any theory which depends on breakage followed by the joining of ends (see Belling 1933). Other theories and explanations have been offered, (Weinstein 1954, 1958, Levine 1956, Fagerlind 1960, Papazian 1960).

The autoradiographic studies of chromosome replication in somatic cells of Bellevalia (cf. Taylor 1957, 1958), genetic studies of the fine structure of the gene, and cytogenetic analyses of meiotic chromatid tetrads are adding additional information. The stage seems to be set for some major advances toward an explanation of crossing over.

Chromosome morphology

The classical studies of the morphology of individual chromosomes of plant species begun by S. Nawaschin 1910 to 1916, (reviewed by Lewitsky 1931); and continued by Taylor (1925), Sharp (1929), McClintock (1929, 1930), Lewitsky and many others have been useful in studies of systematic relationships and in attempts to learn the basic facts governing chromosome behavior and in turn genetical behavior. Eventually it should be possible to set up working models which will account for the internal structure and chemistry of the chromosomes and the genetical material as well as the genetical facts. Improvements in cytological smear techniques, among others the accidental discovery of the use of iron with aceto-carmine (Belling 1921), and that the application of heat greatly improves the staining differentiation of the chromosomes (McClintock 1929, 1930), were responsible for many advances in cytogenetics in the 1930's, and in subsequent years. Smears have practically replaced the paraffin technique for studying chromosomes in somatic tissues (root and shoot tips) and at meiosis in microsporogenesis in plants.

The pioneering studies of the morphology of the chromosomes of maize made by McClintock (1929, 1930), using acetocarmine smears, first for the haploid set at the first post meiotic division of the microspores, later for studies of pairing in the pachytene stages of meiosis led to the establishment of maize as a favorable species for cytogenetic studies. The discovery that in the salivary gland cells of Drosophila melanogaster larvae the chromosomes are multi-stranded, that homologues are paired and the chromomere patterns are distinctive and relatively constant (Painter 1931, Bridges 1935) greatly enhanced the value of Drosophila and certain other species of Diptera for cytogenetic experimentation.

A brief general survey of the differences in gross chromosome morphology at the prophase stages of meiosis or at metaphase in somatic tissues, particularly in plants, will precede a discussion of genetical information as related to these cytological differences.

At the prophase stages of meiosis in different species or within a particular species, the chromosomes may differ in: thickness, total length, position of the centromere or spindle fiber attachment region, chromomere pattern, and in the number and positions of secondary constrictions, satellites, and densely stained regions. Differences within a species may be used to identify the different chromosomes, or to distinguish the two chromosome arms. These are the tools for studies relating genetical information to morphological features. Only in a few species is such information available.

At pachytene of meiosis the chromosomes of certain species, e.g. Hordeum (barley) and Tradescantia, appear to be much thicker than those in others, e.g. maize. Within an individual plant, the chromosomes are usually of about the same thickness.

Total length

Total length of the individual chromosomes at somatic metaphase shows a wide range between species. The chromosomes in fungi in general are very short, e.g. in the phycomycete, Saprolegnia (Mäckel 1928), whereas those in Liliaceae, e.g. Tradescantia, Trillium, Tulipa and Lilium are very long (30u for the longest one in Trillium, Warmke 1937). Alfalfa, clovers, cotton, and rice are a few of those with very short chromosomes. The chromosomes in maize are longer, but still relatively short.

The range within a species is usually not very great but may be useful in distinguishing between the different chromosomes of a given species. For example, the longest chromosome in maize is about 2.2 times the length of the shortest one. Certain species, however, have extremely short ones in addition to the long ones. For example, in D. melanogaster chromosome 4 is about 0.2 micron in length whereas 2 and 3 are about 2.8 microns. A number of species have short chromosomes that are supernumeraries (see page 14). There are many short chromosomes in addition to the long ones in the common fowl, turkey and dove (Sokolow, et al. 1936).

