OBSERVATIONS MADE WITH THE ULTRAVIOLET MICROSCOPE ON THE MINOR SPIRAL OF CHROMOSOMES IN *OSMUNDA*

by

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The ultraviolet microscope, in the present context, has been used as a high resolution instrument comparable to an extra lens on the visual light microscope and not, as is often the case, as a form of microspectrometer for the chemical identification of cell components. From numerous studies by other investigators in the latter field the powerful absorption of ultraviolet light by nucleoproteins is well known. This means that the fine details of chromosome structure are a particularly suitable field for high power microscopy with ultraviolet light since the intensity of photographic contrast obtainable by this means is comparable to that conferred by the use of stains in visual microscopy, while the greater degree of optical resolution corresponding to the reduction of wave length from visual light to ultraviolet permits of a virtual doubling of significant magnification. This improvement is of course trifling compared with the spectacular powers of the electron microscope but the practical difficulties in the handling of the specimen are also correspondingly less. Except for the replacement of the human eye by the camera and for the greater degree of mechanical precision required in the optical equipment, ultraviolet microscopy is so like visual microscopy that the same type of material in almost the usual condition can be utilized. This means that a preparation which has been closely studied with visual light and in which significant detail near the limit of optical resolution is known to exist can be transferred to the ultraviolet microscope and be re-examined with immediate clarification of view once a very few preliminary difficulties have been overcome. Discussion of these difficulties and of the treatment needed to circumvent them for cytological material such as that to be discussed below will be found in MANTON AND SMILES (1943).

The observations with which this paper is concerned were carried out in the summers of 1944 and 1945 after which, for reasons unconnected with the work, it had temporarily to be discontinued. Had this not occurred the particular topic of the minor spiral would have been developed more completely before publication was attempted and some of the photographs which have yielded significant evidence would perhaps have been replaced by others more perfect technically or better suited to mechanical reproduction. Even without this, however, they represent a body of new knowledge which could not have been obtained by the methods prevailing in 1939 and since the field of enquiry is itself only a part of a larger investigation which is about to be resumed under somewhat changed circumstances, incompleteness in the sense of the original programme is perhaps unimportant.

The fern *Osmunda* which has formed the subject of three previous communications

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(MANTON, 1939; MANTON AND SMILES, 1943; MANTON, 1945) is the most favourable cytological material which has so far been encountered among the lower plants and though the chromosomes are both smaller in size and more numerous than those of the most frequently studied Dicotyledons and Monocotyledons, its systematic position as well as its intrinsic merits give it a particular interest. That its intrinsic merits are not negligible is shown by the fact that on certain matters, notably the estimations of chromonema length at leptotene and of direction of coiling in the sister chromatids of a split somatic chromosome, the information available for *Osmunda* is more complete than that for any other plant. For this reason alone the filling in of gaps in our knowledge wherever possible is particularly desirable and the ultimate objective of the whole investigation is to integrate the facts for spiral structure, both quantitative and qualitative, into the morphological descriptions of mitosis and meiosis more fully than has previously been done.

MATERIAL AND METHODS

Though the methods to be used here are essentially those of MANTON AND SMILES (1943), some differences of detail are involved in the cytological treatment of a different tissue. In 1943 the test object was the germinating spore. In the present paper it is spore mother cells at the second meiotic division. The standard treatment for revealing spiral structure in the chromosomes of either meiotic division is to expose the living cells to ammonia, either as vapour or in solution, for a few seconds before fixation. In the first paper of this series (MANTON, 1939) the ammonia treatment had been applied according to the method of SAX AND HUMPHREY (1934) the end product of which is a balsam mount stained in gentian violet. This method has now been replaced by the following.

A fresh smear of mother cells is covered with a drop of 20% alcohol containing ammonia, for ten seconds, the exact strength of ammonia required being determined by trial. The ammoniated alcohol is then drained off and the cells are killed with a drop of acetocarmine which is almost at once replaced by a second drop to remove admixture with the alcohol. This reagent is then left on for long enough for the chromosomes to become visible and the stage and efficacy of the treatment assessed. They are then transferred to acetic alcohol to harden. Two strengths (1:1 and 1:3) of absolute alcohol: glacial acetic acid mixture are conveniently used for this purpose in the latter of which the preparation may remain for anything up to 12 hours. A new drop of acetocarmine is then put on, a coverslip is added and the preparation heated gently over a flame without being allowed to boil. Gentle manual pressure is then applied by passing a finger over a piece of blotting paper placed over the coverslip. This pressure spreads the contents of cells more nearly into one plane, a feature which greatly assists observation with visual light and is indispensible with ultraviolet. At the same time the action of the reagent tends to enlarge all dimensions of the chromosomes without thereby introducing any other detectable artifact. This also facilitates observation although some of this enlargement is reversible and is lost when the acetic acid is replaced by other reagents. Once the heat and pressure have been applied the preparation is virtually finished and it may either be examined at once with visual light, or it may be treated as an ordinary acetocarmine smear and transferred by stages to balsam, or it may be incorporated in a cellulose nitrate film and transferred bodily to a quartz slide ready for the ultraviolet microscope. The details of the latter process are given in

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MANTON AND SMILES (1943) under the heading of WELCH's Durofix transfer method.

