Outline Studies in Biology

Plant Cytogenetics

D.M. Moore

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OUTLINE STUDIES IN BIOLOGY

Editor's Foreword

The student of biological science in his final years as an undergraduate and his first years as a graduate is expected to gain some familiarity with current research at the frontiers of his discipline. New research work is published in a perplexing diversity of publications and is inevitably concerned with the minutiae of the subject. The sheer number of research journals and papers also causes confusion and difficulties of assimilation. Review articles usually presuppose a background knowledge of the field and are inevitably rather restricted in scope. There is thus a need for short but authoritative introductions to those areas of modern biological research which are either not dealt with in standard introductory textbooks or are not dealt with in sufficient detail to enable the student to go on from them to read scholarly reviews with profit. This series of books is designed to satisfy this need. The authors have been asked to produce a brief outline of their subject assuming that their readers will have read and remembered much of a standard introductory textbook of biology. This outline then sets out to provide by building on this basis, the conceptual framework within which modern research work is progressing and aims to give the reader an indication of the problems, both conceptual and practical, which must be overcome if progress is to be maintained. We hope that students will go on to read the more detailed reviews and articles to which reference is made with a greater insight and understanding of how they fit into the overall scheme of modern research effort and may thus be helped to choose where to make their own contribution to this effort. These books are guidebooks, not textbooks. Modern research pays scant regard for the academic divisions into which biological teaching and introductory textbooks must, to a certain extent, be divided. We have thus concentrated in this series on providing guides to those areas which fall between, or which involve, several different academic disciplines. It is here that the gap between the textbook and the research paper is widest and where the need for guidance is greatest. In so doing we hope to have extended or supplemented but not supplanted main texts, and to have given students assistance in seeing how modern biological research is progressing, while at the same time providing a foundation for self help in the achievement of successful examination results.

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Plant Cytogenetics

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Preface

One of the great unifying themes of biology during the past seventy years or so has been provided by genetics and cytology, which have together furnished a firm basis for understanding the materials and processes upon which the variation and evolution of all living organisms depends. Many recent texts have, quite rightly, stressed those tenets of cytogenetics common to most eukaryotes and it might be wondered, therefore, why animals and plants have been considered in separate volumes of this series. There are two principal reasons for this. Firstly, there is now so much cytogenetical information on plants and animals that any attempt to even outline the subject in a single slim volume would result in an unacceptably superficial treatment. Secondly, whilst acknowledging the common cytogenetical foundations of all organisms, stemming in part from the virtually ubiquitous genetic material, DNA, it is sometimes forgotten that plants and animals are different, with different evolutionary opportunities and, in many instances, they have utilized their common genetical and chromosomal endowment to adopt different evolutionary strategies, which are reflected in their patterns of variation. Consequently, some cytogenetical processes are better observed in animals, others in plants. In this book I have attempted to outline some of the features of the structure and behaviour of chromosomes, which still encompass the central enigmas of cytogenetics, and have then continued by looking at various chromosmal mechanisms found in plants and their role in generating and canalizing the variation which, as a student of taxonomy and evolution, is the real reason for my interest in cytogenetics. Of necessity, such an outline as this is selective, even when concerned almost entirely with flowering plants as in this case, but I hope that the references supplied will encourage the reader to consult the original sources which span the history of cytogenetics and which, above all, permit an appreciation of the large amount of work that has already been carried out and of the great task still ahead.

I should like to thank Professor V.H. Heywood for his extremely helpful comments on the completed text. I am also very grateful to Mrs Abigail Gillett and Mrs Rosa Husain for skilfully transcribing my handwriting into an orderly typescript. Finally, thanks are due to my wife and children for tolerating me while I wrote it.

D.M.M.

1 The beginnings of cytogenetics

1.1 Rise of the chromosomes

Cytology, the scientific study of cells, had its beginnings in the 17th century, when the first microscopes were used by Hooke (1635–1703), Grew (1641–1712) and Malpighi (1628–1694) to make the initial observations which eventually led to the theory of Schleiden and Schwann (1838–1839) that the cell was the basic unit of structure and function in all living organisms. The general introduction of compound microscopes about this time permitted rapid progress in cytology so that by 1858 Remak and Virchow could suggest that all cells arose from the division of pre-existing cells. Increasing appreciation of the importance of the nucleus led, with the observations of Hertwig [1[(1875) on sea urchin eggs, to the recognition of its role in fertilization and cell-division.

