefore to show that the plants under observation are truly dioecious.

It was hoped that the seeds might germinate and produce plants, and that these might provide more conclusive evidence in favour of dioecism or monoecism, but this hope has not been fulfilled. The facts so far obtained seem to indicate that the variety of Hydrocharis morsus-ranae in the grounds of Royal Holloway College is probably dioecious. This, considered in relation to the findings of other investigators, would seem to show that the plant may be monoecious in some districts and dioecious in others, but whether this is really so remains to be determined.

SUMMARY.

A. Seed formation in Hydrocharis morsus-ranae.

1. The development of the microsporangium and megasporangium, nuclear division in the pollen grain and the development of the embryo sac have been investigated in an endeavour to find the cause of the commonly reported lack in England of fruits and seeds. The divisions are found to be normal in all respects and to follow the usual sequence. Interesting deviations from normal development are as follows;

a). The generative nucleus divides into two male nuclei inside the grain, which is therefore shed in a 3-nucleate condition.

b). In about 30 - 40% of the ovules examined, two or more megaspore mother cells are produced in the young nucellus and enter into the prophase of meiosis together. They reach pachytene, and then all except one usually degenerate at this stage, whilst one completes the meiotic divisions.

c). The majority of the embryo sacs examined pass through the normal stages of development, but a great number of sacs show irregular numbers of nuclei instead of the usual seven or eight expected, and they are arranged in groups of three, four or five instead of in normal order. It is presumed that these would probably not produce embryos. The percentage of such sacs was particularly high in 1931, when it was as

much as 50 - 60%, and it is suggested that it may be increased during colder summers. This may help to account for the alleged lack of seed production in these seasons.

d). About 10% of the pollen is defective, and it is suggested that this percentage may also be increased during colder summers.

2. Pollen tubes have been seen growing through the micropyle and discharging male nuclei, therefore although fertilisation has not been seen, it is presumed that it occurs normally in properly developed sacs.

3. Young embryos were found in the autumn of 1931 and 1932 and the stages in their development are described. Ripe fruits and seeds were obtained in 1932 and 1933, in contrast with the usual reports of their rarity. Of the seeds produced, however, a large percentage appear to be non-viable, as they shrivel on drying, although conclusive tests have not yet been made. It is suggested that seeds are set in England during very hot summers but not in colder ones, when the normal production of pollen and embryo sacs may be interfered with.

B. <u>The dioecious or monoecious habit of Hydrocharis morsus-ranae</u>.

Various investigators have described the plant as dioecious or monoecious. In the variety under observation, male and female plants have never been found connected

together, which indicates that it is dioecious. Experiments, which are unfortunately incomplete, support this view.

It is possible that Hydrocharis morsus-ranae may be dioecious in certain districts but monoecious in others.

LITERATURE CITED.

1920 Arber,A. : Water Plants; Cambridge, 45-46.

1903 Coulter,J.M. and Chamberlain,C.J. : Morphology of Angiosperms; New York, p.188.

1889 Engler,A. and Prantl,K. : Die natürlichen Pflanzenfamilien; II, 1, Leipzig, p.242.

1891 Goebel,K. : Pflanzenbiologische Schilderungen; II, 1, Marburg, p.234.

1884 Hooker,Sir J.D. : The student's flora of the British Isles; London, 381-382.

1873 Lindberg,S.O. : Is Hydrocharis really dioecious? Trans. and Proc. Bot. Soc. Edinburgh, 11, p.389.

1929 Tuschnjakova,M. : Untersuchungen über die Kernbeschaffenheit einiger Diözischer Pflanzen; Planta, Archiv für Wissenschaftliche Botanik, Berlin, 7, 427-431.

1895 Warming,E.,transl. Potter,M.C. : A handbook of systematic botany; London, p.283.

1919 Willis,J.C. : A dictionary of flowering plants and ferns; Cambridge, p.334.

OBSERVATIONS ON ARUM MACULATUM.