Before ultraviolet photography can begin the cellulose nitrate film must be dissolved away and the stain as far as possible removed by soaking in 45% acetic acid. A quartz coverslip is then put on and ringed with wax, the mounting medium being either 45% acetic acid or any other convenient transparent liquid. Serial photography right through the specimen at known differences of focal level, usually either 0.1 or 0.2 μ between consecutive exposures, then provides the entire evidence on which observations are based. For the material under discussion a polar view of a plate of chromosomes may contain anything from ten to thirty significant focal levels. For the first working out of the evidence all the focal levels of a successful series are printed by photographic enlargement to a standard magnification of three thousand diameters but for purposes of reproduction in half-tone selected portions of the evidence have been further magnified to four thousand diameters, a degree of enlargement which represents the limit of significant magnification with ultraviolet light in the sense that two thousand or perhaps three thousand is the limit with visual light.

The ultraviolet microscope used has been the Beck-Barnard instrument at the *National Institute for Medical Research, Hampstead*, which carries Zeiss quartz lenses computed to work with the 2750 A Cadmium line supplied by a stationary electrode cadmium spark source. I am deeply indebted to the MEDICAL RESEARCH COUNCIL and to the authorities of the Institute for their generosity in receiving me as a guest whilst working there. I am also personally indebted to Mr. SMILES and Mr. WELCH for technical help in operating the instrument and to my own technical assistant Mr. CLARKE for preparing the prints for publication. I must also acknowledge the assistance from the ROYAL SOCIETY of a small grant towards the cost of frequent journeys to London, which I held during 1944 and 1945.

THE MINOR SPIRAL

The minor spiral in the present context means the spiral revealed by ammonia treatment in the chromosomes of the second meiotic division. In *Osmunda* as in many other plants this spiral differs from that at the first meiotic division (the major spiral) by being narrower in diameter and having a larger number of gyres. It also differs from the spiral of a somatic chromosome in having a smaller number of gyres and therefore it is perhaps important to emphasize the limitation which will be put on the word "minor". It has sometimes by other writers been loosely applied to any manifestation of spiral structure other than the major coil of the first meiotic division; it will, however, on this occasion be strictly confined to the spiral of the second meiotic division only.

A difference in the number of gyres and in diameter, between the spirals at the two meiotic divisions is a very usual though not a universal feature in plants (exceptions include *Trillium* and *Vicia*). Some idea of the extent of the difference may be obtained by a glance at Figs 1–4, which show major and minor spirals in two well known organisms *Tradescantia virginiana* and *Osmunda regalis*, in each case photographed with visual light from a preparation stained in gentian violet after the standard pretreatment of SAX AND HUMPHREY (1934). The photographs are all at the same magnification ($\times$ 3000) for the sake of comparability. This degree of enlargement is clearly excessive for the very large chromosomes of *Tradescantia* (Figs 1 and 2) but is necessary for effective reproduction of the essential details of the smallest object, the minor spiral of

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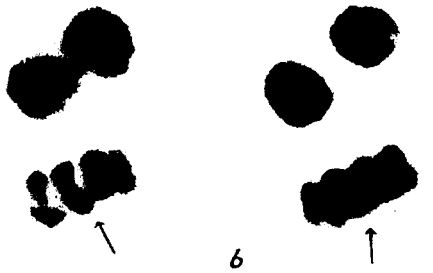

Fig. 1. *Tradescantia virginiana* the major spiral photographed with visual light from a balsam mount stained in gentian violet, × 3000. Two focal levels of an unpaired chromosome with an optical section of a ring pair above.

Fig. 2. The same showing the minor spiral at anaphase of the second meiotic division, for comparison of size with *Osmunda*.

Fig. 3. *Osmunda regalis* the major spiral, treatment and magnification as in Fig. 1.

Fig. 4. The same showing the minor spiral (after MANTON, 1939).

Osmunda in Fig. 4. These four figures express not merely the relative difference between major and minor spirals in the same plant but also the permanent differences of size between the two species. In both, the major spiral falls easily within the province of visual microscopy. In *Osmunda*, however, the minor spiral is so near to the limit of resolution that the need for special methods for observing it is at once apparent. That even with the new methods the minor spiral of *Osmunda* remains uncomfortably small cannot be denied but comparison of Figs 7 and 9 (et seq.) both with each other and with Figs 2 and 4 respectively will show that the technical improvement though not unlimited is sufficiently definite to make all the difference to the study of this particular problem.

CONVENTIONS REGARDING DIRECTION OF COILING

Direction of coiling does not figure largely in the new evidence although some understanding of it is essential, for the reason that unless direction can be demonstrated, in parts at least of the chromosomes under observation, it cannot be certainly known that the features observed are really parts of a spiral. For this reason a word may perhaps be said regarding the conventions used for expressing direction.

As on the previous occasion (MANTON AND SMILES, 1943) the meanings attached to the words right-handed spiral and left-handed spiral will be those of the physical sciences and not those of the biological sciences which use the words in an exactly inverse sense. In the physical sciences a right-handed spiral is that of the ordinary

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Fig. 5. *O. regalis*, early second division not treated for spiral structure, to show gross morphology of the chromosomes. Permanent acetocarmine photographed visual light × 1000 (after MANTON, 1930). Two lost chromosomes in the cytoplasm still show the major spiral of the previous division; other chromosomes long and thin, with minor spiral invisible.

Fig. 6. The same at anaphase of the second division. Note changed shape of the chromosomes though the minor spiral is invisible (after MANTON, 1930).

carpenter's screw and an example of it is visible in Fig. 1 in which (a) is the upper focus and (b) the lower. Had (b) been the upper surface and (a) the lower the spiral would have been left-handed.