During the next few years the role of the chromosomes in the nuclear cycle was realized and to some extent described. In both plants and animals Flemming, van Beneden and Strasburger observed and described mitosis (Fig. 1.1), as well as the salient features of meiosis [6,7], which was more fully described by von Winiwarter [2] (Fig. 1.2), while Balbiani [3] and Carnoy [4] discovered and observed the salivary gland chromosomes of Diptera. Van Beneden showed that during mitosis the daughter halves of the chromosomes pass to opposite poles and that the fertilized egg of *Ascaris* receives an equal number of chromosomes from each parent, a number halved during the meiotic divisions which precede the formation of the gametes so that it remains constant from one generation to the next. Although cytology still remained a branch of either histology or embryology during this period there was, largely because of the influence of Roux [5] and Weismann [6], a gradual acceptance of the idea that the chromosomes were the material basis of heredity. This, then, was the state of knowledge when E.B. Wilson [7] wrote the second edition of his great work, *The Cell in Development and Inheritance,* published in the year that Mendel's genetic discoveries were disinterred and made available to the scientific community.

1.2 Appearance of genetics

Although it is usual to trace the history of genetics back to Aristotle, and even Hippocrates, who recognized that individuals may resemble remote ancestors rather than their parents and that the effects of mutilations are not transmitted to offspring, the foundations of the subject which persisted into modern times were laid during the 18th century. Thus, Kölreuter, who published the results of his extensive crosses on plants between 1761 and 1766, recognized that hybrids were usually intermediate between the parents and that they were often sterile in crosses between widely different forms; he also emphasized the identity of hybrids from reciprocal crosses, while even earlier Robert Fairchild (1719) observed the dominance of double over single flowers in the progeny of crosses in *Dianthus* [8]. The continued accumulation of data on animals and plants derived from gardeners, farmers and sportsmen, during the next hundred years was brought together by Darwin in *The Variation in Animals and Plants under Domestication* (1868), an interesting source of information in which he, like Gaertner (1772–1850) emphasized the greater variability of the second and later generations compared to the first generation resulting from hybridization. Most

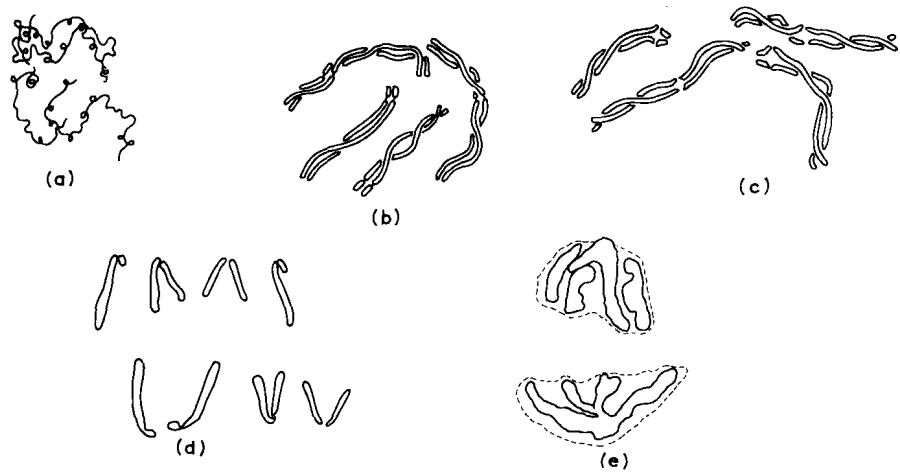

Fig. 1.1 Diagram of mitosis in a plant with 2 pairs of chromosomes; one pair with subterminal and the other with median centromeres. Mitosis is a continuous process but it is usual to recognize four stages – prophase, metaphase, anaphase and telophase. At the onset of mitosis the long, threadlike chromosomes, each composed of two chromatids, become visible in the nucleus. Throughout prophase each chromatid develops 'internal coils' and so becomes shorter, thicker and more readily visible by increased spiralization, except at the centromere and, sometimes, other chromosome-segments (secondary constrictions). During early prophase (a) the chromosomes usually show lax 'relic coils' persisting from the spiralization during the previous division and as they become clearer towards mid-prophase (b) the chromatids are seen to be twisted around each other in 'relational coils'. At the end of prophase, when the chromosomes attain their maximum contraction, the nuclear membrane breaks down and the proteinaceous spindle develops between the two poles of the cell. The centromeres become attached to the spindle and move on to the equator of the cell midway between the poles by metaphase (c). During anaphase (d) the sister centromeres of each chromosome separate and move on the spindle towards the poles with the chromatid (now daughter chromosome) arms trailing behind them. As the daughter chromosomes near the poles they become more aggregated, a nuclear membrane is reorganized around each polar group and the internal coils relax so that the telophase chromosomes (e) become longer, thinner and less visible. As the nuclei enter interphase each daughter cell has an equal complement of chromosomes and genes.

of the observations available did not, however, refer to separate characters, but described the overall features of each individual.

