AS-2320
M.Sc. (Third Semester) Examination, 2013
Paper-: Fifth
FOREST GENETIC DIVERSITY AND CONSERVATION

Time allowed : Three hours

Maximum Marks : 60

Model answer

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Section : A

Q1. All questions are compulsory

1(a). Fill up the blanks [1x1]

(i) Margalef proposed species richness index.

(ii) Convention on Biological Diversity & Agenda 21 advocates strongly for biodiversity inventorying and monitoring.

(iii) World Conservation Union uses biodiversity indicators to determine rare species.

(iv) Exploiter is the indicator of pollution.

(v) Gizzily Bear of North America is considered as an ecosystem engineer.

1(b). True/False [1x1]

(vi) Island biogeography examines the beta diversity of isolated natural communities.

Ans : True

(vii) The karyotypes are used to distinguish protein of species.

Ans : False

(viii) Species migrate to large island in search of resources. This is called rescue effect.

Ans : False

(ix) Random amplified polymorphic DNA markers are most widely used marker in forest trees to date.

Ans : True

(x) Pseudogenes are non-functional copies of genes.

Ans : True

1(c). Short answer [2×1]

(xi) ***Define SSR.***

Simple sequence repeats are 1-6bp long sequences distributed along the chromosomes. These are also known as micro satellites. The number and position of micro satellites vary from individual to individual.

The SSRs analysis has following uses –

- SSRs analysis is used to identify genes associated with specific traits.
- It is used in the construction of genetic maps.
- It is used for the identification of polymorphic genes.
- It distinguishes one organism from others based on the difference in the DNAs.

(xii) ***What is allozyme?***

Allozymes have been the most important type of genetic marker in forestry and are used in many species for many different applications. Allozymes are allelic forms of enzymes that can be distinguished by a procedure called electrophoresis. The more general term for allozymes is isozymes, and refers to any variant form of an enzyme, whereas allozyme implies a genetic basis of the variant form. Most allozyme genetic markers have been derived from enzymes of intermediary metabolism, such as enzymes in the glycolytic pathway. However, conceivably an allozyme genetic marker could be developed from any enzyme.

(xiii***) Name and define restriction enzyme.***

The nuclease enzyme that cuts the DNA at a unique sequence is called restriction endonuclease. They cut the DNA in a non-terminal region. Restriction endonucleases are used to generate rejoinable DNA fragments. They are also called molecular knives, molecular scissors, restriction enzymes or molecular scalpels.

The enzyme breaks two phosphodiester bonds, one in either strand of the duplex DNA to cut the DNA. The 3’ cut end has a free –OH group and 5’ cut end has a phosphate group.

The restriction endonucleases are grouped into three types. The type I and type II restriction enzymes recognize specific sequences in the duplex DNA but cut the DNA far away from the recognition sites. So they are not useful for genetic engineering. The type II restriction endonucleases recognize specific sites and cut the DNA at the recognized sites. So they are of much use in genetic engineering. Eg. Eco RI, Hind III etc.

(xiv) ***Define vulnerable species.***

The species that are under threat such that they may have to be classified as endangered in the near future if causal factors continue to operate. These are species whose populations have been seriously depleted and whose ultimate security is not assured, as well as those species whose populations are still abundant but are under threat throughout their range.

(xv) ***Define EUFORGEN.***

The European Forest Genetic Resources Programme, established in 1995, is a collaborative program among 30 European countries aimed at ensuring the effective conservation and sustainable use of forest genetic resources in Europe. It is coordinated by IGPRI and FAO and funded by the participating nations. EUFORGEN create a forum for forest geneticists to analyze needs, exchange experiences and develop conservation plans for selected species, using both in situ and ex situ methods. The program also contributes to the development of conservation strategies for the ecosystems to which the species belong. The program is divided into five operating networks that focus on conifers, Mediterranean oaks, *Populus nigra,* temperate oaks and beech, and other hardwoods. Individual networks produce work plans that are carried out by participating countries.

Section : B (Attempt any four questions) **(1x10)**

Q2) What is biodiversity? State the importance of biodiversity. What are the threats of biodiversity?

Ans : Biodiversity is the degree of variation of life forms within a given species, ecosystem, biome, or an entire planet. Biodiversity is a measure of the health of ecosystems. Biodiversity is in part a function of climate.

Rapid environmental changes typically cause mass extinctions. One estimate says that less than 1% of the species that have existed on Earth are extinct.

Biodiversity supports many ecosystem services that are often not readily visible. It plays a part in regulating the chemistry of our atmosphere and water supply. Biodiversity is directly involved in water purification, recycling nutrients and providing fertile soils..

Importance of biodiversity

1. Consumptive Value

(a) *Food* –

Food plants exemplify the most fundamental value of biodiversity. Originally, plants were consumed directly from the wild and gathering of wild produce continues throughout the world even today. About 80,000 plants are identified from the wild which are edible. Some 90% of the present crop has originated from the wild variety.

(b) *Medicinal value* – Living organisms provide us with many useful drugs and medicines. About 80% of medicines are extracted from the forest. Eg. Aspirin from Willow bark is used as an anti-inflammatory, Penecillin from Fungus is used as an antibiotic, Quinine from Chincona bark is used for malaria treatment, Vinblastine from Rosy periwinkle plant is used as an anticancer drug etc.

2. Production Value

(a) *Wood based industry* – Wood is one of the few commodities used and traded worldwide that is mainly harvested from wild sources. It is also one of the economically most important commodities in national and international trade. Eg. Ply, furniture, Railway slipper etc.

(b) *Animal based industry –* Animal and other animal products make up another class of commodities of great economic importance in international and national trade that are harvested mainly from wild source. Eg. Apiculture, Sericulture, leather, ivory etc.

3. Economic value

Tourism industry is mainly based on observation of wildlife within protected areas and is a major source of income for many developing countries. Eco-tourism, is now getting more attention and it includes interest in the all species of plants and animals, and forest. Live male lion of 7 years old can generate up to $ 5,15,000. After death, skin value $1,000. Kanyan elephant can generate $1 million, Whale in Queensland cost can generate up to $12 million annually etc.

