Biodiversity, classification and conservation (chapter 18):

- A **species** is a group of organisms with similar morphological, physiological, biochemical and behavioural features, which can breed together naturally to produce fertile offspring, and are reproductively isolated from other species
- An **ecosystem** is a relatively self-contained, interacting community of organisms, and the environment in which they live and with which they interact
- A **niche** is the role of an organism in an ecosystem
- **Biodiversity** can be defined as the degree of variation of life forms in an ecosystem, considered at three different levels:
  - The variation in ecosystems or habitats
  - The number of different species in the ecosystem and their relative abundance
    - Species diversity takes species richness (number of species in a community) and a measure of the evenness of the abundance of the different species
    - Ecosystems with high species diversity tend to be more stable than ones with limited diversity (more able to resist change)
  - The genetic variation within each species
    - Genetic diversity is the diversity of alleles within the genes in the genome of a single species
    - Important in providing populations with the ability to adapt to changes in biotic (caused by living organisms) and abiotic (physical characteristic of a habitat) factors
- Sampling can be random or systematic
- Random sampling using **frame quadrats**:
  - A quadrat is a square frame that marks off an area of ground, or water

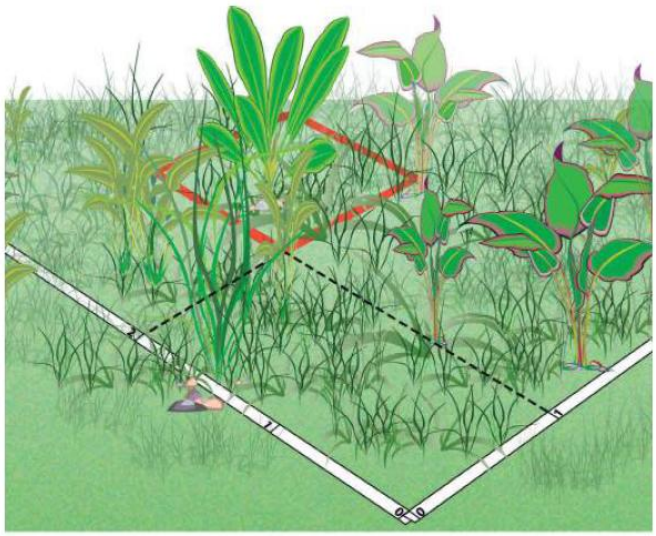


Figure 18.8 In random sampling, quadrats are positioned randomly in an area marked off by measuring tapes. This reduces the chances of bias in sampling the ecosystem.

Figure 18.9 Estimating percentage cover. This 1 m^2 quadrat is divided into 100 small squares to make it easier to make the estimation for each species.

  - Samples must use random sampling technique to avoid any bias (Fig 18.8)
  - Results can be used to calculate **species frequency** (measure of the chance of a particular species being found in a particular quadrat) and **species density** (measure of how many individuals per unit area)
  - Either count the actual number of organisms (e.g. limpets on a rocky sea shore) or estimate the percentage cover of each species (e.g. plant species in meadow)

- Random sampling using **mark-release-recapture** technique**:**
  - First a large number of animals is captured and marked in a way that will not affect its chances of survival (e.g. patch of fur cut away or small spot of paint applied); the number of marked animals recorded, returned to their habitat and left to mix randomly with the rest of the population; given enough time, another large sample is captured; number of marked and unmarked individuals is counted; the proportion of the marked individual used to calculate an estimate of the total number of population

Number caught and marked in first sample	= 247
Number caught in second sample	= 259
Number in the second sample that had been marked	= 16
So the estimated number in the population	$= \frac{247 \times 259}{16}$
	= 3998

- **Simpson's index of diversity**:
  - After collecting data about species abundance, species diversity can then be found using Simpson's index of diversity, *D*

$$D = 1 - \left(\sum \left(\frac{n}{N}\right)^2\right)$$

  - n is the total number of organisms of a particular species
  - *N* is the total number of organisms of all species
  - Values of *D* range from 0 (lowest species diversity) to 1 (highest species diversity)
  - Comparisons should be made on a 'like' for 'like' basis (similar community and organisms

Simpson's Index of Diversity

A sample was made of the animals living on two rocky shores. 10 quadrats were placed on each shore, and the number of animals of each species in each quadrat was counted. The results are shown in the table.

Species	Number of individuals, *n*	
	Shore A	Shore B
painted topshells	24	51
limpets	367	125
dogwhelks	192	63
snakelocks anemones	14	0
beadlet anemones	83	22
barnacles	112	391
mussels	207	116
periwinkles	108	93
total number of individuals, *N*	1107	861

To determine Simpson's Index for shore **A**, calculate $\frac{n}{N}$ for each species, square each value, add them up and subtract from 1. Repeat the procedure for shore **B**.