Total lengths of the chromosomes at metaphase may differ in different tissues. Also in certain tissues they may present a very different appearance. For example, in the salivary gland cells of certain Diptera (Drosophila, Sciara, Bibio, Chironomus (midge), and Culex (mosquito), the chromosomes are paired and each consists of a bundle of many chromonemata (32, 64 or more Painter 1941, Metz 1941, D'Angelo 1950). The chromosomes appear to divide, but the chromatids do not separate. This is somewhat comparable to endomitosis which occurs in certain tissues of plants and animals, in which the chromosomes divide but the cell does not (cf. Lorz 1947 for a general review). The salivary chromosomes in the relaxed state in Drosophila are over 100 times the length at gonial meiotic metaphase, and with maximum stretching for cytological analysis they are about 300 times as long.

Position of the centromere

(Also known by the terms: spindle fiber attachment region (S.F.A.), kinetochore, insertion region, or primary constriction). The centromere may appear to be terminal in the chromosomes of certain species or in certain chromosomes (Cleveland 1949). The X chromosome of Drosophila has a short arm comprised of two small chromomeres (Kaufmann 1934). Telocentric chromosomes that have arisen in experimental cultures of maize are unstable and frequently form isochromosomes (one arm duplicated) (Rhoades 1940). Usually the centromere is not terminal and hence divides the chromosome into two arms, as first emphasized by S. Nawaschin. The ratio of long/short arm lengths is relatively constant for a particular chromosome and may be distinctive for certain ones in the genome, as e.g. in maize and barley. At metaphase of somatic mitosis as seen for example in stained preparations of root tip cells, there is a constriction or apparent gap at that point. In pachytene chromosomes in maize, the centromere is a short, very lightly stained region (not always easily seen) which differs in length for different chromosomes but is constant for a particular one (McClintock 1930). Sansome has suggested that these regions in all chromosomes are homologous and Muller has referred to a 'gene' for the centromere. In view of the above differences this appears unlikely, except in the sense that this is a region which duplicates itself.

In plants of maize with an abnormal chromosome 10 (an added, largely heterochromatic segment on the end of the long arm), secondary centric regions have been observed on the abnormal chromosome 10 and on any other chromosome that has a knob (Rhoades and Vilkomerson 1942, Rhoades 1952).

A polycentric condition may exist in certain species, e.g. Ascaris megalocephala (Walton 1924) and in Tityus (a Brazilian scorpion, Piza 1941 and Rhoades and Kerr 1949). Diffuse or scattered spindle attachments are found in Coccids (Hughes-Schrader, 1948) and also in the plant species Luzula purpurea (wood rush, Malheiros, et al. 1947).

Thus the centromere is one of the most useful features identifying particular chromosomes.

Secondary constrictions and satellites

One or more chromosomes in the genome may have a region at which nucleolar material is organized into a nucleolus (Nawaschin 1927, Heitz 1931, McClintock 1934, Frankel 1937, Håkansson and Levan, 1942, Poulson and Metz 1938). This region is terminal in one chromosome of Sorghum versicolor (Garber 1944). In species in which it is not terminal, there is usually a secondary constriction at that point in the somatic metaphase chromosomes. The segment distal to the secondary constriction is the satellite.

The satellite in maize is composed of four chromomeres at the pachytene stage; but in somatic chromosomes as usually stained it is a small spherical body at the end of the chromosome, not obviously attached. The two satellites in barley (Burnham, et al. 1954) and one in rye (Lima-de-Faria 1952) are longer and appear to be no different from the remainder of the chromosome material. In tomatoes, the satellite appears to be heterochromatic and differs in length in different strains (Lesley 1938).

There is no satellite in Drosophila melanogaster, although the X and Y chromosomes are associated with the nucleolus at a point between bands 20B12 and 20C12 probably between the gene bobbed (bb) and the centromere (Kaufmann 1938). In somatic cells, a secondary constriction sets off a proximal segment that is less than a third the length of the X (Kaufmann 1934).

Hence, in many species, satellites and secondary constrictions serve to identify certain chromosomes.

