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Faculty of Natural and Life Sciences.

Genetics

Course handout dedicated to 2nd Year License students in Agronomical Sciences, and Biology.

Prepared by:

Dr. Fatima Dahlia.

Dr. Soria Barouagui.

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Introduction to genetics

Genetics is the science of heredity. Its applications invade our daily lives. Whether it is prenatal diagnosis, agronomy, biology or forensic science, the precision of genetic methods is appreciated. It is both a science, a scientific theory of biological heredity, and an investigative tool exportable to all fields of biology, in order to undertake the analysis of the most varied phenomena.

Genetics studies the transmission of traits from one generation to the next as well as the passage of genes to traits. The discoveries of sexuality, Mendel's laws, the modalities of fertilization and the formation of gametes, the notions of gene, genotype and phenotype are also important for the history of genetics in all its dimensions.

One of the most fascinating properties of life is that it reproduces itself every generation from single cells such as zygotes (fertilized eggs). This regeneration has existed since the origin of life and all organisms currently living on Earth, from the smallest such as bacteria to the largest such as whales, result from millions of regeneration cycles.

The history of genetics begins with the discovery of sexuality and reproduction. In animals and humans, this discovery is very old, and dates back beyond Antiquity, while in plants it is much more recent, with its discovery at the end of the 17th century by "Rudolf Jakob Camerarius" and its general recognition during the 18th century.

At that time, in plants as in animals, the notions of heredity, fertilization and embryo development were confused in the notion of generation, in the sense of reproduction of individuals. Naturalists, even philosophers, sought to explain the ability of living organisms to reproduce by giving descendants who, overall, resemble them. They were interested at the same time, in a non-distinct way, in reproduction, in the formation of the embryo in animals and in the transmission of characters, but with more interest in the first two points. To witness the emergence of a science of heredity, seen as the study of resemblances between parents and their children, it was necessary to wait until a separation was conceived between reproduction, the development of the embryo and the transmission of characters from generation to generation; this could only really be achieved after the work of "Gregor Mendel" who published in 1865 the result of his experiments on the crosses of strains of peas showing variations transmitted from one generation to the next. Mendel not only provided the experimental results of controlled crosses, but he also deduced the existence of discrete "factors" that transmit developmental information from a parent to its offspring.

In 1900, the Dutch botanist "Hugo De Vries" rediscovered the laws established by Mendel and published in a text entitled "on the law of segregation of hybrids", considered as the birth certificate of genetics even if this name was not given to the new discipline until a few years later. In the same year, almost simultaneously, Carl Correns and Erich "Von Tschermak" obtained the same results as "De Vries". It was then extended to animals by "Lucien Cuenot and William Bateson".

At the beginning of the 20th century, it became evident that the information specifying the development of organisms was contained in the chromosomes of the cell nucleus. The accumulation of information elements, begun in the 1920s, led to the conclusion that DNA is the genetic material.

Chapter 1 :
Qualitative genetics in diploid individuals

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Qualitative or Mendelian genetics explains the hereditary transmission of genes by the chromosomal theory of heredity. Going from simple to complex, monohybridism establishes this law from the study of crosses between individuals that differ in the genetic composition of the alleles of a single locus. Then, plurihybridism extends and generalizes this law from the study of crosses between individuals that differ in the genetic composition of the alleles of several loci. The chromosomal theory of heredity will be established first from genes located on different chromosomes on the same chromosome and genes located on autosomes and then will be generalized later to genes located on sex chromosomes or heterosomes.

1. Terminology

1.1. Chromosome

Morphological support of genetic information, located in the nucleus of cells. They are constant and even in number in all somatic cells of the same individual and in all individuals of the same species. The two chromosomes of the same pair are said to be homologous. The sex chromosomes "X and Y" are called heterosomes while the other chromosomes are called autosomes.

1.1. Gene

A structure of molecular order, constitutive of DNA and therefore of chromosomes, controlling a particular hereditary trait. It is a unit of heredity and a functional unit coding for a polypeptide. It is transmitted by the individual to its offspring.

In a diploid cell, a gene is controlled by 2 alleles while in a haploid cell, a gene is controlled by only 1 allele.

1.2. Allele

Name given to each of the variants of the same gene. Alleles direct the same character but they make it take different forms. Alleles can be :

- ✓ **Allèle létal** : quand il qualifie une anomalie ou tue l'individu porteur. La plupart des gènes létaux sont à l'état récessif, ils ne peuvent donc agir que chez les individus homozygotes au locus concerné.
- ✓ **Dominant allele** : when it masks the expression of the other allele.
- ✓ **Recessive allele** : if its expression is masked by the other gene of the pair.

- ✓ **Codominant allele** : when it is expressed jointly with the other gene of the pair.
- ✓ **Lethal allele** : when it qualifies an anomaly or kills the carrier individual. Most lethal genes are recessive, so they can only act in individuals homozygous at the locus concerned.

1.3. Locus

It is the location or position of a DNA sequence, a gene or a marker on the same chromosome (plural = loci).

1.4. Gamete

Male (spermatozoon or pollen grain) or female (ovum) reproductive cell. Its nucleus contains n chromosomes, so it is a haploid cell.

1.5. Phenotype

Is a measurable or distinctive characteristic of an organism. This characteristic can be visible to the naked eye such as the color or texture of the hair, or requires special tests for its identification, such as a serological test for blood type. The phenotype is the visible expression of the genotype in a given environment.

1.6. Genotype

The set of alleles of an individual constitutes its genotype. It is the genetic constitution, limited to the genes studied in a particular cross. In other words, it is their allelic state that is studied.

1.7. Homozygous

The union of gametes carrying identical alleles at a given locus leads to a homozygous genotype. A homozygote produces only one type of gamete.

Example :

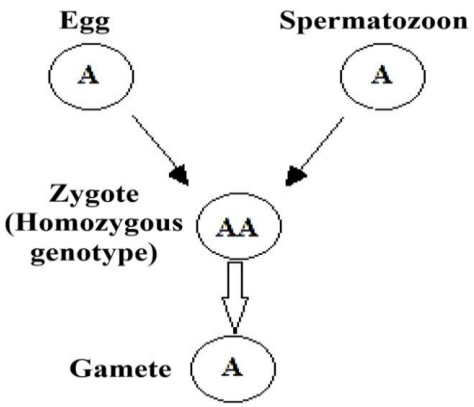


Figure 1 : Homozygous individuals

1.8.Heterozygous

The union of two gametes carrying different alleles, at the same locus, leads to a heterozygous genotype. A heterozygote produces several types of gametes.

Example :

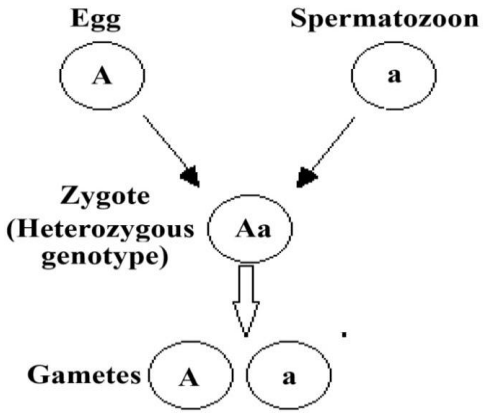


Figure 2 : Heterozygous individuals

1.9.Pure line

A group of individuals with a similar genetic heritage is often referred to as a species, a progeny, a line or a variety. Self-fertilization and crosses between closely related individuals for many generations generally produce a population that is homozygous at virtually all loci.

Crosses between homozygous individuals of a pure line produce homozygous offspring identical to the parents.

Example :

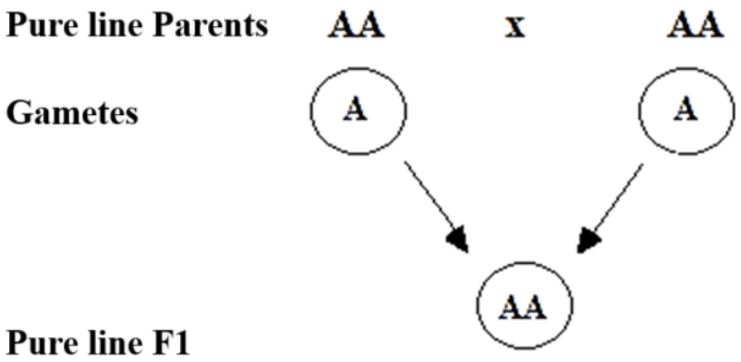


Figure 3 : Pure line individuals

1.10. Hybrid

The term hybrid is synonymous with the term heterozygote. It is the union between individuals that differ by at least one pair of alleles at a locus. The result of this union is an offspring carrying at this same locus the two different alleles of their parents.

Example :

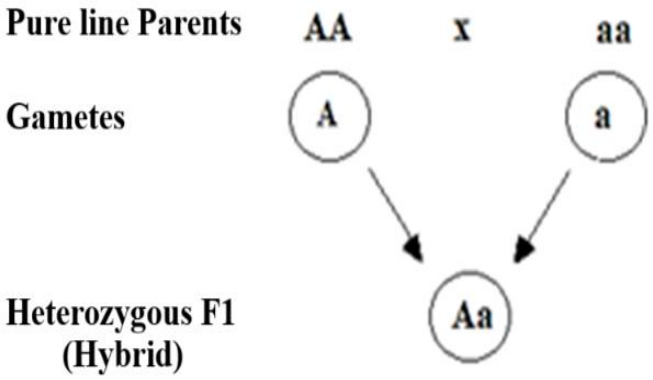


Figure 4 : Hybrid individuals

1.11. Self-fertilization

It is synonymous with autogamy and is the mode of sexual reproduction where the two gametes used come from the same parent.

2. Monohybridism

2.1.Mendelian inheritance

The simplest Mendelian cross involves strains varying for a single trait ; it is therefore referred to as a monohybrid cross. This cross is made between individuals of two pure parental strains each exhibiting one of the two possible phenotypes of the trait.

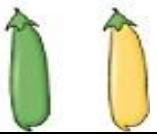
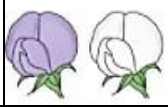
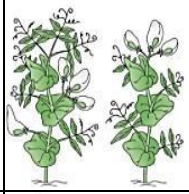
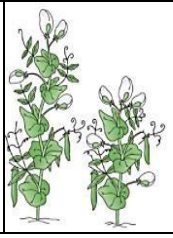
We first observe the first generation resulting from this cross, then we consider the descendants resulting from the self-fertilization of the individuals of this first generation. The parents at the origin of the cross, the parental generation, are designated by P1 ; the first generation resulting from the cross is designated by F1 and the second generation resulting from the self-fertilization of the F1 is designated by F2.

Mendelian inheritance (dominance and recessiveness)

The cross carried out by Mendel between two pure lines of pea plants. One of the plants has the tall phenotype and the other is dwarf phenotype, gives a homogeneous F1 generation of tall phenotype. By letting the F1 individuals self-fertilize, Mendel observes that among 1064 F2 individuals, 787 individuals are tall phenotype and 277 are dwarf phenotype. The dwarf phenotype disappeared in the F1 generation to reappear in the F2 generation ; in contrast, the other parental character (tall), which remains alone in F1, is said to be dominant.

Mendel also performed such crosses with each of the six other characters studied in the pea ; the results are similar to those observed for the size character (Table 1).

Table 1 : Mendel's results on the study of seven phenotypic pairs, associated with the seven characters studied in the pea.

Characters and phenotype pairs associated with character variability			**F1 Phenotype**	**F2 Phenotype**	**Ratio of numbers in F2**
Seed appearance	[Smooth]/[Wrinkled]		100%[Smooth]	[Smooth] : 5474 [Wrinkled] : 1850	2.96 : 1
Seed color	[Yellow]/[Green]		100%[Yellow]	[Yellow] : 6022 [Green] : 2001	3.01 : 1
Pod appearance	[Full]/[Constricted]		100%[Full]	[Full] : 882 [Constricted] : 299	2.95 : 1
Pod color	[Yellow]/[Green]		100%[Green]	[Yellow] : 428 [Green] : 152	2.82 : 1
Flower colour	[Violet]/[White]		100%[Violet]	[Violet]: 705 [White] : 224	3.15 : 1
Flower position	[Axial]/[Terminal]		100%[Axial]	[Axial] : 651 [Terminal]: 207	3.14 : 1
Plant height	[Tall]/[Dwarf]		100%[Tall]	[Tall] :787 [Dwarf]: 277	2.81 : 1

The data from a genetic analysis are often expressed and discussed in the form of ratios of observed or theoretical frequencies. In our example, out of all the P1 and F1 (100% [tall]), the 1064 F2 individuals are divided into 787 [tall] and 277 [dwarf] according to a 2.84 : 1 ratio; or approximately 3 to 1, which amounts to saying that ¾ of the F2 represents the phenotype observed in the F1 offspring while ¼ of the offspring F2 presents the recessive trait that disappeared in F1. The results of the other traits mentioned in table 1 show the recurrence of the ¾ : ¼ ratios.

To explain his observations, Mendel suggested that each observed trait was dependent on a pair of factors constituting particular units of heredity, transmissible from one generation to the next, without being altered and determining, according to their combinations, the possible form of the trait, that is to say the phenotype of the individual.

These precise and reproducible results led to the following interpretations :

- A hereditary factor called a "gene" is necessary to produce a trait ;
- Each plant has a pair of this type of gene ;
- The gene exists in two forms called "alleles", for example **G** for the tall plant and **g** for the short plant ;

A plant can be **G/G**, **g/g** or **G/g**. The slash indicates that the alleles form a pair ;

In a **G/g** plant, the **G** allele dominates so that the phenotype is tall. Therefore, the phenotype of the **G/g** plant defines the **G** allele as dominant and the g allele as recessive ;

During meiosis, the members of a gene pair are equally distributed between the ovules and the pollen grains. This is the law of "segregation of gametes" established by Mendel ;

For this reason, a single gamete contains only one member of the gene pair (a single allele).

Application example

A cross was made between two pure lines of peas; one has plants that have a giant stem and the other has plants that have a dwarf stem (Fig. 5). All the individuals of the F1 have a giant stem. The individuals of the F1 were crossed among themselves. The F2 individuals consisted of 787 plants with a giant stem and 277 plants with a dwarf stem.

Observations

☒ The parents differ by a single trait (stem size) and are pure lines ; they are therefore homozygous ;

☒ The individuals of the F1 have a single phenotype (giant stem), they are therefore similar and 100% homogeneous ;

☒ In F1, there is the appearance of only one parental phenotype (giant stem) to the exclusion of the other (dwarf stem), this means that the giant trait is dominant and the dwarf trait is recessive;

☒ In F2, there is the reappearance of the second parental phenotype (dwarf stem) with low values compared to those of the other phenotype (giant stem).

Genetic representation

Codes : **G** for giant and **g** for dwarf**.**

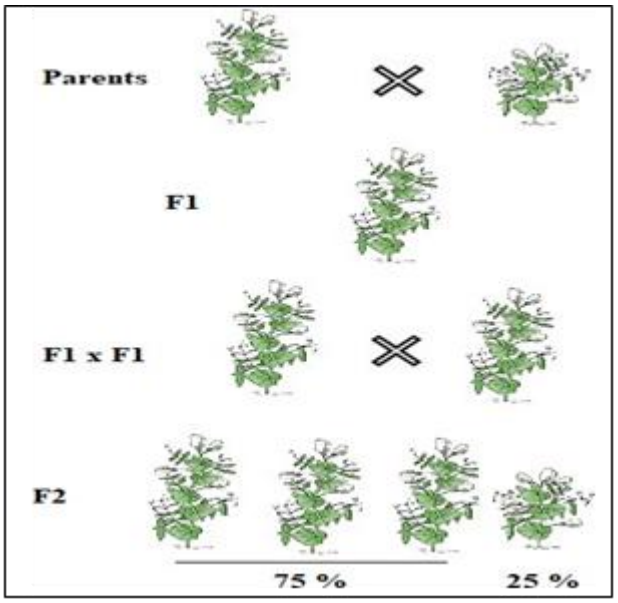
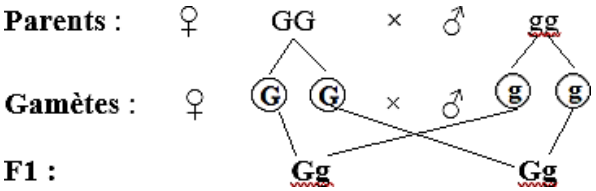


Figure 5 : Cross up to F2 of dwarf and giant pea plants.



Genotypes : **Phenotypes** :

Parents : ♀ GG **Parents :** ♀ [G]

♂ gg ♂ [g]

F1 : Gg **F1 :** [G]

We note that all the individuals of the first generation F1 are of the same genotype and the same phenotype and are therefore 100% similar.

From these results, Mendel established his first law which is the ***law of uniformity of the F1*** which says : "If two pure lines (homozygotes) are crossed for a pair of alleles, all the descendants of the first generation (the heterozygotes F1) are of identical external appearance (phenotype)".

F2 : ♀ F1 × ♂ F1

♀ Gg × ♂ Gg

Gametes : ♀ Ⓖ ⓖ × ♂ Ⓖ ⓖ

♀ \ ♂	Ⓖ	ⓖ
Ⓖ	**GG**	**Gg**
ⓖ	**Gg**	**gg**

Genotypes : **Phenotypes :**

F2 : ¼ GG : ½ Gg : ¼ gg **F2 :** ¾ [G] : ¼ [g]

By calculating the ratios observed in the example, we find that the ratio of plants with giant stems was 787/277 = 2.84 against 277/277 = 1 for plants with dwarf stems.

Comparing the results observed in the example with the theoretical results, we find that the proportions are almost equal.

Observed results	**Theoretical results**
Giant stem [G]: 787 / (787 + 277) = 0.739	**Giant stem [G]:** 3/4 = 0.75
Dwarf stem [g]: 277 / (787 + 277) = 0.260	**Dwarf stem [g]:** 1/4 = 0.25

The seeds produced (by self-fertilization) by the F2 plants were sown the following year and gave the third filial generation F3. Mandel found that the 787 giant-stemmed plants of the F2 were of two kinds since one third of them (262 plants) produced only giant-stemmed plants, while the other two thirds (525 plants) produced a ratio of about 3 giant-stemmed plants to 1 dwarf-stemmed plant. The 277 dwarf-stemmed plants of the F2 were, on the contrary, of a single kind since all of them produced in F3 only dwarf-stemmed plants identical to themselves.

F3 : ♀ F2 × ♂ F2

	♀ GG × ♂GG	♀ Gg × ♂ Gg	♀ gg × ♂ gg
	♀ Ⓖ Ⓖ × ♂ Ⓖ Ⓖ	♀ Ⓖ ⓖ × ♂ Ⓖ ⓖ	♀ ⓖ ⓖ × ♂ ⓖ ⓖ
F3 : Genotypes :	GG	¼ GG : ½ Gg : ¼ gg	gg
Phenotypes :	[G]	¾ [G] : ¼ [g]	[g]

Remarks

Giant-stemmed plants obtained in F2, which give in F3 only giant-stemmed plants, are homozygous (homozygotes produce only one type of gamete) ;

The giant-stemmed plants of the F2, which give in F3 both giant-stemmed plants and dwarf-stemmed plants, are heterozygous (heterozygotes produce several types of gametes ;

The plants of the F2 which have the recessive trait and which are dwarf-stemmed, produce in F3 only dwarf-stemmed plants. They are homozygous. The recessive trait is always homozygous.

From these results, Mendel established his second law which is the ***law of segregation of gametes (purity of gametes)*** and which says: "The two units that form a pair of factors in the homozygous state (GG) or heterozygous (Gg) segregate (or separate) during the formation of gametes, so that each gamete (male or female) contains only one of the two units that forms a

pair of factors".

2.2. Non-Mendelian inheritance (change in genotypic and phenotypic frequencies)

2.2.1. Incomplete or partial dominance (absence of dominance)

In the preceding paragraphs, the examples discussed showed complete dominance. In other words, the phenotype of the F1 generation was identical to that of one of the two parents (the dominant phenotype). This is not always the case. Often the F1 generation is clearly intermediate between the two parents.

There is an absence of dominance (incomplete or partial dominance) when neither of the parental phenotypes appears in F1, but a new phenotype appears which is ***intermediate*** between the two parental phenotypes.

Example

The inheritance of petal color in the four o'clock flower. When two purebred lines with white and red petals, respectively, are crossed, the F1 generation has pink petals rather than red and white petals (Fig. 6). The F2 generation comprises three classes of plants with the ratio 1 white : 2 pink : 1 red. The red-petaled flowers of the F2 crossed among themselves gave in F3 only red-petaled flowers. The white-petaled flowers of the F2 crossed among themselves gave in F3 only white-petaled flowers. The pink-petaled flowers of the F2 crossed among themselves gave in F3 three classes of plants with the ratio 1 white : 2 pink : 1 red.

Interpretation

☒ The parents differ by a single trait (petal color) and are purebred lines ; they are therefore homozygous ;

☒ The individuals of the F1 have a single phenotype (pink petals), they are therefore similar and 100% homogeneous ;

☒ In F1, there is the appearance of a phenotype (pink petals) which is intermediate between the two parental phenotypes (white petals and red petals) ;

☒ In F2, there is the reappearance of the two parental phenotypes (white petals and red petals).

☒ The F2 plants with red petals and those with white petals each gave, in F3, a single phenotype (red or white petals), they are therefore homozygous and each produce a single type of gamete. Whereas, the F2 plants with pink petals gave three classes of plants in F3, they are therefore heterozygous and produce several types of gametes.

Genetic representation

Codes: **R^1** for red petal color; **R^2** for white petal

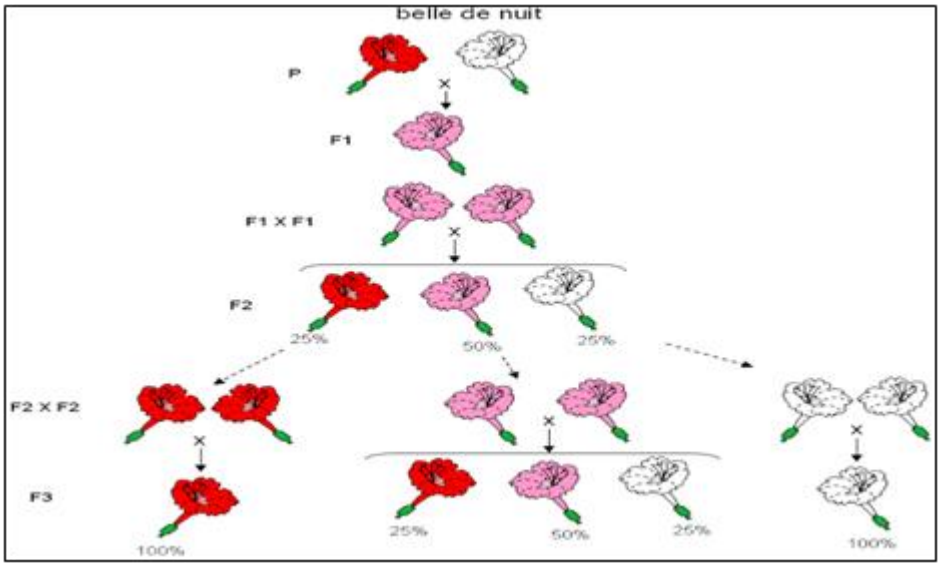


Figure 6: Cross up to F3 of plants with red and white flowers (case of partial dominance).

Parents : ♀ R^1R^1 × ♂ R^2R^2

Gametes : ♀ Ⓡ¹ Ⓡ¹ × ♂ Ⓡ² Ⓡ²

F1 : R^1R^2

F2 : ♀ F1 × ♂ F1

♀ R^1R^2 × ♂ R^1R^2

Gametes : ♀ Ⓡ¹ Ⓡ² × ♂ Ⓡ¹ Ⓡ²

♀ / ♂	Ⓡ¹	Ⓡ²
Ⓡ¹	R^1R^1Ⓡ¹	R^1R^2
Ⓡ²	R^1R^2Ⓡ²	R^2R^2

Genotypes :

Parents : ♀ R^1R^1.

♂ R^2R^2.

F1 : R^1R^2.

F2 : ¼ R^1R^1 : ½ R^1R^2 : ¼ R^2R^2.

F3 : ♀ F2 × ♂ F2

♀ R^1R^1 × ♂ R^1R^1 : Ⓡ¹ Ⓡ¹ × Ⓡ¹ Ⓡ¹ → R^1R^1

♀ R^1R^2 × ♂ R^1R^2 : Ⓡ¹ Ⓡ² × Ⓡ¹ Ⓡ² → ¼ R^1R^1 : ½ R^1R^2 : ¼ R^2R^2

♀ R^2R^2 × ♂ R^2R^2 : Ⓡ² Ⓡ² × Ⓡ² Ⓡ² → R^2R^2

Phenotypes :

Parents : ♀ [R^1].

♂ [R^2].

F1 : [R^1R^2].

F2 : ¼ [R^1] : ½ [R^1R^2] : ¼ [R^2].

F3 : [R^1] → [R^1]

[R^1R^2] → ¼ [R^1] : ½ [R^1R^2] : ¼ [R^2]

[R^2] → [R^2]

The F1 generation corresponds to heterozygotes R^1R^2. In the F2 generation, the genotypic ratio is the same as before (Dominance/Recessiveness). The difference is that here (absence of dominance) the heterozygotes have a new intermediate genotype (pink). While the phenotypic ratio has changed. In the case of dominance and recessiveness, the phenotypic ratio was ¾ : ¼. Whereas, in the case of absence of dominance, the phenotypic ratio is ¼ : ½ : ¼.

Plants with red and white petal phenotypes are purebred (homozygous), because self-fertilization gives, in the next generation, the same phenotype as the mother plants (only one type of gamete produced). Plants with the pink petal phenotype are heterozygous because self-fertilization gives three phenotypic classes in the next generation.

2.2.2. Codominance

It can happen that a heterozygote presents an intermediate phenotype between those of the two homozygotes. Each allele is capable of a certain degree of expression in the face of the other: this is called codominance. It is important to note that if the phenotype of the heterozygote seems to be a mixture of the phenotypes of the homozygotes, each allele nevertheless retains its identity and will segregate normally at meiosis. Under no circumstances is there the appearance of a "mixed" allele.

The difference between incomplete dominance (absence of dominance) and codominance is that in the first case, the characters of the two homozygotes mix together to give an intermediate character in the heterozygote. Whereas, in the second case, the characters of the two homozygotes do not mix and are expressed simultaneously in the heterozygote.

Example

The inheritance of feather color in poultry. When crossing purebred white-feathered hens and purebred black-feathered roosters, the F1 generation was composed of hens and roosters with white and black plumage (Fig. 7). The F2 generation, resulting from the crossing of F1 individuals among themselves, comprises three classes of individuals with the ratio 1 white plumage : 2 white and black plumage : 1 black plumage.

Interpretation

☒ The parents differ by a single trait (feather color) and are purebred lines ; they are therefore homozygous ;

☒ The individuals of the F1 have a single phenotype (white and black feathers), they are therefore similar and 100% homogeneous ;

☒ In F1, there is the appearance of a new phenotype (black and white feathers) which involved the simultaneous appearance of both parental phenotypes (white feathers and black feathers);

☒ In F2, there is the reappearance of both parental phenotypes (white feathers and black feathers).

Genetic Representation

Codes : B^1 for white plumage color

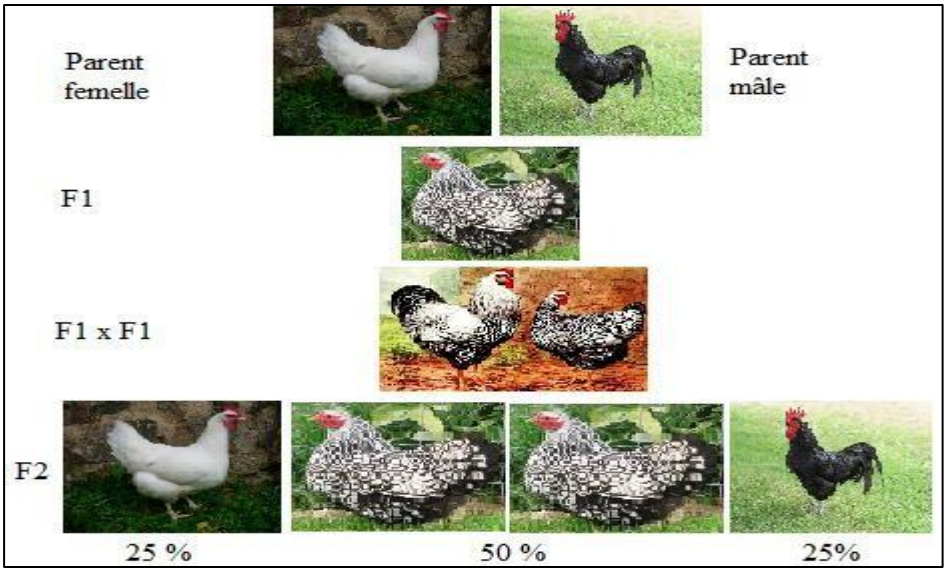


Figure 7: Cross up to F2 of white-feathered hens and black-feathered roosters (case of codominance).

Parents : ♀ B^1B^1 × ♂ B^2B^2

Gametes : ♀ × B^1B^2 ♂ (B²)

F1 : (B¹) (B¹)

F2 : ♀ F1 B^1B^2 × ♂ F1 B^1B^2

♀ × ♂

Gametes : ♀ (B¹) (B²) × ♂ (B¹)

♀ / ♂	(B¹)	(B²)
(B¹)	B^1B^1	B^1B^2
(B²)	B^1B^2	B^2B^2

Genotypes :

Parents : ♀ B^1B^1.
♂ B^2B^2.

F1 : B^1B^2.

F2 : ¼ B^1B^1 : ½ B^1B^2 : ¼ B^2B^2.

Phenotypes :

Parents : ♀ [B^1].
♂ [B^2].

F1 : [B^1B^2].

F2 : ¼ [B^1] : ½ [B^1B^2] : ¼ [B^2].

2.2.3. Test-cross

Knowing that a recessive allele is not expressed in the presence of a dominant allele, for any character represented by two alleles, one dominant "G" and the other recessive "g", the homozygous dominant "GG" and heterozygous "Gg" genotypes are phenotypically indistinguishable.

Thus, for example, a gray mouse can have two phenotypes: "GG" or "Gg", knowing that the character "coat color" is governed by two alleles, one dominant "G" (dark gray coat), the other recessive "a" (white form, albino). In order to determine the genotype of such a gray mouse, it is sufficient to submit it to a test-cross, in other words to cross it with a homozygous recessive individual. The result of the cross, i.e., the observation of the phenotypes of the F1 generation, will allow us to choose between the two possible genotypes of the "tested" individual. Two possibilities are considered for the dark gray mouse of the dominant character:
If its genotype is homozygous, the result is as follows (Fig. 8A):

Parents : ♂ GG × ♀ gg

Gametes : ♂ Ⓖ Ⓖ × ♀ ⓖ ⓖ

F1 : Gg

Genotype F1 : 100 % Gg

Phenotype F1 : 100 % [G]

The homozygous genotype produces only one type of gamete and therefore, only one genotype will be present in F1.

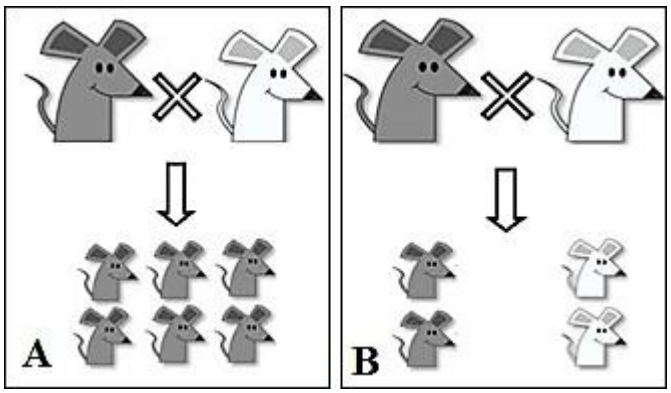


Figure 8 : Cross between gray and white mice: A) purebred, B) gray mice are heterozygous and white mice are homozygous recessive.

If its genotype is heterozygous, the result is as follows (Fig. 8B) :

Parents : ♂ Gg × ♀ gg

Gametes : ♂ Ⓖ ⓖ × ♀ ⓖ ⓖ

♂ / ♀	Ⓖ	ⓖ
ⓖ	Gg	gg

Genotype F1 : ½ Gg : ½ gg
Phenotype F1 : ½ [G] : ½ [g]

The heterozygous genotype produces two types of gametes and therefore, two genotypes and two phenotypes will be present in F1.

Backcross

A backcross, also called a "back cross", is the crossing of a hybrid with one of its parents or with an individual genetically similar to one of its parents, so as to obtain a descendant having a genetic identity closer to that of the parent. This process is used in selective breeding of plants (agriculture, horticulture), in animal breeding and for the production of organisms by gene knockout.

When it comes to crosses between pure lines, crossing the F1 hybrid with the parent carrying the recessive characters, the result is identical to that of the test cross.

Taking the same example of the test-cross, the results of the backcrosses are as follows (Fig. 9):

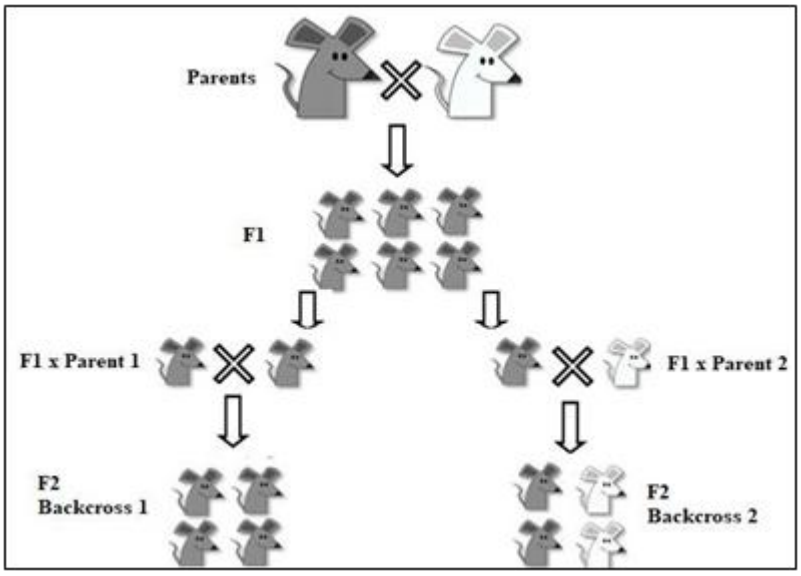


Figure 9 : The two backcrosses between gray and white mice.

Parents : ♂ GG × ♀ gg

Gametes : ♂ Ⓖ Ⓖ × ♀ ⓖ ⓖ

F1 : Gg

Genotype F1 : 100 % Gg **Phenotype F1 :** 100 % [G]

F2 : ♂ F1 × ♀ Parent 1

F2 : ♂ Gg × ♀ GG

♂ Ⓖ ⓖ × ♀ Ⓖ Ⓖ

♀ / ♂	Ⓖ	ⓖ
Ⓖ	GG	Gg

F2 : ♂ F1 × ♀ Parent 2

F2 : ♂ Gg × ♀ gg

♂ Ⓖ ⓖ × ♀ ⓖ ⓖ

♀ / ♂	Ⓖ	ⓖ
ⓖ	Gg	gg

Note

The backcross method is used in plant breeding to incorporate into an otherwise desirable variety **A**, a character that it lacks and that exists in a variety **B**, or sometimes even in another species (for self-pollinators). The method consists of crossing varieties **A** and **B** to introduce the desired character into **A**. The F1 hybrid **AB** is then crossed with variety **A** to produce offspring in which individuals with the desired character will be selected: **A^2B**. This last operation is repeated the number of generations required to integrate the desired character of variety **B** into a fully **A** genome: **A^3B**, **A^4B**, **A^5B**, etc. Moreover, this method was used by Mendel to verify his 2nd law: the law of purity of gametes.

2.2.4. Multiple Allele Series

To study the transmission of genetic material, a pair of alleles is often considered at a specific locus on a chromosome. However, there are many examples where more than two different alleles have been found at the same locus. This is the case, for example, with the inheritance of coat color in domestic rabbits.

Example

In rabbits, the production of certain pigments in the coat of the domestic rabbit is determined by 4 different alleles (Fig. 10):

The wild-type allele "C" produces a wild-type coat (black). This allele "C" dominates all the others in the series.

The allele "c^{ch} " : produces the chinchilla phenotype (a silvery-gray coat) in the homozygous state and gives a pale gray coat color when crossed with a Himalayan or albino

rabbit (here no dominance).

The allele "c^h " : produces the Himalayan phenotype (white with black pigmented extremities of the paws) in the homozygous state and dominates the albino type.

The allele "c^a " : produces the albino phenotype (lacking pigment).

The different combinations of these 4 alleles can produce 10 different genotypes and 5 different phenotypes. The number of different genotypes possible in a series of "n" alleles is given by n(n+1)/2 n= number of alleles. Here it is 4(4+1)/2= 10 genotypes.

☒ The phenotype [C] is represented by the genotypes CC : Cc^{ch} : Cc^h : Cc^a (case of dominance).

☒ The phenotype [c^{ch}] is represented by the genotype $c^{ch}c^{ch}$ (homozygosity).

☒ The phenotypes [$c^{ch}c^a$] and [$c^{ch}c^h$] are represented by the genotypes $c^{ch}c^a$: $c^{ch}c^h$ (case of absence of dominance or partial dominance).

☒ The phenotype [c^h] is represented by the genotypes: c^hc^h (homozygosity) : c^hc^a (case of dominance).

☒ The phenotype [c^a] is represented by the genotype c^ac^a (homozygosity).

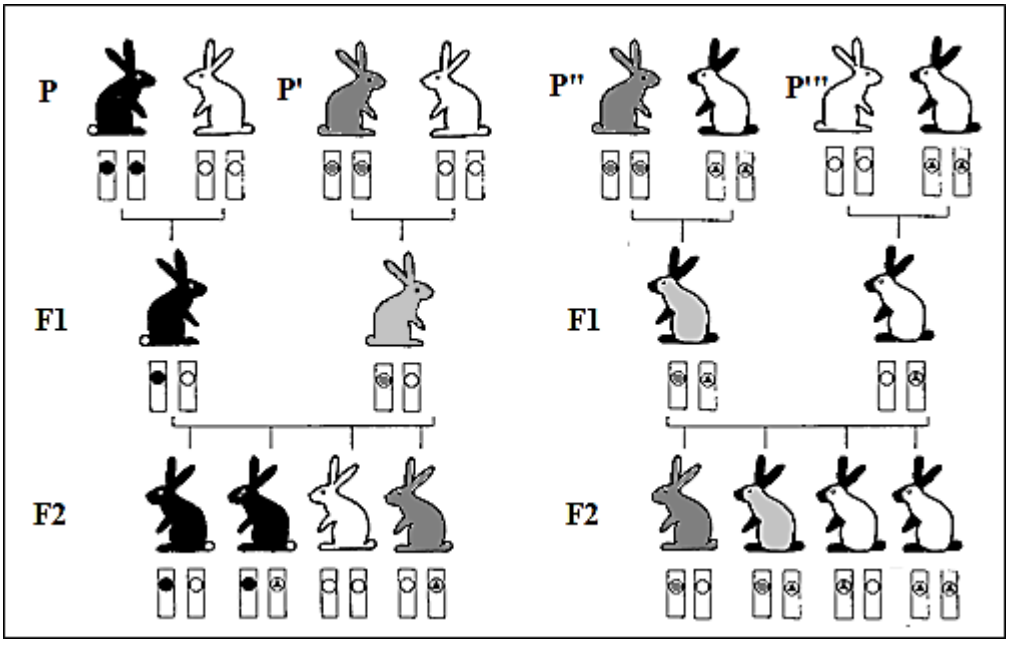


Figure 10 : Transmission of the genetic material coding for coat color in rabbits by several pairs of alleles.

2.2.5. Lethal Genes

Certain alleles manifest only through the death of the individual before maturity, during the prenatal or postnatal period. Such alleles are called ***lethal***. Lethal alleles can be dominant or recessive.

A dominant lethal allele, meaning one that kills both a homozygote and a heterozygote,

can arise through mutation of a normal allele. Nevertheless, such an allele is eliminated from a population as soon as it arises.

A recessive lethal allele kills only individuals homozygous for that allele. Depending on the case, the heterozygote will be apparently normal or will manifest some deficiencies.

Example

The recessive mutation that causes a yellow coat (mutant phenotype) in mice that normally have an agouti coat (normal phenotype). If the mutated allele is in the heterozygous state, it determines the yellow coat color. If the mutated allele is in the homozygous state, it causes the death of the mouse before birth.

Crossing purebred agouti mice produces only agouti mice in F1. Crossing purebred agouti mice with yellow mice resulted in 50% agouti mice and 50% yellow mice in the F1 generation. Crossing yellow mice with each other resulted in ⅔ yellow mice and ⅓ agouti mice in the F1 generation (Fig. 11).

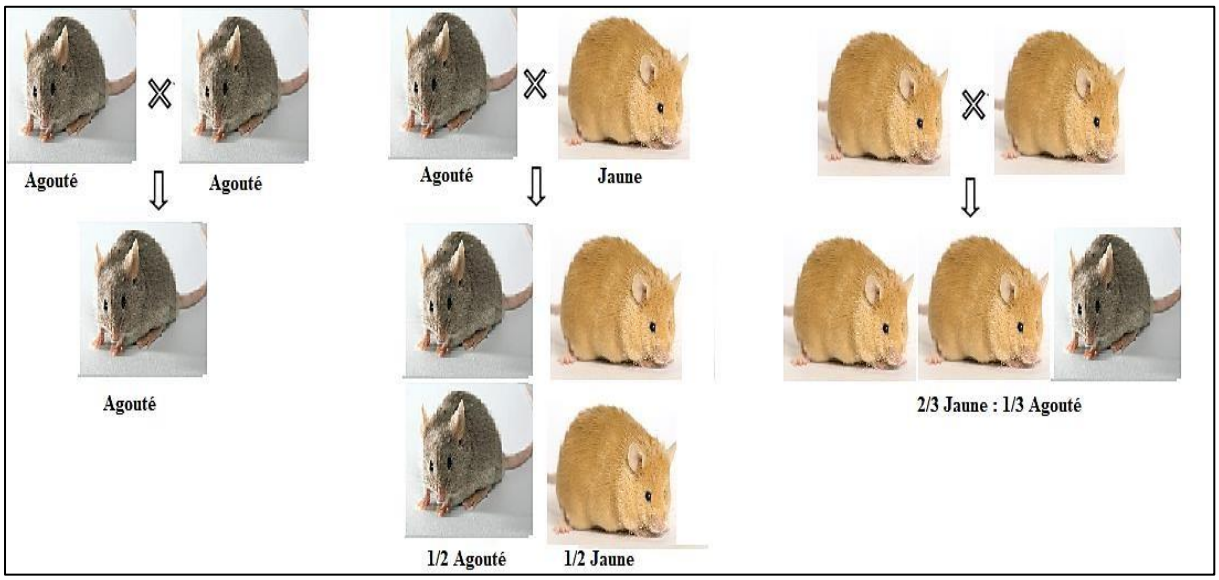


Figure 11 : Cross between purebred agouti mice and mutant yellow mice carrying the lethal allele.

Genetic Representation

Codes : **A for agouti coat and A^j for yellow coat. The genotype of the yellow mouse is AA^j.**

Cross 1

Parents : ♀ AA × ♂ AA

Gametes : ♀ Ⓐ Ⓐ × ♂ Ⓐ Ⓐ

F1 : AA

Genotypes F1 : 100% AA

Phenotypes F1 : 100% [A]

Cross 2

Parents : ♀ AA × ♂ AA^j

Gametes : ♀ Ⓐ Ⓐ × ♂ Ⓐ $Ⓐ^j$

F1 : AA AA^j AA AA^j

Genotypes F1 : ½ AA : ½ AA^j

Phenotypes F1 : ½ [A] : ½ [A^j]

Cross 3

Parents : ♀ AA^j × ♂ AA^j

Gametes : ♀ (A) (A^j) × ♂ (A) (A^j)

F1

♂ \ ♀	(A)	(A^j)
AA (A)	AA	AA^j
AA^j (A^j)	AA^j	A^jA^j **Die before birth**

Genotypes F1 : ⅓ AA : ⅔ AA^j
Phenotype F1 : ⅓ [A] : ⅔ [A^j]

These results can be explained with a single pair of alleles. If we consider coat color, the yellow mutant allele $\mathbf{A^j}$ is dominant over the wild-type agouti allele **A**: heterozygous mice have a yellow coat. However, the yellow mutant allele also behaves as a recessive allele, lethal in the homozygous state. Mice with the $\mathbf{A^jA^j}$ genotype die before birth, so no homozygous yellow mice are obtained.

2.2.6. Sex-Linked Inheritance

a) Sex Chromosome Systems

Homogametic Female and Heterogametic Male

In humans and apparently all other mammals, normal males have an **XY** chromosome constitution and females have an **XX** chromosome constitution. Thus, the female produces only one type of **X** gamete ; she is said to be homogametic. Whereas the male produces two types of gametes, **X** and **Y**, he is said to be heterogametic. The female can be fertilized by either of the two types of chromosomes from the sperm, and since the union of gametes is random, we will have :

Parents : ♀ XX× ♂ XY

Gametes : ♀ (X) (X) × ♂ (X) (Y)

Descendants : ½ XX ½ XY

In certain insects of the order Hemiptera (true bugs) or of the order Orthoptera (grasshoppers, crickets...), males are also heterogametic, but their sperm either contain an X chromosome or are completely devoid of sex chromosomes. Thus, the X chromosome in these males has no homologue, there is no Y chromosome, we will write XO. Females are homogametic XX, while the male is XO : he has an odd number of chromosomes.

Parents : ♀ XX× ♂ XO

Gametes : ♀ (X)(X) × ♂ (X)(O)

Descendants : ½ XX ½ XO

Heterogametic Female and Homogametic Male

This sex determination mechanism is found in a fairly large number of animals, insects in particular in butterflies, moths, caddisflies, silkworms, and in some birds and fish. Females have a chromosome similar to the Y chromosome in humans. In this case, the sex chromosomes are sometimes designated by Z and W instead of X and Y for the female to draw attention to the fact that it is the female that is heterogametic. The female is ZW and the male is ZZ.

In other species, the domestic chicken for example, it is the females that have only one sex chromosome. To mark the difference, males are symbolized by ZZ and females by ZO.

In either case, there will be 50% males and 50% females in the offspring.

b) Sex-Linked Inheritance Proper

A gene is said to be sex-linked when it is located on the X chromosome (mammals, Drosophila, and others...) or on the Z chromosome (analogous to the X in birds and other species where sex is determined by a ZO or ZW mechanism).

In humans, there are certain recessive X-linked diseases which are: color blindness, Duchenne muscular dystrophy, hemophilia (there are no hemophiliac women: it is a lethal condition for women).

X-linked Inheritance

Usually, the two reciprocal crosses involving autosomal traits give identical results. This is not the case for sex-linked traits.

Example

Two reciprocal crosses are performed :

1st cross : When white-eyed females (mutants) are crossed with wild-type males (red eyes), all the male offspring have white eyes like the mother while all the female offspring have red eyes like the father (Fig. 12).

2nd cross : if we perform the reverse cross, i.e., red-eyed females with white-eyed males, we will have offspring composed entirely of red-eyed individuals (Fig. 13)

Interpretation

Observation of the two F1 results shows us that :

- For the first cross, the males inherit the character of the mother while the females inherit the character of their father.
- For the second cross, the offspring is homogeneous, and all the offspring inherit the character of the mother (male and female). This means that the wild-type character (red eyes) of the mother is dominant and that the mutant character (white eyes) of the father is recessive.
- The two crosses do not give the same offspring.
- The distribution of characters is different for male and female offspring.

These observations are sufficient to " diagnose " a case of sex-linked inheritance. In the case of X-linked inheritance, different results are obtained depending on the direction of the cross. This particular type of inheritance is due to the fact that the Y chromosomes do not have alleles homologous to the one at the white locus located on the X chromosome. Very few genes linked to the Y chromosome are known. Males therefore only have one allele for sex-linked traits. This state is called hemizygous. Unlike the homozygous or heterozygous states that the female can present.

1st cross

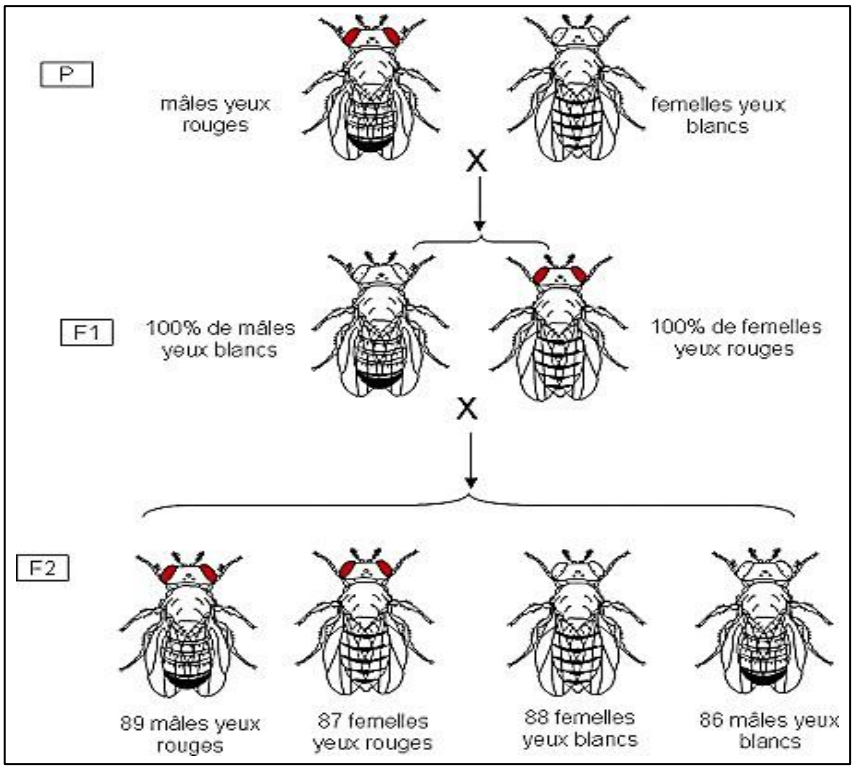
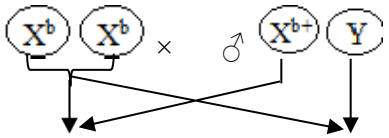


Figure 12 : First reciprocal cross between female Drosophila with white eyes and male Drosophila with red eyes.

Codes : b for the mutant white allele and b^+ for the wild-type red allele

Parents : ♀ X^bX^b × ♂ $X^{b+}Y$

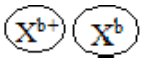
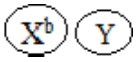
Gametes : ♀  ♂

F1 : $X^{b+}X^b$ X^bY

		Genotypes	Phenotypes
Parents :	♂	$X^{b+}Y$	$[b^+]$
	♀	X^bX^b	[b]
F1 :	♂	X^bY	½[b]
	♀	$X^{b+}X^b$	½$[b^+]$

F2 : ♀ F1 × ♂ F1

F2 : ♀ $X^{b+}X^b$ × ♂ X^bY

Gametes : ♀  × ♂ 

♀ \ ♂	X^b	Y
X^{b+}	$X^{b+}X^b$	$X^{b+}Y$
X^b	X^bX^b	X^bY

	Genotypes	Phenotypes	
F2 :	♂ ¼ $X^{b+}Y$: ½ X^bY	♂ ¼ $[b^+]$: ¼ [b]	½ $[b^+]$: ½ [b]
	♀ ½ $X^{b+}X^b$: ½ X^bX^b	♀ ¼ $[b^+]$: ¼ [b]	

These theoretical results correspond to the observed results in this example.

	Theoretical Results	Observed Results
♂ $[b^+]$	(¼)*100 = **25 %**	(89/350)*100 = **25,43%**
♂ [b]	(¼)*100 = **25 %**	(86/350)*100 = **24,57%**
♀ $[b^+]$	(¼)*100 = **25 %**	(87/350)*100 = **24,85%**
♀ [b]	(¼)*100 = **25%**	(88/350)*100 = **25,15%**

2nd cross (the reciprocal cross)

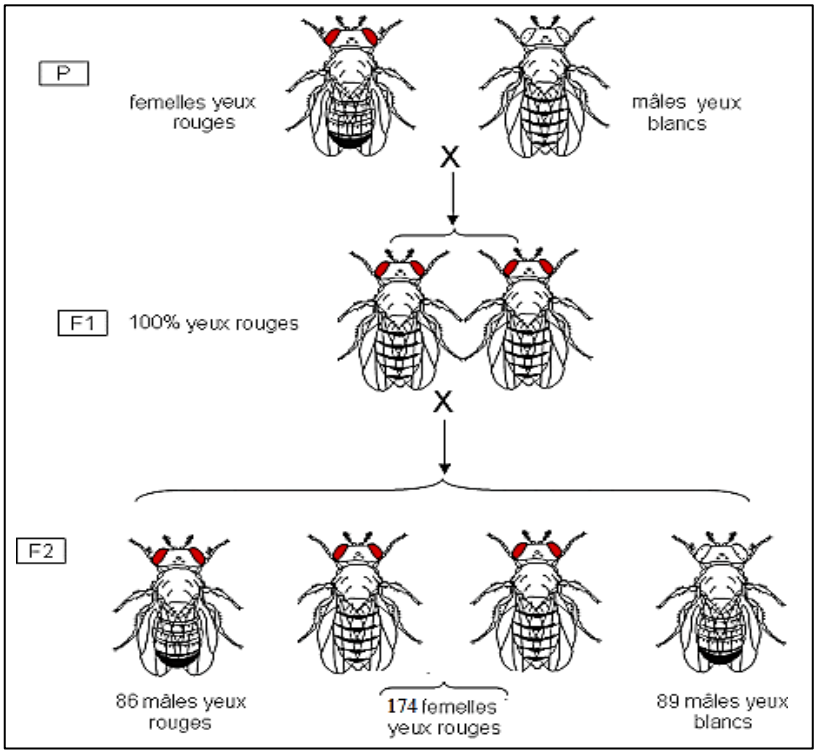


Figure 13 : Second reciprocal cross between female Drosophila with red and white eyes and male Drosophila with white eyes.

Parents : ♀ $X^{b+}X^{b+}$ × ♂ $X^{b}Y$

Gametes : ♀ X^{b+} X^{b+} × ♂ X^{b} Y

F1 : $X^{b+}X^{b}X^{b+}Y$

		Genotypes	**Phenotypes**
Parents :	♂	$X^{b}Y$	[b]
	♀	$X^{b+}X^{b+}$	[b^{+}]
F1 :	♂	$X^{b+}Y$	½[b^{+}]

♀ $X^{b+}X^{b}$ ½[b^{+}]

F2 : ♀ F1 × ♂ F1

F2 : ♀ $X^{b+}X^{b}$ × ♂ $X^{b+}Y$

Gametes : ♀ X^{b+} X^{b} × ♂ X^{b+} Y

♂ / ♀	X^{b+}	Y
X^{b+}	$X^{b+}X^{b+}$	$X^{b+}Y$
X^{b}	$X^{b+}X^{b}$	$X^{b}Y$

	Genotypes	**Phenotypes**	
F2 :	♂ ¼ $X^{b+}Y$: ¼ $X^{b}Y$	♂ ½ [b^{+}] : ½ [b]	¾ [b^{+}] : ¼ [b]
	♀ ¼ $X^{b+}X^{b+}$:¼ $X^{b+}X^{b}$	♀ ½ [b^{+}]	

These theoretical results correspond to the observed results in this example.

