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Observations:

- Variations in beak-shapes of finches
- Unique-shell shapes and sizes of Galapagos tortoises on each island
- In each island, there were unique birds, species and tortoises and no two islands had exactly the same kind of species, though similar to those found in the nearest island
- In a given population of species, e.g., humans, there are individuals with different features (height, color of hair/eyes/skin), these differences within a species, called variations are heritable
- Though the species were different in different islands, based on the environment such as being provided by the island, the species could adapt to that environment.

Conclusions: → distribution of species show continental drift

- Individuals in a population exhibit variable traits: Variations
- Many traits are heritable
- Species can adapt to their environment and develop new traits over time. Species are not fixed and unchanging, but rather can evolve and change over time through natural selection. Individuals with advantageous traits are more likely to survive and reproduce, passing on those traits to their offspring and leading to the gradual evolution of new species
- As population grows in size, eventually the resources become limited. This would result in a

competition among the individuals of a population for the resource; Leading to the "survival of the fittest"

→ Individuals with traits or variations which allow them to best adapt to the environment are most likely to survive & reproduce, & also pass on these favourable characters to the next generation

Explanation:

During his visit to the Galapagos Islands, Darwin observed that finches on different islands had distinct beak shapes that correspond with the type of food available. For example; finches with thinner, pointed beaks were more successful at catching insects, while those with thicker, stronger beaks were better at cracking open seeds. This observation led Darwin to conclude that species could adapt to their environment over time and develop new traits that help them survive and reproduce.

Darwin also observed that Galapagos tortoises on each island had unique shell shapes & sizes. This suggested to him that these tortoises had evolved differently depending on the environment and selective pressures present on each island. For example, tortoises on islands with more vegetation had larger shells, while those on drier islands had smaller shells. This observation led Darwin to conclude that species were not fixed and unchanging, but rather could evolve over time through natural selection.

He suggested that individuals with advantageous traits would be more likely to survive & reproduce, passing on those traits to their offspring. Over time, these small changes could accumulate and lead to the gradual evolution of new species.

→ In 1859, Darwin presented his ideas in "Origin of species by means of natural selection"

* Every species shows an evidence of descent from a previous species with modifications - they all have common ancestor

* The pressure which brings about these modifications or, variations, is the environment in which the organism lives

* This pressure, provided by the environment is what is called the "natural selection"

② → RNA is likely to have evolved first among the three biopolymers (DNA, RNA and proteins)

Reasons

- i) RNA is able to store genetic information & act as an enzyme: RNA is able to perform the functions of both DNA & proteins, making it a likely candidate for the first biopolymer to evolve. It can store genetic information, similar to DNA, and it can also act as an enzyme, similar to proteins. RNA is both capable of storing genetic information & catalyzing chemical reactions. Essential life processes like metabolism, splicing & translation revolve around RNA.
- ii) RNA can self-replicate: RNA has been shown to be able to self-replicate under certain conditions. This ability to self-replicate is a key-feature of life, making RNA a strong candidate for the first-biopolymer to evolve.
- iii) RNA can form spontaneously: RNA can form spontaneously under certain conditions without the need for enzymes or other complex molecules. This means that it's more likely to have formed on its own in the early Earth environment.
- iv) RNA is simpler than DNA & proteins: RNA is a simpler molecule than both DNA & proteins. It is composed of only 4 nucleotides, whereas DNA is composed of 4 nucleotides and proteins are composed of 20 amino acids. This simplicity makes it more likely that RNA could have formed through random chemical reactions in the early Earth environment.

- I) Polymerize itself
- II) Cleave itself
- III) Act as an enzyme and this catalytic activity is not dependent on proteins
- IV) Involved in multiple steps of protein synthesis, even ribosomes are $\frac{2}{3}$ rd RNA
- V) Even DNA synthesis is dependent on RNA

→ RNA evolved all the essential methods for storing and expressing genetic information before DNA came onto the scene. However, single-stranded RNA is rather unstable and is easily damaged by enzymes. By essentially doubling the existing RNA molecule, DNA evolved at a much more stable form to pass genetic information with accuracy. Overall, while there is still debate about the exact sequence of events that led to the evolution of life, RNA is currently the most widely accepted candidate for the first biopolymer to evolve.

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→ Our microbiome refers to the collection of microorganisms that live in and on our bodies, primarily in our gut, skin and oral cavities. The microbiome include bacteria, fungi, viruses & other microorganisms which interact with our bodies in various ways

→ Here are some of the functions of our microbiome:

(i) Helps with digestion: The bacteria in our gut microbiome helps break-down complex-carbohydrates, fiber & other nutrients that our body can't digest on its own. These bacteria also produce vitamins (B12, K for Blood Coagulation, Thiamin & Riboflavin) and other compounds that our bodies need to function properly.

