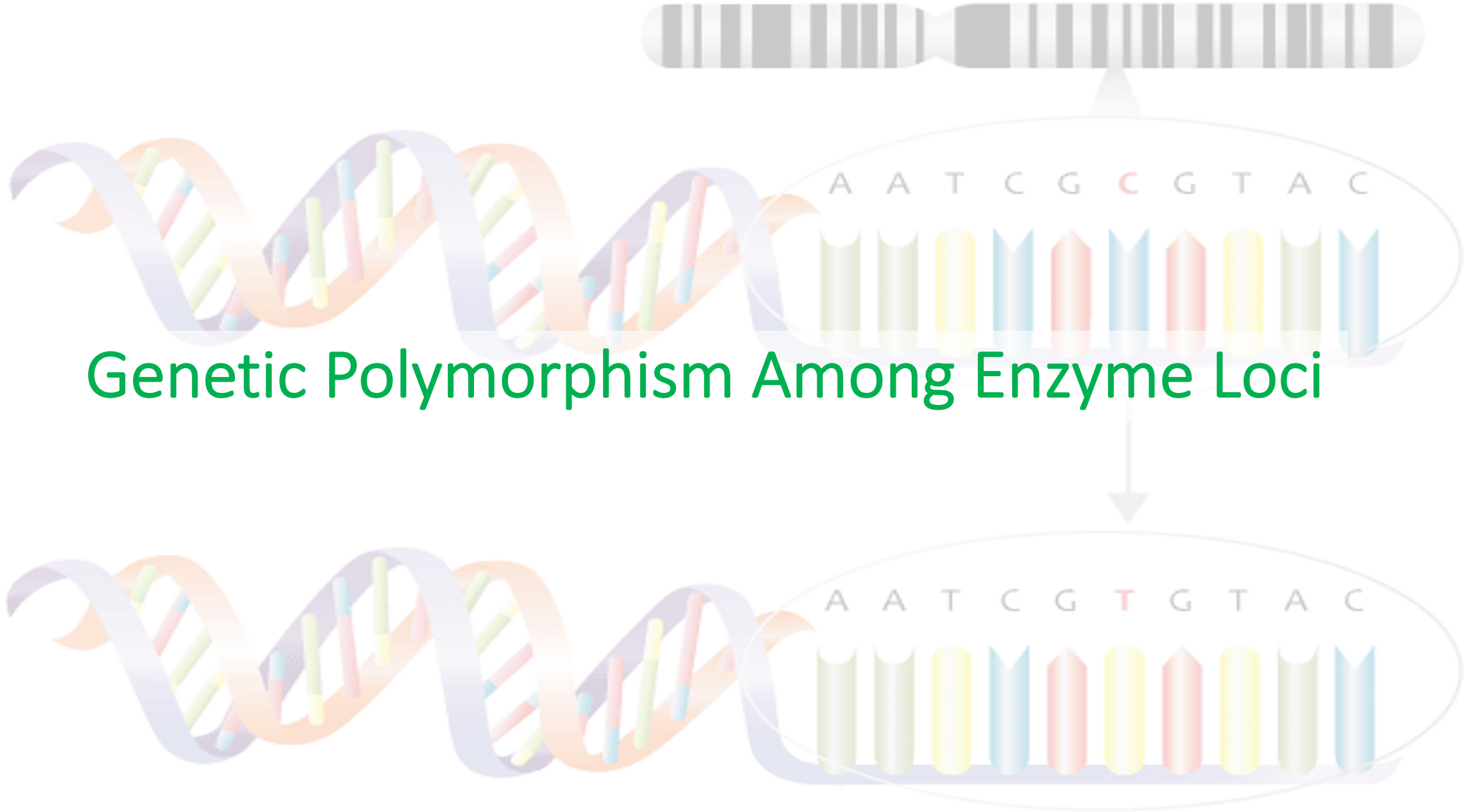


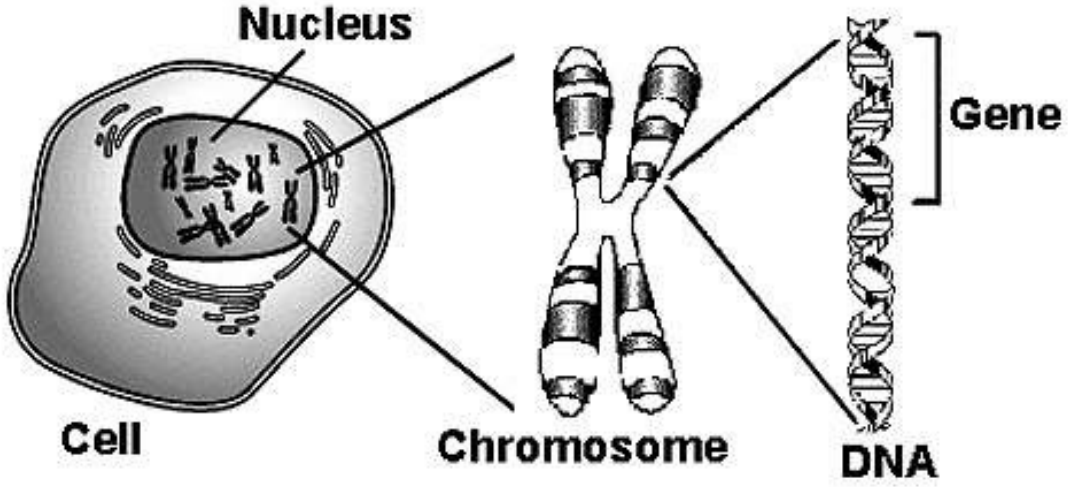
Genetic Polymorphism Among Enzyme Loci

Overview

In the nucleus of each cell, the DNA molecule is packaged into thread-like structures called chromosomes.

Each chromosome has a constriction point called the **centromere**.