Species	Shore A		
	n	$\frac{n}{N}$	$\left(\frac{n}{N}\right)^2$
painted topshells	24	0.022	0.000
limpets	367	0.332	0.110
dogwhelks	192	0.173	0.030
snakelocks anemones	14	0.013	0.000
beadlet anemones	83	0.075	0.006
barnacles	112	0.101	0.010
mussels	207	0.187	0.035
periwinkles	108	0.098	0.010
total number of individuals, *N*	1107	$\sum \left(\frac{n}{N}\right)^2 = 0.201$	

For shore **A**, Simpson's Index of Diversity (*D*) = 1 – 0.201 = 0.799

- Systematic sampling is needed during investigations of species distribution in an area where the physical conditions such as altitude, soil moisture, soil pH, light intensity or soil type change

- Systematic sampling using **line and belt transects**:
  - Randomly select a starting point in a field and lat out a measuring tape in a straight line
  - Sample the organisms that are present along the line (transect), e.g. record the identity of the organisms touching the line at set distances
  - Line transect will provide qualitative data that can be represented as in Fig 18.11
  - Belt transect technique by placing a quadrat at regular intervals along the line and recording the abundance of each species within the quadrat

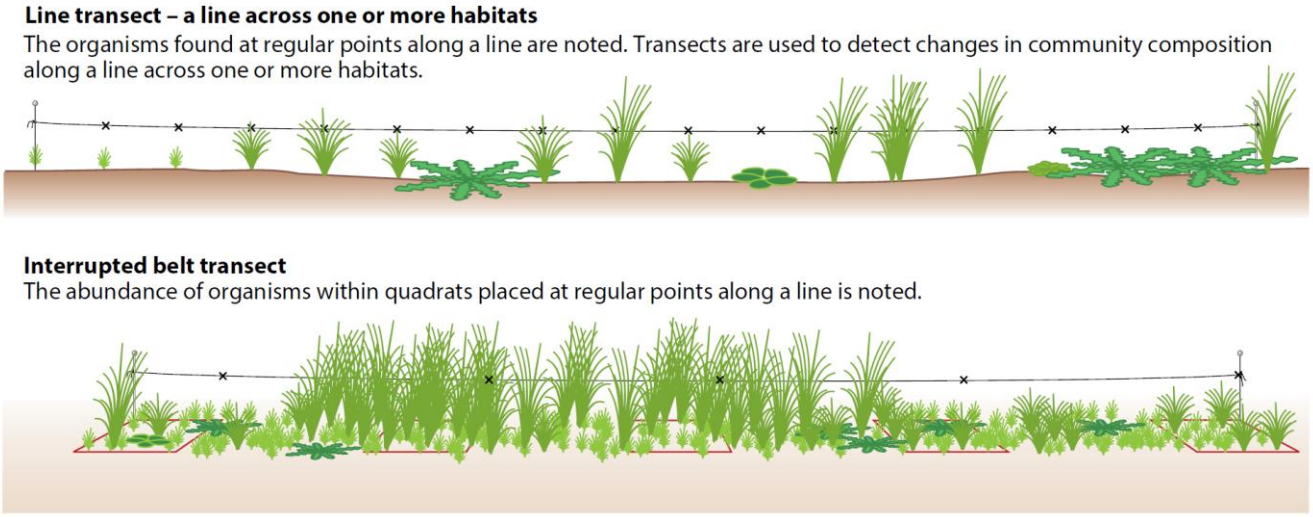


Figure 18.11 Systematic sampling using transects: **a** a line transect, and **b** an interrupted belt transect.

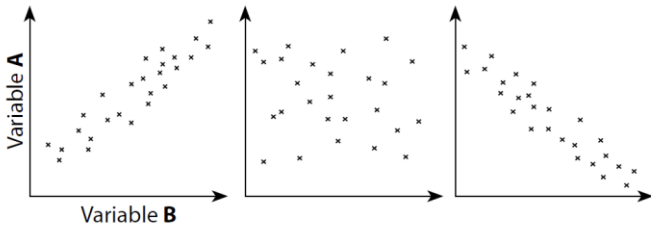


Figure 18.13 Three types of association: **a** a positive linear correlation, **b** no correlation, and **c** a negative linear correlation.

- Spearman's rank correlation and Pearson's linear correlation to analyse the relationships between the distribution and abundance of species and abiotic or biotic factors
- **Pearson's linear correlation**:
  - Null hypothesis: there is no correlation (percentage cover) between A and B
  - Given two species, P and Q in ten different 1 m^2 quadrats:

Quadrat	Number of individuals of species P	Number of individuals of species Q
1	10	21
2	9	20
3	11	22
4	7	17
5	8	16
6	14	23
7	10	20
8	12	24
9	12	22
10	9	19

Table P2.3 Numbers of species **P** and species **Q** in ten quadrats.