Other examples of *right*-handed spirals are the two marked chromatids of the right hand central chromosome of Figs 7, a–d in *Tradescantia*, while a *left*-handed spiral in the smaller chromosomes of *Osmunda* may be seen in the marked chromosome at the bottom of Figs 9a and b. A very clear example of a *change* of direction may be seen in the lower chromatid of the left-hand central chromosome of Figs 7a and b and other examples of all these phenomena will be discussed below.

MORPHOLOGY OF CHROMOSOMES AT THE SECOND DIVISION IN *Osmunda*

A glance at Figs 5, 6 and 8 will sufficiently explain the morphology of the chromosomes of *Osmunda* in features other than spiral structure. At the end of anaphase (Fig. 6) the haploid complement of 22 rod shaped chromosomes of very uniform size and shape is easily seen, the position of the centromere in all but one of the chromosomes being approximately terminal. The position of the centromere is better seen at metaphase since it is then revealed as the place at which the otherwise widely diverging chromatids are attached. In *Tradescantia* the median centromeres give the second division chromosomes at metaphase an X shape (Fig. 7). In *Osmunda* with terminal centromeres the shape approximates to that of a V as may be seen in parts of Fig. 5 and again in Fig. 8 and subsequently. The only chromosome in *Osmunda* possessing a more nearly median centromere is that numbered 2 in Fig. 8. This is also the chromosome previously used for demonstration of the minor spiral in Fig. 4 from which it is clear that the short arm is about half the length of the long one. This chromosome is of importance because it is the only one in the haploid complement which can be separately

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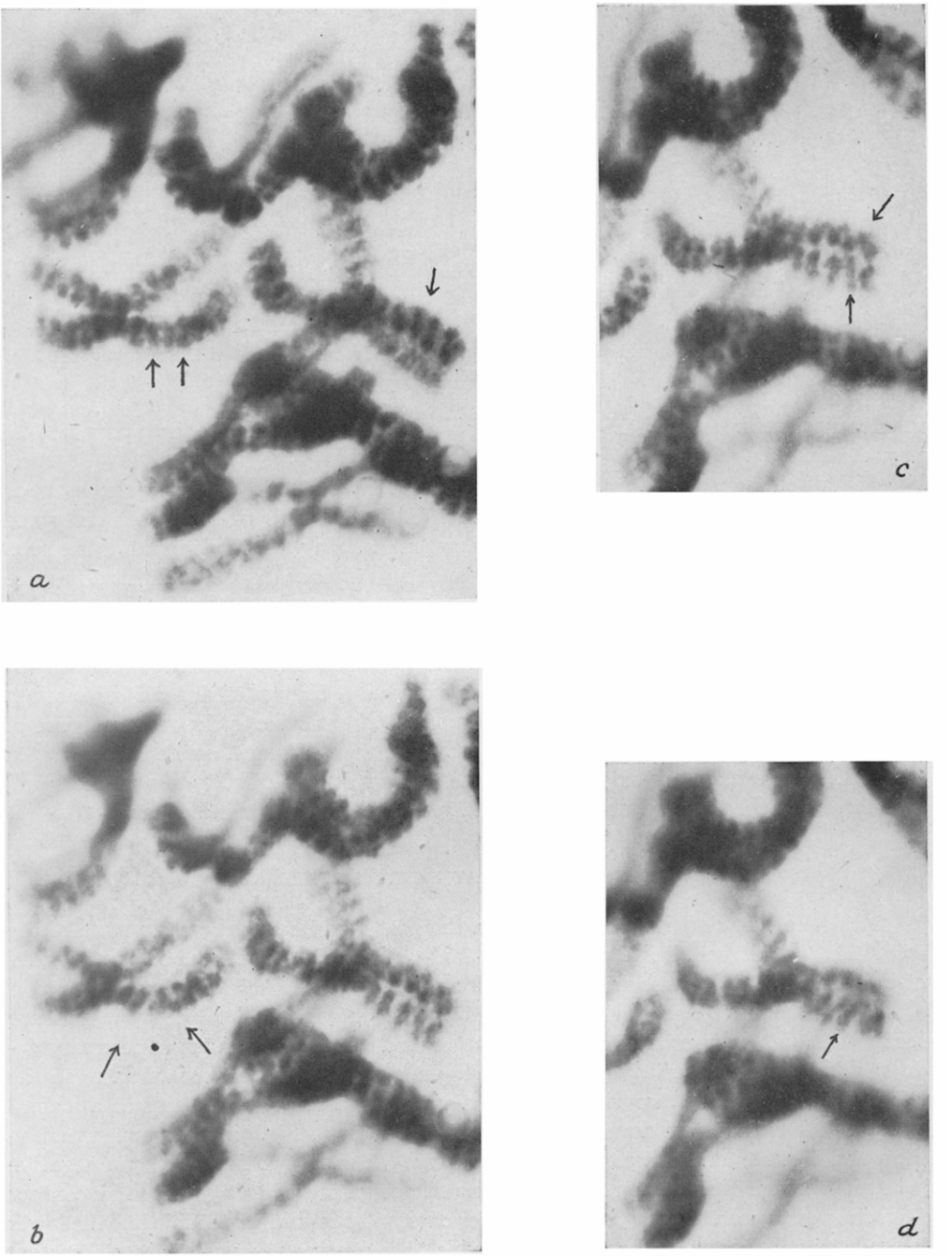

Fig. 7. *Tradescantia virginiana*. High power U.V. photographs of the minor spiral × 4000. Four focal levels 0.4 μ apart showing details of direction of coiling in parts of two chromosomes. In the right-hand central chromosome both chromatids are coiled in right-handed spiral from the centromere to the right-hand end. The left-hand central chromosome shows a change of direction in the lower chromatid. Fig. a the top focus, Fig. d the bottom. (Series 105 exposures 3a, 4a, 4b, 5b).