The location of the centromere on each chromosome gives the chromosome its characteristic shape, and **can be used to help describe the location of specific genes.**

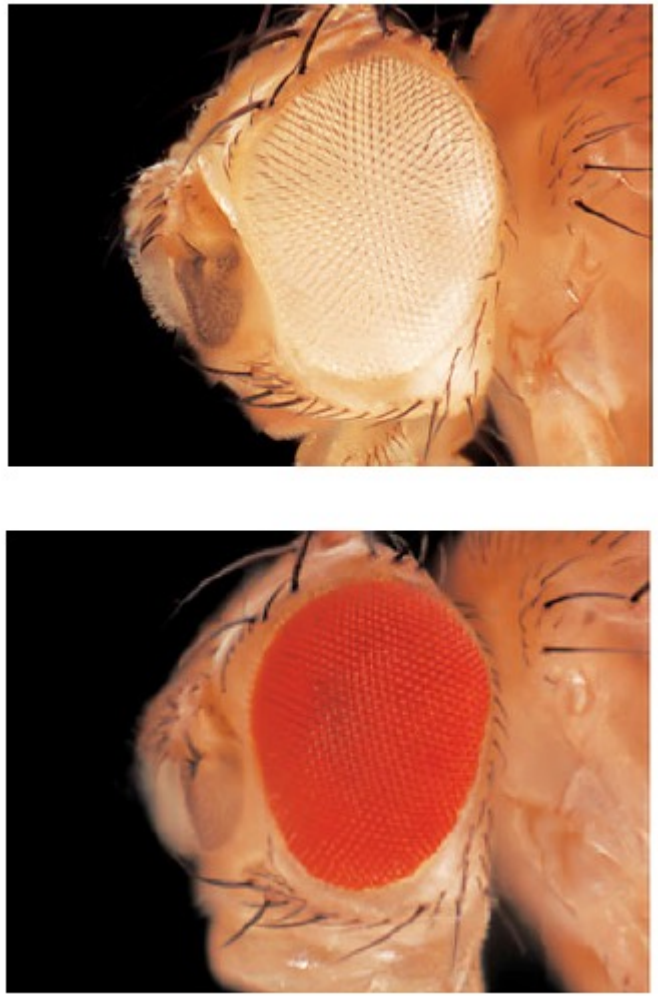


What is genetic variation

Scientists began studying the inheritance of traits in the fruit fly, Drosophila melanogaster.

A white-eyed fly was discovered in a bottle containing normal (wild-type) red-eyed flies.

This variation was produced by a mutation in one of the genes controlling eye color. **Mutations** are defined as any heritable change and are the source of all genetic variation.



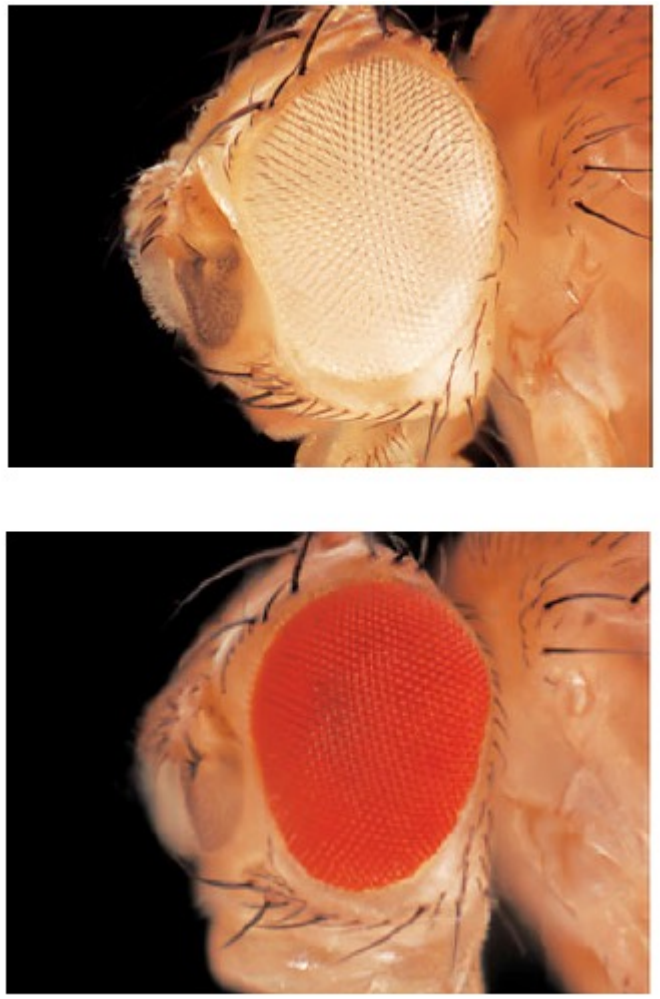
The normal red eye color in *D. melanogaster* (bottom) and the white-eyed mutant (top).

The variant eye color gene discovered in Drosophila is an allele of a gene controlling eye color.

Alleles are defined as **alternative forms of a gene**.

Different alleles may produce differences in the observable features, or phenotype, of an organism.

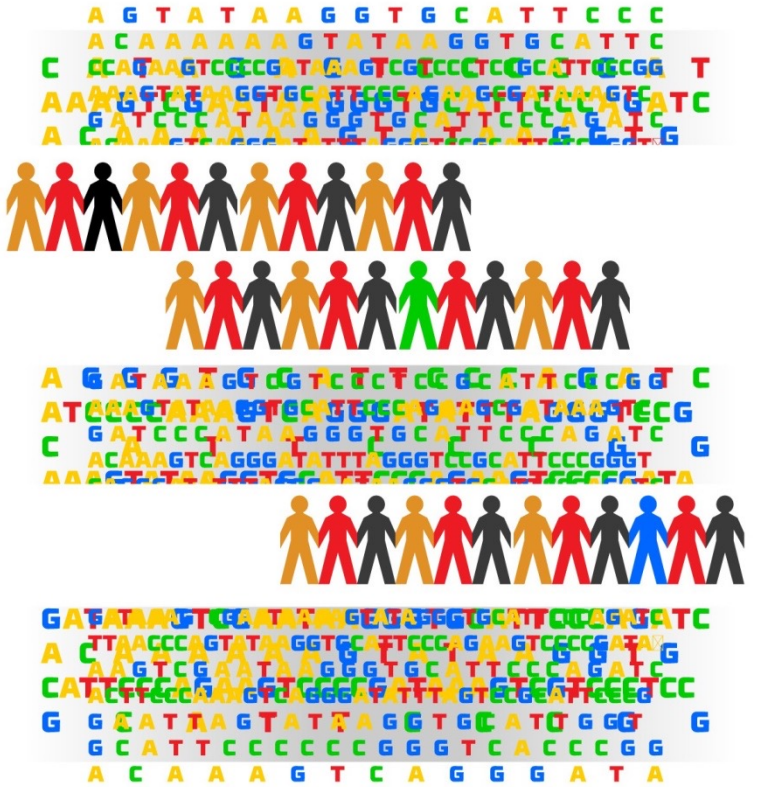
The set of alleles for a given trait carried by an organism is called the **genotype**.



The normal red eye color in *D. melanogaster* (bottom) and the white-eyed mutant (top).

How different is one human genome from another?

We are all very similar, the DNA of most people is **99.9% the same** but the 0.1% of unique DNA, plus the interaction of genetic and environmental factors, **is what leads to our different phenotypic features**.



Polymorphism

In population genetics, the term **polymorphism** **(meaning many forms)** refers to the observation that many traits display variation within a population.