Darkly-staining regions and "knobs"

Many or most of the chromosomes in many species have darkly-stained (heterochromatic) regions on one or both sides adjacent to the centromere. These regions persist in the metabolic nuclei and probably correspond at least in part to what have been designated as pro-chromosomes or chromo-centers (Gregoire 1932, Heitz 1933). They are characteristic of many species of Solanaceae, (e.g. Nicandra physaloides, apple-of-Peru, Janaki-Ammal 1932), of the North American Euoenotheras, (Wisniewska 1935, for Oe. hookeri), and of Impatiens (Smith 1934).

In Drosophila, almost the entire Y chromosome, 1/3 to 2/3 of the X chromosome proximal to the centromere (Painter 1931, Dobzhansky 1932), and regions on both sides adjacent to the centromere in chromosomes 2 and 3 are heterochromatic. These regions are represented in the chromosomes of the salivary gland cells by not more than a few chromomere bands (Hinton 1942). For further discussions on heterochromatin, see Vanderlyn (1949), Schultz (1939, 1947), Barigozzi (1950).

In maize, in addition to the heavily stained regions adjacent to the centromeres in certain chromosomes, there are darkly-stained bodies or "knobs" which may be terminal or subterminal. Their number and position are constant for a particular chromosome in a race, but vary in different races (McClintock 1930, Longley 1939, W. Brown 1949).

They also persist in the metabolic nucleus (D. T. Morgan 1943). Chromosome 10 in certain strains has an additional long densely stained segment on the end of the long arm referred to as abnormal 10 or K10. Hence knobs and other heavily stained regions may be used to distinguish certain arms and chromosomes.

Chromomere pattern

For Drosophila melanogaster, the chromomere pattern has been studied only in the chromosomes of the larval salivary gland cells. The different chromosomes show a characteristic pattern and sequence of cross bands, some heavily, others lightly stained, some a single band or row of dots, others doublets (Painter 1941 and Bridges 1935). Detailed maps of the cross bands were prepared for the X by Bridges (1938), 2R by Bridges and Bridges (1939), and 3L, 3R, and 2L by P. N. Bridges (1941, 1941a, 1942). The numbering and letter designation system makes it easy to describe aberrant chromosome types and gene locations. Detailed maps for several other Drosophila species are available, including pseudoobscura (Tan 1937), subobscura (Frizzi 1941), funebris (Slizynska and Slizynski 1941), and others (Wharton 1943).

Only in certain regions of the maize chromosomes are the chromomere patterns distinctive. There may be a prominent chromomere in certain positions.

A gradient of decreasing chromomere density from the centromere to the distal ends has been described in rye and in Agapanthus by Lima-de-Faria (1952, 1954). In certain species large portions of each chromosome, usually distal to the centromere, may stain very lightly with acetocarmine, termed ghost-regions by McClintock, e.g. in Sorghum vulgare (Garber 1944), and in Lycopersicon (S. Brown 1949, Barton 1951, Gottschalk 1954).

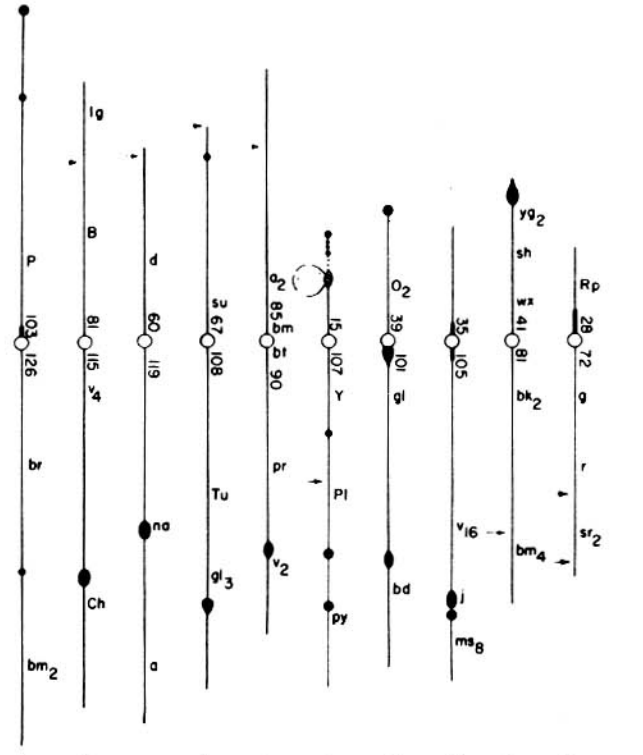
Supernumerary chromosomes

In many species or in occasional individuals of certain species there are, in addition to the longer chromosomes, short supernumerary or accessory ones which vary in number (Müntzing 1958). A few of the plant species in which they have been reported are: maize, Sorghum, Kentucky blue grass, and rye (for a list see Darlington 1937, Table 16). In animals, a noteworthy example is the common fowl (Newcomer 1959).

