Extrachromosomal Inheritance

Unit 2: Extrachromosomal Inheritance (6 lectures)

Chloroplast mutation: Variegation in Four o'clock plant; Mitochondrial mutations in yeast; Maternal effects-shell coiling in snail; Infective heredity- Kappa particles in *Paramecium.*

Chloroplast mutation

PLASTID INHERITANCE

The inheritance pattern of plastid characters due to plasma genes located in plastids is known as ***plastid inheritance.*** Plastid inheritance was the first case of cytoplasmic inheritance to be discovered independently by Correns and Baur in 1908. All our knowledge about plastid inheritance in higher plants is based on studies on chlorophyll variegation in leaves. ***Variegation*** refers to the presence of white or yellow spots of variable size on the green background of leaves. Variegation may be produced by (1) some environmental factors, (2) some nuclear genes and, (3) in some cases, plasma genes. The present discussion shall focus on the inheritance of variegation

due to plasma genes. Mutant plasma genes affecting chloroplast are known in many crop plants, *e.g.*, barley, wheat, jowar, maize, oat, pea, tobacco, tomato, lettuce, sugarbeet, chillies, many ornamental plants etc.

Variegation in Four o'clock plant

MIRABILIS JALAPA

Leaves of *Mirabilis jalapa*, the four o'clock plant, may be green, white or variegated, and some branches may have only green, only white or only variegated leaves. Correns made reciprocal crosses in all combinations among the flowers produced on the three types of branches; results from these crosses are summarised in Table 42.1. When flowers from a green branch were used as female, all the progeny were green irrespective of the phenotype (green, white or variegated) of the branch used as male parent. Similarly, progeny from the crosses involving flowers from white branches as female parent were all white irrespective of the male flowers being from green, white or variegated branches. But in the progeny from all the crosses involving flowers from variegated branches as the female parent, green, white and variegated plants were recovered in variable proportions.

Table 42.1. *A summary of the findings of Correns from various types of crosses in* Mirabilis jalapa.

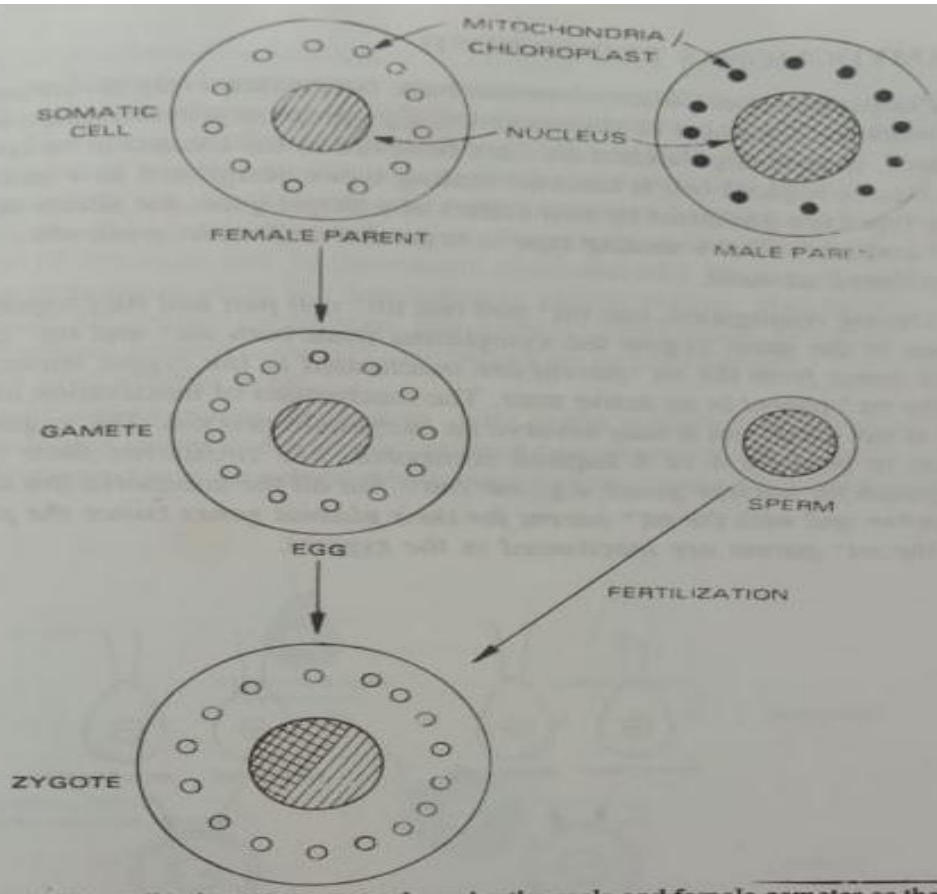
Leaf phenotype of the branch used as the female parent	*Leaf phenotype of the branch used as the male parent*	*Leaf phenotype of the progeny (F_1)*
Green	1. Green	Green
	2. White	Green
	3. Variegated	Green
White	1. Green	White
	2. White	White
	3. Variegated	White
Variegated	1. Green	Green, white and variegated in variable ratios in each of the three cases.
	2. White	
	3. Variegated	

Note : Leaf phenotype of the progeny is the same as that of the female parent. In the progeny from variegated female parents, green, white and variegated progeny are recovered in variable proportions.

Clearly, the phenotype of progeny is the same as that of the female parent, except when the female parent is variegated. These results may be explained by assuming that the plasma gene producing variegation in *M. jalapa* is located in plastids, an assumption made by Correns himself. It is now known that chloroplasts differentiate from proplastids and their differentiation is affected by both nuclear and plasma genes. Proplastids having the mutant plasma gene differentiate into colourless abnormal chloroplasts, while those having the normal allele develop into normal green chloroplasts.

The maternal transmission of plasma genes in higher plants and animals is explained on the basis of unequal contribution by male and female gametes to the cytoplasm of the zygote. In general, egg/egg cell has a considerable amount of cytoplasm which contains several mitochondria and proplastids. On the other hand, male gametes or sperms have only a thin layer of cytoplasm surrounding the nuclei which usually does not contain any mitochondria or proplastids. Further, during fertilization, ordinarily only the sperm nucleus enters the egg cell, and its cytoplasm is left outside the egg cell. As a result, the mitochondria and proplastids present in the zygote are derived from the female parent only (Fig. 42.1). Therefore, plasma genes located in these organelles will also be derived from the female parent only.

Now we return to the results from the various crosses in four o'clock. Green leaves obviously have normal chloroplasts, while white leaves have only abnormal ones. In variegated leaves, green sectors have normal plastids, while white sectors have abnormal ones. Egg cells produced on a green branch will have normal proplastids, while sperms produced on green, white or variegated branches will contribute no plastids to the zygote. Therefore, progeny from the three crosses using flowers from the green branches as females will all be green. Similarly, progeny from the three crosses involving flowers on the white branches as female will all be white since the zygotes from these crosses will receive only the abnormal proplastids through egg cells. However, cells in branches having variegated leaves may have only normal (corresponding to the green sectors), only abnormal (corresponding to the white sectors) or both the types (mixed cells) of proplastids. Egg cells from the 'only normal' cells will have only normal proplastids, while those from the 'only abnormal' cells will have abnormal proplastids only. However, egg cells derived from the mixed cells will have both the types of proplastids. Zygotes from these three types of egg cells, therefore, will develop into green, white and variegated seedlings, respectively.