Fig. 8. *Osmunda regalis*. General view of the cell used for the early stage of the minor spiral. Low power U.V. photograph × 500.

identified, the shapes of all the others being so much alike as to make them for practical purposes indistinguishable.

If half-chromosomes (chromatids) only are considered close comparison between Figs 5 and 6 will make clear another detail of difference, this time between early and late stages of the second division. At early metaphase (Fig. 5) the chromatids are relatively longer and thinner than they appear to be at anaphase (Fig. 6) and this difference can be shown to be caused by changes occurring during metaphase itself, early stages such as Fig. 8 being like Fig. 5, but late stages of metaphase such as those of Figs 16–18 being indistinguishable from anaphase except for the position of the chromatids on the spindle. Demonstration that the basis of this apparent change of dimensions lies in a change in the number and size of the gyres of spiral structure is the main purpose of this paper. It is, however, important to notice that the difference can be demonstrated by all the more usual cytological techniques which do not directly reveal the spiral and there can therefore be no question that the appearance is an artifact.

THE MINOR SPIRAL AT EARLY METAPHASE

A general view of the cell from which the best evidence on the early state of the spiral has been derived has already been introduced as Fig. 8 and some enlarged details of individual chromosomes will now be discussed.

Chromosome 1 (Figs 9 and 10) provides a useful introduction to the spiral and two focal levels of it are reproduced in two types of print in the hope that the necessary details will survive mechanical reproduction in one or other if not in both. Fig. 9, on the left of the page, shows two views in ordinary photographic enlargements at a magnification of four thousand diameters, the upper focal level, Fig. 9a being uppermost on the page. Fig. 10, on the right of the page, shows the same two focal levels in negative prints, the image of these being reversed since the negative print is obtained by contact from the positive. In all four prints the upper chromatid is uninterpretable but the lower is far better. It contains about 14 gyres which are fairly evenly coiled in a direction which, for a considerable distance, can be diagnosed as right handed. Of the two focal levels required for determination of direction the lower, Fig. b, is the more distinct but in both views the direction of slope of the gyres is unequivocal and is indicated by the angle at which the arrows have been drawn in Figs 9a and b.

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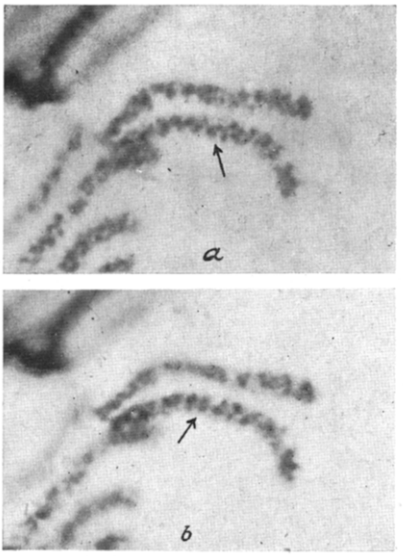

Fig. 9. Detail of chromosome 1. High power U.V. × 4000. Two focal levels 0.4 μ apart. The lower chromatid alone interpretable, with about 14 gyres of a right handed spiral. (Series 102 exposures 3a, 4b).

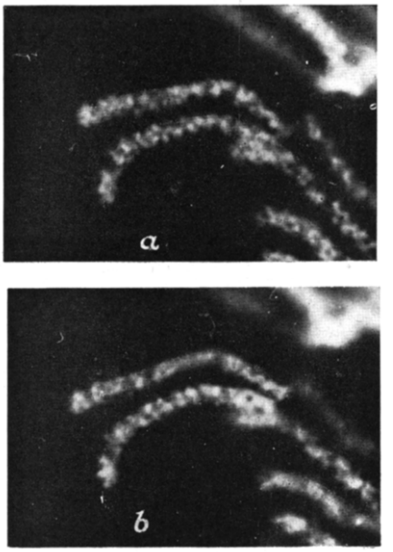

Fig. 10. The same as the preceding but negative print and therefore inverted.

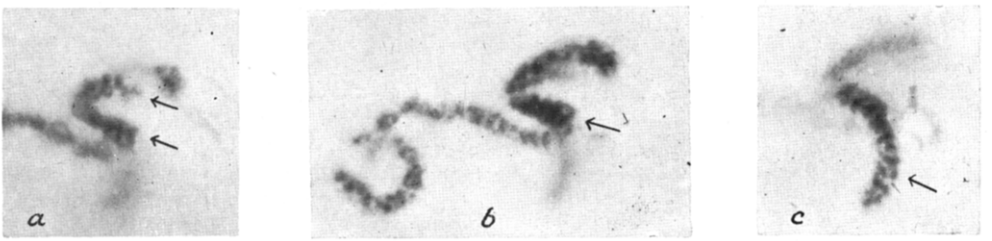

Fig. 11. Detail of chromosome 2 to show aproximate number of gyres in the same chromosome as that of Fig. 4. The long arm (Fig. c) has ten gyres, the short arm (Fig. a and b) has four. High power U.V. photograph × 4000. (Series 102 exposures 2a, 3b, 9a).