Historically, polymorphism first referred to the **variation in traits** that are observable with the **naked eye**.

Polymorphisms in color and pattern have long attracted the attention of population geneticists. These include studies involving yellow and red varieties of the elder-flowered orchid, and brown, pink, and yellow land snails.

Polymorphism in the Hawaiian happy-face spider.

Genes → Traits

These three spiders are members of the **same species** and carry the **same genes**.

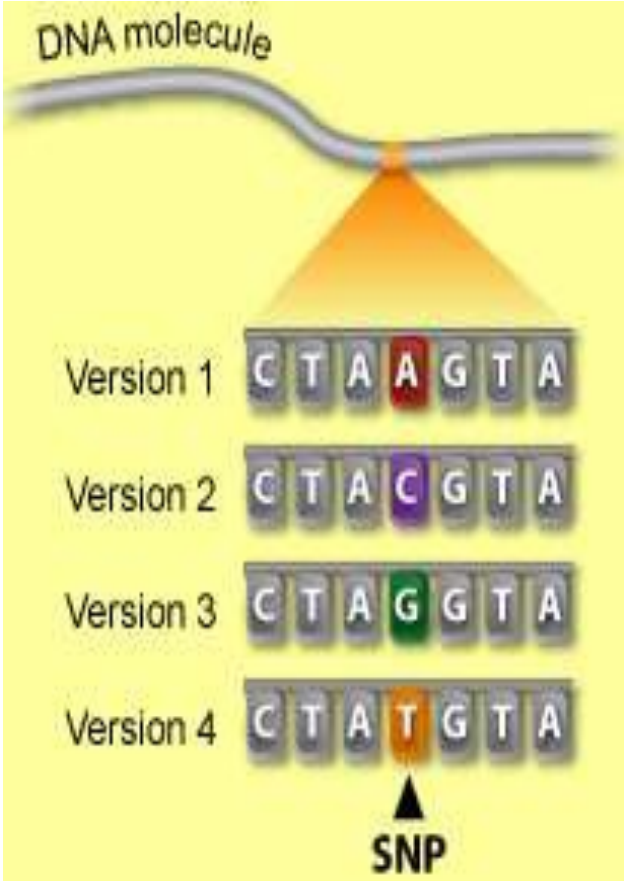
However, several **genes** that affect pigmentation patterns **<u>are polymorphic</u>**, **meaning** that more than one allele occurs in each gene within the population.

This **polymorphism** within the Hawaiian happy-face spider population produces members that look **quite different from each other**.

What is genetic polymorphism ?

Polymorphism variant of a gene or noncoding region that has two or more alleles .

Molecular geneticists use this term to **describe a variant of a locus** within a population of organisms that has two or more alleles.



Mutation and Genetic polymorphism

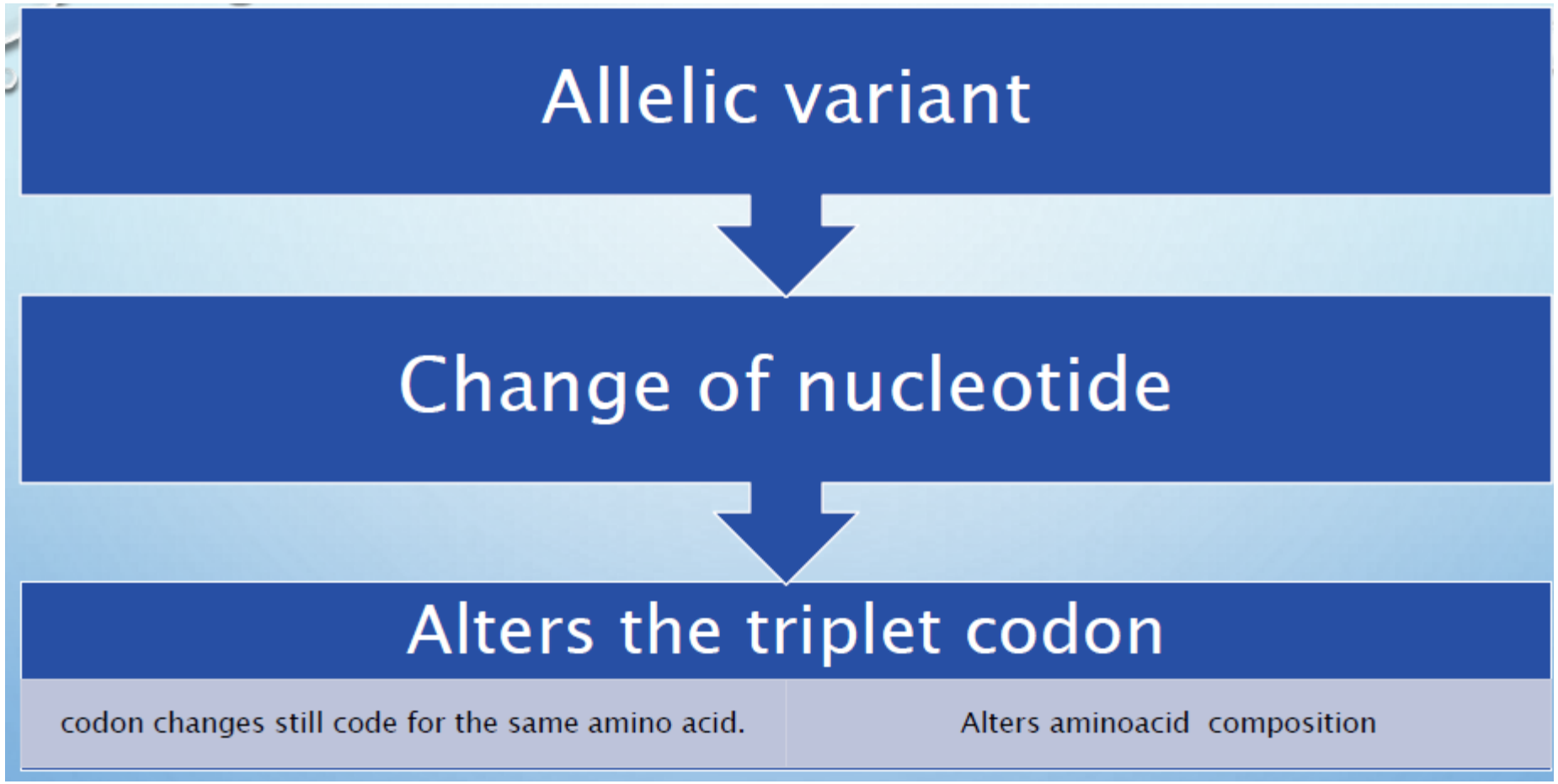
When a **nucleotide** change is **very rare**, and not present in many individuals, it is often called a **mutation**.