This plant was chosen for investigation as it is monoecious and the spike of close-growing flowers provides convenient material for investigation. A survey of the literature reveals the fact that it has never been studied in detail, although other members of the Araceae family have received close attention. Arum spp. are briefly referred to by Hofmeister (1861), but neither Campbell (1900) nor Gow (1908) in their study of the Araceae family describe Arum itself.

MATERIAL AND METHODS.

Abundant material is always available in the grounds of Royal Holloway College and the surrounding neighbourhood, and fixations were made at frequent intervals from October 1931 to June 1932, and during April and May 1933. Therefore all stages have been obtained, from the development of the archesporium in the anthers and ovules to the formation of fairly large embryos. It has been impossible, however, during these years to cut and examine even half of this material. The greater part of the time has been given up to Scilla nonscripta and Hydrocharis morsus-ranae, as these proved more interesting subjects.

The fixatives and stains used and the methods employed were similar to those described for Scilla nonscripta. The stages of meiosis in the pollen mother cells and the

divisions in the pollen grain were studied by means of pollen smears, supplemented by paraffin sections. Meiosis in the megaspore mother cells and the development of the embryo sac and embryo have been studied from paraffin sections, obtained by embedding and cutting small pieces of the flowering spike. Sections were cut from 16 - 18μ, the most convenient thickness for the examination of nuclei.

OBSERVATIONS.

Somatic mitosis.

Metaphase plates have been examined in sections of root tips. There are thirty-two small chromosomes, with either terminal, subterminal or median attachment constrictions, but with no outstandingly characteristic shapes.

Development of the pollen mother cells and pollen grains.

The archesporium in the anther is well defined at the beginning of October. Stages of meiosis have been obtained from January to March, the stage reached in different flower spikes varying quite considerably on the same day. All stages have been obtained of the first and second divisions of meiosis and they follow the usual sequence, but details of chiasma formation are difficult to interpret. Apparently one or two chiasmata are formed in each bivalent. In contrast to Scilla nonscripta and Hydrocharis morsus-ranae, nucleolar budding has not been found in this plant. Walls are laid down between the nuclei after the first and second divisions, and the angles assumed by them are irregular, as

in Scilla nonscripta and Hydrocharis morsus-ranae.

The pollen grains thus formed, gradually round off from one another and rest for a few weeks in a uninucleate condition. The nucleus of each grain then divides twice in fairly rapid succession, the chromosomes being more easily distinguished in these haploid nuclei. The first division results in the formation of a larger tube nucleus which stains lightly and a smaller generative nucleus which stains deeply. The two nuclei are in separate cells, with a delicate membrane between them. The generative nucleus then divides again into two small chromatic male nuclei, as in Hydrocharis morsus-ramae. In this 3-nucleate condition, the grain is shed.

Development of the megaspore mother cell and embryo sac.

An alternation in the time of active division stages in the nuclei of the pollen mother cell and megaspore mother cell has been observed in this plant, as in Scilla nonscripta. The nucleus of the pollen mother cell reaches pachytene at the same time as the megaspore mother cell nucleus, but it then completes the two divisions of meiosis, whilst the nucleus of the megaspore mother cell remains in pachytene. When pollen grains have been formed, meiosis is then completed in the ovule. This alternation in time, however, is not such an invariable feature in A. maculatum as it is in S. nonscripta, for spikes of Arum have occasionally been cut with both the megaspore mother cells and pollen mother cells in

active stages of meiotic division at the same time. Similarly the nuclear divisions in the pollen grain may sometimes precede the division in the embryo sac, but many cases have been found in which such precedence is not shown.'

Stages of meiosis in the megaspore mother cell follow the normal sequence and a linear row of four megaspores is produced, of which the one furthest from the micropyle always becomes the embryo sac, whilst the other three degenerate fairly rapidly, leaving a cap for a short time upon the young embryo sac.