Chromosome 2 (Fig. 11) is less perfect than the preceding and direction of coiling can at no point be determined in it. The number of gyres, however, can be determined fairly clearly in the long arm (Fig. c) and approximately in the short arm, the two chromatids of which are contained in Figs a and b. This chromosome is of interest because it is identifiable by the relative length of the short arm as the same individual as that already described on p. 574 and in Fig. 4. In comparison with Fig. 4, however, the gyres are very nearly twice as numerous, there being 10 instead of 5 in the long arm and 4–5 instead of 3 in the short. This represents a total of 14–15 gyres in the whole chromosome instead of the 8 recorded in Fig. 4.

That all the other chromosomes present have numbers of gyres of the same order is perhaps demonstrated by Fig. 12 which shows an enlarged view of a considerable portion of the left hand nucleus of Fig. 8, some additional details of which appear in Figs 13, 14 and 15.

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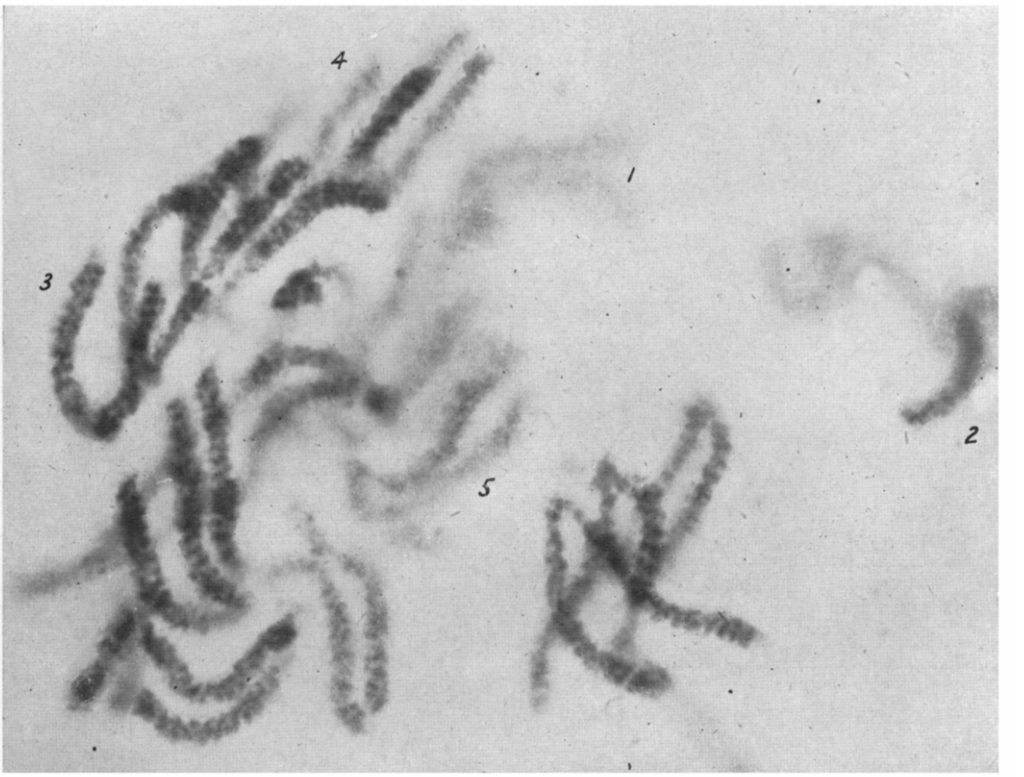

Fig. 12. Enlarged view of part of the left hand nucleus of Fig. 8. High power U.V. photograph × 4000. (Series 102 exposure 11a).

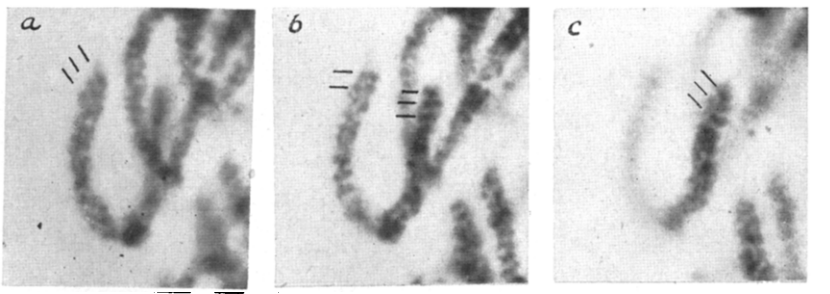

Fig. 13. Three focal levels 0.4 μ apart to show direction of coiling at the distal ends of the two chromatids of chromosome 3. Ink lines mark the slope of the gyres. Fig. a is the top-most focus. Direction is opposite in the two chromatids. × 4000 (Series 102 exposures 14b, 11a, 9b).