In contrast to mutations, **genetic polymorphisms** are usually considered normal variants in population.

When a **specific allele** occurs in **at least 1% of the population**, it is said to be a **genetic polymorphism.**

Polymorphism can be observed:

- At the level of the whole individual (**phenotype**)
- In variant forms of **proteins and blood group substances** (**biochemical polymorphism)**
- In morphological features of **chromosomes (chromosomal polymorphism)**
- At the level of DNA in differences of nucleotides (**DNA polymorphism)**.


Allelic variant
Change of nucleotide
Alters the triplet codon
codon changes still code for the same amino acid.
Alters aminoacid composition

What is the underlying cause of polymorphism?

1. **Deletion** and **duplication** of millions of base pairs of DNA.

2. **Changes** in one or a few **bases** in the DNA located between genes or within exons.

3. **Sequence changes** may also be located in the coding sequence of genes themselves and result in different protein variants that may lead in turn to different phenotypes.

Types of polymorphism

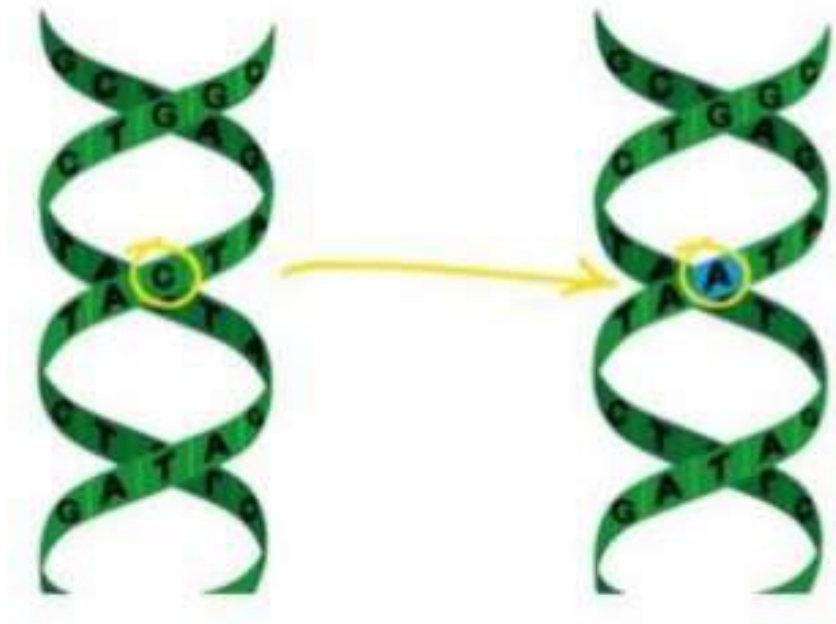
1. Single-nucleotide polymorphism (SNP)

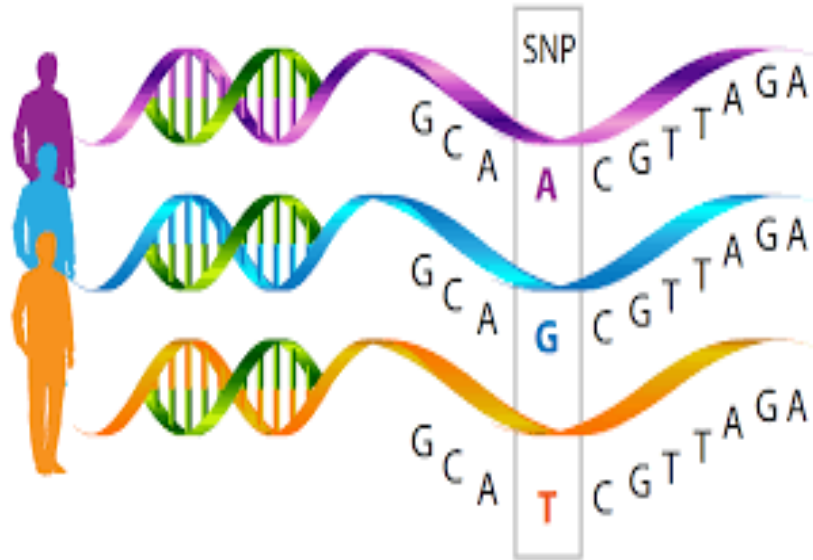
The **most common** form of polymorphisms is the single nucleotide polymorphism, which is a **change in a single base pair** (bp) in the genomic DNA.

Single nucleotide polymorphisms can affect **gene function**.

For example, a single nucleotide polymorphism located in a promoter region may influence the amount of mRNA produced.

Genetic Polymorphism (SNP):





Single Nucleotide Polymphormisms (SNPs) are single base differences in otherwise identical DNA sequences.

Allele 1: C

TGATCTTG . . . . . TGCCAGTT . . . . . CCGTAGCGAA

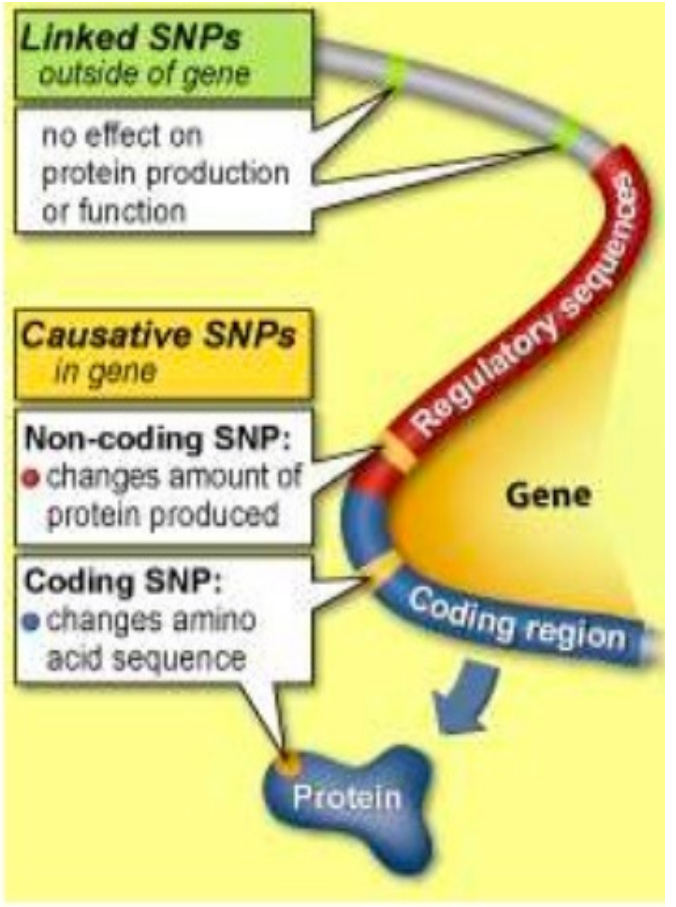
TGATCTTG . . . . . TGCTAGTT . . . . . CCGTAGCGAA

Allele 2: T

Coding region SNPS

A. Synonymous: The substitution causes no amino acid change to the protein it produces. SNP in which both alleles produce the same polypeptide sequence is known as synonymous Sometimes called silent mutation.