Those in maize, designated as 'B' chromosomes, have a terminal or nearly terminal centromere (McClintock 1933, Darlington and Upcott 1941). Except for a short euchromatic segment they are heavily-stained (heterochromatic). They do not pair with any of the primary A-chromosomes; and have no similarity to any portions of them except for a superficial resemblance to the additional terminal segment on chromosome 10 found in certain strains as mentioned earlier. In somatic metaphase the 'B' chromosomes are short and club-shaped. At one time those in rye were thought to be fragments of a normal chromosome. In rye they are not heterochromatic.

Idiograms

Careful measurements of chromosome lengths, positions of primary and secondary constrictions, and satellites have been made based on somatic metaphase chromosomes, especially from metaphase stages in root tips of plants (Lewitsky 1931). They furnish a general picture of chromosome morphology which is useful in comparing species. For individual chromosomes with about the same dimensions it is difficult or impossible to be certain that the same chromosome is being measured in different cells. As we shall see later, interchanges and other aberrations may be used to distinguish such chromosomes in cytological studies.



1	2	3	4	5	6	7	8	9	10	
229	196	179	175	175	122	140	140	122	100	Total
1.23	1.42	2.0	1.63	1.07	7.1	2.6	3.0	20	2.6	Long / Short

FIG. 1. Pachytene morphology of the 10 chromosomes of zea mays. The lengths are relative (after McClintock and Burnham), based on a length of 100 for chromosome 10. The positions of additional knobs as found by McClintock are indicated by arrows.

The relative lengths of the ten chromosomes of maize at the pachytene stage are shown in Figure 1. Differences in total length, arm ratio; or position, size or shape of knob serve to distinguish many of the chromosomes. Chromosome 6 has a satellite and nucleolus organizer. Prominent chromomeres in certain regions serve for identification also.

The relation of morphological features to genetic information

Length

Do differences in chromosome length within a species correspond to differences in genetic map length? Information in Drosophila melanogaster on cytological length of the different chromosome arms in the salivary gland cells, counts of the numbers of bands in the salivaries (Bridges 1942), the genetic map lengths of the chromosomes (Bridges and Brehme 1944), and the meiotic length (Cooper 1950) are summarized in Table 1.

The data in Table 1 indicate that differences in total genetic length and in arm lengths correspond roughly to differences in relative genetic map length, except for chromosome 4. A perfect relationship is not expected since there are relatively long heterochromatic regions with few genes, and with low crossover values. A closer relationship might be expected between map length and

Table 1. Genetic map lengths and chromosome arm lengths in Drosophila melanogaster

		Chromosome length				No. of bands in salivaries		
			Salivary chrom.				max. stretch	
Chromosome	Genetic map length	at meiosis meta. I	lax	med. stretch	max. stretch	med. str.	total	doublets =1
I	66+4*	1.8	140	220	414	725	1011	698
IIL	55.0		135	205	370	584	803	607
IIR	53.0	2.6	148	245	446	660	1136	854
IIIL	46.0		153	210	424	542	884	688
IIIR	60+4*	3.2	180	280	519	697	1178	915
IV	0.2	0.2	9	15	46**	50	137**	88**
TOTAL			765	1175	2219	3258	5149	3850

* Added for distance beyond the last gene.

** Slizynski, (1944).

salivary chromosome length; since as mentioned earlier, the heterochromatic regions are represented by few bands in the salivary chromosomes. Likewise the relationship between chromosome length and number of genes is not likely to be close unless there are few or no long heterochromatic regions. Similar comparisons in maize are possible only for a few of the chromosome arms, as shown in Table 2. For the other arms either the position of the centromere in the map is not known with certainty, or there is no gene known to be close to the end of the arm.

