Discussions in CYTOGENETICS

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by

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FOREWORD

This book furnishes detailed discussions which include methods, theoretical expectations, summaries of published results, and possible applications or uses in further experimentation or in breeding. The first chapter includes a brief summary of basic information, and special aspects of chromosome behavior and the life cycle as a background for the discussions that follow. The next eight chapters fall into two groups: 1. changes in chromosome structure, and 2. changes in chromosome number. It concludes with a chapter on sex determination and one on apomixis.

It is intended as an advanced course which follows courses in cytology and genetics. It is a supplement rather than a substitute for other books in the field. The goal of the course is to aid in attaining a working knowledge which will enable the student to read and understand the published research as it appears; and to plan his own experiments. The study questions and particularly the problems in Appendix 1 are important aids in attaining this goal. Problems 4 and 5 are recommended as a basic introduction. A critical reading of original papers which includes an analysis of the methods used and the interpretation of the results is recommended also. As an aid in their selection, an abbreviated list of references is furnished in Appendix 2. These represent typical papers rather than an attempt to survey a particular topic. Laboratory study of slides prepared from material illustrating various types of chromosome aberrations is essential also.

There is a need for research in all areas of a particular field. In pursuing a current fad in research there is a danger that other important areas may be neglected. Advances in each contribute to the whole. Throughout the book, attention is called to problems on which information is lacking and to special uses or applications which should furnish needed information.

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I am grateful for the generosity of the many individuals and publishers in granting permission to use published tables and illustrations. The citation that accompanies each indicates its source. Material from the Maize Genetics and the Barley Newsletters was used with the permission of the authors. Errors there undoubtedly are, I will appreciate it if they are called to my attention.

C. R. B.

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CHAPTER 1 INTRODUCTION

Cytogenetics is the correlated study of genetics and cytology. This is a natural outgrowth of the fact that chromosomes are the vehicles of that portion of heredity which follows Mendelian laws. Chromosome behavior furnishes the mechanism for the observed genetic segregation and breeding behavior. There are striking differences in gross morphology of the chromosomes between and within species. How these are reflected in our information on linkage maps and breeding behavior is therefore important to geneticists and to breeders.

This chapter includes a brief summary of what is known about linkage, followed by discussions of differences in the various gross morphological features of the chromosomes, the relation of these to various aspects of genetics including linkage and linkage maps; certain features of the life cycles in plants and animals in relation to cytological and breeding behavior, and certain general facts about chromosome behavior which are basic to an understanding of the cytogenetical behavior of chromosome aberrations and changes in number. To furnish a background for those discussions, the following summary of our current information about linkage is presented.

Linkage information

1. Linkage between characters is observed only when the corresponding genes are located on the same chromosome. Exceptions:

   a. "affinity", a loose association between genes on different chromosomes (see 14 below);
   b. physiological association, characters that are an end result of the same sequence of physiological processes; or in interspecific crosses only combinations of chromosomes that approximate the parental combinations may be physiologically compatible.

2. Recombination values between different genes may vary from less than 1% to 50%; but are relatively constant for any two linked pairs.

3. When several genes show linkage with each other, and their recombination values are used as a measure of distance between them; the genes can be arranged in linear order and the values represented on a "linkage map". Linkage maps are constructed by using 1% recombination as the unit of map distance. Fisher and his school have used "1 centimorgan" as the unit. The recombination values between markers are corrected for the undetected doubles, using the Kosambi formula (Kosambi 1944). The resulting map is supposed to represent the actual crossover frequencies. It seems unlikely that the proper correction for all species and all regions of the chromosomes can be arrived at by a formula.

4. There are as many linkage groups as there are pairs of chromosomes.

5. Genetic exchanges have had cytological exchange between the two members of the chromosome pair, as shown by using heteromorphic pairs marked cytologically at two points and studying the genetic crossovers cytologically (in Drosophila, Stern 1931; in Zea, Creighton and McClintock 1931 and Brink and Cooper 1935).