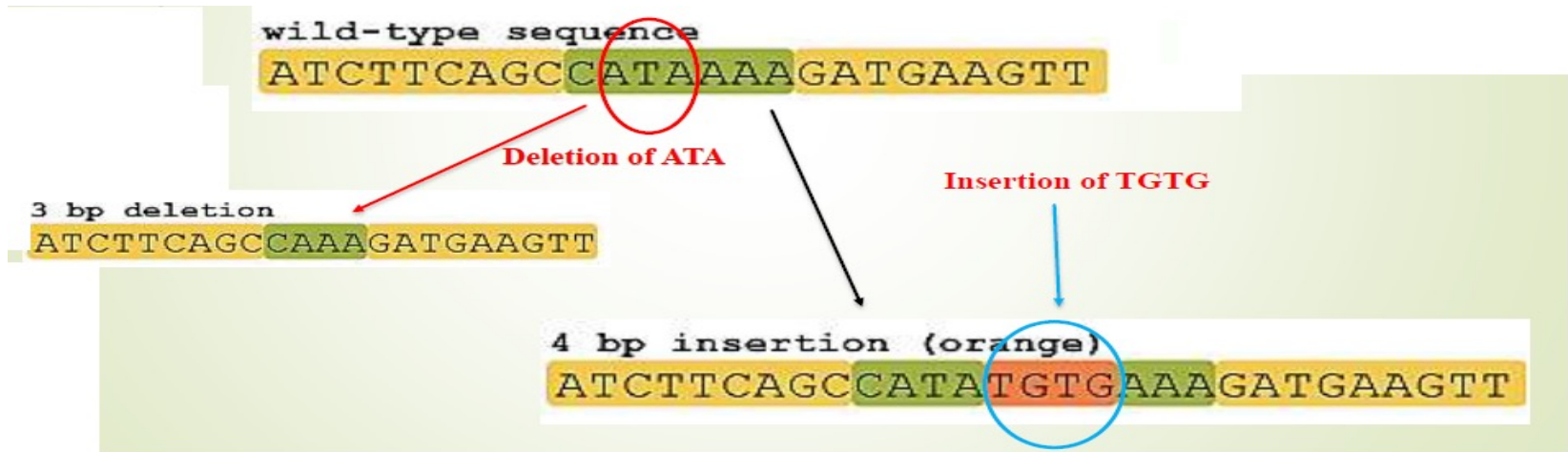
B. Non Synonymous: The substitution results in an alteration of the encoded amino acid. If different polypeptide sequence is produced they are non - synonymous. A non- synonymous change may either be missense or nonsense, where a missense change results in different amino acid, while a nonsense change results in a premature stop codon.



2. Structural variants (Insertions and deletions)

Insertion-deletion polymorphisms or indels, is a type of genetic variation in which **a specific nucleotide sequence** is **present** (Insertion) or **absent** (deletion).

This type is widely spread across the genome. An **indel in the coding region** of a gene that multiples of 3 nucleotides **result** in a protein with extra amino acids (**insertion**) or loss of amino acids (**deletion**) or is not a multiple of 3 nucleotides **results** in a **frameshift mutation**; shifting the reading frame and the DNA transcript sequence may now code for an entirely different set of amino acids or result in a premature stop codon, altering the protein structure and function.

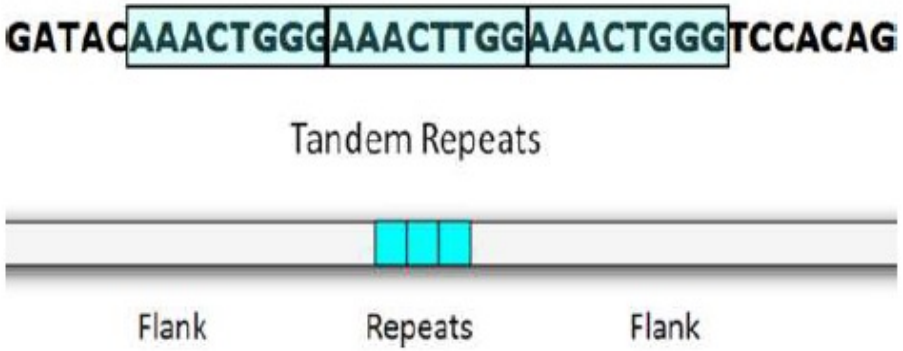

wild-type sequence
ATCTTCAGCCATAAAAGATGAAGTT
Deletion of ATA
3 bp deletion
ATCTTCAGCCAAAGATGAAGTT
Insertion of TGTG
4 bp insertion (orange)
ATCTTCAGCCATATGTGAAAGATGAAGTT

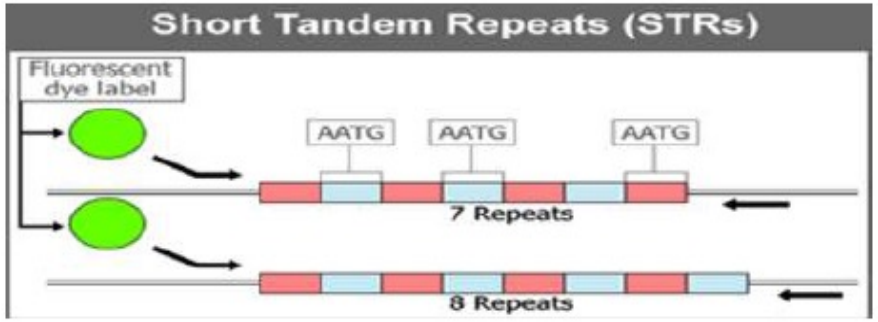
3.Repeated sequence

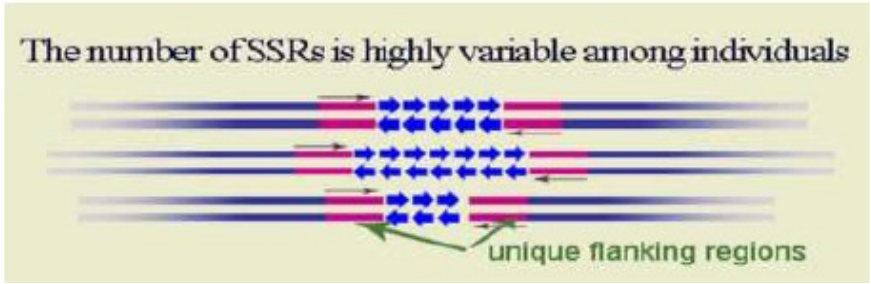
Tandem repeats: copies which lie adjacent to each other, either directly or inverted.