The nucleus in the embryo sac now divides three times in fairly rapid succession, and the typical polarised 8-nucleate sac is thus obtained. A 7-nucleate sac follows upon the fusion of the two polars, the fusion occurring slightly towards the micropylar end of the sac.

Germination of the pollen grain and fertilisation.

The spathes of certain flower buds have been carefully slit open, the top of the spike, including the stamens, removed and the ovaries pollinated artificially. They have then been tied up in muslin bags and fixed at certain intervals of time. Other ovaries from withered and withering flowers have also been fixed and subsequently examined. The pollen grains germinate on the hairy stigma and the pollen tubes grow down the short style to the ovule, where they pass through the micropyle. The two small and deeply stainable male nuclei pass more or less unchanged down the tube

although they become rather more oval in shape. The tube nucleus accompanies them. They are then discharged into the embryo sac through one of the synergids. A few doubtful cases of fertilisation have been found, but have not been studied in detail, especially as in the larger embryo sacs it is extremely difficult to obtain a good fixation. Mucilage is produced and seems to interfere with the penetration of the fixative. The fertilised egg and triple fusion nucleus rest for a time.

Development of the endosperm and embryo.

The triple fusion nucleus divides and produces from eight to sixteen endosperm nuclei before the fertilised egg divides, but it has not been possible with these small nuclei to see whether the nuclear complements contributed by the male and polar nuclei maintain their separate identity, as they do in Scilla nonscripta. It is stated by Campbell for other members of the Araceae family and by Hofmeister for Arum spp. that the endosperm is cellular, but it does not seem to be the case in this material of Arum maculatum, though the fixative often causes the cytoplasm to collect into strips, which give the superficial appearance of cell membranes. The embryo resembles that of other members of the Araceae family (Campbell) in its massive character and lack of suspensor. The lack of suspensor is supposed by Campbell to be due to the fact that the endosperm is formed mainly at the top of the sac, so that the young embryo is

already in close contact with its food supply. A characteristic feature of the embryo sac of Arum maculatum after fertilisation is the prolonged persistence of one synergid, which becomes enlarged, has a very active appearance and may be concerned in some way with the passing of food to the young embryo.

LITERATURE CITED.

1900 Campbell,D.H. : Studies on the Araceae; I; Ann. Bot., 14, 1-25.

1903 Id. : Studies on the Araceae, II, The embryo sac and embryo of Aglaonema and Spathicarpa; Ibid, 17, 665-687.

1908 Gow,J.E. : Studies in Araceae; Bot. Gaz., 46, 35-42.

1861 Hofmeister,W.F: Neue Beiträge zur Embryobildung; Monocotyledons; Leipzig.

Summary of observations on other Monocotyledons.

Sagittaria sagittifolia.

The plant is monoecious. The cells have large clear nuclei, and large characteristic chromosomes. It is therefore a promising species for investigation but work on it has been abandoned owing to lack of material and time.

In the pollen mother cells stages of meiosis from pachytene to diakinesis have been obtained. A few stages of nuclear division in the pollen grain have been found and 2 - nucleate pollen grains have been shown to exhibit a characteristic difference in size and staining power of the tube and generative nuclei. The pollen grain is shed in the 2 - nucleate condition.

8 - nucleate embryo sacs have been cut, and sacs showing stages in the fusion of the two polar nuclei. Double embryo sacs have been found.

Younger ovules have been fixed, but have not been examined.

Ruscus aculeatus.

Flowers of this plant apparently vary from the hermaphrodite to the dioecious condition. The sixteen chromosomes are large and characteristic, but have not been studied in detail.

Stages in the first division of meiosis of the pollen

mother cell have been obtained, together with all stages of pollen grain division. All stages in the development of the microsporangium and megasporangium, pollen grain and embryo sac have been fixed, but have not been examined.

Sparganium ramosum and Typha latifolia.

Flower buds of these plants have been cut and examined, but the chromosomes proved so numerous and small that the material was discarded as unsuitable for further investigation.