	Theoretical Results	**Observed Results**
♂ [b^{+}]	(¼)*100 = **25 %**	(86/349)*100 = **24,64%**
♂ [b]	(¼)*100 = **25 %**	(89/349)*100 = **49,86%**
♀ [b^{+}]	(½)*100 = **50 %**	(174/349)*100 = **25,15%**

Y-linked inheritance

In humans and mammals, the male transmits his Y to his sons only. A gene can therefore only manifest itself in males and is said to be: Holandric. In humans, there are a few examples:

Shapes of the toes.

A gene for hair on the outer part of the ear.

A gene that determines the sex SRY located on the Y chromosome and is transmitted to his sons.

Particular types of sex-linked inheritance

The fact that they pair during meiosis indicates that they contain at least some homologous segments. Genes located on homologous segments are said to be incompletely sex-linked or partially sex-linked, because they can recombine by crossing over just like genes located on autosomes. The region where the two chromosomes pair is called the pseudo-autosomal region. On the other hand, genes located on the non-homologous segment of the X chromosome are called completely sex-linked genes (Fig. 14).

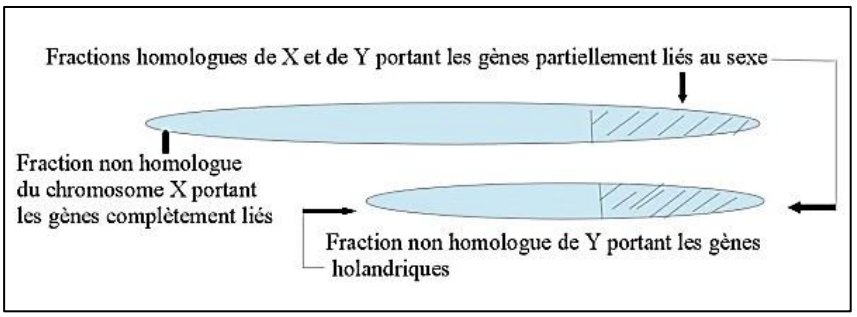


Figure 14: Genes located on homologous segments incompletely sex-linked or partially sex-linked.

3. Dihybridism

Mendel undertook, as a natural extension of crosses involving a single trait (monohybridism), crosses designated as dihybridism because they simultaneously involve two traits, therefore two pairs of phenotypes and two pairs of allelic factors.

Dihybridism is the cross between two individuals belonging to two homozygous (pure) lines that differ from each other by two traits or two genes, therefore two pairs of alleles located on autosomes (non-sex chromosomes).

If the two genes that code for the two different traits are located on different chromosomes, we speak of independent genes. But if the two genes that code for the two different traits are located on the same chromosome, the genes are said to be linked.

3.1. Independent genes

3.1.1. Mendelian inheritance (dominance and recessiveness)

Mendel studied the cross between two varieties of pure lines of peas that differ in two characteristics which are the appearance of the seeds (smooth and wrinkled) and the color of the seeds (yellow and green). He crossed a variety of peas with smooth, yellow seeds with a variety of peas with wrinkled, green seeds. In the first generation F1, all the seeds were smooth and yellow (Fig. 15). The second generation F2, resulting from the crossing of F1 individuals among themselves (self-fertilization), gave the following results :

315 plants produce yellow and smooth grains.

108 plants produce yellow and wrinkled grains.

101 plants produce green and smooth grains.

32 plants produce green and wrinkled grains.

Interpretation

☒ This is a cross between two individuals belonging to two pure lines that differ by two pairs of alleles or two genes : it is a dihybridism.

☒ The first generation F1 is homogeneous and similar with the manifestation of a single parental phenotype. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents. On the other hand, this indicates that the traits that appear in F1 are the dominant traits, so the yellow trait is dominant over the green trait and the smooth trait is dominant over the wrinkled trait.

☒ In the second generation F2, there is the appearance of the two parental phenotypes (yellow and smooth ; green and wrinkled) in addition to two other new phenotypes which are intermediate between the parental phenotypes (yellow and wrinkled ; green and smooth). These phenotypes are said to be recombined.

☒ The values obtained in F2 correspond to the following proportions : 56.65% [yellow and smooth] : 19.44% [yellow and wrinkled] : 18.16% [green and smooth] : 5.75% [green and wrinkled].

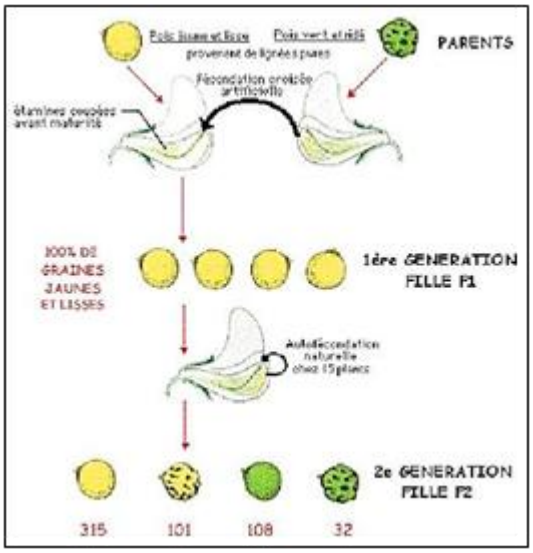


Figure 15: Cross between two varieties of pure lines of peas that differ in two characteristics (appearance and color of the seeds).

Genetic representation

Codes : **L for smooth seeds; l for wrinkled seeds; J for yellow seeds and j for green seeds.**

Parents : ♀ JJLL × ♂ Jjll

Gametes : ♀ (JL) (JL) (JL) (JL) × ♂ (jl) (jl) (jl) (jl)

F1 : JjLl

Genotypes : **Phenotypes**

Parents : ♀ JJLL **Parents** : ♀ [JL]

♂ jjll ♂ [jl]

F1 : JjLl **F1** : [JL]

F2 : ♀ F1 × ♂ F1

♀ JjLl × ♂ JjLl

Gametes : ♀ (JL) (Jl) (jL) (jl) × ♂ (JL) (Jl) (jL) (jl)

♂ / ♀	(JL)	(Jl)	(jL)	(jl)
(JL)	JJLL	JJLl	JjLL	JjLl
(Jl)	JJLl	JJll	JjLl	Jjll
(jL)	JjLL	JjLl	jjLL	jjLl
(jl)	JjLl	Jjll	jjLl	jjll

Genotypes

4/16 JjLl : 1/16 JJLL : 1/16 JJll : 1/16 jjLL : 1/16 jjll : 2/16 JJLl : 2/16 JjLL : 2/16 Jjll : 2/16 jjLl.

Phenotypes

[**JL**] : 4/16 + 2/16 + 2/16 + 1/16 = 9/16. [**Jl**] : 2/16 + 1/16 = 3/16.

[**jL**] : 2/16 + 1/16 = 3/16. [**jL**] : 1/16.

Nine genotypes are obtained with four phenotypes. The proportions of the phenotypic classes are 9/16 : 3/16 : 3/16 : 1/16 which correspond respectively to 56.25% : 18.75% : 18.75 % : 6.25%. These theoretical results found by Mendel correspond to the observed results found in this example.

In this example, the segregation of the seed appearance alleles is independent of the segregation of the color alleles, since the chromosomes behave as independent entities during meiosis.

There are two other methods to find the genotypes and phenotypes of the second generation F2.

Method of the table of genotypes and phenotypes.

Genotypes

F2 : ♀ F1 × ♂ F1
♀ JjLl × ♂ JjLl

If we only consider the 1st gene (seed color) separately (Jj x Jj), we will have the genotypes with the proportions of a monohybrid of the F2: ¼ JJ: 2/4 Jj: ¼ jj. And if we only consider the 2nd gene (seed appearance) separately (Ll x Ll), we will have the genotypes with the proportions of a monohybrid of the F2: ¼ LL: 2/4 Ll: ¼ ll. The proportions of the possible F2 genotypes for the two genes are given by the product of the genotypes of the first gene with that of the second gene as shown in the following table:

Table 2 : Genotype table method.

	¼ JJ	**2/4 Jj**	**¼ jj**
¼ LL	1/16 JJLL	2/16 JjLL	1/16 jjLL
2/4 Ll	2/16 JJLl	4/16 JjLl	2/16 jjLl
¼ ll	1/16 JJll	2/16 Jjll	1/16 jjll

Phenotypes

The same type of reasoning is applied to the phenotypes (table 3). If we consider the 1st gene (seed color) separately (Jj x Jj), we will have the phenotypes with the proportions of a monohybrid of the F2 : ¾ [J] : ¼ [j]. And if we consider the 2nd gene (seed appearance) separately (Ll x Ll), we will have the phenotypes with the proportions of a monohybrid of the F2 : 3/4[L] : 1/4[l].

Table 3 : Phenotype table method

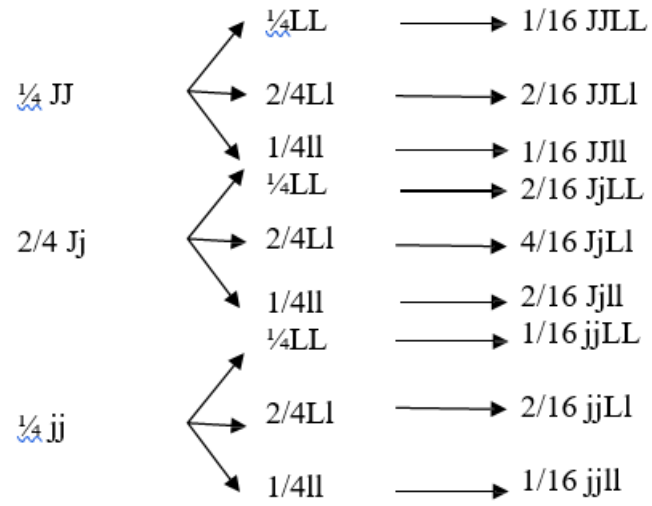
	¾ [J]	**¼ [j]**
¾ [L]	9/16 [JL]	3/16 [jL]
¼ [l]	3/16 [Jl]	1/16 [jl]

Dichotomous method or Branched system

Genotypes

1st gene (seed color) : ¼ JJ : 2/4Jj : 1/4jj.

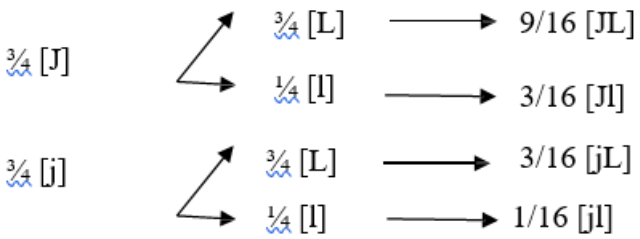
2nd gene (seed appearance) : ¼LL : 2/4Ll : 1/4ll.



Phenotypes

1st gene (seed color) : ¾ [J] : ¼ [j].

2nd gene (seed appearance) : ¾ [L] : ¼ [l]



3.1.2. Non-Mendelian Inheritance (change in genotypic and phenotypic frequencies)

3.1.2.1. Absence of Dominance (Incomplete dominance)

Example 1

A cross was made between two purebred radish lines that differ in two traits. The female line had red, long fruits and the male line had white, round fruits (Fig. 16). All fruits harvested in the first generation F1 were red and oval. The F1 individuals crossed among themselves give 1000 plants in F2 divided into six phenotypes according to the following results:

189 plants with red, long fruits;

375 plants with red, oval fruits;

186 plants with red, round fruits;

64 plants with white, long fruits;

125 plants with white, oval fruits;

61 plants with white, round fruits;

Interpretation

☒ This is a cross between two individuals belonging to two pure lines that differ by two pairs of alleles or two genes : it is a dihybridism.

☒ The first generation F1 is homogeneous and similar. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents.

☒ In the first generation F1, all fruits have a red color which is identical to the color of the female parent and have an oval shape which is intermediate between the shapes of the parents. It is therefore a case of dominance and recessiveness for the first character (red dominant : white recessive) and a case of incomplete dominance (absence of dominance) for the second character.

☒ In the second generation F2, there is the appearance of the two parental phenotypes (red and long : white and round) in addition to four other new phenotypes which are intermediate between the parental phenotypes (red and oval ; red and round ; white and long ; white and oval). These phenotypes are called recombined.

Genetic Representation

Codes

» R for red radish ;

» r for white radish ;

» L^1 for long radish ;

» L^2 for round radish.

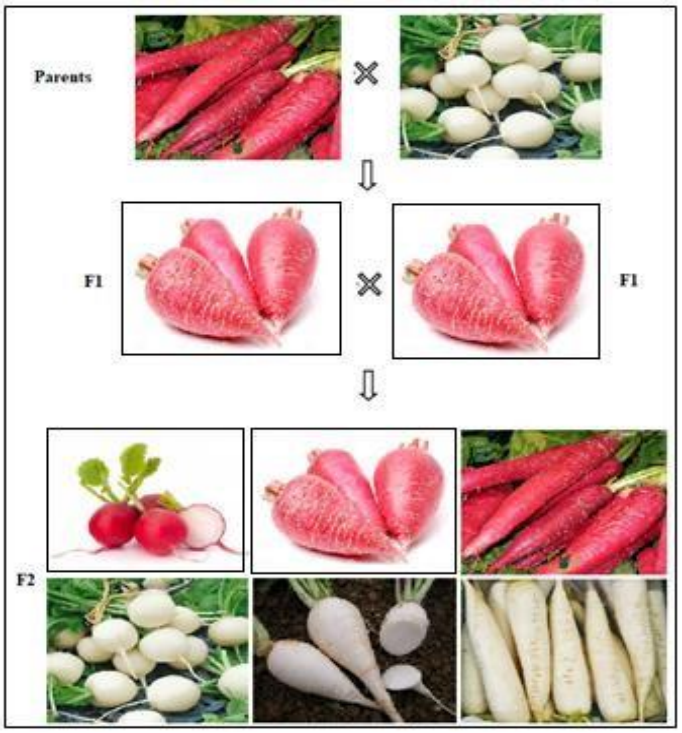


Figure 16: Cross up to F2 between two radish lines that differ in two characteristics: fruit color (dominance/recessiveness) and fruit shape (absence of dominance).

Parents : ♀ RRL^1L^1 × ♂ rrL^2L^2

Gametes : ♀ RL^1 RL^1 RL^1 RL^1 × ♂ rL^2 rL^2 rL^2 rL^2

F_1 :

F_2 : ♀ F_1 RrL^1L^2 x ♂ RrL^1L^2

Gametes : ♀ RL^1 RL^2 rL^1 rL^2 ♂ RL^1 RL^1 RL^2 rL^1 rL^2

♂ / ♀	RL^1	RL^2	rL^1	rL^2
RL^1	RRL^1L^1	RRL^1L^2	RrL^1L^1	RrL^1L^2
RL^2	RRL^1L^2	RRL^2L^2	RrL^1L^2	RrL^2L^2
rL^1	RrL^1L^1	RrL^1L^2	rrL^1L^1	rrL^1L^2
rL^2	RrL^1L^2	RrL^2L^2	rrL^1L^2	rrL^2L^2

Genotypes :

4/16 RrL^1L^2 :

2/16 RRL^1L^2 : 2/16 RrL^1L^1: 2/16 rrL^1L^2 : 2/16 RrL^2L^2 :

1/16 RRL^1L^1 : 1/16 RRL^2L^2: 1/16 rrL^1L^1 : 1/16 rrL^2L^2.

Phenotypes :

[$\mathbf{RL^1}$] : 2/16 + 1/16 = 3/16. [$\mathbf{rL^1}$] : 1/16.

[$\mathbf{RL^1L^2}$] : 4/16 + 2/16 = 6/16. [$\mathbf{rL^1L^2}$]: 2/16.

[$\mathbf{RL^2}$] : 2/16 + 1/16 = 3/16. [$\mathbf{rL^2}$]: 1/16.

Nine genotypes are obtained with six phenotypes. The proportions of the phenotypic classes are 3/16 : 6/16 : 3/16 : 1/16 : 2/16 : 1/6 which correspond respectively to 18.75% : 37.5% : 18.75% : 6.25% : 12.5% : 6.25%. These theoretical results correspond to the results observed in this example.

	Theoretical results	**Observed results**
[$\mathbf{RL^1}$]	(3/16)*100 = **18,75%**	(188/1000)*100 = **18,8%**
[$\mathbf{RL^1L^2}$]	(6/16)*100 = **37,5%**	(375/1000)*100 = **37,5%**
[$\mathbf{RL^2}$]	(3/16)*100 = **18,75%**	(186/1000)*100 = **18,6%**
[$\mathbf{rL^1}$]	(1/16)*100 = **6,25%**	(63/1000)*100 = **6,3%**
[$\mathbf{rL^1L^2}$]	(2/16)*100 = **12,5%**	(125/1000)*100 = **12,5%**
[$\mathbf{rL^2}$]	(1/16)*100 = **6,25%**	(61/1000)*100 = **6,1%**

Example 2

Another cross was made between two radish lines that differ in two traits (fruit color and shape). The female line had purple, long fruits and the male line had white, round fruits (Fig. 17). All fruits harvested in the first generation F1 were mauve and oval. The F1 individuals crossed among themselves give 3000 plants in F2 divided into nine phenotypes according to the following results :

188 plants with purple, long fruits ;

376 plants with purple, oval fruits ;

187 plants with purple, round fruits ;

374 plants with mauve, long fruits ;

750 plants with mauve, oval fruits ;

373 plants with mauve, round fruits ;

189 plants with white, long fruits ;

377 plants with white, oval fruits ;

186 plants with white, round fruits.

Interprétation

☒ This is a cross between two individuals belonging to two pure lines that differ by two pairs of alleles or two genes : it is a dihybridism.

☒ The first generation F1 is homogeneous and similar. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents.

☒ In the first generation F1, all the fruits have a mauve color which is intermediate between the colors of the two parents and have an oval shape which is also intermediate between the shapes of the two parents. This is therefore a case of incomplete dominance (absence of dominance) for both traits.

☒ In the second generation F2, there is the appearance of the two parental phenotypes (purple and long : white and round) in addition to seven other new phenotypes which are intermediate between the parental phenotypes (purple and oval; purple and round; mauve and long; mauve and oval; mauve and round; white and long; white and oval). These phenotypes are said to be recombined.

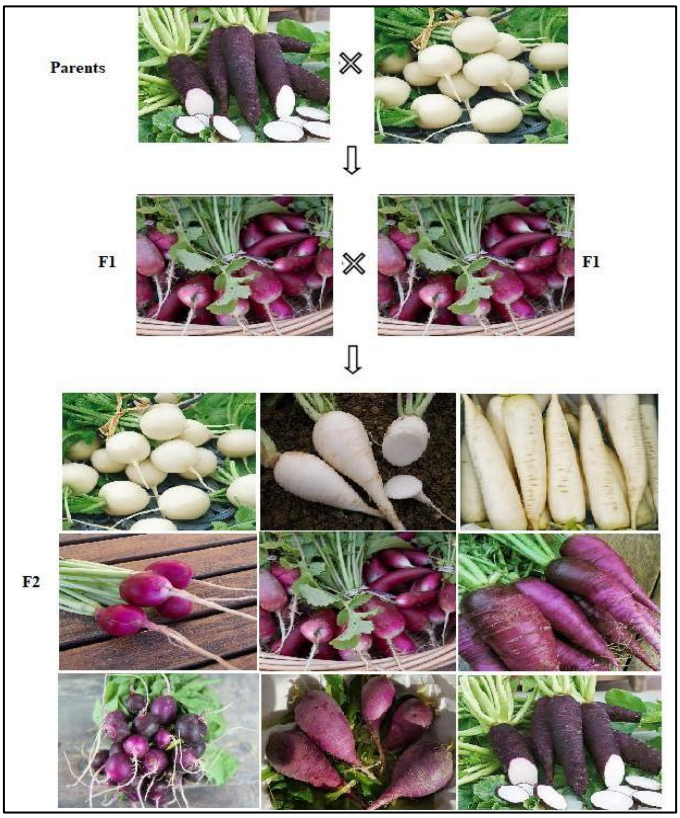


Figure 17: Cross up to F2 between two radish lines that differ in two characteristics: fruit color and fruit shape (case of absence of dominance).

Genetic representation

Codes : **V for purple radish; V' for white radish;** L^1 **for long radish and** L^2 **for round radish.**

Parents : ♀ VVL^1L^1 × ♂ $V'V'L^2L^2$

Gametes : ♀ (VL^1) (VL^1) (VL^1) (VL^1) × ♂ $(V'L^2)$ $(V'L^2)$ $(V'L^2)$ $(V'L^2)$

F1 : $VV'L^1L^2$

F2 : ♀ F1 × ♂ F1

♀ $VV'L^1L^2$ × ♂ $VV'L^1L^2$

Gametes : ♀ (VL^1) (VL^2) $(V'L^1)$ $(V'L^2)$ ♂ (VL^1) (VL^2) $(V'L^1)$ $(V'L^2)$

♂ / ♀	(VL^1)	(VL^2)	$(V'L^1)$	$(V'L^2)$
(VL^1)	VVL^1L^1	VVL^1L^2	$VV'L^1L^1$	$VV'L^1L^2$
(VL^2)	VVL^1L^2	VVL^2L^2	$VV'L^1L^2$	$VV'L^2L^2$
$(V'L^1)$	$VV'L^1L^1$	$VV'L^1L^2$	$V'V'L^1L^1$	$V'V'L^1L^2$
$(V'L^2)$	$VV'L^1L^2$	$VV'L^2L^2$	$V'V'L^1L^2$	$V'V'L^2L^2$

Genotypes :

4/16 VV'L^1L^2 : 2/16 VVL^1L^2 : 2/16 VV'L^1L^1 : 2/16 V'V'L^1L^2 : 2/16 VV'L^2L^2 : 1/16 VVL^1L^1 : 1/16 VVL^2L^2 : 1/16 V'V'L^1L^1: 1/16 V'V'L^2L^2.

Phenotypes :

1/16 [**V L^1**]	2/16 [**VV' L^1**]	1/16 [**V' L^1**] :
2/16 [**V L^1 L^2**]	4/16 [**VV' L^1 L^2**]	2/16 [**V' L^1 L^2**]
1/16 [**V L^2**]	2/16 [**VV' L^2**]	1/16 [**V' L^2**].

Nine genotypes are obtained with nine phenotypes. The proportions of the phenotypic classes are 1/16 : 2/16 : 1/16 : 2/16 : 4/16 : 2/16 : 1/16 : 2/16 : 1/6 which correspond respectively to: 6.25% : 12.5 % : 6.25% : 12.5 % : 25% : 12.5% : 6.25% : : 12.5 % : 6.25%.

These theoretical results correspond to the results observed in this example.

	Theoretical results	**Observed results**
[**VL^1**]	(1/16)*100 = **6,25%**	(188/3000)*100 = **6,26%**
[**V L^1L^2**]	(2/16)*100 = **12,5%**	(376/3000)*100 = **12,54%**
[**V L^2**]	(1/16)*100 = **6,25%**	(187/3000)*100 = **6,24%**
[**VV'L^1**]	(2/16)*100 = **12,5%**	(374/3000)*100 = **12,46%**
[**VV' L^1 L^2**]	(4/16)*100 = **25%**	(750/3000)*100 = **25%**
[**VV' L^2**]	(2/16)*100 = **12,5%**	(373/3000)*100 = **12,44%**
[**V' L^1**]	(1/16)*100 = **6,25%**	(189/3000)*100 = **6,3%**
[**V' L^1 L^2**]	(2/16)*100 = **12,5%**	(377/3000)*100 = **12,56%**
[**V' L^2**]	(1/16)*100 = **6,25%**	(186/3000)*100 = **6,2%**

Remark

When it was a case of dominance and recessiveness, nine genotypes and four phenotypes in proportions 9:3:3:1 were obtained. But when the interactions between genes differed (dominance/recessiveness : absence of dominance or absence of dominance for both characters), it is remarkable that the number of genotypes did not change (nine genotypes) but the number and proportions of phenotypic classes changed. The number of phenotypes went from four to six for the first case and from four to nine for the second case.

3.1.2.2. Codominance

Example 1 :

A cross was made between two purebred horse lines that differ in two characteristics. The female line had a solid white coat and blond hair and the male line had a black coat and black hair (Fig. 18). All the descendants of the first generation F1 had a black and white coat and black hair. The individuals of the F1 crossed with each other give 50 foals in F2 divided between six

phenotypes according to the following results :

- 9 foals with black coat and black hair ;
- 18 foals with black and white coat and black hair ;
- 10 foals with white coat and black hair ;
- 3 foals with black coat and blond hair ;
- 6 foals with black and white coat and blond hair ;
- 4 foals with white coat and blond hair ;

Interpretation

☒ It is a cross between two individuals belonging to two pure lines which differ by two pairs of alleles or two genes : it is a dihybridism.

☒ The first generation F1 is homogeneous and similar. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents.

☒ In the first generation F1, all the descendants have a black and white coat where there is the manifestation of the color of both parents and have black hair identical to that of the male parent. This is therefore a case of codominance for the first character (white and black are codominant) and a case of dominance and recessiveness for the second character (dominant black : recessive blond).

☒ In the second generation F2, there is the appearance of the two parental phenotypes (white coat and blond hair : black coat and black hair) in addition to four other new phenotypes (black and white coat with black hair ; black coat with white hair ; black and white coat with blond hair ; white coat with black hair). These phenotypes are said to be recombined.

Genetic Representation

Codes

» N for black coat ;

» n for white coat ;

» R^1 for black hair ;

» R^2 for blond hair ;

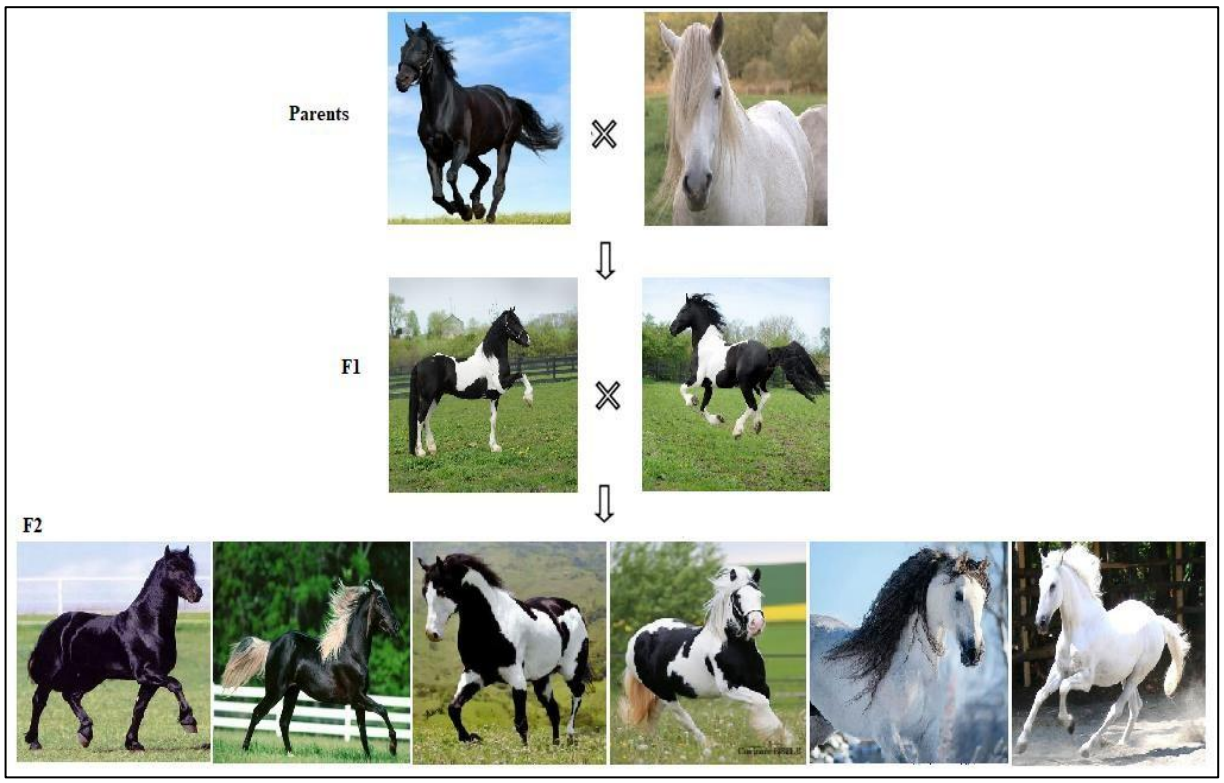


Figure 18: Cross up to F2 between two breeds of horses that differ by two characteristics : hair color (dominance/recessiveness) and coat color (codominance).

Parents: ♀ NNR^1R^1 × ♂ nnR^2R^2

Gametes: ♀ $(NR^1)(NR^1)(NR^1)(NR^1)$ × ♂ $(nR^2)(nR^2)(nR^2)(nR^2)$

F1 : NnR^1R^2

F2 : ♀ F1 × ♂ F1

♀ NnR^1R^2 × ♂ NnR^1R^2

Gametes : ♀ $(NR^1)(NR^2)(nR^1)(nR^2)$ × ♂ $(NR^1)(NR^2)(nR^1)(nR^2)$

♂ / ♀	(NR^1)	(NR^2)	(nR^1)	(nR^2)
(NR^1)	NNR^1R^1	NNR^1R^2	NnR^1R^1	NnR^1R^2
(NR^2)	NNR^1R^2	NNR^2R^2	NnR^1R^2	NnR^2R^2
(nR^1)	NnR^1R^1	NnR^1R^2	nnR^1R^1	nnR^1R^2
(nR^2)	NnR^1R^2	NnR^2R^2	nnR^1R^2	nnR^2R^2

Genotypes :

4/16 NnR^1R^2 : 2/16 NNR^1R^2 : 2/16 NnR^1R^1: 2/16 nnR^1R^2 : 2/16 NnR^2R^2 : 1/16 NNR^1R^1 : 1/16 NNR^2R^2: 1/16 nnR^1R^1: 1/16 nnR^2R^2.

Phenotypes :

[$\mathbf{NR^1}$] : 2/16 + 1/16 = 3/16. [$\mathbf{nR^1}$] : 1/16.
[$\mathbf{NR^1R^2}$] : 4/16 + 2/16 = 6/16. [$\mathbf{nR^1R^2}$]: 2/16.
[$\mathbf{NR^2}$] : 2/16 + 1/16 = 3/16. [$\mathbf{nR^2}$]: 1/16.

Nine genotypes are obtained with six phenotypes. The proportions of the phenotypic classes are 3/16: 6/16: 3/16: 1/16: 2/16: 1/6 which correspond respectively to 18.75%: 37.5%: 18.75%: 6.25%: 12.5%: 6.25%. These theoretical results correspond to the observed results in this example.

	Theoretical results	**Observed results**
[$\mathbf{NR^1}$]	(3/16)*100 = **18,75%**	(9/50)*100 = **18%**
[$\mathbf{NR^1R^2}$]	(6/16)*100 = **37,5%**	(18/50)*100 = **36%**
[$\mathbf{NR^2}$]	(3/16)*100 = **18,75%**	(10/50)*100 = **20%**
[$\mathbf{nR^1}$]	(1/16)*100 = **6,25%**	(3/50)*100 = **6%**
[$\mathbf{nR^1R^2}$]	(2/16)*100 = **12,5%**	(6/1000)*100 = **12%**
[$\mathbf{nR^2}$]	(1/16)*100 = **6,25%**	(4/1000)*100 = **8%**

Example 2

A cross was made between two radish lines that differ in two characteristics (fruit color and shape). The female line had red and long fruits and the male line had white and round fruits (Fig. 19). All the fruits harvested in the first generation F1 were red and white and oval in shape. The individuals of the F1 crossed among themselves give 1500 plants in F2 divided into nine phenotypes according to the following results :

- 94 plants with red and long fruit ;
- 188 plants with red and oval fruits ;
- 93 plants with red and round fruits ;
- 187 plants with red and white and long fruit ;
- 374 plants with red and white and oval fruits ;
- 188 plants with red and white and round fruits ;
- 95 plants with white and long fruit ;
- 188 plants with white and oval fruits ;
- 93 plants with white and round fruits.

Interpretation

☒ It is a cross between two individuals belonging to two pure lines that differ by two pairs of alleles or two genes : it is a dihybridism.

☒ The first generation F1 is homogeneous and similar. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents.

☒ In the first generation F1, all the fruits have a red and white color which is the manifestation of the two parental colors and have an oval shape which is intermediate between the shapes of the two parents. It is therefore a case of codominance for the first character and a case of incomplete dominance (absence of dominance) for the second character.

☒ In the second generation F2, there is the appearance of the two parental phenotypes (red and long : white and round) in addition to seven other new phenotypes which are intermediate between the parental phenotypes (red and oval ; red and round ; red and white and long ; red and white and oval ; red and white and round ; white and long ; white and oval). These phenotypes are called recombined.

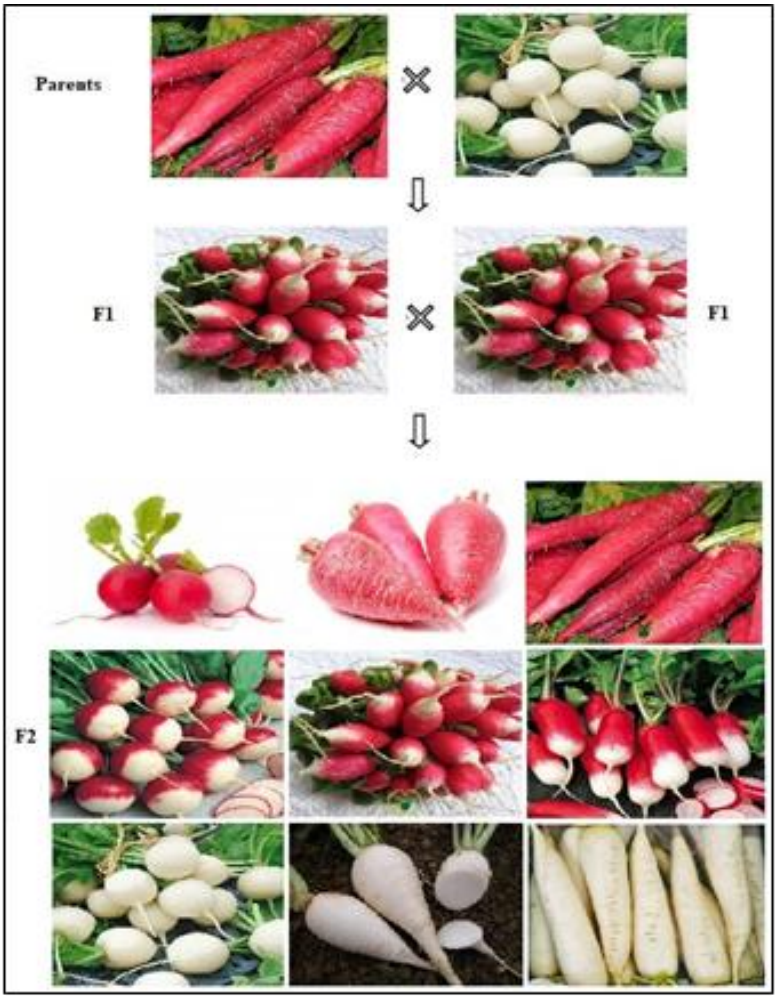


Figure 19: Cross up to F2 between two radish lines that differ by two characteristics: fruit color (codominance) and fruit shape (absence of dominance)

Genetic representation

Codes : R for red radish ; R' for white radish ; L^1 for long radish and L^2 for round radish.

Parents : ♀ RRL^1L^1 × ♂ $R'R'L^2L^2$

Gametes : ♀ RL^1 RL^1 RL^1 RL^1 × ♂ $R'L^2$ $R'L^2$ $R'L^2$ $R'L^2$

F1 : $RR'L^1L^2$

F2 : ♀ F1 × ♂ F1

♀ $RR'L^1L^2$ × ♂ $RR'L^1L^2$

Gametes : RL^1 RL^2 $R'L^1$ $R'L^2$ RL^1 RL^2 $R'L^1$ $R'L^2$

♀ \ ♂	RL^1	RL^2	$R'L^1$	$R'L^2$
RL^1	RRL^1L^1	RRL^1L^2	$RR'L^1L^1$	$RR'L^1L^2$
RL^2	RRL^1L^2	RRL^2L^2	$RR'L^1L^2$	$RR'L^2L^2$
$R'L^1$	$RR'L^1L^1$	$RR'L^1L^2$	$R'R'L^1L^1$	$R'R'L^1L^2$
$R'L^2$	$RR'L^1L^2$	$RR'L^2L^2$	$R'R'L^1L^2$	$R'R'L^2L^2$

Genotypes :

4/16 $RR'L^1L^2$: 2/16 RRL^1L^2 : 2/16 $RR'L^1L^1$: 2/16 $R'R'L^1L^2$: 2/16 $RR'L^2L^2$: 1/16 RRL^1L^1 : 1/16 RRL^2L^2 : 1/16 $R'R'L^1L^1$: 1/16 $R'R'L^2L^2$.

Phenotypes :

1/16 [**RL^1**] : 2/16 [**RL^1L^2**] : 1/16 [**RL^2**] : 2/16 [**$RR'L^1$**] : 4/16 [**$RR'L^1L^2$**] : 2/16 [**$RR'L^2$**] : 1/16 [**$R'L^1$**] : 2/16 [**$R'L^1L^2$**] : 1/16 [**$R'L^2$**].

Nine genotypes are obtained with nine phenotypes. The proportions of the phenotypic classes are 1/16: 2/16: 1/16: 2/16: 4/16: 2/16: 1/16: 2/16: 1/6 which correspond respectively to: 6.25%: 12.5%: 6.25%: 12.5%: 25%: 12.5%: 6.25%: : 12.5%: 6.25%.

These theoretical results correspond to the observed results in this example.

	Theoretical results	**Observed results**
[**RL^1**]	(1/16)*100 = **6,25%**	(94/1500)*100 = **6,26%**
[**R L^1 L^2**]	(2/16)*100 = **12,5%**	(188/1500)*100 = **12,54%**
[**R L^2**]	(1/16)*100 = **6,25%**	(93/1500)*100 = **6,2%**
[**RR' L^1**]	(2/16)*100 = **12,5%**	(187/1500)*100 = **12,46%**
[**RR' L^1 L^2**]	(4/16)*100 = **25%**	(374/1500)*100 = **24,94%**
[**RR' L^2**]	(2/16)*100 = **12,5%**	(188/1500)*100 = **12,53%**
[**R' L^1**]	(1/16)*100 = **6,25%**	(95/1500)*100 = **6,34%**
[**R' L^1 L^2**]	(2/16)*100 = **12,5%**	(188/1500)*100 = **12,53%**
[**R' L^2**]	(1/16)*100 = **6,25%**	(93/1500)*100 = **6,2%**

Remark

The results of codominance and incomplete dominance (absence of dominance) are similar. In both cases the proportions of genotypes and phenotypes are the same.

3.1.2.3. Backcross

As already mentioned, the backcross is a cross between an individual of the first generation F1 and a parental structure or an individual of the same genotype as the parents.

Example

In this part, the first example of dihybridism will be used to illustrate the two results of a backcross.

Mendel crossed a variety of smooth, yellow peas with a variety of wrinkled, green peas. Both varieties were homozygous. The F1 individuals, which are all yellow and smooth seeded plants, were crossed with both starting parents (Fig. 20). The cross between the F1 individuals and the parent carrying the dominant traits (yellow and smooth) gave, in F2, only plants with yellow and smooth seeds. While the cross between individuals of the F1 offspring and the parent carrying the recessive alleles gave, in F2, four different phenotypes, including two parental types and two recombined, with the following values :

- 245 plants with yellow and smooth seeds ;
- 248 plants with yellow and wrinkled seeds ;
- 241 plants with green and smooth seeds ;
- 246 plants with green and wrinkled seeds.

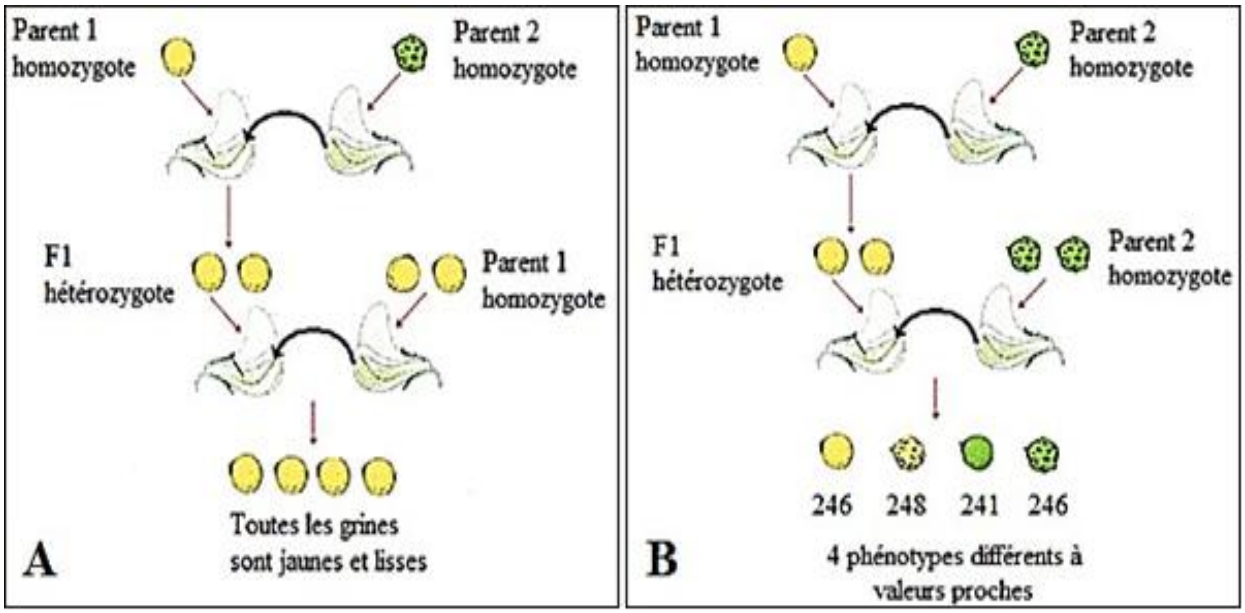


Figure 20: First cross (A) and second cross (B) backcross of two pea lines that differ in two traits (seed color and appearance).

Genetic representation

Codes : L for smooth seeds ; l for wrinkled seeds ; J for yellow seeds and j for green seeds.

Parents : ♀ JJLL × ♂ Jjll

Gametes : ♀ (JL) (JL) (JL) (JL) × ♂ (jl) (jl) (jl) (jl)

F1 : JjLl

Genotypes : **Phenotypes**

Parents : ♀ JJLL **Parents** : ♀ [JL]

♂ jjll ♂ [jl]

F1 : JjLl **F1** : [JL]

First backcross

F2 : ♀ F1 × ♂ Parent 1

♀ JjLl × ♂ JJLL

Gametes : ♀ (JL) (Jl) (jL) (jl) × ♂ (JL) (JL) (JL) (JL)

♂ / ♀	(JL)	(Jl)	(jL)	(jl)
(JL)	JJLL	JJLl	JjLL	JjLl

Genotypes : **Phenotypes :**

¼ JJLL : ¼ JJLl : ¼ JjLL : ¼ JjLl. 100% [JL]

Second backcross

F2 : ♀ F1 × ♂ Parent 2

♀ JjLl × ♂ JJLL

Gametes : ♀ (JL) (Jl) (jL) (jl) × ♂ (jl) (jl) (jl) (jl)

♂ / ♀	(JL)	(Jl)	(jL)	(jl)
JjLl (jl)		Jjll	jjLl	jjll

Genotypes : **Phenotypes :**

¼ JJLL : ¼ JJLl : ¼ JjLL : ¼ JjLl. ¼ [JL] : ¼ [Jl] : ¼ [jL] : ¼ [jl]

For this second case, four genotypes are obtained with four phenotypes. The proportions of the phenotypic classes are ¼: ¼ : ¼: ¼ which correspond respectively to 25%: 25%: 25%: 25%.

These theoretical results correspond to the observed results in this example.

	Theoretical results	Observed results
[**JL**]	(1/4)*100 = **25%**	(245/980)*100 = **24,95%**
[**Jl**]	(1/4)*100 = **25%**	(248/980)*100 = **25,33%**
[**jL**]	(1/4)*100 = **25%**	(241/980)*100 = **24,59%**
[**jl**]	(1/4)*100 = **25%**	(246/980)*100 = **25,13%**

3.1.2.4. Test-cross

Considering the previous example, the yellow and smooth seeds obtained in F2 have the phenotype [JL] but their genotypes can be either JJLL, if the plant is homozygous, or JjLl, if the plant is heterozygous. Visually, one can never distinguish between seeds of genotype JJLL and those of genotype JjLl. To do this, we must use the test cross (Fig. 21) to find out if the seeds are from homozygous (JJLL) or heterozygous (JjLl) plants.

Test cross :

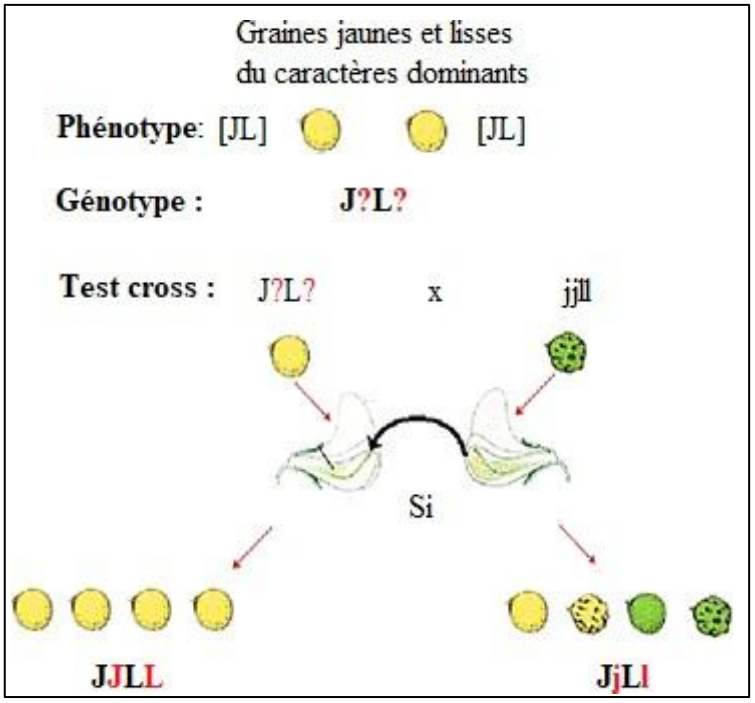


Figure 21: Test cross in peas to specify the homozygosity or heterozygosity of genotypes.

Plants from seeds with yellow and smooth phenotype [JL] and unknown genotype (JJLL or jjll) are crossed with plants from seeds carrying the recessive traits (jjll).

If, from this cross, we obtain only one phenotype, it means that each parent provides only one type of gamete and therefore both parents are homozygous and their first generation F1 is similar and homogeneous (Mendel's first law);

If, from this cross, we obtain several phenotypes, it means that the parent carrying the dominant traits provides several types of gametes and therefore it is heterozygous. The parent carrying the recessive traits is always homozygous.

Genetic representation

Yellow and Smooth [JL]

J?L?

If the genotype is JJLL, we will find :

♀ JJLL× ♂ jjll

♀ (JL)(JL)(JL)(JL) × ♂ (jl)(jl)(jl)(jl)

F1 : JjLl

Genotype F1 : JjLl

Phenotype F1 : [JL]

If the genotype is JjLl, we will find :

♀ JjLl × ♂ jjll

♀ (JL)(Jl)(jL)(jl) × ♂ (jl)(jl)(jl)(jl)

♂ \ ♀	(JL)	(Jl)	(jL)	(jl)
(jl)	JjLl	Jjll	jjLl	jjll

Genotype F1 : ¼ JjLl : ¼ Jjll : ¼ jjLl : ¼ jjll

Phenotypes F1 : ¼[JL]: ¼[JL]: ¼[JL]: ¼[JL]

Remark

When the genes are independent, the cross between a double heterozygote and a double recessive homozygote (test cross) gives four phenotypes in equal proportions (25%: 25%: 25%: 25%).

3.1.2.5. Lethal genes

In the previous dihybrid crosses, when the interactions between genes are other than dominance and recessiveness, the genotypic and phenotypic frequencies change. Now, we will check the genotypic and phenotypic frequencies in the presence of a recessive lethal allele. To do this, we consider the same example used in monohybridism (agouti mice and yellow mice) which will be associated with another trait.

Example

A cross between a purebred agouti mouse with red eyes and a yellow mouse with black eyes carrying the recessive lethal allele in the heterozygous state gives in the first generation F1, 39 agouti mice with black eyes and 41 yellow mice with black eyes. To obtain the second generation F2, the following crosses were performed (Fig. 22):

☒ ***First cross :*** the agouti mice with black eyes from F1 crossed with each other give in F2, 117 agoutis mice with black eyes and 43 agoutis mice with red eyes.

☒ ***Second cross :*** the agouti mice with black eyes from F1 are crossed with the yellow mice with black eyes from F1. The results obtained in F2 are as follows: 61 agouti mice with black eyes; 19 agouti mice with red eyes; 59 yellow mice with black eyes and 21 agouti mice with red eyes.

☒ ***Third cross :*** the yellow mice with black eyes from F1 crossed with each other give in F2, 31 agouti mice with black eyes ; 9 agoutis mice with red eyes ; 58 yellow mice with black eyes and

22 yellow mice with red eyes.

Interpretation

☒ This is a cross between two that differ by two pairs of alleles or two genes : it is a dihybridism.

☒ The first generation F1 is not homogeneous (the F1 consists of two different genotypes). This is due to the heterozygosity of a pair of alleles in the yellow parent with black eyes which provides two types of gametes.

☒ In the first generation F1, all individuals have black eyes, which leads to the understanding that the black eye trait is dominant and the red eye trait is recessive.

☒ Since the first generation F1 consists of two different genotypes, three crosses are necessary to obtain the second generation F2.

☒ At the end of the first cross between agouti mice with black eyes, all the mice are agouti which leads to confirm the homozygosity of these mice for this first trait. However, two eye colors are obtained indicating heterozygosity for this trait with dominance of black over red. The individuals are divided into two phenotypes in the following percentages : 73.125% agouti mice with black eyes and 26.875% agouti mice with red eyes.

☒ The offspring obtained by the second cross are distributed among four phenotypes in the following percentages : 38.125% agouti mice with black eyes ; 11.875% agouti mice with red eyes ; 36.875% yellow mice with black eyes and 13.125% yellow mice with red eyes.

☒ The offspring obtained by the third cross are also distributed among four phenotypes in the following percentages : 25.83% agouti mice with black eyes ; 7.5% agouti mice with red eyes ; 48.33% yellow mice with black eyes and 18.34% yellow mice with red eyes.

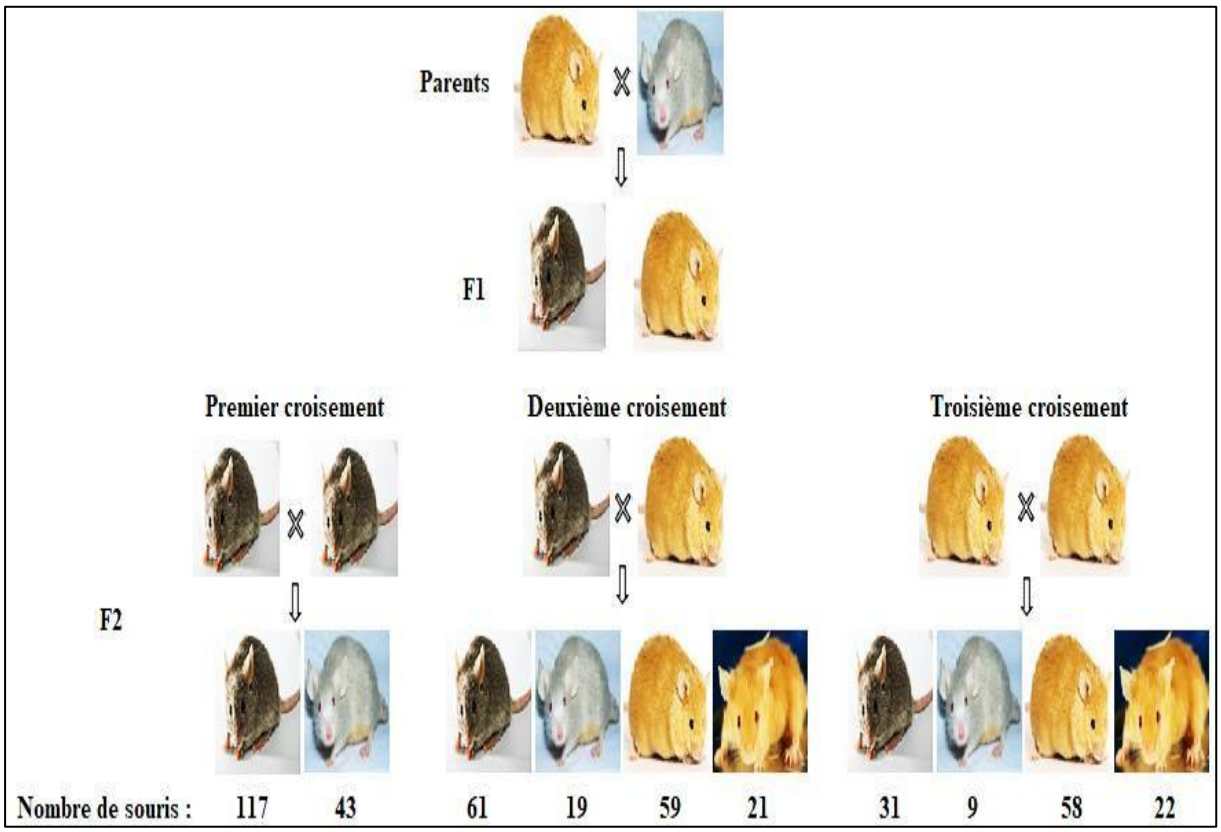


Figure 22: Cross between purebred agouti mice with red eyes and mutant yellow-coated mice with black eyes carrying the lethal allele.

Genetic representation

Codes: A **for agouti coat;** A^j **for yellow coat;** N **for black eye and** n **for red eye.**

Parents : ♀ AAnn × ♂ AA^jNN

Gamètes : ♀ (An) (An) (An) (An) × ♂ (AN) (AN) (A^jN) (A^jN)

$AANn$ AA^jNn

Genotypes Phenotypes

Parents : ♀ AAnn
♂ AA^jNN
F1 : ½ AANn : ½ AA^jNn

Parents : ♀ [An]
♂ [A^jN]
F1 : ½ [AN] : ½ [A^jN]

First cross

Parents : ♀ AANn × ♂ AANn

Gametes : ♀ (AN) (AN) (An) (An) × ♂ (AN) (AN) (An) (An)

♂ \ ♀	(AN)	(An)
(AN)	AANN	AANn
(An)	AANn	AAnn

Genotypes **Phenotypes**

¼ AANN : ½ AANn : ¼ AAnn ¾ [AN] : ¼ [An]

These theoretical results correspond to the observed results in this example.