4. Ecosystem Value

The most significant service of biodiversity to human being is the intake of carbon dioxide and release of oxygen in order to maintain atmosphere equilibrium. It has an important role in conservation of essential characteristics of soil. It affects the degree of rainfall in a region. Forest prevents droughts, floods and soil erosion. It absorbs heat and protects us from severe radiation. It also acts as natural habitats of wildlife, both plants and animals. Beside In fact, forest saves our lives along with other resources.

5. Ethical Value

Moral justification for conservation of biodiversity is based on the belief that species have a moral right to exist, independent of our need for them. Ethical values are deep rooted within human culture, religion and society. But those who look on cost benefit analysis, they overlook these ethical values. Eg. (a) Plants – Tulsi, Pepal, Lotus etc. and (b) Animal – Snake, Porcupine, tiger etc.

Threat to biodiversity

Habitat destruction :

Factors contributing to habitat loss are: overpopulation, deforestation, pollution (air pollution, water pollution, soil contamination) and global warming or climate change.

Deforestation

Deforestation on a human scale results in decline in biodiversity, and on a natural global scale is known to cause the extinction of many species. The removal or destruction of areas of forest cover has resulted in a degraded environment with reduced biodiversity. Forests support biodiversity, providing habitat for wildlife; moreover, forests foster medicinal conservation. With forest biotopes being irreplaceable source of new drugs (such as taxol), deforestation can destroy genetic variations (such as crop resistance) irretrievably.

Human overpopulation :

It is self-evident that the massive growth in the human population through the 20th century has had more impact on biodiversity than any other single factor.

Developmental activities

Deforestation and forest degradation, through agricultural expansion, conversion to pastureland, infrastructure development, destructive logging, fires etc., account for nearly 20% of global greenhouse gas emissions, more than the entire global transportation sector and second only to the energy sector. It is now clear that in order to constrain the impacts of climate change within limits that society will reasonably be able to tolerate, the global average temperatures must be stabilized within two degrees Celsius. This will be practically impossible to achieve without reducing emissions from the forest sector, in addition to other mitigation actions.

Introduced and invasive species :

Human activities have frequently been the cause of invasive species circumventing their barriers, by introducing them for food and other purposes. Human activities therefore allow species to migrate to new areas (and thus become invasive) occurred on time scales much shorter than historically have been required for a species to extend its range.

Genetic pollution:

Endemic species can be threatened with extinction through the process of genetic pollution, i.e. uncontrolled hybridization, introgression and genetic swamping.

Overexploitation:

Overexploitation occurs when a resource is consumed at an unsustainable rate. This occurs on land in the form of overhunting, excessive logging, poor soil conservation in agriculture and the illegal wildlife trade.

Climate change :

Global warming is also considered to be a major threat to global biodiversity. For example coral reefs which are part of the biodiversity hot spot. It will be lost in 20 to 40 years if global warming continues at the current trend.

Q3) What are in-situ and ex-situ conservation? How biodiversity could be conserved by it?

Ans : Conservation is the management by which the sustainable use of resources can be managed for the present generation. It includes in-situ and ex-situ conservation which are as follows -

(A) In-situ Conservation

This includes conservation of species within their natural and native area. There are three different types of in-situ conservation which are as follows -

(a) Protected areas :

An area declared under the Wild Life (Protection) Act, 1972 for the protection of wildlife in India. As on today, there are four types of protected areas in India namely-

(i) National Park :

A large area which received highest level of protection under Section 35 of the Wild Life (Protection) Act, 1972 for the conservation of Wildlife is called National Park. There are about more than 100 National Park in India. In Chhattisgarh, there are 4 national parks. These are the area where biodiversity especially the wildlife species are received highest level of protection.

(ii) Wildlife Sanctuary :

A large area which received medium level of protection under Section 18A of the Wild Life (Protection) Act, 1972 for the conservation of Wildlife is called Wildlife Sanctuary. There are about more than 500 wildlife sanctuaries in India. In Chhattisgarh, there are 11 wildlife sanctuaries. These are the area where biodiversity especially the wildlife species are received medium level of protection.

(iii) Conservation Reserve :

A small area owned by the government located very adjacent to a national park or wildlife sanctuary which received medium level of protection under Section 36A of the Wild Life (Protection) Act, 1972 for the conservation of Wildlife is called Conservation Reserve. These are the area where biodiversity especially the wildlife species are received medium level of protection.

(iv) Community Reserve :

A small area *owned by the local people* located very adjacent to a village area which received medium level of protection under Section 36C of the Wild Life (Protection) Act, 1972 for the conservation of Wildlife is called Community Reserve. These are the area where biodiversity especially the wildlife species are received medium level of protection.

(b) Reserve Areas

(i) Biosphere Reserve

A biosphere reserve is a kind of conservation reserve (forested area) created to protect the *biological and cultural diversity* of a region while promoting sustainable economic development.

A biosphere reserve is a unique kind of protected area that differs from a Protected Area (National Park, Wildlife Sanctuary, Conservation Reserve and Community Reserve). There are three very different, but equal, aims:

- *conservation* of genetic resources, species, and ecosystems;
- scientific *research and monitoring*; and
- promoting *sustainable development* in communities of the surrounding region.

(ii) Tiger Reserve

A large area which received highest level of protection under the Wild Life (Protection) Act, 1972 for the conservation of tiger and their habitat is called tiger reserve. There are about 42 tiger reserves in India.

(iii) Elephant Reserve

A large area which received highest level of protection under the Wild Life (Protection) Act, 1972 for the conservation of elephant and their habitat is called tiger reserve. There are about 32 tiger reserves in India.

(c) Other area

(i) Sacred grove

A sacred grove or sacred woods are any grove of trees of special religious importance to a particular culture. In India, sacred groves are scattered all over the country. Around 14,000 sacred groves have been reported from all over India.