. 42.1. Unequal contribution of the cytoplasm by the male and female gametes as the basis of cytoplasmic inheritance in higher plants and animals.

ble 42.2. *Variation in the frequencies of green, white and variegated progeny from the different green × white crosses in* Pelargonium zonale.

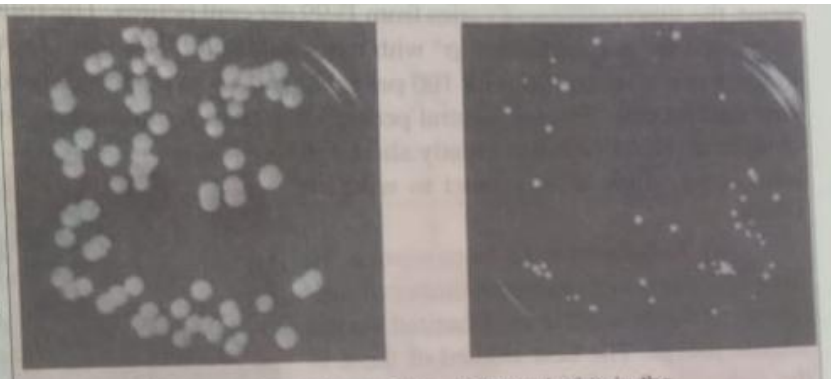
Cross	*Frequency (%) in progeny*		
	Green	*Variegated*	*White*
FoS* × FS	71.7	26.2	2.1
FS × FoS*	54.8	2.0	56.3
FS* × FS	69.0	27.2	3.8
FS × FS*	51.3	4.0	44.7
FoS* × DV	88.3	11.7	0
DV × FoS*	76.0	17.7	6.3
FS* × DV	79.9	18.9	1.2
DV × FS*	70.9	20.7	8.4
JCM* × FS	56.9	32.9	10.2
FS × JCM*	53.5	2.3	44.2

e parent contributing white plastids
Flower of Spring; FoS = Foster's Seedlings;
= Dolly Varden; JCM = Mrs. J.C. Mappin
commercial varieties of *Pelargonium*)

Mitochondrial mutations in yeast

other ingre...

(c) Extra-nuclear inheritance by mitochondria. The most important work on the genetics of mitochondria done in yeast which was initiated by the discovery of petite mutants by **B. Ephrussi** (1953). Subsequently mt DNA was studied in several organisms including plants and animals.



A comparison of normal vs. petite colonies in the yeast *Saccharomyces cerevisiae*.

(i) Petite in yeast. Yeast, *Saccharomyces cerevisiae*, are single-celled ascomycetes fungi. In the life cycle, diploid and haploid adult alternates, the former reproducing by asexual meiospores called **ascospores**, the latter by **isogametes**. The **petite** mutants in yeast fail to grow on carbon source such as glucose and produce smaller colonies (the "littles") when grown on sugars such as glucose. Since this difference can be observed only when such yeast cultures are kept in a oxygen-containing environment; so it is concluded that petite mutants have a defective aerobic respiratory mechanism. In

other words, slow growth of petite can be attributed to yeast cells utilization of less efficient fermentation process. These petites differ from wild type, called **grande** and are characterized by their insensitivity to inhibitors of aerobic pathways (such as cyanide), (ii) absence of cytochromes a_1, b and a number of other changes in mitochondrial respiratory enzymes; (iii) incomplete development of mitochondria; and (iv) lack of stainability of petite mitochondria.

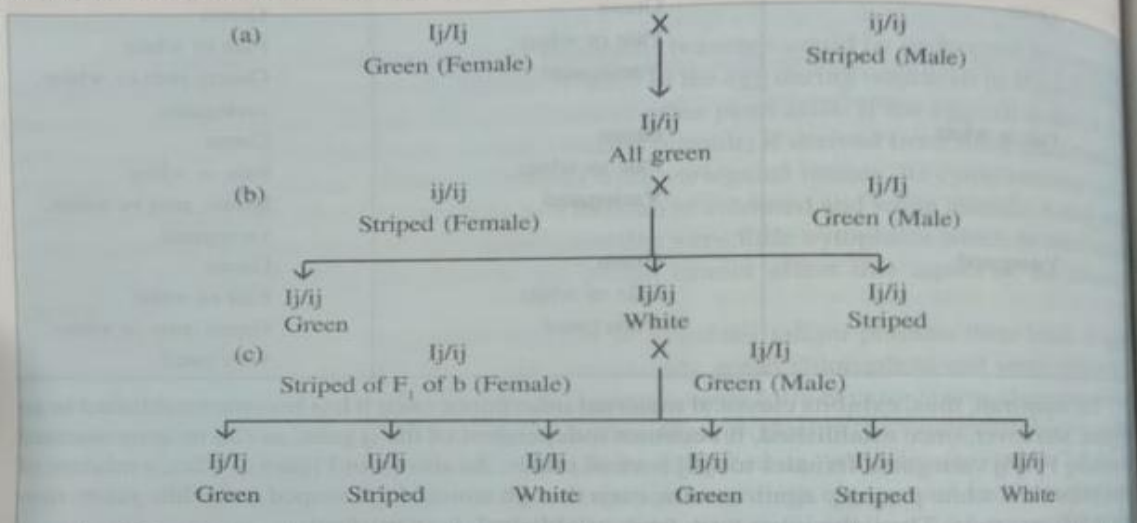


Fig. 17.5. (a) Cross between green (normal) and striped (iojap) plants. (b) Reciprocal of cross (a) (c) Cross of F_1 striped females (of cross b) to normal (green) males.

The petite mutants can be **segregational**, *i.e.*, they follow mendelian segregation and, therefore presumably controlled by chromosomal genes. They may also be **vegetative**, *i.e.*, non-segregational or extra-chromosomal. The genetic basis of petite character is a cytoplasmic factor ρ+ (rho) which may be absent or defective in petites. Thus, a vegetative petite can be **neutral** (ρ^0) which completely lacks ρ^+ or it may be **suppressive** (ρ^-) having a defective ρ^+. The neutral petites are not transmitted while suppressive petites are transmitted to a fraction of vegetative diploid progeny. In various strains of yeast, the suppressiveness varies from 1–99 per cent petites. The following two lines of evidences have suggested the association of ρ^+ with mitochondrial DNA (mt DNA); (1) **Ethidium bromide**, which induces petite mutations with 100 per cent efficiency, causes degradation of mt DNA after prolonged exposure of cells. In fact, neutral petites have been found lacking in mt DNA. (2) Supressive petites contain mt DNA which is greatly altered in base composition with respect to wild mt DNA.

Maternal effects-shell coiling in snail

MATERNAL EFFECTS

The development of some characters in several organisms is either governed or markedly influenced by the genotype of the female parent; this is known as ***maternal effect***. Although, such characters are governed by nuclear genes, they still show the following features: (1) reciprocal differences in F_1; (2) which, in most cases, disappear in F_2; and (3) a considerably smaller variation in F_2 as compared to that in F_3. In some extreme cases, there may be no phenotypic segregation in F_2; in such cases, phenotypic segregation is evident only in the F_3 generation. However, in most cases, the effects of segregation are apparent in F_2 itself, but the range of variation in the trait observable in the F_2 is relatively much smaller than that in the F_3. Many important characteristics of both plants and animals show maternal effects, of which some examples are described below.