	Theoretical Results	**Observed Results**
[AN]	(¾)*100 = **75%**	(117/160)*100 = **73,125%**
[An]	(¼)*100 = **25%**	(43/160)*100 = **26,875%**

Second cross

Parents : ♀ AANn × ♂ AA^{j}Nn

Gametes : ♀ (AN) (AN) (An) (An) × ♂ (AN) (An) (A^{j}N) (A^{j}n)

♂ \ ♀	(AN)	(An)	(A^{j}N)	(A^{j}n)
(AN)	AANN	AANn	AA^{j}NN	AA^{j}Nn
(AN)	AANN	AANn	AA^{j}NN	AA^{j}Nn
(An)	AANn	AAnn	AA^{j}Nn	AA^{j}nn
(An)	AANn	AAnn	AA^{j}Nn	AA^{j}nn

Genotypes : 2/16 AANN : 4/16 AANn : 2/16 AAnn : 2/16 AA^{j}NN : 4/16 AA^{j}Nn : 2/16 AA^{j}nn.

Phenotypes : 6/16 [AN] : 2/16 [An] : 6/16 [A^{j}N] : 2/16 [A^{j}n].

These theoretical results correspond to the observed results in this example.

	Theoretical Results	**Observed Results**
[AN]	(6/16)*100 = **37,5%**	(61/160)*100 = **38,125%**
[An]	(2/16)*100 = 1**2,5%**	(19/160)*100 = **11,875% %**
[A^{j}N]	(6/16)*100 = **37,5%**	(59/160)*100 = **36,875%**
[A^{j}n]	(2/16)*100 = 1**2,5%**	(21/160)*100 = **13,125%**

Second cross

Parents : ♀ $AA^{j}Nn$ × ♂ $AA^{j}Nn$

Gametes : ♀ (AN) (An) ($A^{j}N$) ($A^{j}n$) × ♂ (AN) (An) ($A^{j}N$) ($A^{j}n$)

♂ / ♀	(AN)	(An)	($A^{j}N$)	($A^{j}n$)
(AN)	AANN	AANn	$AA^{j}NN$	$AA^{j}Nn$
(An)	AANn	AAnn	$AA^{j}Nn$	$AA^{j}nn$
($A^{j}N$)	$AA^{j}NN$	$AA^{j}Nn$	$A^{j}A^{j}NN$	$A^{j}A^{j}Nn$
($A^{j}n$)	$AA^{j}Nn$	$AA^{j}nn$	$A^{j}A^{j}Nn$	$A^{j}A^{j}nn$

die before birth

Genotypes : 1/12 AANN : 2/12 AANn : 1/12 AAnn : 2/12 $AA^{j}NN$: 4/12 $AA^{j}Nn$: 2/12 $AA^{j}nn$.

Phenotypes : 3/12 [AN] : 1/12 [An] : 6/12 [$A^{j}N$] : 2/12 [$A^{j}n$].

These theoretical results correspond to the observed results in this example.

	Theoretical Results	**Observed Results**
[AN]	(3/12)*100 = **25%**	(31/120)*100 = **25,83%**
[An]	(1/12)*100 = **8,33%**	(9/120)*100 = **7, 5% %**
[$A^{j}N$]	(6/12)*100 = **50%**	(58/120)*100 = **48,33%**
[$A^{j}n$]	(2/12)*100 = **16,67%**	(22/120)*100 = **18,34%**

These results are explained by two pairs of alleles. Considering coat color, the yellow mutant allele **A^{j}** is dominant over the wild-type agouti allele **A** : heterozygous mice have a yellow coat. However, the yellow mutant allele also behaves as a recessive allele, lethal in the homozygous state. Mice with the **$A^{j}A^{j}$** genotype die before birth, so no homozygous yellow mice are obtained. Considering eye color, there is a dominance of black color over red color.

The phenotypic frequencies, this time too, are different from the 9:3:3:1 ratio obtained by Mendel, for independent genes, when there is only a dominance and recessiveness relationship between normal alleles.

3.1.2.6. Epistasis

Epistasis is a form of gene interaction in which one gene influences the phenotypic expression of another non-allelic gene such that the phenotype effectively depends on the first and not the second gene when both are present in the genotype.

The gene located at a locus that suppresses or masks the action of a gene located at another locus is called ***epistatic***. The gene or locus whose expression has been suppressed or

modified is called ***hypostatic***.

The classic 9:3:3:1 phenotypic proportions observed in the F2 of a dihybrid can be modified (in proportions). These modifications are due to interactions between genes involved in the same biochemical pathways. When there is epistasis between two loci, there are always fewer than four phenotypic classes usually found in the F2 of a bifactorial cross.

There are six commonly described classes of epistasis, three of which are represented by 3 phenotypes (12:3:1 ; 9:3:4 ; 9:6:1) and the other three have only 2 phenotypes (9:7 ; 15:1 ; 13:3).

a) Simple dominant epistasis (12:3:1)

If the dominant allele of a locus (gene) A is responsible for the expression of a certain phenotype regardless of the allele present at the other locus (B, b), locus A is said to be epistatic to locus B. This epistasis is dominant because allele A can be expressed in the presence of B and b. The alleles of the hypostatic locus (B, b) will only be able to express themselves in homozygous recessive individuals (aa) for the locus (a). Thus, the genotypes A-B- and A-bb will produce the same phenotype, while aaB- and aabb will produce two other different phenotypes. The classic 9:3:3:1 ratio becomes 12:3:1 (table 4).

Table 4 : Modification of the classic phenotypic ratio: simple dominant epistasis.

Genotypes	**A-B-**	**A-bb**	**aaB-**	**aabb**
Classic Ratios	9	3	3	1
Simple dominant epistasis	12		3	1

Example

The coat color of dogs depends on the action of at least two genes. At one locus, a dominant epistatic inhibitor of coat color pigment (I) prevents the expression of color alleles at another locus, producing white coat color, matching independently. When the recessive state exists at the inhibitor locus (ii), the alleles of the hypostatic locus can be expressed, (iiB-) producing black and (iibb) producing brown. The dihybrid cross between heterozygous white dogs gives in F1, 32 dogs divided into three phenotypes as follows: 23 white puppies; 7 black puppies and 2 brown puppies (Fig. 23).

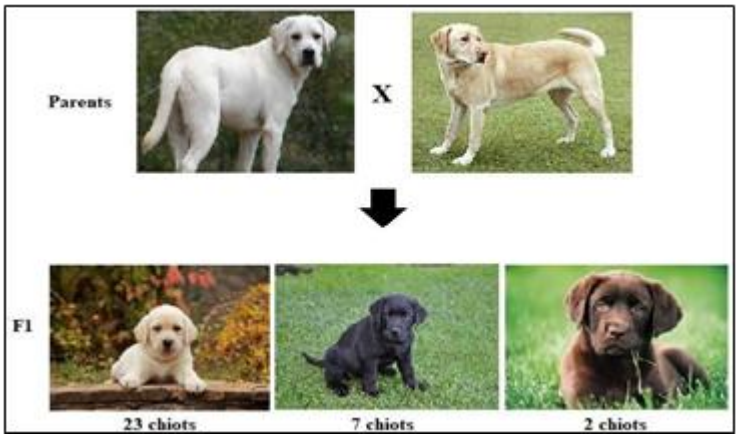


Figure 23: Dihybrid cross between heterozygous white dogs (simple dominant epistasis)

Interpretation

☒ The presence of the allele (I) in the dominant state masks the expression of the two alleles (B) and (b). This is a case of dominant epistasis. The allele (I) is epistatic, while the alleles (B) and (b) are hypostatic (Fig. 24).

☒ The presence of the allele (i) in the recessive state does not mask the expression of the other alleles (B) and (b). Allele (B) determines the black coat color whether homozygous or heterozygous, while allele (b) determines, in the homozygous state, the brown coat color. Therefore, it is clear that allele (B) is dominant over allele (b) which is recessive.

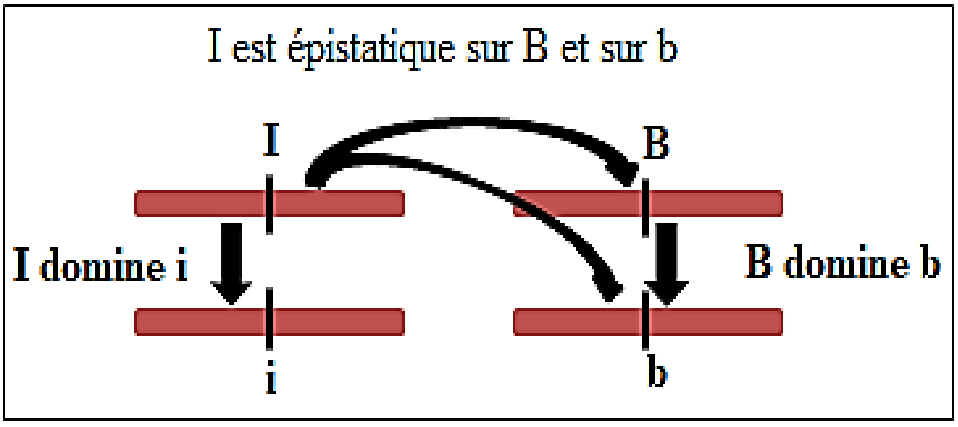


Figure 24: Simple dominant epistasis

Genetic representation

Codes : I for the dominant epistatic allele and **i** for the recessive allele (both alleles determine white coat color) ; **B** for black coat color and **b** for brown coat color.

Parents : ♂ IiBb × ♀ IiBb

Gametes : ♂ IB Ib iB ib ♀ IB Ib iB ib

♀ \ ♂	IB	Ib	iB	ib
IB	IIBB	IIBb	IiBB	IiBb
Ib	IIBb	IIbb	IiBb	Iibb
iB	IiBB	IiBb	iiBB	iiBb
ib	IiBb	Iibb	iiBb	iibb

Genotypes

4/16 IiBb; 1/16 IIBB; 1/16 IIbb; 1/16 iiBB; 1/16 iibb; 2/16 IIBb; 2/16 IiBB; 2/16 Iibb; 2/16 iiBb.

Phenotypes

12/16 [I-] includes [IB] and [Ib] (because I is epistatic to B and b); 3/16 [iB]; 1/16 [ib].

The genotypes IIBB, IIBb, IiBB, IiBb, IIbb, Iibb have the same phenotype. They are white. These theoretical results correspond to the observed results in this example.

	Theoretical Results	**Observed Results**
[I-] [iB]	(12/16)*100 = **75%**	(23/32)*100 = **71,88%**
[ib]	(3/16)*100 = 1**8,75%**	(7/32)*100 = **21,87% %**
	(1/16)*100 = **6,25%**	(2/32)*100 = **6,25%**

b) Simple recessive epistasis (9:3:4)

If the recessive genotype of a locus (aa) prevents the expression of the alleles of the locus (B), the genotype aa is said to exert recessive epistasis on locus B (B/b). The alleles of the hypostatic locus B (B/b) will only be able to express themselves in the presence of the dominant allele A. The genotypes aaB- and aabb have the same phenotype, while the genotypes A-B- and A-bb express two different phenotypes. Thus, the phenotypic proportions ; 9:3:3:1 become 9:3:4 (table 5).

Table 5 : Modification of the classic phenotypic proportions : simple recessive epistasis

Genotypes	**A-B-**	**A-bb**	**aaB-**	**Aabb**
Classic Ratios	9	3	3	1
Simple recessive epistasis	9	3	4	

Example

Two different genes determine coat color in rats. The presence of the recessive allele (b) in the homozygous state determines a white coat color in rats regardless of the combination of alleles of the other gene. However, the presence of the recessive allele in the heterozygous state determines a black coloration (if B-N-) or cream (if B-nn) depending on the allelic combination of the other gene. A cross between purebred black rats and purebred albino rats produced an F1 composed only of black rats (Fig. 25). The cross between the F1 rats

produced the following offspring: 14 cream rats, 47 black rats and 19 albino rats.

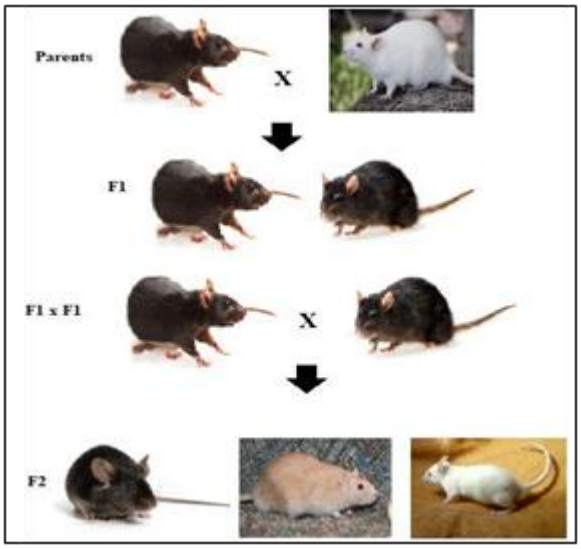


Figure 25: Cross between purebred black rats and albino rats (simple recessive epistasis).

Interpretation

☒ Two different genes are involved in determining the coat color of rats : this is a case of dihybridism.

☒ The first generation F1 is homogeneous and similar. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents.

☒ The presence of the (B) allele in the dominant state does not mask the expression of the other alleles (N) and (n). The (N) allele determines the black coat color whether in the homozygous or heterozygous state, while the (n) allele determines, in the homozygous state, the cream coat color.

☒ We can present (N) and (n) as alleles that can only determine the production of their respective pigments with the collaboration of (B).

☒ We can imagine that (N) and (n) are each responsible for the production of the precursor of a pigment and that these precursors (functional enzymes) require the presence of a substance or enzyme that activates them, the production of which is under the control of (B). (b) in the homozygous state does not allow the production of this complementary substance or this enzyme. This implies that (bb) prevents the expression of (N) and (n). (b) is therefore epistatic on (N) and (n). Since (b) is recessive to (B), the epistasis it determines is said to be recessive (Fig. 26).

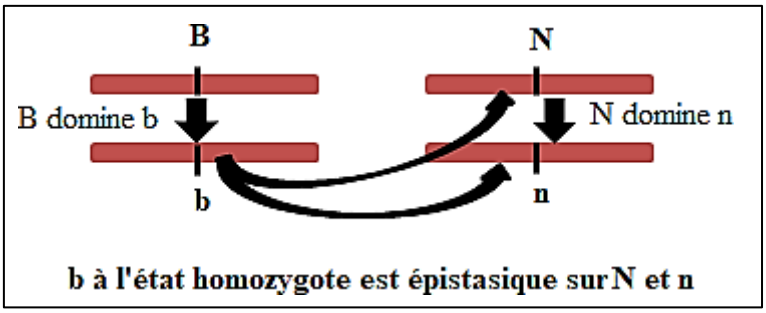


Figure 26: Simple recessive epistasis.

Genetic representation

Codes : b for the recessive epistatic allele and **B** for the dominant allele (both alleles determine the white coat color) ; **N** for the black coat color and **n** for the cream coat color.

Parents : ♂ BBNN × ♀ bbnn

Gametes : ♂ (BN) (BN) (BN) (BN) ♀ (bn) (bn) (bn) (bn)

F1 : BbNn

F2 : ♂ F1 × ♀ F1

F2 : ♂ BbNn × ♀ BbNn

Gametes : ♂ (BN) (Bn) (bN) (bn) ♀ (BN) (Bn) (bN) (bn)

♂ / ♀	(BN)	(Bn)	(bN)	(bn)
(BN)	BBNN	BBNn	BbNN	BbNn
(Bn)	BBNn	BBnn	BbNn	Bbnn
(bN)	BbNN	BbNn	bbNN	bbNn
(bn)	BbNn	Bbnn	bbNn	bbnn

Genotypes : 4/16 BbNn ; 1/16 BBNN ; 1/16 BBnn ; 1/16 bbNN ; 1/16 bbnn ; 2/16 BBNn ; 2/16 BbNN ; 2/16 Bbnn; 2/16 bbNn.

Phenotypes : 9/16 [BN] ; 3/16 [Bn] ; 4/16 [b-] which groups [bN] and [bn] (because bb is epistatic on N and n).

The genotypes bbNN, bbNn, bbnn have the same phenotype. They are white.

These theoretical results correspond to the results observed in this example.

	Theoretical results	Observed results
[BN]	(9/16)*100 = **56,25%**	(47/80)*100 = **58,75%**
[Bn] [b-]	(3/16)*100 = **18,75%**	(14/80)*100 = **17,5% %**
	(4/16)*100 = **25%**	(19/80)*100 = **23,75%**

c) Double dominant epistasis with cumulative effect (9:6:1)

When the presence of a dominant allele (in the homozygous or heterozygous state) at either locus (but not both at the same time) results in the same phenotype (A-bb and aaB- produce the same phenotype), the phenotypic distribution becomes 9:6:1. The dominant alleles when present together produce a phenotype with a cumulative effect. Recessive alleles in the homozygous state (aabb) produce a different phenotype (table 6).

Table 6 : Modification of the classical phenotypic ratio : double dominant epistasis with cumulative effect.

Genotypes	**A-B-**	**A-bb**	**aaB-**	**aabb**
Classical ratios	9	3	3	1
Double dominant epistasis with cumulative effect	9	6		1

Example

The red coloration of wheat grains is due to the R-B- genotype. The white coloration is due to the double recessive genotype rrbb. The R-bb and rrB- genotypes give brown grains. A homozygous red variety crossed with a white variety gives red grains in F1 and in F2 (Fig. 27): 907 red grains: 602 brown grains and 101 white grains.

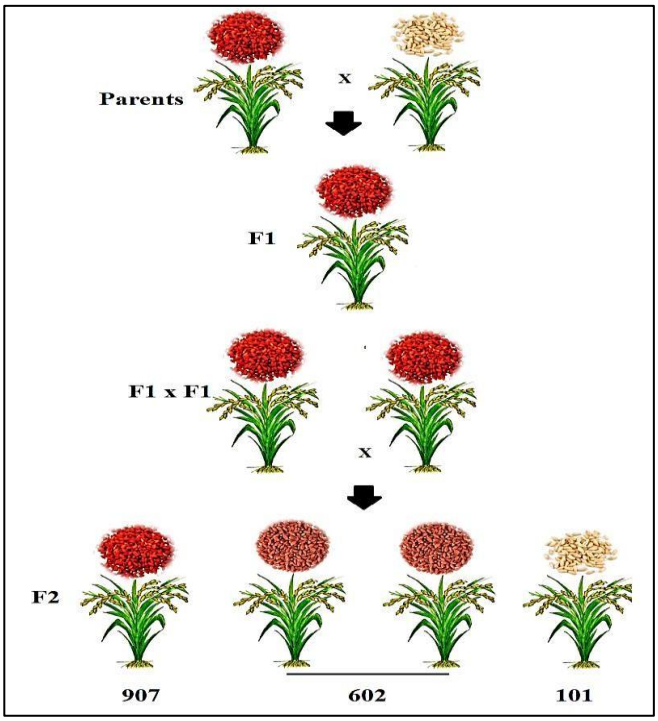


Figure 27: Cross between homozygous red wheat varieties and white seeds (double dominant epistasis with cumulative effect).

Interpretation

☒ Two different genes are involved in determining the color of wheat grains: this is a case of dihybridism.

☒ The first generation F1 is homogeneous and similar. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents.

☒ The R allele and the B allele, when found alone with recessive alleles, determine the brown coloration. They are expressed by the same phenotype separately but when they are together, they are expressed by another cumulative phenotype (red). The R allele is epistatic on bb and the B allele is epistatic on rr (Fig. 28).

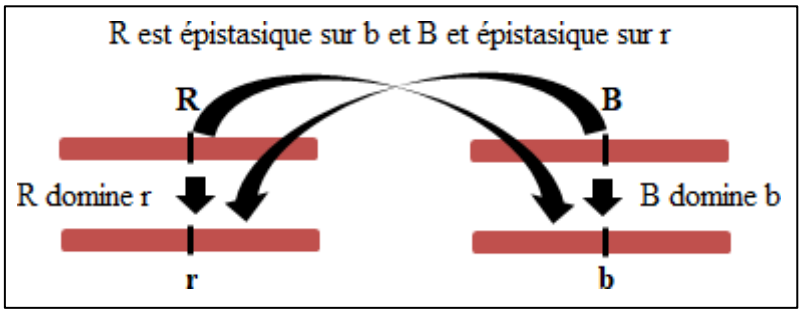


Figure 28: Double dominant epistasis with cumulative effect.

Genetic representation

Codes : R and B for the brown color of the grains, B-R- for the red color and bbrr for the white color of the grains.

Parents : ♂ RRBB × ♀ rrbb

Gametes : ♂ RB RB RB RB × ♀ rb rb rb rb

F1 : RrBb

F2 : ♂ F1 × ♀ F1

F2 : ♂ RrBb × ♀ RrBb

Gametes : ♂ RB Rb rB rb × ♀ RB Rb rB rb

♀ \ ♂	RB	Rb	rB	rb
RB	RRBB	RRBb	RrBB	RrBb
Rb	RRBb	RRbb	RrBb	Rrbb
rB	RrBB	RrBb	rrBB	rrBb
rb	RrBb	Rrbb	rrBb	rrbb

Genotypes : 4/16 RrBb ; 1/16 RRBB ; 1/16 RRbb ; 1/16 rrBB ; 1/16 rrbb ; 2/16 RRBb ; 2/16 RrBB ; 2/16 Rrbb; 2/16 rrBb.

Phenotypes : 9/16 [RB] ; 6/16 [Rb] et [rB]; 1/16 [rb]

Les génotypes RRbb, rrBB, Rrbb et rrBb ont le même phénotype. Les grains sont marron. Ces résultats théoriques correspondent aux résultats observés dans cet exemple.

	Theoretical results	**Observed results**
[RB] [Rb]	(9/16)*100 = **56,25%**	(907/1610)*100 = **56,34%**
[rB]	(6/16)*100 = **37,5%**	(602/1610)*100 = **37,39%**
[rb]	(1/16)*100 = **6,25%**	(101/1610)*100 = **6,27%**

d) Double dominant epistasis without cumulative effect (15 : 1)

If the dominant alleles at each of the two loci are expressed by the same phenotype without cumulative effect, we speak of double dominant epistasis. A is epistatic on b and B is epistatic on a. the classic allelic frequencies in this case become 15:1 (table 7).

Table 7 : Modification of the phenotypic ratio: double dominant epistasis without cumulative effect.

Genotypes	**A-B-**	**A-bb**	**aaB-**	**aabb**
Classical ratios	9	3	3	1
Double dominant epistasis without cumulative effect	15			1

Example

The plant of the genus Capsella, commonly called \"shepherd's purse\", produces a seed capsule, the shape of which depends on two independently segregating genes, represented by the symbols (A) and (B). Dihybrid plants with ovoid capsules and triangular capsules are interpollinated (Fig. 29). All the plants of the first generation F1 all had triangular capsules. The F1 plants are interpollinated with each other and the second generation F2 is composed of 940 plants with triangular capsules and 60 plants with ovoid capsules.

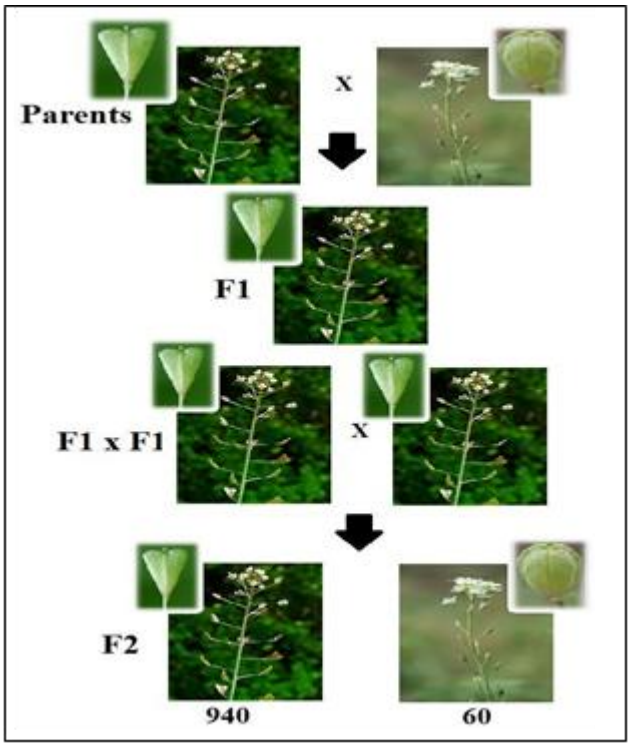


Figure 29: Dihybrid cross between shepherd's purse plants with ovoid capsules and triangular capsules (double dominant epistasis without cumulative effect).

Interpretation

☒ Two different genes are involved in determining the shape of the seed capsules of shepherd's purse: this is a case of dihybridism.

☒ The first generation F1 is homogeneous and similar. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents.

☒ The A allele and the B allele alone or together are expressed by the same phenotype (triangular capsules). The A allele is epistatic on bb and the B allele is epistatic on aa (Fig. 30).

Genetic representation

Codes : A and B for the dominant epistatic alleles which determine the triangular shape of the capsule. a and b for the hypostatic alleles which, in the homozygous state, determine the ovoid shape of the capsule**.**

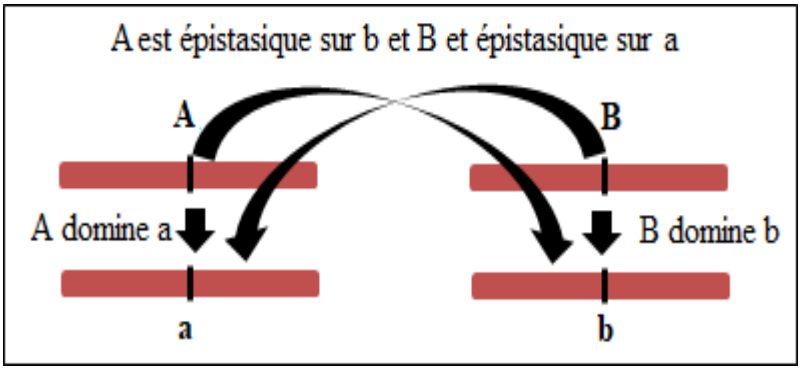


Figure 30: Double dominant epistasis without cumulative effect

Parents : ♂ AABB × ♀ aabb

Gametes : ♂ (AB) (AB) (AB) (AB) × ♀ (ab) (ab) (ab) (ab)

F1 : AaBb

F2 : ♂ F1 × ♀ F1

F2 : ♂ AaBb × ♀ AaBb

Gametes : ♂ (AB) (Ab) (aB) (ab) × ♀ (AB) (Ab) (aB) (ab)

♀ \ ♂	(AB)	(Ab)	(aB)	(ab)
(AB)	AABB	AABb	AaBB	AaBb
(Ab)	AABb	AAbb	AaBb	Aabb
(aB)	AaBB	AaBb	aaBB	aaBb
(ab)	AaBb	Aabb	aaBb	aabb

Genotypes : 4/16 AaBb ; 1/16 AABB ; 1/16 AAbb ; 1/16 aaBB ; 1/16 aabb ; 2/16 AABb ; 2/16 AaBB ; 2/16 Aabb; 2/16 aaBb.

Phenotypes : 15/16 [AB]/[Ab]/[aB] ; 1/16 [ab]

The genotypes AaBb, AABB, AAbb, aaBB, aaBb, AABb, AaBB, Aabb have the same phenotype. The capsules are triangular. These theoretical results correspond to the results observed in this example.

	Theoretical results	**Observed results**
[AB] [Ab] [aB]	(15/16)*100 = **93,75%**	(940/1000)*100 = **94%**
[ab]	(1/16)*100 = **6,25%**	(60/1000)*100 = **6%**

e) Double recessive epistasis (9 : 7)

When the two recessive homozygous genotypes give identical phenotypes, the phenotypic ratios of the F2 become 9 : 7. The genotypes aaB-, A-bb and aabb give the same phenotype. When the dominant alleles are present together, each complements the other, and a different phenotype is obtained (table 8).

Table 8 : Modification of the classic phenotypic ratio: double recessive epistasis

Genotypes	**A-B-**	**A-bb**	**aaB-**	**aabb**
Classical ratios	9	3	3	1
Double recessive epistasis	15	7		

Example

Two varieties of peas (Lathyrus odoratus) with white flowers are crossed. The F1 consists only of purple flowers (Fig. 31) and in F2 we obtain 96 individuals of which 53 plants have purple flowers and 43 plants have white flowers.

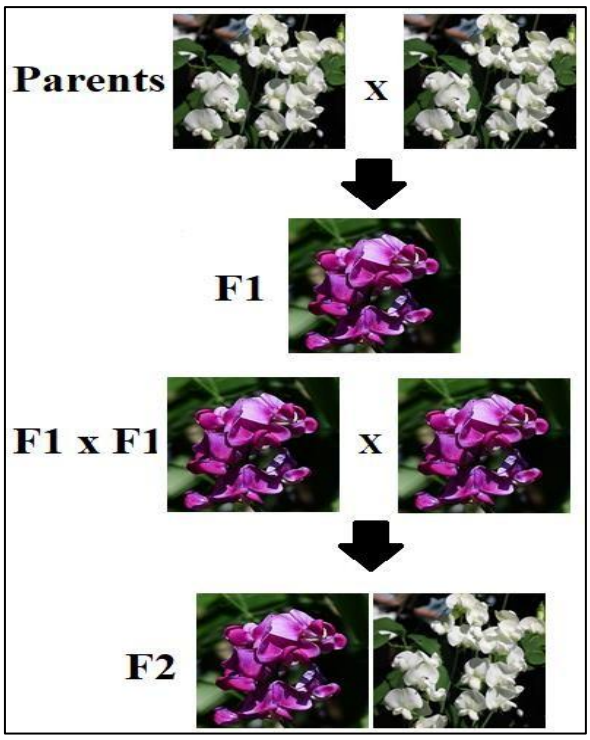


Figure 31: Cross of two varieties of peas (*Lathyrus odoratus*) with white flowers (double recessive epistasis).

Interpretation

☒ The first generation F1 is 100% homogeneous, all with purple flowers, the parents are therefore pure, Mendel's first law is verified.

☒ The second generation F2 indicates that this is a dihybridism with interaction between two genes that code for the same phenotype.

☒ Instead of finding four phenotypes, we have only two in different proportions, which leads us to say that the interaction is of the epistatic type. The observed ratios are (53/96) and (43/96) which correspond to the ratios; 9/16 and 7/16, indicating that this is a double recessive epistasis. (b) the homozygous state prevents pigmentation or the action of (P) therefore (b) is epistatic on (P). (p) in the homozygous state also prevents pigmentation or the action of (B), it is therefore epistatic on (B). (p) and (b) are expressed by the same phenotype, while the two dominant alleles together determine a new phenotype (Fig. 32).

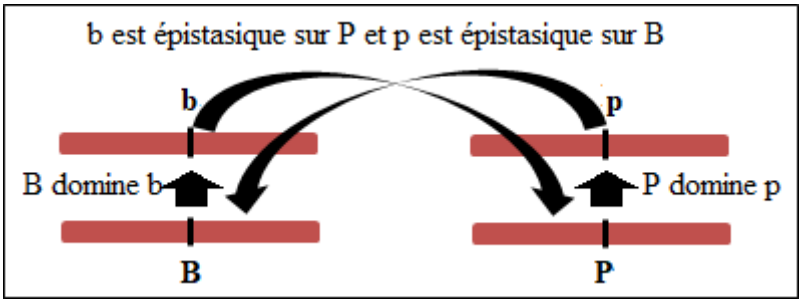


Figure 32: Double recessive epistasis

Genetic representation

Codes : B and P for the hypostatic alleles determining purple pigmentation and b and p for the epistatic alleles determining the absence of pigmentation (white).

Epistasis begins at the outset with parents who have white flowers and are homozygous.

Parents : ♀ bbPP × ♂ BBpp

Gametes : ♀ (bP) (bP) (bP) (bP) ♂ (Bp) (Bp) (Bp) (Bp)

F1 : BbPp

F2 : ♂ F1 × ♀ F1

F2 : ♂ BbPp × ♀ BbPp

Gametes : ♂ (BP) (Bp) (bP) (bp) ♀ (BP) (Bp) (bP) (bp)

♀ \ ♂	(BP)	(Bp)	(bP)	(bp)
(BP)	BBPP	BBPp	BbPP	BbPp
(Bp)	BBPp	BBpp	BbPp	Bbpp
(bP)	BbPP	BbPp	bbPP	bbPp
(bp)	BbPp	Bbpp	bbPp	bbpp

Genotypes : 4/16 BbPp ; 1/16 BBPP ; 1/16 BBpp ; 1/16 bbPP ; 1/16 bbpp ; 2/16 BBPp ; 2/16 BbPP ; 2/16 Bbpp; 2/16 bbPp.

Phenotypes : 9/16 [BP] ; 7/16 [bp]/[Bp]/[bP].

The genotypes, BBpp, Bbpp, bbPP, bbPp and bbpp have the same phenotype. The flowers are white. The genotypes grouping two dominant alleles together (BBPP, BbPp, BBPp and BbPP) determine the purple flower phenotype.

For the purple pigments to be produced, two complementary substances are needed, one being produced by P and the other by B. Thus, there will be the production of purple pigments by

all genotypes constituted by at least one P and one B.

The genotypes Bbpp and BBpp have only one functional enzyme B and cannot achieve pigmentation.

The genotypes bbPp and bbPP have only one functional enzyme P and cannot achieve pigmentation.

The bbpp genotype has no functional enzyme and cannot synthesize the purple pigment. This is referred to as double recessive epistasis and also complementation because the presence of two dominant alleles P and B together is necessary to produce the purple pigment.

These theoretical results correspond to the results observed in this example.

	Theoretical results	**Observed results**
[AB]	(9/16)*100 = **56,25%**	(53/96)*100 = **55,2%**
[Ab] [aB] [ab]	(7/16)*100 = **43,75%**	(43/96)*100 = **44,8%**

f) Dominant and recessive epistasis (13 : 3)

When the same phenotype is obtained either by the presence of a dominant genotype at one locus (A-), or by the presence of the recessive genotype at the other locus (bb), only two phenotypes are observed in F2. The dominant allele A is epistatic to allele B and allele b is epistatic to allele a. In this case, individuals : A-B-, A-bb and aabb have the same phenotype, and individuals aaB have another phenotype. Therefore, the classic phenotypic frequencies become 13:3 instead of 9:3:3:1 (table 9).

Table 9 : Modification of the classic phenotypic ratio: dominant and recessive epistasis.

Genotypes	**A-B-**	**A-bb**	**aabb**	**aaB-**
Classical ratios	9	3	1	3
dominant and recessive epistasis.	13			3

Example

In chickens, a cross between a white Leghorn rooster and a white Wyandotte hen is expressed by an entirely white F1. The cross between individuals of the F1, an F2 composed of 261 white individuals and 59 pigmented individuals is obtained (Fig. 33).

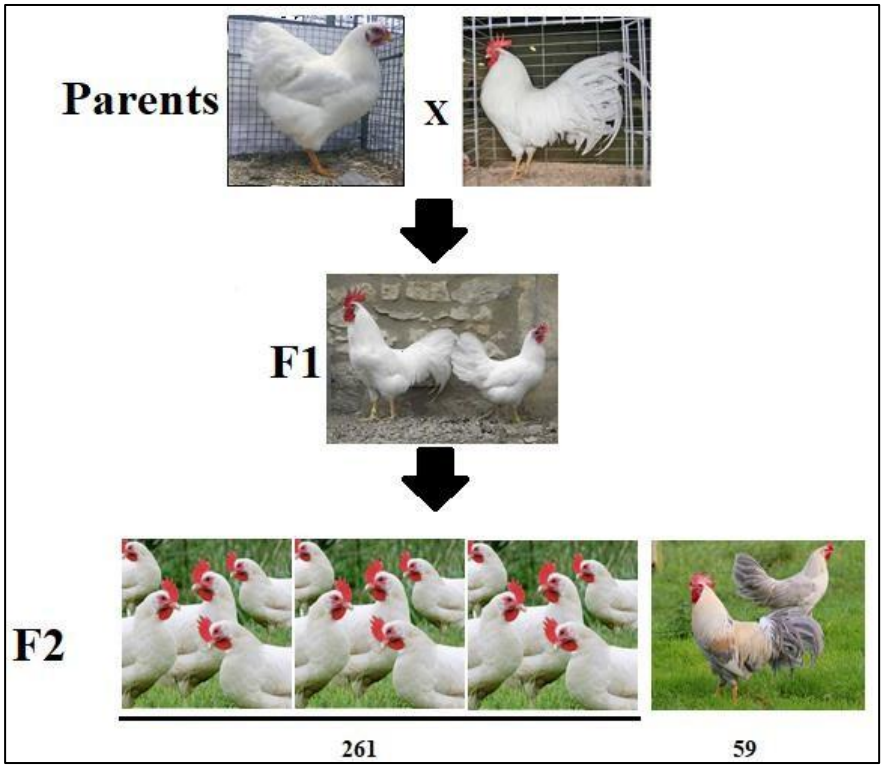


Figure 33: Cross between a white Leghorn rooster and a white Wyandotte hen (dominant and recessive epistasis).

Interpretation

☒ The first generation F1 is 100% homogeneous, all individuals are white, the parents are therefore pure, Mendel's first law is verified.

☒ The second generation F2 indicates that this is a dihybridism with interaction between two genes that code for the same phenotype.

☒ Instead of finding four phenotypes, we have only two in different proportions, which leads us to say that the interaction is of the epistatic type. The observed ratios are (261/320) and (59/320) which correspond to the ratios; 13/16 and 3/16, indicating that this is a dominant and recessive epistasis. (p) the homozygous state prevents pigmentation or the action of (b) therefore (p) is epistatic on (b). (B) in the homozygous and heterozygous state prevents pigmentation or the action of (P), it is therefore epistatic on (P). (B) and (p) are expressed by the same phenotype, while the recessive hypostatic allele (b) in the homozygous state determines with the dominant hypostatic allele (P) a new phenotype.

☒ The 13:3 ratio appears to be a modification of the 9:3:3:1 dihybrid ratio in which all genotypes containing at least one B (numbering 12) and the aabb genotype produce the same white phenotype, while the others numbering 3 produce a pigmented plumage. These results can be interpreted by assuming 2 epistases, one dominant and the other recessive (Fig. 34).

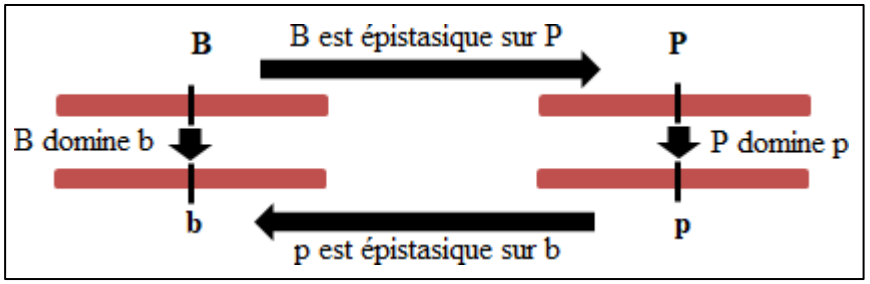


Figure 34: Dominant and recessive epistasis

Genetic representation

Codes : B and p for the hypostatic alleles determining the white color and b and P for the epistatic alleles determining the presence of a different pigmentation.

The epistasis begins initially with parents who have white feathers and are homozygous.

Parents : ♀ BBPP × ♂ bbpp

Gametes : ♀ (BP) (BP) (BP) (BP) × ♂ (bp) (bp) (bp) (bp)

F1 : BbPp

F2 : ♂ F1 × ♀ F1

F2 : ♂ BbPp × ♀ BbPp

Gametes : ♂ (BP) (Bp) (bP) (bp) ♀ (BP) (Bp) (bP) (bp)

♀ \ ♂	(BP)	(Bp)	(bP)	(bp)
(BP)	BBPP	BBPp	BbPP	BbPp
(Bp)	BBPp	BBpp	BbPp	Bbpp
(bP)	BbPP	BbPp	bbPP	bbPp
(bp)	BbPp	Bbpp	bbPp	bbpp

Genotypes : 4/16 BbPp ; 1/16 BBPP ; 1/16 BBpp ; 1/16 bbPP ; 1/16 bbpp ; 2/16 BBPp ; 2/16 BbPP ; 2/16 Bbpp; 2/16 bbPp.

Phenotypes: 13/16 [BP]/[bp]/[Bp]; 3/16 [bP].

The genotypes, BBpp, Bbpp, and bbpp, BBPP, BbPp, BBPp and BbPP have the same phenotype. The feathers are white. The genotypes grouping the two hypostatic alleles together (bbPP, bbPp) determine the phenotype of the pigmented feathers.

These theoretical results correspond to the results observed in this example.

	Theoretical results	Observed results
[AB] [Ab] [ab]	(13/16)*100 = **81,25%**	(261/320)*100 = **81,56%**
[aB]	(3/16)*100 = **18,75%**	(59/320)*100 = **18,44%**

3.2. Linked genes

When two or more genes are located on the same chromosome, they are physically linked. They can be linked on one of the autosomes or on the sex chromosome. Genes located on different chromosomes are distributed in the gametes independently of each other (Mandel's law of independent segregation). Genes located on the same chromosome will tend to stay together during gamete formation. Therefore, the results of the test cross of dihybrid individuals will be different, depending on whether these genes are linked or carried by different chromosomes.

When genes are independent, they segregate independently into gametes during meiosis and the test cross gives four products in equal proportions. Similarly, the proportions between parental and recombinant types are equal. On the other hand, when genes are linked, they tend to stay together during gamete formation and only separate if there is crossing over. The proportions of recombinants will therefore be lower than those of the parents.

3.2.1. *Crossing over*

a) Simple *crossing over*

During meiosis, each chromosome duplicates, thus giving two identical sister chromatids. Homologous chromosomes pair up (synapsis) and crossing over takes place between non-sister chromatids. This phenomenon involves the breaking of the ligation of only two of the four strands, at any point on the chromosome. In the following figure, the crossing over takes place in the region located between loci A and B.

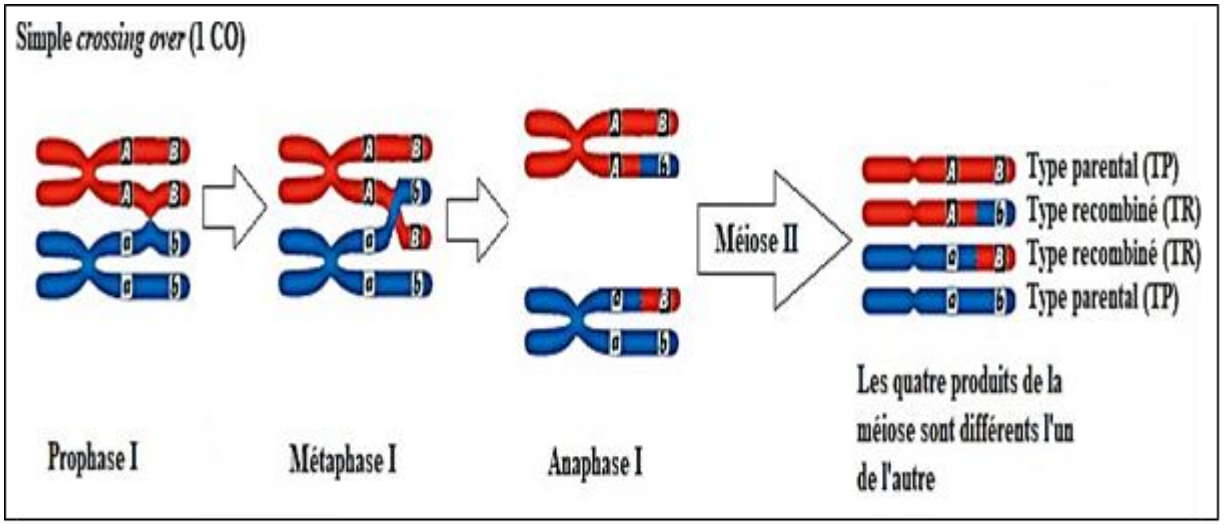


Figure 35: Simple crossing over between loci A and B, involving two non-sister chromatids.

Two of the products of meiosis (AB and ab) have the same linkage profile as that of the parental chromosomes. These come from the chromatids that have not undergone crossing over: they are said to be **non-recombinant**, therefore of **parental types**. The other two products of meiosis (Ab and aB), resulting from a crossing over, are rearranged compared to the parental linkage profile: they are said to be **recombinant**.

In the absence of crossing over, all the products of meiosis are of the parental type. If there is no crossing over, there is no separation between the linked genes on the same chromosome and consequently, there will be no more recombination (Fig. 36).

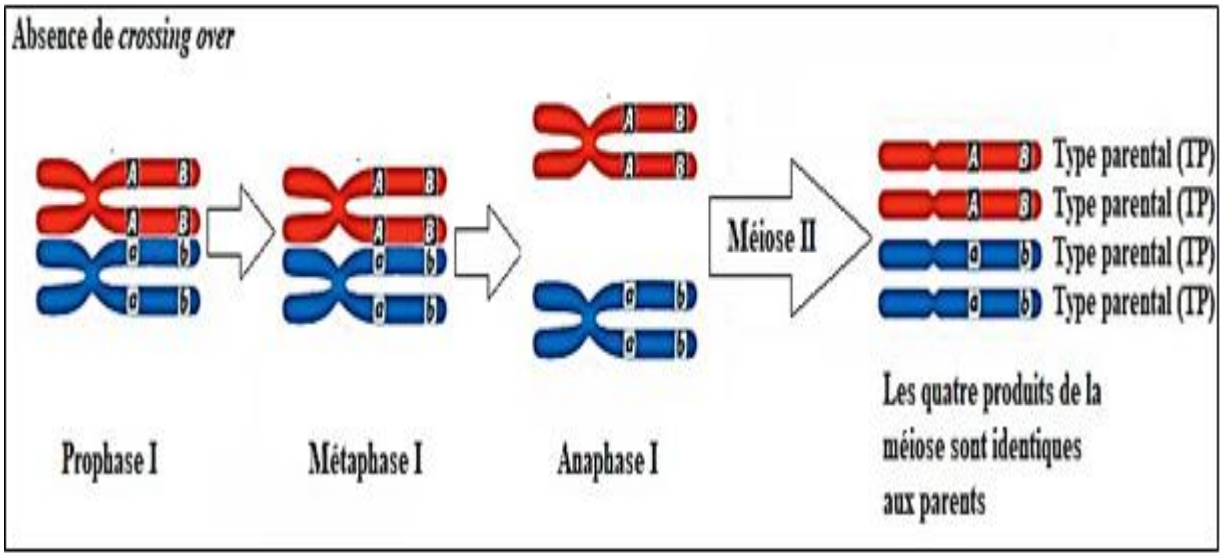


Figure 36: Absence of crossing over between loci A and B.

The alleles of dihybrid individuals at two linked loci can be associated in two different ways (Fig. 37):

If the two dominant (or wild) alleles are on one chromosome and the two recessive (or mutant) alleles are on the other chromosome (AB/ab), the linkage type is said to be in **coupled phase** or in ***Cis* position**.

When the dominant allele of one locus and the recessive allele of the other are on one chromosome and the remaining two are on the other chromosome (Ab/aB), the linkage type is said to be in **repulsion phase** or in ***Trans* position**.

Note : Recombinant and parental gametes are of different types depending on how these genes are linked in the parents.

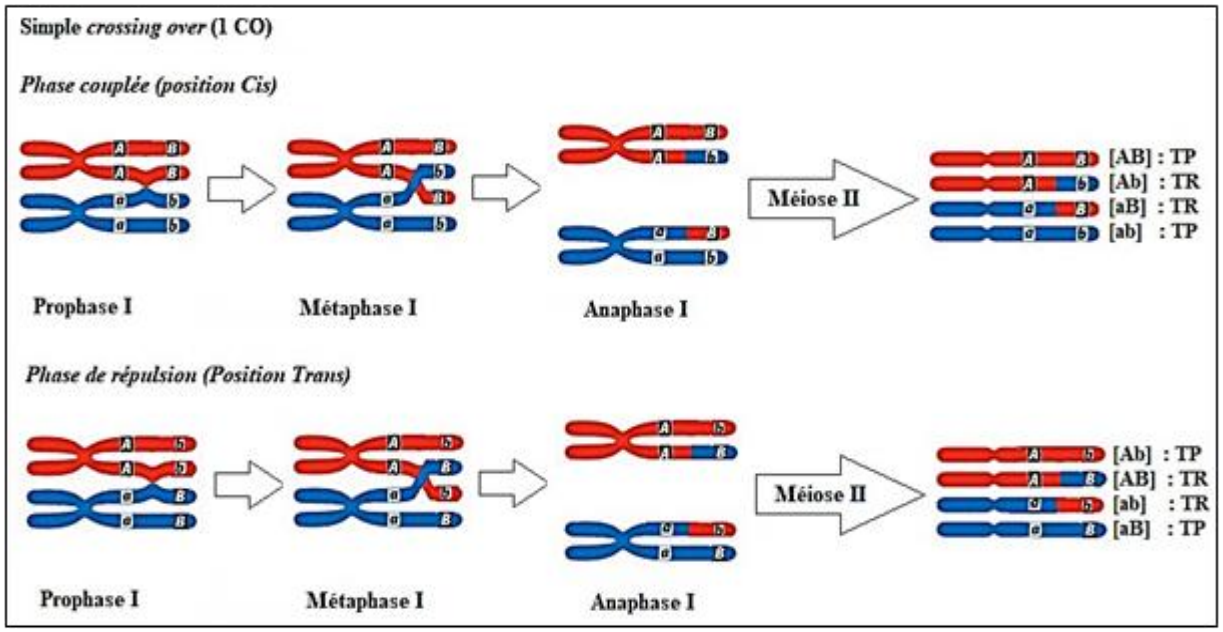


Figure 37 : Different associations between alleles : Coupling phase (*Cis*) and repulsion phase (*Trans*).

b) Multiple *crossing over*

When two crossovers occur between two genetic markers, the phenotypes of the offspring are all of the parental type (Fig. 38). To detect this double crossing over, a third locus (G) is used and must be located between the two previous ones (A and B). In this case, the double crossovers give two recombinant phenotypes with the two parents.

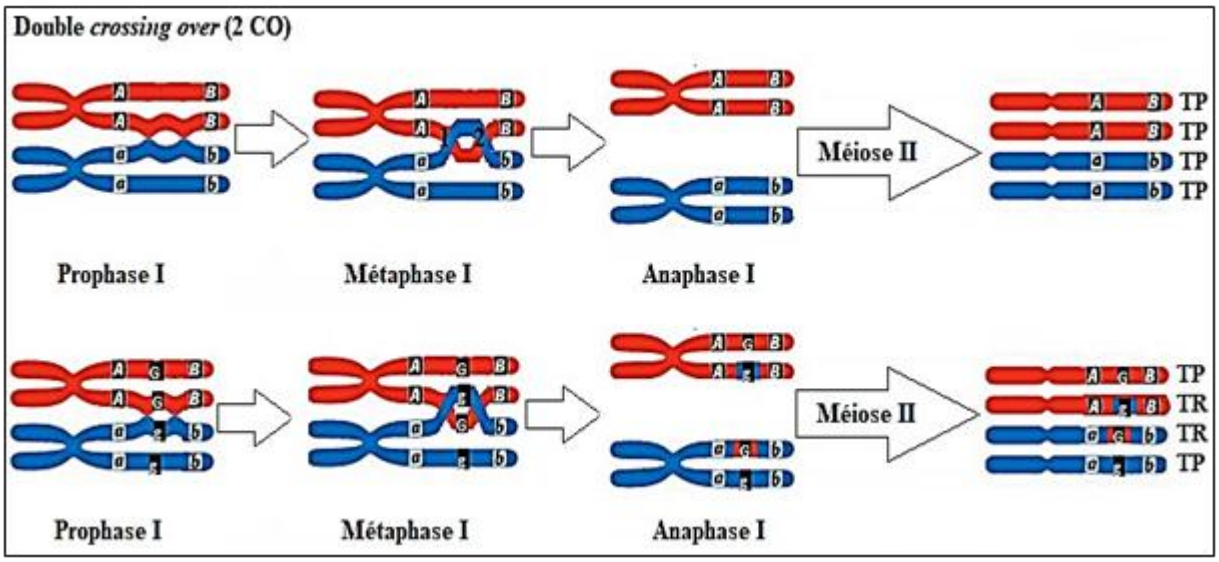


Figure 38: Double crossing over between loci A and B, involving two non-sister chromatids.

An odd number of crossovers (1, 3, 5, 7, etc.) between two loci will give a new genetic recombination between these two markers and the products of this meiosis are two parental types and two recombinant types (Fig. 35) While, the even number of crossovers (2, 4, 6, 8, etc.) between two loci will only give phenotypes of parental types.

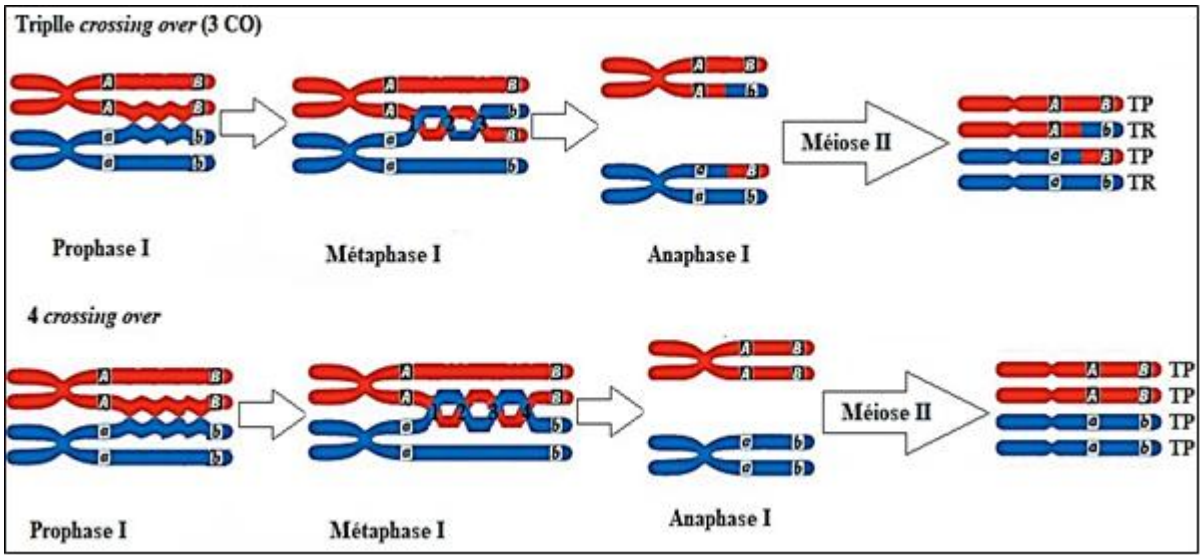


Figure 39: Multiple crossing over.