The sacred grove acts as reservoirs of rare fauna, and more often rare flora, amid rural and even urban settings. However they are not protected under federal legislation. Some NGOs work with local villagers to protect such groves. Each grove is associated with a presiding deity, and the groves are referred to by different names in different parts of India. They were maintained by local communities with hunting and logging strictly prohibited within these patches.

(B) Ex-situ Conservation

It is the conservation of selected rare plants and animals in places outside their natural habitat. These include offsite collection and gene banks. Important areas under this conservation are:

(a) Gene bank: Gene banks are a type of bio repository which preserves genetic material. In plants, this could be by freezing cuts from the plant, or stocking the seeds. In animals, this is the freezing of sperm and eggs in zoological freezers until further need. With corals, fragments are taken which are stored in water tanks under controlled conditions.

In plants, it is possible to unfreeze the material and propagate it, however, in animals; a living female is required for artificial insemination. While it is often difficult to utilize frozen animal sperm and eggs, there are many examples of it being done successfully.

Types of gene banks

(i) Seed bank

The seed bank preserves dried seeds by storing them at a very low temperature. Spores and pteridophytes are conserved in seed banks, but other seedless plants, such as tuber crops cannot be preserved this way. The largest seed bank in world is the Millennium Seed Bank housed at the Wellcome Trust Millennium Building (WTMB), located in the grounds of Wakehurst Place in West Sussex, near London.

(ii) Tissue bank

In this technique buds, meristematic cells etc are conserved through particular light and temperature arrangements in a nutrient medium. This technique is used to preserve seedless plants and plants which reproduce asexually.

(iii) Cryobank

In this technique, a seed or embryo is preserved at very low temperatures. It is usually preserved in liquid nitrogen at -196°C. This is helpful for the conservation of species facing extinction.

(iv) Pollen bank

This is a method in which pollen grains are stored. Using this technique, plants with one set chromosome can be made.

(v) Field gene bank

This is a method of planting plants for the conservation of gene. For this purpose ecosystem is artificially constructed. Through this method one can compare the difference among plants of different species and can study it in detail. It needs more land, adequate soil, weather, etc.. Germ plasma of important crops is conserved through this method. 42,000 varieties of rice are conserved in the Central Rice Research Institute in Orissa.

(b) Arboretum: An arboretum (plural: arboreta) in a narrow sense is a collection of trees only. Related collections include a fruticetum (from the Latin *frutex*, meaning *shrub*), and a viticetum, a collection of vines. More commonly, today, an arboretum is a botanical

garden containing living collections of woody plants intended at least partly for scientific study. An arboretum specializing in growing conifers is known as a pinetum. Other specialist arboreta include salicetums (willows), populetums and quercetums.

(c) Zoos and Safari Parks

A zoo (short for zoological park or zoological garden, and also called a menagerie) is a facility in which animals are confined within enclosures, displayed to the public, and in which they may also be bred. The abbreviation "zoo" was first used of the London Zoological Garden, which opened for scientific study in 1828 and to the public in 1847. The number of major animal collections open to the public around the world now exceeds 1,000, around 80 percent of them in cities.

Some zoos keep fewer animals in larger, outdoor enclosures, confining them with food and fences, rather than in cages are called Safari Park. These are also known as zoo parks and lion farms, allow visitors to drive through them and come in close contact with the animals.

(d) Botanical Garden

A botanical garden is a well-tended area displaying a wide range of plants labeled with their botanical names. It may contain specialist plant collections

Botanical gardens are often run by forest department, universities or other scientific research organizations, and often have associated herbaria and research programmes in plant taxonomy or some other aspect of botanical science. In principle, their role is to maintain documented collections of living plants for the purposes of scientific research, conservation, display, and education, although this will depend on the resources available and the special interests pursued at each particular garden. By doing this, the biodiversity is conserved.

Disadvantages of in-situ conservation

- It requires large area for the conservation of species.
- It requires huge man power for the protection of area
- It require huge fund to maintain the area
- Maintaining the fringe villager and hunter is very difficult.

Disadvantages of ex-situ conservation

- In order to prevent genetic drift, the population in captivity has to be larger than that they have been maintaining in most of the cases.
- Animals in captivity may undergo genetic adaptation to their artificial conditions and thus become useless for the natural habitats.
- Animals in captive population may lose their knowledge of the natural environment. This makes them incapable to live in the wild.
- The ex-situ populations may represent only a limited portion of the gene pool of the species.
- Ex-situ conservation efforts require a regular supply of resources, funds and other moral supports.
- Ex-situ conservation efforts are usually concentrated in one or few places. There are possibilities that the entire population of endangered species may be wiped out in a single stroke of natural calamity.

Q4) Write Short Notes on

(a) Karyotypic variation

A karyotype is the number and appearance of chromosomes in the nucleus of a Eukaryotic cell. The term is also used for the complete set of chromosomes in a species or an individual organism.

Karyology

The study of whole sets of chromosomes is sometimes known as karyology. The chromosomes are depicted (by rearranging a microphotograph) in a standard form, it is known as a karyogram or ideogram; in pair ordered by size and position of centromere for chromosomes of the same size.

The basic number of chromosomes in the somatic cells of an individual or a species is called the somatic number and is designated as 2n. Thus, in human 2n=46. In the germ –line (the sex cell) the chromosome number is n. So in normal diploid organism, autosomal

chromosomes are present in two copies. There may or may not be sex chromosomes. Polyploid cells have multiple copies of chromosomes and haploid cells have single copy.

Importance of karyotypic analysis

The study of karyotype is important for cell biology and genetics and the results may be used in evolutionary biology. Karyotype can be used for much purpose- such as to study chromosomal aberrations, cellular function, taxonomic relationship and information about past evolutionary events.

Staining

The study of karyotype is made possible by staining. Usually a suitable dye such as Giemsa is applied after cells have been arrested during cell division by a solution called Colchicine. For human, white blood cells are used most frequently because they are easily induced to divide and grow in tissue culture. Sometimes observation may be made on non-dividing (interface) cells. The sex of an unborn fetus can be determined by observation of interface cells.