An extreme example of maternal effect is known in the snail *Limnaea*. In this snail, the direction of coiling of its shell is controlled by a single nuclear gene *D/d*; the dominant allele *D* produces right-handed coiling, while its recessive allele *d* produces left-handed coiling. *The direction of shell coiling in an individual is governed by the genotype of its female parent and not by its own genotype.* As a result, reciprocal

crosses show differences in coiling in F_1 and there is no phenotypic segregation in F_2; the phenotypic effect of segregation is observable in F_3 only.

Crosses between females with left-handed coil (*dd*) and males having right-handed coil (*DD*) produce F_1 progeny (*Dd*) with left-handed coil, since the genotype of the female parent is *dd*. In F_2, segregation of *Dd* produces three genotypes (*DD*, *Dd*, *dd*) in the ratio 1 : 2 : 1. But the F_2 snails with *DD*, *Dd* as well as *dd* genotypes exhibit right-handed coiling since their female parent has the genotype *Dd* which determines right-handed coiling in the progeny (irrespective of the genotypes of the progeny). The F_3 progeny from the F_2 individuals with the genotypes *DD* and *Dd* will show right-handed coiling, while those from *dd* F_2 individuals will exhibit left-handed coiling of their shells; this produces the typical 3 : 1 ratio (right-handed : left-handed) in F_3 (Fig. 43.1).

The reciprocal cross (♀ right-handed, *DD* × ♂ left-handed, *dd*), on the other hand, yields right-handed coiling in the F_1 (*Dd*) as well as in the three genotypes, 1 *DD* : 2 *Dd* : 1 *dd*, obtained in the F_2. But in F_3, 2/3 of the progenies show right-handed coiling since they are derived from F_2 individuals having the genotypes *DD* and *Dd*. The remaining 1/3 of the F_3 progenies exhibit left-handed coiling since their female parents had the genotype *dd*; this yields the typical monohybrid ratio of 3: 1 in the F_3.

The features of inheritance of coiling of *Limnaea* shells may be summarised as follows: (1) F_1s from reciprocal crosses show differences in coiling pattern, (2) homogeneous coiling in F_2, *i.e.*, no segregation in F_2, and (3) appearance of the typical 3 : 1 ratio in F_3 in place of F_2. The 3 : 1 ratio (although observable only in F_3) clearly indicates that coiling of shells is governed by a single nuclear gene. But the segregation of this nuclear gene is apparently delayed by one generation. In fact, the segregation of nuclear gene *Dd* is normal and occurs in the F_2 generation itself, as is the case in all other cases of mendelian inheritance. But the phenotypic effects of this segregation are visible only one generation after the actual segregation of the alleles, *i.e.*, in the F_3. This is because *the direction of coiling in this snail is determined by the plane or the direction of the first mitotic division of the zygote. The plane of the first division, on the other hand, is determined by some substances already present in the egg cell.* Obviously, these substances are produced by the female parent; as a result, they would produce the phenotype appropriate for the maternal genotype. Further, genotype of, the zygote itself has no effect on the plane of first division and, consequently, on the direction of coiling since its gene products are not involved in determining this trait. As a result, *the direction of coiling in an individual is governed by the genotype of its female parent. Therefore, phenotypes appear one generation later than the appearance of the concerned genotypes, producing delayed segregation in* F_3.

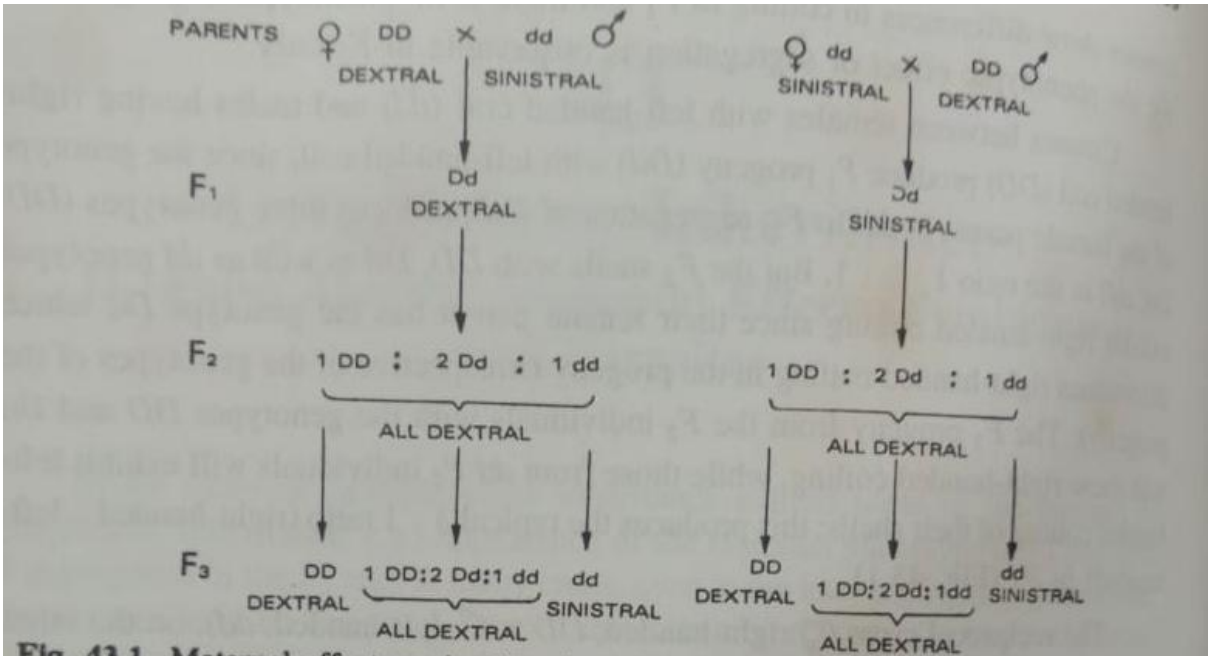


Fig. 43.1. Maternal effect on the direction of shell coiling in *Limnaea* (a snail), and the delayed segregation for coiling. The coiling of an individual is determined by the genotype of its maternal parent.