3.2.2. Linkage

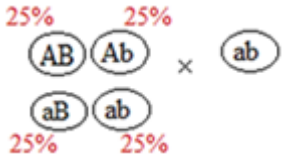
The word linkage is used to designate the phenomenon determined by the location of several genes on the same chromosome. Such genes are not independent of each other but are, on the contrary, linked. The constitution of living beings, however, makes linkage a quantitative phenomenon that can be complete or incomplete.

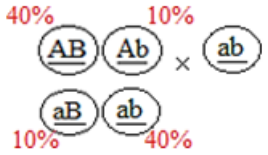
Linkage is complete when linked genes never separate. In this case, there is no crossing over between non-sister chromatids and therefore there is no recombination. Linkage is incomplete when linked genes can separate but less frequently than independent genes. This separation between linked genes is done by crossing over.

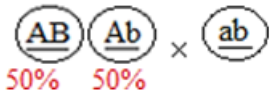
Note : Linkage is most often studied through the test cross.

Table 10 and Figure 40 represent, respectively, the results of three test crosses and the formation of gametes of a dihybrid with, on the one hand, independent genes and, on the other hand, linked genes (complete and incomplete linkage).

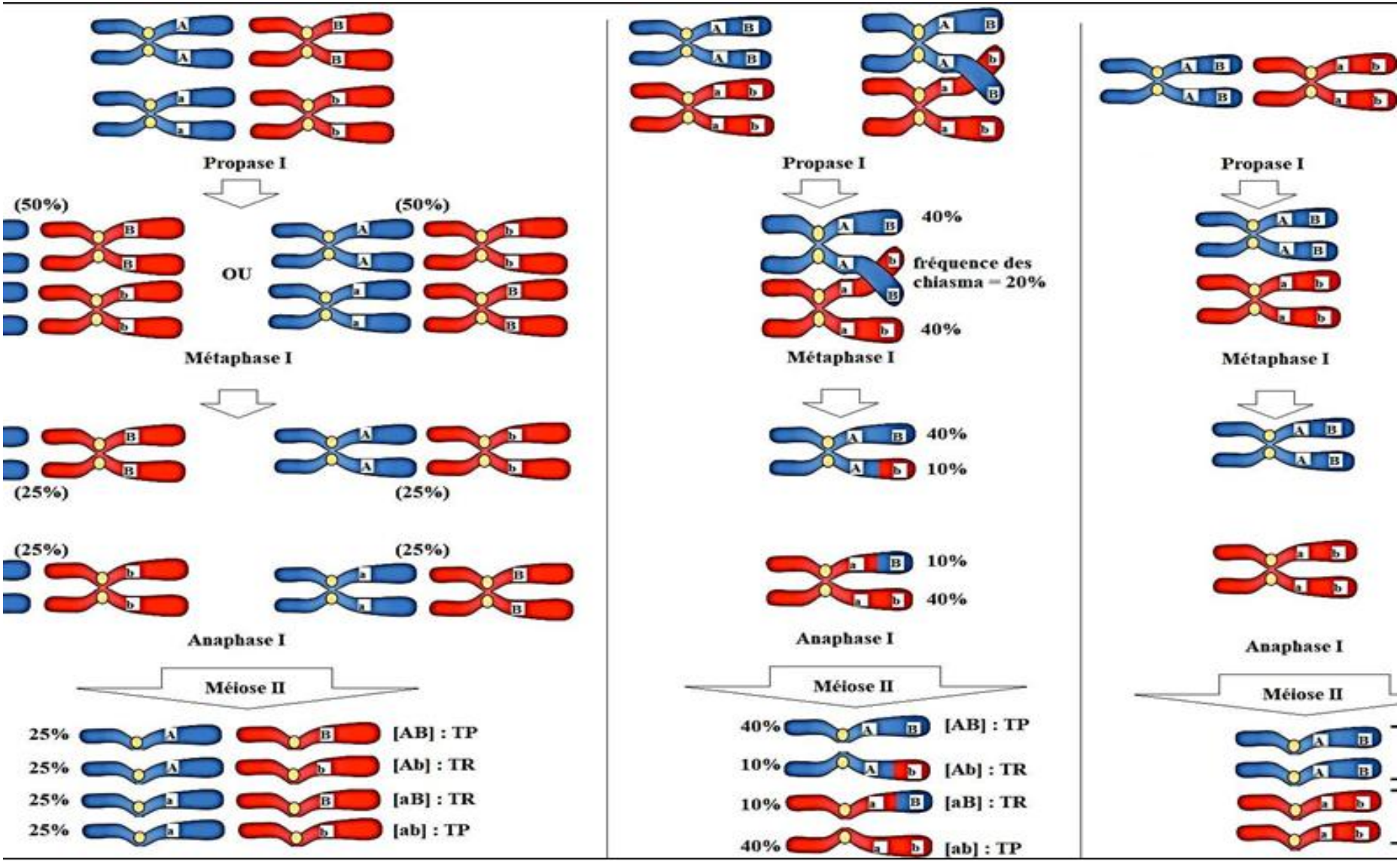
Table 10: Results of test crosses involving independent and linked genes.

<table>
<tr>
<th>Independent Genes</th>
<th>Incomplete Linkage</th>
<th>Complete Linkage</th>
</tr>
<tr>
<td>
Parents : AaBb x aabb


Gametes :
<table>
<tr><th></th><th>AB</th><th>Ab</th><th>aB</th><th>ab</th></tr>
<tr><td>ab</td><td>AaBb</td><td>Aabb</td><td>aaBb</td><td>aabb</td></tr>
</table>
Genotypes:
¼ AaBb
¼ Aabb
¼ aaBb
¼ aabb
Phenotypes:
25% [AB]
25% [Ab]
25% [aB]
25% [ab]
</td>
<td>
Parents : $\frac{A\ B}{a\ b}$ x $\frac{a\ b}{a\ b}$


Gametes :
<table>
<tr><th></th><th>40%
AB</th><th>10%
Ab</th><th>10%
aB</th><th>40%
ab</th></tr>
<tr><td>ab</td><td>$\frac{A\ B}{a\ b}$
40%</td><td>$\frac{A\ b}{a\ b}$
10%</td><td>$\frac{a\ B}{a\ b}$
10%</td><td>$\frac{a\ b}{a\ b}$
40%</td></tr>
</table>
Genotypes:
40% $\frac{A\ B}{a\ b}$ 10% $\frac{A\ b}{a\ b}$
10% $\frac{a\ B}{a\ b}$ 40% $\frac{a\ b}{a\ b}$
Phenotypes:
40% [AB]
10% [Ab]
10% [aB]
40% [ab]
</td>
<td>
Parents : $\frac{A\ B}{a\ b}$ x $\frac{a\ b}{a\ b}$


Gametes :
<table>
<tr><th></th><th>50%
AB</th><th>50%
ab</th></tr>
<tr><td>ab</td><td>$\frac{A\ B}{a\ b}$
40%</td><td>$\frac{a\ b}{a\ b}$
40%</td></tr>
</table>
Genotypes:
50% $\frac{A\ B}{a\ b}$
50% $\frac{a\ b}{a\ b}$
Phenotypes:
50% [AB]
50% [ab]
</td>
</tr>
</table>



Independent Genes **Incomplete Linkage** **Complete Linkage**

Figure 40: Chromosomal representation of gamete formation in the case of independent genes and in the case of complete and incomplete linkage.

The symbolism used in this table is often used in genetics and requires explanation :

The independence of genes is indicated by writing the alleles of each pair above and below separate horizontal lines, one for each pair of chromosomes.

Linkage or the location of more than one gene on the same chromosome is indicated by writing the alleles of these genes above and below a common horizontal line.

Examination of these three test crosses reveals that linked genes do not behave like independent genes. The number and frequencies of phenotypic classes obtained following a test cross differ from one cross to another :

When the genes are independent, four phenotypic classes in equal proportions are obtained following a test cross.

When the genes are linked and there is a possibility of crossing over between non-sister chromatids, four phenotypic classes with unequal proportions are obtained following the test cross. The parental phenotypes, which are derived from chromatids not affected by crossing over, have the highest percentages (e.g., 40% each). Whereas, the recombinant phenotypes, which are derived from chromatids affected by crossing over, have the lowest percentages (e.g., 10% each). The percentage of recombinants corresponds to the percentage of crossing over between non-sister chromatids.

When the genes are linked and there is no crossing over between the non-sister chromatids, two phenotypic classes of parental types, with equal proportions, are obtained following the test cross. The absence of crossing over determines the absence of recombination. This case is found exclusively in the male Drosophila and in the female silkworm.

3.2.3. Genetic map

a) Linear order of linked genes and determination of the distances separating these genes

Example

In a cross between a wild-type strain of Drosophila with long wings and red eyes with a mutant strain with vestigial wings and cinnabar eyes, all individuals of the F1 are wild-type. The F1 females, crossed with mutant males, give, in F2, 200 wild-type Drosophila, 206 mutant Drosophila, 19 Drosophila with long wings and cinnabar eyes, and 26 Drosophila with vestigial wings and red eyes (Fig. 41 A).

In another dihybrid cross between two strains of Drosophila where there is always the character of wing size associated with the character of body color, the following results are obtained. In F1, following the cross of a wild-type strain with long wings and a brown body with a mutant strain with vestigial wings and a black body, all individuals are wild-type. The F1 females, crossed with mutant males, give, in F2, 152 wild-type Drosophila, 147 mutant Drosophila, 35 Drosophila with long wings and a black body, and 31 Drosophila with vestigial wings and a brown body (Fig. 41 B).

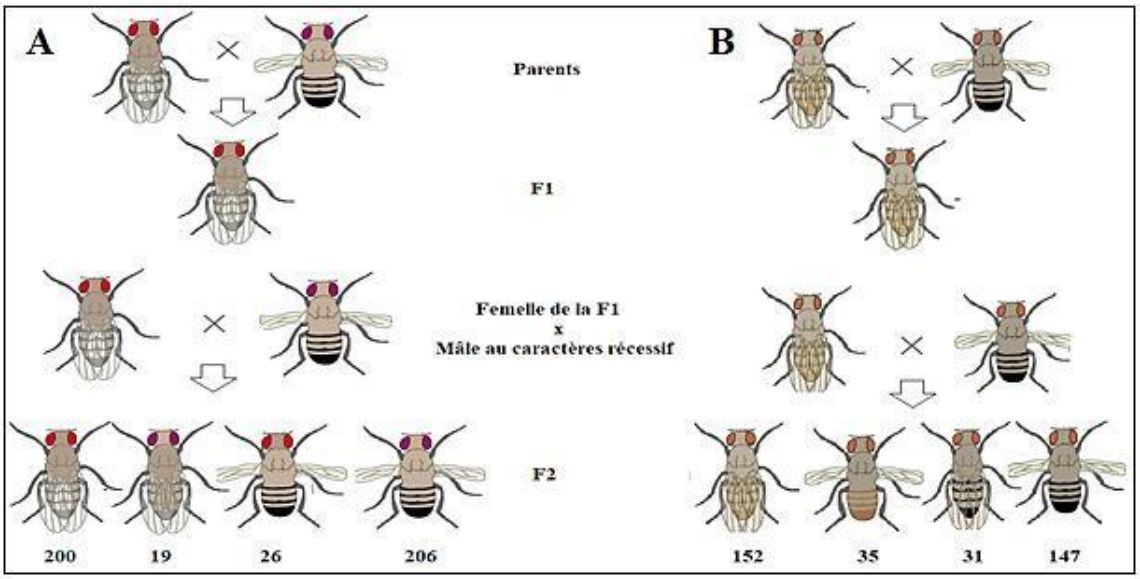


Figure 41: Cross between Drosophila strains: A) wild-type strain with long wings and red eyes and mutant strain with vestigial wings and cinnabar eyes ; B) wild-type strain with long wings and brown body and mutant strain with vestigial wings and black body.

Interpretation

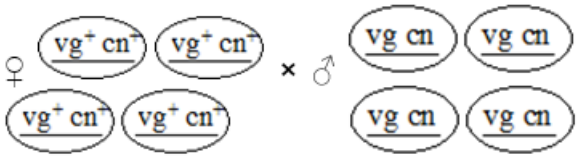
The results obtained in these two test crosses indicate that there is incomplete linkage in each case since the offspring are constituted by four classes of individuals and the two parental classes (wild-type phenotype and mutant phenotype) have the most important values, while the recombinant classes have low values.

Genetic representation

Codes : vg for vestigial wings and $\mathbf{vg}^{+}$ for long wings ; **cn**: for cinnabar eyes and $\mathbf{cn}^{+}$ for red eyes; **n**: for black body and $\mathbf{n}^{+}$ for brown body.

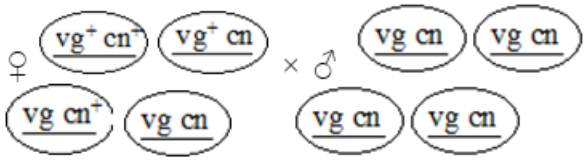
Test cross 1

♀ $\frac{vg+\ \ cn+}{vg+\ \ cn+}$ x ♂ $\frac{vg\ \ \ cn}{vg\ \ \ cn}$



F1 : $\frac{vg+\ \ cn+}{vg\ \ \ cn}$

♀ $\frac{vg+\ \ cn+}{vg\ \ \ cn}$ x ♂ $\frac{vg\ \ \ cn}{vg\ \ \ cn}$



	vg+ cn+	vg+ cn	vg cn+	vg cn
vg cn	$\frac{vg+cn+}{vg\ \ cn}$	$\frac{vg+cn}{vg\ \ cn}$	$\frac{vg\ \ cn+}{vg\ \ cn}$	$\frac{vg\ \ cn}{vg\ \ cn}$

Genotypes :

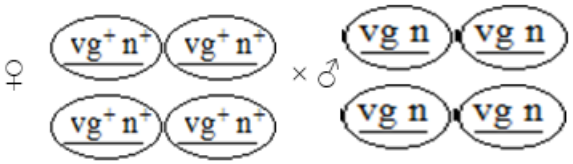
$\frac{vg+cn+}{vg\ \ cn}$ $\frac{vg+cn}{vg\ \ cn}$ $\frac{vg\ \ cn+}{vg\ \ cn}$ $\frac{vg\ \ cn}{vg\ \ cn}$

Phenotypes :

[vg+ cn+] : [vg+ cn] : [vg cn+] : [vg cn]

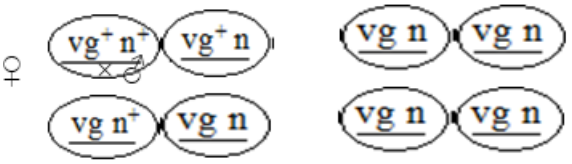
Test cross 2

♀ $\frac{vg+\ \ n+}{vg+\ \ n+}$ x ♂ $\frac{vg\ \ \ n}{vg\ \ \ n}$



F1 : $\frac{vg+\ \ n+}{vg\ \ \ n}$

♀ $\frac{vg+\ \ n+}{vg\ \ \ n}$ x ♂ $\frac{vg\ \ \ n}{vg\ \ \ n}$



	vg+ n+	vg+ n	vg n+	vg n
vg n	$\frac{vg+n+}{vg\ \ n}$	$\frac{vg+n}{vg\ \ n}$	$\frac{vg\ n+}{vg\ \ n}$	$\frac{vg\ n}{vg\ \ n}$

Genotypes :

$\frac{vg+n+}{vg\ \ n}$ $\frac{vg+n}{vg\ \ n}$ $\frac{vg\ n+}{vg\ \ n}$ $\frac{vg\ n}{vg\ \ n}$

Phenotypes :

[vg+ n+] : [vg+ n] : [vg n+] : [vg n]

- ✓ In the first test cross, the recombination percentage is: [(19+26)/(200+19+26+206)]*100 = 9.9%
- ✓ In the second test cross, the recombination percentage is: [(35+31)/(152+35+31+147)]*100 =18.1%.

Therefore, between the vg and cn loci, there is 9.9% recombination, while between the locus of this same vg and that of n, the recombination percentage is almost twice as high as in the first case, i.e., 18.1%. These facts can be interpreted as follows:

Linked genes are distributed in a linear order along the chromosomes.

Crossing over occurs randomly along the chromosomes, which means that in a large number of meiocytes all the different loci of a chromosome will be involved in crossing over as frequently as each other.

The frequency of crossing over between two given loci is therefore higher the further apart these loci are, or in other words, the frequency of crossing over is directly proportional to the distance separating these two loci.

From the interpretation given above, the realization of about twice as many recombinations between vg and n than between vg and cn expresses a distance twice as considerable between vg and n than between vg and cn.

b) Gene Mapping

Since *crossing over* occurs randomly along the entire length of the chromosome, determining the frequency of this phenomenon via the percentage of genetic recombinations that are produced in a heterozygous individual with respect to certain linked genes can therefore be used to calculate the distances separating these genes and to map them (Fig. 42). The appropriate unit of distance to prepare these maps is obviously one percent recombination, a unit called a centimorgan. The data obtained in the previous example allow us to calculate the distances between the three genes and to map them as follows:

Distance between genes = Percentage of recombinaition

= (Sum of recombinant individuals / Total number of individuals)*100

Distance between vg and cn = $[(19 + 26)/451] * 100 = 9.9\ cM$

Distance between vg and n = $[(35 + 31)/365] * 100 = 18{,}1\ cM$

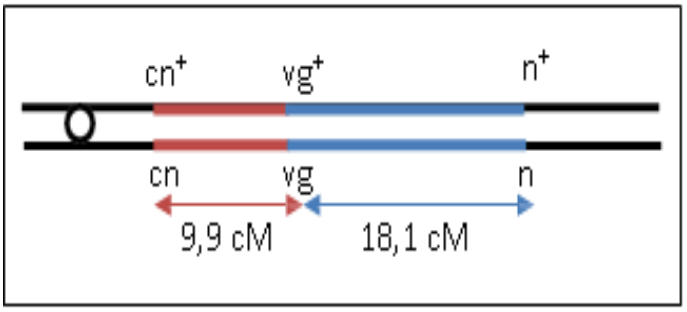


Figure 42: Genetic map

3.2.4. Sex-linked inheritance

Morgan crossed two pure lines (homozygotes) of Drosophila for two traits. The female line had red eyes and long wings and the male line had white eyes and miniature wings. The first generation F1 consisted only of flies with red eyes and long wings. The cross between the F1 flies gave an F2 composed of (Fig. 43):

- 626 Drosophila with red eyes and long wings, including 126 male flies and 500 female flies.
- 124 Drosophila with red eyes and miniature wings, including 60 male flies and 64 female flies.
- 127 Drosophila with white eyes and long wings, including 65 male flies and 62 female flies.
- 123 Drosophila with white eyes and miniature wings, including 62 male flies and 61 female flies.

However, by performing the reciprocal cross, Morgan noticed that the results were different. He noticed by crossing males with red eyes and long wings and females with white eyes and miniature wings, that all the female flies of the F1 had red eyes and long wings and that all the male flies of the F1 had white eyes and miniature wings. The F1 flies were crossed with each other. The percentage of crossing over in this cross was 17%. The resulting F2 was composed of 2300 flies distributed as follows (Fig. 44):

- 965 Drosophila with red eyes and long wings, including 281 male flies and 284 female flies.
- 206 Drosophila with red eyes and miniature wings, including 104 male flies and 102 female flies.
- 185 Drosophila with white eyes and long wings, including 94 male flies and 91 female flies.
- 944 Drosophila with white eyes and miniature wings, including 472 male flies and 472 female flies.

The cross between female Drosophila with normal (grey) body color and long wings with male Drosophila with black bodies and miniature wings gave only Drosophila with normal (grey) body color and long wings. Crossing the F1 females with the black-bodied, miniature-winged males gave the following results in F2 (Fig. 45):

- 192 Drosophila with normal (grey) body color and long wings.
- 208 Drosophila with normal (grey) body color and miniature wings.

- 203 Drosophila with black bodies and long wings.
- 195 Drosophila with black bodies and miniature wings.

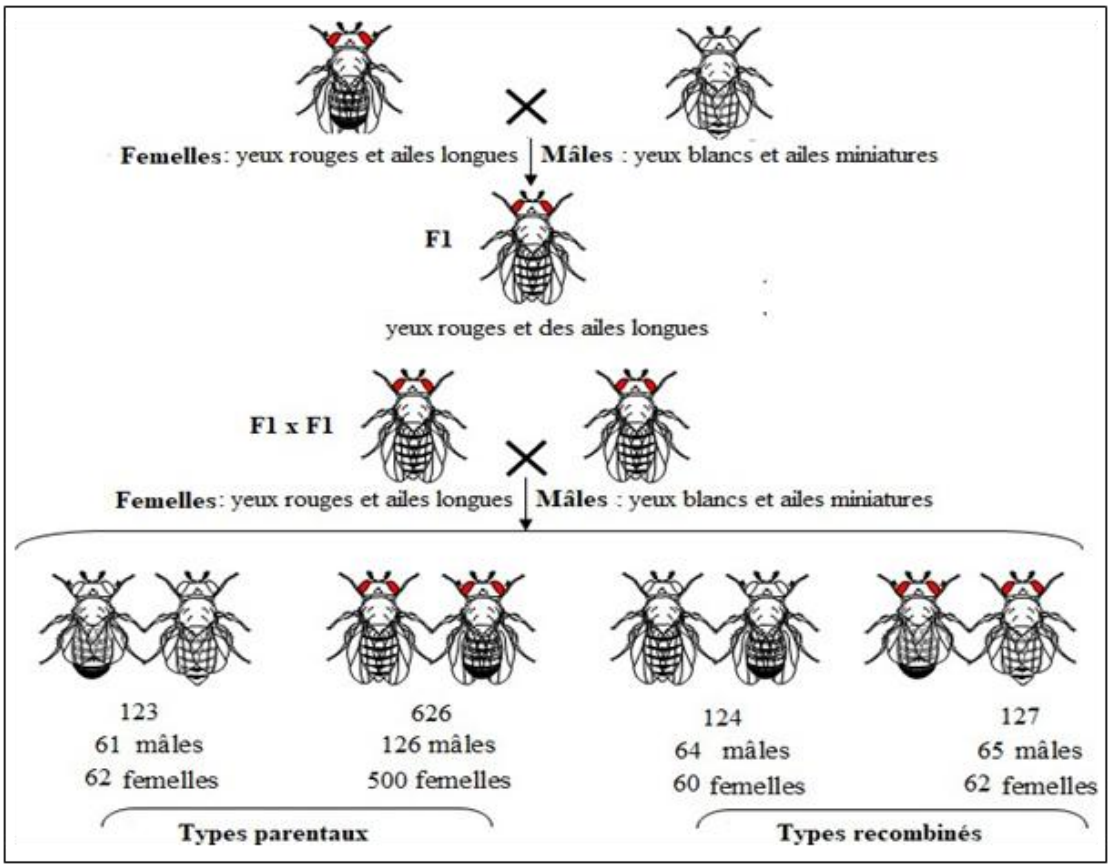


Figure 43: Cross between female line with red eyes and long wings and male line with white eyes and miniature wings.

Interpretation of the first cross

☒ The cross was carried out between two lines of Drosophila that differ in two characteristics (eye color and wing length). It is a dihybrid cross.

☒ The first generation F1 is homogeneous and similar, confirming the homozygosity of the parents, and is characterized by the wild type of the female parent (red eye and long wing), which are the dominant traits.

☒ In the second generation F2, there is the manifestation of four different phenotypes, two of which are parental types and two are recombinant types. The highest values are recorded in the wild phenotype (red eye and long wing), which is characterized by a high number of females compared to males.

☒ The four phenotypes are represented by the following percentages : 62.6% for Drosophila with red eyes and long wings; 12.4% for Drosophila with red eyes and miniature wings; 12.7% for Drosophila with white eyes and long wings; and 12.3% for Drosophila with white eyes and miniature wings.

Genetic Representation

Codes

XX for female sex, XY for male sex, b^+ for red eye color, b for white eye color, m^+ for long wings and m for miniature wings.

Parents : ♀ $X^{m+b+}X^{m+b+}$ × ♂ $X^{mb}Y$

Gametes : ♀ X^{m+b+} X^{m+b+} X^{m+b+} X^{m+b+} × ♂ X^{mb} Y

F1 : $X^{m+b+}X^{mb}$ $X^{m+b+}Y$

Genotypes **Phenotypes**

Parents : ♀ $X^{m+b+}X^{m+b+}$; ♂ $X^{mb}Y$ ♀ $[m^+b^+]$; ♂ [mb]

F1 : ♀ $X^{m+b+}X^{mb}$; ♂ $X^{m+b+}Y$ ♀ $[m^+b^+]$; ♂ $[m^+b^+]$

F2 : ♀ F1 × ♂ F1

F2 : ♀ $X^{m+b+}X^{mb}$ × ♂ $X^{m+b+}Y$

Gametes : ♀ X^{m+b+} X^{m+b} X^{mb+} X^{mb} × ♂ X^{m+b+} Y

♂ \ ♀	X^{m+b+}	X^{m+b}	X^{mb+}	X^{mb}
X^{m+b+}	$X^{m+b+}X^{m+b+}$	$X^{m+b+}X^{m+b}$	$X^{m+b+}X^{mb+}$	$X^{m+b+}X^{mb}$
Y	$X^{m+b+}Y$	$X^{m+b}Y$	$X^{mb+}Y$	$X^{mb}Y$

Genotypes F2:

♀ ⅛ $X^{m+b+}X^{m+b+}$; ⅛ $X^{m+b+}X^{m+b}$; ⅛ $X^{m+b+}X^{mb+}$; ⅛ $X^{m+b+}X^{mb}$.

♂ ⅛ $X^{m+b+}Y$; ⅛ $X^{m+b+}Y$; ⅛ $X^{m+b+}Y$; ⅛ $X^{m+b+}Y$.

Phenotypes F2 :

50% ♀ $[m^+b^+]$.

♂ ⅛ $[m^+b^+]$: ⅛ $[m^+b]$: ⅛ $[mb^+]$: ⅛ [mb].

In general, ⅝ $[m^+b^+]$: ⅛ $[m^+b]$: ⅛ $[mb^+]$: ⅛ [mb].

The observed results correspond to the theoretical results.

	Theoretical Results	**Observed Results**
$[m^+b^+]$	(⅝)*100 = **62,5%**	(626/100)*100 = **62,6%**
$[m^+b]$	(⅛)*100 = 1**2,5%**	(124/100)*100 = **12,4% %**
$[m\ b^+]$	(⅛)*100 = 1**2,5%**	(127/100)*100 = **12,7%**
[mb]	(⅛)*100 = 1**2,5%**	(123/100)*100 = **12,3%**

Interpretation of the second cross (reciprocal)

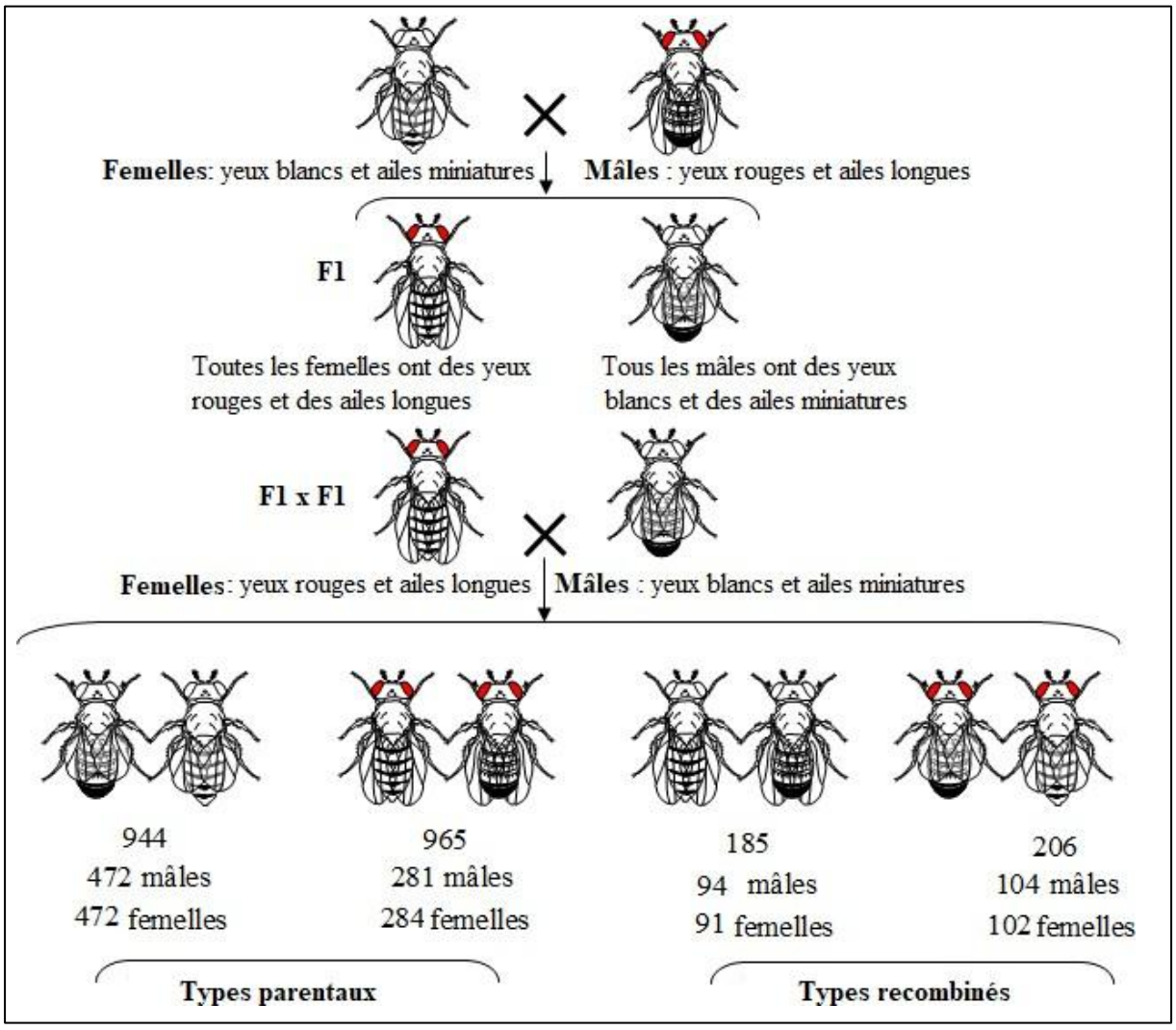


Figure 44: Cross between a male line with red eyes and long wings and a female line with white eyes and miniature wings.

☒ The cross was carried out between two lines of Drosophila that differ in two characteristics (eye color and wing length). It is a dihybrid cross.

☒ The first generation F1 is not homogeneous. The phenotypes of males and females are not identical. We also note that the females take on the red eye color and long wing size and that the males take on the white eye color and miniature wing size. This indicates that these two characteristics are related to sex and that the genes responsible for these characteristics are located on the X chromosome.

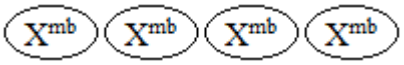
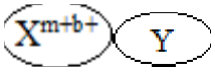
☒ The second generation F2, is obtained by crossing double heterozygous females and homozygous males carrying the recessive traits (test cross) which gives four different phenotypes, two of which are parental types and two phenotypes are recombinant types. The highest values are recorded in the parental phenotypes (red eye and long wing; white eye and

miniature wing) which confirms that the genes are carried by the same chromosome (which is the X chromosome).

☒ The four phenotypes are represented by the following percentages: 41.95% for Drosophila with red eyes and long wings; 8.95% for Drosophila with red eyes and miniature wings; 8.05% for Drosophila with white eyes and long wings and 41.05% for Drosophila with white eyes and miniature wings.

Genetic Representation

Parents : ♀ $X^{mb}X^{mb}$ × ♂ $X^{m+b+}Y$

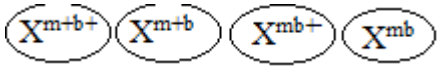
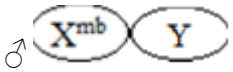
Gametes : ♀  × ♂ 

F1 : $X^{m+b+}X^{mb}$ $X^{mb}Y$

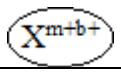
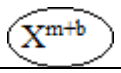
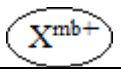
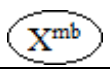
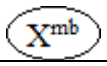
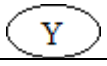
Genotypes	**Phenotypes**
Parents : ♀ $X^{mb}X^{mb}$; ♂ $X^{m+b+}Y$	♀ [mb] ; ♂ $[m^+b^+]$
F1 : ♀ $X^{m+b+}X^{mb}$; ♂ $X^{mb}Y$	♀ $[m^+b^+]$; ♂ [mb]

F2 : ♀ F1 × ♂ F1

F2 : ♀ $X^{m+b+}X^{mb}$ × ♂ $X^{mb}Y$

Gametes : ♀  × ♂ 

41.5% 8.5% 8.5% 41.5%

♂ \ ♀				
	$X^{m+b+}X^{mb}$	$X^{m+b}X^{mb}$	$X^{mb+}X^{mb}$	$X^{mb}X^{mb}$
	$X^{m+b+}Y$	$X^{m+b}Y$	$X^{mb+}Y$	$X^{mb}Y$

Genotypes F2

♀ 20,75% $X^{m+b+}X^{mb}$; 4,25% $X^{m+b}X^{mb}$; 4,25% $X^{mb+}X^{mb}$; 20,75% $X^{mb}X^{mb}$.

♂ 20,75% $X^{m+b+}Y$; 4,25% $X^{m+b}Y$; 4,25% $X^{mb+}Y$; 20,75% $X^{mb}Y$.

Phenotypes F2

♀ 20,75% $[m^+b^+]$; 4,25% $[m^+b]$; 4,25% $[mb^+]$; 20,75% [mb].

♀ 20,75% $[m^+b^+]$; 4,25% $[m^+b]$; 4,25% $[mb^+]$; 20,75% [mb].

In general, 41,5% $[m^+b^+]$: 8,5% $[m^+b]$: 8,5% $[mb^+]$: 41,5% [mb].

The observed results correspond to the theoretical results.

Theoretical Results		Observed Results
[m^+b^+]	**41,5%**	(965/2300)*100 = **41,95%**
[m^+b]	**8,5%**	(206/2300)*100 = **8,95%**
[$m\ b^+$]	**8,5%**	(185/2300)*100 = **8,05%**
[mb]	**41,5%**	(944/2300)*100 = **41,05%**

Les deux croisements réciproques ont montré des résultats différents quelque soit en F1 ou F2. Cela confirme que les deux gènes responsables de l'expression des caractères de la couleur des yeux et de la taille des ailes sont localisés sur le chromosome X.

Note

The two reciprocal crosses showed different results whether in F1 or F2. This confirms that the two genes responsible for the expression of the eye color and wing size traits are located on the X chromosome.

Interpretation of the third cross

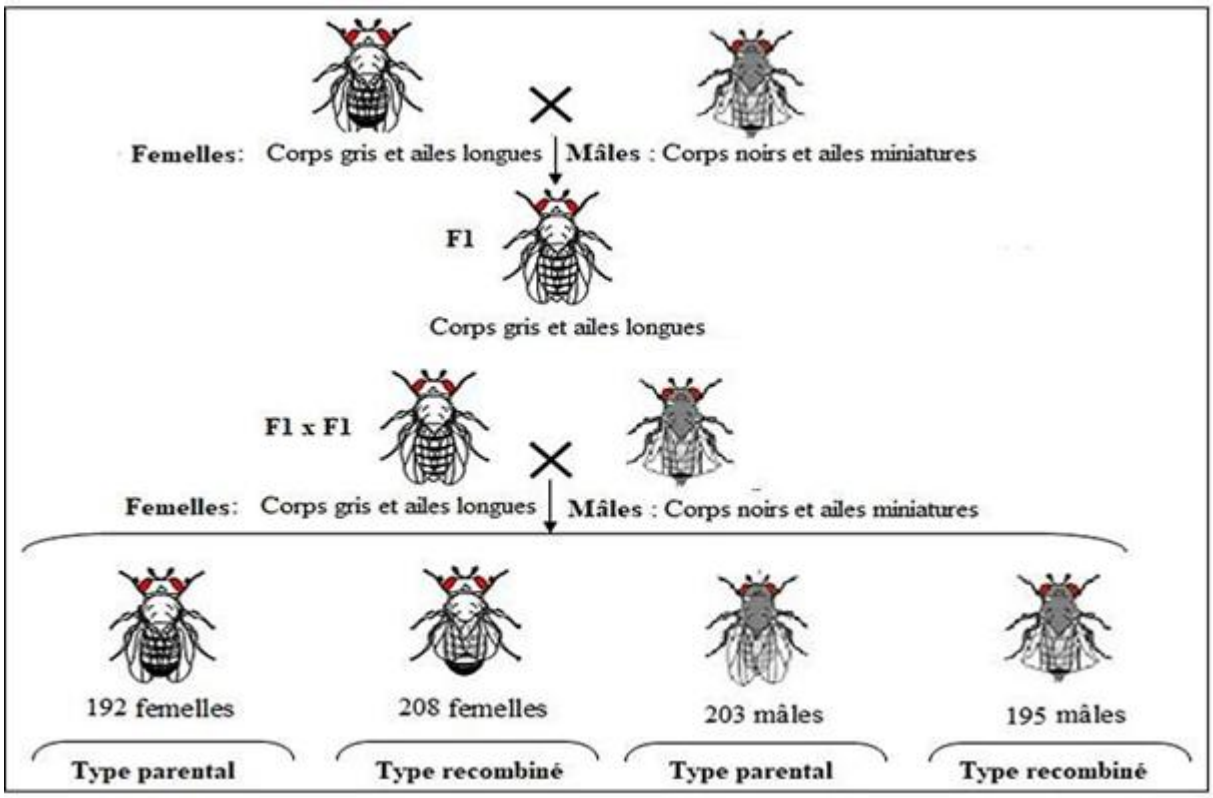


Figure 45: Cross between female Drosophila with gray bodies and long wings with male Drosophila with black bodies and miniature wings.

☒ The cross was carried out between two lines of Drosophila that differ in two characteristics (body color and wing length). It is a dihybrid cross.

☒ The first generation F1 is homogeneous and similar, confirming the homozygosity of the parents, and is characterized by the wild-type character of the female parent (gray body and

long wing), which are the dominant characters.

☒ In the second generation F2, there is the manifestation of four different phenotypes, two of which are parental types and two phenotypes are recombinant types. The highest values are recorded in the wild-type phenotype (red eye and long wing), which is characterized by a high number of females compared to males.

☒ The second generation F2 is obtained by crossing double heterozygous females and homozygous males carrying the recessive traits (test cross), which gives four different phenotypes, two of which are parental types and two phenotypes are recombinant types. The values observed for all phenotypes are close, indicating that the two genes in question are independent (located on different chromosomes). We have already concluded previously that the gene responsible for wing size is located on the X chromosome, so the gene responsible for body color is located on a normal autosomal chromosome.

☒ The four phenotypes are represented by the following percentages: 24.06% for Drosophila with gray bodies and long wings; 26.06% for Drosophila with gray bodies and miniature wings; 25.44% for Drosophila with black bodies and long wings and 24.44% for Drosophila with black bodies and miniature wings.

Genetic Representation

Codes

XX for female sex, XY for male sex, n^+ for gray body color, n for black body color, m^+ for long wings and m for miniature wings.

Parents : ♀ $n^+n^+X^{m+}X^{m+}$ × ♂ nnX^mY

Gametes : ♀ (n^+X^{m+}) (n^+X^{m+}) (n^+X^{m+}) (n^+X^{m+}) × ♂ (nX^m) (nY)

F1 : $n^+nX^{m+}X^m$ $n^+nX^{m+}Y$

Genotypes	**Phenotypes**
Parents : ♀ $n^+n^+X^{m+}X^{m+}$; ♂ nnX^mY	♀ $[n^+m^+]$; ♂ [nm]
F1 : ♀ $n^+nX^{m+}X^m$; ♂ $n^+nX^{m+}Y$	♀ $[n^+m^+]$; ♂ $[n^+m^+]$

F2 : ♀ F1 × ♂ nnX^mY

F2 : ♀ $n^+nX^{m+}X^m$ × ♂ nnX^mY

Gametes : ♀ (n^+X^{m+}) (n^+X^m) $(n\ X^{m+})$ (nX^m) × ♂ (nX^m) (nX^m) (nY) (nY)

		n^+X^{m+}	n^+X^m	nX^{m+}	nX^m
♀	nX^m	$n^+nX^{m+}X^m$	$n^+nX^mX^m$	$nnX^{m+}X^m$	nnX^mX^m
♂	nY	$n^+nX^{m+}Y$	n^+nX^mY	$nnX^{m+}Y$	nnX^mY

Genotypes F2

♀ ⅛ $n^+nX^{m+}X^m$; ⅛ $n^+nX^mX^m$; ⅛ $nnX^{m+}X^m$; ⅛ nnX^mX^m.

♂ ⅛ $n^+nX^{m+}Y$; ⅛ n^+nX^mY; ⅛ $nnX^{m+}Y$; ⅛ nnX^mY.

Phenotypes F2

♀ ⅛ $[n^+m^+]$: ⅛ $[n^+m]$: ⅛ $[nm^+]$: ⅛ [nm]

♂ ⅛ $[n^+m^+]$: ⅛ $[n^+m]$: ⅛ $[nm^+]$: ⅛ [nm].

In general, ¼ $[n^+m^+]$: ¼ $[n^+m]$: ¼ $[nm^+]$: ¼ [nm].

The observed results correspond to the theoretical results.

	Theoretical Results	**Observed Results**
$[n^+m^+]$	(¼)*100 = **25%**	(192/798)*100 = **24,06%**
$[n^+m]$	(¼)*100 = **25%**	(208/798)*100 = **26,06% %**
$[nm^+]$	(¼)*100 = **25%**	(203/798)*100 = **25,44%**
[nm]	(¼)*100 = **25%**	(195/798)*100 = **24,44%**

Note

The observed results are very close to the results obtained by a test cross when the genes are independent. This confirms that the gene responsible for body color and the one responsible for wing size are located on different chromosomes.

4. Polyhybridism

The term polyhybrid represents individuals heterozygous for two or more traits (two genes or more). The fundamental laws of genetics concern all individuals, whether they are monohybrids or polyhybrids.

4.1. Trihybridism: Case of dominance (independent genes)

Example

Two Drosophila belonging to two homozygous lines differing from each other by three traits are crossed. The female is wild-type and has a brown body, long wings, and red eyes, and the male is mutant and has a black body, vestigial wings, and brown eyes. The F1 individuals all have a wild-type phenotype (Fig. 46). Crossing the F1 individuals with each other gave an F2 composed of :

- 269 Drosophila with brown bodies, long wings, and red eyes ;
- 91 Drosophila with brown bodies, long wings, and brown eyes;
- 89 Drosophila with brown bodies, vestigial wings, and red eyes ;
- 31 Drosophila with brown bodies, vestigial wings, and brown eyes;
- 92 Drosophila with black bodies, long wings, and red eyes;
- 28 Drosophila with black bodies, long wings, and brown eyes ;
- 29 Drosophila with black bodies, vestigial wings, and red eyes ;
- 11 Drosophila with black bodies, vestigial wings, and brown eyes.

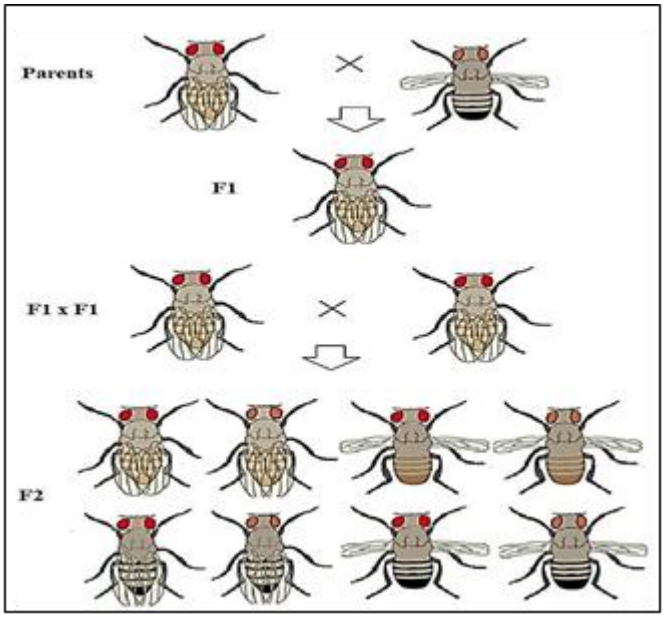


Figure 46: Cross between two Drosophila belonging to two homozygous lines differing from each other by three traits (body color, wing size, and eye color).

Interpretation

 This is a cross between two individuals belonging to two pure lines that differ by three pairs of alleles or two genes : it is a trihybridism.

 The first generation F1 is homogeneous and similar with the manifestation of only one parental phenotype. This leads, on the one hand, to confirm Mendel's first law (law of resemblance) and confirms the homozygosity of the parents. On the other hand, it indicates that the traits that appear in F1 are the dominant traits, so the brown body trait is dominant over the black body trait, the long wing trait is dominant over the vestigial wing trait, and the red eye trait is dominant over the brown eye trait.

 In the second generation F2, there is the appearance of the two parental phenotypes (wild-type and mutant) in addition to six other new phenotypes that are intermediate between the parental phenotypes. These phenotypes are called recombined.

 The values obtained in F2 correspond to the following proportions: 42.19% [brown, long, and red]: 14.07% [brown, long, and brown]: 14.07% [brown, vestigial, and red]: 4.68% [brown, vestigial, and brown], 14.07% [black, long, and red]: 4.68% [black, long, and brown]: 4.68% [black, vestigial, and red]: 1.56% [black, vestigial, and brown].

Genetic Representation

Codes : n^+ for brown body and **n** for black body ; **vg^+** for long wings and **vg** for vestigial wings; **b^+** for red eyes and **b** for brown eyes.

Parents: ♀ $n^+n^+v\ ^+v\ ^+b^+b^+$ × ♂ nnv v bb

Gametes: ♀ $n^+v_g{}^+b^+$ × ♂ nv_gb

F1 : $n^+nvg^+vgb^+b$

Genotypes :

Parents : ♀ $n^+n^+v\ ^+v\ ^+b^+b^+$

♂ nnvgvgbb

F1 : $n^+nvg^+vgb^+b$

Phenotypes :

Parents : ♀ $[n^+v\ ^+b^+]$

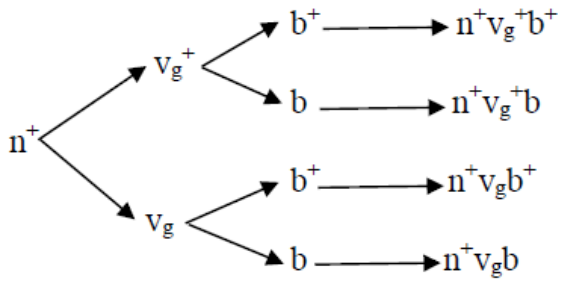
♂ [nvgb]

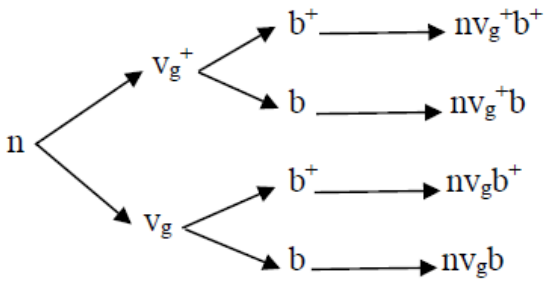
F1 : $[n^+v\ ^+b^+]$

F2 : ♀ **F1** × ♂ **F1**

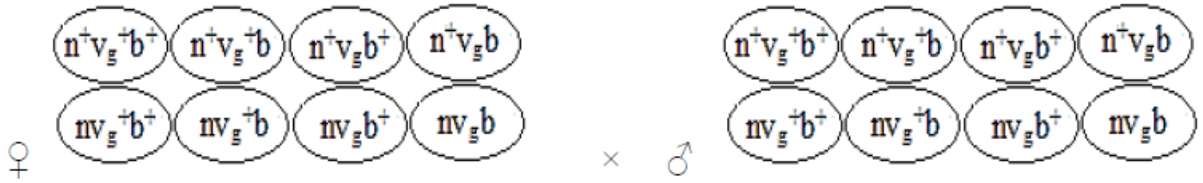
♀ $n^+nv\ ^+v\ b^+b$ × ♂ $n^+nv\ ^+v\ b^+b$

Gametes: to know the number of gametes produced by a trihybrid, we use the Branched system which allows us to easily determine, without error, all the types of gametes. We will therefore have:



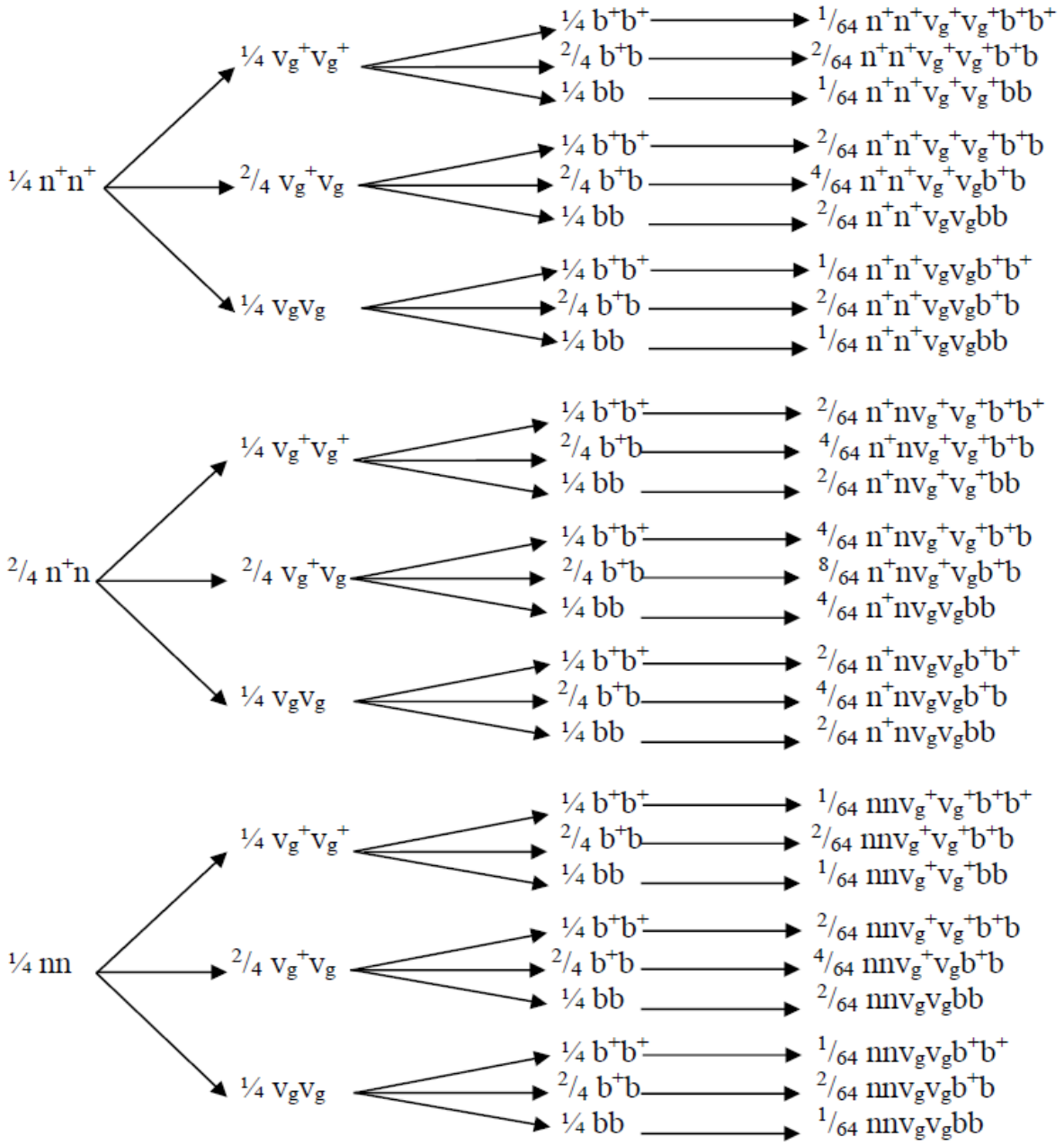


Gametes :

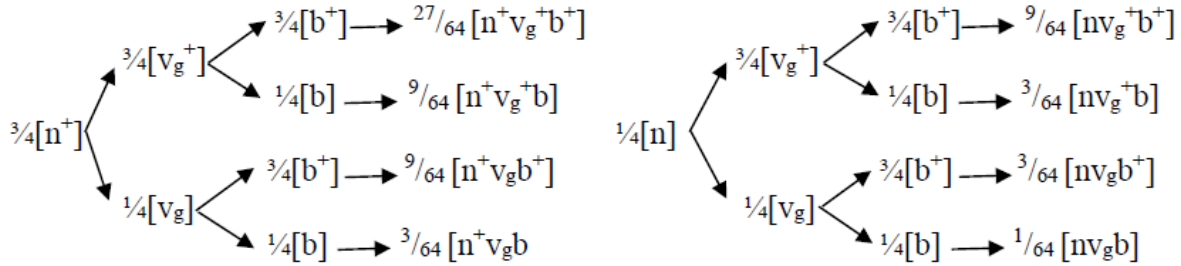


We will have 8 different gametes, and the study of the F2 gives us 27 genotypes and 8 phenotypes. The determination of the genotypes is always done by the same method, so we will have :

Genotypes :



Phenotypes :



Note

The numbers of gametes, phenotypes, and genotypes of the F2 in the case of dominance, vary not randomly but according to the degree of hybridism which corresponds to series. These series are represented in each case by a general formula (Table 11).

Table 11: Variation in the number of gametes, phenotypes, and genotypes of the F2 according to the degree of hybridity.

Degrees of hybridity	Number of gametes	Number of phenotypes	Number of genotypes	Number of combinations between F1 gametes
Monohybrid (1)	$2^1 = 2$	$2^1 = 2$	$3^1 = 3$	$4^1 = 4$
Dihybrid (2)	$2^2 = 4$	$2^2 = 4$	$3^2 = 9$	$4^2 = 16$
Trihybrid (3)	$2^3 = 8$	$2^3 = 8$	$3^3 = 27$	$4^3 = 64$
Tetrahybrid (4)	$2^4 = 16$	$2^4 = 16$	$3^4 = 81$	$4^4 = 256$
Pentahybrid (5)	$2^5 = 32$	$2^5 = 32$	$3^5 = 243$	$4^5 = 1024$
N	2^n	2^n	3^n	4^n

4.2. Test cross

4.2.1. Independent Genes

Example

The females of the F1 from the previous example are crossed with mutant males with black bodies, vestigial wings, and brown eyes. The F2 is composed of:

- ✓ 93 Drosophila with brown bodies, long wings, and red eyes;
- ✓ 91 Drosophila with brown bodies, long wings, and brown eyes
- ✓ 90 Drosophila with brown bodies, vestigial wings, and red eyes;
- ✓ 92 Drosophila with brown bodies, vestigial wings, and brown eyes;
- ✓ 92 Drosophila with black bodies, long wings, and red eyes;
- ✓ 95 Drosophila with black bodies, long wings, and brown eyes;
- ✓ 89 Drosophila with black bodies, vestigial wings, and red eyes;
- ✓ 91 Drosophila with black bodies, vestigial wings, and brown eyes.