This was the background against which Mendel began in 1856 the series of experiments which he reported to the Brno Natural History Society in 1865 and published in their proceedings the following year. Mendel was able to formulate his well-known concepts because he did not consider most of the characteristics of the organisms he was studying but concentrated on a few, well-defined characters. He counted, and kept on counting, the number of individuals with different characters derived from each cross he made and, of great importance, he believed that single pollen grains fertilized single egg cells, and took the trouble to test this (with *Mirabilis*), a fact not known until shortly before his time and not generally recognized by contemporaries such as Darwin even then. The resultant Mendelian laws of inheritance depended upon the occurrence of material factors, later termed genes by Johannsen [9], whose nature was not understood but which occurred singly in gametes and doubly in zygotes and which could have alternative states or allelomorphs [10] (alleles).

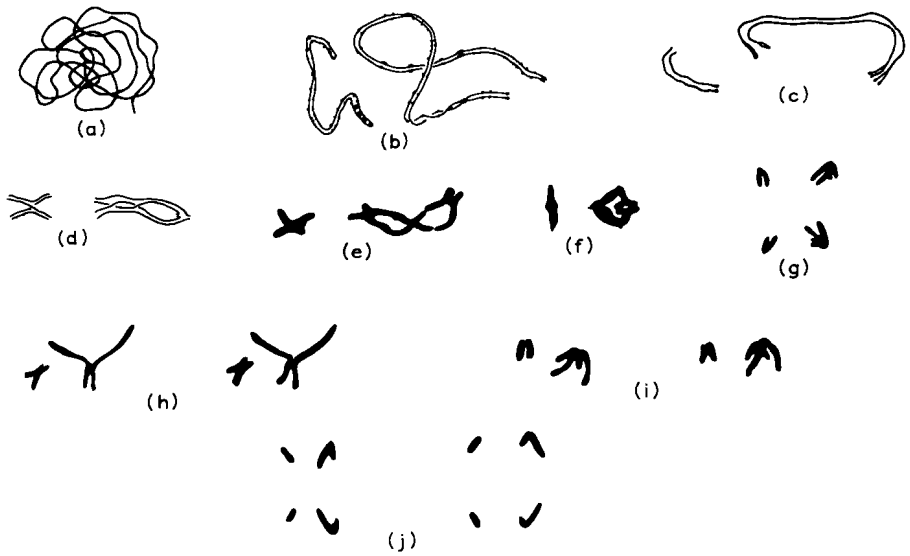

Fig. 1.2 Diagram of meiosis in a plant with 2 pairs of chromosomes. There are 2 consecutive divisions, each of 4 stages; first prophase is divided into 5 stages. At the onset of meiosis, *leptotene* (a), the chromosomes appear single (they are seen to be double in some electron micrographs); the tightly coiled chromomeres give them a beaded appearance. During *zygotene* (b) homologous chromosomes pair exactly to form bivalents. The chromomeres disappear in *pachytene* (c), homologous chromosomes twist relationally around each other and coil internally to become shorter and thicker. The 2 chromatids of each chromosome may be seen where coiling is lax but they are clear during *diplotene* (d) as the homologues move apart to reveal the chiasmata. These apparently form by breakage and reunion of non-sister chromatids and may move distally ('terminalization'). At *diakinesis* (e) the chromosomes are almost fully contracted, sister chromatids become less distinct and the centromeres may be visible. Later the nucleolus disappears, the nuclear membrane breaks down, the spindle forms and *prophase-I* is completed. By *metaphase-I* (f) the bivalents lie with the centromeres equidistant from the spindle equator. At *anaphase-I* (g) the centromeres move polewards trailing the chromosome arms, and sister-chromatids are widely separated as their attraction lapses. After *telophase-I* there may or may not be an *interphase* before the second meiotic division begins. By prophase-II (h) the chromosomes may have lost their major coils and be longer and thinner or they may still be contracted; the sister-chromatids are widely separated and attached only at the double centromere. At *metaphase-II* (i) the paired centromeres move to the spindle equators and at *anaphase-II* (j) the centromeres disjoin and the chromosomes move to the poles to form four groups at *telophase-II*, each with half the parental number.