6. Based on a critical examination of the theory of linear order, the following six basic propositions are statements of observed facts of breeding behavior and are not postulates for any particular theory. These have been selected from the 17 listed by Jennings (1923). Thoughtful consideration will show that each is true.

   a. "If two genes show with one another a low crossover value, they show nearly the same values, low or high, with any other gene".
   b. If for any three genes, A, B and C; crossover values between A-B and B-C are first determined, the value of AC will be either a little less than the sum of these two values (order of genes = ABC); or a little more than their difference (order of genes = ACB).

   Based on linkage data from a backcross involving several loci simultaneously, e.g. $\frac{A\ B\ C\ D\ E}{a\ b\ c\ d\ e}$ x a b c d e; the following are true:

   c. In a considerable proportion of the offspring, no crossing over has occurred between any of the genes.
   d. Among the remainder, the most frequent combination is that in which one point in the linear series of genes separates them into two groups of loci, the members of one group showing recombination with all the members of the other group. These are the single crossovers. For example, such a crossover in the segment BC is expected to produce ABcde and abCDE.
   e. In a much smaller number of individuals, two points in the linear series of genes separate them into three groups of loci such that all the genes between the two points show recombination with all the genes outside the two points. These are the double crossovers. For example, simultaneous crossovers in the segments BC and DE are expected to produce ABcdE and abCDe, in which cd are combined with ABE and CD with abe.
   f. In such a linear series of genes, breaks at two points that are close together do not occur. This is interference. One measure of interference between two segments is calculated as: observed % of doubles/(total % of crossing over in region 1) (total % in region 2 expressed as a decimal). This is termed a coincidence value. If the observed numbers are used, $c = \frac{DN}{AB}$ where D = observed number of doubles, and A and B represent the total number of recombinations observed in regions 1 and 2 respectively and N is the total number.

7. Interference decreases as the distance between the two regions increases. Coincidence values, in general, range between 0 and 1.0, i.e. from complete interference to none. Values above 1 have been reported in Neurospora (Lindegren and Lindegren 1942) for regions symmetrically located in opposite chromosome arms. Repetition of these experiments set up in the same manner is needed.

8. In Drosophila and in maize, there is no interference across the centromere; i.e. between opposite arms of the chromosome. Genes in segments close to the centromere but on opposite sides still show close linkage. Based on counts of chiasma frequencies in chromosomes with distinguishable arms, there is no interference across the centromere in Fritillaria chitralensis (Bennett 1938) and in Uvularia perfoliata (Barber 1941); but there is interference in Culex pipiens (mosquito), (Patau, 1941 and Callan and Montalenti 1947), in Dicranomyia trinotata (Patau 1941), and in Petunia violacea but not

in P. axillaris (Callan and Montalenti 1947). In Trillium (Huskins and Newcombe 1941 and Newcombe 1941) and in Anilocra (Callan 1940), coincidence between arms is above 1.

9. In multiple-point backcross experiments in Drosophila involving a total map length of a little over 100, the ratio of recovered non-crossover chromosomes, chromosomes with a single crossover, and ones with two or more crossovers is roughly 1:2:1 (27.4:46.4:26.2) as shown in the following tabulation:

	X chromosome[1]	Chromosome 2[2]	Chromosome 3[3]
Total map length in experiment	71.1	86.7	102.26
Non-crossovers	41.0%	30.7%	27.4%
Single crossovers	46.8	42.2	46.4
Double crossovers	11.9	19.7	22.9
Triple crossovers	0.4	1.6	3.1
Quadruple crossovers	0.0	0.1	0.2
Total population	16,136	5,284	5,009

[1] Based on 9-point data from Bridges (Weinstein 1936). For 7-point data of Bridges and Olbrycht and Anderson based on 26,911 flies see Anderson and Rhoades (1931).
[2] Based on 8-point control data from Graubard (1932).
[3] Based on 8-point control data from Redfield (1930).

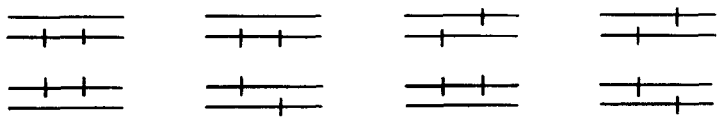
Analyses of the interference relations from the same data reveal that as the distance between crossover points increased coincidence rose to a value of 1 (no interference), and did not deviate significantly from 1 with further increases in the distance. (Formulas for calculating standard error of coincidence were presented by Muller and Jacobs-Muller 1925). These relations apply only to the data considered as recovered strands. The data for the X-chromosome have been calculated as chromatid tetrad frequencies, assuming no chromatid interference (Weinstein 1936). Further analysis of these frequencies indicates that the crossover points in the different types of exchange tetrads tend to occur at certain modal positions (Charles 1938), (cf. also Stephens 1961, 1962).