Observation

Six different characteristics of karyotypes are usually observed and compared-

i) Differences in absolute sizes of chromosomes. Chromosomes can vary in absolute size by as much as twenty fold between genera of the same family. Eg. The Legumes (*Lotus tenuis*) and *Vicia foba* each have six pairs of chromosomes, yet *V. foba* chromosomes are many times larger. These differences probably reflect different amounts of DNA duplications.

ii) Differences in the position of centromere. Those differences probably came about through translocation.

iii) Differences in relative size of the chromosomes. These differences probably arose from segmental interchange of unequal length.

iv) Differences in basic number of chromosomes. These differences could have resulted from successive unequal translocation which removed all the essential genetic materials from a chromosome, permitting its loss without penalty to the organism or through fusion.

Human have one pair fewer chromosomes than the great apes. Human chromosome appears to have resulted from two ancestral chromosomes and many of the genes of those two original chromosomes have been translocated to other chromosomes.

v) Difference in number and position of satellite. Satellites are small bodies attached to a chromosome by a thin thread.

vi) Difference in degree and distribution of heterochromatic regions.

A full account of karyotype may therefore include the number, type, shape and banding of the chromosome as well as other cytogenetic information.

Variation is often found :-

i) Between the sexes.

ii) Between the germ-line and between gametes and the rest of the body.

iii) Between the members of population.

iv) In abnormal individuals.

(b) Neutral allele model

The Neutral allele model is a mathematical model for calculating genetic mutations. The Japanese geneticist Motoo Kimura and American geneticist James F. Crow (1964) introduced the Neutral allele model, an attempt to determine for a finite diploid population what proportion of loci would be homozygous. This was, in part, motivated by assertions by other geneticists that more than 50 percent of *Drosophila* loci were heterozygous, a claim they initially doubted. In order to answer this question they assumed first, that there were a large enough number of alleles so that any mutation would lead to a different allele (that is the probability of back mutation to the original allele would be low enough to be negligible); and second, that the mutations would result in a number of different outcomes from neutral to deleterious.

They determined that in the neutral case, the probability that an individual would be homozygous, *F*, was:

$$F = \frac{1}{4N_e u + 1}$$

where *u* is the mutation rate, and N_e is the effective population size. The effective number of alleles *n* maintained in a population is defined as the inverse of the homozygosity, that is

$$n = \frac{1}{F} = 4N_e u + 1$$

which is a lower bound for the actual number of alleles in the population.

If the effective population is large, then a large number of alleles can be maintained. However, this result only holds for the *neutral* case, and is not necessarily true for the case when some alleles are subject to selection, i.e. more or less fit than others, for example when the fittest genotype is a heterozygote (a situation often referred to as over dominance).

In the case of overdominance, because Mendel's second law (the law of segregation) necessarily results in the production of homozygotes (which are by definition in this case, less fit), this means that population will always harbour a number of less fit individuals, which leads to a decrease in the average fitness of the population. This is sometimes referred to as *genetic load*, in this case it is a special kind of load known as *segregational load*. Crow and Kimura showed that at equilibrium conditions, for a given strength of selection (*s*), that there would be an upper limit to the number of fitter alleles (polymorphisms) that a population could harbour for a particular locus. Beyond this number of alleles, the selective advantage of presence of those alleles in heterozygous genotypes would be cancelled out by continual generation of less fit homozygous genotypes.

These results became important in the formation of the neutral theory, because neutral (or nearly neutral) alleles create no such segregational load, and allow for the accumulation of a great deal of polymorphism. When Richard Lewontin and J. Hubby published their groundbreaking results in 1966 which showed high levels of genetic variation in Drosophila via protein electrophoresis, the theoretical results from the infinite alleles model were used by Kimura and others to support the idea that this variation would have to be neutral.

Q5) Write about the various techniques to assess the genetic diversity.

The genetic diversity of plants could be assessed by different markers basically a genetic or molecular marker. Genetic or molecular marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed. A genetic marker may be a short DNA sequence.

There are three important markers that are used in genome analysis

(1) ***Morphological markers***: These markers are dependent on environmental and physical factors. These markers are most important in investigation in plant breeding field. But they are time consuming, labor intensive and large numbers of plants are required.

(2) ***Biochemical markers***: Here monoterpenes, allozymes etc are used. These are fairly easy to apply and standard protocols are also available.

(3) ***Molecular markers***: The variant DNA fragment that gives some information about gene of interest is called molecular marker. These are of two types: (a) Non-PCR based (b) PCR based

(a) Non-PCR based marker:

Restriction Fragment Length Polymorphism

The variation in the restriction DNA fragment length between individual of a species is called RFLP. The RFLP method involves the following steps:

i) Sample collection: Here tissues or cells are collected to extract their DNA.

ii) DNA isolation: With the help of standard procedure, DNA of the collected samples are isolated and purified to get genomic DNA.

iii) Restriction Digestion with restriction enzyme.

Genomic DNA of each sample is cut with restriction enzymes like EcoRI, Hind III etc. The restriction digest is divided into two halves: one half is used for DNA detection and other is used for probe making.

iv) Electrophoresis : The digested genomic DNA of all the samples are loaded into separate wells in polyacrylamide gel and subjected to electrophoresis. According to size, DNA fragments are separated.

v) Blotting of DNA: The DNA is transferred to Nitrocellulose filter (Southern blotting) by using blotting setup.

vi) Making Genomic DNA Probes

One half of genomic DNA digest from each sample is electrophoresed to separate the DNA fragments. Fragments of 0.5-2.0kb are extracted from the gel and cloned in pUC21 or pBluScript vector to construct rDNAs. These rDNAs are amplified by introducing them into bacterial host and the amplified rDNA are reisolated from the bacteria. The target DNA fragments are excised from the rDNA by using the same restriction enzyme that was used for cutting the DNA. They are purified by electrophoresis.