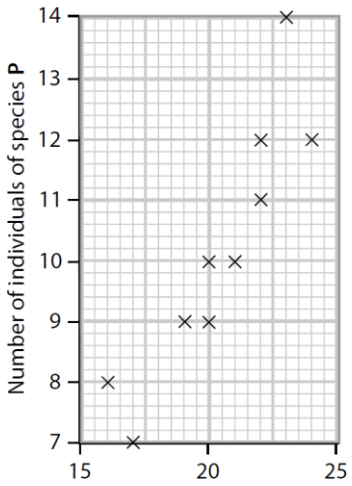


Figure P2.8 Scatter graph of the data in Table P2.3.

- Initially, plot a scatter graph (e.g. Fig P2.8, showing a possibility of a linear correlation between them, hence to make sure, Pearson's linear correlation test is carried out)
- (this test can only be used if the data are interval data and are normally distributed)
- Formula given by:

$$r = \frac{\sum xy - n\bar{x}\bar{y}}{ns_x s_y}$$

where:

- r is the correlation coefficient
- x is the number of species **P** in a quadrat
- y is the number of species **Q** in the same quadrat
- n is the number of readings (in this case, 10)
- $\bar{x}$ is the mean number of species **P**
- $\bar{y}$ is the mean number of species **Q**
- s_x is the standard deviation for the numbers of **P**
- s_y is the standard deviation for the numbers of **Q**

The value should always work out between -1 and +1

A value of +1 means total positive correlation between your two sets of figures.
A value of −1 means total negative correlation between your two sets of figures.
A value of 0 means there is no correlation.

1 Calculate $x \times y$ for each set of values.

Quadrat	Number of species P, x	Number of species Q, y	xy
1	10	21	210
2	9	20	180
3	11	22	242
4	7	17	119
5	8	16	128
6	14	23	322
7	10	20	200
8	12	24	288
9	12	22	264
10	9	19	171
mean	$\bar{x} = 10.2$	$\bar{y} = 20.4$	$\sum xy = 2124$
$n\bar{x}\bar{y}$	$10 \times 10.2 \times 20.4 = 2080.8$		
standard deviation	$s_x = 2.10$	$s_y = 2.55$	

2 Calculate the means for each set of figures, $\bar{x}$ and $\bar{y}$.

3 Calculate $n\bar{x}\bar{y}$. Here, $n = 10$, $\bar{x} = 10.2$ and $\bar{y} = 20.4$, so $n\bar{x}\bar{y} = 10 \times 10.2 \times 20.4$

4 Add up all the values of xy, to find $\sum xy$.

5 Now calculate the standard deviation, s, for each set of figures. The method for doing this is shown in Table P2.1 on page 498.

6 Now substitute your numbers into the formula and calculate r.

$$r = \frac{\sum xy - n\bar{x}\bar{y}}{ns_x s_y}$$
$$= \frac{2124 - (10 \times 10.2 \times 20.4)}{10 \times 2.10 \times 2.55}$$
$$= \frac{2124 - 2080.8}{53.55}$$
$$= \frac{43.2}{53.55}$$
$$= 0.81$$

Table P2.4 Calculating Pearson's linear correlation for the data in Table P2.3.

- The value of r lies close to 1, hence there is a positive correlation between the numbers of species P and the numbers of species Q

- **Spearman's rank correlation**:
  - To find out whether there is a correlation between two sets of variables, when they are not normally distributed

- Initially, plot a scatter graph, to see whether there may be a correlation
- Given two species, R and S in 10 quadrats:

Quadrat	Number of species R	Number of species S
1	38	24
2	2	5
3	22	8
4	50	31
5	28	27
6	8	4
7	42	36
8	13	6
9	20	11
10	43	30

Table P2.5 Numbers of species **R** and species **S** found in 10 quadrats.

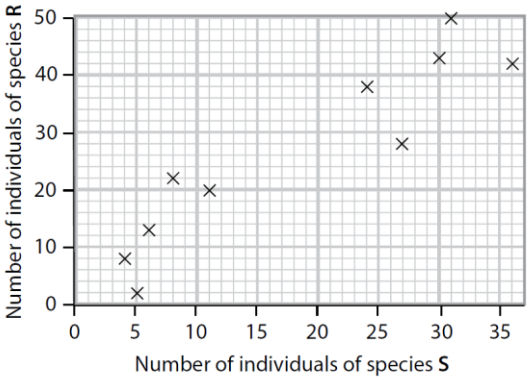


Figure P2.9 Scatter graph of the data in Table P2.5.

