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.
   c. pseudolinkages in interchange heterozygotes.

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 obtained 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), all expressed as decimals. 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	47.8	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).

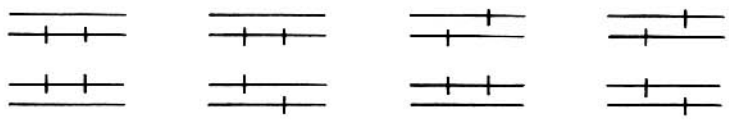
10. Experiments using attached-X females in Drosophila (Anderson 1925, Bridges and Anderson 1925), trisomic plants in maize (Rhoades 1933), and the spores from individual asci (tetrad analysis) in Neurospora (Lindegren 1933) have revealed the following:

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 spores of one allelic type 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).

In maize there is evidence that sister strand crossing over occurs.

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 with examples in various organisms 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). In the ♀ pigeon crossing over does not occur in the sex chromosome, and is greatly reduced in the autosomes.

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 reported 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~~ usual 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.