The DNA fragments are radiolabelled with p^{35} isotope incorporated nucleotide by nick translation or random primer extension or end labelling. As a result, genomic probes are formed. In some cases, non-radioactive labelling is also recommended.

vii) Nucleic Acid Hybridization

DNA blotted nitrocellulose membrane is kept immersed in a hybridization solution containing genomic DNA probes to bring out hybridization. After hybridization, unbound probes are washed out of the membrane.

viii) Autoradiography

Images of radioactive probes are captured on an X-ray film using autoradiography. The autoradiogram shows DNA bands in distinct lanes each of which is characteristic of an individual.

Application

They can be applied in diversity and phylogenetic studies ranging from individuals within species or closely related species as well as in gene mapping studies.

(b) PCR based –

Random Amplified Polymorphic DNAs (RAPDs)

The set of DNAs generated by the random PCR is called random amplified polymorphic DNA.

Major steps of RAPD Analysis

The RAPD analysis involves the following steps-

i) Sample cells or tissues are collected from individuals to be distinguished from one another.

ii) Genomic DNA of each and every sample is isolated by using a standard procedure.

iii) Each genomic DNA is separately treated with Taq polymerase, a primer, dATP, dTTP, dCTP, dGTP and polymerization buffer. The primer is 9-10 base long so that it is often called decaoligonucleotide primer.

iv) All these reaction mixtures are kept separately in a PCR equipment to do repeated cycles of polymerase reactions.

v) As a result of PCR amplification, each reaction tube contains random amplified polymorphic DNA fragments. Each of these DNA fragment has a flanking primer at its 5' end. Ethidium bromide is added to it.

vi) The RAPDs thus obtained from the samples are separately loaded into wells of an agarose or polyacrylamide gel and molecular weight markers are added to one well.

vii) The loaded DNAs are electrophoresed to separate the DNA fragments based on their size.

viii) The electrophoresed gel is examined under a UV light illuminator to detect light bands and photographed.

Application: They are used in gene mapping studies

(c) **Variable Number of Tandem Repeats (VNTRs)**

DNA of eukaryotic organisms has several repeating units of short sequences called tandem repeats.

The variation in the number of tandem repeats between two or more individuals is called variable number of tandem repeats (VNTRs). The VNTRs are used as genetic markers to detect polymorphic genes on chromosomes and to distinguish one individual from others.

The VNTRs analysis involves the following steps –

i) DNA is isolated from the individuals (say for example 5 individuals) using a standard procedure.

ii) It is cut into small pieces using a restriction enzyme separately.

iii) A primer is designed in such a way that it binds on the 5' end of the tandem repeat on the DNA.

iv) The primer is labelled with radioactive nucleotide or fluorescent tag and mixed with the restriction digest.

v) To this reaction mixture, dATP, dCTP, dGTP and dTTP are added. Tag polymerase is added to it to proceed DNA polymerization.

vi) This reaction mixture is taken in a screw capped tube and kept in a thermocycler of PCR apparatus. In this way, DNA of the five individuals are kept in the PCR apparatus in separate tubes.

vii) PCR apparatus is programmed to proceed the PCR reactions. The primer anneals with the tandem repeats. Simultaneously, DNA polymerase adds complementary nucleotides one by one to the primer to form a daughter DNA. Thus the tandem repeats are replicated in the PCR.

viii) The PCR products are heated to 72^0C for 2 minutes and loaded in separate wells of a polyacrylamide gel. Molecular weight markers are loaded in one well. The samples are electrophoresed to separate the DNA fragments.

ix) After electrophoresis, the images of DNA bands are captured on photographic film using a transilluminator. If the primer was radiolabelled, autoradiography is used to get the autoradiogram.

x) The image is developed and used to detect variant fragments in relations to position of bands on the gel. These variant fragments distinguish one individual from other.

(d) Simple Sequence Repeats (SSRs)

Simple sequence repeats are 1-6 bp long sequences distributed along the chromosomes. These are also known as micro satellites. The number and position of micro satellites vary from individual to individual.

The SSRs analysis involves the following steps –

i) DNA is isolated from different individuals of a population.

ii) The DNAs are separately cut into small fragments using a restriction enzyme.

iii) Primer of 18-25 bp is synthesized and its end is labelled with a fluorescent dye.

iv) Primer, dATP, dCTP, dGTP, dTTP and Taq polymerase are added to it.

v) The reaction mixtures are taken in separate tubes and kept in a thermocycler of PCR apparatus.

vi) The PCR machine is programmed to do PCR reaction for the selective amplification of the DNA fragments.

vii) The PCR products are electrophoresed in separate lanes along with markers in one lane.

viii) Images of DNA bands are photographed.

ix) These images are visualized and analyzed automatically using a powerful computer.

Uses of SSRs analysis

i) SSRs analysis is used to identify genes associated with specific traits.

ii) It is used in the construction of genetic maps.

iii) It is used for the identification of polymorphic genes.

iv) It distinguishes one organism from others based on the difference in the DNAs.

(e) **Amplified Fragment Length Polymorphism (AFLP)**

The AFLP detects the presence or absence of a fragment but not the lengths of the fragments.

AFLP involves the following steps –

i) DNA is isolated from two or more individuals of a population using a standard procedure.

ii) Each of these DNAs is cut with two restriction enzymes to generate short DNA fragments. One enzyme that prefers 6 bp target sites (Eg. EcoRI) and another enzyme that cuts at 4 bp target sites (Eg. Mse 1) are chosen to cut the DNA. This treatment generates short DNA fragments with less than 1 kb size for every PCR application.

iii) The DNA is heated to denature the duplex strand into single strands.

iv) EcoR 1 adaptor and Mse 1 adaptor are added to the DNA. The EcoR 1 adaptor binds with one end of the DNA fragment having EcoR 1 cut end. The Mse 1 binds with Mse 1 cut end of the other strand.

v) Primers that can bind with EcoR 1 and Mse 1 site are synthesized in such a way as to have radioactive p^{32}. The EcoR 1 primer is attached with three selective bases and Mse 1 primer is attached with one selective base.

vi) PCR is carried out with the restriction digest and the two primers for the selective amplification of DNA fragments.

vii) The PCR products thus obtained (from two individuals) are electrophoresed in separate lanes on a polyacrylamide gel.

viii) The images of DNA bands on the gel are captured on an X-ray film by means of autoradiography.

ix) The autoradiogram is visualized to resolve the bands and interpret the findings.