As is well-known, the work of Mendel was overlooked for 35 years. During this time Darwin's theory of evolution was further developed and a preoccupation with continuous variation turned to a greater interest in discontinuous variation by such workers as de Vries, Bateson, Galton and Haacke, while there was wider acceptance of Weismann's view that an individual's hereditary endowment, carried in the chromosomes, is halved in each gamete. Thus, although there was no general agreement on the hereditary mechanism and the properties of the chromosomes needed much clarification, the stage was set for due recognition of Mendel's

work when it was rediscovered and the results confirmed by Correns, de Vries and von Tchermak [11] in 1900.

1.3 Chromosomal theory of inheritance

Although the relationship between chromosomes and genes was suspected at the time Mendel's work was rediscovered, it took a further three years to establish the interpretation which has persisted to the present. During that period Montgomery (1901) and Sutton (1902), working on grasshoppers, showed that chromosomes occur in distinct pairs, often of recognizable shape and size, and that synapsis involves the union of maternal and paternal chromosomes, while Winiwarter (1901) concluded, from his studies of meiosis in rabbit ovaries, that bivalents in the first meiotic division resulted from the chromosomes pairing side-by-side and not end to end as believed by Weismann and others [2]. Boveri (1902) showed, from his studies of polyspermy in the fertilization of sea-urchin eggs, that the chromosomes of an individual were not equivalent to one another and that a full complement is necessary for normal development of the cell. Correns and Cannon, both in 1902, pointed out the close parallelism between Mendelian segregation and chromosome reduction, concluding that the genes are on the chromosomes; but they, like de Vries a year later, were incorrect in many suppositions, such as their view that maternal and paternal chromosomes went to opposite poles during meiosis [11]. In the same years two papers by Guyer showed an understanding that random assortment between different pairs of chromosomes would give the independent assortment of genes required by Mendel, although the cytological demonstration was not made until 1913 (Carothers [12], Fig. 1.3). It was, however, Sutton who, in 1903 brought together the data from cytology and genetics to clearly show the role of the chromosomes in heredity and hence to firmly establish the field of cytogenetics. Boveri, in a paper published the same year, advanced many of the same ideas so that the hypothesis

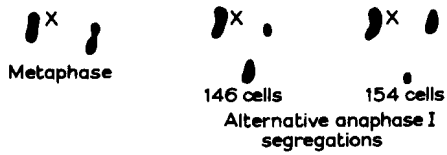

Fig. 1.3 Independent segregation of unpaired X chromosome and heteromorphic pair of large and small chromosomes observed by Carothers [12] at meiotic anaphase in *Brachystola.*

correlating gene and chromosome transmission is known as the 'Sutton-Boveri Hypothesis'.

Basically, the hypothesis is as follows:—

1. In somatic cells there are two similar groups of chromosomes, one of maternal and one of paternal origin. This occurrence of chromosomes in homologous pairs parallels the occurrence of genes in pairs.

2. The chromosomes retain a morphological individuality throughout the various cell-divisions; genes show a similar continuity.

3. During meiosis homologous pairs of chromosomes are brought together and then the members of each pair segregate into different germ cells independently of the members of other pairs; Mendelian genes segregate independently at some time prior to gamete formation.

4. Each chromosome, or chromosome-pair, has a definite role in the life and development of the individual.

In addition to establishing the relationship between genes and chromosomes, Sutton recognized that there must be non-independent assortment of some genes (*linkage*) otherwise, as he noted, 'the numbers of distinct characters ... could not exceed the number of chromosomes'.

The association of a particular inherited character with a particular chromosome was made between 1901 and 1906 by McClung, Stevens, Wilson and others who showed that in Hemiptera and Orthoptera, females have one more chromosome than the males [3]. This so-called X-chromosome occurs in all eggs but in only 50% of sperm so that half of the

resultant zygotes are XX and female, while half are X0 and male. The presence of a small Y-chromosome, partially homologous with the X-chromosome, in males of beetles, insects, mammals and other groups confirmed the same pattern – that the sex chromosomes of the male gametes determine the sex of the progeny – while the discovery of the reverse situation, female heterozygosity, in birds and lepidoptera confirmed the importance of chromosomes in sex determination.