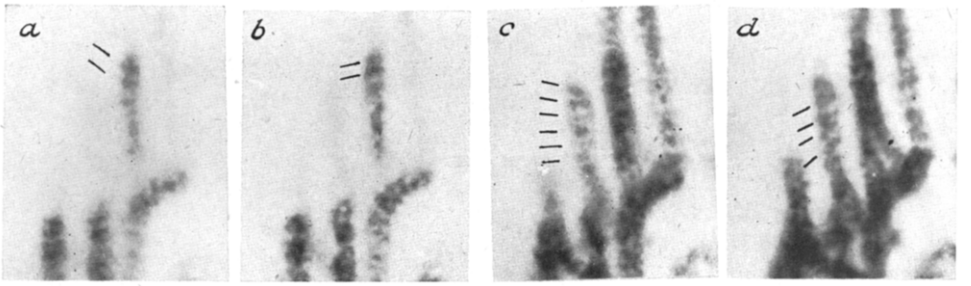

Fig. 14. Four focal levels 0.4 μ apart to show direction of coiling in the distal ends on the two chromatids of chromosome 4. Ink lines mark the slope of the gyres. Fig. a is the uppermost focus, the rest serially. Direction is similar in the two chromatids. × 4000. (Series 102 exposures 7b, 9b, 11b, 13b).

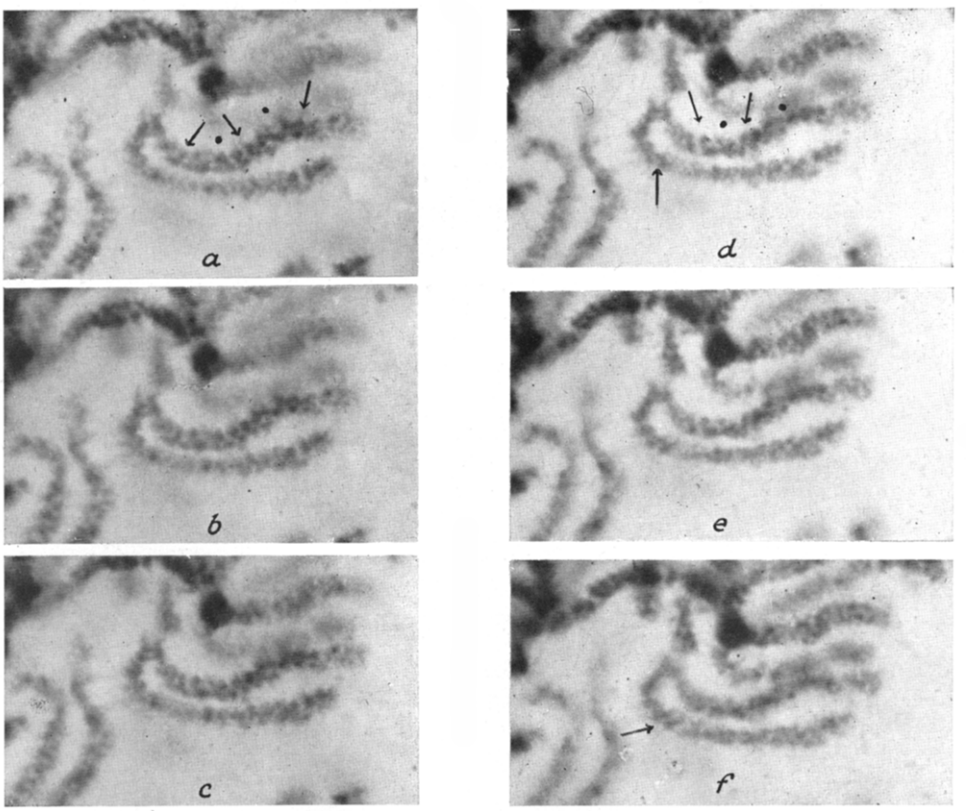

Fig. 15. Details of chromosome 5. U.V. × 4000, six focal levels 0.1 μ apart Fig. a the highest Fig. f the lowest. The upper chromatid contains two changes of direction, the lower chromatid is uninterpretable except for two gyres at the centromere end. (Series 102 exposures 6a–9a).

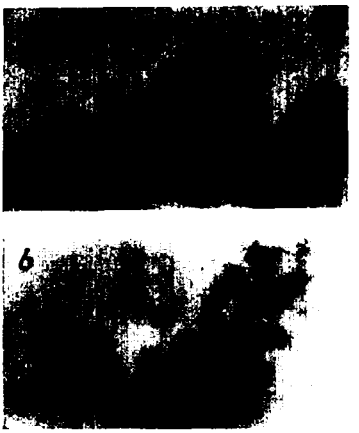

Fig. 16. Late metaphase of the second division in *Osmunda*, two focal levels of parts of one cell photographed with visual light × 2000 to show reduction of number of gyres per chromosome. Detail of one chromosome visible in Fig. 17.

Fig. 17. Detail of one chromosome from field of Fig. 16b high power U.V. photograph × 4000. The blurred outline is due to an opacity of the cytoplasm produced unintentionally by treatment with clove oil. The reduced number of gyres and the increased diameter of the coil in comparison with the earlier state is nevertheless visible. (Series 60 exposure 3a).

Fig. 18. Two focal levels through sister nuclei at late metaphase of the second meiotic division in *Osmunda* to show reduced number of gyres. Visual light photographs × 2000.

Figs 13 and 14 are inserted primarily for further details of direction of coiling. In Fig. 13 three focal levels through chromosome 3 are represented from which direction can be determined in the last two gyres of both chromatids. In the left hand chromatid (Figs 13 a and b) these coils are left-handed but in the right hand chromatid (Figs 13 b and c) they are right-handed; these two chromatids are therefore coiled in *opposite* directions. In Fig. 14, chromosome 4 shows exactly the inverse condition; the distal portions of the two chromatids are *similarly* coiled, namely in a left-handed direction.