10. Experiments using attached-X females in Drosophila (Anderson 1925, Bridges and Anderson 1925), trisomic plants in maize (Rhoades 1933), and the spores from individual asci (tetrad analysis). In Neurospora (Lindegren 1933) have revealed the following:

    a. Crossing over occurs when the chromosomes are double-stranded (so-called 4-strand crossing over). As a result, an ascus in which a crossover has occurred between linked genes usually has two parental and two crossover type spores that are complementary to each other. Exceptional asci occur occasionally with three of one allele and one of the other. New combinations may occur within complex loci which are not regularly associated with recombination of outside adjacent markers. A kind of mis-copying or a copy-choice mechanism has been suggested (cf. Beadle 1957a, Freese 1957).
    b. Results can be explained if the first division is reductional at the centromere. For regions distal to the crossover nearest the centromere, the first division is equational. Evidence that equational separation at division I occurs at the centromere in Trillium was presented by Matsuura (1957), but has not been confirmed.

c. At a given level, the exchange is between only two of the four chromatids.
d. The observed results in Drosophila agree closely with those expected if sister strands do not crossover.
e. Assortment of the chromosomes is determined at the centromere (from attached-X experiments).

11. When a tetrad of chromatids is considered, double crossing over (in two regions) may occur in the following ways, each of which is classified on the basis of the number of strands involved in crossing over:



2-strand 3-strand 3-strand 4-strand

If each is equally likely to occur, the expected ratio is 1 (2-strand): 2 (3-strand): 1 (4-strand) double.

Interference may be of two types:
a. Chiasma interference (between crossovers in the two regions).
b. Chromatid interference - (i.e. if two strands crossed over at a given point, these two might be more likely to crossover at a second point or less likely than expected by chance).

12. From the attached-X experiments in Drosophila, Beadle and Emerson (1935) found that the ratio of 2- : 3- : 4-strand doubles tended to deviate from the 1:2:1 ratio expected from randomness, but approximated this ratio.

13. The Lindegrens and their group consider as established facts the presence of chromatid interference and localized crossing over that is symmetrical on opposite sides of the centromere (coincidence values above 1), based on Neurospora (Lindegren and Lindegren 1942, Shult and Lindegren 1957). These have not been confirmed by others working on the same organism.

14. Linkage-like loose associations between markers in independent chromosomes have been reported in crosses between laboratory stocks of mice (Michie 1953, 1955, M. Wallace 1953, 1958, 1959). The proposed explanation is that centromeres originally from one parent tend to pass to the same pole. The term "affinity" has been applied to the phenomenon. A correlation between the genetic behavior of non-homologous chromosomes in Saccharomyces was reported by Shult and Lindegren (1957).

15. Crossing over values may be modified by a number of agents. In Drosophila, the sensitive regions are near the centromeres, with little or no effect in other regions. The effective agents and the changes in crossing over are as follows:

a. Low and high temperatures increased genetic crossing over without a change in coincidence values (Plough 1917, Plough and Ives 1935).
b. Crossing over and coincidence values are lower in older individuals in Drosophila (Bridges 1915, Stern 1926, Rendel 1958).
c. In general, X-rays increased crossing over in Drosophila (Muller 1925).
d. Crossing over may or may not be lower in the heterogametic sex. In Drosophila there is no crossing over in autosomes, or sex chromosomes in the ♂ as first reported by Morgan (1912, 1914).
e. Internal physiological differences may have an effect. Differences in crossing over in ♂ and ♀ organs on the same plant, in general higher in the ♂ , have been re-

ported in Primula (Altenburg 1916, also discussed by Gowen 1919), and in Pisum (deWinton 1928). In maize this is true for regions proximal to the centromere (Rhoades 1941, Clark 1956, Burnham 1949).

f. General vigor may affect crossing over. In maize crossing over tended to be lower in the more vigorous plants (Stadler 1926).

g. Recombination values may be modified by the application of certain chemicals and enzymes. RNA-ase and ethylenediaminetetra-acetic acid (EDT) increased recombination values in one of the two tested regions of the X-chromosome in Drosophila (Kaufmann, et al. 1957). Genetic recombination in Escherichia coli K-12 has been modified when Hfr donor bacteria were treated with the thymine analogue 5-bromodesoxyuridine under conditions of thymine starvation and mated to untreated recipient bacteria (Folsome 1960).