The association of a particular gene with a particular chromosome was demonstrated by Morgan [14], who showed that the inheritance of the recessive allele (*w*) for white-eye in *Drosophila* paralleled that of the X-chromosome (Fig. 1.4). Conclusive evidence that the white locus was situated on the X-chromosome was provided by Bridges [15], who found that sometimes a cross between a white-eyed female and a red-eyed male gave an occasional white-eyed female or red-eyed male among the F_1 progeny. This was found to be due to non-separation of the X-chromosomes at meiosis in the female so that, exceptionally, eggs with either two or no X-chromosomes were produced. The consequences of this are shown in Fig. 1.5, the XXY constitution of the white-eyed females being confirmed cytologically. With this and other genes present on the X-chromosomes, Bridges found the correlation between genetic-

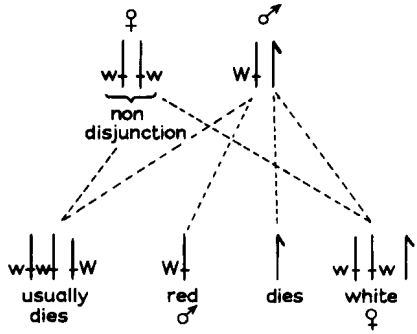

Fig. 1.5 Results of cross between white-eye female (*ww*), with non-disjunction of X-chromosomes, and red-eye male *Drosophila.*

al and chromosomal inheritance to be exact, thus providing the first critical evidence that genes are on chromosomes.

1.4 Linkage, crossing-over and chromosome maps

As noted above, Sutton pointed out that if there were more gene loci than chromosomes, a fact since abundantly demonstrated in all plants and animals studied at all intensively, then his theory would not permit the Mendelian law of independent segregation to apply to genes located on the same chromosome. The data of Bateson and Punnet [16] on Sweet Peas provided genetical evidence of this linkage,

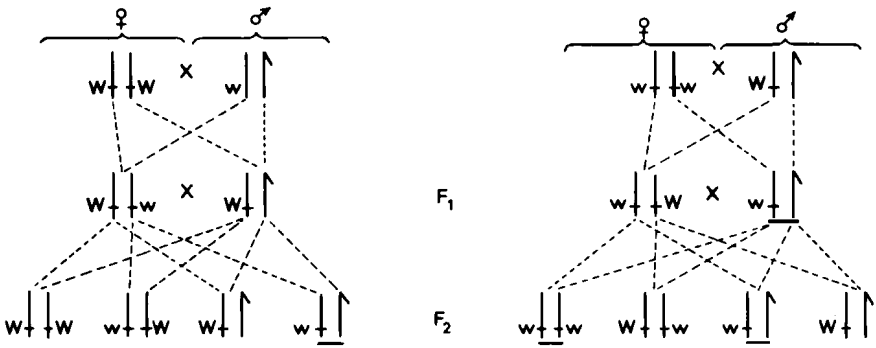

Fig. 1.4 Inheritance of alleles for white (*w*) and red (*W*) eyes in *Drosophila.* Reciprocal crosses show parallel transmission of *W* allele and X-chromosome (+). Y-chromosome indicated by +. White eye phenotypes underlined.

while Sturtevant [17] demonstrated the linear arrangement of genes on the chromosome and initiated the use of the 3-point testcross for mapping the loci, both soon brought to cytological reality by Painter's [18] manipulation of salivary-gland chromosomes and Muller's [19] discovery that X-rays can simultaneously mutate genes and alter chromosomal structure. Finally, Creighton and McClintock [20] demonstrated the correlation between genetical recombination and cytological crossing-over (Fig. 1.6) in maize and so brought into prominence a cytogenetical mechanism which is still not fully understood more than 40 years later.

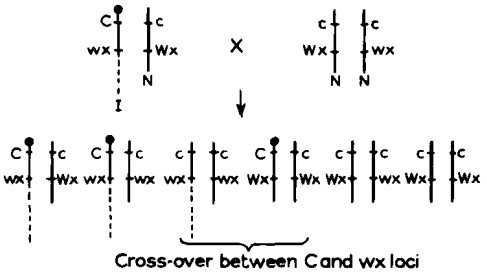

Fig. 1.6 Cross in maize demonstrating correlation between recombination and crossing over. One plant is heterozygous for a chromosome (I) with a terminal knob and a long extra segment (- - - -) and a normal chromosome (N), and for alleles determining waxy (*wx*) or starchy (*Wx*) and coloured (*C*) or colourless (*c*) endosperm.