- Now rank each set of data, with the largest number ranked as 1; calculate the differences in rank, *D*, by subtracting the rank of species S from species R; then square each of these values; add them together to find total difference squared:

Quadrat	Number of species R	Rank for species R	Number of species S	Rank for species S
1	38	7	24	6
2	2	1	5	2
3	22	5	8	4
4	50	10	31	9
5	28	6	27	7
6	8	2	4	1
7	42	8	36	10
8	13	3	6	3
9	20	4	11	5
10	43	9	30	8

Table P2.6 Ranked data from Table 2.5.

Quadrat	Rank for species R	Rank for species S	Difference in rank, *D*	D^2
1	7	6	1	1
2	1	2	−1	1
3	5	4	1	1
4	10	9	1	1
5	6	7	−1	1
6	2	1	1	1
7	8	10	−2	4
8	3	3	0	0
9	4	5	−1	1
10	9	8	1	1
				$\sum D^2 = 12$

Table P2.7 Calculating $\sum D^2$ for the data in Table P2.5.

The formula for calculating Spearman's rank correlation coefficient is:

$$r_s = 1 - \left(\frac{6 \times \sum D^2}{n^3 - n}\right)$$

where:

r_s is Spearman's rank coefficient

$\sum D^2$ is the sum of the differences between the ranks of the two samples

n is the number of samples

$$r_s = 1 - \left(\frac{(6 \times 12)}{(10^3 - 10)}\right)$$
$$= \frac{1 - 72}{1000 - 10}$$
$$= \frac{1 - 72}{990}$$
$$= 1 - 0.072$$
$$= 0.928$$
$$= 0.93 \text{ (to 2 decimal places)}$$

- 0.93 is the correlation coefficient
- Using the values in Table P2.8 to find the critical value (0.05 baseline)

n	5	6	7	8	9	10	11	12	14	16
Critical value of r_s	1.00	0.89	0.79	0.76	0.68	0.65	0.60	0.54	0.51	0.51

Table P2.8 Critical values of r_s at the 0.05 probability level.

- n = 10, the critical value = 0.65 (calculated value is much greater), hence there is a significant correlation between the number of species R and the numbers of species S

- Taxonomy is the study and practice of classification which involves placing the organisms in a series of taxonomic units, or taxa, creating a hierarchy:

Taxon	African bush elephant	Hibiscus
domain	Eukarya	Eukarya
kingdom	Animalia	Plantae
phylum	Chordata	Angiosperms
class	Mammalia	Dicotyledonae
order	Proboscidea	Malvales
family	Elephantidae	Malvaceae
genus	*Loxodonta*	*Hibiscus*
species	*Loxodonta africana*	*Hibiscus rosa-sinensis*

Table 18.4 The classification of African bush elephants and hibiscus plants.

- The prokaryotes are divided between the domains Bacteria and Archaea (many live in extreme conditions); eukaryotes are placed into the domain Eukarya

- **Domain Bacteria**:
  - Prokaryotic cells (no nucleus)
  - DNA exists as circular 'chromosome' and does not have histone proteins associated with it
  - Plasmids (smaller circular molecules of DNA) are often present
  - No membrane-bound organelles (e.g. mitochondria, E.R., Golgi body, chloroplasts) are present
  - Ribosomes (70 S) are smaller than in eukaryotic cells
  - Cell wall is always present and contains peptidoglycans
  - Cells divide in binary fission (not mitosis)
  - Usually exist as single cells or small group of cells
- **Domain Archaea (archaeans)**:
  - Prokaryotic cells (no nucleus)
  - DNA exists as circular 'chromosome' and does not have histone proteins associated with it
  - Plasmids are often present
  - No membrane-bound organelles are present
  - Ribosomes (70 S) are smaller than in eukaryotic cells, but have features similar to those of eukaryotic ribosomes
  - Cell wall is always present, but does not contain peptidoglycans
  - Cells divide in binary fission (not mitosis)
  - Usually exist as single cells or small group of cells
  - Metabolism similar to bacteria, but transcription common with eukaryotes
- **Domain Eukarya**:
  - Cells with nuclei and membrane-bound organelles
  - DNA in the nucleus arranged in linear chromosomes with histone proteins
  - Ribosomes (80 S) in the cytosol larger than prokaryotes'; chloroplasts and mitochondrial DNA have 70 S ribosomes
  - Chloroplast and mitochondrial DNA is circular as in prokaryotes
  - May be unicellular, colonial and multicellular organisms
  - Cell division by mitosis
  - Method of reproduction: asexual and sexual