16. Studies indicate that desoxyribose nucleic acid (DNA) carries the primary genetic information. Watson and Crick (1953, 1953a) proposed a double helical structural formula for DNA. As described by Beadle (1957), "the two polynucleotide chains of the helix are complementary in base sequence and are hydrogen-bonded together through their inwardly directed purine and pyrimidine bases. The four base pairs are adenine-thymine, thymine-adenine, guanine-cytosine and cytosine-guanine. Genetic specificity is presumed to reside in base-pair sequence". Various proposals have been made as to the mode of replication and the manner in which crossing over takes place (Schwartz 1954).

Work by Taylor (1957) indicates the two strands are different in a directional sense.

17. Multiple alleles. -- At certain loci, e.g. at the red vs. white eye locus in Drosophila, there is a series of mutants which behave as alleles when crossed with each other, i.e. the F_1 is not wild type and there is one factor segregation in F_2. Tests on a very large scale have shown that the hybrids between certain members of such series occasionally produce a wild type exception, or there is other evidence that they may be at different sites (Oliver and Green 1944). When the hybrid is heterozygous for an adjacent genetic marker on each side of the locus, these exceptions are shown to have been accompanied by crossing over between the adjacent markers. Further tests showed that the complementary crossover type carrying both recessives also occurred. The double heterozygote in repulsion (trans-arrangement), e.g. $\frac{w^a +}{+ w^{ch}}$ is not wild type; whereas in coupling (cis-) $\frac{w^a w^{ch}}{+ +}$, it is wild type. Here the visual test for strict allelism fails. This behavior has been termed "position pseudoallelism" (Lewis 1948, 1951, 1954) and also the cis-trans or Lewis effect. As Benzer (1955, 1956) has stated it "the classical 'gene' served as a unit of genetic recombination, of mutation, and of function." That they require different definitions is shown by the results described above and by Benzer's studies involving several hundred rII mutants of the T4 bacterial virus. If the trans arrangement has the mutant phenotype, the two mutants are assumed to be defective in the same functional unit to which Benzer applied the term "cistron". The entire group of rII mutants could be grouped in two cistrons. The mutants belonging to each cistron could be placed in linear order based on recombination results. Some failed to give as much as $10^{-3}\%$ recombination, i.e. none in 100,000. The smallest non-zero recombination between two groups of mutants was $10^{-2}\%$, i.e. .01%. The unit of recombination or "recon", is then defined by Benzer as "the smallest element that is interchangeable by genetic recombination". Rough calculations based on the DNA content of a T4 virus particle suggest that "the genetic material is divisible by recombination down to the level of one or a few nucleotide pairs".

A unit of mutation, or "muton" is defined by Benzer as the "smallest element that, when altered, can give rise to a mutant form of the organism". It can be caused by alteration of only a few or many nucleotide pairs.

Hence, if the 'gene' is considered as the functional unit, we find that mutation may occur at different sites within the gene, and that recombination is possible between certain of the sites.

Whether every locus has such a complex structure remains to be seen. Every multiple allelic series that has been adequately tested, in Drosophila, mice, Aspergillus, and maize has shown the same general behavior (cf. Dunn 1956, Pontecorvo 1956 and Laughnan 1955). Thus the phenomena of linkage and crossing over may be considered at two levels; one which ignores the probable fine structure of the loci can be used to explain the visual results and in planning breeding experiments, and the other which considers the fine structure. The latter gives a better understanding of what actually occurs, and must be used in planning new approaches and to explain seemingly anomalous results.