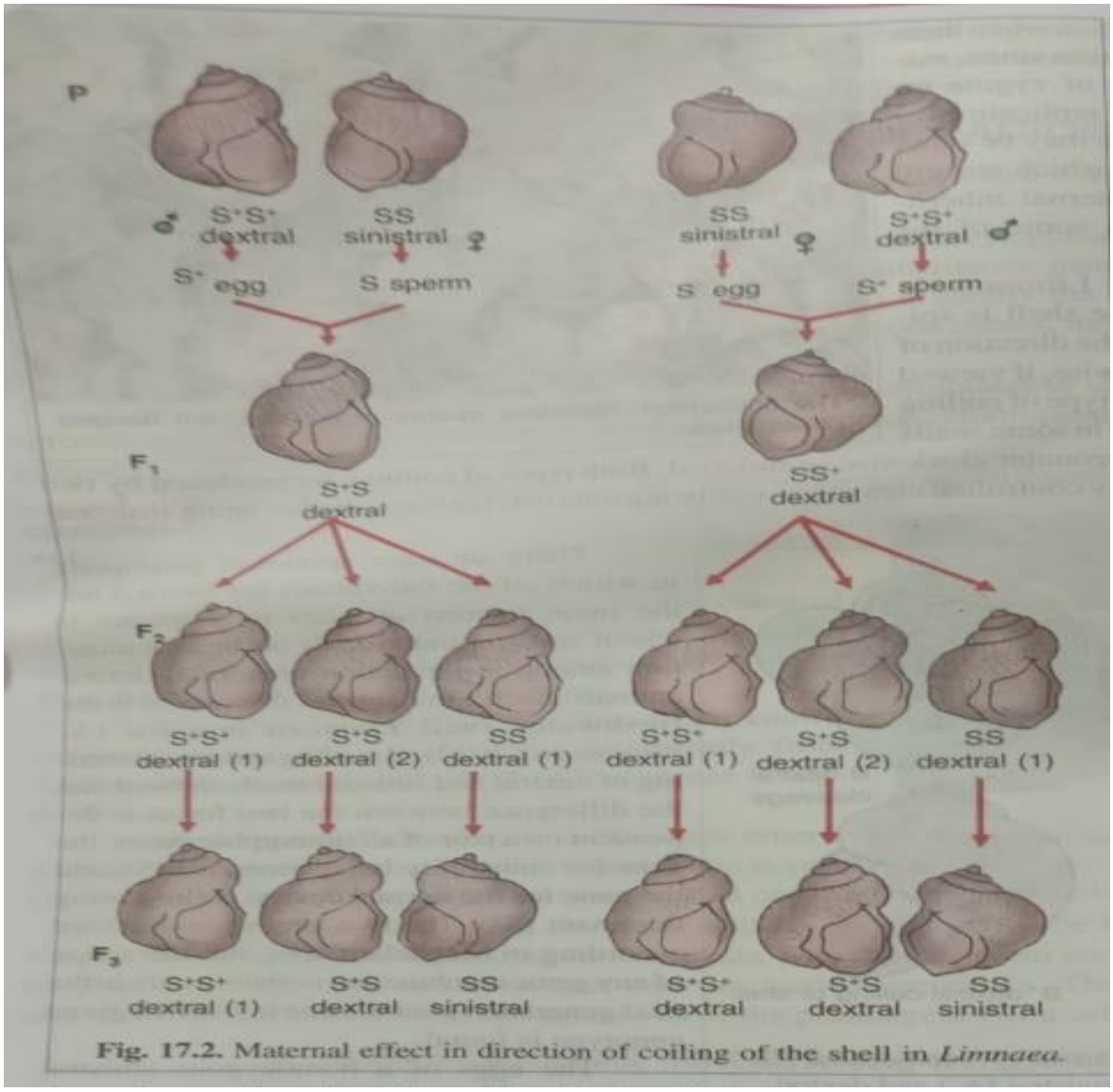
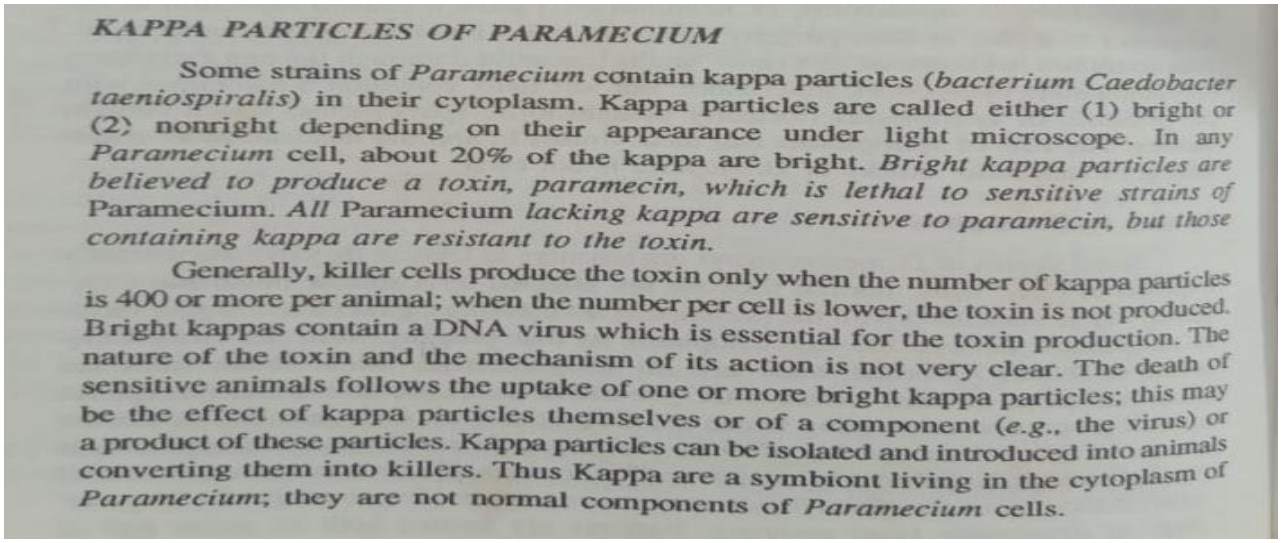


Fig. 17.2. Maternal effect in direction of coiling of the shell in *Limnaea*.

Infective heredity- Kappa particles in *Paramecium*



KAPPA PARTICLES OF PARAMECIUM

Some strains of *Paramecium* contain kappa particles (*bacterium Caedobacter taeniospiralis*) in their cytoplasm. Kappa particles are called either (1) bright or (2) nonright depending on their appearance under light microscope. In any *Paramecium* cell, about 20% of the kappa are bright. *Bright kappa particles are believed to produce a toxin, paramecin, which is lethal to sensitive strains of* Paramecium. *All* Paramecium *lacking kappa are sensitive to paramecin, but those containing kappa are resistant to the toxin.*

Generally, killer cells produce the toxin only when the number of kappa particles is 400 or more per animal; when the number per cell is lower, the toxin is not produced. Bright kappas contain a DNA virus which is essential for the toxin production. The nature of the toxin and the mechanism of its action is not very clear. The death of sensitive animals follows the uptake of one or more bright kappa particles; this may be the effect of kappa particles themselves or of a component (*e.g.*, the virus) or a product of these particles. Kappa particles can be isolated and introduced into animals converting them into killers. Thus Kappa are a symbiont living in the cytoplasm of *Paramecium*; they are not normal components of *Paramecium* cells.

Before discussing the transmission of kappa particles, it will be beneficial to understand the different modes of reproduction in *Paramecium*. These protozoa reproduce both asexually (binary fission) and sexually (autogamy and conjugation).