Q6) Mention about the Biological Diversity Act, 2002.

Ans : The Biological Diversity Act was enacted in 2002 and Biological Diversity Rules were notified in 2004 by the Government of India to conserve, sustainably use, fairly and equitably share the benefits arising out of the use of India's biological resources and associated knowledge. Details of the Biological Diversity Act, 2002 is given below -

CHAPTER - I

Preliminary

1. Short title, extent and commencement

This Act may be called the Biological Diversity Act, 2002. It extends to the whole of India.

2. Definitions- include various definitions. Some of them area-

Biological diversity -means the variability among living organisms from all sources and the ecological complexes of which they are part, and includes diversity within species or between species and of eco-systems

Biological resources- means plants, animals and micro-organisms or parts thereof, their genetic material and by-products (excluding value added products) with actual or potential use or value, but does not include human genetic material;

CHAPTER - II

Regulation of Access to Biological Diversity

3. **Certain persons not to undertake Biodiversity related activities without approval of National Biodiversity Authority**

(1) No person referred to in sub-section (2) shall, without previous approval of the National Biodiversity Authority, obtain any biological resource occurring in India or knowledge associated thereto for research or for commercial utilization or for bio-survey and bio-utilization.

Results of research not to be transferred to certain persons without approval of National Biodiversity Authority

4. No person shall, without the previous approval of the National Biodiversity Authority, transfer the results of any research relating to any biological resources occurring in, or obtained from, India for monetary consideration or otherwise to any person who is not a citizen of India or citizen of India who is non-resident as defined in clause (30) of section 2 of the Income-tax Act, 1961 or a body corporate or organization which Is not registered or incorporated in India or which has any non-Indian participation in its share capital or management.

7. No person, who is a citizen of India or a body corporate, association or organization which is registered in India, shall obtain any biological resource for commercial utilization, or bio-survey and bio-utilization for commercial utilization except after giving prior intimation to the State Biodiversity Board concerned: - Provided that the provisions of this section shall not apply to the local people and communities of the area, including growers and cultivators of biodiversity, and *vaids* and *hakims*, who have been practicing indigenous medicine.

CHAPTER - III

National Biodiversity Authority

Establishment of National Biodiversity Authority

8. The National Biodiversity Authority shall consist of the following members - a Chairperson, three ex officio members to be appointed by the Central Government (one representing the Ministry dealing with Tribal Affairs and two representing the Ministry

dealing with Environment and Forests of whom one shall be the Additional Director General of Forests or the Director General of Forests), seven ex officio members to be appointed by the Central Government to represent different Ministries of the Central Government (Agricultural Research and Education, Biotechnology, Ocean Development, Agriculture and Cooperation, Indian Systems of Medicine and Homoeopathy, Science and Technology and Scientific and Industrial Research), five non-official members to be appointed from amongst specialists and scientists

CHAPTER - IV

Functions and Powers of the National Biodiversity Authority

18. **Functions and powers of National Biodiversity Authority**

The National Biodiversity Authority may grant approval for undertaking any activity referred to in sections 3, 4 and 6.

The National Biodiversity Authority may advise the Central and State Governments matters relating to the conservation of biodiversity, sustainable use of its components and equitable sharing of benefits arising out of the utilization of biological resources.

CHAPTIER - V

Approval by the National Biodiversity Authority

19. Any person referred. to in sub-section (2) of section 3 who intends to obtain any biological resource occurring in India or knowledge associated thereto for research or for commercial utilization or for bio-survey and bio-utilization or transfer the results of any research relating to biological resources occurring in, or obtained from, India, shall make application in such form and payment of such fees as may be prescribed, to the National Biodiversity Authority.

20. No person who has been granted approval under section 19 shall transfer any biological resource or knowledge associated thereto which is the subject matter of the said approval except with the permission of the National Biodiversity Authority.

CHAPTER - VI

State Biodiversity Board

22. **Establishment of State Biodiversity Board**

The Board shall consist of the following members, namely- a Chairperson, not more than five ex officio members to be appointed by the State Government to represent the concerned Departments of the State Government, not more than five members to be appointed from amongst experts in matters relating to conservation of biological diversity, sustainable use of biological resources and equitable sharing of benefits arising out of the use of biological resources.

23. **Functions of State Biodiversity Board**

The functions of the State Biodiversity Board shall be to–advise the State Government, subject to any guidelines issued by the Central Government, on matters relating to the conservation of biodiversity, sustainable use of its components and equitable sharing of the benefits arising out of the utilization of biological resources;

CHAPER - VII

Finance, Accounts and Audit of National Biodiversity Authority

26. Grants or loans by the Central Government

27. Constitution of National Biodiversity Fund

28. Annual report of National Biodiversity Authority

29. Budget accounts and audit

30. Annual Report to be laid before parliament

CHAPTER - VIII

Finance, Accounts and Audit of State Biodiversity Board

31. Grants of money by State Government to State Biodiversity Board

32. Constitution of State Biodiversity Fund

CHAPTER - IX

Duties of the Central and the State Governments

36. Central Government to develop National strategies plans. Etc., for conservation, etc., of biological diversity

The Central Government shall develop national strategies, plans, programmes for the conservation and promotion and sustainable use of biological diversity including measures for identification and monitoring of areas rich in biological resources, promotion of *in situ*, and *ex situ*, conservation of biological resources, incentives for research, training and public education to increase awareness with respect to biodiversity.

37. Biodiversity heritage sites

The State Government, in consultation with the Central Government, may frame rules for the management and conservation of all the heritage sites.