Interpretation

In the second generation F2, all the phenotypes obtained (parental and recombined) have close values. The values obtained in F2 correspond to the following proportions: 12.69% [brown, long, and red]: 12.41% [brown, long, and brown]: 12.28% [brown, vestigial, and red]: 12.55% [brown, vestigial, and brown], 12.55% [black, long, and red]: 12.96% [black, long, and brown]: 12.15% [black, vestigial, and red]: 12.41% [black, vestigial, and brown].

Genetic Representation

Codes

- **n^+** for brown body;
- **n** for black body;
- **vg^+** for long wings;
- **vg** for vestigial wings;
- **b^+** for red eyes;
- **b** for brown eyes.

F2 : ♀ **F1** × ♂ nn vgvg bb

♀ $n^+nv^+v\ b^+b$ × ♂ nnv v bb

Gametes : ♀ $n^+v_g^+b^+$ $n^+v_g^+b$ $n^+v_gb^+$ n^+v_gb $nv_g^+b^+$ nv_g^+b nv_gb^+ nv_gb × ♂ nv_gb

	$n^+v_g^+b^+$	$n^+v_g^+b$	$n^+v_gb^+$	n^+v_gb	$nv_g^+b^+$	nv_g^+b	nv_gb^+	nv_gb
nv_gb	$n^+nvg^+vgb^+b$	n^+nvg^+vgbb	$n^+nvgvgb^+b$	$n^+nvgvgbb$	$nnvg^+vgb^+b$	$nnvg^+vgbb$	$nnvgvgb^+b$	$nnvgvgbb$

Genotypes : ⅛ $n^+nvg^+vgb^+b$; ⅛ n^+nvg^+vgbb ; ⅛ $n^+nvgvgb^+b$; ⅛ $n^+nvgvgbb$; ⅛ $nnvg^+vgb^+b$; ⅛ $nnvg^+vgbb$; ⅛ $nnvgvgb^+b$; ⅛ nnvgvgbb.

Phenotypes : ⅛ $[n^+vg^+b^+]$; ⅛ $[n^+vg^+b]$; ⅛ $[n^+vgb^+]$; ⅛ $[n^+vgb]$; ⅛ $[nvg^+b^+]$; ⅛ $[nvg^+b]$; ⅛ $[nvgb^+]$; ⅛ [nvgb].

The theoretical results indicate a frequency of ⅛ for each phenotype which corresponds to 12.5%. These theoretical results are close to the observed results in the example.

Note : when the genes are independent, the test cross gives in F2 phenotypes in equal proportions, whatever the degree of hybridity.

4.2.2. Linked Genes

Example

A cross was carried out between two pure strains of Drosophila melanogaster. The cross involves three recessive mutant alleles for three genes linked to the X chromosome, respectively responsible for body color yellow (y), eye color white (w), and eye shape echinus (ec). Hemizygous males for the three wild-type alleles are crossed with homozygous females for the three corresponding recessive alleles. This cross produced an F1 generation

comprising heterozygous females for the three pairs of alleles, so they are all wild-type, and hemizygous males carrying the recessive traits. The F1 females are crossed with mutant males carrying recessive alleles for the three traits. The F2 is composed of eight phenotypes with the following values:

4759 Drosophila of phenotype [y^+ w^+ ec^+] ; 4685 Drosophila of phenotype [y w ec] ; 80 Drosophila of phenotype [y w^+ ec^+] ; 70 Drosophila of phenotype [y^+ w ec] ; 193 Drosophila of phenotype [y w ec^+] ; 207 Drosophila of phenotype [y^+ w^+ ec]; 3 Drosophila of phenotype [y w^+ ec^+] ; 3 Drosophila of phenotype [y w^+ ec];

Interpretation

» The F1 is composed of two different phenotypes distributed between males and females despite the parents being homozygous, this indicates that the genes are located on the X chromosome (therefore they are linked) ;

» Phenotypically, all F1 females are [wild-type] and all males are [yellow, white, echinus]. Given the genotype of the parents, the mutant alleles of the three genes are on one of the X chromosomes and the wild-type alleles are on the other chromosome ;

» In males, each gamete will contain either an X chromosome carrying the mutant alleles of the three genes, or a Y chromosome, which does not contain any of the loci considered ;

» It is a test cross that gave the F2. In F2, 8 different phenotypes are obtained, two of which are parental phenotypes [y^+ w^+ ec^+] and [y w ec] and six recombinant phenotypes. The values of the parental classes are higher compared to those of the recombinant classes, this confirms that the genes are linked and located on the same chromosome.

» The F2 phenotypes due to the absence of crossing over are determined by the parental combination of alleles present in the gametes of the F1 female (Fig. 43). In this case, each gamete contains either the wild-type alleles or the mutant alleles of the three genes, depending on the X chromosome of the F1 female not affected by crossing over. After segregation, there will therefore be production, in equal proportions, of the two types of gametes and therefore the two parental phenotypes of the F2. These complementary F2 phenotypic classes are called reciprocal classes.

» The phenotypes due to the absence of crossing over are the most easily recognizable because they are the most numerous in the offspring (Fig. 47). The parental phenotypes [y^+ w^+ ec^+] and [y w ec] alone represent 94.4% of the F2 offspring.

» The second easily detectable category is represented by the phenotypes resulting from a double crossing over. These phenotypes are the least numerous due to their low probability of

occurrence (Fig. 47). This category results from two independent and simultaneous crossing over events. The two corresponding reciprocal phenotypes are [y^+ w ec^+] and [y w^+ ec] and represent only 0.06% of the offspring.

» The four remaining phenotypic classes represent the two categories resulting from a single crossing over. The reciprocal phenotypes [y w^+ ec^+] and [y^+ w ec] result from a single crossing over between the yellow and white loci and constitute 1.5% of the offspring.

»The reciprocal phenotypes [y^+ w^+ ec] and [y w ec^+] result from a single crossing over between the white and echinus loci and constitute 4% of the offspring.

4.3.Gene Mapping

Example

A cross is made between two pure lines of corn. The female plants have the phenotype [pr^+ v bm] and the male plants have the phenotype [pr v^+ bm^+].

All F1 offspring are of the wild-type phenotype [pr+ v+ bm+]. The F1 male plants are then crossed with female plants of phenotype [pr v bm]. The F2 consists of 8 phenotypes distributed as mentioned below:

Table 12: Details of the cross performed between the two pure lines of corn.

Offspring phenotypes	Staff	Total and percentage	Types of exchanges
[pr^+ v bm] [pr v^+ bm^+]	230 237	467 42,1%	No *crossing over* (NCO)
[pr^+ v^+ bm] [pr v bm^+]	82 79	161 14,5%	Simple *crossing over* (SCO)
[pr^+ v bm^+] [pr v^+ bm]	200 195	395 35,6%	Simple *crossing over* (SCO)
[pr v bm] [pr^+ v^+ bm^+]	44 42	86 7,8%	Double *crossing over* (DCO)

Interpretation

Homozygous parents that differ by three pairs of alleles give a homogeneous F1 with dominance of wild-type characters. This is a trihybridism with verification of Mendel's first two laws.

The F2 is obtained following a test cross (a cross between triple heterozygous individuals and a homozygous recessive). This F2 consists of 8 phenotypes with different values with a predominance of parental phenotypes, thus indicating that the genes are linked.

To draw the genetic map, we must first determine the order of the genes and then calculate the distances that separate them.

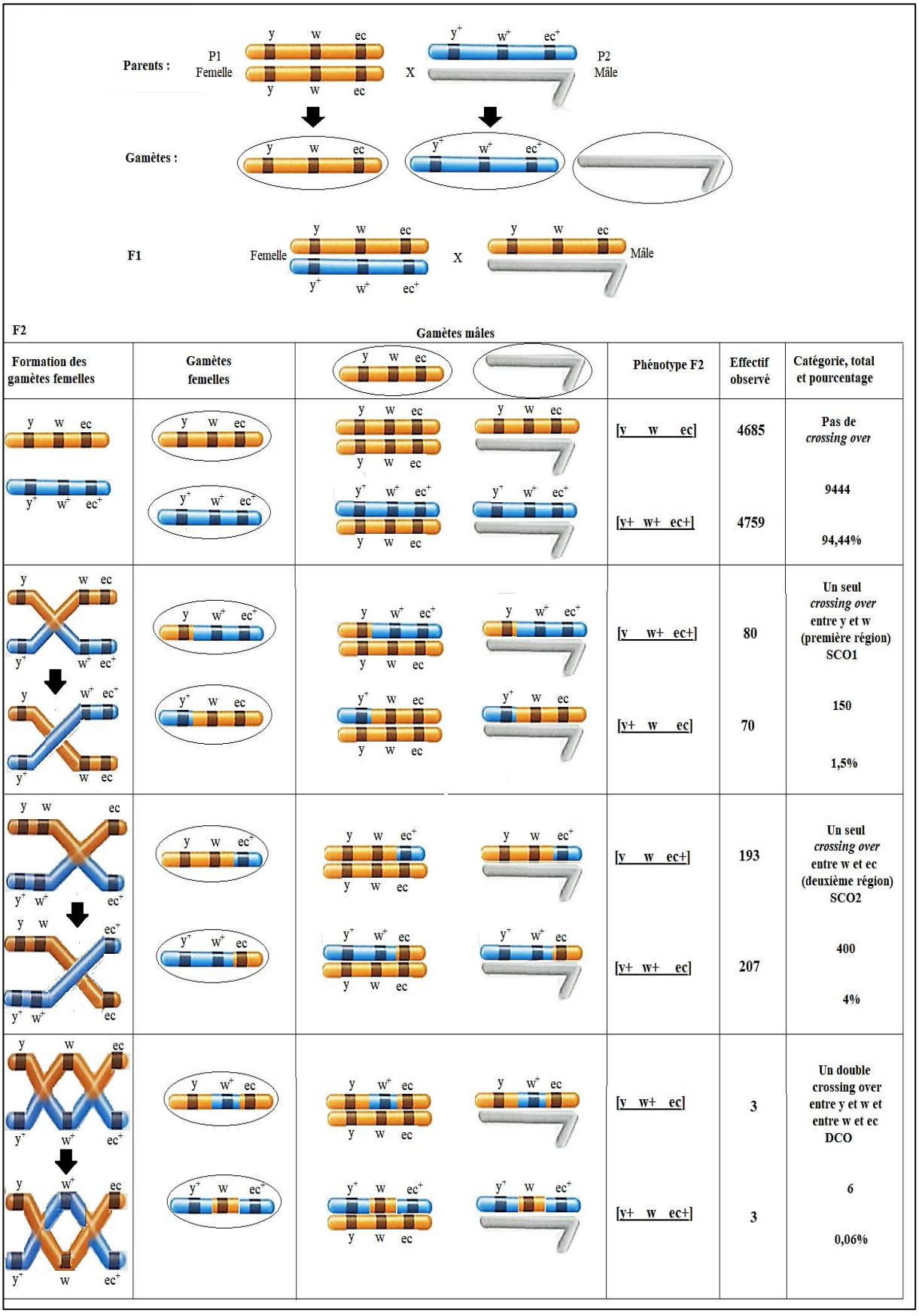


Figure 47: Three-point cross involving the genes yellow (y+ or y), white (w+ or w) and echinus (ec+ ec) in Drosophila melanogaster.

a) Determination of the gene order

In most mapping experiments, the gene order is not known and constitutes another variable in the analysis.

To determine the order of the genes, we rely on the phenotypes resulting from a double crossover. We know that following a double crossover, the allele placed in the middle will change location when compared to the heterozygous parental genotype.

In this example, the two reciprocal phenotypes resulting from a double crossover and which are represented by the lowest values are : [pr v bm] and [pr^+ v^+ bm^+]. By comparing these two phenotypes to the reciprocal parental phenotypes [pr^+ v bm] and [pr v^+ bm^+], we notice that the pr^+ and pr alleles have exchanged places, so these two alleles are in the middle and are associated with the alleles (v^+/v) and (bm^+/bm) which are located at the extremities.

Therefore, we can establish the true order of the genes which is v pr^+ bm and v^+ pr bm^+.

b) Estimation of genetic distances

The genetic distance, which corresponds to the frequency of crossovers, is the percentage of recombinant individuals.

Since we already know the order of the genes, it is sufficient to estimate the distance between the v^+ pr alleles and between the pr bm^+ alleles.

For the two pairs of alleles v/v^+ ; pr^+/pr only, the parental forms are [v pr^+]/[v^+ pr] and the recombinant forms are: [v pr]/[v^+ pr^+]. Therefore, only phenotypes that have a combination of [v pr] ou [v^+ pr^+] are included in the calculation.

Distance between v^+ pr = Percentage of recombinants

= [(82 + 79 + 44 + 42)/1109]*100

= 22.27%

= 22.27 cM

Or directly the distance between v^+pr = 14.5% + 7.8% = 22.3%

For the two pairs of alleles pr+/pr ; pr/pr+ only, the parental forms are [pr+ bm]/[pr bm+] and the recombinant forms are: [pr bm]/[pr + bm+]. Therefore, only phenotypes that have a combination of [pr bm] or [pr + bm+] are included in the calculation.

Distance between pr bm$^+$ = Percentage of recombinants

= [(200 + 195 + 44 + 42)/1109]*100

= 43.37%

= 43.37cM

Or directly the distance between v$^+$pr = 35.6% + 7.8% = 43.4%. The genetic map is represented in figure 48.

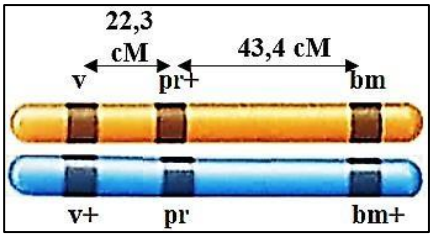


Figure 48: Genetic map of the three pairs of alleles v/v+ ; pr+/pr and bm/bm+ in corn.

4.4. Genetic interference

Once the distances between the genes are known, we can predict the expected frequency of multiple exchanges such as doubles *crossing overs*. Thus, in the previous example on corn, the distance between v and pr$^+$ is 22,3 recombination units and that of pr$^+$ and bm is 43,4 recombination units. If single *crossing over* occur independently, the theoretical frequency of double crossovers is (DCOth) :

DCOth = 22,3% × 43,4% = 9,7% = 0,097

In most genetic mapping experiments, the observed frequency of double *crossovers* (DCOobs) is lower than the theoretical frequency of double *crossovers* (DCOth). In the corn example, only 7,8% of double *crossing overs* are observed instead of the expected 9,7%. This is explained by a phenomenon called interference, which occurs when a crossover produced in one chromosomal region inhibits a second event in a nearby region.

To calculate the disparities resulting from interference, we can calculate the coefficient of coincidence (C):

C = (DCOobs / DCOth) = (0,078/0,097) = 0,804

Once (C) is calculated, we can quantify the interference (I) using the equation:

I = 1 – C = 1 – 0,0804 = 0,196.

✓ If the interference is total (I = 1) and the coefficient of coincidence is zero (C = 0), this indicates that there are no observed double crossovers (DCOobs = 0). In this case, we will

obtain six genotypes instead of eight in the F2, because the two reciprocal phenotypes resulting from a double crossover have a value of 0. They do not exist.

- ✓ If there are fewer double crossovers than expected, DCOobs < DCOth, the coefficient of coincidence is less than 1 and the interference will have a positive value.
- ✓ If there are more double crossovers than expected, DCOobs > DCOth, the coefficient of coincidence is greater than 1 and the interference will have a negative value.
- ✓ If all double *crossing overs* are observed, DCOobs = DCOth, the coefficient of coincidence is equal to 1 and the interference will be zero.

4.5.Frequency analysis

In genetics, it is often necessary to calculate expected frequencies and numbers of particular genotypes. For example, when predicting the outcome of a Mendelian cross or when predicting the frequency of a particular genotype in a population. In science, it is not enough to predict, but we must also test.

Examples

Mendelian genetics predicts that couples consisting of normally pigmented individuals heterozygous for the albinism gene will have offspring comprising three times as many normally pigmented individuals as albino individuals. However, we cannot expect the four children from such parents to consist of exactly 3 normal : 1 albino. This is not what Mendelian theory claims. What it argues is that each child born to such parents has a three in four chance of being normally pigmented and a one in four chance of being albino. In fact, this theory predicts that some of these offspring will exhibit a distribution other than 3:1 (table 13).

Note that [A] : is the normally pigmented individual, [a]: the albino individual (A dominates a). Thus, the 3:1 distribution is only one of the possible distributions.

Table 13: Possible offspring of a normal couple heterozygous for the albinism gene.

Different categories of offspring of 4 children from unions : Aa x Aa	**Number of children with phenotype**	
	Normal [A]	**Albino [a]**
1st case	4	0
2nd case	3	1
3rd case	2	2
4th case	1	3
5th case	0	4

In a cross we can know the proportion of flowers produced in the F2: ¼ red, $^{2}/_{4}$ pink, ¼ white, but we cannot predict what color each plant will produce, we have to examine it to know.

Probability theory allows us to calculate what we expect to happen. So, the probability (p) of something happening is the number of times the event occurs.

(a) divided by the total number of times it was possible for it to occur (n).

a) **Summation rule:** It applies to mutually exclusive events.

Examples

The dice can roll either a 5 or a 6, but not both, so what is the probability of rolling a 5 or a 6 ?

Each of these events has a probability of 1/6. We add them together to get the probability of rolling a 5 or a 6.

P(5 ou 6) = 1/6 + 1/6 = 2/6 ou bien 1/3. [P(5 ou 6) = P(5) + P(6)].

An example in genetics would be a cross where we would expect to getflowers: ¼ red : $^{2}/_{4}$ pink : ¼ white. The probability that any flower is red or pink is = ¼ + $^{2}/_{4}$ = ¾.

b) **Multiplication rule :** It applies to independent events that occur in a specific order.

Examples

What is the probability of getting two 6s when rolling two dice ?

The roll of the first dice has no effect on the roll of the second. The probability of getting a 6 on the first roll is $^{1}/_{6}$. The same applies to the second roll, since it is independent.

P(6 et 6) = P(6) x P(6) = $^{1}/_{6}$ x $^{1}/_{6}$ = $^{1}/_{36}$.

If two people who are heterozygous for a gene responsible for a recessive genetic disease (phenylketonuria "PKU") marry, if they have three children, what is the probability (the risk) that they will all be affected ?

The probability that a child is homozygous for the recessive allele is ¼, but the probability that all three are affected is : P(all 3) = ¼ x ¼ x ¼ = $^{1}/_{64}$.

c) **Calculation of probabilities (combinations of the two) :** The rules of summation and multiplication are often combined.

Examples :

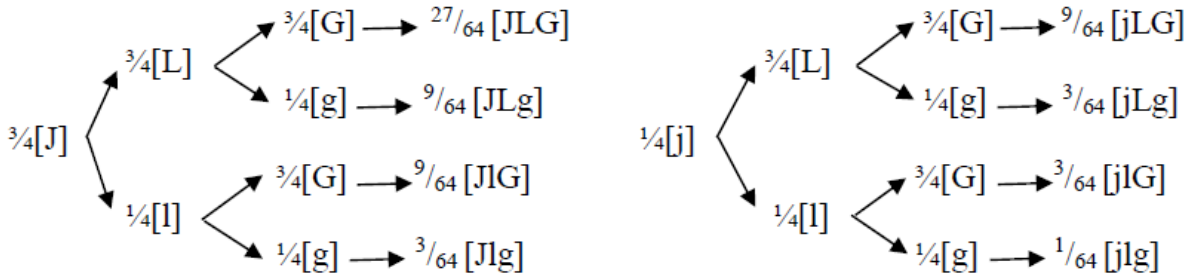
What is the probability that two children will have one boy and one girl ?

This is the probability that the first is a boy, multiplied by the probability that the second is a girl (½ x ½ = ¼) added to the probability that the first is a girl and the second is a boy (also ¼). So that the combination boy – girl, in no particular order, has a probability of ¼ + ¼ = 2/4 or ½.

Combinations are different ways of achieving the same result. There are two combinations that allow two babies to be a girl or a boy.

If we cross a line of yellow (J) and smooth (L) peas with gray bark (G) with a line carrying recessive traits: green (j), wrinkled (l), white bark (g) peas. What is the probability that in F2 we obtain green, smooth peas with barkgray?

The simplest way to calculate the frequency of independent event combinations (genotypes and phenotypes) is to use probability trees : Branché system. Here we have :



The answer is ¾ smooth x ¼ green x ¾ gray bark = 9/64.

The probabilities of all possible phenotypes sum to 64/64 = 1. The same principle can be applied to genotypes.

Chapter 2 :
Qualitative genetics in haploid individuals

Chapter 2: Qualitative Genetics in Haploid Individuals

1. Life cycle

Any organism with sexual reproduction is characterized by its life cycle, that is to say the alternation between two processes which characterizes sexual production.

The duration of the haploid and diploid phases varies depending on the organism. There are 3 types of organisms.

1.1. Diplobiontic organisms

Diplobiotic organisms are organisms that have a diploid phase that is longer than the haploid phase. In this type of cycle, gametes are the only haploid cells. Meiosis involves the formation of gametes, and the union of the gametes produces a diploid zygote.

Example : higher animals and plants.

1.2. Haplobiontic organismes

Organisms that have a haploid phase that is longer than the diploid phase are called haplobiontic organisms.

Example: filamentous fungi.

1.3.Haplodiplobiontic organismes

These are organisms whose haploid phase is as long as the diploid phase. This type of cycle is present in many yeasts.

Example: *Saccharomyces Crevisiae*

2. Haploid cycle of *Neurospora crassa*

It is a filamentous fungus of the Ascomycetes family with a haploid chromosome number of 7. The vegetative apparatus is called: **mycelium** and it is composed of filaments called **hyphae**. The end of the hypha can condense to give spores called **conidia** which germinate and in turn produce new hyphae by asexual (vegetative) multiplication (Fig. 49).

Neurospora crassa is also hermaphrodite, it contains 2 types of sexual gametes called either (+) and (-) or (**A**) and (**a**) whose union is necessary for sexual reproduction to occur.

- **Conidia:** also called **microconidia**, represent the **male** organ. They detach from their environment and can be transported by the wind or water and will attach themselves to the receptor filaments of the female organ which migrate towards the ascogonia.
- **Ascogones :** represent the **female** organ which is surrounded by receptor filaments.

They are pear-shaped.

The mycelia that are **(A)** or **(a)** which are of sign (+) or (-) will form, by 2 types of alleles, the sexual reproduction provided that these 2 nuclei are of opposite signs and will fuse. A micro-conidium (A) can only fertilize an ascogone (a) or vice versa.

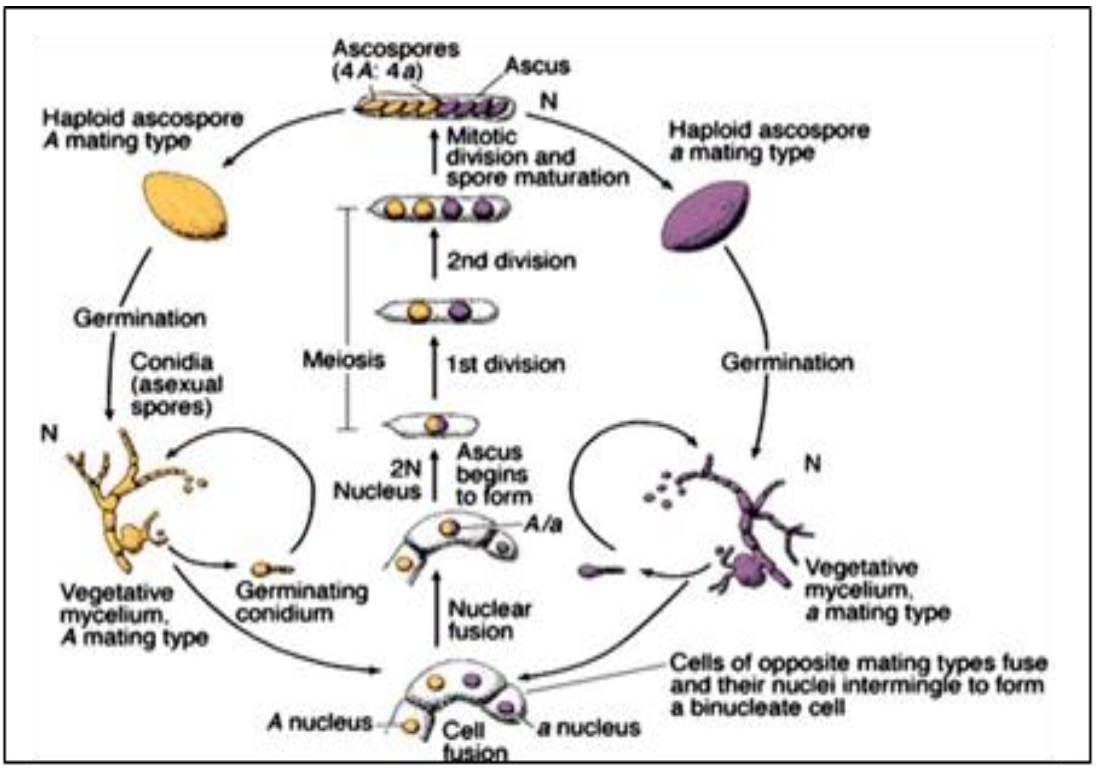


Figure 49: Haplobiontic cycle of *Neurospora crassa*

Fertilization leads to the formation of a dicaryotic filament (diploid) in which each segment contains a haploid nucleus (A) and a haploid nucleus (a). The dicaryotic filaments grow and differentiate into semi-fructifications called **"proto-perithecia" (figure 2)**; the cells that are at the origin of the asci finally undergo true karyogenesis, that is to say the fusion of the two haploid cells (A and a).

The protoperithecium transforms into a **"perithecium"**: in each ascogone, a normal meiosis leads to the reduction of the number of chromosomes and the formation of 4 cells with n chromosomes (Fig. 46).

An additional mitosis will take place and thus 8 haploid nuclei are formed which are called: **ascospores** and which are contained in **Asci** (Fig. 50).

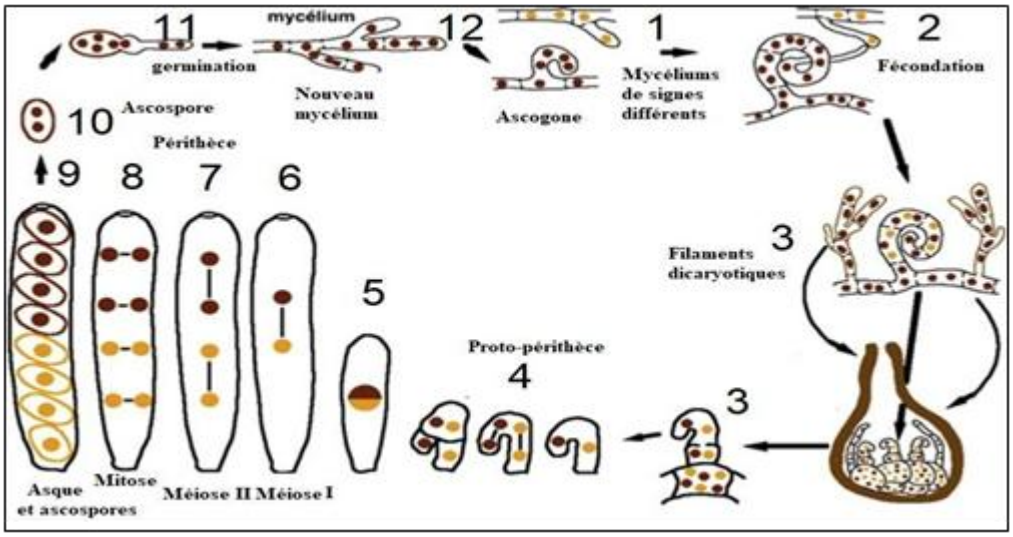


Figure 50: Sexual reproduction in *Neurospora crassa*

3. Segregation of an Allele Pair in *Neurospora crassa*

When two strains carrying different alleles (here A and a) are crossed, 6 types of asci are obtained, including :

- 02 asci are pre-reduced asci.
- 04 asci are post-reduced asci.

3.1. Segregation at the first meiotic division

There is pre-reduction when the alleles segregate (separate) from each other at the **first** division of meiosis. When there is **no chiasma (no *crossing-over*)** between the centromere and the gene studied, at Anaphase I, the alleles separate on either side (**A** towards one pole and **a** towards the other pole) and after meiosis, we will have 4 cells, then with mitosis, we will have the 8 **ordered** ascospores (Fig. 51).

3.2. Segregation at the second meiotic division

If a *crossing over* occurs between the gene and its centromere, then two chromatids are joined at the same centromere, one carrying the allele (A) and the other the allele (a). At the end of anaphase I, the alleles (A) and (a) always remain together and the separation between the two alleles occurs during anaphase II (Fig. 52).

The random orientation of the two alleles at the two metaphases of meiosis determines the production of four different types of joint orientations which are expressed by the four types of post-reduced asci (Types III, IV, V and VI) (Fig. 52).

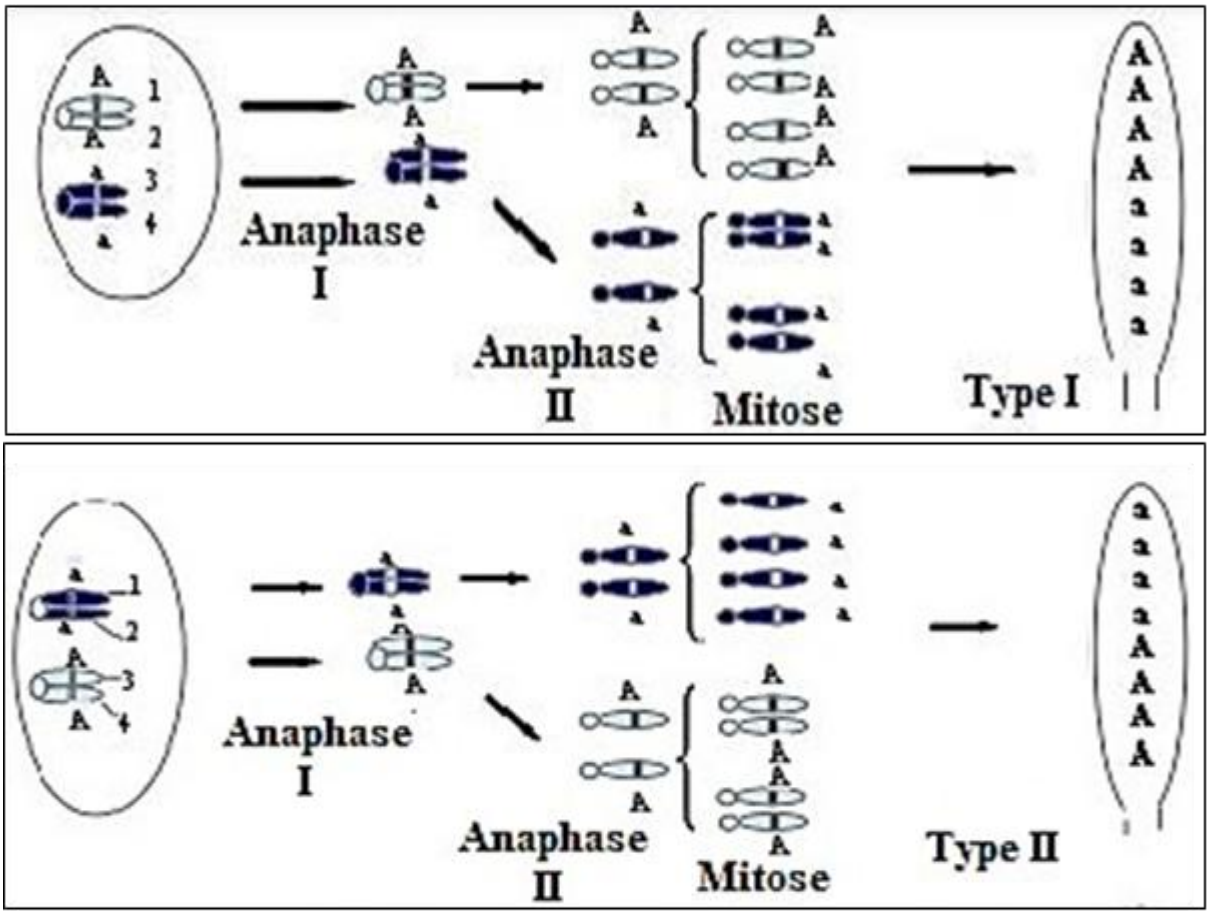


Figure 51 : Formation of pre-reduced asci of Type I and type II in the absence of *Crossing-over*

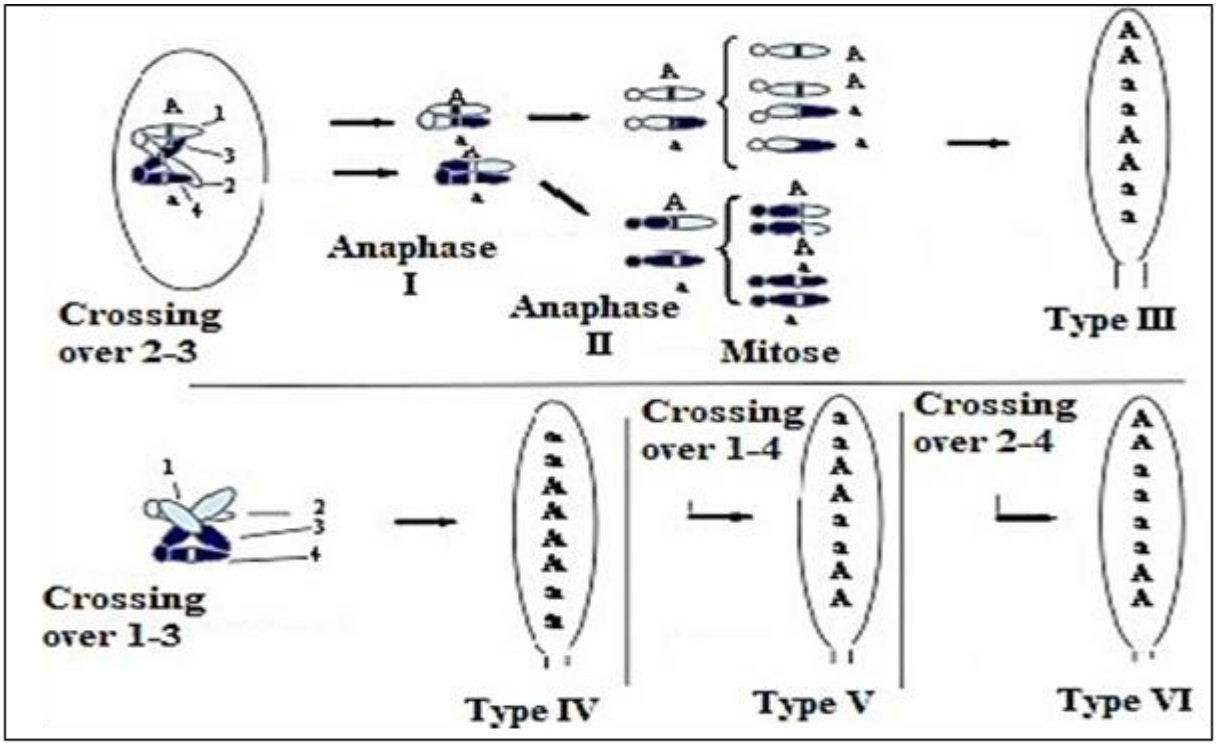


Figure 52: Formation of pre-reduced asci of Types III, IV, V and VI in the presence of Crossing-over between non-sister chromatids (2-3 ; 1-3; 1-4 and 2-4).

3.3. Estimating distances between the gene and its centromere (ordered tetrad)

The frequency of crossing-over between the gene and its centromere is a function of the distance separating this gene from the centromere. Thus, if the percentage of post-reduced asci is a measure of the intensity of the linkage, it must be remembered, however, that a crossing over only affects two chromatids and therefore out of the four products of meiosis, there are only two that contain a rearranged (recombined) chromatid. Therefore, the percentage of post-reduced asci must be divided by 2 to obtain the percentage of recombined chromatids.

Distance = ½ * (percentage of post-reduced asci)

= ½ * (Number of post-reduces asci /Total) * 100

Example

A strain of *Neurospora crassa* requiring Methionine (a^-) is crossed with another wild-type strain (a^+). The results of the F1 are shown in the table below.

Type I	Type II	Type III	Type IV	Type V	Type VI
A	a	A	a	A	a
A	a	A	a	A	a
A	a	a	A	a	A
A	a	a	A	a	A
a	A	A	a	a	A
a	A	A	a	a	A
a	A	a	A	A	a
a	A	a	A	A	a
126	**132**	**10**	**11**	**11**	**12**

Calculating the distance between gene A and its centromere.

Distance = ½ * (percentage of post-reduced asci)

= ½ * (Number of post-reduces asci /Total) * 100

= ½ * [(10 + 11 + 11 + 12) / 302] * 100

= 7.38% which corresponds to 7,38 cM.

4. Segregation of two allele pairs in Chlamydomonas

4.1. Independent segregation

The analysis of genetic data from haploid organisms makes it possible to distinguish between linkage or genetic independence of 2 genes, and then to calculate the distance between them once the linkage is established.

We consider a tetrad analysis in Chlamydomonas, if we accept that the 4 products of meiosis are not ordered and do not undergo an additional mitosis, the general principles mentioned in Neurospora crassa are applicable to Chlamydomonas.

To compare independence and linkage, we look at 2 theoretical mutant alleles a and try at distinct loci in Chlamydomonas. Suppose that 100 tetrads obtained in the cross: **try a** (x) + **A**, between a strain of sign a and auxotrophic for tryptophan (i.e. the strain is unable to synthesize tryptophan) and a second strain of sign A and prototrophic for tryptophan (i.e. the strain can synthesize tryptophan) gives the following results (figure 2.7). The tetrads are divided into 3 possible distribution types:

- ✓ All type I tetrads have 2 try a spores and 2 +A spores are called **parental ditype (PD)**.
- ✓ Type II tetrads have 2 + a spores and 2 try A spores are called : **non-parental ditype or recombinant ditype (NPD or RD)**.
- ✓ Type III tetrads have one spore of each possible genotype and are therefore called : **tetratype (T)**.

Remark

It is clear that the allelic pairs can be located on the same chromosome, or on different chromosomes ; the proportions of PD, NPD and T depend on this location (Fig. 53):

☒ If the 2 allelic pairs are located on different chromosomes, which implies independent segregation (independent genes), we find, in addition to a certain number of tetratypes, an equal number of PD and NPD

☒ DP and DNP type tetrads are therefore simply the consequence of the fact that the two pairs of homologous chromosomes, divided into chromatids and paired, are placed on the equatorial plate of metaphase I independently of each other and 2 positions are possible with identical probabilities: DP = DNP.

4.2.Genetic linkage

We consider another tetrad analysis in chlamydomonas, assuming that 100 tetrads obtained in the cross : ab (x) a^+b^+ give the following results (Fig. 54), the tetrads are also divided into 3 types of distribution (DP, DNP and T) :

- ✓ If the 2 allelic pairs are on the same chromosome, and if there is no C.O (absolute linkage), we should obviously expect not to find only DPs.
- ✓ If the linkage between the two alleles a and b is not absolute (presence of C.O), the number of DNPs is less than the number of DPs and there are a certain number of Tetratypes.

Remark

It is clear that DNPs are infrequent, they are the result of a rare phenomenon (2 C.O). The unequal proportions between DP and DNP show that the genes are linked.

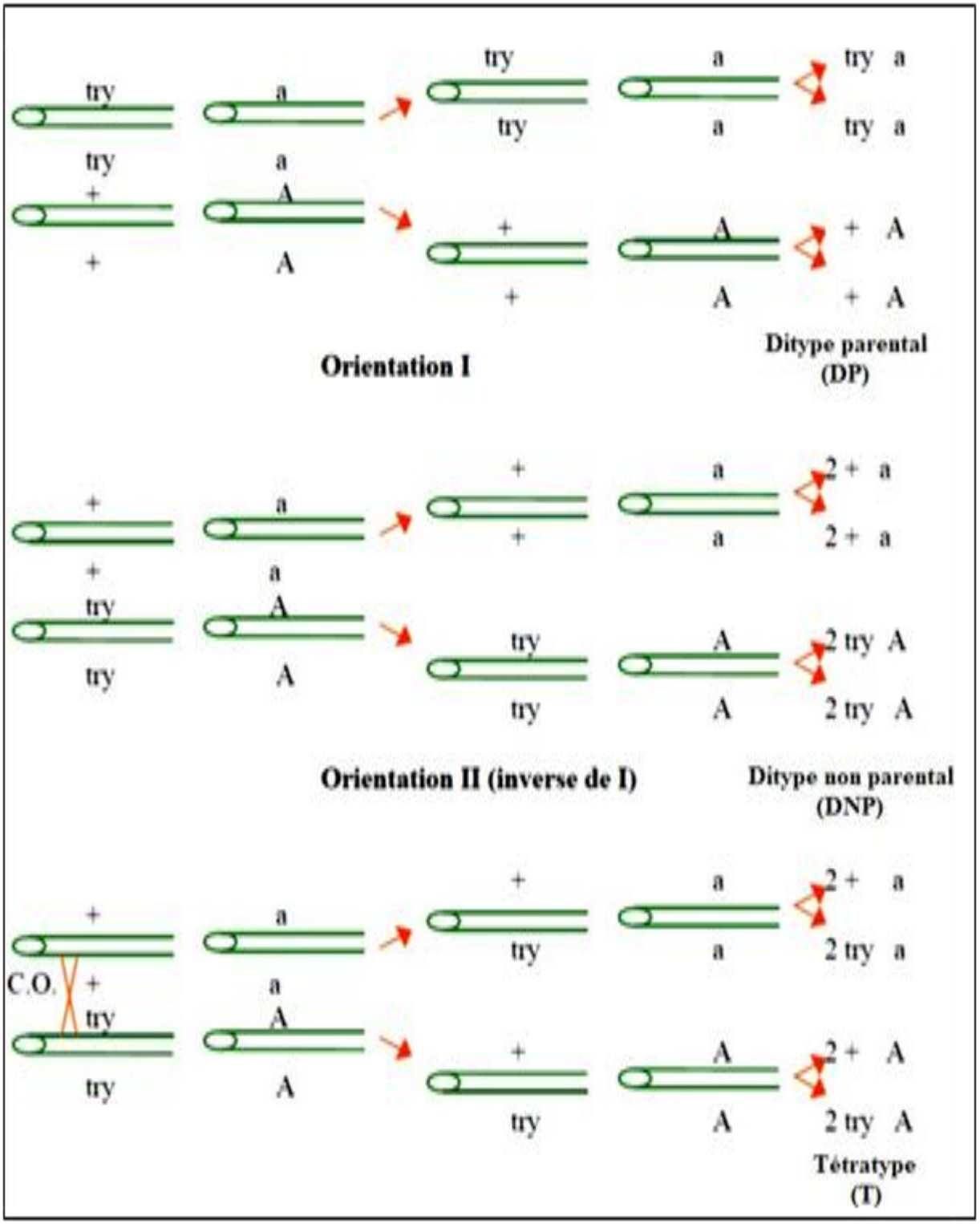


Figure 53: Independent segregation (independent genes)

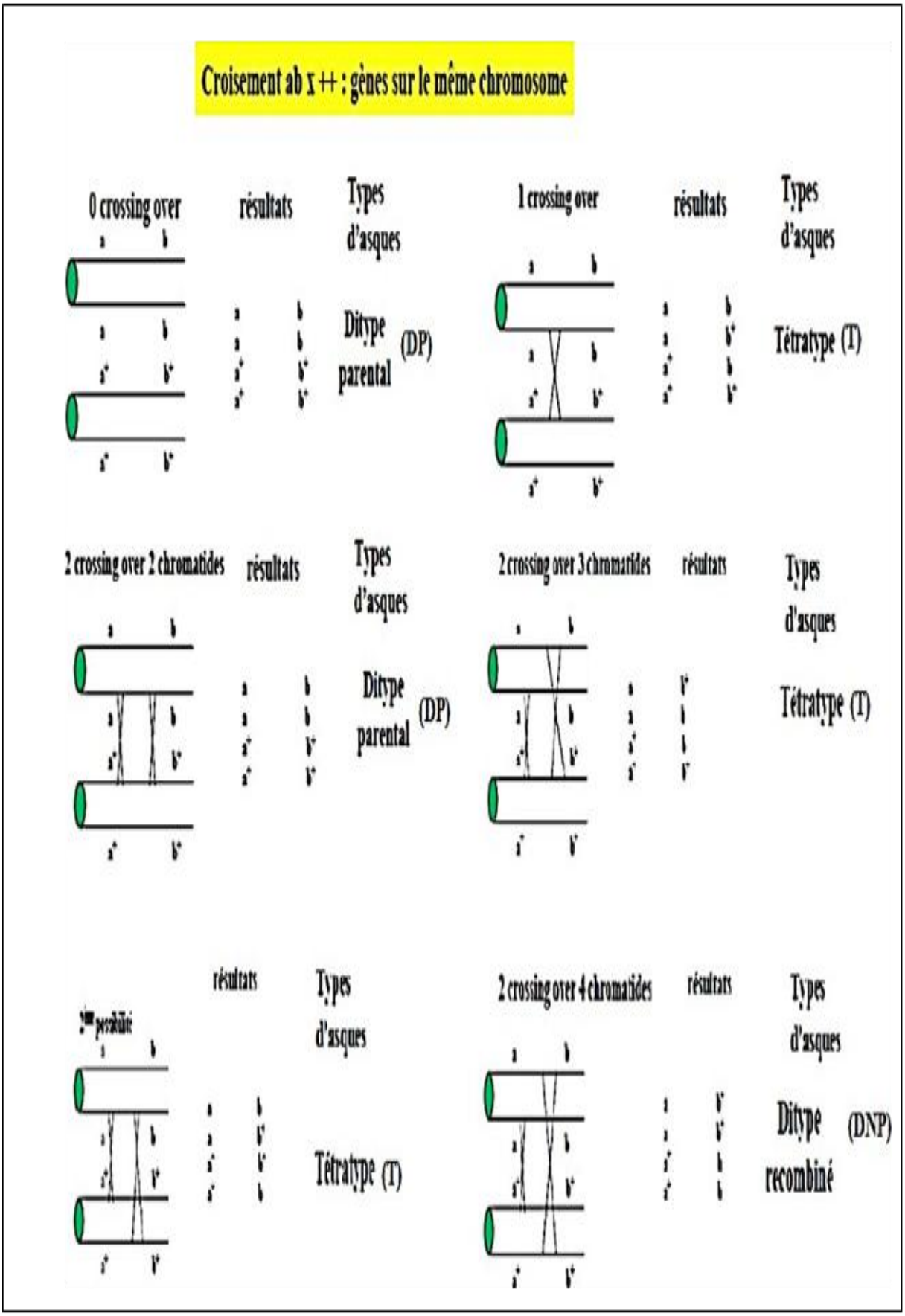


Figure 54: Transmission of two characters whose genes are carried by the same chromosome.

We calculate the distance between two genes in the case where they are located on the same chromosome and this is verified when DP > DNP.

To determine the distance between two genes, it is sufficient to know that tetratype tetrads (T) contain 2 recombinant spores (1/2) and 2 parental spores (1/2) and that non-parental ditype tetrads (DNP) contain only recombinant spores. So the distance which is the percentage of recombinants is :

Distance between 2 genes = [(DNP + ½ T) / Total] ∗ 100

Exemple

In Chlamydomonas, a cross is made between a strain requiring Arginine (Arg^-) and not para-aminobenzoic acid (Pab^+) with a strain not requiring Arginine (Arg^+) and requiring para-aminobenzoic acid (Pab^-). We obtain 03 types of tetrads are :

119 (DP)		**1 (DNP)**		**71 (T)**	
Arg^-	Pab^+	Arg^-	Pab^-	Arg^-	Pab^+
Arg^-	Pab^+	Arg^-	Pab^-	Arg^-	Pab^-
Arg^+	Pab^-	Arg^+	Pab^+	Arg^+	Pab^-
Arg^+	Pab^-	Arg^+	Pab^+	Arg^+	Pab^+

Distance = [(DNP + ½ T) / Total] ∗ 100

= [(1 + ½ (71)) /(119 + 1 + 71)] * 100

= 19,1% which corresponds to 19,1 cM.

5. Bacterial genetics

5.1. Generalities on bacteria

A bacterium is a single-celled organism (prokaryote) of small size, variable morphology which has its own characteristics.

The size of a bacterium varies between 1 to 10 µm. The weight of a bacterium is about 10-12 g. It contains 70% water. Based on dry weight, a bacterium is made up of proteins (55%), lipids (10%), lipopolysaccharides (3%), peptidoglycan (3%), ribosomes (40%), RNA (20%) and DNA (3%).

The chromosome of the prokaryotic cell is located in an irregularly shaped region called the nucleoid. The chromosome is most often unique. It is the medium of genetic information. It is a circular (sometimes linear) double helix formation, supercoiled thanks to topoisomerases. Length 1 mm.

Plasmids are double-stranded DNA molecules that replicate independently of the chromosome, can integrate into it, and are transmissible. They carry fertility traits (F factor),

antibiotic resistance (R factor), bacteriocins (Col plasmids), virulence, antiseptic resistance, metabolic traits, among others. Plasmids can give a selective advantage to the bacterium. Plasmids can be spontaneously eliminated from the host cell.

Transposable elements are fragments of DNA that move around the bacterial genome by transposition, hence the name transposon. The transposon is unable to replicate itself. The simplest transposable elements are insertion sequences (IS) with a short DNA sequence.

5.2. Reproduction in bacteria

Reproduction, always asexual in bacteria, can be done in two ways: by binary fission of cells or scissiparity, each cell dividing by partitioning into two other cells which lengthen while remaining united and constitute more or less elongated chains or rods, or else separate and constitute distinct organisms. This mode of reproduction, which merges with growth, is the normal mode, the one that continues indefinitely when the micro-organism is kept in its usual environment where it finds the conditions of existence that are most favorable to it. During division, DNA duplicates as well as other constituents.

The second mode of reproduction is that by endogenous or exogenous spores, which only occurs in certain circumstances, when the nutrient medium is exhausted by desiccation or by lack of the nutritive principles essential for the growth of the organism.

The inner protoplasm of each cell is then concentrated in the form of spores which constitute small, highly refractile, rounded, shiny granules, formed of protoplasm surrounded by a thick membrane whose two layers are called exospore and endospore. The presence of these spores gives the bacteria a swollen shape at the point where the spore has formed, either in the middle (*fusiform bacteria, e.g. Clostridium*), or at one end (*Helobacteria*), or at both ends (*Dispora*). The formation of two spores in the same cell, always then very elongated, is a rather rare occurrence.

Spores resist desiccation and very high or very low temperatures, which vary according to the species, much better than the bacteria from which they originate, and can thus remain for a very long time without losing their vitality and the ability to germinate. If, after this time, they again encounter the liquid medium favorable to their multiplication, they germinate there and give rise to a new colony of bacteria. Each spore swells at the expense of the liquid which soaks it, breaks its thick exospore and lengthens into a filament sometimes perpendicular to the long axis (*Bacillus subtilis*), sometimes, and this is the most common case, directed in the same direction (*Bacillus amylobacter*), and thus reproducing the

primitive form of the mother bacterium. This filament lengthens, partitions and multiplies again by scissiparity, until the exhaustion of the nutrient liquid forces it to provide new spores.

5.3. Genetic variation in bacteria

Bacterial DNA can be subject to variations which result in the appearance of hereditary differences in the permanent structures and/or functions of bacteria. Genetic or genotypic variations (the genotype is the set of genetic determinants carried by a cell) result from a mutation, a transformation, a conjugation, the acquisition of a plasmid, a transduction, in short, a change in the nature of one or more genes. Genetic variations must be distinguished from phenetic or phenotypic variations (the phenotype is the set of observable properties of a cell). The former affect the bacterial genome in its nucleotide sequence while the latter affect the behavior of the bacterium.

Phenotypic variations resulting from the adaptation of a whole bacterial population with the same genotype to various external conditions are reversible, not transmissible to offspring but specific (not random). Their mechanism is related to the activity of genes which can be regulated by more or less complex systems: induction as in the lactose operon; repression as in the tryptophan operon.

5.3.1. Genetic variations by mutation

Mutation is a change, spontaneous or caused by a mutagenic agent, hereditary (stable), sudden (discontinuous), rare (10^{-6} à 10^{-9}) and independent in the characters of a bacterium, and which is linked to a modification of the bacterial genome (DNA). There is no difference in nature between the mutation of a eukaryotic cell and that of a prokaryotic cell.

Any change in the nucleotide sequence of a gene constitutes a mutation. The nucleotide sequence can change in two ways, either by substituting one base pair for another following an error during replication, or by breaking the sugar-phosphate backbone of the DNA with loss, addition or inversion of DNA between the two breaks.

Any change in the nucleotide sequence of a gene constitutes a mutation. This change can be done :

» Either by substitution (exchange) of one base pair for another base pair, this mutation occurs at points we will speak of point mutations.

» Either by breaking the sugar-phosphate backbone of the DNA molecule with loss, addition or inversion of DNA sequences between the two breaks.

a) Substitutions

Substitution is the replacement of one nucleotide by another in the primary structure of the nucleic acid. A substitution can lead to very different results after translation.

It depends on its position relative to the reading frame. Transversions resulting from the substitution of one nucleotide for another from another chemical family (Purine ↔ pyrimidine); cause more mutations than transitions. Indeed, the latter result from the substitution of one nucleotide for another but from the same chemical family (purine ↔ purine or pyrimidine ↔ pyrimidine).

A substitution in a codon can result in the same amino acid : it is said to be synonymous (degeneracy of the genetic code). When the amino acid is different : it is said to be non-synonymous or missense. If the amino acid is replaced by an amino acid belonging to the same chemical family, we will speak of a conservative missense mutation, on the other hand, if the amino acid is different, we will speak of a non-conservative missense mutation. It will be all the more deleterious for the functions of the protein as the new amino acid will be different from the one that should have been translated.

As it can result in a termination codon : it is said to be nonsense. The translated protein will be truncated at this location.

b) Transposition

Transpositions result from the incorporation of nucleic acids synthesized outside the genome, by a transposase which incorporates these nucleic acids into the cell's genome, which increases the chances of producing new characters. These nucleic acids can be :

Segments of DNA encoding resistance to antibiotics (example Tn5) or heavy metals, these transposons are widely used for *in vitro* mutagenesis.

5.3.2. Genetic variations by transfer of genetic material

The bacterium can be subject to genetic variations other than mutation. These can result from the transfer of genetic material from one bacterium to another by processes as different as transformation, transduction and conjugation.

These gene transfers in bacteria are unidirectional, most often partial (1 to 2% of the transferred genome) and of low efficiency (recombination frequency of the order of 10-6). The donor generally gives up a small part of its DNA to the recipient. Thus, we will not obtain complete zygotes but rather partial zygotes or merozygotes.

a) Transformation

By definition, "natural" or physiological transformation is the first known model of genetic material transfer, which is fixed and absorbed by recipient bacteria, said to be in a state of competence.