Asexual Reproduction

Paramecium reproduces asexually by means of binary fission (Fig. 43.3). Each animal has two diploid micronuclei and a large macronucleus. *The macronucleus*

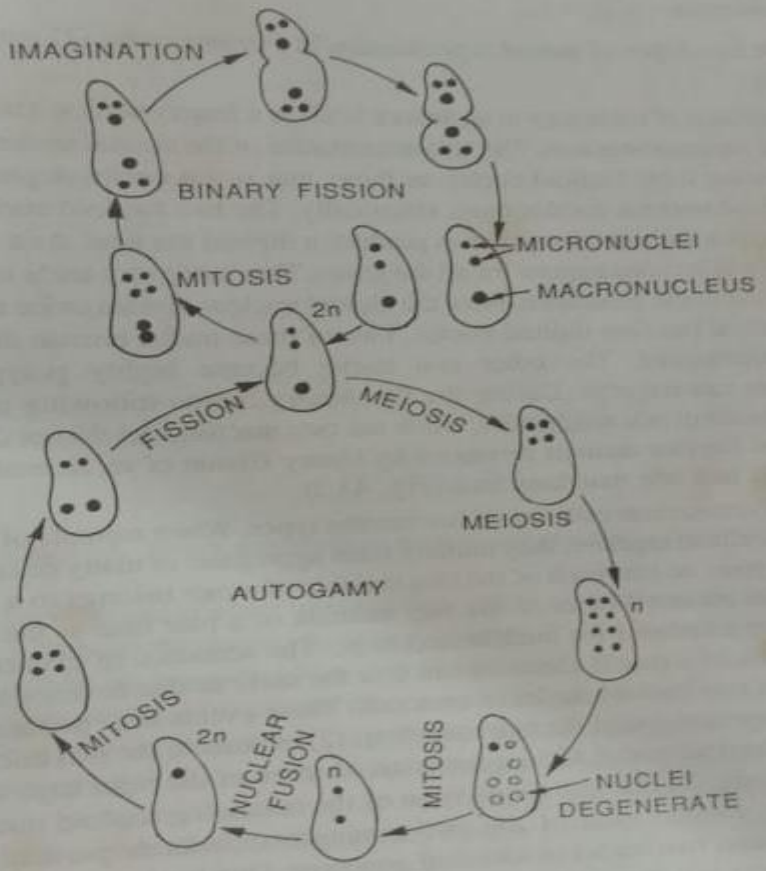


Fig. 43.2. Binary fission and autogamy in *Paramecium*.

governs the phenotype of the animals while the micronuclei participate in reproduction. During binary fission, the two micronuclei of each animal (*Paramecium*) undergo one mitotic division to yield a total of four diploid nuclei. About the same time, the macronucleus constricts in the middle and divides into two parts. Two

diploid nuclei (produced from the micronuclei) and one half of the macronucleus move to the anterior portion of the animal. The remaining two micronuclei and the other half of the macronucleus migrate to the posterior part of the cell. Now the plasma lemma begins to ivaginate around the middle of each animal. The constriction due to invagination progressively increases and finally divides the cell into two daughter cells each of which is identical to the parent animal in its genotype. Thus all the animals obtained through binary fission of a single animal are identical with each other and with the parent animal. A *Paramecium* may divide by binary fission once every six hours under optimum conditions.

Sexual Reproduction

There are two types of sexual reproduction in *Paramecium:* (1) autogamy and (2) conjugation.

Autogamy. Initiation of autogamy in an animal leads to a fragmentation and ultimately degeneration of its macronucleus. The two micronuclei of the animal undergo meiotic division, producing eight haploid nuclei; of these, any seven nuclei degenerate. The remaining haploid nucleus divides once, mitotically. The two haploid nuclei, having identical genotypes, thus obtained fuse to produce a diploid nucleus. As a result, this diploid nucleus will be homozygous for all the genes. Thus autogamy leads to complete homozygosity in a single generation. Now the diploid nucleus divides twice mitotically so that each animal has four diploid nuclei. Two of these nuclei remain diploid and function as micronuclei. The other two nuclei become highly polyploid and differentiate into macronuclei. During the first binary fission following autogamy, the two micronuclei divide mitotically, while the two macronuclei do not divide. As a result, each of the two animals produced by binary fission of an animal receives two micronuclei and one macronucleus (Fig. 43.2).

Conjugation. *Paramecium* cells are of two mating types. When animals of different mating types are mixed together, they initially form aggregates of many cells. Finally, they assort into pairs so that each of the two animals of a pair belongs to a different mating type. The plasma lemma of the two animals of a pair fuse at the point of contact providing a channel for nuclear exchange. The sequence of events in each of the two animals of a pair is identical and it is the same as that in autogamy upto the production of two haploid nuclei in each cell. These events may be summarised as folllows: (1) degeneration of the macronucleus, (2) meiosis in the two micronuclei producing eight haploid nuclei, (3) degeneration of seven of the eight haploid nuclei produced by meiosis, (4) one mitotic division of the remaining haploid nucleus. At the end of these events, each of the two conjugants (animals participating in conjugation) contains two nuclei of identical genotype. One haploid nucleus of one animal now migrates into the other animal and *vice-versa*, that is, the two conjugants exchange one haploid nucleus. Thus at the end of conjugation, each of the two animals has two haploid nuclei: one of these nuclei is derived from its own micronucleus, while the other nucleus is contributed by the other animal of the pair. The two nuclei in each animal fuse to produce a single diploid nucleus; obviously, the two animals of a pair will have diploid nuclei of an identical genotype (Fig. 43.3).

are not lethal to sensitive animals but it is the toxin liberated by the killer cells in the culture medium which produces the lethal effect. When killer animals are transferred into a fresh medium, the toxic level of paramecin is produced only after several hours. Therefore, when killer and sensitive cells, excluding the culture medium, are transferred into a fresh medium, the sensitive cells survive in the fresh medium since there is little or no toxin in the medium.

Kappa particles, like other bacteria, multiply through fission. However, their multiplication in the cytoplasm of an animal depends on the presence of a dominant nuclear gene *K* in the animal. Kappa particles are able to multiply only in the animals having the genotype *KK* or *Kk*. In *kk* animals, kappa are unable to multiply; as a consequence, they are eliminated through dilution following several binary fissions

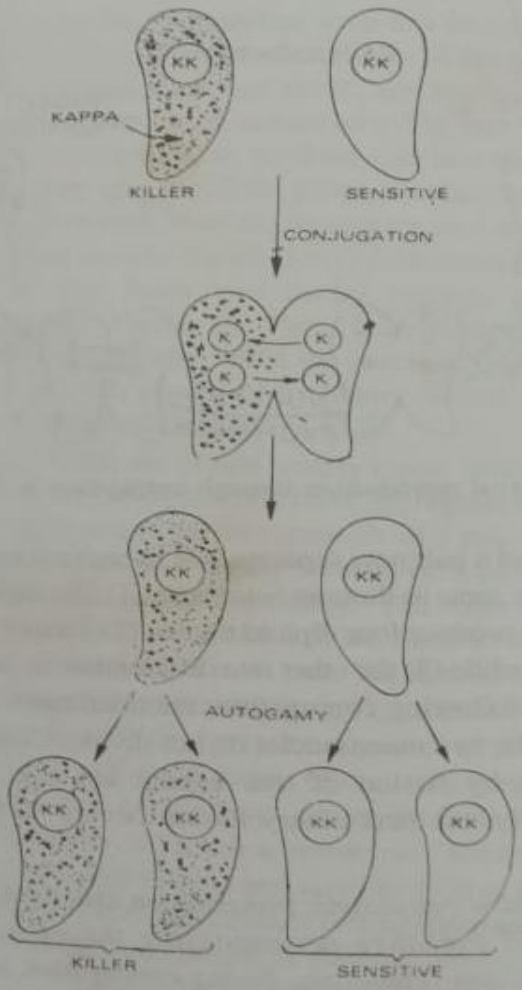


Fig. 43.4. Conjugation of *Paramecium* without the exchange of cytoplasm. Progeny from one exconjugant are killers, while those from the other are sensitives. For convenience, only one of the two micronuclei is shown, and the macronucleus is not shown.

of such animals. Thus killer animals with *kk* genotype ultimately become sensitive due to a loss of their kappa particles.