A tandem repeat polymorphism consists of a series of nucleotides that are repeated in tandem (i.e., one time after another).

The polymorphism consists of the number of repeats.



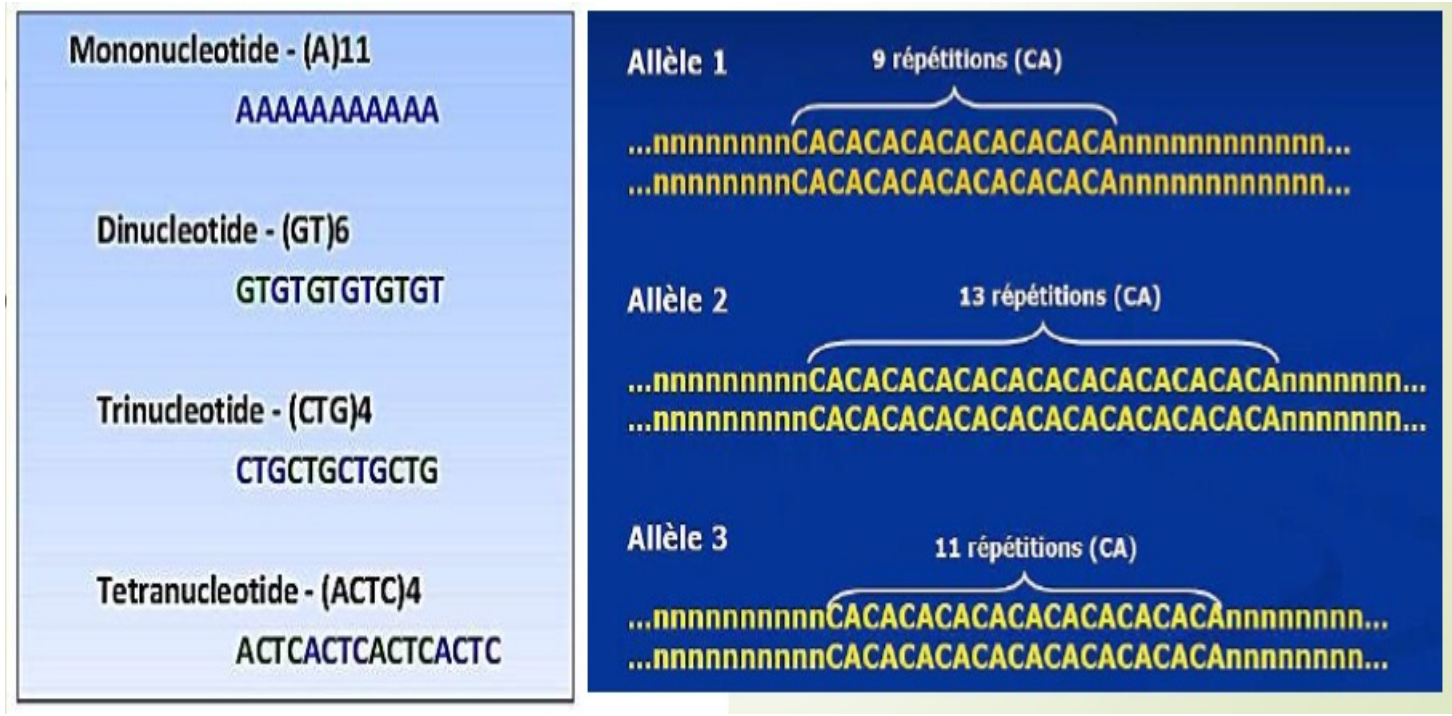




Microsatellites variation (tandem repeat of 1 – 4 base pair bp)

Also known as the **Simple Sequence Repeats (SSRs),**and **single tandem repeats (STR).** Microsatellites are **stretches** of DNA, consisting of tandemly repeating mono-, di-, tri-, tetra-, and pentanucleotide units, which are arranged through the genome of most eukaryotic species.

They are typically neutral, codominant and are used as molecular markers.