- 4 kingdoms of Eukarya: kingdom Protoctista, Fungi, Plantae and Animalia
- **Kingdom Protoctista**:
  - Any eukaryote that is not fungus, plant or animal is a protoctist
  - Features:
    - Eukaryotic
    - Mostly single-celled, or exist as groups of similar cells
    - Protozoa – having animal-like cells (no cell wall)
    - Algae – having plant-like cells (cellulose cell walls and chloroplasts)
- **Kingdom Fungi**:
  - Eukaryotic
  - No chlorophyll (no photosynthesis)
  - Heterotrophic nutrition – use organic compounds made by other organisms as their source of energy and source of molecules for metabolism
  - Reproduce by means of spores
  - Simple body form (may be unicellular or made up of long threads called **hypae** (with or without cross walls))
  - Have cell walls made up of chitin or other substances (not cellulose)
  - Does not have cilia or flagella
- **Kingdom Plantae**:
  - Multicellular eukaryotes with cells that are differentiated to form tissues and organs
  - Few types of specialised cells
  - Some cells have chloroplasts and photosynthesise
  - Cells have large, often permanent vacuoles for support
  - Autotrophic nutrition
  - Cell walls are always present (made of cellulose)
  - Cells may have flagella
- **Kingdom Animalia**:
  - Multicellular eukaryotes with many different types of specialised cells
  - Cells that are differentiated to form tissues and organs
  - No chloroplasts (no photosynthesis)
  - Small and temporary cell vacuoles (e.g. lysosomes and vacuoles)
  - Heterotrophic nutrition
  - No cell walls
  - Communication is by the nervous system
  - Cells may have cilia or flagella
- **Viruses**:
  - Acellular – no cellular structure like bacteria and fungi
  - No features traditionally use for classification
  - Infectious but has no metabolism
  - Their taxonomic system is based on the type of nucleic acid they contain (DNA or RNA), and whether the nucleic acid is single-stranded or double-stranded (DNA and RNA)

Nucleic acid	Number of strands	Example	Host organism	Disease
DNA	1	canine parvovirus type 2	dogs	canine parvovirus
		African cassava mosaic virus	cassava plants	mosaic disease
	2	varicella zoster virus (VZV)	humans	chickenpox
RNA	1	rotavirus	humans	gastroenteritis
		morbillivirus	humans	measles
		tobacco mosaic virus (TMV)	tobacco, tomato, pepper	mosaic disease
	2	human immunodeficiency virus	humans	HIV/AIDS

- Major threats to the biodiversity of aquatic and terrestrial ecosystems:
  - Habitat loss and degradation of the environment
    - E.g. clearing of land for agriculture housing, transport and industry removes vegetation, leading to loss of habitats completely or **habitat fragmentation** (divided into small areas); **deforestation** causes severe land degradation due to soil erosion once the vegetation is removed
  - Climate change
    - Industrialisation and combustion of fossil fuels led an increase in the concentrations of carbon dioxide and methane in the atmosphere (greenhouse gases), causing disruptions in migration patterns of aquatic life, acidification of oceans (coral reefs and molluscs), increase in ocean temperature causes coral bleaching, rise in sea levels, and increase in frequency of natural catastrophes
  - Excessive use of fertilisers and industrial and domestic forms of pollution
    - Enters food chain and causes disruptions, due to severe impacts on different species
    - Non-biodegradable plastics
    - Fertilisers provide extra nutrients for producers (e.g. algae), algal bloom causing eutrophication and increases toxicity; reduces biodiversity of coral reefs
    - Air pollution – acid rain (combustion of high sulfur content fuels), causing destruction of vegetation and acidification of aquatic ecosystems, decreasing biodiversity
  - Overexploitation and unsustainable use of resources
    - E.g. overfishing, removal of valuable trees by logging companies and poaching
  - Effects of invasive **alien species** on native species, especially endemics
- Reasons for the need to maintain biodiversity:
  - Moral and ethical issues
  - Ecological reasons
    - The higher the diversity of an ecosystem, the less likely for it to be unbalanced by change in environmental conditions or threats
    - All organisms in an ecosystem interact in many ways, if one key species disappears, the whole community will be affected
    - Loss of drugs and fuels derived from living organisms
  - Aesthetic reasons
    - Ecotourism (wildlife) provides many countries' income and employment
  - Social and commercial reasons
    - Decrease in genetic variety due to selective breeding of uniform, high yielding crops and genetic modified crops – leading to major impacts due to diseases
    - Diversity of microbes as a source of many useful products: antibiotics and enzymes
- Roles of conserved areas, zoos, 'frozen zoos', botanic gardens and seed banks in protecting endangered species:

❖ **Conserved areas** (national parks and marine parks) – controlled by the government of a country and protected by legislation:
  - Maintaining the natural habitat, hence all the 'life support systems' are provided
  - Protecting of the whole ecosystems threatened by human developments