Sometimes, cytoplasmic exchange does occur during conjugation in *Paramecium*. When a cytoplasmic exchange takes place during conjugation between a killer and a sensitive animal, both exconjugants receive kappa particles (Fig. 43.5). If both killer and sensitive animals have the nuclear genotype *KK*, both the exconjugant animals will have the genotype *KK* and will become killers. Therefore, autogamy in such exconjugants produces only *KK* progeny, all of which retain kappa and the killer trait (Fig. 43.5 situation A).

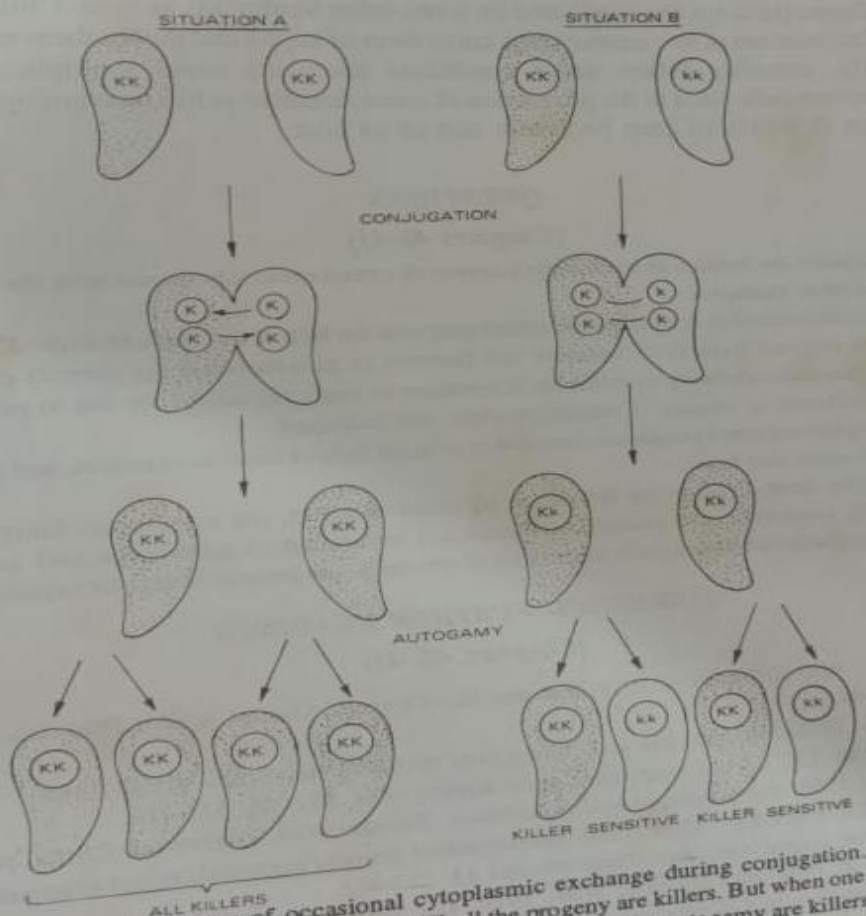


Fig. 43.5. Consequences of occasional cytoplasmic exchange during conjugation. When both animals are homozygous *KK*, all the progeny are killers. But when one animal is *KK* and the other *kk*, 50% of the progeny following autogamy are killers, while the rest (50%)

When the killer animal has the genotype *KK* and the sensitive animal is *kk*, the two exconjugants (animals after conjugation) will have the genotype *Kk*. If cytoplasmic exchange occurs in such a conjugation, the two animals will receive kappa particles and, therefore, will become killers. Autogamy in these exconjugants produces two types of progeny: 50% of the progeny are *KK* and the rest (50%) are *kk* (autogamy produces homozygosity for all the nuclear genes in a single step). Kappa particles will multiply normally in animals of the genotype *KK* and they will remain killers. However, kappa will be unable to multiply in animals having the genotype *kk*; as a result, kappa will disappear from such animals after several cell divisions (binary fissions). Therefore, 50% of the animals following autogamy in the exconjugants are killers, while the remaining 50% of the cells become sensitive (Fig. 43.5, situation B).

Kappa particles are eliminated by some other treatments as well. Culturing of killer *Paramecium* at low temperatures cures them of kappa and makes them sensitive. Similarly, culturing killers under conditions favouring rapid multiplication of *Paramecium* cells leads to the production of some sensitive cells since multiplication of kappa is unable to keep pace with that of its host.

Fine structure of gene

Unit 5: Fine structure of gene (6 lectures)

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Classical vs molecular concepts of gene

THE CHANGING CONCEPT OF 'GENE'

According to the classical concept, gene is the unit of function, recombination and mutation. Thus gene is considered to control the inheritance of one character (*unit of function*), to be indivisible by recombination (*unit of recombination*) and to be the smallest unit capable of mutation (*unit of mutation*).

It was soon realized that a gene does not produce a character by itself, although it may exercise the major control on its development. During the 1940's it became evident that a *a gene controlled a single biochemical reaction by directing the production of a single enzyme.*

But it was soon discovered that *one gene produces a single polypeptide and not one enzyme.* This is because a single protein may consist of more than one polypeptide. It was further established that DNA is the genetic material, that a single gene has several sites capable of mutation (the smallest unit capable of mutation is called *muton* after Benzer), and that recombination occurs within genes (the unit of structure not subdivisible by recombination was termed as *recon* by Benzer; see, later).

It can be shown that muton and recon represent a single nucleotide/base pair of a DNA molecule. However, in practice, mutations occurring in several different neighbouring nucleotides may map at the same site due to the limits of resolution through recombination test. Ordinarily it is not possible to separate recombination values showing very small differences from each other unless impractically large progeny populations are effectively screened. Therefore, many mutations, although affecting deferent mutons (and hence recons), may map at the same site giving the impression that a recon is composed of several mutons; this is more likely in eukaryotes than in prokaryotes since in the case of latter very large populations can be effectively screened.

Thus the gene may now be defined as a *segment of DNA which codes (contains the information) for a single polypeptide (the functional unit). At the operational level, the alleles of a single gene do not show complementation in a cis-trans test, while those of different genes show complementation.* Clearly, the operational approach defines the *cistron* of Benzer, which is regarded by most geneticists as a synonym for gene.

It may be pointed out that the above two approaches (functional and operational) define a *structural gene* (a gene coding for a polypeptide) only; but many genes either code for RNA only (*e.g.*, genes for rRNA, tRNA and possibly chromosomal RNA) or do not code for any gene product and act as binding sites for various molecules for the regulation of gene action (*e.g.*, promoter and operator genes).