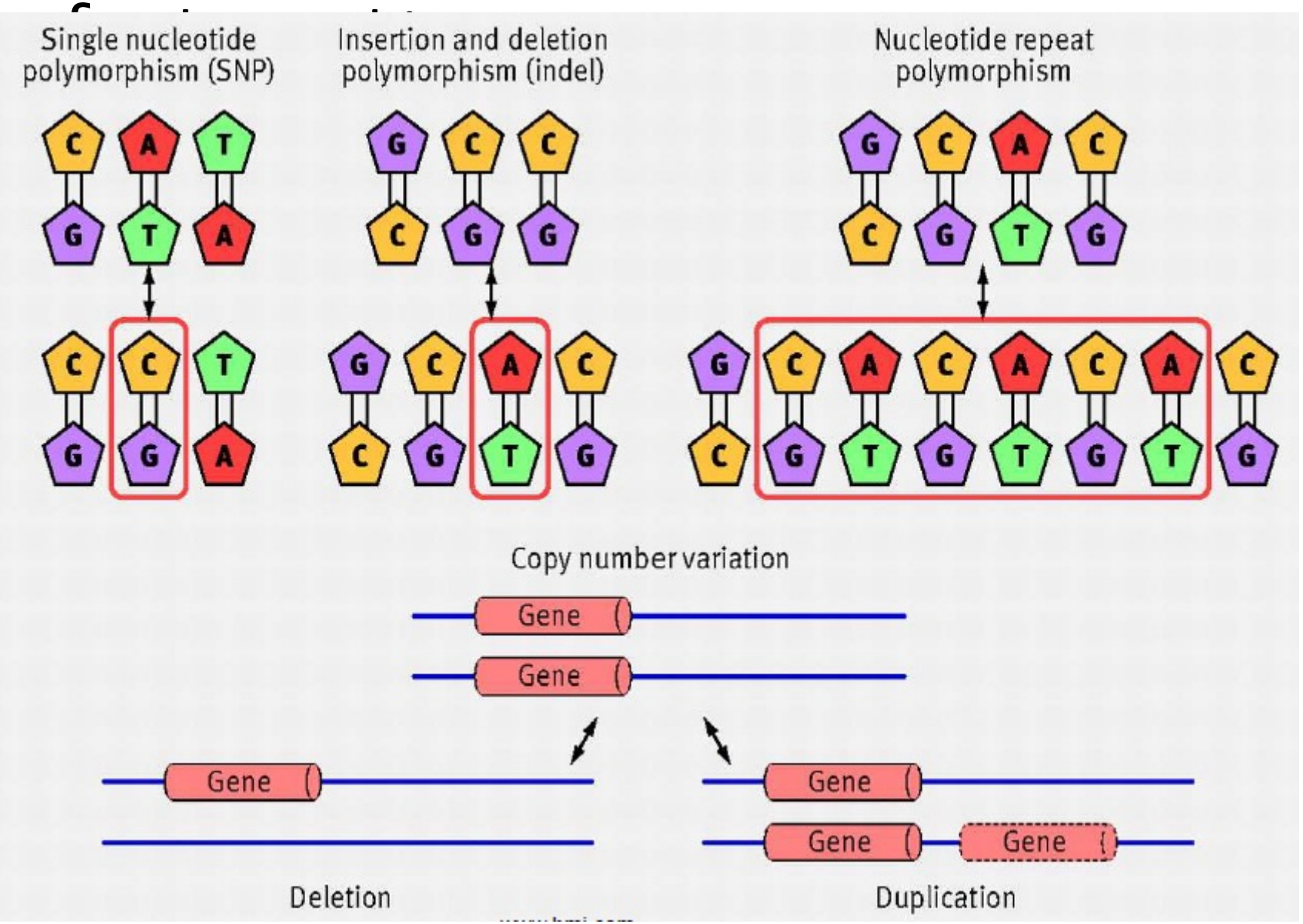


4. Copy number variants (CNVs)

Copy number variants (CNVs) are submicroscopic **structural variations** that are due to deletion, duplication, and replicative transposition.

If the variation in copy number occurs in tandem, it is referred to as variable number of tandem repeats (VNTRs).

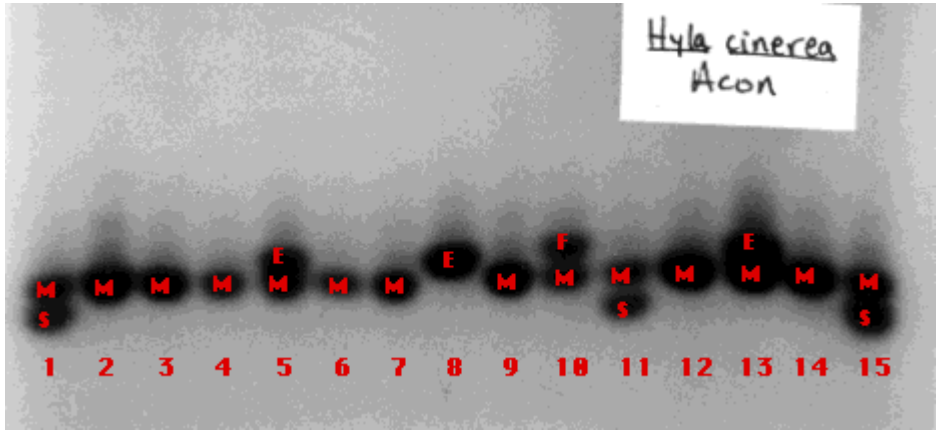
Types


Single nucleotide polymorphism (SNP)
Insertion and deletion polymorphism (indel)
Nucleotide repeat polymorphism
Copy number variation
Gene
Gene
Gene
Gene
Gene
Gene
Deletion
Duplication

Genetic polymorphisms in human metabolizing enzymes

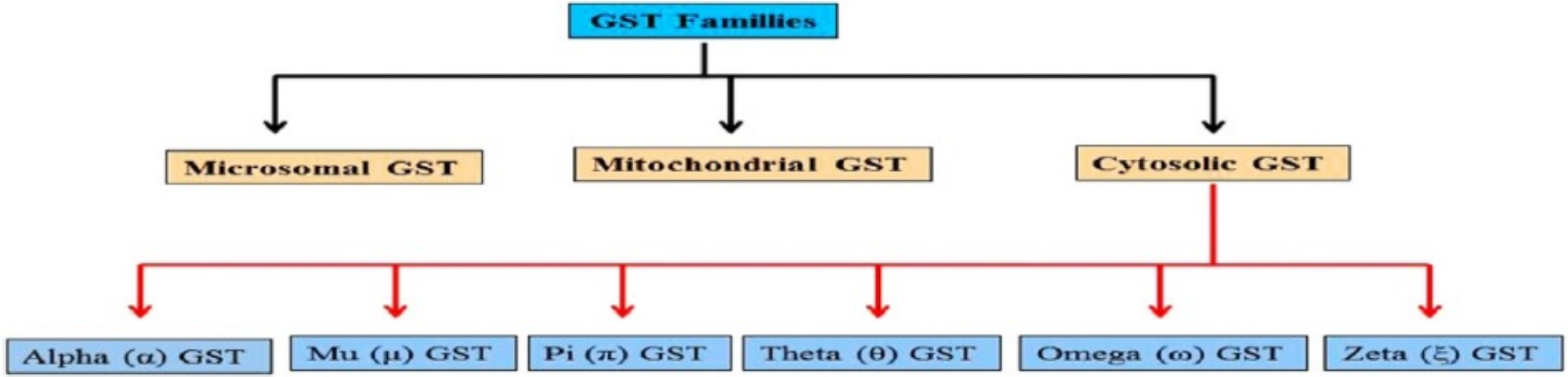
- Enzymes are frequently polymorphic.
- A population may contain two or more variants of an enzyme encoded by a single locus.
- The variants **differ** slightly in their **amino acid sequence** and often this causes them to migrate differently under electrophoresis.
- By treating the gel with the substrate for the enzyme, its presence can be visualized.

Electrophoresis of tissue extracts from 15 different green treefrogs (Hyla cinerea) reveals 4 allelic versions of the enzyme **aconitase** (one of the enzymes of the citric acid cycle). The 4 alleles can be distinguished by the speed with which their protein product migrates



Genetic polymorphism of glutathione S-transferases

- Glutathione S-tranferases (GSTs) are phase II drug metabolizing enzymes, they play crucial role in detoxification of environmental pollutants, carcinogens, drugs, xenobiotics and oxidative stress products.
- Genetic differences in expression and activity of GSTs are due to the existence of polymorphic alleles which encode them.
- GST genetic polymorphism is the main reason for many neurological dysfunctions.
- GST has over expressed in epileptic brain and pi (π) GST has used to predict stroke; mu (μ) and pi (π) GST are over expressed in Alzheimer's disease (AD).