In 1928, Frederick Griffith demonstrated that the subcutaneous inoculation of mice with a mixture of heat-killed encapsulated (virulent) pneumococci and live non-encapsulated (non-virulent) pneumococci leads to fatal septicemia with live encapsulated pneumococci. There has therefore been a transformation or "reversion" of the non-encapsulated (R) pneumococci into encapsulated (S) pneumococci. In 1944, Avery Mac Leod and McCarty demonstrated that the "transforming principle" is bacterial DNA. They succeed in reproducing the transformation *in vitro* in the presence of strongly polymerized DNA. The transforming activity is lost in the presence of deoxyribonuclease.

The transfer, which is partial and limited to a few bacterial species, results in the recipient bacterium acquiring new stable and transmissible genetic characteristics. The factors that affect transformation are :

» DNA size : The double-stranded DNA required for transformation must be at least 5 X 105 daltons.

» Recipient competence : Some bacteria are able to absorb DNA naturally. However, these bacteria only take up DNA at a specific time in their growth cycle when they produce a specific protein called competence factor. This state of competence only appears in a fraction of the bacterial population.

The state of competence develops, in Gram-positive bacteria, when the bacterium excretes a species-specific activator, which binds to the surface of the bacterium. There is then synthesis of a DNA-binding protein, an autolysin and an endonuclease. The bound DNA is then partially hydrolyzed and then converted into a single-stranded fragment (Fig. 51A). In Gram-negative bacteria, the state of competence is also related to the synthesis of a wall activator which is excreted by the bacterium in the exponential growth phase in H. influenzae, or in the stationary phase as is the case with Acinetobacter.

- Appearance of the state of competence (Fig. 55A);
- Fixation then penetration;
- Integration of donor DNA into the recipient bacterium's genome by homologous recombination (Fig. 55B).

Transformable bacteria are capable of fixing DNA from multiple sources but are only capable of forming genetic recombinations if the donor bacterium and the recipient bacterium are genetically very close. This relative specificity is linked to the homology of endogenous and exogenous nucleotide sequences.

Note : Donor DNA binds to the wall at receptor sites, under strict conditions of cellular metabolism, pH, temperature and osmolarity.

Although transformation only allows the transfer of a small fraction of the bacterial genome (<1 %), is of relative efficiency (the transfer frequency is of the order of 10^{-4} to 10^{-6}) and is limited to a few bacterial species, it is of great theoretical and practical interest.

Transformation has made it possible to understand the mechanism of capsule synthesis, the genetic control of antibiotic resistance, the establishment of genetic maps, etc...

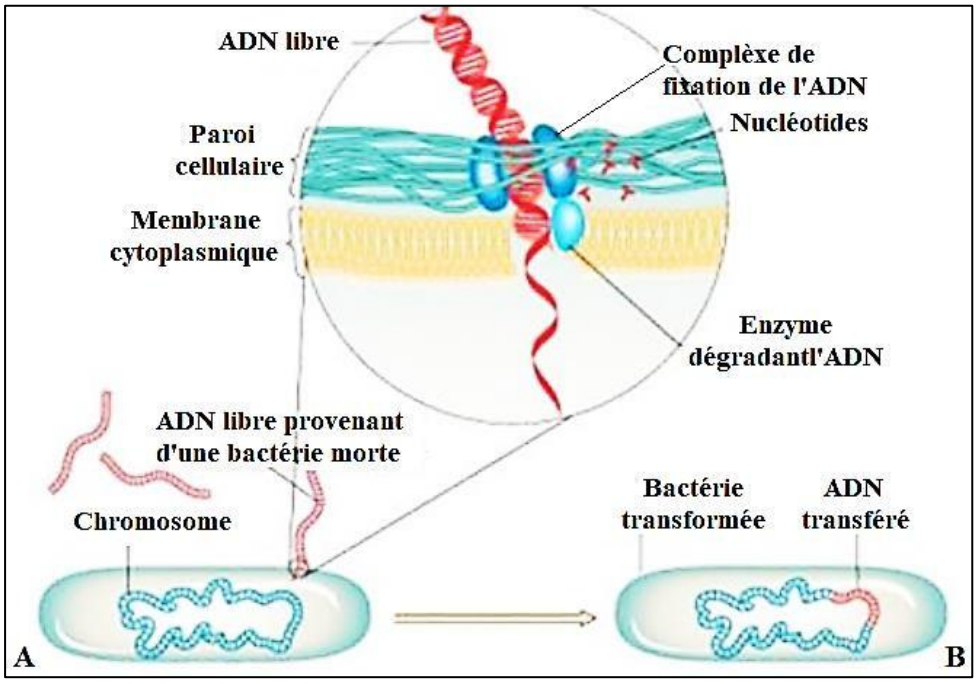


Figure 55: Bacterial transformation, (a) binding of naked DNA, (b) integration of the DNA fragment after homologous recombination.

b) Conjugation

The experiment of Lederberg and Tatum (1946) is at the origin of the discovery of conjugation. In a liquid culture medium, these authors mixed two types of auxotrophic mutants of *E. coli*. After several hours of contact between the mutants, Lederberg and Tatum isolated *E.coli* T^+ L^+ M^+ B^+ (approximately 100 per 108 *E. coli*) and concluded that recombination had occurred with a low frequency (10^{-6}) and further required contact between

the two types of auxotrophic mutants.

The transfer of chromosomal DNA by conjugation only occurs between bacteria of the same species (specificity), and especially in Gram-negative bacteria such as enterobacteria (*E. coli*, *Salmonella ssp* and *Pseudomonas aeruginosa*).

The transfer of the sex factor, which is unidirectional, is the first known plasmid. The genetic information it carries codes for the biosynthesis of sex pili, for its possible insertion into the bacterial chromosome and for its transfer of the latter to recipient bacteria (F^-).

Chromosomal transfer is only possible after pairing by a pair of donor and recipient bacteria. It first involves the sex pili (2 to 3 per F^+ bacterium) which recognize the receptors on the surface of the F^- bacteria by their ends and attach to them, then retract by bringing the two bacteria closer together. They thus allow their contact and the formation of a cytoplasmic bridge of 100 to 300 μm through which chromosomal transfer will take place.

All the characters encoded by the chromosome can be transferred. Indeed, the F factor can be integrated into the bacterial chromosome at certain sites. In this position, it allows the transfer of chromosomal genes close to these sites from one bacterium to another, but rarely transfers the factor itself (Fig. 56).

The F factor can remain autonomous in the cytoplasm. In this position, it only transmits the F factor to the recipient bacterium, but no chromosomal genes. During the transition from the integrated state to the autonomous state, the F factor can carry bacterial genes with it. The result is an F' plasmid that contains these genes and is capable of transferring them to a recipient bacterium of new genes : this is F-duction or sex-duction.

If the genes transferred by the F' factor integrate into the chromosome of the recipient bacterium, it is said that legitimate (chromosomal) recombination has occurred. If they do not integrate, they become true mobile genes from one bacterium to another.

Some plasmids are capable of ensuring their own transfer by conjugation. They are then called conjugative plasmids.

Conjugation F^+ x F^-

Conjugation is a transfer of DNA between a donor bacterium and a recipient bacterium, which requires contact and pairing between the bacteria, and relies on the presence in the donor or male bacterium of a sex or fertility factor (F factor). This allows the synthesis of sex pili and gives polarity to the chromosome. The F factor usually resides on a plasmid.

Donor strains are called F^+ while those lacking it are called F^- (Fig. 56).

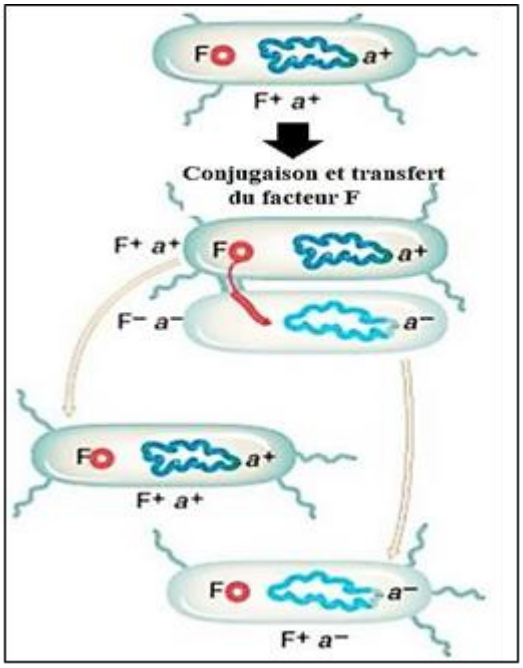


Figure 56: Conjugation F+ x F^-

Once the cytoplasmic bridge is formed, genetic transfer can begin. It initially only involves one strand of DNA, which allows the integrity of the donor bacterium's genome to be restored by an asymmetric replication process. This asymmetric replication process takes place very close to the cytoplasmic bridge and involves a specific replication site.

A single-stranded tail is generated by an endonuclease at the oriT site, the 5' end migrates to the recipient cell, and can be converted into the double-stranded form by the synthesis of a complementary strand (Fig. 57). While the DNA on the 3' side will be replicated continuously by complementarity, the 5' end is replicated discontinuously by Okazaki fragments.

This rolling circle model (Fig. 57) also applies to the replication of DNA bacteriophages, which will first give rise to a long concatemer that will then be cleaved into phage units.

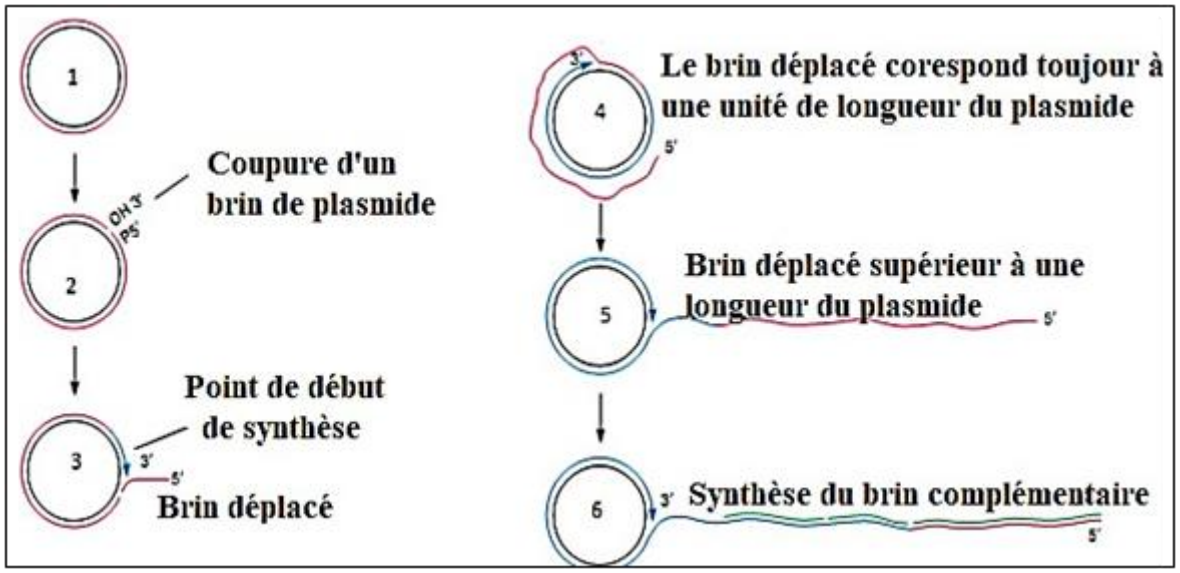


Figure 57: « Rolling circle » plasmid replication model.

Conjugation Hfr x F⁻

An Hfr strain (High frequency of recombination) results from the integration of the F factor into the chromosome. The insertion of the F factor is done by crossing-over (Fig. 58). Recombination occurs between two IS sites (RSS).

Hfr x F^- conjugation is a chromosomal DNA transfer that is unidirectional, oriented, progressive and sometimes total (Fig. 58), has many similarities with extrachromosomal (plasmid) DNA transfer. The 'female' bacterial cell that has just conjugated with a 'male' bacterial cell and contains a fragment of male DNA (after recombination) is called an exconjugant.

Conjugation F'X F⁻

When F inserts between the ton and lac genes in a repetitive IS1 sequence, it gives an Hfr strain. The abnormal excision of F, by recombination with another IS2 element, includes the lac locus. The result is F' lac. When the latter is transmitted to another (recipient) cell by F'x F^- conjugation, the result is a partial diploid F'lac^+ / lac^-.

Note : The F plasmid does not have a single insertion site, it can recombine with several IS.

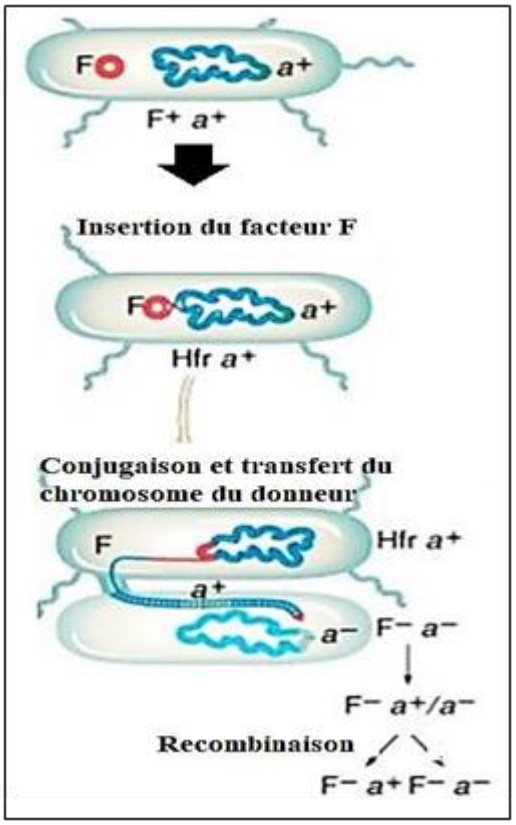


Figure 58: Conjugation Hfr x F-

c) Transduction

The transfer of genetic material from one bacterium to another, using a bacterial virus (bacteriophage, phage) as a vector, is called transduction. This aspect of transfer will be discussed later in the bacteriophage transduction section.

6. Virus genetics

6.1. General information on viruses and bacteriophages

It is by biological necessity that viruses are parasites. They do not have the ability to synthesize their components independently. To reproduce, they must introduce their genome into a living host cell. All viruses are small particles made up of a protein-rich protective envelope that facilitates their transport from one cell to another. Their genetic material is either DNA or RNA, but never both at the same time.

Bacteriophages (or phages) are viruses that infect bacteria. They have played, and continue to play, a fundamental role in bacterial genetics and molecular genetics in general. For more than thirty years (1940-1973), they were valuable tools for studying the consequences of

introducing new genetic material into a cell. Thus, studies on phages have helped in understanding the structures and expression of genes, the assembly of complex biological structures and the transfer of genetic material between bacteria.

A phage consists of a nucleic acid « chromosome » (DNA or RNA), surrounded by a protein wall : the capsid. The whole forms a nucleocapsid.

Most bacteria are susceptible to attack by bacteriophages. These are used in lysotyping, and there are two types :

- ✓ Virulent phages : follow a lytic cycle.
- ✓ Temperate phages : follow a lysogenic cycle, remain integrated into bacterial DNA in the form of a prophage (do not destroy their hosts).

6.2. Bacteriophage life cycle

6.2.1. Lytic cycles

The most studied phages are those that multiply in *E. coli* or very closely related bacteria. They have been arbitrarily named T1, T2, T4, P1, F1, M13 or l for example. A phage infects a bacterium by attaching to a specific receptor on the bacterial surface. Once attached, the phage injects its genetic material into the cytoplasm. Some of the phage genes are expressed immediately (early genes) after the DNA is injected into the bacterium, using enzymes present in the cytoplasm of the bacterium. These early genes encode enzymes necessary for the replication of phage DNA into a large number of copies. The late genes then begin to be expressed. These are mainly genes that encode the proteins needed to produce new phage capsids. Once the various phage components are assembled, the bacterial wall is destroyed (this is called bacterial lysis), releasing a large number of new phages into the surrounding environment. Each of them is capable of infecting a new bacterium, allowing a new biological cycle of the phage (Fig. 55).

6.2.2. Lysogenic cycles

In the 1920s, it was observed that some bacterial strains appeared resistant to certain phages. However, these resistant bacteria caused the lysis of other bacteria when the cultures were mixed. These resistant bacteria are called lysogenic. Around the mid-1940s, André Lwoff showed that a lysogenic bacterium is a bacterium that contains a viral genome, inserted at a specific point in its own chromosome. The presence of phage DNA integrated into the bacterial chromosome, called a prophage, protects the bacteria from infection by another phage (superinfection). This immunity remains stable and can be transmitted to daughter bacterial

cells. The prophage is therefore duplicated in the same way as other regions of the bacterial chromosome. In a small fraction of lysogenic cells, the production of infectious phages can be observed. This is referred to as the induction of the prophage of lysogenic bacteria. This process obviously deprives the cell of its immunity, lyses the bacteria, and releases the infectious phages into the medium. The latter can infect any non-lysogenic bacteria present in the culture medium. Subsequent studies showed that various agents, such as ultraviolet radiation or certain chemicals, could induce lysis of a significant fraction of a population of lysogenic bacteria (Fig. 59).

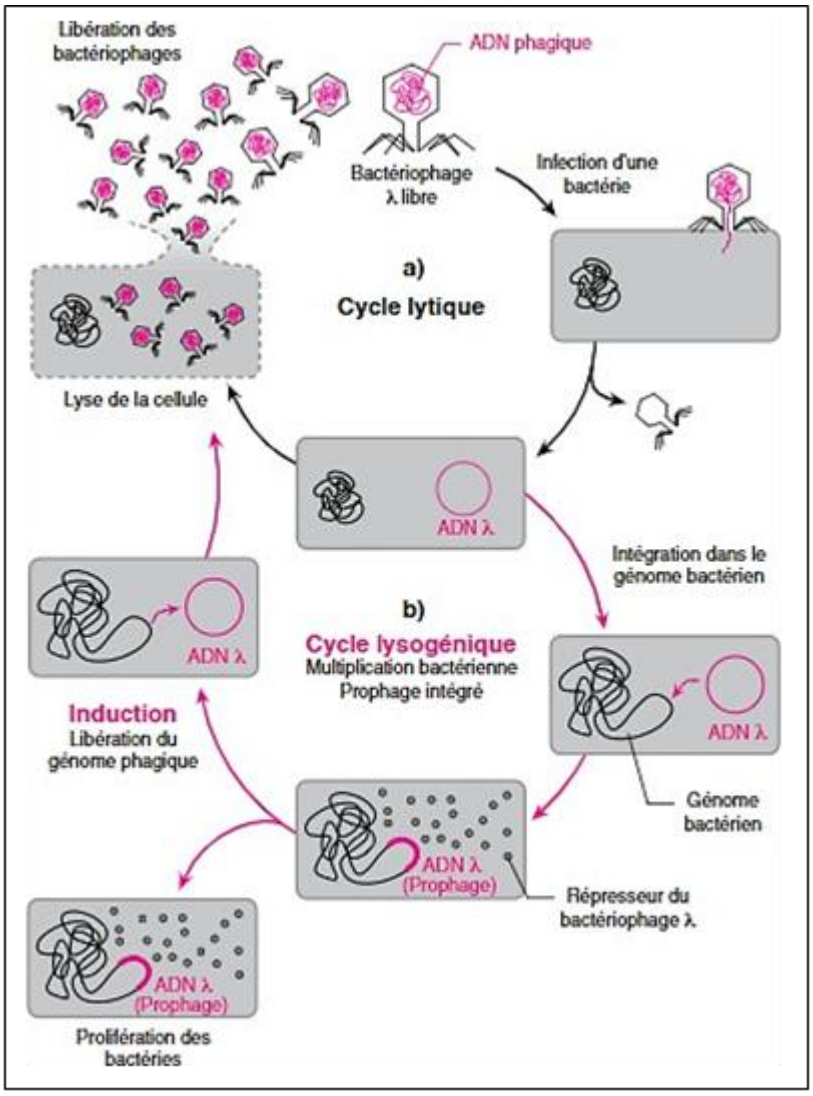


Figure 59: Lytic cycle of a bacteriophage (a) and lysogenic cycle (b).

6.3. Transduction in bacteriophages

In 1951, J. Lederberg and N. Zinder, working on the bacterium *Salmonella*, showed that traits could be passively transmitted from one bacterium to another via a bacteriophage.

They called this phenomenon transduction.

6.3.1. Generalized Transduction

A typical transduction experiment consists of infecting a leu^+ bacterium with a transducing phage specific to this bacterium. The development of this phage in the bacteria leads to their lysis, which releases a large number of progeny. The majority of these phages are identical to the infecting phage. Exceptionally, the capsid contains a fragment of bacterial chromosome DNA integrated into the phage DNA molecule. When a culture of leu^- bacteria is infected with the lysate containing all the phages, some of those that have « taken on board » a small fragment of bacterial chromosome introduce the leu^+ gene into the recipient bacteria. By recombination, the leu^+ allele can substitute for the leu^- allele and the bacterium is transduced for this trait. Any fragment of the donor bacterium's DNA carrying a genetic marker can be transduced in this way. This is called generalized transduction (Fig. 60).

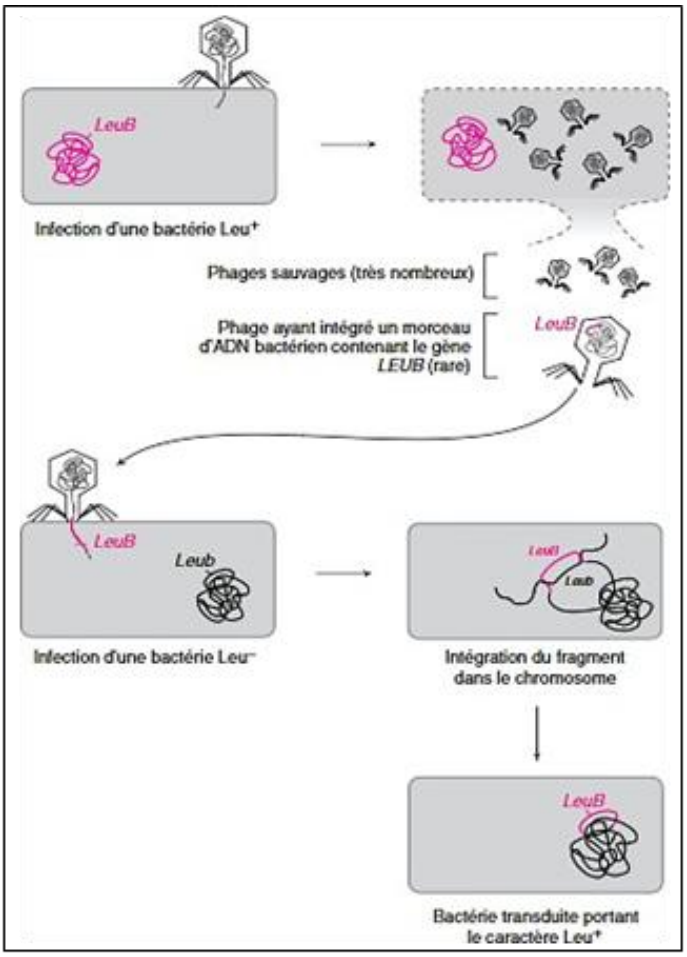


Figure 60: Mechanism of generalized transduction (The *leuB* gene codes for ß-isopropylmalate dehydrogenase, an enzyme involved in the leucine biosynthesis pathway. The mutated allele of this gene is denoted *leub*).

6.3.2. Specialized Transduction

There is another type of transducing phage that carries only a restricted and well-defined part of the bacterial chromosome due to the insertion of the transducer at a specific position on the bacterial chromosome. This phenomenon is called specialized transduction.

The lambda phage, a temperate phage, is a good example of a specialized transducer. Induction of the lambda prophage of the lysogenic *E. coli* bacterium leads to the production of certain defective particles due to incorrect excision of the prophage. The phage genome takes with it a fragment of E. coli chromosomal DNA while some phage genes remain in the bacterial chromosome (Fig. 61). This abnormal phage DNA, carrying gal or bio bacterial genes close to the integration site, can be packaged and infect other bacteria. If a normal phage particle also infects the bacterium (double infection) ldgal can integrate into the host genome at the specific lambda phage attachment site. Thus, the gal genes are transduced into the second infected bacterium.

The sequences called att « attachment » containing the specific sites on the phage DNA (attP) and on the bacterial chromosome (attB) contain only a very short region of homology. The integration reaction is catalyzed by the Int (integrase) enzyme synthesized by the lambda bacteriophage in abundance at the beginning of infection. An accessory protein from *E. coli* called IHF (Integration Host Factor) also participates in this catalysis. The integration of phage 1 is reversible and its excision requires a second enzyme Xis (excisionase) also synthesized by the phage. The formation of a complex between the integrase and Xis catalyzes the excision of the prophage, which allows the reconstitution of intact bacterial and phage DNA (Fig. 61).

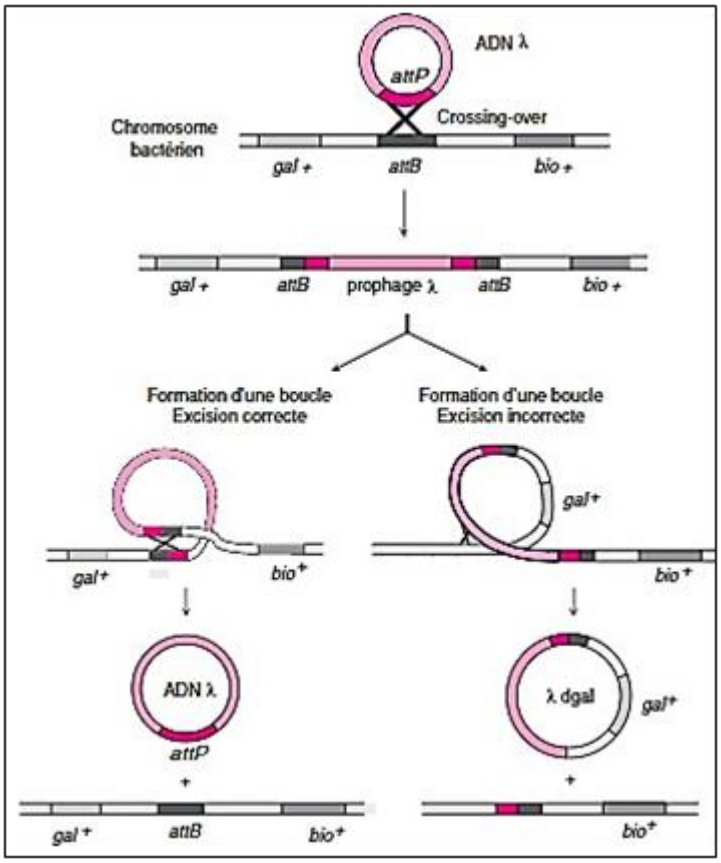


Figure 61: Integration of phage λ and mechanism of specialized transduction.

Chapter 3 :
Molecular Basis and Function of the Genome

Chapter 3: Molecular Basis and Function of the Genome

Introduction

Genetics is a branch of biology that studies heredity, that is, the transmission of biological traits from one generation to the next. It explores the mechanisms by which genetic information is transmitted and expressed in living organisms. Genetics helps us understand how biological characteristics and traits are inherited and manifested in individuals.

Genes are specific segments of DNA located on chromosomes, and they contain the instructions for the production of proteins, the basic building blocks of life.

Genetics also studies genetic variations, which are differences in the DNA sequence between individuals. These variations can be responsible for the observed differences in physical characteristics, diseases, and susceptibility to diseases in individuals. Genetic studies help identify genes involved in particular diseases, study the mechanisms underlying genetic diseases, and assess the genetic risks associated with certain conditions.

Modern genetics uses advanced techniques such as DNA sequencing to analyze genetic sequences and identify genetic variations. It also uses statistical tools to study patterns of trait transmission and to assess genetic and environmental influences on observed characteristics.

Molecular biology is a discipline at the heart of genetics. It focuses on the study of the molecular processes that govern life and determine the characteristics of organisms. It is primarily concerned with biological macromolecules, such as DNA (deoxyribonucleic acid), RNA (ribonucleic acid), and proteins. These molecules play an essential role in the storage, transmission, and expression of genetic information and are responsible for cellular structure and function.

DNA is the molecule that contains the genetic information of an organism. Molecular biology studies the structure of DNA, how it is replicated during cell division, and how it is transcribed into RNA. RNA, in turn, plays a crucial role in protein synthesis as a molecular messenger that carries genetic information from the cell nucleus to the ribosomes, the cellular machines responsible for protein synthesis.

Molecular biology also examines the mechanisms of RNA translation into proteins, which is the process by which amino acids are assembled to form specific proteins. It studies how proteins are structured and function in cells, as well as the processes of gene expression regulation that control when and where genes are turned on or off. Molecular biology has

important implications in many fields, including medicine, agriculture, biotechnology, and basic research. It helps us understand the genetic causes of diseases, develop gene therapies, improve crops and species, and generate new knowledge about life and its mechanisms.

Molecular biology uses a variety of experimental techniques to study these processes, including DNA cloning, PCR (polymerase chain reaction) to amplify specific DNA sequences, DNA sequencing to determine the order of nucleotide bases in a DNA fragment, gel electrophoresis to separate DNA or protein molecules based on their size and charge, and various genetic manipulation techniques to study gene functions. These techniques give molecular biology the ability to identify possible genetic mutations that affect the genome.

Molecular biology plays an essential role in many areas of biology, medicine, and scientific research. It helps us understand the molecular mechanisms of genetic diseases, develop new gene therapies, and design drugs that target specific proteins involved in diseases. It is also used in biotechnology research, genomics, drug development, and other related fields.

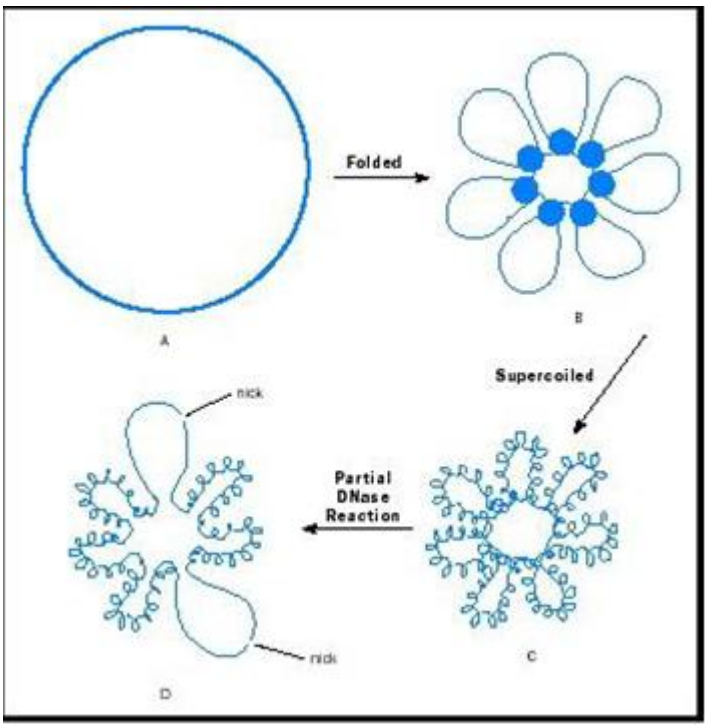
1. Chromosome: Carrier of Genetic Information

All cellular life forms have gene-carrying structures which are chromosomes. However, chromosomes must be considered separately in the two main groups of living organisms : prokaryotes and eukaryotes.

1.1. Prokaryotes

The bacterial chromosome is formed by a single DNA double helix ; this double helix is neither surrounded nor protected, it measures about 1 mm in length, and it is circular (Fig.62). The DNA molecule forming the chromonema occupies a very small area, about 1µm in diameter. The bacterial chromosome must therefore be folded very stably in a space that is not even limited by a nuclear membrane.

The chromonema consists of a number of loops (40 to 50) held together by RNA (coiling). The chromosome forms a continuous molecule through all these loops. Each loop can also maintain other secondary coils (supercoiling). Each turn of this supercoiling would count about 400 base pairs. The compact form of the chromonema could be the result of 3 superimposed structures (Fig. 62).



A : *primary structure : 2 polynucleotide chains*

B : *secondary structure : Helix folded on itself, more or less 50 loops*

C : *tertiary structure : Each loop = solenoid*

Figure 62 : Chromosome structure in prokaryotes (Escherichia coli)

1.2. Eukaryotes

These are contained within a nucleus. They can be visualized by light microscopy, after staining and during nuclear division, during mitosis. Between two cell divisions, i.e., during interphase, examination of the nucleus does not allow individual chromosomes to be characterized : they are too extended, they then appear as a network of very fine filaments forming chromatin. It is necessary to wait for this network of filaments to coil, fold, and contract to make the first observations. This contraction is maximal at metaphase (Fig. 63).

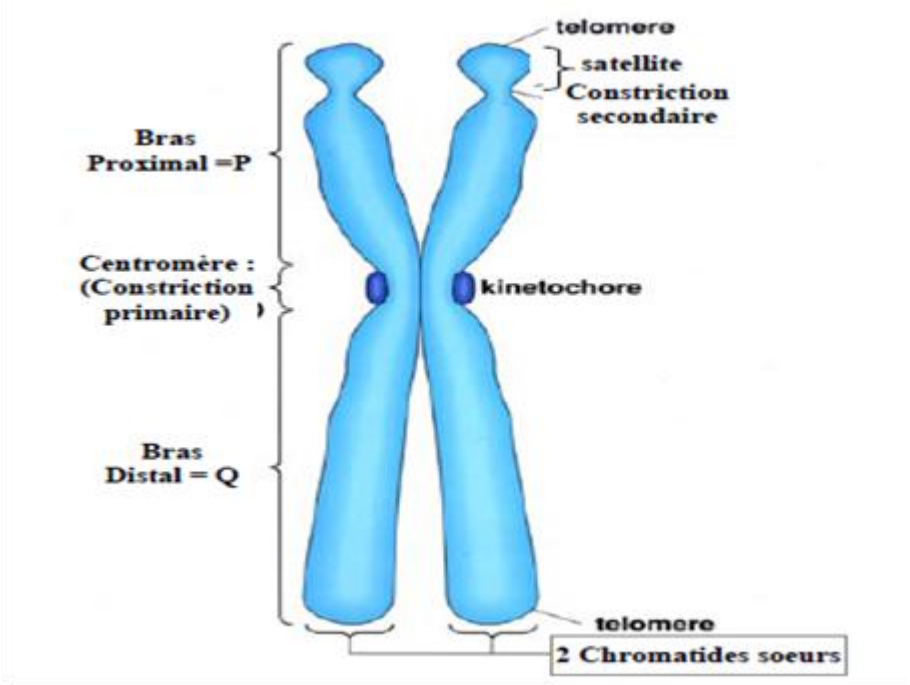


Figure 63 : Chromosome structure in eukaryotes

1.3. The external constitution of the chromosome and some essential identification characteristics

It is established from the observation of chromosomes in mitosis, when they exhibit a sufficiently advanced spiralization of the chromatin filaments : it is this spiralization that makes their morphological study possible. It is composed of :

1.3.1. Chromomeres

At the beginning of mitosis, before the condensation of the chromosome is too important, it is observed that certain regions have a more pronounced thickening. These areas, which stain more strongly, are called chromomeres. The distribution of chromomeres is common for a given chromosome and allows it to be characterized.

1.3.2. Chromatids

Each mitotic chromosome, in metaphase, appears to be constituted by the association of two subunits, joined along their entire length ; these are the chromatids (Fig. 63).

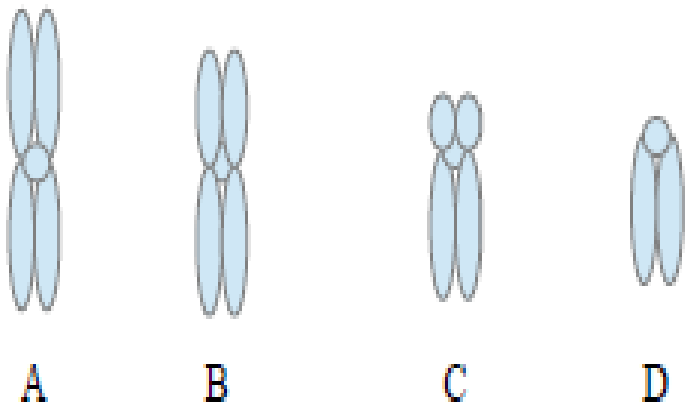
1.3.3. Constrictions

a) ***Primary constriction:*** the centromere corresponds to a zone where the two chromatids are less thick and where they are more closely joined. It separates the chromosome into two arms, its position is always the same for a given chromosome and characterizes it (the centromere occupies a different place from one type of

chromosome to another). A centromeric index is defined : **length of the short arm / total length of the chromosome.**

Different morphologies of chromosomes are distinguished according to the location of the centromere (Fig. 64):

- ✓ **Metacentric chromosome :** the centromere is near the middle so that the two arms are equal (P=Q). It is median.
- ✓ **Submetacentric chromosome :** The centromere is located in a submedian position so that it separates a short arm, called the proximal arm, and a long arm called the distal arm.
- ✓ **Telocentric chromosome :** The centromere is located in a terminal position. The proximal arm is practically punctiform.
- ✓ **Acrocentric chromosome :** when the proximal arm is very small. The centromere is close to the end of the chromosome ; it is in a subterminal position.



A : metacentric ; **B :** submetacentric ; **C :** acrocentric ; **D :** telocentric

Figure 64 : The different types of chromosomes (morphology)

b) Secondary constriction : these are also zones where the chromatids are less thick, therefore weakly stainable. Their positions are constant for a given chromosome, so they are good identification markers.

Nucleolar organizers : these are also secondary constrictions but correspond to the chromosomal zone where, during interphase, the nucleoli are formed. These constrictions are often located in a subterminal position on one of the arms of the chromosome, isolating on it a small terminal portion called a satellite (Fig. 63). In a given cell, as many nucleolar organizers are observed on the mitotic chromosomes as there are nucleoli in the interphase nucleus. The

nucleolus contains DNA, RNA, and proteins ; it appears in the nucleus in interphase as a spherical mass.

1.3.4. Chromosome Bands

It is possible to reveal characteristic bands on the chromosome (Fig. 65). Indeed, the use of staining techniques with fluorescent substances or staining after enzymatic digestion or heat denaturation highlights transverse bands. These techniques have revealed additional details in the structure of the chromosome. Each chromosome reveals a specific profile of light bands alternating with dark bands. Homologous chromosomes have identical profiles. The dark bands indicate a high density of DNA following a significant contraction of coiled fibers (B fiber, heterochromatin). While the light bands indicate a low density of DNA (A fiber, euchromatin).

1.3.5. Telomeres

Ends of chromosomes. The function of the telomere is to protect the ends of the chromosome from degradation.

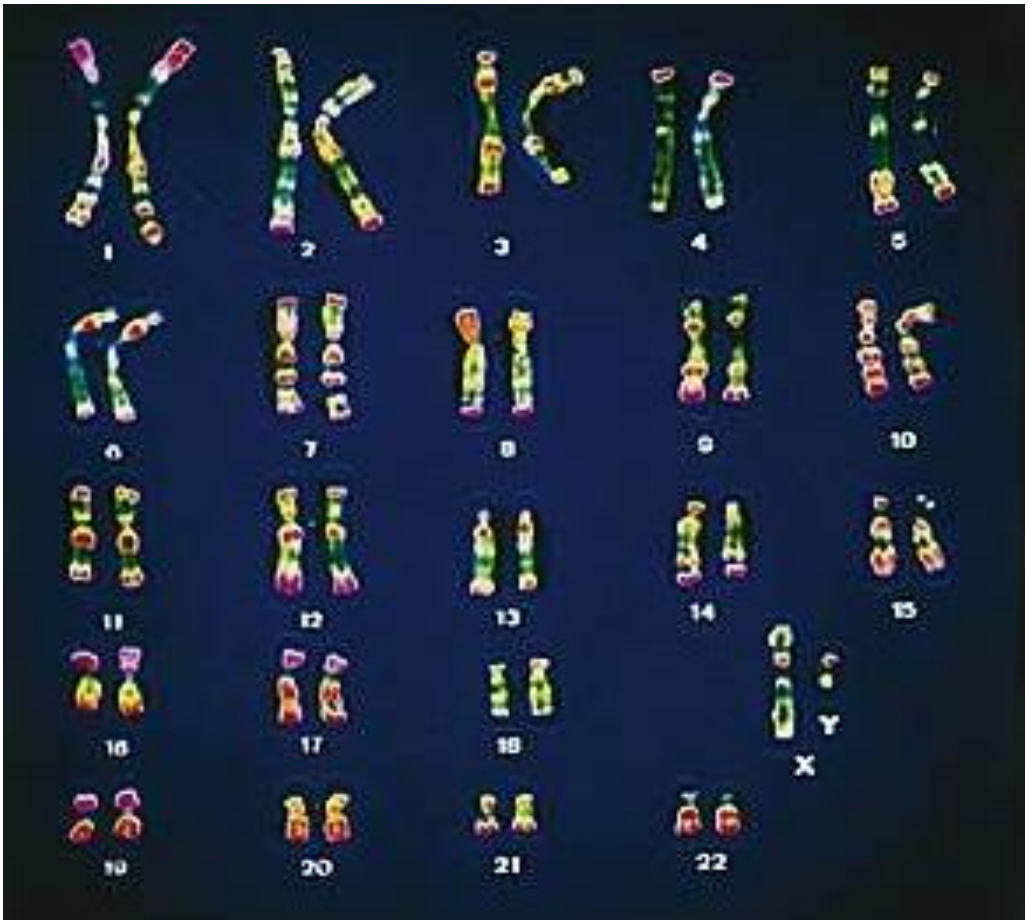


Figure 65 : Human karyotype with a representation of chromosome bands (2n = 46 chromosomes).

1.4. Karyotype

The number, size, and shape of chromosomes are characteristic of a species ; the different pairs of chromosomes of a given cell can be classified according to their particular characteristics, thus producing the karyotype of the species to which this cell belongs. This karyotype within the same species can vary: following genetic accidents caused by mutation (loss or addition of chromosomal segments, doubling of the number of chromosomes), or malfunction during nuclear division (loss or gain of chromosomal units). The karyotype can vary depending on the sex of the individual (Fig. 65).

The size of mitotic chromosomes is extremely variable. For the same phase of mitosis, it is constant for each type of chromosome in a cell of a given species.

The number of chromosomes varies widely among species. For each species, the chromosomes are grouped in homologous pairs. Each species has in its somatic cells 2 sets of n different chromosomes. Each set is called the haploid set of n chromosomes and the total number of chromosomes in the cell, 2n, corresponds to the diploid number. Example: the fruit fly (Drosophila) has 8 chromosomes (4 pairs, n = 4). Human cells (46 chromosomes, 23 pairs of chromosomes, n = 23). In humans, the haploid set of chromosomes is n = 23 and the diploid set is 2n = 46.

1.5. Heterosomes and autosomes

In many species, one of the chromosome pairs can be constituted in one of the sexes by morphologically dissimilar chromosomes. These **heterosomes** are involved in determining the sex of the individual, for this reason they are also called **sex chromosomes.** The other pairs, which are always made up of two similar chromosomes, are called **autosome pairs** (Fig. 4).

1.6. The internal or finer constitution of the chromosome

Chromosomes are composed of DNA, proteins, and a small amount of RNA (in transit to the cytoplasm).

The association of DNA and proteins that constitutes chromosomes is called chromatin. Chromosomes correspond to the most compact form of chromatin. The proteins are represented by two classes : histones and non-histone proteins.

1.6.1. Histones

These are basic proteins, particularly rich in arginine, lysine, and alanine (very stable). They play a crucial role in the ordered packaging of this very long DNA molecule within a

nucleus a few micrometers in diameter. They are classified into two groups :

Nucleosomal histones : which allow the formation of the nucleosome. The nucleosome consists of two complete turns of DNA wrapped around a core formed by 4 histones, called H2A, H2B, H3, and H4. The nucleosome gives chromatin a «beads on a string» appearance (Fig. 66).

H1 histones : are larger proteins and are responsible for stacking nucleosomes.

1.6.2. Non-histone proteins

In a chromosome, DNA is not only packaged with histones into repetitive and regular nucleosomes but is also folded by other proteins into a series of loops. These are acidic proteins (topoisomerase II).

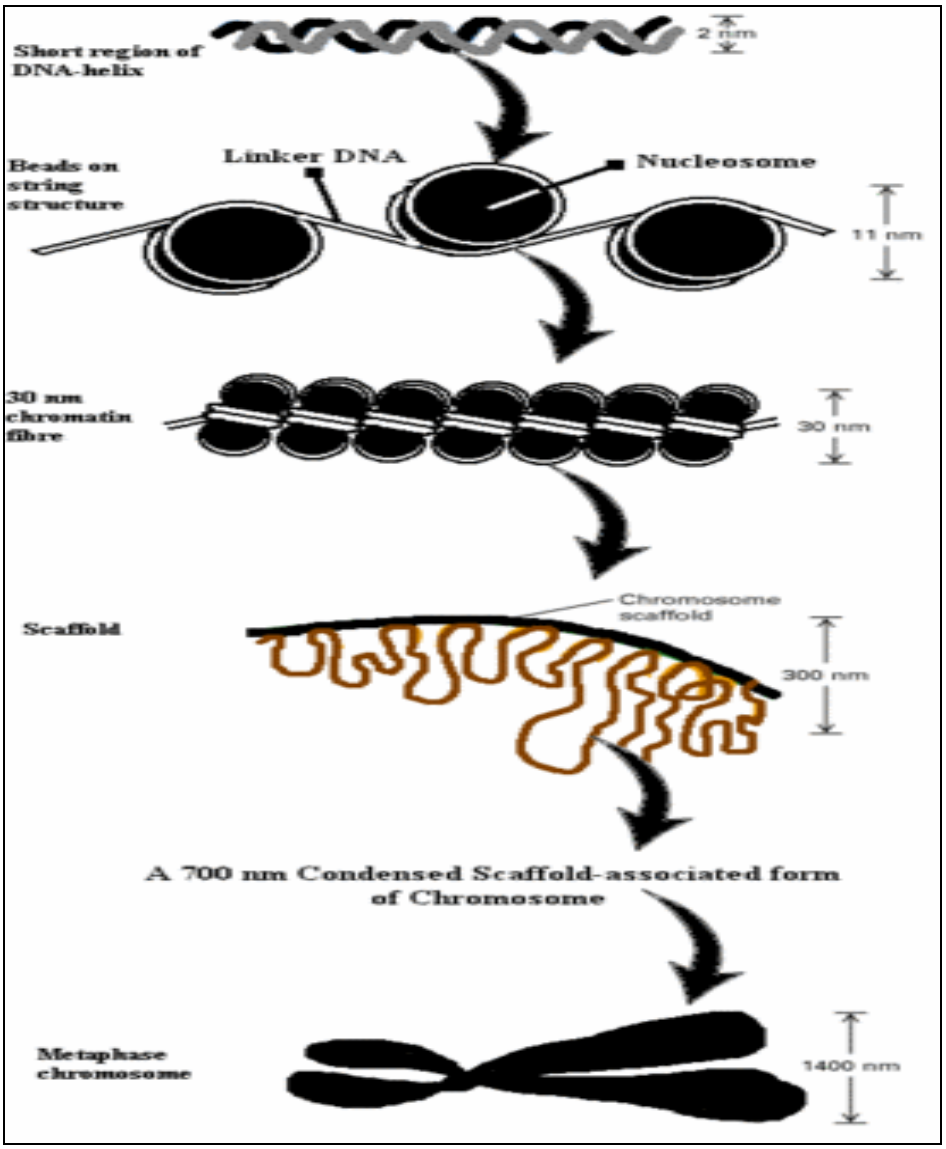


Figure 66 : Internal or finer constitution of the chromosome

2. Cell divisions

The typical life cycle of cells is often divided into two main phases : the growth phase and the division phase (Fig. 67).

Growth phase : It is also called the interphase. It is composed of three main stages :

- ✓ ***G1 phase (Gap 1) :*** During this stage, the cell grows and performs its specific functions. It also accumulates the nutrients necessary for future cell division.
- ✓ ***S phase (Synthesis) :*** During this stage, the cell's DNA is replicated. Each chromosome is copied to form two identical sister chromatids, connected by a centromere.
- ✓ ***G2 phase (Gap 2) :*** The cell continues to grow and prepare for cell division. It synthesizes additional proteins and organelles to support future division.
- ✓ **Cell division (M) :** is the process by which a cell divides to give rise to two daughter cells. There are two main types of cell division : mitosis and meiosis.

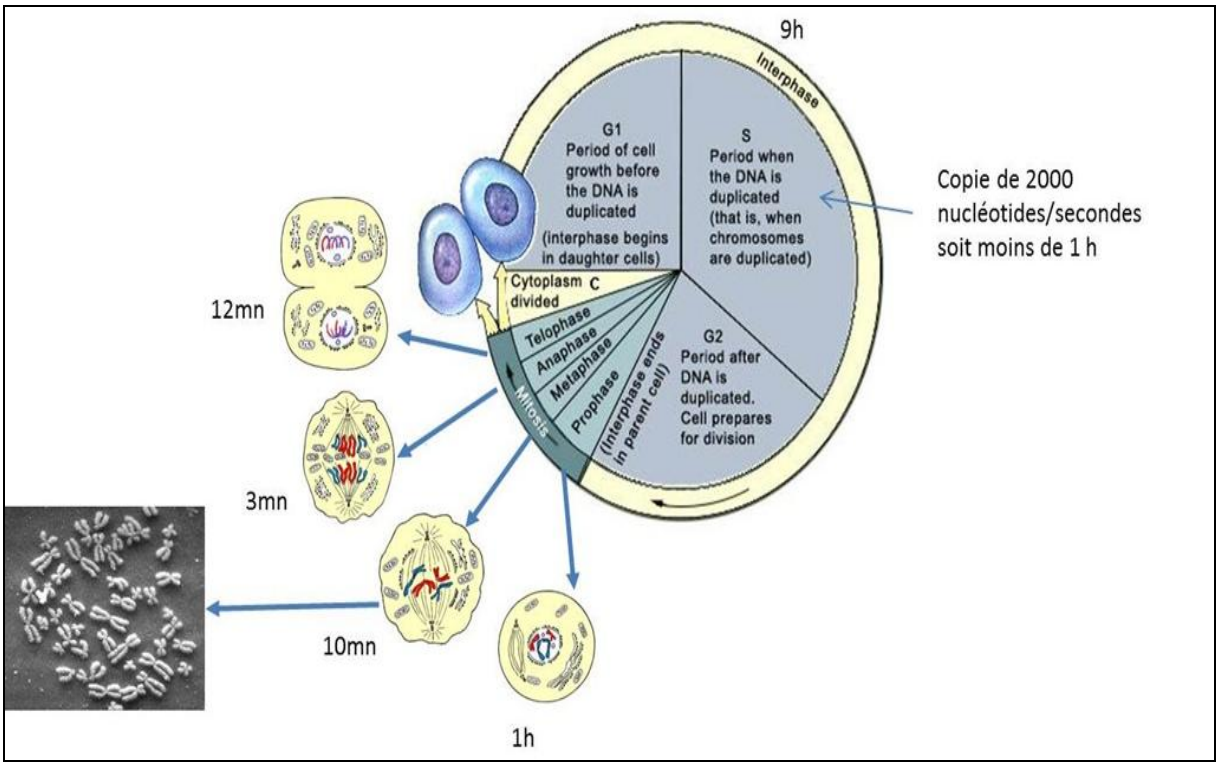


Figure 67 : Typical life cycle of a human cell (example : Stomach cell)

2.1. Mitosis

Mitosis is the mechanism by which the chromosome content of a somatic cell (i.e., the cells of a eukaryote that are not destined to become sex cells) remains constant during successive cell divisions. It allows a mother cell to divide into two identical daughter cells, by

making an exact copy of each chromosome.

During this division there is a division of the :

- Nucleus or **karyokinesis**, also called **karyodiérèse**.
- Rest of the cell or **cytokinesis**, also called **cytodiérèse**.

During interphase the nucleus is swollen and the chromosomes are not individualized. Indeed, the latter are very long and tangled, forming a chromatin network. It is possible to observe the nucleolus at this stage (Fig. 68).

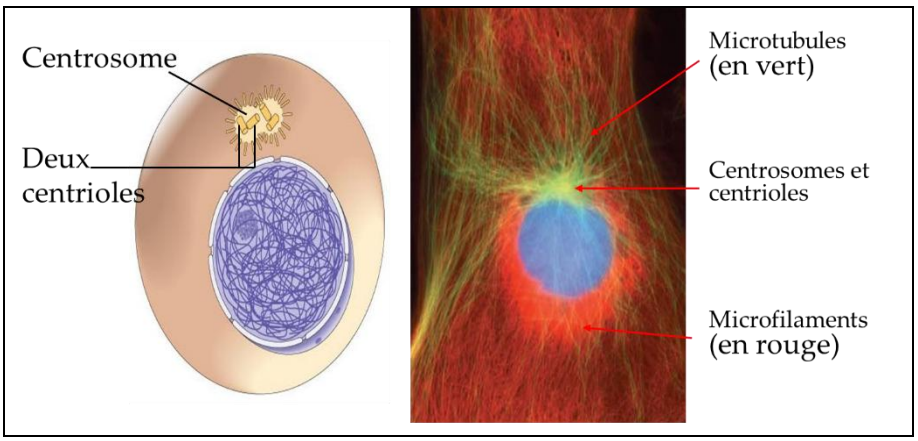


Figure 68 : End of interphase (G2 phase) : Lung cell of Taricha granulosa (a newt with 22 chromosomes)

Mitosis consists of 5 successive phases :

2.1.1. Prophase

a) Nuclear phenomenon

During the transition phase from G2 to M phase. There is a transformation of chromatin into filaments forming the chromosomes. Each chromosome is composed of two sinuous, intertwined nucleosomal fibers, united by the centromere. The cell becomes spherical, H1 histones cause chromosomal condensation. Thus, the chromosomes become shorter, thicker, and visible under a light microscope and appear to be formed of two chromatids joined by a centromere. Each chromosome approaches the nuclear envelope, leaving an empty centro-nuclear space. The nucleolus decreases in size and disappears completely (Fig. 69).

b) Cytoplasmic phenomenon

The main microtubule organizing center (MTOC) in most animal cells is called the

centrosome. It is located at one of the nuclear poles and is bathed in a dense substance. The latter contains a pair of centrioles that separate during the G1 phase of the cell cycle, and each centriole replicates during the S phase. During prophase, the centrosome is divided into 2, and each daughter centrosome becomes the focus of a star-shaped aster of microtubules. Two asters are thus formed, which gradually migrate towards the opposite poles of the nucleus, while the microtubules lengthen. At the end of prophase, the asters are each located at one of the opposite poles of the nucleus (Fig. 69).

Note : Not all microtubule organizing centers contain centrioles. In the mitotic cells of higher plants, for example, microtubules originate from poorly defined dense regions that are completely devoid of centrioles.