(ii) Supports immune system: The microbiome plays a crucial role in regulating our immune system. The bacteria in our gut microbiome help train our immune system to recognize and respond to pathogens, while also preventing harmful bacteria from taking hold in our gut.

(iii) Influences mental health: Recent research has shown that the microbiome can influence our mood & mental health. The gut-brain axis is a 2-way communication system that involves the microbiome and disruptions to this system have been linked to mental-health disorders like depression & anxiety.

(iv) protects against pathogens: Our skin & mucous membranes are home to a diverse community of microorganisms that help protect us from harmful pathogens. These microorganisms compete with harmful bacteria for space & nutrients, preventing them from taking hold & causing infection.

⑤ Regulates metabolism: The microbiome has been shown to play a role in regulating metabolism and body-weight. Changes in the composition of microbiome have been linked to conditions like obesity & type-2 diabetes
→ Determines the effect of medicines
↳ Eg: paracetamol

→ Our gut microbiome acts like an extra organ, helping us to digest molecules in our food that we couldn't break down ourselves, even can steal genes in order to help us digest exotic food. It trains the immune system and also influences your behaviour

④

① Given the mRNA sequence:

5' AUGGUGGCCUUAU CAU UAGGGGCUU 3'

Divide the sequence into codons of 3 nucleotides

AUG GUG GCC UAU CAU UAG GGG CUU

From the codon-usage table:

AUG - Methionine

GUG - Valine

GCC - Alanine

UAU - Tyrosine

CAU - Histidine

UAG - stop codon

GGG - Glycine

CUU - Leucine

Amino acid-sequence of the polypeptide encoded by the above mRNA sequence is:

Met Val Ala Tyr His STOP Gly Leu

→ So, we ignore the STOP codon. Hence the resultant Encoding is:

Met-Val-Ala-Tyr-His

ii) A single-base (point) mutation changing the twelfth base of the mRNA sequence from U to A would result in a different codon being formed. The original codon at this position is "UAU" [coding for Tyrosine], but with A instead of U mutation, the new codon becomes "UAA" (coding for STOP)

Amino acid sequence of the polypeptide encoded by the above mRNA sequence is:

AUG GUG GCC UAA CAU UAG GGG CUU



Met Val Ala STOP His STOP Gly Leu

→ So, we ignore the STOP codon, Hence the resultant encoding is:

Met-Val-Ala

iii)

On making the required change, the new mRNA sequence:

5' AUG CGU GGC CUA UCA UUA GGG GCU 3'

Divide the sequence into codons of 3 nucleotides:

AUG CGU GGC CUA UCA UUA GGG GCU

From the codon usage table:

AUG - Methionine

CGU - Arginine

GGC - Glycine

CUA - Leucine

UCA - Serine

UUA - Leucine

GGG - Glycine
GCU - Alanine

→ Amino acid sequence of the polypeptide encoded by the above mRNA sequence is

Met-Arg-Gly-Leu-Ser-Leu-Gly-Ala

⑤ Restriction endonucleases are a type of endonuclease that recognize & cleave DNA at specific sequences called recognition sites. The recognition site is usually a short DNA sequence, usually 4 to 8 base pairs long and can be palindromic.

By using the same restriction endonuclease to cut both the vector & insert DNA, the two can be joined together end-end.

SARS-COV-2 is novel coronavirus which is an RNA virus, its genetic material is composed of a single-stranded RNA molecule that is approximately 30,000 bases long.

Restriction endonucleases recognize & cleave DNA, but not RNA. The recognition site for endonuclease is GGATCC, which is specific to DNA. However, RNA differs from DNA in that it doesn't contain the base thymine (T) but instead has uracil (U).

Therefore, the BamHI restriction endonuclease cannot recognize or cleave the genomic RNA of the SARS-COV-2 virus (can't cut) ★

Furthermore, even if the SARS-COV-2 genome was double-stranded DNA, BamHI wouldn't be an appropriate restriction endonuclease to use. BamHI recognizes & cuts the sequence GGATCC, which is a 6-base pair sequence that occurs on average once every 4096 base pairs of DNA. The SARS-COV-2 genome is approximately 30,000 base pairs in length, and therefore only contains a few potential BamHI recognition sites.
↳ unlikely to produce useful fragments

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② No, the two restriction enzymes BamHI & BclI will not result in the same number of fragments in a random DNA sequence. This is because they recognize different sequences of nucleotides, and it is possible that one enzyme recognizes a sequence that the other enzyme does not. Additionally, the enzymes may cut the DNA between different bases, resulting in different sized fragments. Therefore, the resulting DNA fragments will be different for each enzyme, even when cutting the same DNA sequence.