Table 2. Comparisons of pachytene length and genetic map length in maize (based on the genetic map by Rhoades (1958b) and on the pachytene map in Figure 1). S = short arm, L = long arm.

Chromosome arm	Relative length	Genetic map length
2S	81	74+
5L	90	65+
9S	41	59+

It appears that the 9S arm with half or less of the length of 2S or 5L has a longer genetic map than expected from proportionality.

Within a species, differences in chromosome length that do not reflect differences in genetic map length, or in number of genes, may result from differences in the extent of heterochromatin or from the presence of duplications. Species differences in chromosome length at metaphase of meiosis may be accounted for by differences in degree of coiling of the chromonemata or in the amount of relational coiling of the chromosomes.

In hybrids between species whose chromosomes differ greatly in length, the length is usually intermediate, but in a few the great differences are maintained, e.g. in the hybrids Godetia deflexa x G. bottae (Håkansson 1943) and Mahonia aquifolium x Berberis sargentiana (Levan 1944). In these hybrids, length is seemingly controlled by the genotype of the individual chromosomes whereas in the others length is controlled by the entire genotype, i.e. by the interaction of the genes from both parents. How might the linkage maps in the two types of hybrid compare with those in the original parents?

Centromere

As noted above, a centromere divides the chromosome and hence also the linkage map into two arms. In chromosomes with diffuse spindle attachments induced fragments behave normally at meiosis (Hughes-Schrader and Ris 1941), whereas in species with a localized centromere, fragments without a centromere (said to be acentric) do not proceed to either pole. Hence the centromere is important in chromosome kinetics. Other forces are important also, as we shall see later, since under some circumstances homologous centromeres pass to the same pole. The centromere is subject to occasional misdivision which results in a telocentric fragment (one arm missing), or in an isochromosome (one arm duplicated) (Darlington 1939, 1940).

The centromere region is involved in a number of important genetic relations. First, the chromosomes separate reductionally at the centromere at Division I of meiosis. This was shown in Drosophila by Anderson (1925) and Bridges and Anderson (1925); and in maize by Rhoades (1933) and by McClintock (1934). It appears to be true in Neurospora crassa since on this assumption, logical linkage maps that include the centromere can be

constructed. Second, crossing over per unit of physical length is lower in regions adjacent to the centromere. One result is that the genes in these regions are clumped in the linkage maps for the three long chromosomes of Drosophila melanogaster. Since these regions are heterochromatic, the clumping might be an effect of either the centromere or the adjacent regions or both. The shift of a region to a position near the centromere of chromosome 4 reduced the crossing over in that region (Beadle 1932a). This and other experiments in Drosophila designed to distinguish between the two effects indicate that the centromere itself causes a reduction in crossing over (Mather 1939). The genes are clumped also in various other regions, e.g. at the distal end of the X, at one end of 2, and in one other region of 3, obviously not because of proximity to a centromere.

In maize there is a cluster of genes around P, su, and bm_1, in chromosomes 1, 4 and 5 respectively, regions near a centromere. Third, the effects of age, temperature and X-rays on crossing over in Drosophila are greatest in regions proximal to the centromere. Mather's experiments (1939) suggested that the temperature effect might be an effect of heterochromatin rather than a centromere effect. Fourth, as mentioned earlier, simultaneous crossing over on opposite sides of the centromere occurs without interference in Drosophila. It is possible that this is because the regions are so long physically, that interference would not be expected, as suggested by Brown (1940, page 42). The fact remains that in breeding experiments interference is not observed. (See page 2, item 8, for the behavior in other species).

Secondary constriction

There are no data on interference across a secondary constriction. Translocations in Drosophila with one break in the X between the centromere and the nucleolus organizing region should make this experiment possible. A similar experiment might be performed in maize by using chromosomal interchange stocks in which the satellite of chromosome 6 has been replaced by a gene-bearing segment of another chromosome, but there are no genes known with certainty to be in the short arm of 6. McClintock (1941, page 75) has shown that in Zea irregular behavior including breakage and bridge formation occurs following crossing over in the short arm of chromosome 6. If this is the usual behavior, the region between the centromere and the nucleolar organizer region should have become short genetically during the course of evolution.