- Limiting human activities (strict limits on hunting, grazing farm animals or other activities that might affect animals and plants living there)
- Alien species and invasive plants removed
- Raises awareness of important issues (education)
- Incentives to indigenous people
- Population monitoring

❖ **Zoos**:
- Captive breeding programmes
- Reintroduction programmes (difficulty in readjusting to the wild)
- Increase in genetic diversity

❖ <u>(assisted reproduction is a solution to interbreeding problems)</u>

✓ Sperm bank (transferring of species in between zoos):
- Collection and freezing of semen from males
  - Checked for sperm activity; diluted with a buffer solution and albumen; stored into straws, into liquid nitrogen

✓ **Artificial insemination** (AI) – embryo transfer and surrogacy – solves the problem of males and females who do not show any courtship behaviour and will not mate:
- Straw placed into warm water causing 'active' sperms; placed into a catheter; and inserted into the vagina; through the cervix into the uterus, leading to ovulation
- Following AI, the resulting embryo 'flushed out' of the uterus and transferred to other females (**surrogate** mothers) that have had hormonal treatment to prepare them for pregnancy
- **Embryo transfer** protects the endangered animal from the risks of pregnancy and means that she can be a source of many offspring

✓ **In vitro fertilisation** (IVF):
- Oocytes (eggs) are collected by inserting a needle into the ovaries and withdrawing some mature follicles; kept in culture medium for a short time; mixed with semen in vitro; then divides to form embryos; frozen (similar to sperm) until a surrogate mother becomes available
- The resulting oocytes divide to form embryos; cultured then placed into a female species
- Eggs (oocytes) and embryos are stored much like sperm

❖ '**Frozen zoo**':
- Holds genetic resources in the form of sperm, eggs and embryos from many endangered animals until they might be needed
- Materials can be kept for a long period of time

❖ **Botanic garden**:
- Seeds or cutting are collected from species in the wild; used to build up the population of plants to be reintroduced to their natural habitats
- Roles of botanic gardens:
  - Protect endangered plant species
  - Research methods of reproduction and growth
  - Research conservation methods for new habitats
  - Reintroduce species to habitats where it is extinct or rare
  - Educate public on the roles of plants

❖ **Seed bank**:
- National and international organisations collect and store seeds from the world's plants (seeds from the same species collected from different sites, to contain a

good proportion of the total gene pool for that species) to prevent losses in genetic diversity in crop plants in cases of disasters

- Culling (killing of animals) and contraceptive methods (birth control and chemical contraceptives – targeting zona pellucid (layer of glycoproteins around the egg) by stimulating an immune response producing antibodies against it) used to prevent overpopulation of protected and non-protected species
- Explain the reasons for controlling alien or invasive species:
  - Successful predators with few controls
  - Compete effectively with native organisms that occupy the same niche
  - Introduce diseases that spread to similar organisms that have never been exposed to the pathogens
  - May cover large areas of water, blocks sunlight from reaching aquatic plants, reducing the oxygen concentration of the water, killing the fishes
  - Outcompetes native species by reducing the space in which they can grow
  - E.g. the Burmese pythons which invaded the Everglades National Park in Florida, which feeds on a wide variety of mammals and birds, competing with native predators, where humans are its only predator
- Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES):
  - Controls the trade in endangered species, and any products such as furs, skins and ivory – countries signing an agreement
  - Does this by considering the evidence presented to it about endangered species and assigns them to one of the three Appendices (most endangered, less endangered and request of the country)
  - Species are reviewed by expert committees and the list is growing
- World Wide Fund for Nature (WWF):
  - Campaign group for wildlife
  - Largest non-governmental organisation (NGO) specialising in conservation
  - Funds conservation projects, publicises environmental issues and campaigns to save ecosystems from degradation and species from extinction
- Restoring degraded habitats:
  - Conservation involves restoring degraded areas by natural catastrophes or human activities
  - Famers decide to plant trees on land that is no longer needed for food and production or has become degraded by overuse
  - Replanting of mangrove forests to coastal erosions, providing protections against storm damages
  - Tree planting projects by NGOs
  - Reclamation projects such as Eden Project in Cornwall UK

Genetic engineering (Chapter 19):