THE FINE STRUCTURE OF GENES

The fine structure of a gene is essentially the linkage map of its various mutant alleles. In this map, the sites of mutational changes in different alleles of the same gene are determined on the basis of the frequency of recombination among these alleles. Thus the fine structure map of a gene is essentially comparable to the linkage map of a chromosome [except for the number of genes involved (one in the case of gene fine structures, and several in the case of chromosome maps)]. Understandably, the values of crossing over observed in the studies on fine structure of a gene are very low (*e.g.*, 0.001 or even 0.0001%); therefore, extremely large progeny populations have to be effectively screened for efficient identification of the crossover products. For this reason, fine genetic analysis is more convenient in prokaryotes than in eukaryotes, and is more readily accomplished in the case of conditional lethal alleles which permit a very efficient recovery of the crossover products.

Cis-Trans complementation test for functional allelism

CIS-TRANS TEST

In a *cis-trans test*, the phenotypes produced in *cis-* and *trans-* heterozygotes for two mutant alleles are compared with each other. In a *cis-heterozygote*, both the mutant alleles are located in the same chromosome and their wild type alleles are present in the homologous chromosome, *i.e., the mutant alleles are linked in the coupling phase* (Fig. 24.2). *Cis-heterozygotes are expected to produce the wild type phenotype (unless the mutant alleles are dominant or codominant) irrespective of whether the two mutant alleles are located in the same gene or in two different genes.*

On the other hand, the two mutant alleles are located in the homologous chromosomes, *i.e.*, one mutant allele is located in each homologue, in the case of *trans-heterozygotes. Clearly, the mutant alleles are linked in the repulsion phase. Trans-heterozygotes are expected to produce the mutant phenotype if the two alleles are located in the same gene. But if they are situated in two different genes, the wild type phenotype would be obtained.*

Thus by comparing the phenotypes produced in *cis-* and *trans-*heterozygotes for any two mutant alleles it is possible to decide if they are located in the same gene or in two different genes. *They are placed in the same gene if their cis-heterozygotes produce the wild type phenotype, while their trans-heterozygotes have the mutant phenotype. But if both their cis- and trans-heterozygotes have the wild type phenotype, they are considered to be located in two different genes.*

Complementation Test

Obviously, for a clear-cut interpretation of the results from a *cis-trans* test, data from both *cis-* and *trans-*heterozygotes must be available. But ordinarily the construction, identification and recovery of the chromosomes having both the mutant alleles for producing *cis-*heterozygotes is very difficult, time-taking and costly; in

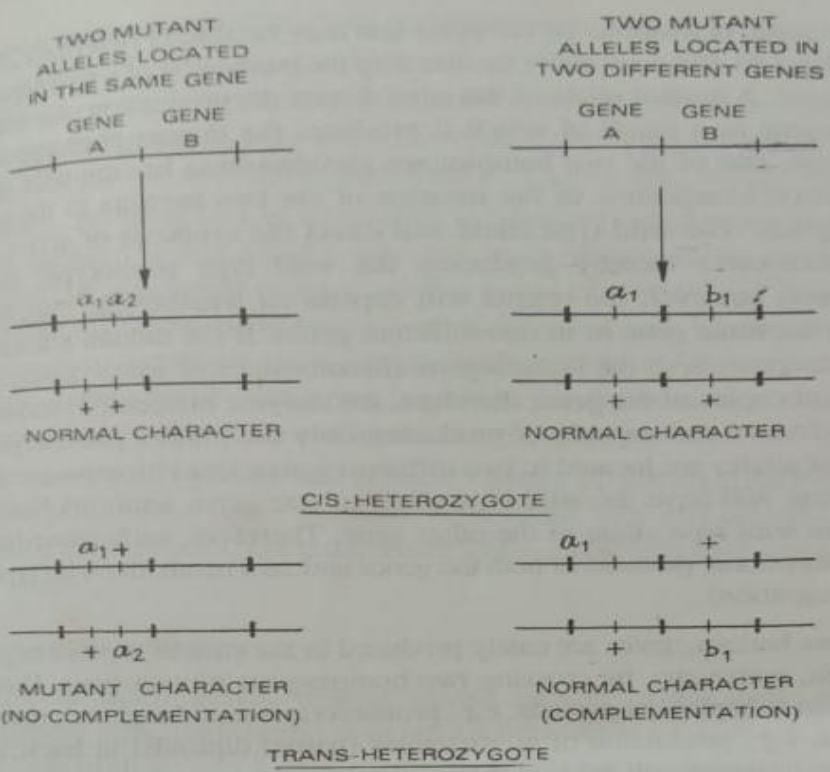

Fig. 24.2. Classification of multiple allels as 'true multiple alleles' or as 'pseudoalleles' on the basis of the *cis-trans* test, *i.e.*, comparison of the phenotypes produced by *cis*- and *trans*-heterozygotes for the two mutant alleles. This classification is based on the concept that mutant alleles located in the same gene do not show complementation; this is generally true in prokaryotes, but in eukaryotes several noteworthy exceptions are known.

many cases it may not be feasible. Therefore, the production of *cis*-heterozygotes is generally omitted, and data from only *trans*-heterozygotes are available. In such a study, *if trans-heterozygote has the mutant phenotype the two mutant alleles are placed in the same gene. But if it has the wild type phenotype, the mutant alleles are considered to be located in two different genes.*

The production of wild type phenotype in a *trans*-heterozygote for two mutant alleles is known as *complementation*, and such a study is called *complementation test*. The results from complementation tests are precise, highly reliable and they permit an operational demarcation of genes as follows: *mutant alleles located in the same gene do not show complementation, while those located in different genes show complementation.* Since, this delineation of a gene is based on complementation test, which is the *trans* portion of the *cis-trans* test, Benzer proposed the term *cistron* (derived from *cis-trans*) for such a unit. However, *most geneticists now use the term gene in the same sense as cistron*; the latter term is only occasionally used.

The Basis for Complementation Test

The basic philosophy of *cis-trans* test may be simply described as follows. A gene produces its effect primarily by directing the production of a polypeptide (protein) or an enzyme. A mutant allele of the gene directs the production of a defective form of the enzyme as a result of which it produces the mutant phenotype. In the *cis*-heterozygote, one of the two homologous chromosomes has the wild type allele(s) of the gene(s) irrespective of the location of the two mutants in the same or two different genes. The wild type allele will direct the synthesis of active enzyme (or protein) molecules thereby producing the wild type phenotype. In the *trans*-heterozygote, however, the results will depend on whether the mutant alleles are located in the same gene or in two different genes. If the mutant alleles are located in the same gene, both the homologous chromosomes of *trans*- heterozygotes will have mutant copies of the gene; therefore, the enzyme molecules produced by them will be defective and capable of producing only the mutant phenotype. But if the two mutant alleles are located in two different genes, one chromosome of the *trans*-heterozygote will have the wild type allele of one gene, while its homologue will contain the wild type allele of the other gene. Therefore, such *trans*-heterozygotes will have functional products of both the genes and, as a result, the wild type phenotype (*complementation*).

Trans-heterozygotes are easily produced in the case of diploid organisms, like *Drosophila*, maize etc., by crossing two homozygous mutant lines. However, thier construction in haploid organisms, *e.g.*, prokaryotes, many fungi etc., requires special techniques, *e.g.*, production of merozygotes (partial diploids) in bacteria, infection of a single bacterial cell by two different mutant phage particles etc.