38. Power of Central Government to notify threatened species

Without prejudice to the provisions of any other law for the time being in force, the Central Government, in consultation with the concerned State Government, may from time to time notify any species which is on the verge of extinction or likely to become extinct in the near future as a threatened species and prohibit or regulate collection thereof for any purpose and take appropriate steps to rehabilitate and preserve those species.

CHAPTER - X

Biodiversity Management Committees

41. Constitution of Biodiversity Management Committee

Every local body shall constitute a Biodiversity Management Committee within its area for the purpose of promoting conservation, sustainable use and documentation of biological diversity including preservation of habitats, conservation of land races, folk varieties and cultivars, domesticated stocks and breeds of animals and microorganisms and chronicling of knowledge relating to biological diversity.

CHAPTER - XI

Local, Biodiversity Fund

42. Grants to Local Biodiversity Fund

43. Constitution of Local Biodiversity Fund

44. Application of Local Biodiversity Fund

45. Annual Report of Biodiversity Management Committees

46. Audit of accounts of Biodiversity Management Committees

47. Annual report, etc, of the Biodiversity Management Committee to be submitted to district Magistrate

CHAPTER - XII

Miscellaneous

55. Penalties

Whoever contravenes or to or abets the contravention of the provisions of section 3 or section 4 or section 6 shall be punishable with imprisonment for a term which may extend to five years, or with fine which may extend to ten lakh rupees and where the damage caused exceeds ten lakh rupees such fine may commensurate with the damage caused, or with both.

60. **Power of Central Government to give directions to State Government**

The Central Government may give directions to any State Government as to the carrying into execution in the State of any of the provisions of this Act or of any rule or regulation or order made there under.

63. **Power of State Government to make rules**

The State Government may, by notification in the Official Gazette, make rules for carrying out the purposes of this Act.

64. **Power of remove difficulties**

The National Biodiversity Authority shall, with the previous approval of the Central Government, by notification in the Official Gazette, make regulations for carrying out the purposes of this Act.

65. Power to remove difficulties

If any difficulty arises in giving effect to the provisions of this Act, the Central Government may, by order, not inconsistent with the provisions of this Act, remove the difficulty: Provided that no such order shall be made after the expiry of a period of two years from the commencement of this Act.

Every order made under this section shall be laid, as soon as may be, after it is made, before each House of Parliament.

Q7) Discuss about genetic erosion.

Genetic erosion is the process by which the removal or loss of genes takes place. It is very clear that the gene will loss one time it will never get back as such. So the genetic erosion is a severe form of loss of good genes from the nature.

Cause of genetic erosion

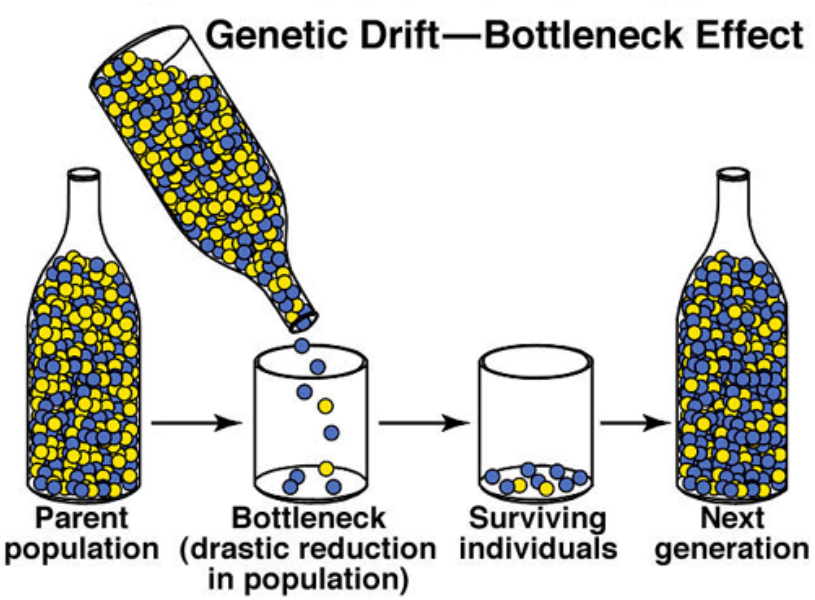
There are several causes of genetic erosion but it is broadly divided in two categories-

(a) Technical cause :

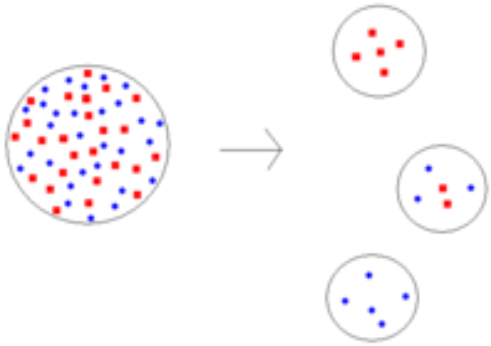
i) *Genetic drift* – Genetic drift is an evolutionary force operating in small populations. It was described by SEWALL WRIGHT in 1931. It changes the gene frequency in small population purely by chance. The gene frequency will continue to fluctuate until one allele is lost and other is fixed. In small population, heterozygous gene pairs tend to become homozygous by chance. Genetic drift tends to preserve or destroy genes without distinction, whether favourable, neutral or unfavourable.

ii) *Bottle Neck Effect* – This term was coined by STEBBINS. A population may be formed of thousands of individuals; but in the next season, only a few individuals may survive. This few individuals form the progenitors for the population in the next season. This yearly cyclic phenomena of population is called bottle neck effect. Due to this effect, following could happen :-

- Sperm count will reduce
- Vigour is less
- Low productivity
- Chances of extinction



iii) *Founder Effect* – In population genetics, the founder effect is the loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population. It was first fully outlined by Ernest Mayr in 1942, using existing theoretical work by those such as Sewall. As a result of the loss of genetic variation, the new population may be distinctively different, both genotypically and phenotypically, from the parent population from which it is derived.