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2 Chromosome structure

Although deoxyribosenucleic acid (DNA) had been shown to be largely localized in the chromosomes (it also occurs in mitochondria and other cell organelles) by the specific staining techniques of Feulgen and Rossenbeck [1] it was a further 20 years before Avery, Macleod and McCarty demonstrated it to be the primary hereditary material. When, in 1953, Watson and Crick proposed their double helix, which would permit accurate pairing and duplication of DNA, as well as suggesting how mutation might occur, the central problems in cytogenetics seemed to be solved [2]; indeed, studies of the 'chromosome' (*genophore*) [3] in bacteria and other prokaryotes give credence to this. However, the chromosomes of most animals and plants (*eukaryotes*) each contain much more DNA, arranged in a linear and not a circular fashion. Furthermore, unlike genophores, the DNA in chromosomes is regularly and intimately associated with histone molecules, although the manner of their arrangement is still largely unresolved and constitutes one of the major problems of cytogenetics. Information on chromosome structure is derived from four sources:– (a) light microscopy, using bright field or phase contrast illumination; (b) electron microscopy; (c) cytochemistry; (d) genetic behaviour.

2.1 Chromonemata and chromatids

2.1.1 Gross structure

The most generally accepted basic units of chromosomal organization appear in the interphase nucleus as a series of fine threads visible only by interference microscopy. Once the cell commences division these chromonemata shorten and increase in volumes as they coil so that they become visible by phase-contrast microscopy or, following fixation and staining with fuchsin, or carmine, or orcein etc., with the ordinary light microscope. They are shown to be associated in pairs, attached to a single centromere, to form the chromosome; during this visible phase they are known as chromatids. Exceptionally, the chromosome may be polytene and consist of many chromatids laterally opposed, as in the salivary glands of *Drosophila* and other insects, and also some plant cells (Section 3.5).

During meiosis the chromonemata exhibit a chromomeric pattern, which is not seen in mitotic division. The chromomeres appear at first prophase as a series of darker staining 'beads' on the chromonemata. They may be of uniform size, show a regular gradation with larger knobs near the centromere and smaller spots towards the chromosome ends, as in tomato, or form a less distinct size-gradient, as in rye and *Salvia viridis* [4,5]. The basic pattern of chromomeres is characteristic for each chromosome. La Cour and Wells [6] have shown from light and electron microscope studies of leptotene chromosomes in *Tulbaghia, Fritillaria* and *Lilium,* that the chromomeres are borne eccentrically to the chromosome axis. As meiotic prophase proceeds the chromomeres increase in size and decrease in number as they tend to merge into one another, the number diminishing in proportion to the chromosome length. This is explained by the generally accepted view that the chromomeres are coiled portions of the chromonemata; in *Tradescantia,* for example, the chromomeres increase in size until they become the visibly distinct coils of late prophase.

2.1.2 Ultrastructure

Although, as noted above, the problem of how the DNA molecule, with its fundamental genetical properties, is integrated into the architecture of the chromosome is still incompletely resolved, histochemical and electron microscope studies are gradually clarifying the picture. The study of electron micrographs of mitotic and meiotic chromosomes from various plants and animals led to the conclusion that the chromosome is built like a cable with numerous identical strands [7,8]. Subsequent work, which has employed both electron microscopic study of sections and of nuclei (spread on an air-water interface, picked up on carbon-coated grids, fixed and dried by, for example, amylacetate), has confirmed that the chromosome is composed of fibrils [9]. The reported diameters of these have varied from 3–50 nm, the most frequent range being 10–20 nm, but their length cannot be determined. However, Ris and others have shown that the diameter of the fibrils varies considerably, depending upon the use of different buffers during fixation [10], and there is now considerable evidence from electron microscopy and X-ray diffraction data that fibre diameters are about 10 nm [11,12].