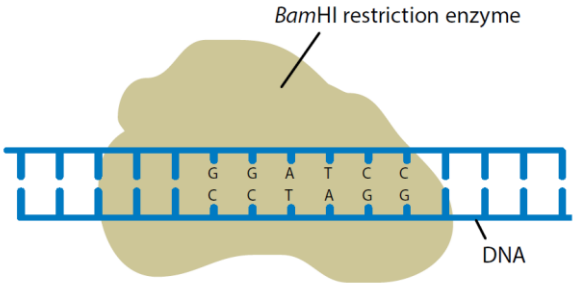
- Genetic engineering involves the extraction of genes from one organism, or the synthesis of genes, in order to place them in another organism (of the same or another species) such that the receiving organism expresses the gene product
- **Recombinant DNA**: DNA made by joining pieces from two or more different sources creating a transgenic organism or a genetically modified organism (GMO)
- An overview of gene transfer:
  1. The gene that is required is identified, may be cut from a chromosome; made from mRNA by reverse transcription or synthesised from nucleotides

2. Multiple copies of the gene are made using polymerase chain reaction (PCR)
3. Gene is inserted into a **vector** which delivers the gene to the cells of the organism
4. Vector takes gene into the cells
5. Cells have new gene identified and cloned

- 'Tools' needed for these steps:
  - Enzymes: restriction endonucleases, ligase and reverse transcriptase
  - Vectors: **plasmids**, viruses and liposomes
  - Genes coding for fluorescent or easily stained substances as markers
- **Restriction endonucleases** are a class of enzymes from bacteria which recognise and break down viral DNA of viruses known as bacteriophages (phages), preventing any viral infection
  - Each restriction enzymes binds and cuts a specific site / base sequence of DNA giving sticky ends (short lengths of unpaired bases) which can form hydrogen bonds with complementary sequences of bases on other pieces of DNA cut with the same restriction enzyme
  - When long pirces of DNA are cut with restriction enzyme, there will be a mixture of different lengths, hence to find a specific piece of DNA, gel electrophoresis and gene probes are required to separate lengths of DNA; then multiple copies of the required piece of DNA can be made using the polymerase chain reaction (PCR)

Restriction enzyme	Restriction site	Site of cut across DNA	Source of enzyme
*Eco*RI	5′ –GAATTC– 3′ 3′ –CTTAAG– 5′	–G | AATTC– –CTTAA | G–	*Escherichia coli*
*Bam*HI	5′ –GGATCC– 3′ 3′ –CCTAGG– 5′	–G | GATCC– –CCTAG | G–	*Bacillus amyloliquefaciens*
*Hind*III	5′ –AAGCTT– 3′ 3′ –TTCGAA– 5′	–A | AGCTT– –TTCGA | A–	*Haemophilus influenzae*
*Hae*III	5′ –GGCC– 3′ 3′ –CCGG– 5′	–GG | CC– –CC | GG–	*Haemophilus aegyptius*

Table 19.1 Four restriction enzymes and their target sites.



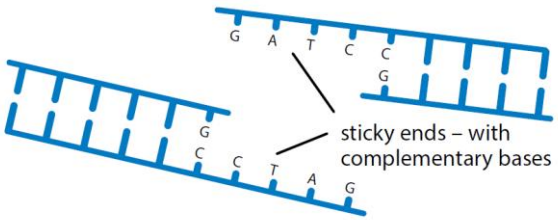


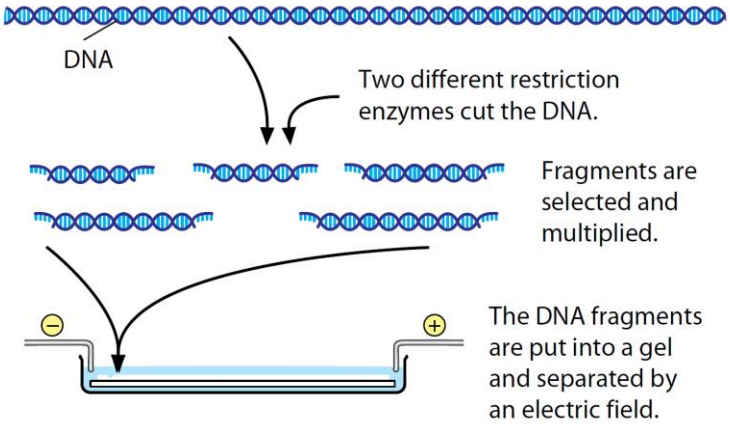
Figure 19.2 The restriction enzyme, *Bam*HI, makes staggered cuts in DNA to give sticky ends.