18. How crossing over occurs is not known. As seen at meiosis, (diplotene and later stages) chromosome pairs have one to several chiasmata, each chiasma being the point at which the four chromatids appear to change partners. Two theories have been advanced to explain the origin of chiasmata, the two-plane or classical theory, and the one-plane or chiasmatype theory. According to the classical theory (Robertson 1916, Wenrich 1917, Bělař 1928) the separation between pairs of chromatids is along the synaptic plane in some segments (one of these at the centromere) and along the equational plane in others. When adjacent segments open out along different planes, a chiasma results. Sister strands are associated on one side, non-sisters on the other. A variant of this, the neo-classical theory as proposed by Matsuura and Haga (1942) assumes that the plane of separation between pairs of chromatids is a matter of chance at every point including the centromere.

According to the one-plane theory (Janssens 1924, Belling 1928, Darlington 1930, 1931), the opening out is along the synaptic plane only. Chiasmata are formed because crossing over has already occurred. Sister strands are associated on both sides of the chiasma. The number of chiasmata in the early stages should correspond to the number of crossovers. As meiosis proceeds, terminalization of the chiasmata occurs and may reduce the number that can be observed.

Crossing over, according to the two-plane theories, occurs subsequent to chiasma formation and the number of crossovers might not correspond to the number of chiasmata.

Two theories have been advanced to explain how crossing over occurs. According to both, the chromosomes are relationally coiled about each other in the early stages of meiosis. Based on Belling's scheme, as the gene string is replicated the new string will be made up of segments whose templates were different members of the chromosome pair. Since 4- and 3-strand doubles are known to occur, there must be some exchanges in which the original gene-strings do not remain intact. Belling assumed the genes replicate first, followed by the formation of fibers which could connect with adjacent genes in the same string or with genes belonging to non-sister strings.

According to the proposal by Darlington (1935a, b, 1936), as the relationally coiled chromosomes become double, torsion develops at various points until breakages occur which permit the strands to unravel, the joining of broken ends results in a crossover if the ends belong to non-sister strands. Objections have been raised to

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 (Mackel 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 (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.

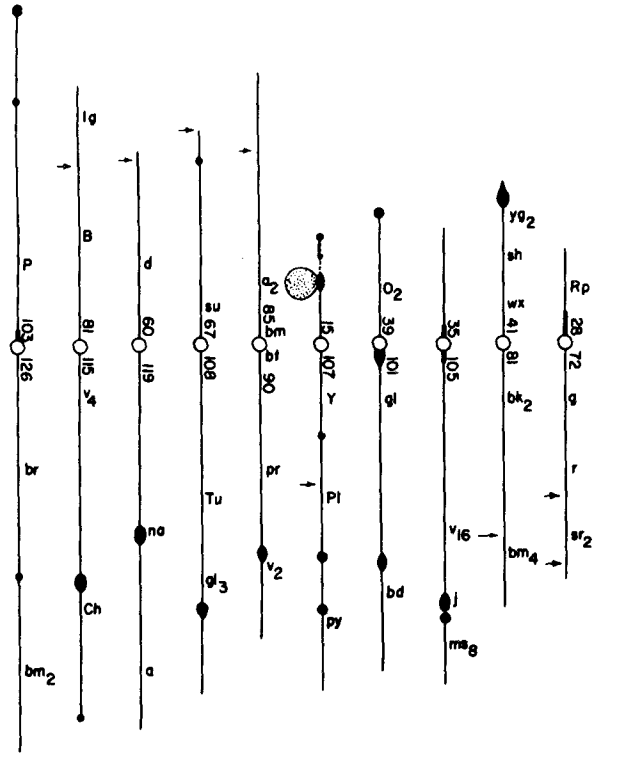


FIG. 1. Pachytene morphology of the 10 chromosomes of zea mays. The lengths are relative (after McClintock and Burnham), based on a length of 100 for chromosome 10. The positions of additional knobs as found by McClintock are indicated by arrows.

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	Genetic map length	Chromosome length at meiosis meta. I	Salivary chrom. lax	Salivary chrom. med. stretch	Salivary chrom. max. stretch	No. of bands in salivaries med. str.	No. of bands in salivaries max. stretch total	No. of bands in salivaries max. stretch 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).