That changes of direction can occur along the length of a chromosome can be shown in *Osmunda* no less certainly than in *Tradescantia* although the best single example from the point of view of reproducible clarity belongs to a cell at a slightly

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Fig. 19. Two focal levels 0.4 μ apart through one of the nuclei of the preceding specimen, U.V. photograph × 4000. A change of direction very distinctly shown in the chromosome marked by the arrow, especially in the lower focal level (Fig. b). (Series 67 exposures 4a, 5b).

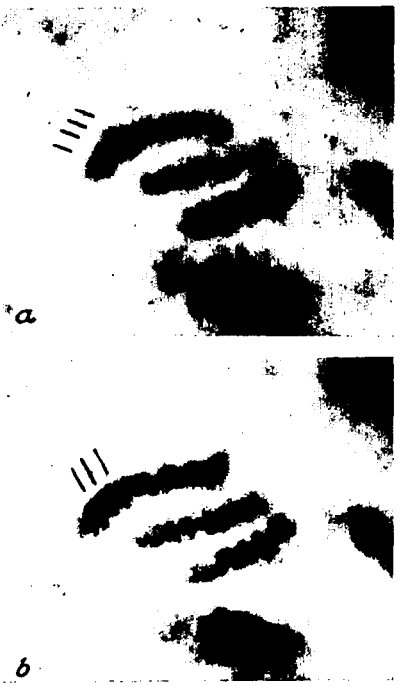

Fig. 20. Anaphase chromosomes from the same preparation as Fig. 17 with gyres of a right-handed spiral visible opposite the ink lines. U.V. photograph × 4000. (Series 72 exposures 11a, 12b).

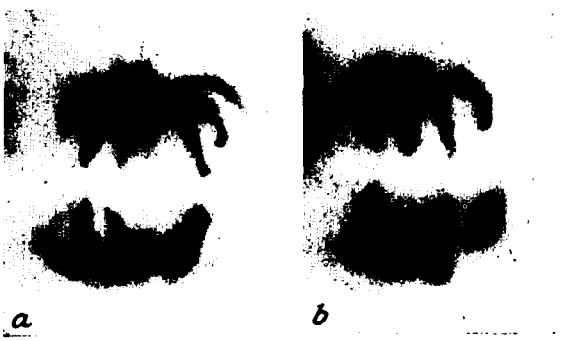

Fig. 21. Anaphase at the same stage as the preceding in another preparation. Two focal levels photographed with visual light × 2000 to show reduced number of gyres as in Fig. 4 and Fig. 20.

later stage than the one under discussion. It may, however, be helpful to look at the marked chromatid of Fig. 19 and to compare it with the marked chromatid of Figs 7a and b. In both cases the dead point of a change of direction appears in one focal level and the intervening lines in the other; both levels being of course required for full demonstration of the change. In the *Tradescantia* chromosome it so happens that the view containing the dead point is the upper focal level (Fig. 7a). In the *Osmunda* chromosome the comparable view is the lower focal level (Fig. 19b), the lines joining the dead point to the gyres on the two sides of it being visible in Fig. 19a.

Comparable evidence of changes of direction in the cell of Fig. 8 is contained in Fig. 15 although the photographs are more difficult to reproduce. The sequence of photographs in this case represents differences of focal level of 0.1 μ and as before the significant directions are indicated by arrows drawn parallel to the slope of the gyres. The lower chromatid is uninterpretable except for two gyres near the centromere which are coiled in a right-handed direction (Fig. 15 d–f). The upper chromatid can, however, be analysed for almost its entire length, at least in the original prints. At the centromere

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end (Figs 15 a and d) it is also right-handed but about a third of the way down the chromosome direction changes and the middle region of about four gyres is left-handed after which direction changes again. The distal end of this chromatid cannot be clearly determined but the total number of gyres in its whole length is again of the order of 14.

THE MINOR SPIRAL AT LATE METAPHASE AND ANAPHASE

Late metaphase is represented here by two cells both of which were obtained rather early in the work before great experience had been gained with the ultraviolet microscope. Both were studied in a preliminary way with visual light, some photographs being reproduced in Figs 16a and b and 18a and b. In both cases the transfer to a quartz slide was made without mishap but subsequent photography was somewhat marred by technical mistakes which were later avoided. In particular the use of clove oil as a medium for preliminary observation with visual light though successful for that purpose as Fig. 16 will illustrate has a deplorable effect on subsequent transparency to ultraviolet and the mistiness of Fig. 17 caused by the opacity of the cytoplasm was wholely due to this treatment.

Owing to these and other defects the ultraviolet pictures (Figs 17 and 19) of late metaphase are less informative than would otherwise have been the case although, in default of better, they have some value for purposes of comparison with the earlier stage. The visual light photographs (Figs 16 and 18) are, however, more extensively reproduced since, for many purposes they are the more informative. In particular the demonstration of the number of gyres per chromosome is best obtained from these photographs. This number, as may be seen in several places in Figs 16 and 18 is not more than 8 per chromatid and in some chromosomes may be slightly less. In the ultraviolet photographs, even that of Fig. 17, this number of gyres is faintly discernible although these photographs are of greater interest for the comparison they give of the diameter of the spiral at late metaphase; in contrast to the earlier stage of Fig. 15, etc., it is markedly wider.