The results from complementation (or cis-trans) tests are clear-cut when the mutants used in the study are nonpolar (see, next section), recessive, and produce either no gene product (protein or enzyme) or totally defective gene products. Mutants ideal for complementation test are, deletion mutants in which a large segment of the gene is deleted, frame-shift mutants and nonsense mutants (in eukaryotes only).

The results from complementation tests are likely to be ambiguous if the active product of the gene in question is a multimer (made up of two or more polypeptides: these polypeptides may be homologous, that is products of a single gene, or they may be nonhomologous, *i.e.*, products of two or more different genes), or when mutations causing single amino acid substitutions in the protein/enzyme are used in the study.

Limitations of Complementation Test

Complementation (or *cis-trans*) test does not provide useful information in the following situations.

1. Where the mutant alleles are dominant or codominant.
2. When the mutant alleles located in the same gene show complementation.
3. In cases where the mutant alleles are polar mutations.

4. When the gene in question does not produce a 'diffusible' gene product, *e.g.*, protein.

A valid *cis-trans* test is possible only when the *cis*-heterozygote produces the wild phenotype, which is possible only when the mutant alleles are recessive. As a result, *cis-trans test cannot be applied to dominant or codominant mutant alleles.*

Similarly, *cis-trans* test is applicable to 'nonpolar' mutations only. A *polar mutation*, in addition to producing a defective gene product of the concerned gene, interferes with the expression of other genes located on one side of this gene. Such mutations generally occur in the operons of prokaryotes (see, Chapter 36), and are, in fact, nonsense (polypeptide chain-terminating) mutations. Since a polar mutation suppresses the expression of other genes located in its neighbourhood, it cannot show complementation with the mutant alleles of such genes.

Some genes, *e.g.*, operator and promotor genes, do not code for a polypeptide or an enzyme (a diffusible gene product). As a result, they can act only in the *cis*-position; hence they cannot show complementation. Therefore, such genes are called *'cis-acting genes'*.

Intragenic Complementation

In case of many genes, mutant alleles located in the same gene show complementation, which is referred to as *intragenic complementation*. It is generally observed in case of those genes whose active products are multimers of homologous polypeptides. Such a multimer molecule may be either a homomultimer or a heteromultimer. A *homomultimer* is composed of two or more polypeptides produced by a single allele of the gene, while in a *heteromultimer* polypeptides produced by both the alleles of a gene are present. *In general, homomultimer molecules produced by mutant alleles are inactive, but often heteromultimer molecules are partially or completely active.* In many cases of intragenic complementation, the active form of the enzymes produced by the *trans*-heterozygotes have been purified and shown to be heterodimers or heterotetramers of the mutant polypeptides produced by the two mutant alleles of the same gene. The reasons for activity of such heteromultimers are not clearly understood, but this definitely involves the three-dimensional organisation of biologically active protein molecules.

In general, amorphic mutations yield clear-cut results from complementation test. But data from complementation tests on hypomorphic mutants are usually ambiguous due to intragenic complementation; this is the case both in prokaryotes and eukaryotes. An *amorphic mutation* leads to a complete loss of function of the concerned gene product, *i.e.*, the gene product is either absent or totally inactive. But in the case of a *hypomorphic mutation*, the gene product is partially active, although the level of its activity is markedly lower than that of the wild type gene product. In general, *hypomorphic mutations are likely to show intragenic complementation, while amorphic mutations are unlikely to do so.*

Structure of Phage T4

THE FINE STRUCTURE OF A GENE IN A PROKARYOTE

The most extensive fine structure map of a locus constructed to date is that of the r^{II} locus of T_4 phage of *E. coli* due to Seymour Benzer. T_4 is similar to T_2 in morphology, is an obligate parasite like all viruses, and contains a chromosome of about 200,000 bp (base pairs) long, which is packed within its head. When a T_4 phage particle infects a cell of *E. coli*, the bacterial cell lyses in about 20-25 minutes liberating 200-300 progeny phage particles.

When *E. coli* cells are plated (in an agar medium in petriplates) in sufficiently large numbers they produce a uniform confluent growth or 'lawn'. If individual T_4 particles are placed on the surface of an agar medium seeded with a lawn of *E. coli* cells, each phage particle would initiate a chain of infection-lysis so that all the *E. coli* cells in the immediate vicinity of the phage particles will be lysed. This leads to the development of clear zones, called *plaques*, in the lawn of bacterial cells. The plaques produced by the wild type T_4 particles are relatively small with fuzzy or turbid margins called *halos*. The halos are produced due to a phenomenon called *lysis inhibition*, which is a delay in the lysis of T_4-infected *E. coli* cells as a consequence of its subsequent infection by another T_4 particle.

rII Locus.

The R^{II} Locus

Several mutants of T_4 do not exhibit lysis inhibition, and produce relatively large plaques with clear margins; these mutants are called *rapid lysis* mutants and are denoted by *r*. Most of the *r* mutants map in one of the three distinct loci called r^{I}, r^{II} and r^{III}. Mutants in the r^{II} locus are easily recognised due to their inability to multiply in *E. coli* strain K12(λ), which has the chromosome of phage λ integrated in its chromosome. However, r^{II} mutants grow rapidly in other strains of *E. coli*, *e.g.*, strain B and K12 (lacking the λ chromosome). Thus r^{II} mutants are conditional lethals as they are unable to grow in K12(λ); this property was exploited by Benzer for a fine genetic analysis of the r^{II} locus.

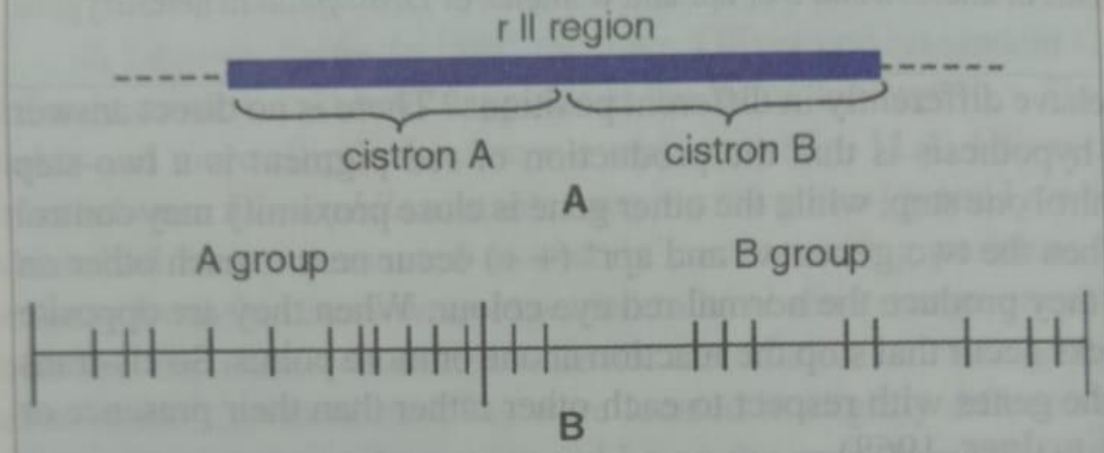

Fig. 11.7. A—rII region of T_4 phage showing two cistrons; B–Gene map of rII locus.