In the figure shown, the original population has nearly equal numbers of blue and red individuals. The three smaller founder populations show that one or the other colour may

predominate (founder effect), due to random sampling of the original population. It leads to loss of valuable genes from a population.

iv) *Serial Founder Effect* - Serial founder effects have occurred when populations migrate over long distances. Such long distance migrations typically involve relatively rapid movements followed by periods of settlement. The populations in each migration carry only a subset of the genetic diversity carried from previous migrations. As a result, loss of some valuable genes may occur from the original population.

(v) *Inbreeding depression* – Inbreeding depression is the reduced fitness in a given population as a result of breeding of related individuals. This causes genetic erosion. It is often the result of a population bottleneck. In general, the higher the genetic within a breeding population, the less likely it is to suffer from inbreeding depression. Inbreeding depression seems to be present in most groups of organisms, but varies across mating systems.

Breeding between closely related individuals, called inbreeding, may on one hand result in more recessive deleterious traits manifesting themselves, because the genomes of pair-mates are more similar: recessive traits can only occur in offspring if present in both parents genomes, and the more genetically similar the parents are, the more often recessive traits appear in their offspring. Consequently, the more closely related the breeding pair is, the more homozygous deleterious genes the offspring may have, resulting in very unfit individuals.

vi) Less sex ratio- It is also responsible for the genetic erosion. Technically the sex ration should be 3:1 (F/M). If the ration is less than this, it is also the reason for genetic erosion.

(b) General cause

These are the following cause of genetic erosion –

i) Habitat destruction – Habitat destruction either by natural or anthropogenic cause is the reason of genetic erosion.

ii) Deforestation – Deforestation for the construction purpose is snatching the home or loss of organism. When they die in this way genetic erosion can take place.

iii) Pollution – Pollution is the big reason for genetic erosion. It may be by the industry of pesticides. Some pesticides like DDT is checking the formation of egg shell in the bird. Thus the death rate is high and it leads to the genetic erosion.

iv) Population pressure – There is rapid increase in population which results an increase in the rate of genetic erosion.

v) Isolation – Habitat isolation is also the problem of genetic erosion.

Methods for checking genetic erosion

To control the genetic erosion, there are following measures can be applied—

(a) In-situ gene conservation -

In this method, genes are conserved in their natural habitat. Eg. National Park, Wildlife Sanctuary etc.

(b) Ex-situ conservation –

In this method, gene can be conserved outside of their natural habitat. Eg. Gene Bank, Botanical garden etc.

(c) Other methods –

There are the following way by which the genetic erosion can be reduced-

- Afforestation
- Population control
- Pollution control
- Habitat management

Q8) Write Short Notes on

(a) Factors affecting island biogeography

Island biogeography is a field within biogeography that examines the factors that affect the species richness of isolated natural communities. The theory was developed to explain

species richness of actual islands. It has since been extended to mountains surrounded by deserts, lakes surrounded by dry land, fragmented forest and even natural habitats surrounded by human-altered landscapes. Now it is used in reference to any ecosystem surrounded by unlike ecosystems. The field was started in the 1960s by the ecologists Robert MacArthur and E.O. Wilson, who coined the term *island biogeography*, as this theory attempted to predict the number of species that would exist on a newly created island.

A number of factors that affect the island biogeography are:

- Degree of isolation (distance to nearest neighbour, and mainland)
- Length of isolation (time)
- Size of island (larger area usually facilitates greater diversity)
- The habitat suitability which includes:
    - Climate (tropical versus arctic, humid versus arid, etc.)
    - Initial plant and animal composition if previously attached to a larger land mass (e.g. marsupials, primates)
    - The current species composition
- Location relative to ocean currents (influences nutrient, fish, bird, and seed flow patterns)
- Serendipity (the impacts of chance arrivals)
- Human activity

It had been realized the potential application of island biogeography concept to the field of conservation biology. The idea that reserves and national park formed islands inside human-altered landscapes (habitat fragmentation), and that these reserves could lose species as they 'relaxed towards equilibrium' (that is they would lose species as they achieved their new equilibrium number, known as ecosystem decay) caused a great deal of concern. This is particularly true when conserving larger species which tend to have larger ranges.

Island biogeography theory also led to the development of habitat corridors as a conservation tool to increase connectivity between habitat islands. Habitat corridors can increase the movement of species between parks and reserves and therefore increase the

number of species that can be supported, but they can also allow for the spread of disease and pathogens between populations, complicating the simple proscription of connectivity being good for biodiversity.

In species diversity, island biogeography most describes allopatric speciation. Allopatric speciation is where new gene pools arise out of natural selection in isolated gene pools. Island Biogeography is also useful in considering sympatric speciation, the idea of different species arising from one ancestral species in the same area. Interbreeding between the two differently adapted species would prevent speciation, but in some species, sympatric speciation appears to have occurred.

(b) IUCN categories of protected area.

A protected area is defined as "an area of land and / or sea especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means" (IUCN, 1994). The main purpose for establishment and management of protected area are identified as :-

- Scientific research
- Wilderness protection
- Preservation of species and genetic diversity
- Maintenance of environmental services
- Protection of specific natural and cultural features
- Tourism and recreation
- Education
- Sustainable use of resources from natural ecosystem
- Maintenance of cultural and traditional attributes.

Based on the possible mix of priorities that can be generated by the management objectives, protected area have been grouped into following six categories:-

Category –I : Strict Nature Reserve/ Wilderness Area – Managed mainly for science or wilderness protection (includes two sub-categories : Strict Nature Reserve – Ia and Wilderness Area – Ib).

Category – II : National Park – Managed for ecosystem protection and recreation.

Category – III : National Monument – Managed mainly for conservation of specific natural features.

Category – IV : Habitat / Species Management Area – Managed mainly for conservation through management intervention.

Category – V : Protected Landscape/ Seascape – Managed mainly for landscape / seascape.

Category –VI : Managed Resource Protected Area – Managed mainly for the sustainable use of natural systems.