- There are three major GST families are widely distributed in living things.
- Both cytosolic and mitochondrial GST families comprise soluble enzymes, but they are not related to each other in other properties
- Third family belongs to membrane associated proteins those involved in eicosanoid and glutathione (MAPEG) metabolism, therefore designated them as microsomal GSTs.
- Both cytosolic and mitochondrial GSTs have certain similarities in the structural protein folding , but MAPEG enzymes have no structural similarities with remaining two families

Genetic polymorphisms of GST

1- Alpha (α) class GST

- There are five alpha (α) GST genes have been identified: **GSTA1, GSTA2, GSTA3, GSTA4 and GSTA5**, they located **on chromosome six**
- Gene-specific probes and real-time PCR methods shows that the GSTA1, GSTA2 and GSTA4 are **distributed** widely in **all tissues**, and GSTA3 is very **rare** but GSTA5 is **not detected**.
- GSTA1 may play key role in the cellular mechanisms that protect from the by-products of lipid peroxidation.

- GSTA1 may play key role in the cellular mechanisms that protect from the by-products of lipid per-oxidation.
- GSTA1 catalyse the **conjugation** of the **myelosupressive agent** such as busulfan with **GSH**
- But base variation of GSTA1 alleles under the condition of polymorphism shows the low expression and altered biotransformation.

2- Mu (μ) class GST

- There are five mu (μ) GST genes: GSTM1, GSTM2, GSTM3 GSTM4 and GSTM5, these genes have been mapped to chromosome 1.
- GSTM1 variant alleles are correlated to tumor size and the there is an association of GSTM1 null allele with head and neck carcinomas has discovered.

3- Pi (π) class GST

- There is only one member of the GSTP has widely known, located on chromosome 11, express in all tissues that includes red blood corpuscles but little amounts found in the liver
- The GSTP expression levels increased in many tumours.
- GSTP polymorphism got much attention in recent decades and due its contribution in the progression of **Parkinson's disease** that caused by numerous environmental factors including xenobiotic agents and pesticides.

4- Theta (Θ) class GST

- Theta (Θ) class GST comprise two isoenzymes such as GSTT1 and GSTT2
- They located on chromosome 22
- 46% of myelodysplastic syndrome patients have null GSTT1 than 16% of controls.
- The GSTT2 contains 244 amino acids and share 55% similarity in sequence with GSTT1
- SNPs in the promoter region of this gene may confer greater risk of colorectal cancer.

5- Omega (Ω) class GST

- There are two members of omega class GSTs: GSTO1 and GSTO2 those loci exist on chromosome 10
- Number of polymorphisms has reported in the GSTO genes in coding and noncoding regions.
- The role of GSTO1 locus in AD shows a **positive relation** between AD and an uncommon polymorphism which shows major functional effect.

6- Zeta (ζ) class GST

- The zeta GST located on chromosome 14
- it is abundant in liver and kidney proximal convoluted tubules
- Zeta GST act as maleylacetoacetate isomerase in catabolic pathway of phenylalanine and tyrosine
- decrease in catalytic activity leads to metabolic diseases like type I tyrosinaemia and phenylketonuria, so that GSTZ polymorphisms have many clinical consequences.

Genetic polymorphisms of cytochrome P450 enzymes

- **The cytochrome P450 (CYP) enzymes** group of enzymes in smooth endoplasmic reticulum of hepatocytes and epithelial cells of small intestines.
- The main function of CYP450 is oxidative catalysis of various endogenous and exogenous substances
- They have important role in biotransformation of xenobiotics and drugs metabolism
- CYP450 are implicated in phase I metabolism of 80% of drugs currently in use, including anticancer drugs.

- CYP enzymes are also important in the synthesis of many beneficial substrates, such as **steroid hormones** (such as estrogen and testosterone), **fatty** acids, and **sterols** (such as cholesterol and bile acids).
- So far, 57 different CYP genes in 18 protein families have been identified in the human genome.
- One of the most studied roles of CYP enzymes is their involvment in **drug metabolism.**
- These proteins control the speed at which drugs are broken down, and the length of time that the drugs are present in the body.

- There are 57 active CYP genes in the human genome
- The majority of the P450 isoforms are expressed in the liver, while some are expressed in other tissues such as central nerve system, gastrointestinal tract, lung, trachea, nasal and olfactory mucosa and adrenal gland.
- **In particular**, three families of P450s: CYP1, CYP2 and CYP3 are responsible for 75–80 % of the phase I metabolism and 65–70 % of the clearance of clinically used drugs
- **The most important P450s for drug metabolism include** CYP3A4, CYP2D6, CYP2C9 and CYP2C19.

1- CYP3A subfamily accounts for more than 50 % of all CYP-dependant drug metabolism and substantial interindividual variability in CYP3A activity was observed.

24 Polymorphisms identified so far did not explain this variability as no correlation was found between the genotype and the phenotype.

Induction and inhibition by drugs and some food constituents seem to be clinically more relevant, as they increase or decrease CYP3A drug metabolism

2- CYP2D6 is one of the most studied P450 and the molecular basis of the interindividual variability that may lead to decreased or increased CYP2D6 activity is well understood.

- CYP2D6 genetic polymorphism is the major determinant of its activity and the metabolic capacity (phenotype) can be predicted by genotyping.
- CYP2D6 is involved in the metabolism of 20–25 % of all drugs in the clinical use, among them many antidepressants, antipsychotics, antiepileptics, antiarythmics and others

3- CYP2C9 comprises 20 % of all cytochromes P450 in the liver and participates in metabolism of about 10 % to 20 % of commonly prescribed drugs such as antithrombotic and hypoglycemic agents, non-steroidal anti-inflammatory drugs.

Human CYP2C9 gene is highly polymorphic.

Besides the wild-type CYP2C9*1 allele the two most common variant alleles in Caucasian populations are CYP2C9*2 and CYP2C9*3

- It was demonstrated in vitro and in vivo that these two allelic variants of CYP2C9 gene influenced the metabolic activity of the enzyme.
- In accordance with other studies indicate that in order to achieve the optimal therapeutic effect patients with one polymorphic CYP2C9 allele required 44.5 % lower warfarin dose, while patients with two polymorphic CYP2C9 alleles required 66.4 % lower warfarin doses as compared to the patients with two normal alleles.

4- CYP2C19 is involved in metabolism of about 5 % of all drugs and is particularly important for metabolism of proton pump inhibitors and antidepressants.

It has actually been shown that the cure rate of acid-related ulcers and gastroesophageal reflux disease is higher in PMs.

Besides two common deficiency alleles CYP2C19*2 and *3, a novel allele CYP2C19*17 that confers increased activity has been identified recently.

This allele was reported to have a frequency of 18 % in the Swedish population and it is likely that therapeutic response to proton pump inhibitors and antidepressants will be reduced in the homozygous carriers.