Although a detailed consideration of the relationship between DNA and histones in the chromosomes of plants and other eukaryotes is beyond the scope of this book, and is indeed as yet unresolved, it is worth pointing out that X-ray and chemical data [12,13] suggest that chromatin fibrils are composed of a series of repeating units consisting of tightly packed DNA and associated protein, alternating with more extended DNA and associated protein. Electron micrographs of chromatin fibrils following formaldehyde fixation show a bead-like appearance. The thickened 'beads' are about 7 nm in diameter, which is compatible with a globular histone tetramer (diameter 4–5 nm) associated with a double helix of DNA (diameter *c.* 2 nm, [12,14]). Cleavage of chromatin by certain nucleases produces pieces of DNA comprising about 200 base pairs, or multiples thereof, and it is suggested that this is consistent with biochemical and X-ray data on the size of the repeating units. There is, then, some evidence that the chromatin fibril is a flexibly jointed chain of repeating units; this flexibility would permit the extensive coiling and folding of which the chromatin fibril is known to be capable.

The relationship between the apparent multiple fibrillar structure of the chromonemata and the classical evidence of the chromatid as the basic unit of cytogenetics has long proved difficult to reconcile. Although some workers [15] have considered the chromatids to consist of a single, strongly folded and coiled chromatin fibril there is considerable evidence to support the view that the chromatid contains at least 2 DNA duplexes [16]. Half-chromatids have been reported from light microscopic studies in *Endymion, Haemanthus* and *Vicia* while following irradiation there is cytogenetical evidence of subchromatid breaks and recombination (Section 3.2, 3.3). Reconciliation between these two apparently conflicting situations may depend upon the observation that, in studies of the synaptinemal complex during pachytene (Section 3.3), only a part of the chromatin is involved in recombination. Perhaps, therefore, the multiple structure is a form of genetic insurance policy, but much cytogenetical and cytochemical information is still needed to fully understand the ultrastructure of the chromosome.

2.2 Centromeres, telomeres and chromosome form

2.2.1 Centromeres

The centromere (kinetochore) is the most conspicuous feature of most chromosomes, appearing from mitotic prometaphase to anaphase as a region which, because it does not coil, is a weakly stained 'primary constriction' distinguished from the thicker, darker staining chromosome arms. The distinctness of the centromere varies a great deal between different organisms but it can generally be enhanced by

pre-treatment with mitotic inhibitors, such as paradichlorobenzene and colchicine, before staining. Although the position of the centromere is constant for a given chromosome it can vary between them thus providing a valuable marker for describing the chromosome complement. Although more elaborate classifications have been proposed [17], it is customary to distinguish three principal chromosome types based on the position of the centromere:–
metacentric – the median centromere separates two arms of approximately equal length;
acrocentric – the interstitial centromere separates two arms of obviously unequal length;
telocentric – the centromere is terminal to give a one-armed chromosome.

There has been much discussion as to whether the centromere is ever truly terminal but, even if this is so, there are certainly many cases in which one arm is not visible and the chromosomes are apparently telocentric (Fig. 2.1b; Section 5.3).

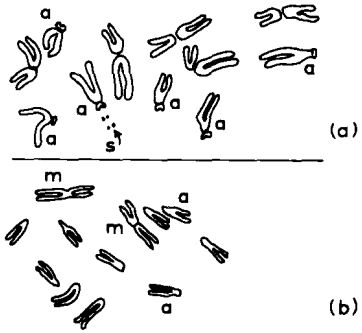

Fig. 2.1 Chromosome types in karyotypes of two plant species. (a) *Callisia fragrans* ($2n = 12$) –6 metacentrics + 6 acrocentrics (a), one with satellites (s). (Drawn from Jones and Jopling, [11].) (b) *Oxalis dispar* ($2n = 12$) –2 metacentrics (m) + 2 acrocentrics (a) + 8 telocentrics. (Drawn from Marks, [53].)

Whilst examining cleavage divisions in the salamander, Metzner described differential staining of small bodies within the centromere [18]. These 'Leitkörperchen' have subsequently been reported in many animals and plants and variously referred to as polar granules, attachment chromomeres and kinetochores [19,20,21]. There has been a tendency to use the terms centromere and kinetochore synonymously for the primary constriction but nowadays the term kinetochore is reserved for the structures within the centromere by which the chromosomes are moved during cell division (Section 3.3). Lima-de-Faria [22] showed that the centromere contained two to five pairs of darker staining chromomeres joined by uncoiled chromonematal fibrils (Fig. 2.2). This symmetrical organization gives two mirror images about a plane passing