The anaphase condition is represented in visual light by Fig. 21 and in ultraviolet by Fig. 20. The two views of one cell contained in Fig. 21 compare directly with the isolated chromosome of Fig. 4 with the addition that the chromosomes in Fig. 20 are seen in position at the poles. Fig. 21 on the other hand is the only ultraviolet series yet obtained of this stage. It is from another cell in the same preparation as that of Figs 18 and 19 and although far from perfect it is just possible to determine direction of coiling in the distal end of the topmost chromosome and the number of gyres (approximately 8) in the lowermost chromosome. The diameter in all three of these chromosomes contrasts strikingly with the much narrower condition of the spiral in Fig. 15, etc.

DISCUSSION

It would therefore seem that the gyres of spiral structure are not constant through the whole of a division but that they are reduced in number and increased in diameter as the division proceeds. At first sight this is a very peculiar type of development for which a causal mechanism is by no means self evident, yet it is a phenomenon which has been described by other workers, notably by SWANSON (1942, 1943) for the first meiotic division and the pollen tube mitosis in *Tradescantia*, and also by SPARROW (1942) for

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the pollen grain mitosis of *Trillium*. Its detection now in the second meiotic division of *Osmunda* suggests that it is a general and perhaps an essential feature of the spiralization cycle over a very large part of the plant kingdom.

It is not profitable at this stage to enquire fully into the interpretation of these facts since it is hoped to amplify them shortly in several directions. One special aspect of the problem should, however, perhaps be given brief mention to avoid the risk of profitless speculation; this is the question of chromonema length. In earlier communications (especially MANTON, 1939 and 1945) some fairly precise numerical comparisons of chromonema length were made between a limited number of selected stages of mitosis and meiosis in *Osmunda*. From this it was shown that very considerable changes of length occur during the prophases of the first meiotic division, of a kind which could not be detected in the course of a somatic division. These need not be further discussed here since the present communication does not specifically deal with either of the particular divisions referred to; the previous observation that the chromonema at leptotene or early pachytene is four times as long as it appears to be at the fully spiralized stages of late metaphase or anaphase of either meiotic division is therefore unaffected. When however the comparison is extended to include the early stages of the second meiotic division it might be asked whether, for this division, some modification of the previous position might be required.

Without attempting a final answer to this question at the present stage, it may nevertheless be said at once that such evidence as is available is negative. The accurate measurement of chromosome diameter, which is essential for calculation of chromonema length in the fully spiralized condition, is impossible with the particular techniques used here since these are known to introduce artificial changes which cannot be precisely allowed for. Rough comparisons however, which can for example be made between any of the Figs 9–15 and Figs 17, 19, or 20 show that as gyre number is approximately halved the diameter of the spiral is approximately doubled, indicating either *no* change in chromonema length as gyres are eliminated or at most only a slight shrinkage, too small to be registered with the existing methods and certainly less than 50%. It seems therefore necessary to conclude not only that there is so far no conflict between the new data and previous conclusions for this organisms but also that some physical property other than changes of chromonema length must be primarily involved in the causal mechanism of gyre elimination.

SUMMARY

A reduction of the number of gyres per chromosome has been demonstrated to occur during the course of the second meiotic division in *Osmunda*, from approximately 14 at early metaphase to approximately 8 at late metaphase and anaphase.

The diameter of the coil increases as the number of gyres is reduced which suggests that large changes of chromonema length are not involved.

Direction of coiling has been studied in a preliminary way as a necessary part of the demonstration of the reality of the spiral at the various stages. Right-handed and left-handed directions have both been found and corresponding parts of attached chromatids can be coiled either in similar or in opposite directions. Changes of direction can occur more than once along a chromosome arm.

RÉSUMÉ

On a démontré qu'il se produit une diminution du nombre des cercles par chromosome pendant la durée de la seconde division méiotique dans *Osmunda*. Ce nombre passe d'environ 14 au début de la

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métaphase à environ 8 à la fin de la métaphase et à l'anaphase. Le diamètre de l'enroulement augmente lorsque le nombre de cercles diminue, ce qui permet de penser qu'il ne se produit pas de grandes variations dans la longueur des chromonèmes. La direction de l'enroulement a été étudiée d'une manière préliminaire comme une phase nécessaire de la démonstration de la réalité de la spirale aux différents stades. On a trouvé des enroulements à droite et des enroulements à gauche, et des parties correspondantes de chromatides liés peuvent être enroulées, soit dans une direction similaire, soit dans une direction opposée. Des changements de direction peuvent se produire plus d'une fois le long d'une branche de chromosome.

ZUSAMMENFASSUNG

Es wurde bewiesen, dass während der zweiten meiotischen Teilung in *Osmunda* die Anzahl der Windungen per Chromosom abnimmt, und zwar von ungefähr 14 zu Beginn der Anaphase bis etwa 8 am Ende der Metaphase und während der Anaphase.

Der Durchmesser des Knäuels nimmt zu, während die Zahl der Windungen abnimmt, was darauf schliessen lässt, dass die Chromonemalänge nicht stark verändert wird.

Eine vorläufige Untersuchung der Wicklungsrichtung wurde unternommen, da sie einen notwendigen Teil des Beweises zur Existenz von Spiralen in den verschiedenen Stadien bildet. Es wurden sowohl Wicklungen nach rechts wie nach links beobachtet und einander entsprechende Teile von zusammenhängenden Chromatiden können entweder gleichsinnig oder entgegengesetzt gewickelt sein. Entlang einem Chromosomarm kann mehr als einmal Richtungswechsel eintreten.

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