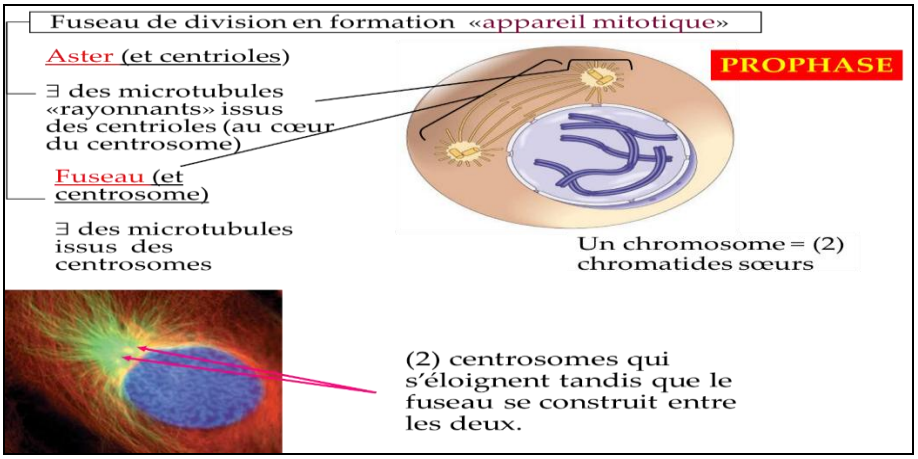


Figure 69 : Prophase of mitosis

2.1.2. Pre-metaphase

It begins with the sudden rupture of the nuclear envelope, which disperses into membrane vesicles that are indistinguishable from pieces of the endoplasmic reticulum. These vesicles remain visible around the spindle during mitosis. The spindle microtubules penetrate the nuclear region. Specialized protein complexes called kinetochores undergo maturation on each centromere and bind to certain spindle microtubules which are then called kinetochore microtubules. The remaining microtubules in the spindle are called polar microtubules, while those located outside the spindle are called astral microtubules. Kinetochore microtubules extend in opposite directions from the two sister chromatids of each chromosome, exerting tension on the chromosomes which are animated by a disordered movement (Fig. 70).

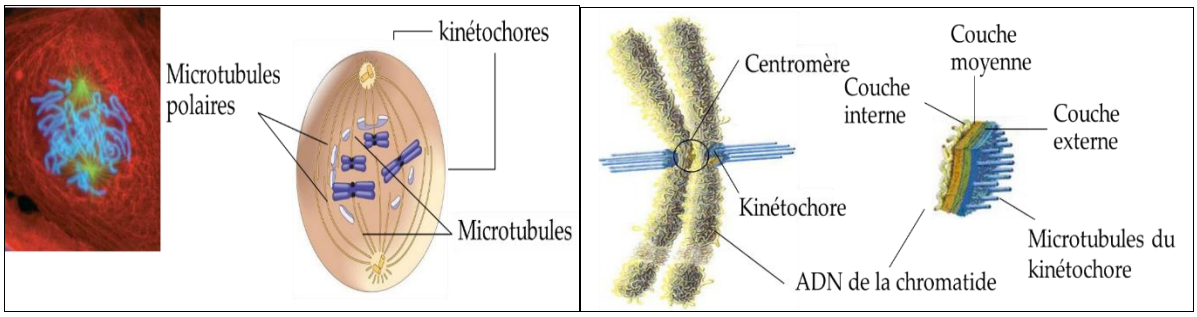


Figure 70 : Pre-metaphase of mitosis

2.1.3. Metaphase

The kinetochore microtubules finally align the chromosomes in a plane located in the middle of the cell called the equatorial plate (the chromosomes are located at the equator of the cell). Each chromosome is held under tension at this equatorial plate (Fig. 71).

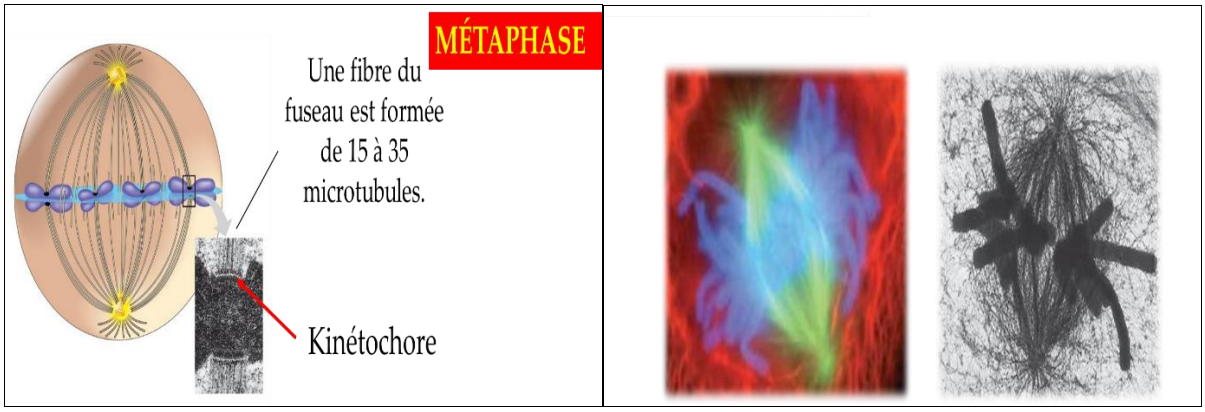


Figure 71: Metaphase of mitosis

2.1.4. Anaphase

Anaphase begins when the kinetochores separate after the centromere divides along its longitudinal axis, allowing each chromatid to be pulled towards one of the spindle poles it faces. Thus, the kinetochore microtubules shorten, causing each chromatid or daughter chromosome to move towards one of the cell's poles. The polar microtubules lengthen and the two poles of the spindle move further apart (Fig. 72).

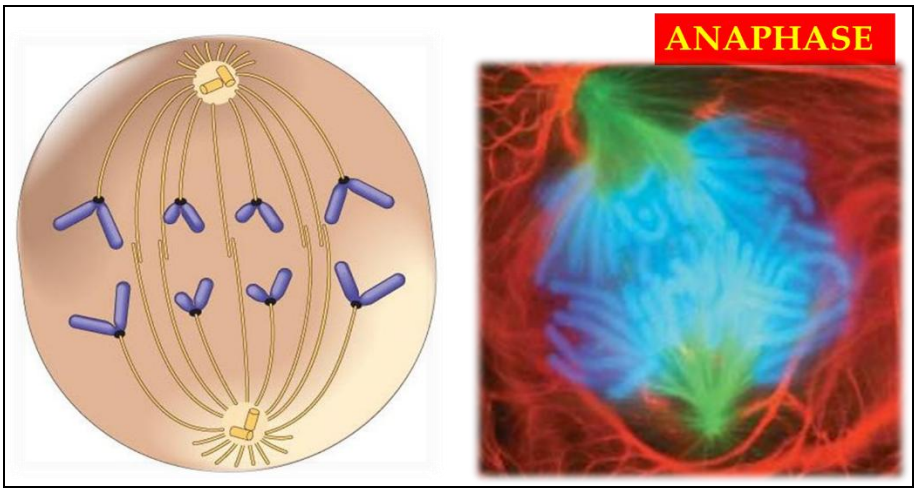


Figure 72: Anaphase of mitosis

2.1.5. Telophase

It begins with the cessation of chromosome migration, which regroup at the cell poles into a compact mass. Nuclear reconstruction begins. The chromosomes become less compact, they unwind. The nuclear envelope reconstitutes itself from the fragments that adhere to the chromosomes and from the endoplasmic reticulum which, during mitosis, appears in the form of vesicles located outside the spindle. The nucleoli reappear from the nucleolar organizers of certain chromosomes (Fig. 73).

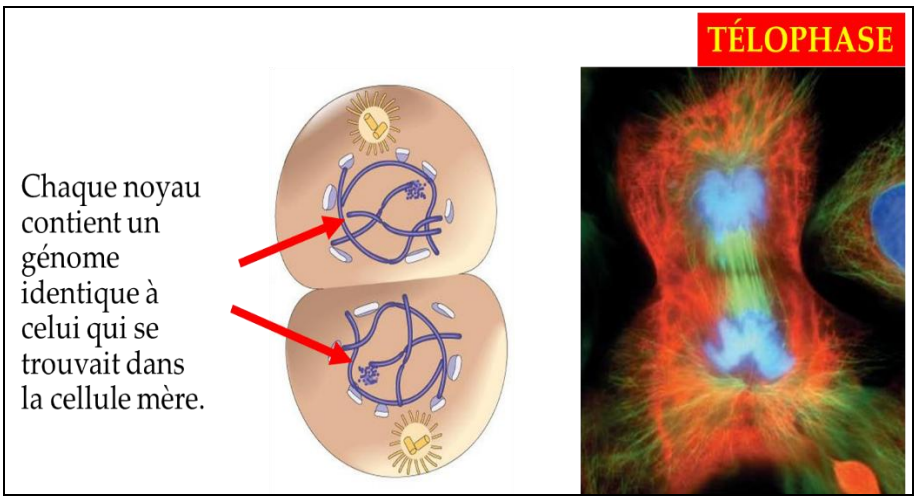


Figure 73: Telophase of mitosis

2.1.6. Cytokinesis

a) In animals

The cytoplasm divides by a process known as cleavage. The plasma membrane invaginates around the center of the cell perpendicular to the spindle axis, and between the two daughter nuclei to form the annular furrow which gradually deepens until the daughter

cells separate.

The cytoplasmic structures that were found at the end of prophase on the periphery of the cell spindle are randomly distributed in the daughter cells (Fig. 74).

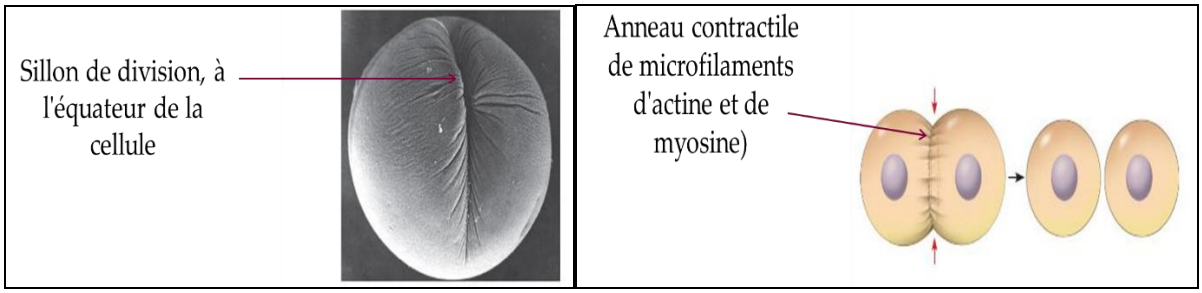


Figure 74: Cytokinesis during the division of an animal cell.

b) In plants

The majority of cells in higher plants are surrounded by a rigid cell wall and the cytokinesis mechanism is very different from the one we have just described for animal cells. The cytoplasm is divided inside the plant cell, by the development of a new cell wall between the two daughter cells, rather than by the pinching of the two daughter cells. In addition, cytokinesis occurs at the same time as telophase (Fig. 75).

The new transverse wall or cell plate begins to assemble in a plane located between the two daughter nuclei and in association with the residual polar microtubules of the spindle, which form a cylindrical structure called the **phragmoplast**. Small vesicles derived from the Golgi apparatus (filled with cell wall precursors in contact with the microtubules on each side of the phragmoplast) are transported along the microtubules until they reach the equatorial region. They fuse to form the membrane : **the early cell plate** (Fig. 75). The molecules that these vesicles release assemble inside the cell plate to form pectin, hemicellulose and other constituents of the primary cell wall. This wall extends laterally to reach the original cell wall by the same mechanism as before. Thus, we will have separation of the two daughter cells. Cellulose microfibrils are deposited inside the cell plate to complete the wall. There are elements of the endoplasmic reticulum that often remain trapped on either side of the plate. These are then transformed into plasmodesmata, the complex pores that cross the mature cell wall and interconnect the cytoplasms of all plant cells.

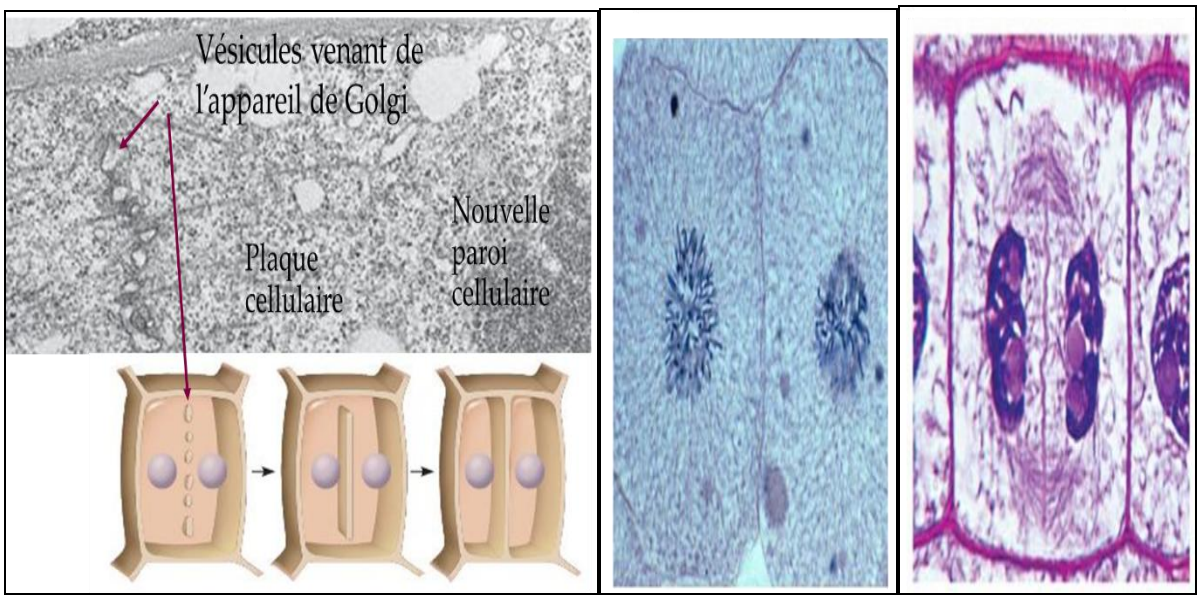


Figure 75: Cytokinesis during plant cell division.

2.2. Meiosis

Meiosis is the mechanism by which a diploid cell gives rise to four haploid daughter cells. It consists of two successive divisions, called :

- Meiosis I or reductional division, because it halves the number of chromosomes.
- Meiosis II or equational division, it is a mitotic division and resembles mitosis.

2.2.1. Meiosis I

2.2.1.1. Prophase I

The most complex phase is prophase I, which is itself subdivided into 5 stages : leptotene, zygotene, pachytene, diplotene and diakinesis (Fig. 76).

a) Leptotene stage

Begins when each chromosome passes from its interphase conformation to the state of long, thin and stretched filaments. Each chromosome is attached by its two ends to the nuclear envelope giving a particular structure called : attachment plaque. Although each chromosome is duplicated (during interphase) and consists of two sister chromatids, these chromatids are closely joined and cannot be observed at this stage (Fig. 76).

b) Zygotene stage

There is pairing of homologous chromosomes. It begins with a bringing together of the homologous ends of the two chromosomes at the nuclear envelope which progresses like a zipper, aligning the chromosomes opposite each other. In some cases the pairing process can

begin in internal regions of the chromosomes and progress towards the ends, producing the same type of alignment. Each gene would be juxtaposed with its homologous gene located on the opposite chromosome. Each resulting pair of chromosomes is called **a bivalent**, but since each homologous chromosome of the pair consists of 2 closely joined sister chromatids, we speak of **a tetrad** (Fig. 76).

c) Pachytene Stage

As soon as pairing is complete along the entire length of the chromosome, the cells are said to have entered pachytene. The chromosomes appear thicker. Indeed, chromosome condensation continues during this stage, the chromosomes continue to shorten and thicken (Fig. 76).

d) Diplotene Stage

The dissociation of the pairs marks the beginning of this stage, we can observe the duplication of each of the two chromosomes. The homologous chromosomes of each bivalent move away from each other at a certaine distance.

However, each bivalent remains connected at one or more chiasmata which show the sites where a crossing-over has taken place. Crossing-over is a phenomenon that takes place during pachytene or zygotene, but it is only during the diplotene stage that the consequences are observed. The formation of crossing-over constitutes one of the essential differences between meiosis and mitosis (where they occur only rarely) (Fig. 76).

e) Diakinesis

The cell immediately passes from the diplotene stage to diakinesis, a stage that transitions to metaphase, when the chromosomes condense, thicken and detach from the nuclear envelope. The four separate chromatids of each bivalent are clearly visible. The sister chromatids of each pair are connected by their centromeres, while the non-sister chromatids that have undergone crossing-over are connected by the chiasmata (Fig. 76).

Note : this prophase I differs from that of mitosis in that the homologous chromosomes pair up. Each chromosome pair is called a bivalent (or tetrad).

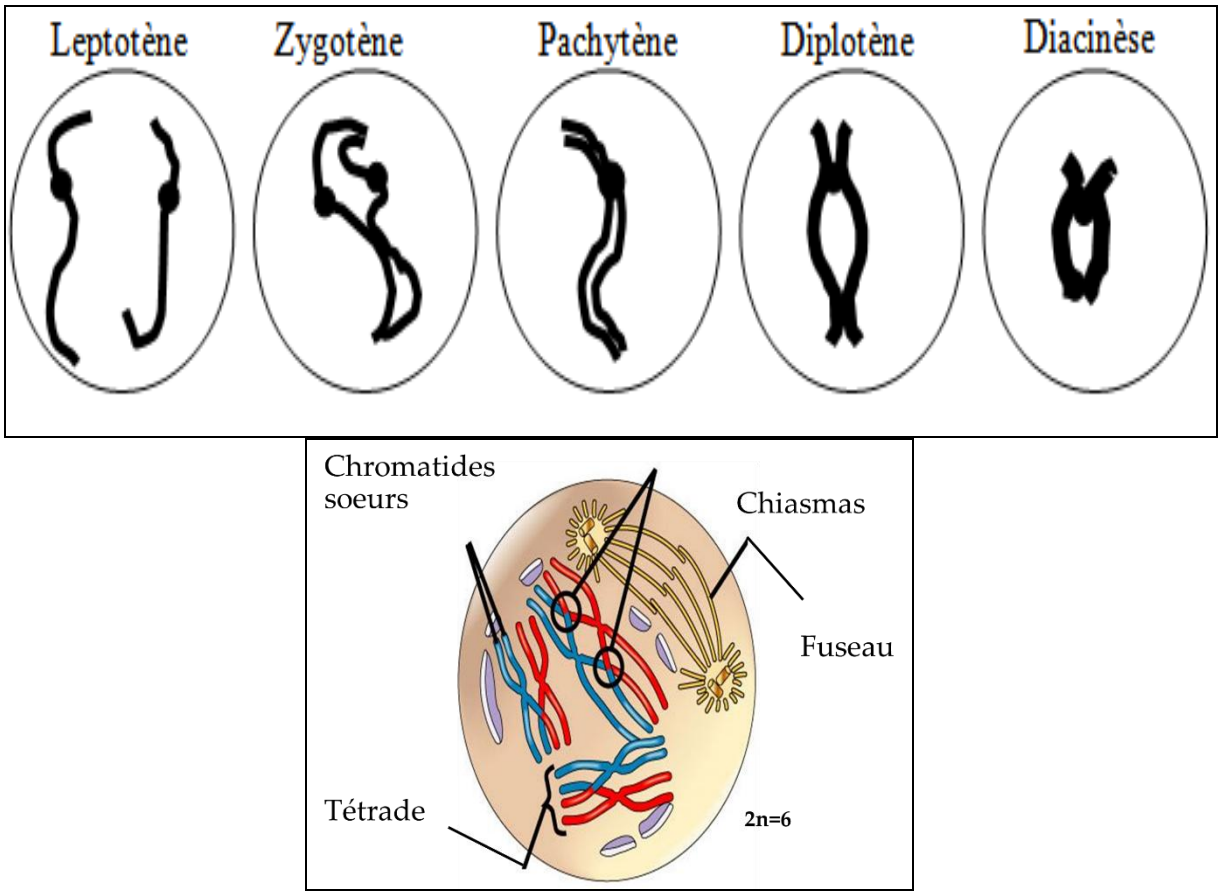


Figure 76: Prophase I of meiosis.

2.2.1.2. Metaphase I

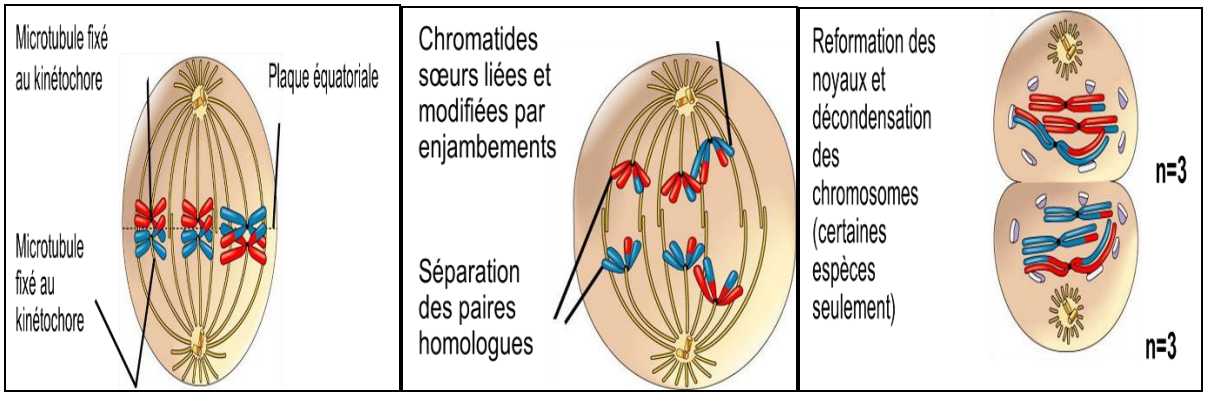
The nuclear membrane and nucleoli disappear and the spindle becomes clearly visible. The bivalents reach the equator of the cell to form an equatorial plate. The two centromeres of a homologous chromosome pair attach to fibers from opposite poles of the spindle. The two kinetochores of each centromere are oriented towards the same pole of the spindle (Fig. 77A).

2.2.1.3. Anaphase I

The two duplicated homologues (each consisting of two chromatids) separate from each other and move towards opposite poles. The centromeres do not divide during this phase. This absence of division constitutes another essential difference with mitosis. The chromatids remain united, so each centromere will carry two chromatids towards one or the other of the cell poles (Fig. 77B).

2.2.1.4. Telophase I

When the two groups of chromosomes reach the poles, a nuclear membrane forms around each one, the achromatic spindle disappears with the division of the mother cell into two ‘’n’’ chromosome or haploid daughter cells (Fig. 77C).



(A) **(B)** **(C)**

Figure 77: Metaphase I (A), Anaphase I (B) and Telophase I (C) of meiosis.

Note : The two divisions of meiosis are generally separated from each other by an interphase, during which the chromosomes transform into chromatin but do not duplicate ; This means that the duplication of chromosomes (S phase) is absent here.

2.2.2. Meiosis II

2.2.2.1. Prophase II

The chromosomes have a contracted appearance and their number corresponds to the haploid complement (Fig. 78).

2.2.2.2. Metaphase II

The chromosomes are positioned on the equator of the cells to form the equatorial plate. Often, at this stage, the chromatids partially separate from each other instead of remaining closely together as in mitosis (Fig. 78).

2.2.2.3. Anaphase II

The centromeres divide, releasing the sister chromatids which migrate towards the opposite poles of the cells, as in normal mitosis (Fig. 78).

2.2.2.4. Telophase II

Formation of the nuclear membrane around each group of chromosomes. Division of the rest of the cell or cytokinesis. This results in 4 haploid cells (Fig. 78).

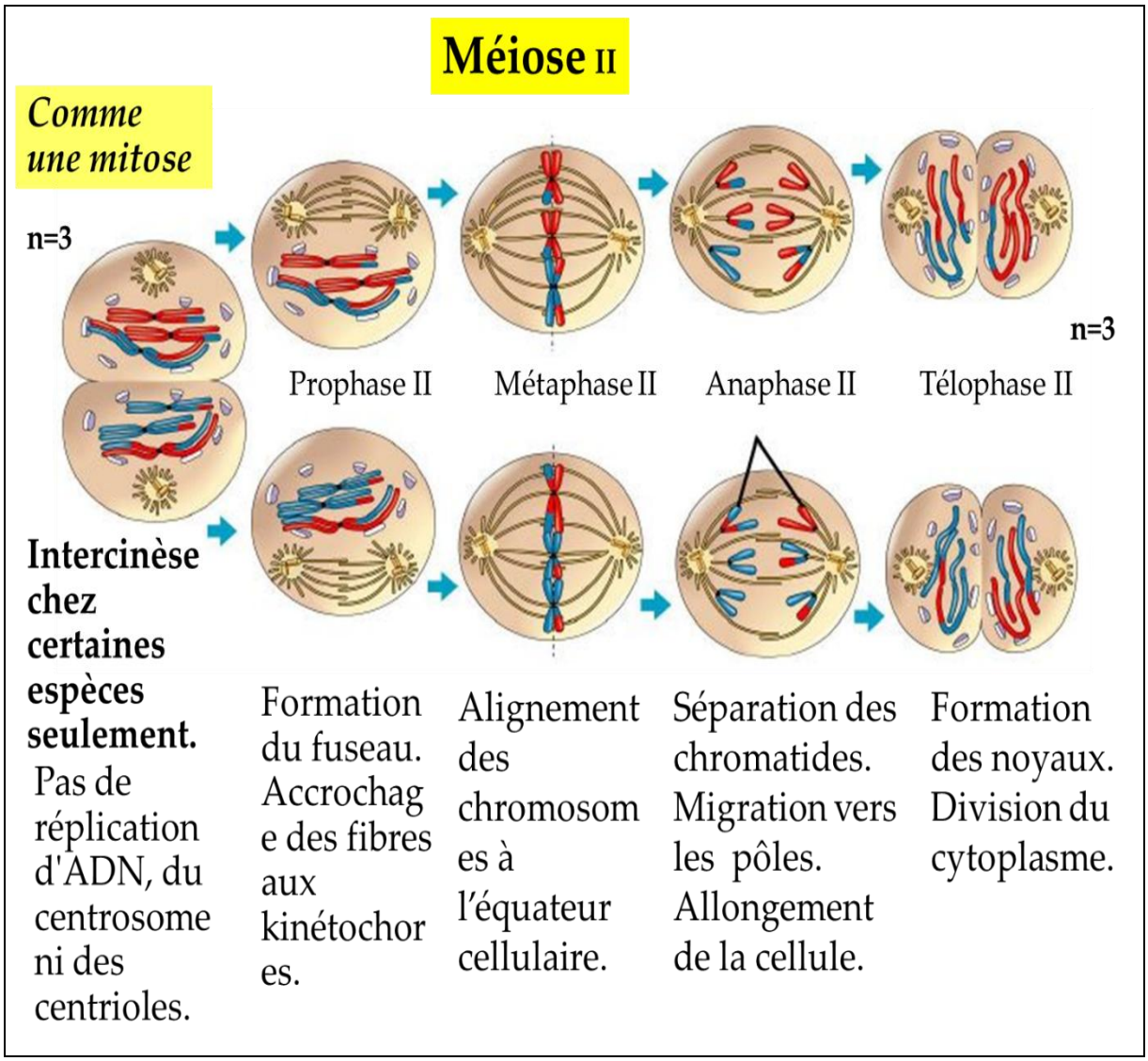


Figure 78: Stages of the second division of meiosis.

2.2.3. Consequences of meiosis

a) Interchromosomal shuffling

The random distribution of maternal and paternal homologous chromosomes between daughter cells during meiotic division I creates new chromosomal associations. Indeed, each gamete receives a different mixture of maternal and paternal chromosomes, this is interchromosomal shuffling.

Meiosis therefore achieves a mixing of the genes from each parent by the sole action of the random migration of chromosomes. By this process alone, an individual could in principle produce 2n genetically different gametes where « n » is the haploid number of chromosomes (Fig. 79).

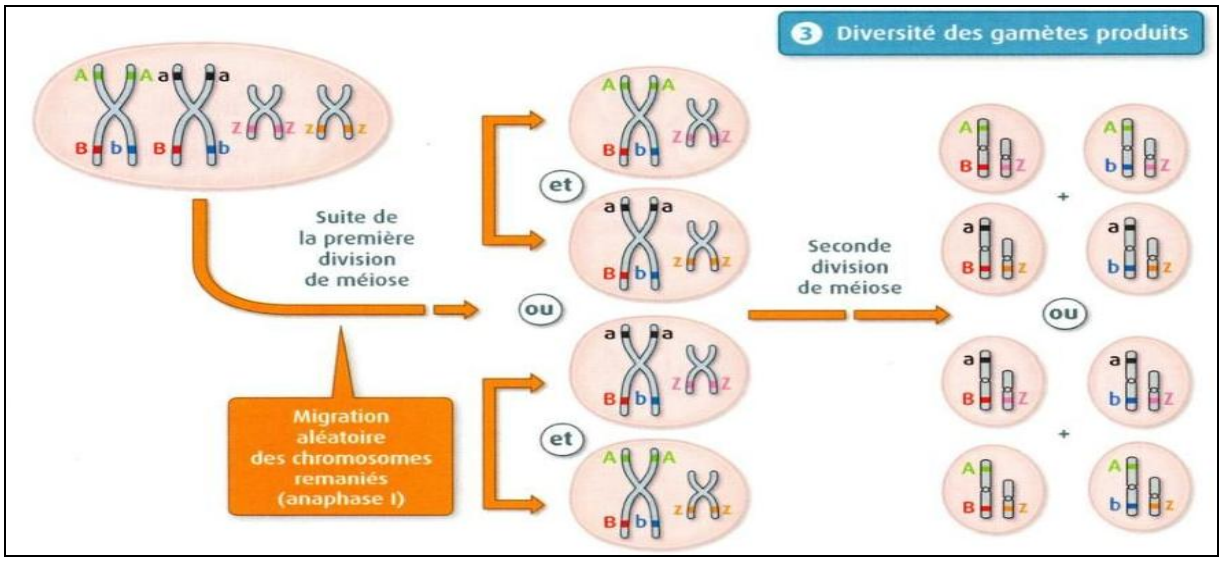


Figure 79: Interchromosomal shuffling

b) Intrachromosomal shuffling

These are crossovers that can occur at any point on the chromosomes. Crossing-over involves the breaking of 2 segments of non-sister chromatids and their cross-linking resulting in a recombination of genes. It takes place during the long meiotic prophase I (pachytene or zygotene). Recombination increases genetic variation by generating new gene associations (Fig. 80).

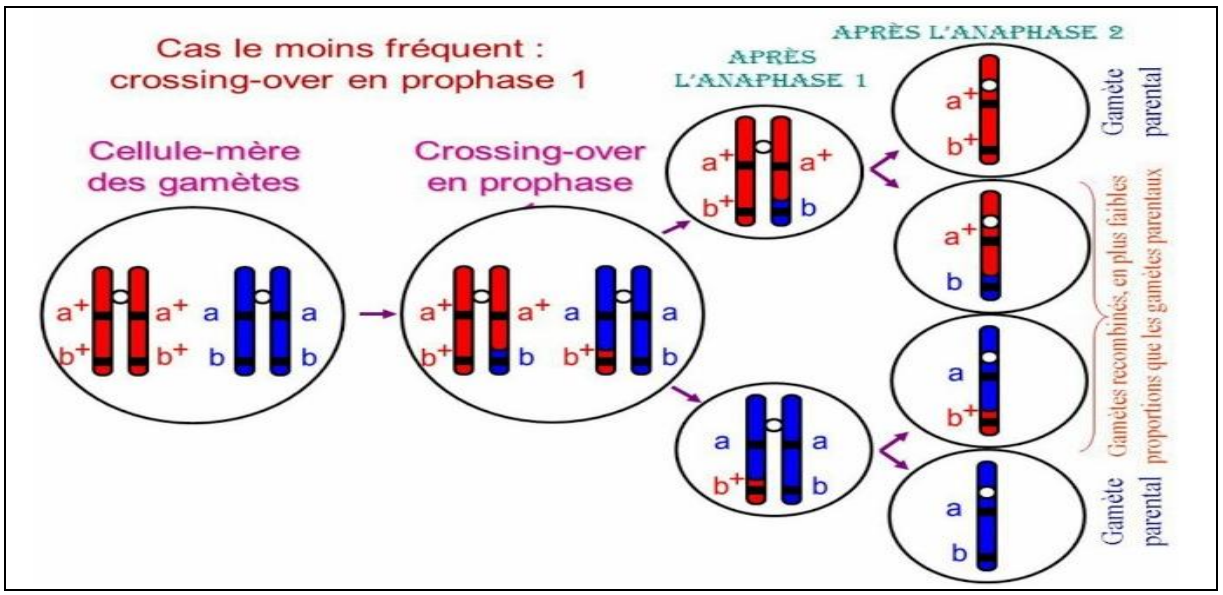


Figure 80: Intrachromosomal shuffling

Conclusion : Meiosis is a process that generates new genetic variability, notably by these two mechanisms : random distribution of chromosomes of paternal and maternal origin and modification of the structure of chromosomes by *crossing-over*.

3. Gametogenesis

Gametogenesis is the set of processes that transform initial germ cells or gonia into cells capable of being fertilized. These cells capable of being fertilized are called **gametes**. In animals, the term spermatogenesis specifically refers to gametogenesis in the male sex and the term oogenesis refers to that which occurs in the female sex. In plants, on the other hand, the corresponding terms are microsporogenesis (male) and megasporogenesis (female).

3.1. In animals

In all vertebrate embryos, certain cells are chosen very early in development as gamete precursors. These primordial germ cells migrate to the developing gonads (ovaries in females and testes in males), after the period of mitotic proliferation, they undergo meiosis and differentiate into mature gametes : eggs and sperm.

3.1.1. Spermatogenesis

This is the set of processes that transform the initial germ cell or spermatogonium, diploid, into a highly specialized cell, the spermatozoa, haploid and capable of fertilization. Spermatogenesis takes place in the seminiferous tubules of the testicle. It takes place in several stages :

Multiplication of spermatogonia, which allows the number of stem germ cells to be conserved. The spermatogonia undergo a growth phase and will be designated as diploid primary spermatocytes (Fig. 81).

Meiosis, which will cause the cells to pass from the diploid stage to the haploid stage. Thus, the primary spermatocytes divide by meiosis (Fig. 81):

- ✓ In meiosis I, 2 haploid secondary spermatocytes are obtained.
- ✓ In meiosis II, the latter will give 4 haploid spermatids.

Differentiation into sperm : the spermatids undergo maturation and give rise to spermatozoa. Cells with a very reduced cytoplasm possessing a long whip-like tail. Thus, each spermatid, a rounded cell with normal cytoplasm, will then gradually differentiate during spermiogenesis into a small flagellated cell with reduced cytoplasm (differentiation of a flagellum, elimination of most of the cytoplasm and reorganization of cytoplasmic organelles).

3.1.2. Oogenesis

It takes place in the ovaries from stem cells (oogonia). In the female fetus, the

oogonia, diploid germ cells of the ovaries, multiply rapidly by mitosis. In women, for example, when the female fetus is 5 and a half months old, its two ovaries contain about 7 million oogonia. The oogonia enter a period of growth and begin meiosis :

- ✓ ***The first meiotic division*** begins, the oogonia transform into primary oocytes, and will be blocked at this stage towards the end of prophase I (Fig. 81). Many primary oocytes degenerate before birth. Those that remain occupy the cortical region of the immature ovary. At birth, a female already has a predetermined stock of primary oocytes. Unlike males who have a continuous production. These primary oocytes remain at this stage immobilized in prophase I for a period that can range from a few days to several years, depending on the species. The next phase of oocyte development is called oocyte maturation, and generally does not begin until sexual maturity (in women, at puberty). The primary oocyte is activated and begins to grow each month and continues meiosis I. It will give 2 haploid cells of unequal size, a small one called the first polar body, and a large one, the secondary oocyte.
- ✓ ***In meiosis II***: the first polar body usually produces 2 polar bodies even smaller than itself and the secondary oocyte divides again asymmetrically to give the mature egg and a second small polar body (both are haploid). In most vertebrates, oocyte maturation continues until metaphase of meiosis II and then stops ; it is this cell and not a functional egg that is expelled at the time of ovulation. If no sperm penetrates the secondary oocyte, it degenerates. On the other hand, if a sperm penetrates, the secondary oocyte completes meiosis II. This results in a large egg and a tiny second polar body.

What you need to know is that oogenesis generally produces 3 tiny polar bodies with almost no cytoplasm and one large egg. All these cells are haploid, but only one is functional (Fig. 81).

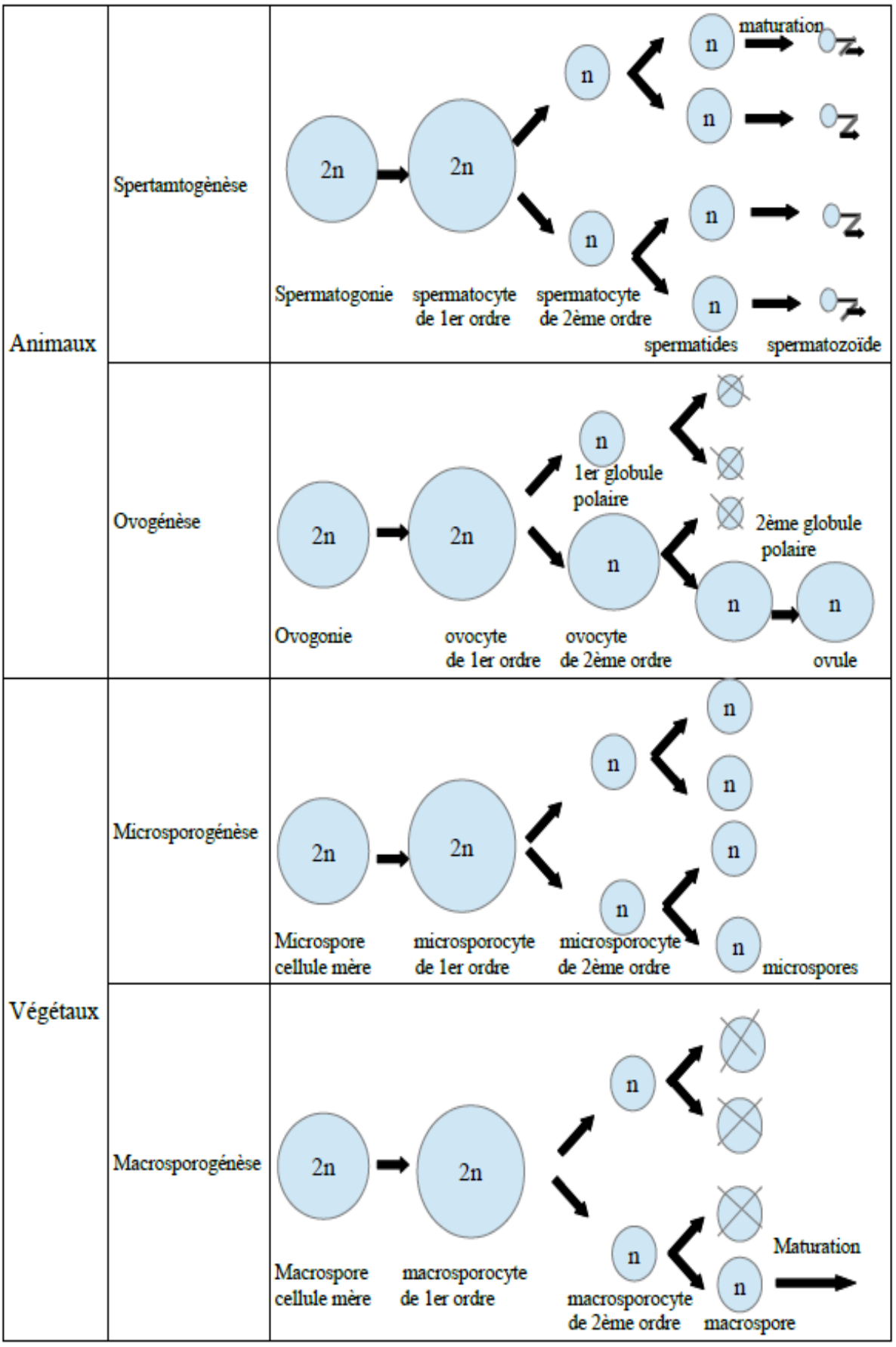


Figure 81: Gametogenesis in higher animals and flowering plants.

3.2. In plants (angiosperms)

3.2.1. Microsporogenesis

The diploid mother cells of the microspores are located in the anthers. These diploid mother cells undergo a growth phase and will be called primary microsporocytes. These cells undergo meiosis (Fig. 81) :

After meiosis I, a primary microsporocyte gives 2 haploid cells, these are the microsporocytes II.

After meiosis II, 4 haploid microspores are obtained: the microspore undergoes a first mitosis which ends with the production of 2 cells of unequal volume, a small one called the spermatogenic cell and a large one called the vegetative cell (the 1st bathes in the cytoplasm of the 2nd). The pollen grain is blocked at the 2 haploid cell stage (Fig. 82).

Note : during germination, the vegetative cell does not divide, but the spermatogenic cell undergoes a second mitosis which determines the formation of 2 male gametes. The whole forms the male gametophyte.

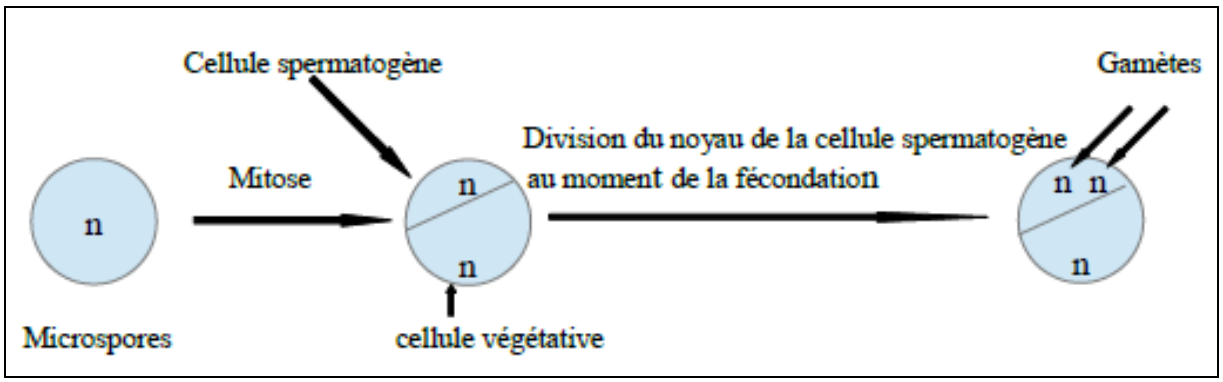


Figure 82: Genesis of the male gametophyte

3.2.2. Macrosporogenesis (megasporogenesis)

In the ovary, a diploid cell : **megasporocyte** or **macrosporocyte**, undergoes meiosis and gives 4 haploid cells: these are the **megaspores** or **macrospores**, 3 of which degenerate (Fig. 20). The surviving megaspore undergoes 3 successive mitoses without division of the cytoplasm and results in a giant cell with eight haploid nuclei or **embryonic sac**. The embryonic sac is surrounded by the integuments. At one end of this sac there is an opening : **the micropyle** through which the pollen tube penetrates. On the micropyle side, 3 nuclei are grouped together, two of which are called **synergids** (which degenerate after fertilization) and the 3rd located in the center is the only one that can be fertilized, develops to give the **oosphere**. On the opposite side, 3 nuclei (antipodes) which also degenerate. In the middle

there are **2 polar nuclei**, these are diploid nuclei (Fig. 83).

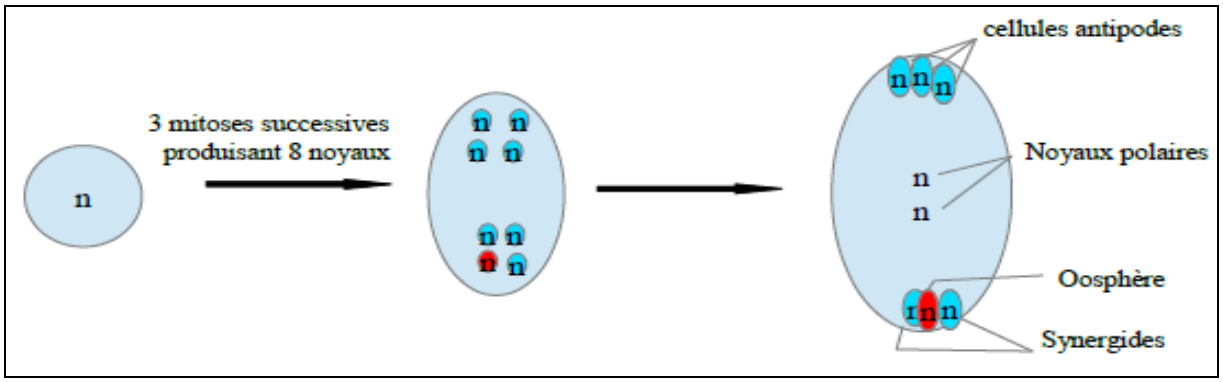


Figure 83: Genesis of the female gametophyte

4. Fertilization

4.1. In animals

Once released, the sperm, like the egg, are destined to die in a few minutes or a few hours if they do not meet to fuse during fertilization. Thanks to fertilization, the egg and the sperm are saved from degeneration. Thus, fertilization is the union of a female gamete with a male gamete (sperm). Only the head of the sperm penetrates the egg, the tail remains outside and degenerates. Once fertilized, the egg is called a zygote and has 2n chromosomes. The latter undergoes successive mitoses giving many cells which organize themselves to form the tissues and organs of the new individual.

4.2. In plants (double fertilization)

Pollen grains from the anthers are carried by the wind or by insects to the stigma.

Upon contact with the stigma, the pollen grain hydrates and germinates, producing a pollen tube (it is the vegetative nucleus that allows it to germinate) which penetrates the style. In the pollen tube, the vegetative nucleus descends first, followed by the 2 sperm nuclei. The pollen tube grows and reaches the embryonic sac through the micropyle. The vegetative nucleus degenerates and the two sperm nuclei are expelled into the embryonic sac. One of the sperm nuclei fuses with the oosphere giving a diploid zygote which will develop into an embryo. The other sperm nucleus fuses with the polar nuclei to give a triploid nucleus (3n) which by successive mitosis will form the endosperm (Fig. 84A; Fig. 84B).

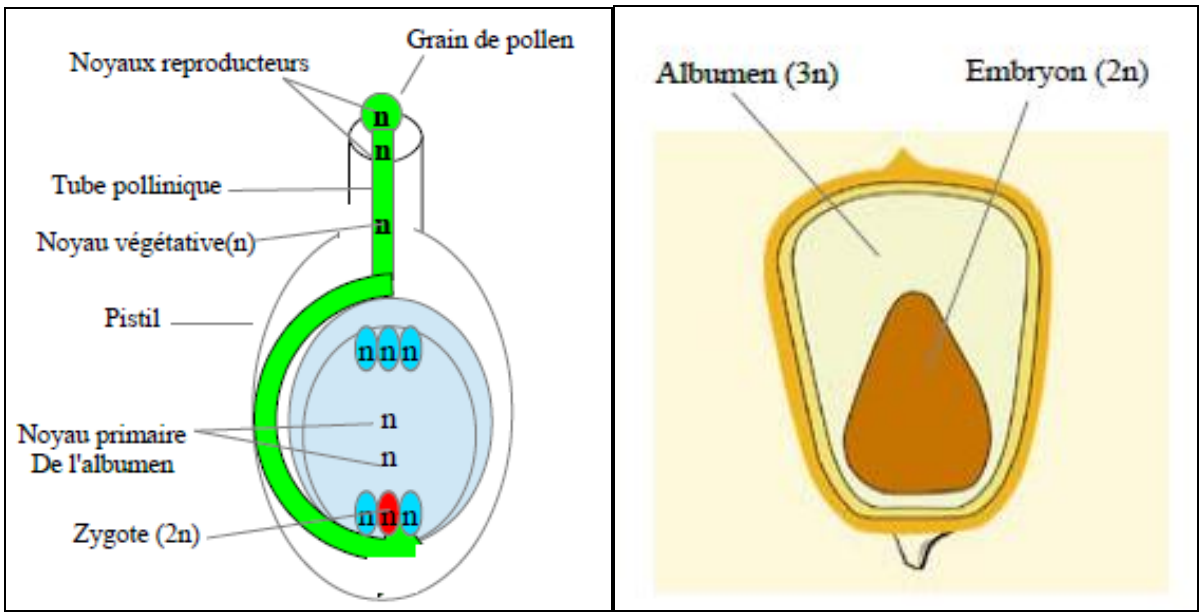


Figure 84: Double fertilization phenomenon in flowering plants (A), and Diagram of a corn seed showing an embryo (2n) and an endosperm (3n) (B)

4.3. Consequences of fertilization

Fertilization is the fusion of two haploid gametes, paternal and maternal, allowing the formation of a new individual with new combinations. In this process, genomes are shuffled and recombined to produce individuals that possess new combinations of genes. The advantage provided by fertilization is genetic diversity.

5. Genetic Material

Nucleic acids are chemical substances that exist not only in the nucleus, but also in the cytoplasm of cells. There are 2 types :

- ✓ **DNA :** deoxyribonucleic acid, is mainly located in the nucleus of cells in eukaryotes and in certain cellular organelles such as chloroplasts and mitochondria. On the other hand, in prokaryotes, DNA floats directly in the cytoplasm.
- ✓ **RNA :** ribonucleic acid : mainly found in the cytoplasm of cells.

5.1. Composition

Nucleic acids are very long molecules formed by the repetition of subunits called nucleotides. A nucleotide itself is formed of three elements : Base, Phosphoric Acid, and Ose (sugar).

5.1.1. Bases

There are two possible types of bases (Figures 85 and 86) :

a) **Pyrimidine bases :** represented by thymine (T), cytosine (C), and uracil (U).

b) **Purine bases :** all have a purine nucleus and are represented by adenine (A) and guanine (G).

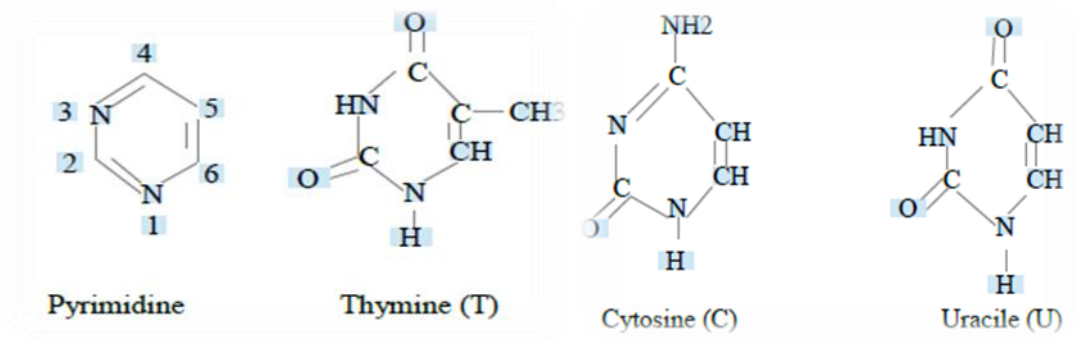


Figure 85: The pyrimidine base nucleus and the 3 pyrimidine bases

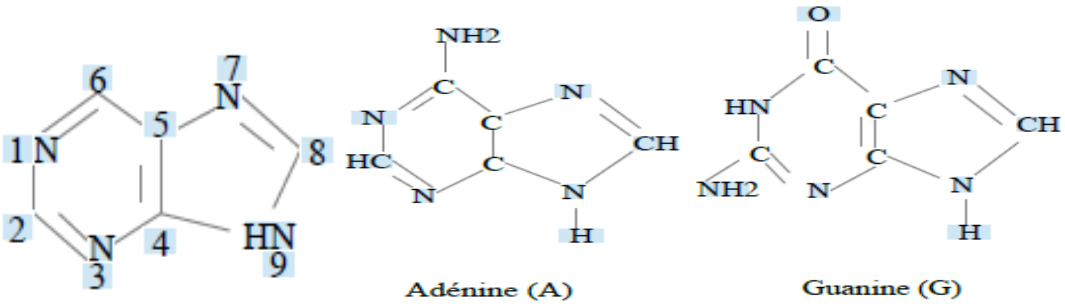


Figure 86: The purine base nucleus and the 2 purine bases

5.1.2. Ose (Sugar)

There are two types of oses in nucleic acids (Figure 87) :

Ribose : it is a 5-carbon ose.

Deoxyribose : it is also a 5-carbon ose but with one oxygen molecule missing compared to ribose.

5.1.3. Phosphoric Acid

It is a triacid ; two of the three acid functions are esterified in DNA or RNA.

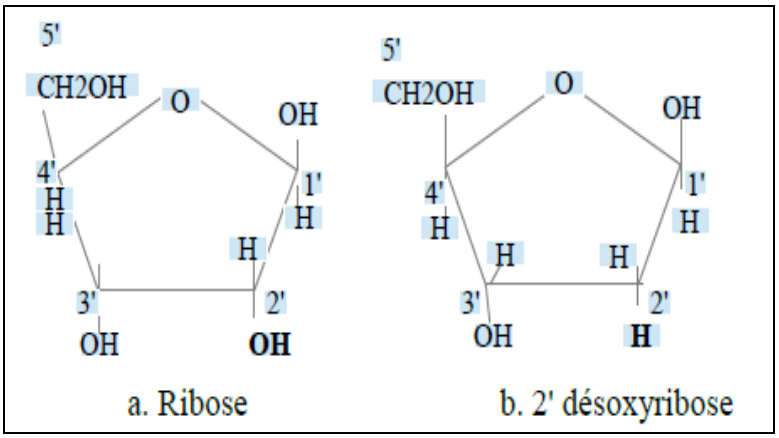


Figure 87: The two types of oses in nucleic acids

A nucleic acid chain has two ends :

- ✓ One containing the phosphate group with 2 free acid functions is called the 5'p end.
- ✓ The other containing a free OH in 3' on the ose, is called the 3'-OH end.

A nucleic acid chain is always read in the 5'p to 3'OH direction.

5.2. Characteristics of DNA

Three characteristics are specific to DNA and differentiate it from RNA :

- ➢ **Ose :** it is deoxyribose for DNA and ribose for RNA.
- ➢ **Bases :** DNA contains 4 bases which are A, C, G, and T, while in RNA, the base T is replaced by the base U.
- ➢ **The two nucleotide chains**, whereas RNA is formed of a single strand (chain) of nucleotides. These two DNA chains have 3 properties :
  - ✓ **Antiparallel :** means that the 2 DNA strands are parallel but in opposite directions, from 5'p to 3'OH for one strand and the other from 3' to 5'.
  - ✓ **Complementary :** the rule of complementarity is as follows : opposite A is T with two hydrogen bonds and opposite C is G with 3 hydrogen bonds. The ratio **A+G/T+C=1**, so there are as many A as T and as many C as G.
  - ✓ **Helical :** this equal distribution of A and T on the one hand, and C and G on the other hand, therefore implies a particular structure of DNA in a double helix.

6. The Phenomenon of Replication

Replication is the duplication of DNA after cell division.

6.1. Characteristics of Replication

Replication is said to be semi-conservative. This means that on the two strands of any DNA molecule, there is always : an old DNA strand that comes from one of the 2 parental DNA strands, and a new, newly formed strand (Fig. 88).