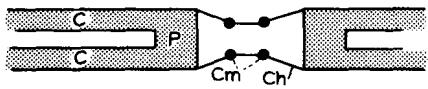

Fig. 2.2 Lima-de-Faria's [22] model of the centromere. Pairs of centromeric chromomeres (Cm) are connected to each other and to the proximal regions (P) joining the sister chromatids (C) by the chromatonemal fibrils (Ch).

through the centre of the centromere and this structure has been reported in many plants, including *Allium cepa* and *Tradescantia* spp. [23]. However, electron microscopy of sectioned material shows that practically all metaphase chromosomes can be interpreted as having one more or less circular kinetochore per chromatid [24,25,26]. In species of *Tradescantia, Ornithogalum, Rhoeo* and *Allium* the kinetochores, when stained by Giemsa techniques (Section 2.3), appear during mid-prophase to prometaphase. They are about 0.5 μm in diameter and sometimes appear to be attached to remnants of spindle fibres. By metaphase the two kinetochores, one per chromatid, are clearly separated and lie laterally on opposite sides of the centromeres [27]. The kinetochore is believed to be rich in repetitive DNA, which would permit its division, suggested by observations on chromosome structural changes (Chapter 5), but it would seem that no more than two of

Lima-de-Faria's 'centromeric chromomeres' can be kinetochores.

In some organisms the centromere is unlocalized so that many points along the chromosome can function as kinetochores and it has been shown that the diffuse or polycentric chromosomes of, for example, *Luzula* and *Cyperus* do not possess kinetochores of the kind described above [28].

2.2.2 Telomeres

If chromosomes are fractured by X-rays, for example, the resulting segments may fuse again; however, they will not fuse with the ends of chromosome arms, which themselves cannot fuse with each other. This has led to the consideration that the chromosome is terminated by a telomere which confers polarity upon it. The telomere has been shown [29] to be a compound structure consisting of several differentiated segments; in rye, for example, it is composed of at least 2 pairs of chromomeres and intercalary fibrils. Breakage within this region should still give stable chromosome ends, as has been demonstrated in rye and maize. The compound structure of the telomere is very like that of the centromere and they are further shown to share a number of properties relating to their cycle of division and behaviour during meiosis (Sections 3.3, 3.4).

2.2.3 Secondary constrictions and satellites

In many chromosome complements at least one pair of chromosomes is seen to have an unspiralized, non-staining region additional to the centromere. This secondary constriction frequently occurs near the end of the chromosome so that the segment beyond the constriction is small and is then termed a satellite or trabant, joined to the rest of the chromosome by the satellite stalk. The only known function of the secondary constriction is that of nucleolar organization and it is believed that the nucleoli, involved in protein synthesis, are controlled by specific loci associated with the secondary constriction; in maize this nucleolar organizing element is at the base of the satellite stalk and it has been shown that, following fragmentation by X-irradiation, both sub-units remain functional [30], thus indicating a compound structure as in centromeres and telomeres.

The number of satellited chromosomes in the complement varies in different organisms and is not always parallelled by the number of nucleoli visible at prophase, possibly because of their coalescence. Similarly, chromosomes associated with nucleolar organization may not possess satellites, as in *Nothoscordum inutile,* for example, in which the four chromosomes concerned with this activity seem to have compound constrictions with both centromeric and nucleolar organizing functions, while in several *Trillium* species, the nucleolar organizer appears to be terminal [31].

A further point to remember when using satellites to characterize features of a chromosome complement is that the secondary constriction can vary greatly during the course of mitosis. Thus, during prometaphase the satellites are joined to the chromosome arm by a long slender satellite stalk, which is readily fractured during squash preparations, while at metaphase, particularly following pretreatment with drugs such as oxyquinoline, the stalk can be so short as to make it difficult to determine the presence of the satellite [32]. Further, homologous chromosomes can differ in the size of their satellites. The nucleolar organizer of one species may be dominant to that of another species so that in hybrids the chromosomes of the 'weaker' set may not show the satellite present in the parent.

2.3 Euchromatin and heterochromatin

The standard sequence of condensation (at its maximum during metaphase – anaphase) and elongation (greatest during interphase), described (Figs. 1.1, 1.2) for the chromonemata during the nuclear cycle is known as the eucycle and the chromatin which follows this sequence is termed euchromatin. However, some chromosome segments, and even whole chromosomes, have a different cycle so that they are more condensed and deeper stained.