Satellites

Thus far no gene has been found in the satellite but an intensive search has not been made. Based on appearance under the microscope, there is no reason not to expect them in barley, maize or rye.

Knobs

There is no evidence on the presence or absence of genes in the knobs. In maize, recombination in the Lg_2-A region (lg = liguleless, A = anthocyanin) is reduced when the subterminal knob in 3 is heterozygous (Rhoades and Dempsey 1957). Recombination values between albino plant (wd), the most distal marker, and waxy (wx), the gene nearest the centromere (both genes in the short arm of 9) in plants heterozygous for a knobless, small, medium, or large terminal knob on 9, were 26.9, 17.7 and 12.7% respectively (Kikudome 1959). Hence the larger the knob, the lower the recombination value. In the presence of the knob on abnormal 10, crossing over for the same region was increased, the corresponding values being 31.5, 26.8 and 29.2 (avg. of 2 values) respectively. The suppressing effect of the knobs on crossing over was suppressed by the presence of abnormal 10.

When plants heterozygous for the abnormal chromosome 10 were crossed as ♀ parents, about 70% of the offspring had the abnormal chromosome 10 (Longley 1945, Rhoades 1942). For other chromosomes heterozygous for knobs in the same plants there was also preferential segregation of the knobbed over the knobless member of the pair. This preferential segregation was thought to be occasioned by the neocentromere activity which first appeared at metaphase I in chromosomes carrying a knob (Rhoades and Vilkomerson 1942, Rhoades 1952). Genetic markers on the chromosomes with knobs also showed preferential segregation (Longley 1945, Kikudome 1959, Emmerling 1959). There was no false linkage, however, between genetic markers on two chromosome pairs that were also heterozygous for knobs. Evidence presented by Rhoades (1958) gives some support to his hypothesis that "the formation of heterozygous dyads as a result of crossing over is an essential antecedent to preferential segregation", the knobbed chromosome being segregated to the basal or functional megaspore.

Heavily stained segments adjacent to the centromere

The loci of a few typical mutant genes have been found in the heterochromatic regions adjacent to the centromeres in Drosophila, but the number is low. Mather (1943, 1949) suggested that genes with minute but cumulative effects might be located in the heterochromatin. The genes in heterochromatic regions may be overly active in laying down material as suggested by Schultz (1939).

Individuals that have chromosome aberrations involving one break in heterochromatin frequently show variegation for characters determined by genes adjacent to the breaks (Schultz 1936). The effect decreases for genes that are progressively farther away. Addition of extra heterochromatin in the form of an additional Y may almost suppress the variegation. The expression of genes in some cases has been shown to depend on the balance between eu- and heterochromatin. Hence, there seems to be some physiological activity from heterochromatin, and it is not inert (Muller, et al. 1937). The low frequency of typical genes is not a satisfactory test for heterochromatin, since a region that is duplicated will give a similar result.

Crossing over in these regions is low in relation to their physical length.

Chromomere pattern

The linear order of the chromomere pattern corresponds to the linear order of the genes. To what extent each chromomere corresponds to a gene is not known, but there is some evidence that a chromomere may represent more than one gene (McClintock 1941). She also found as much as 10 to 15% recombination in a region having only a few chromomeres near the end of chromosome 2 in maize. Evidence from complex loci indicates that occasional recombination may occur within what had formerly been designated as a gene.

Supernumerary chromosomes

Variation in the number of supernumerary or 'B' chromosomes in maize does not modify the segregation for genetic characters. However, large numbers of 'B' chromosomes have a general adverse effect on vigor in maize (Randolph 1941), and in rye, (Müntzing 1948). The centromeres of these chromosomes divide but fail to disjoin at certain nuclear divisions following meiosis. In maize this occurs at the second post-meiotic division of 90 to 100% of the microspores, but usually not in any of the divisions of the megaspores (Roman 1947, also see page 105). Non-disjunction occurs in rye at the first post-meiotic division of both kinds of spores (Müntzing 1945). Supernumeraries in Sorghum purpureo-sericeum have been reported to cause additional nuclear divisions in the generative nucleus of the microspores (Darlington and Thomas 1941).