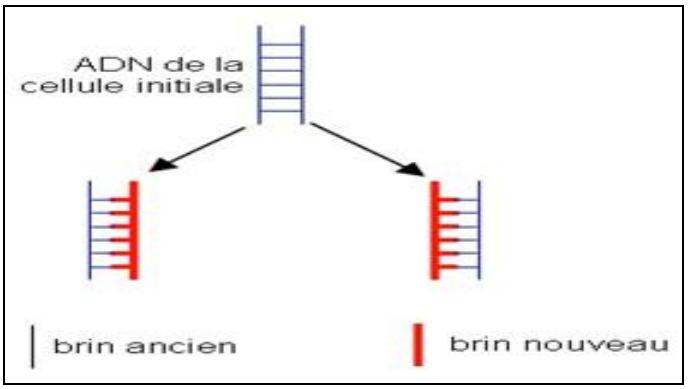


Figure 88: Semi-conservative replication

6.2. Elements Necessary for Replication

- **Parental DNA:** Replication always starts from a DNA template called template DNA.
- **Nucleotides:** Since DNA is composed of nucleotides, the presence of deoxyribose, the four bases (A, G, C, T) in triphosphate form ATP, GTP, CTP, TTP is necessary.
- **Enzymes**
  - ✓ ***DNA helicase:*** its role is to separate the two DNA strands after hydrolysis of the hydrogen bonds.
  - ✓ ***RNA polymerase:*** its role is to synthesize a first RNA primer and form the new 5' end.
  - ✓ ***DNA polymerase III:*** its role is to lengthen the new DNA strand.
  - ✓ ***DNA polymerase I:*** its role is to hydrolyze the RNA primers and replace them with DNA.
  - ✓ ***DNA ligase:*** its role is to join the DNA primers together.
  - ✓ ***DNA Telomerase:*** exists only in Eukaryotes. Its role is to replicate the linear ends of DNA.

6.3. The Mechanism of Replication (in general) :

Replication begins at a specific point on the chromosome called the « Origin of Replication» or « initiation point». Replication occurs in the 5'→3' direction in a complementary and antiparallel manner.

The progression of replication involves the unwinding of the parental double helix with the intervention of : DNA helicase and SSB proteins (**S**ingle **S**trand **B**anding Protein) which bind the DNA strands and prevent them from re-spiraling.

RNA polymerase or primase : capable of starting a nucleic acid chain (DNA or RNA) by coupling 4 to 12 RNA nucleotides. DNA polymerase III will then take over, lengthening the primer but with DNA this time.

Note : replication is continuous for one strand, it is called the « leading» or « advanced» strand, and discontinuous for the other strand which is called « lagging».

Replication for the discontinuous strand is done by the successive addition of small DNA fragments, called « OKAZAKI Fragments» which bind to the primer formed by the primase. These fragments are synthesized in the opposite direction of the general direction of progression (antiparallel). The RNA primers will then be destroyed, hydrolyzed and replaced by DNA thanks to the action of DNA polymerase I. Finally, the DNA fragments are joined together by DNA ligase (Fig. 89).

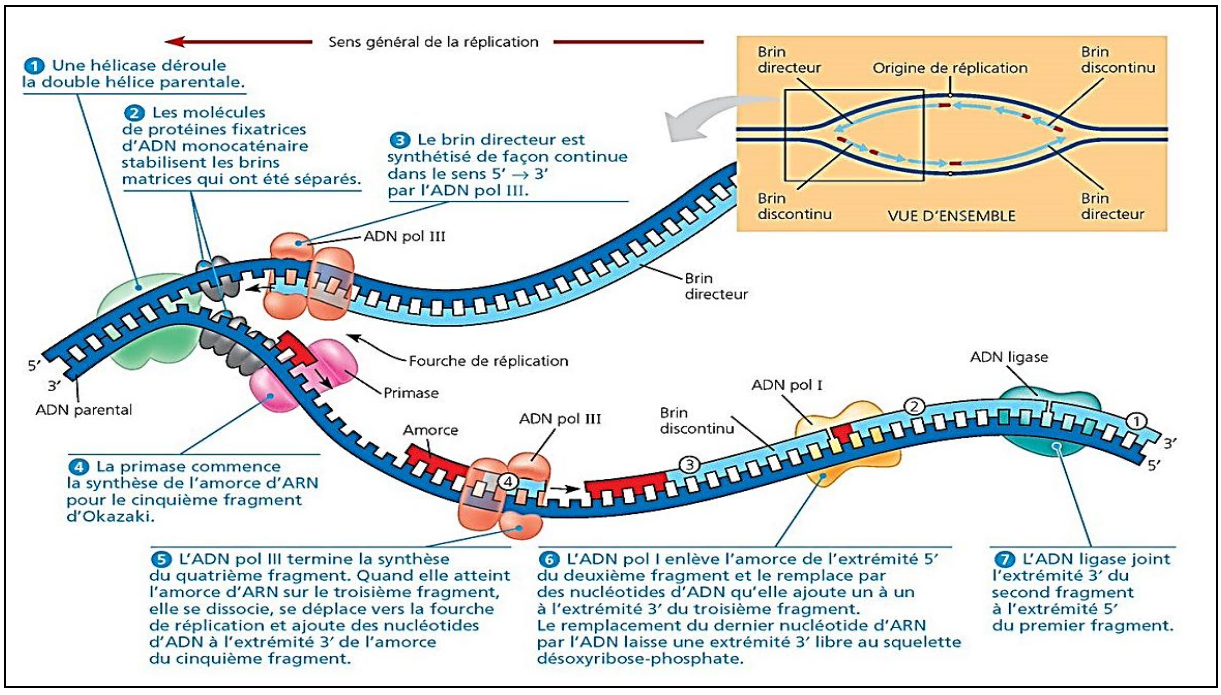


Figure 89: Mechanism of replication

The differences that exist between eukaryotic and prokaryotic replication are illustrated in the following table :

Table 14 : Differences between eukaryotic and prokaryotic replication.

Prokaryotes	Eukaryotes
Replication begins at 1 initiation point.	There are multiple origins called "replicons" and replication starts in multiple places at the same time because the DNA chain is too long.
OKAZAKI fragments are 1000 to 2000 bases in size.	OKAZAKI fragments are 100 to 200 bases in size.
The chromosomes of prokaryotes are circular, replication begins at a single point and progresses in a simple way to reach the starting point.	The chromosomes of eukaryotes are linear. Their ends cannot be completelyreplicated by discontinuous replication. It requires a new enzyme (DNA telomerase) to replicate these ends.

7. Protein Synthesis

Protein synthesis takes place in two main steps : transcription and translation.

7.1. Transcription

Transcription is the phenomenon by which RNAs are synthesized. Three classes of RNA are produced by transcription of DNA in both prokaryotes and eukaryotes. These classes are represented by :

- **mRNA :** carrying the message from DNA that is intended to be translated into proteins at the ribosomes.
- **rRNA :** which, associated with proteins, forms ribosomes, particles on which proteins are synthesized.
- **tRNA :** which carries amino acids to the ribosomes and chooses the location that each must occupy.

The transcription of mRNA is followed by translation (translated into proteins), however, tRNA and rRNA are not.

Notes :

- ✓ Not all DNA is transcribed, but only certain portions of DNA called «genes» (Fig. 90).
- ✓ Only one of the two DNA strands is copied, but it is not always the same strand.
- ✓ RNA is produced using the template strand also called the positive sense strand or coding strand.
- ✓ The synthesis of an mRNA takes place in the 5'→3' direction in an antiparallel manner with respect to the transcribed DNA strand and in a complementary manner.

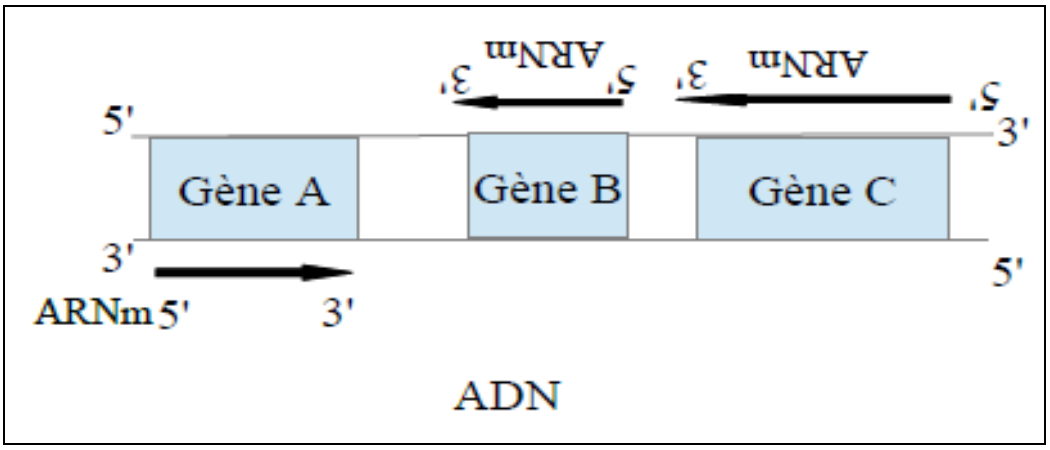


Figure 90: DNA transcription: only one of the 2 DNA strands is transcribed

7.1.1. Elements Necessary for Transcription

- ➢ **Nucleotides :** they must contain the bases A, C, G and U in active form or in triphosphate form : ATP, CTP, GTP and UTP.
- ➢ **RNA polymerase :** the enzyme that allows nucleotides to be joined together to form mRNA.
- ➢ **DNA template :** essential for making an mRNA, to have a DNA template.

7.1.2. Steps of transcription

Transcription is divided into 3 phases: initiation, elongation and termination. However, there are some differences between eukaryotes and prokaryotes.

a) Initiation

Transcription cannot start randomly, but must be limited to the beginning of a gene. The start signal for transcription is « the promoter ». RNA polymerase can recognize these promoters where it will bind to initiate transcription. The following table shows the

differences in the initiation of transcription between eukaryotes and prokaryotes.

Table 15 : Differences between the initiation of transcription in eukaryotes and prokaryotes.

Prokaryotes	Eukaryotes
Transcription start signal	
There are two promoters : • **Sequence « -35 »** : located approximately 35 nucleotide pairs before the transcription start point. It is represented by TTGACA. • **Sequence « -10 »** : Located approximately 10 nucleotide pairs before the transcription start point. Represented by TATATT.	There are three promoters : • TATA box. • CCAAT box. • GC box. These promoters are located approximately 100 nucleotide pairs before the transcription start point.

b) Elongation

During elongation, RNA polymerase moves along the DNA molecule, breaking hydrogen bonds and unwinding the double helix as it progresses. The enzyme adds ribonucleotides to the 3' end of the DNA molecule with an order of addition determined by the order of the bases on the template strand.

c) Termination : end of transcription

Transcription ends non-randomly at specific points located after the end of the coding sequence. The following table shows the differences in the termination of transcription between eukaryotes and prokaryotes.

Table 16: Differences between the termination of transcription in eukaryotes and prokaryotes.

Prokaryotes	Eukaryotes
End of transcription signal	
Termination occurs at sequences called **palindromes**, which are symmetrical about their midpoints, so that the first half of the sequence is followed by its exact complement in the second half. The first half pairs with the second half to form a so-called hairpin structure, which is thought to be responsible for stopping transcription.	The gene end signal is the sequence read on the untranscribed DNA strand called the "sense" strand : **AATAAA**, a sequence called the **"end signalpolyadenylation".** RNA polymerase recognizes this signal on the DNA but continues to transcribe beyond it. Transcripts will end with the signal AAUAAA followed by a certain number of nucleotides.

mRNA Maturation

The transcribed mRNA must go through 3 maturation steps before passing into the cytoplasm. These steps are (Fig. 91) :

- Capping : is the formation of a Cap at the 5' end to protect it ;
- Polyadenylation : is the formation of a tail at the 3' end made up of approximately 250 Adenine nucleotides. Its role is to protect the 3' end ;

In eukaryotes, mRNAs are produced by transcription of protein-coding genes by RNA polymerase II. However, eukaryotic genes have a discontinuous structure. Indeed, a gene includes :

  - ✓ Exons that contain the (hereditary) information and which will generally be expressed (by being translated into proteins).
  - ✓ Introns (or intervening sequences), which are interspersed in the middle of the information-containing part. They will be transcribed but will not be translated (the role of these DNA sequences that are not expressed is still unknown). "They can be represented as the advertisement that interrupts a film".

- The mRNAs produced by transcription of the intron and exon sequences are called pre-mRNA (precursor mRNA) or primary transcript. Before being translated into protein, the pre-mRNA undergoes a series of maturation events that transform it into a mature mRNA. Maturation obviously takes place in the nucleus. It is during maturation (called «processing» by Anglo-Americans, which means « treatment », « transformation ») that the pre-mRNA will undergo excision-splicing to finally give the mRNA.

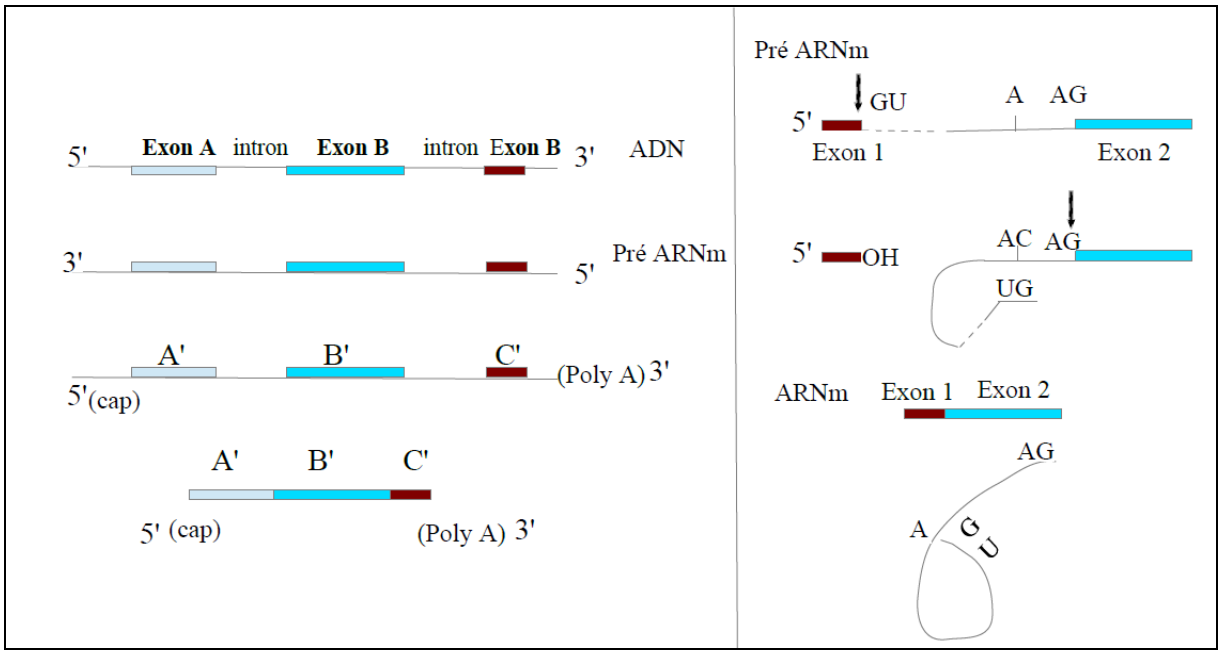


Figure 91: mRNA Maturation

7.2. Translation

Translation is the mechanism by which mRNA is decoded. It is the second step in protein synthesis. It follows the transcription of DNA into mRNA. For any translation, we need a dictionary, this dictionary is the genetic code.

7.2.1. The Genetic Code (3-letter code)

The only variable part of an mRNA is the nucleobases. There are 4 different bases AUGC and 20 different amino acids.... So how can 4 bases code for 20 amino acids ?

A 3-letter code means that 3 nucleotides (a set also called a triplet) or codon carried on the mRNA will be translated to position an A.A. There are 64 codons available and out of the 64 codons we find (Fig. 92):

3 codons (UAA, UAG, UGA) are nonsense codons that cannot be translated into an amino acid. These codons are in fact stop signals and are called « stop codons ».

There are 61 codons left for 20 amino acids. Apart from 2 cases, Met and Trp, coded by a single codon, the other 18 are coded by several codons from 2 to 6 (e.g.: The 6 codons of Leu.).

Characteristics of the Genetic Code

- **Universal :** the code is the same in all living organisms, whether it is an animal, a plant, a bacterium or a virus. Which is extraordinary !!!

- **Degenerate (degeneracy on the 3rd base) :** the code is said to be degenerate because the same AA can be coded by several different codons. It should be noted that in most cases the triplets coding for the same AA differ from each other only by the third base.

1 ère base	2ème base				3ème base
	U	C	A	G	
U	UUU, UUC } Phe UUA, UUG } Leu	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr UAA, UAG } Stop	UGU, UGC } Cys UGA } stop UGG } Trp	U C A G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His CAA, CAG } Gln	CGU, CGC, CGA, CGG } Arg	U C A G
A	AUU, AUC, AUA } Ile AUG } Met	ACU, ACC, ACA, ACG } Thr	AAU, AAC } Asn AAA, AAG } Lys	AGU, AGC } Ser AGA, AGG } Arg	U C A G
G	GUU, GUC, GUA, GUG } Val	GCU, GCC, GCA, GCG } Ala	GAU, GAC } Asp GAA, GAG } Glu	GGU, GGC, GGA, GGG } Gly	U C A G

Figure 92: The Genetic Code

7.2.2. Location of Translation

Translation takes place in the cytoplasm at the ribosomes.

7.2.3. Elements Necessary for Translation

- **Amino acids :** the peptide chain is made up of a succession of several AAs linked to each other by amide bonds.
- **mRNA :** the sequence of AAs in a peptide chain must be in a certain order. This can be achieved thanks to mRNAs.
- **tRNA :** is indeed linked on one side by its 3' CCA end to the amino acid and on the other

side by its anticodon to the mRNA (weak hydrogen bond between the complementary bases of the anticodon and codon). The AAs in the cytoplasm will not arrive free on the ribosome, but bound to the tRNA that carries them (aminoacyl-tRNA) (Fig. 93).

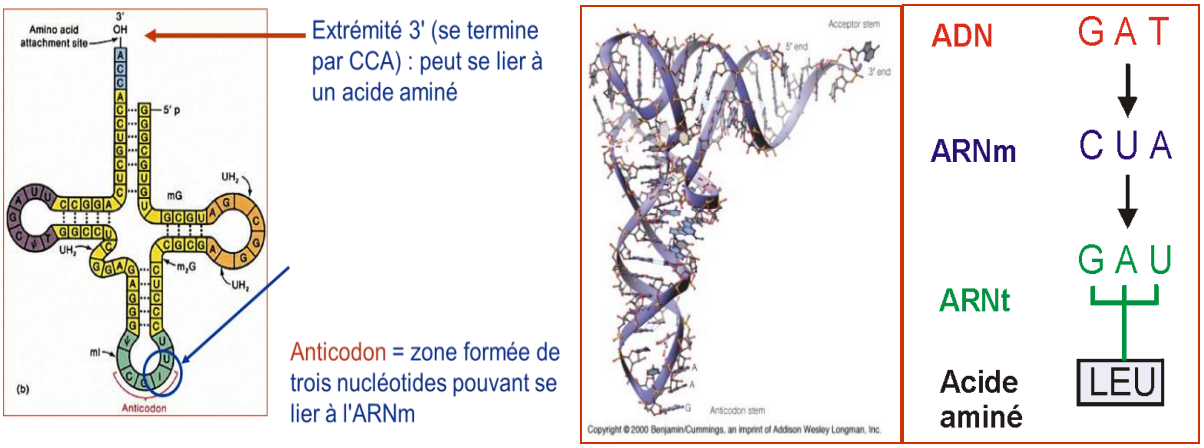


Figure 93: tRNA

7.2.4. The Different Stages of Translation

There are three main stages of translation :

a) Initiation

Just before translation begins, the ribosome is not formed. The 2 ribosomal subunits are indeed dissociated and free in the cytoplasm. At the initiation phase, the small subunit binds to the mRNA at **a specific point** (table 17) located upstream of AUG. Once bound, the small subunit migrates along the mRNA until it encounters the AUG. Then, before the large subunit binds, the tRNA takes its place and then the large subunit settles with the first amino acid, thus allowing the initiation of translation. In eukaryotes, AUG codes for methionine (Fig. 94A). Whereas, this amino acid is linked to formic acid in prokaryotes and methionine is therefore formylated (f-methionine) (Fig. 94B).

Table 17: Specific points (recognition sites) of translation initiation in prokaryotes and eukaryotes.

Prokaryotes	**Eukaryotes**
Translation start signal	
The Shine Dalgarno sequence (5'AGGAGGU3').	The Cape

Note : Ribosomes have two sites :

A site (amino acid site) where the tRNA carrying the amino acid will come ;

P site (peptidyl site) for the tRNA carrying the peptide chain being elongated.

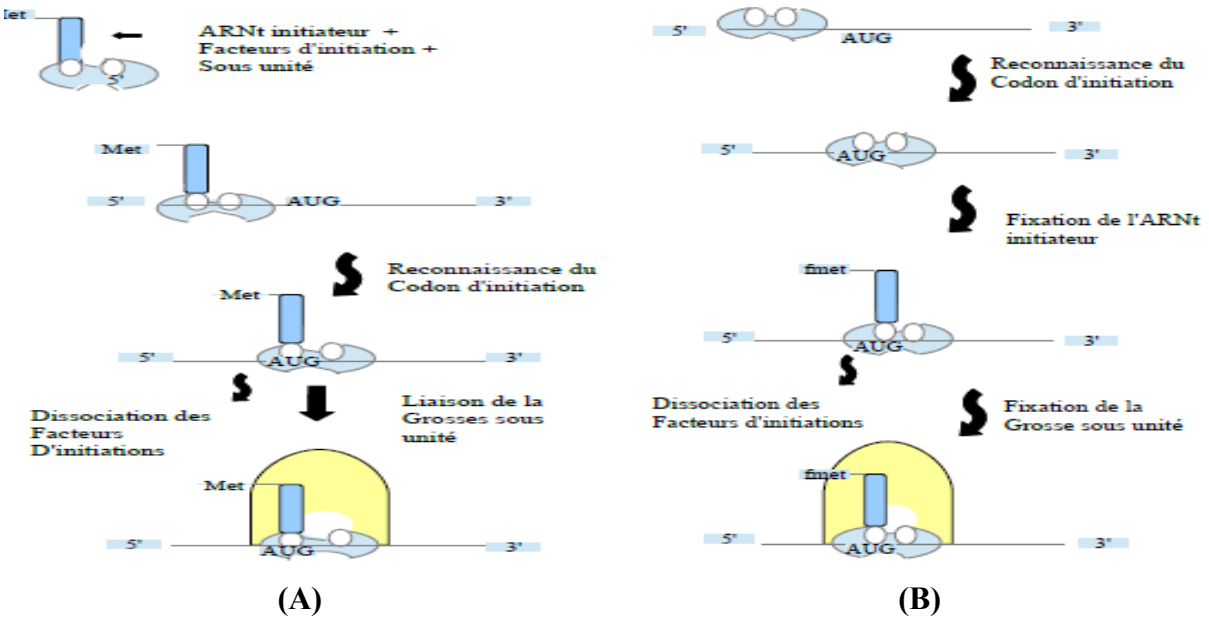


Figure 94 : Initiation of translation in eukaryotes (A) and prokaryotes (B).

b) Elongation

After initiation, the first amino acid is then in place. It will now be necessary, during the next phase called elongation, to form a peptide bond for each AA to be attached, i.e. for each peptide bond to be made, the same cycle containing 3 steps is described each time (Fig. 95; Fig. 96):

Step 1 : attachment of a new aminoacyl-tRNA :

In the ribosome, the second tRNA comes with amino acid n°2 in the A site of the large subunit. It is codon n°2 placed on the mRNA after the AUG codon which therefore determines the choice of the second anticodon and therefore the second AA.

Step 2 : peptide bond formation :

There is a break between methionine and the first tRNA which is ejected, this is where the peptide bond is formed between COOH of the first AA and NH2 of the second carried by tRNA n°2. But in fact the COOH of the 1st AA was not free since it was already engaged in a bond with the tRNA. The formation of the peptide bond giving the peptide and the detachment of the 1st tRNA occur simultaneously. Recall that the enzyme involved in this

bond is called peptidyl transferase. At this stage, a dipeptide has therefore been formed which is located in the A site. It is carried by tRNA n°2, the ejection of the 1st tRNA has freed the P site.

Step 3 : translocation :

The ribosome will move one step (one codon) on the mRNA in the 5' to 3' direction. A new codon is now in front of the A site. Simultaneously, tRNA N°2 carrying the dipeptide has passed from the A site to the P site, it has changed compartment hence the name translocation but it is still in front of its codon n°2.

Many cycles follow one another with the same steps : attachment of AA-tRNA to the ribosome, formation of the peptide bond and finally translocation.

During elongation, the peptidyl transferase will cut, transfer, and weld each time, thus lengthening the peptide by one amino acid.

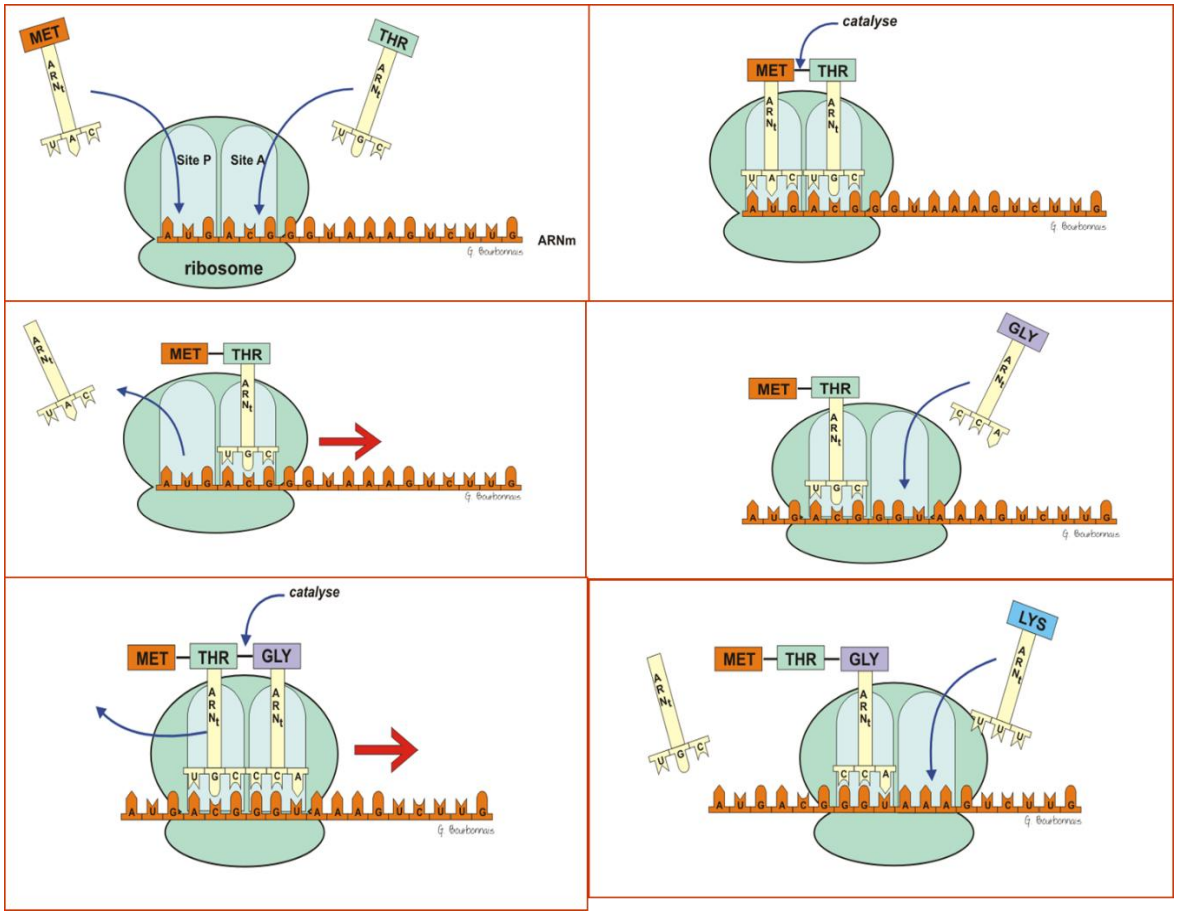


Figure 95: Translation Elongation

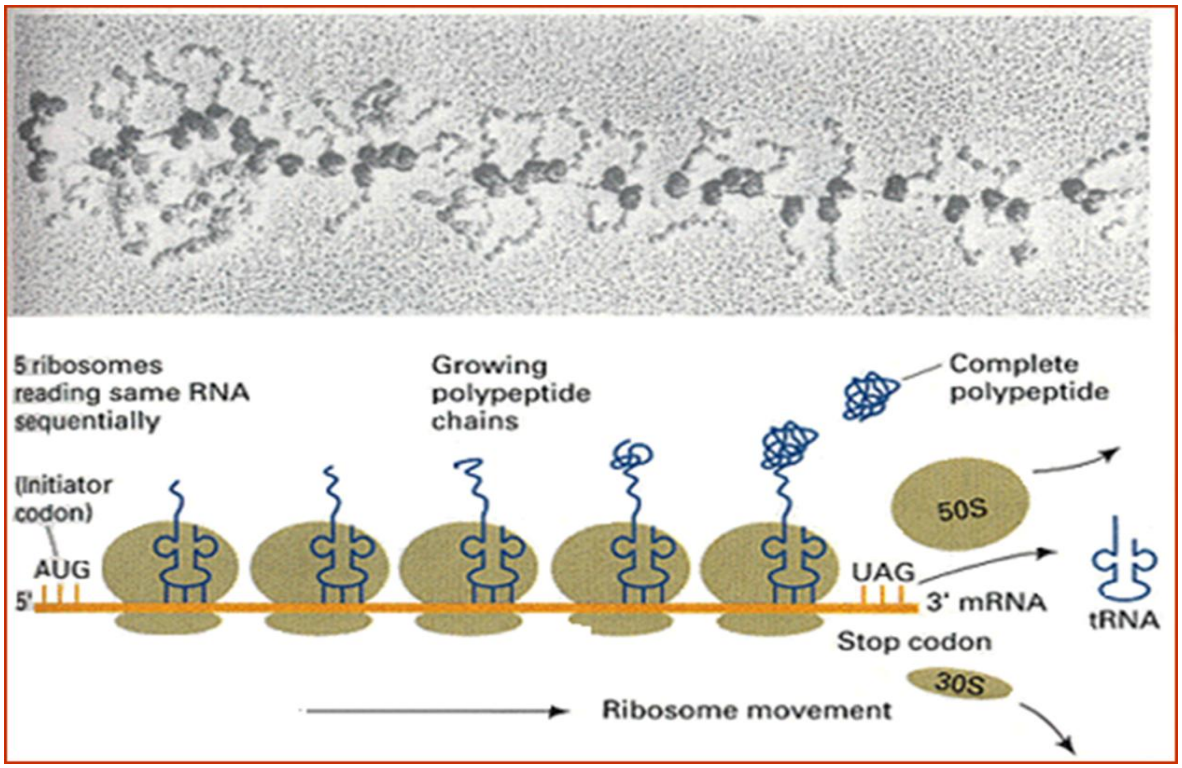


Figure 96 : mRNA Translation

c) Termination

The end of translation occurs when the ribosome, moving along the mRNA, finds a stop codon : UAA, UAG or UGA. These codons do not code for any AA. There is no tRNA with an anticodon complementary to any of these three codons. There will then be a break between the last tRNA and the peptide chain. The ester bond linking this last tRNA to the last AA is hydrolysed, thus releasing the peptide chain (Fig. 96). It is the peptidyl transferase that would make this last cut.

8. Genetic mutations

If recombination increases variation, mutation is the primary creator of variation. Indeed, one of the sources of hereditary variations is mutation, a genetic event creating modifications in transmissible information. Mutation is a rare phenomenon (the rate is 10^-5 to 10^-7 spontaneous mutations per generation). It can be of spontaneous (natural) or induced origin (thanks to mutagenic agents). Also, the classification of mutations can be made according to the dimension of the biological element affected.

8.1.Gene Mutation

The DNA sequence of a gene determines the amino acid sequence of the protein it encodes. However, a modification in the DNA sequence leads to a modification in the amino

acid sequence. This transformation can affect the functioning of the protein, which can have deleterious effects on the organism.

8.1.1. DNA Mutation

8.1.1.1. Definition

Mutations are accidents in the copying of purine (A, G) or pyrimidine (T, C) bases that occur most often during DNA replication. The newly synthesized DNA is no longer the exact replica of the parental DNA.

8.1.1.2. Causes of DNA mutations

The copying accident during DNA replication can be :

- ✓ A miscopied base : this is ***a substitution*** mutation
- ✓ When a purine is replaced by another purine or a pyrimidine by a pyrimidine, it is a transition.
- ✓ When a purine is replaced by a pyrimidine or vice versa, it is a transversion.
- ✓ A forgotten base : this is ***a deletion*** mutation.
- ✓ An added base : this is ***an insertion*** mutation.

8.1.2. RNA mutations

8.1.2.1. Definition

Copying accidents can also occur during transcription. But, in this case, the problems are less serious because the modified mRNA molecules remain few compared to those that are not, and, moreover, there is no transmission of these errors to the next generation.

8.1.2.2. Causes of RNA mutations

Depending on the case, the synthesized protein will or will not be very different from the protein initially encoded by the non-mutated gene.

a) Mutation without reading frame shift

- ***Silent mutations*****:** this type of mutation affects the 3rd base of a codon but does not change the encoded amino acid. Indeed, the resulting codon codes for the same amino acid. For example : UUU is replaced by UUC. Both these codons code for phenylalanine. It is a substitution that has no consequence.
- ***Conservative mutations*****:** a codon coding for an amino acid is replaced by a codon giving an amino acid from the same group. For example : AAA (lysine) is mutated to AGA

(arginine). Lysine and arginine belonging to the same amino acid group (basic). This mutation is most often without consequence.

- ***Missense mutations*:** a codon is replaced by a codon giving a chemically very different amino acid. For example: AAG (lysine) is mutated to GAG (Glu). Lysine is a basic amino acid while glutamic acid (Glu) is an acidic amino acid. This results in a protein that is most often abnormal, resulting in the appearance of a different character in the individual's phenotype.
- ***Nonsense mutations or mutations affecting the stop codon***: the mutation transforms a codon coding for an amino acid into a stop codon. The mutation leads to a premature stop of mRNA translation, thus giving a shorter protein. For example : UGC which codes for Cysteine is mutated to UGA which is a stop codon. If the error occurs at the beginning of the peptide chain, the consequences are serious. But if the error occurs towards the end of the chain, it can be negligible. Conversely, a stop codon can be transformed into a codon coding for an amino acid. This will result in a longer protein.

b) Mutation with reading frame shift

These are due to the insertion or deletion of one or more bases which cause a shift in the reading of the triplets. This alters the reading phase. These mutations are serious if the phase shift occurs at the beginning of the gene. In this case, a completely different protein is obtained (figure 97).

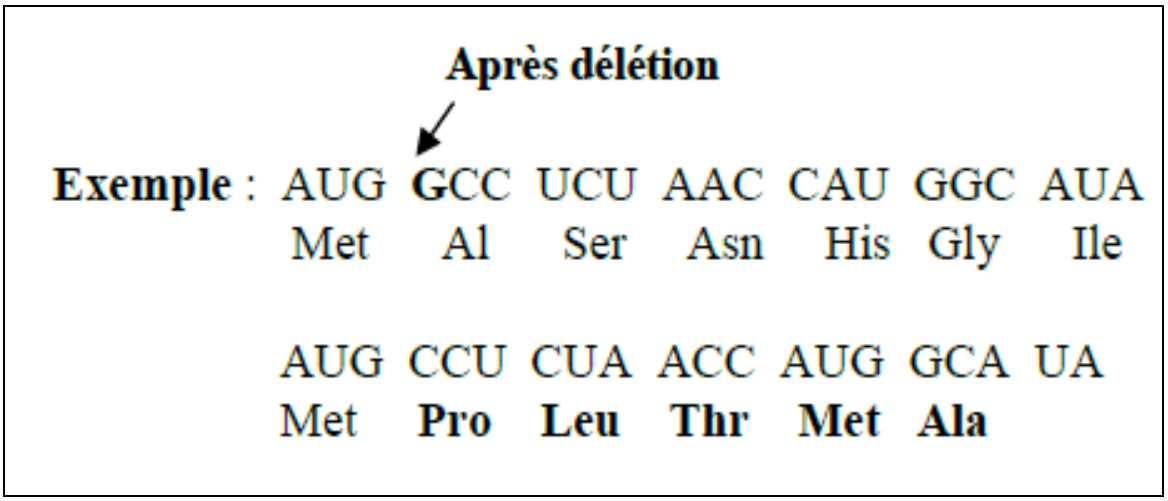


Figure 97: Reading Frame Shift

Note : The most frequently observed mutations in pathology are transversions, frameshifts, and deletions.

8.2. Chromosomal mutations

These are modifications of greater amplitude and different nature occurring on the chromosome, the support of the genes. These mutations can affect the number of chromosomes (aneuploidy) and the structure of chromosomes.

8.2.1. Mutations affecting the number (Aneuploidy)

There is aneuploidy whenever there are one or more chromosomes in excess or less than the natural diploid complement. It is an abnormality in the number of chromosomes (Table 18). There are two types of aneuploidy (by excess and by default).

a) Aneuploidy by default

This is when one or more chromosomes are missing (less) :

If one chromosome is missing (2n - 1), the corresponding chromosome exists in only one copy : this is ***monosomy***.

If two members of a chromosome pair are absent, it is ***nullisomy*** (2n - 2).

b) Aneuploidy by excess

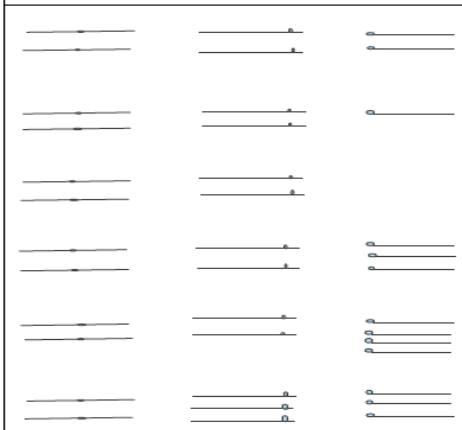
This is when one or more chromosomes are in excess (more) :

If a chromosome is extra (2n + 1), it is ***trisomy*** (i.e., when one of the chromosome types is represented 3 times).

When one of the chromosome types is represented by 4 units, it is a ***tetrasomic***.

When two types of chromosomes are represented by 3 units, we speak of double ***trisomy***.

Table 18: Numerical chromosomal variations: only part of the genome (Aneuploidy)

Garniture chromosomique de l'espèce	Caractéristiques
	individu diploïde normal 2n = 6 chromosomes. Monosomique : 2n -1 = 5 chromosomes. Nullisomique : 2n -2 = 4 chromosomes. Trisomique : 2n+1 = 7 chromosomes. Tétrasomique : 2n+2 = 8 chromosomes. Double trisomique :2n+1+1= 8 chromosomes

c) Origin of the anomaly (causes of Aneuploidy)

Aneuploid variations all result from mitotic or meiotic abnormalities and are observed in both diploids and polyploids. They are produced by the non-disjunction of chromosomes. Non-disjunction corresponds to the inability of chromosomes to separate (disjunction) during the 1st division of meiosis. It can also be due to sister chromatids that do not separate, either at the time of the second division of meiosis or at mitosis (Fig. 98). The two joined chromosomes or chromatids migrate to one of the poles and will be included in a daughter cell. Thus, when at meiosis I the two homologues of a pair of chromosomes go to the same pole, two gametes are produced, one gamete with n+1 and one gamete with n-1 chromosomes. The union of a normal gamete with a gamete at n+1 gives a trisomic zygote (2n+1) and the union of a normal gamete with a gamete at n-1 gives a monosomic zygote (2n-1).

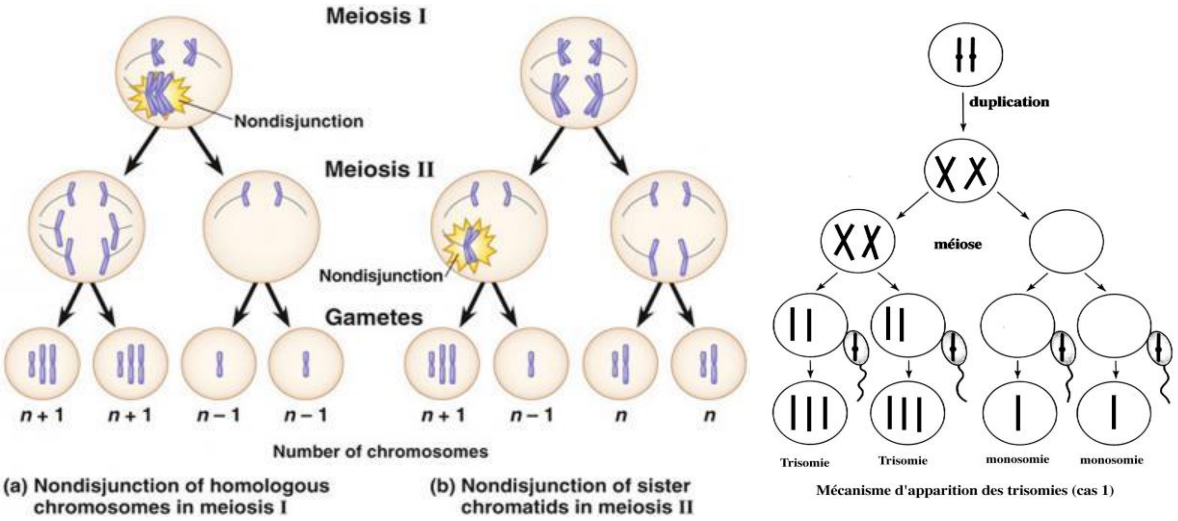


Figure 98: Aneuploidy

d) Some consequences of Aneuploidy

The viability and fertility of aneuploids are generally reduced in animals and plants. They cause changes in gene dosages, and physiological function is more or less disrupted depending on the chromosome involved. Furthermore, nullisomy is deleterious. Here are some examples :

- ✓ In Drosophila, monosomic IV and trisomic IV are fertile and viable. However, monosomics and trisomics of chromosomes II and III are not viable.
- ✓ Individuals with trisomy 13 **(Patau syndrome)** live 130 days on average. While for trisomy 21, individuals live longer **(Down syndrome)**.
- ✓ Monosomy X **(Turner syndrome)** with a karyotype of 45 chromosomes instead of 46 (44

autosomal chromosomes and a single X chromosome) represents about 5% of human chromosomal aberrations. Out of 40 zygotes carrying monosomy X, only one develops to birth with frequent congenital heart defects, especially aortic and renal. An important element is the absence of ovarian function.

- ✓ Trisomy X or additional Y. In men (47, XXY**) Klinefelter syndrome** : they are tall, secondary sexual characteristics are underdeveloped or absent, and infertility is due to an abnormality in spermatogenesis. The presence of an additional Y (47, XYY) is not expressed by a particular phenotype. Similarly, in girls carrying 3X, some of them have intellectual disabilities with learning difficulties, particularly a delay in language acquisition.

8.2.2. Mutations affecting chromosome structure

The structure of chromosomes can itself be accidentally modified. This modification is due to a chromosomal break. It results in losses of genetic material or rearrangements of gene sequences.

a) Causes of structural mutations

- ✓ **Deficiencies or deletions:** This is the loss of a chromosome fragment. The fragment that does not have a centromere will be lost during the next cell division.
- ✓ **Duplications:** A portion of a chromosome exists in 2 copies, often arranged in tandem.
- ✓ **Inversions:** Inversion occurs when the chromosome segment between the 2 breakpoints is reversed. This results in a balanced rearrangement with no net loss of chromosomal material. The inverted segment may include the centromere.
- ✓ **Insertion:** A part of one chromosome attaches to another chromosome or even centromeric fusion of two chromosomes (Robertsonian translocation).
- ✓ **Translocation:** Exchange of segments between non-homologous chromosomes (reciprocal translocation).

b) Consequences of structural mutations

- ➢ **Deficiency or deletion :** In humans, the deficiency of the short arm of chromosome 5P is known, leading to ***''cris du chat'' syndrome***, which owes its name to the characteristic cries of infants. It results in intellectual disability and growth retardation.
- ➢ **Duplication :** the duplication of a chromosomal segment 5Q in humans is an unbalanced

structural anomaly. It results in individuals who have a prominent forehead, a small mouth, and drooping eyelids.

- **Inversions and translocations :** correspond to different situations from the previous ones, insofar as the genome is not quantitatively modified. Carriers generally express a normal phenotype : only their karyotype can possibly detect these anomalies. It is at the level of the offspring that the consequences of these translocations manifest themselves.

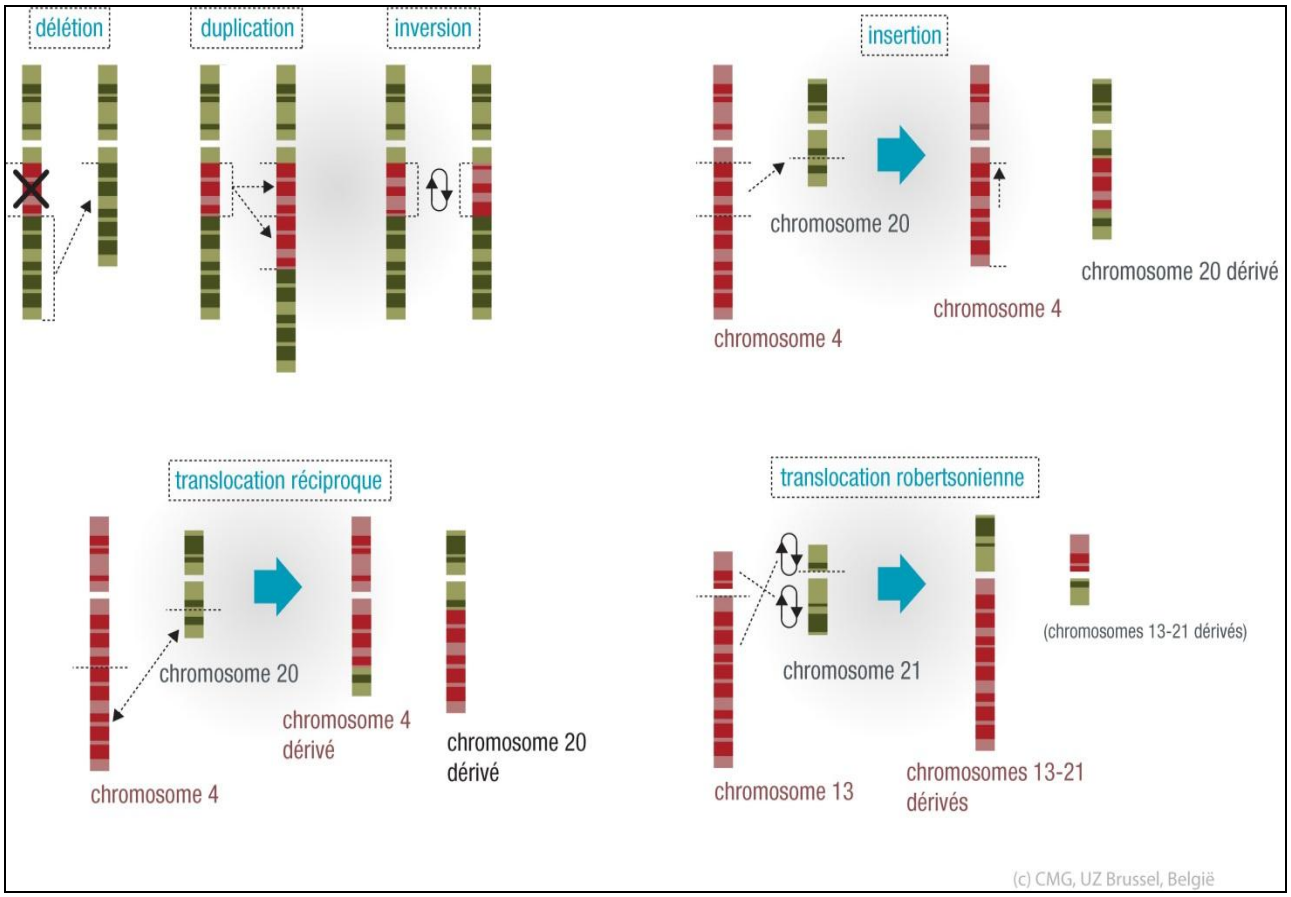


Figure 99: Categories of structural variations of chromosomes in a diploid individual

Example

Translocation of chromosome 21 onto a chromosome of groups 14 and 15 observed in humans : In carrier parents, during meiosis, the 14-21 chromosome pairs with chromosome 14 on the one hand and with chromosome 21 on the other. At anaphase, there is normal disjunction between chromosomes 14-21 and 14, which each migrate to a pole, but the remaining chromosome 21 can migrate to either pole. Four possible gametes are obtained, only one of which is normal (14+21), one carrying the translocation (14-21), one deficient for this chromosome (21) and one carrying the supernumerary chromosome 21 (14-21 + 21). Following fertilization involving the normal gamete (14+21) of the other parent, we obtain

either a normal descendant, or a phenotypically normal but carrying the translocation, or a monosomic 21 (lethal), or a Down syndrome individual who, although possessing 46 chromosomes, actually has 3 copies of chromosome 21 (explanation in progress).

8.3. Genomic mutations : Euploidy

Genomic mutations are variations in the degree of ploidy or numerical variations in the (whole) genome. They occur accidentally and can thus lead to a number of chromosomes in some normally diploid individuals that is different from that characteristic of the species. There are 2 types of Euploidy : haploidy and polyploidy.

8.3.1. Haploidy

a) Definition

When the genome is represented once, it is called haploidy or monoploidy (figure 5). Haploidy is a normal condition in many lower organisms (many algae, mosses and fungi) where the most important phase of the life cycle is haploid. In addition, haploids are quite common in some insects of the order Hymenoptera (wasps or bees).

On the other hand, in normally diploid plants and animals, there is occasional production of haploid individuals. These individuals, with rare exceptions, generally do not reach the adult stage, and when they do, they will be highly sterile. Thus, the natural haploid mutation leads to a sharp drop in viability, and selection generally leads to the elimination of carrier individuals.

b) Origin of this mutation

In some species (asparagus, pepper, citrus) the seeds sometimes give several seedlings simultaneously. This is an anomaly that results from the parallel development of a normal diploid embryo and a haploid embryo resulting from the parthenogenetic growth of a cell in the embryonic sac (example: synergid), this is called **polyembryony**.

When a male gamete enters the oosphere and does not fertilize it, there is no fusion of the male and female nuclei, but the oosphere develops by parthenogenesis and gives a haploid embryo.

8.3.2. Polyploidy

a) Definition

Polyploidy is the multiplicity of the genome from one species to another in the same

genus due to a mutation. Polyploidy is the state of cells that have more than 2 sets (copies) of chromosomes. Indeed, when the genome is represented 3 times or more, it is said that there is polyploidy (table 19).

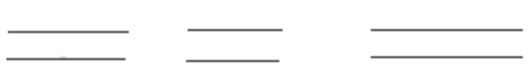
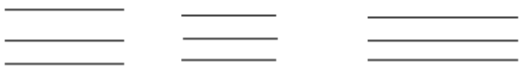
It is frequent in plants and has been observed in several genera : Gossypium (cotton); Trifolium (clovers); Triticum (wheat); Solanum (potato), etc.

b) Origin of this mutation

It can result naturally from an anomaly of mitosis or meiosis. It is therefore spontaneous.

- **From mitosis** by the formation of a mitotic restitution nucleus, that is to say a single nucleus with a tetraploid number of chromosomes instead of two diploid nuclei, following the suppression of a mitotic anaphase (therefore somatic doubling).
- **From meiosis** by the production of unreduced gametes, i.e. with a diploid number of chromosomes.
    - ✓ If two unreduced gametes meet, a tetraploid individual is formed; 2x (♀) ⊗ 2x (♂) = 4X (rare phenomenon).
    - ✓ If a reduced gamete unites with an unreduced gamete, or if a reduced gamete is fertilized by two spermatozoa, a triploid individual is produced; 2x (♀) ⊗ x (♂) = 3X.

Table 19: Numerical chromosomal variations: the entire genome: Euploidy

Garniture chromosomique de l'espèce	Nombre de chromosome	Représentation du génome	Degré de ploïdie
	6	2 fois	Diploïde
	1	1 fois	Haploïde ou monoploïde
	9	3 fois	Triploïde
	12	4 fois	Tétraploïde

8.4. Cytoplasmic Mutations

The cytoplasm plays an important role in heredity. It is known that certain cytoplasmic organelles contain genetic material analogous to that of the nucleus (DNA and RNA) and are endowed with genetic continuity, meaning they can be transmitted to offspring. These are mainly mitochondria and chloroplasts. These genes have their own transmission. In a cross, both parents contribute equally to the nuclear genome of the zygote. However, the cytoplasmic contribution of the father and mother is generally unequal.

In higher animals, the egg provides almost all of the cytoplasm and the sperm almost none. Consequently, the genes of cytoplasmic organelles are generally transmitted exclusively by the mother. Mitochondrial inheritance is therefore maternal (uniparental).

In about 2/3 of higher plants, the chloroplasts of the male parent (contained in the pollen grains) do not enter the zygote, so that the DNA of the chloroplasts, like that of the mitochondria, is transmitted maternally. In other plants, the pollen's chloroplasts enter the zygote and chloroplast inheritance is biparental.

In addition to mutations in the genes carried by nuclear chromosomes, mutations can also occur in mitochondria and chloroplasts.

8.4.1. Mitochondrial Mutations

They are a significant cause of human genetic diseases. Diseases caused by mitochondrial genes have two characteristics :

- ✓ **Maternal transmission :** a mitochondrial pathology is never transmitted from a sick father to his descendants, but from a sick mother to all her descendants. Example **:** mitochondrial encephalopathy.
- ✓ **Heteroplasmy :** cells contain many mitochondria. In some mitochondrial diseases, each mitochondrion carries the causative mutation (homoplasmy), but in other cases, each cell has a mixed population of mutant and normal mitochondria (heteroplasmy).

8.4.2. Chloroplast Mutations

Variegated plants that exhibit white patterns or stripes on their leaves are often due to a mixture of normal chloroplasts and chloroplasts carrying a mutation that leads to the inability to synthesize chlorophyll. In a zygote, at each cell division, the chloroplasts are randomly separated during the growth and development of the plant. Thus, there will be the production of alternating green and white patches on the leaves, the green ones containing the

normal chloroplasts, while the white patches contain the defective chloroplasts.

Example : maternal transmission in the four o'clock plant (figure 100). The color of the chloroplasts of this plant determines the color of the different branches. Variegated branches are mosaics of all-white or all-green cells. The flowers can come from green, white or variegated branches, but when they are crossed, it is the ovule that determines the color of the branch in the resulting plant. For example, if the ovule comes from a flower on a white branch, regardless of the origin of the pollen, the resulting plant will have white branches, which proves maternal transmission.

Figure 100: The Four O'Clock Plant.

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