If random sequence doesn't imply restrictions on frequencies of A, T, G, C

If random sequence → imposes equal frequencies of bases

(Yes), the length of the enzyme recognition sequence determines the number of fragments of a random DNA sequence it produces.

The length of recognition sequences of the enzyme *Bal II* and *BamH I* are equal. So, we can conclude that 2 enzymes result in the same no. of fragments in a random DNA sequence joining 2 fragments of DNA is easy if both of them can be cut with the same enzyme as cutting generates complementary ends.

because size of pattern is same and all 6-mers will occur with the same frequency in a random DNA sequence

$$P = 1/4^6$$

(b) The advantage of having such a pair of RE's is that they can increase the chance of finding a suitable restriction site for cutting out a specific DNA region of interest isoschizomers. BamHI recognizes the sequence 5'-GGATCC-3' and Bcl II recognizes the sequence 5'-TGATCA-3'. Both enzymes generate a four-base overhang with the sequence 5'-GATC-3'. DNA cloning

For Example: If we want to insert a specific of DNA into a plasmid vector, they can use BamHI to cut the plasmid at a specific loci, creating sticky ends. Then, they can use Bcl II to cut the desired DNA fragment at different location, also creating sticky ends, the DNA fragment can be easily inserted into the plasmid vector and ligated using DNA ligase.

G|GATCCTTAGTA|GATCTG|GATCCA|GATCT

Other advantages include:

- i) Flexibility in cloning strategy: Since the sticky ends are same, DNA fragment generated by either enzyme can be easily ligated together, allowing for more flexibility in cloning strategies.
- ii) Increased efficiency: Having a pair of restriction enzymes with the same sticky ends can increase cloning efficiency.
- iii) Facilitates directional cloning: Directional cloning is the process of inserting a DNA fragment into a vector in a specific orientation. Using a pair of restriction enzymes with the same sticky ends make it easier to perform directional cloning.
- iv) Facilitates swapping of DNA fragments: Having a pair of restriction enzymes with the same sticky ends can make it easier to swap DNA fragments b/w different vectors.

- Site-directed mutagenesis
- Generation of chimeric proteins
- construction of expression libraries
- construction of recombinant viruses

⑦ Cloning and PCR are two methods commonly used to make copies of DNA, but they differ in their approach and application. Here are some advantages & limitations of cloning over PCR

⇒ Advantages of cloning over PCR:

- i) Cloning can produce large quantities of a specific DNA fragment. PCR, on the other hand can produce only a limited amount of DNA, typically upto a few micrograms per reaction
- ii) Cloning can be used to produce multiple copies of a gene/DNA fragment in a vector that can be easily propagated in bacterial/yeast cells. This allows for the long-term storage of the cloned DNA fragment, and it can be retrieved & propagated as needed
- iii) Cloning allows for manipulation & modification of the cloned DNA fragment, including the insertion of new sequences or mutations, which can be useful for functional analysis

iv) Cloning is less prone to errors compared to PCR, which can introduce errors during the amplification process due to the use of thermostable DNA polymerase and other factors

Limitations of cloning over PCR: (2-4 days)
4 hrs for PCR

i) Cloning is time-consuming & labor-intensive process that requires specialized knowledge & skills

ii) Cloning typically involve the use of restriction enzymes and ligases, which can introduce sequence bias and limitations in the choice of restriction sites

iii) Cloning can be limited by the size of the DNA fragment that can be cloned, which is typically up to a few kilobases

iv) Cloning requires the use of competent bacterial/yeast cells which can be challenging to obtain, & it may be suitable for certain types of DNA, such as genomic DNA

PCR can be done outside
in an *in vitro* solution
without living cells

→ For creating clones, restriction enzymes are required whereas, PCR doesn't require any restriction enzyme

cloning replicates DNA
within the cell

→ Cloning generates 2 clones from one parent at a time at a time whereas PCR generates multiple copies of DNA segment

→ Microgram quantity of DNA is required for cloning whereas, only a nanogram quantity of DNA is required to PCR

→ One common source of error in PCR is mispriming and inconsistent ligation reactions in the case of cloning