"In extreme cases the nuclear division may give four to five generative nuclei and thereby kill the pollen grain, much as the excessive mitosis of a tumour may kill an animal" (Darlington and Mather 1949). Supernumerary chromosomes in rye bring about lowered fertility (Müntzing 1946).

Special consequences of differences in gametogenesis in higher plants and animals

At this point, the meiotic and post-meiotic cycles in higher plants and animals will be reviewed paying special attention to differences which affect the transmission of certain chromosomal aberrations; and also affect the results from radiation experiments. Although there are many variations of the meiotic and post-meiotic cycle in higher plants (see Maheshwari 1950, page 86, ff), the sequences shown in Figures 2 and 3 are found in many of our crop plants. Meiosis; micro- and megasporogenesis. Each microspore-mother-cell (pollen-mother-cell or P. M. C.) in the anthers undergoes two meiotic divisions to produce a quartet of microspores, as shown in Figure 2A.

Likewise the megaspore-mother cell in the ovary undergoes two meiotic divisions but produces a linear quartet of megaspores as shown in Figure 3A.

Each of the four spores from microsporogenesis, and each of the four from megasporogenesis, has one of the chromatids from the tetrad of chromatids present originally in each of the pairs of chromosomes and hence contains the n number of chromosomes. Reduction is completed during the two meiotic divisions. In many species (dicotyledons especially), both nuclear divisions are completed before cell division occurs. In other species (for example the cereals) cell division occurs at the end of the first and second meiotic divisions. In these latter the first and second division planes may be distinguished in the spore quartet immediately after division II. This has been useful in cytological studies of crossing over and chromosome segregation (page 75).

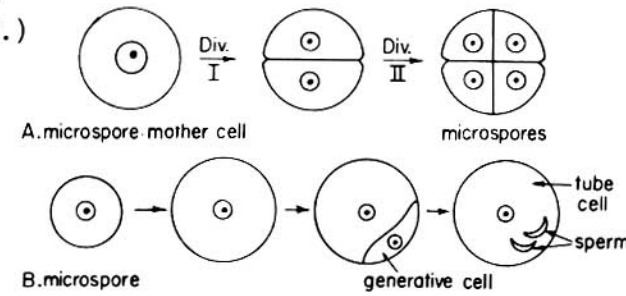


FIG. 2. Schematic diagrams:
A. Microsporogenesis in a species in which cytokinesis occurs after division I.
B. Post-meiotic divisions of the microspore to form the pollen grain.

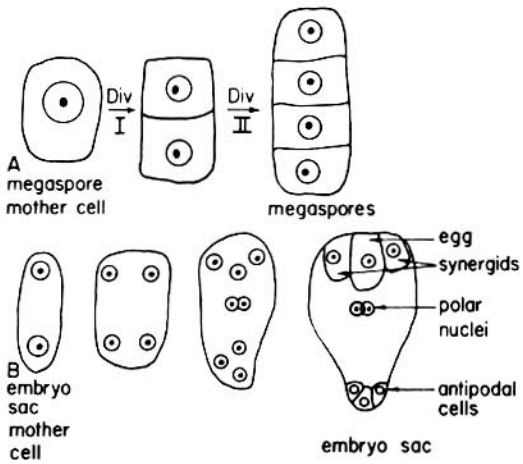


FIG. 3. Schematic diagrams:
A. Megasporogenesis.
B. Post-meiotic divisions in the development of the embryo sac (megagametophyte). Usually the functional megaspore is the one at the chalazal end of the quartet but in certain species it is the one at the micropylar end.

Post meiotic (somatic) divisions and subsequent events in plants

THE MALE GAMETOPHYTE. As shown in Figure 2B, the microspore divides to produce the tube and generative cell. This is the first post meiotic division. The generative cell then divides to form the two