- Inserting a gene into a plasmid (small, circular pieces of double-stranded DNA) vector:
  - To get the plasmids, bacteria is treated with enzymes to breakdown their cell walls
  - The 'naked' bacteria is then spun in a centrifuge
  - The circular DNA plasmid is cut open using a restriction enzyme (the same enzyme used to cut out the gene, to have complementary sticky ends)
  - The opened plasmids and the lengths of DNA are mixed together causing pairing of sticky ends
  - **DNA ligase** is used to link together the sugar-phosphate backbones of the DNA molecule and the plasmid, producing recombinant DNA
- Properties of plasmids that allow them to be used in gene cloning:
  - Small / circular, piece of double-stranded DNA that have multiple origins of replication, a high copy number and replicates independently

- Easy to extract from bacteria
- Can be cut using restriction endonuclease
- Gene / DNA can be inserted and can be taken up by bacteria
- May contain genes for antibiotic resistance, which is used as marker genes that helps in identifying transformed bacteria
- Acts as a vector and may carry promoter

- Gene coding from human insulin can be obtained and inserted into a plasmid vector:
  - obtain mRNA from β cells of islets of Langerhans of pancreas
  - **Reverse transcriptase** reverses transcription using mRNA as a template to make a single-stranded cDNA
  - DNA polymerase used to make cDNA double stranded
  - Sticky ends created using **restriction endonuclease**
  - Obtain plasmids and cut with the same restriction endonuclease to produce complementary sticky ends
  - cDNA (insulin gene) mixed with plasmid and **DNA ligase** used to seal nicks in sugar-phosphate backbone
- Advantages of treating diabetics with human insulin produced by rDNA technology:
  - Identical to that produced by body, hence no immune response (rejection)
  - There is no need for animal insulin, as extraction will be costly and complex
  - For religious and ethical reasons
  - Human insulin produced is pure, hence no risk of diseases
  - Production is very efficient and mass produced, hence always available
- Advantages of treating diabetics with factor VIII for the treatment of haemophilia produced by rDNA technology:
  - Identical to that produced by body, hence no immune response (rejection)
  - There is no need for donated blood, expensive and limited
  - Risks of infections, e.g. HIV
  - Production is very efficient and mass produced, hence always available
- Advantages of treating diabetics with adenosine deaminase for treating (SCID) produced by rDNA technology:
  - Identical to that produced by body, hence no immune response (rejection)
  - There is no need for animal insulin, as extraction will be costly and complex
  - For religious and ethical reasons
  - Human insulin produced is pure, hence no risk of diseases
  - Production is very efficient and mass produced, hence always available
- Genes for fluorescent (e.g. GFP (green fluorescent protein) which glows bright green when UV light shone onto it) or easily stained substances as markers in gene technology
- Promoters need to be transferred with desired genes when producing a genetically modified cell due to positional problems (e.g. gene may insert in any of the chromosomes, gene may be within an, intron / non-coding DNA, gene may share promoter with host gene that does not get switched on in this cell)
- Movement of charged molecules during electrophoresis depends on:
  - Net overall charge – highly charged molecules move faster
  - Size – smaller molecules move faster
  - Composition of gel – size of pores determines the speed of movement

- Electrophoresis used in genetic fingerprinting:



A radioactive probe is added to bind to the invisible bands of DNA, so they can blacken an X-ray film

Figure 19.10 Genetic profiling.

  - Variable number tandem repeats (VNTRs) sequence – a region of DNA that varies between different people
  - The quantity of DNA increased by polymerase chain reaction (PCR) to make copies of DNA
  - DNA fragmented by restriction enzymes/endonucleases loaded into wells in **agarose gel** at negative end (cathode end), and attracted towards the anode when a direct current is applied, due to the negatively charged phosphate groups of DNA
  - The smaller, shorter fragments will move faster
  - Buffer should be used to keep a constant pH
  - The pieces transferred to absorbent paper placed on top of the gel
  - Heated to separate the two strands in each DNA molecule
  - Probes containing a radioactive isotope added and placed on an X-ray film
  - The radiation emitted from the proves creates a pattern of dark stripes
- The principles of the polymerase chain reaction (PCR)
  - Method for rapid production of a large number of copies of a particular length of DNA (amplification of DNA), where only small sample of DNA is needed
  - DNA is denatured and separated into two strands by heat (95 °C)
  - Primer DNA added to the ends of the single-stranded DNA molecules, process known as **annealing** (60°C –65°C)
  - *Taq* polymerase (taken from microorganisms that have evolved to live in hot environments, causing it to be heat stable (does not denature hence does not have to be replaced during each cycle) and has a high optimum temperature hence process is efficient) replicates template strand by complimentary base pairing (70°C –75°C)
  - Heated again to separate strands, where process is repeated
- Microarrays used in the analysis of genomes and in detecting mRNA in studies of gene expression:
  - Probes are short lengths of single-stranded DNA which are complementary to the DNA being tested for
  - Many copies of one type of probe placed in each cell of the microarray
  - Single-stranded DNA made from cDNA, which is made from mRNA, using reverse transcriptase, from fragmented and denatured (to form single-stranded DNA) DNA
  - Target DNA labelled with fluorescent tags and hybridises with the probes on the microarray
  - Unbound target DNA, washed off

- UV light used to detect presence of hybridised probes

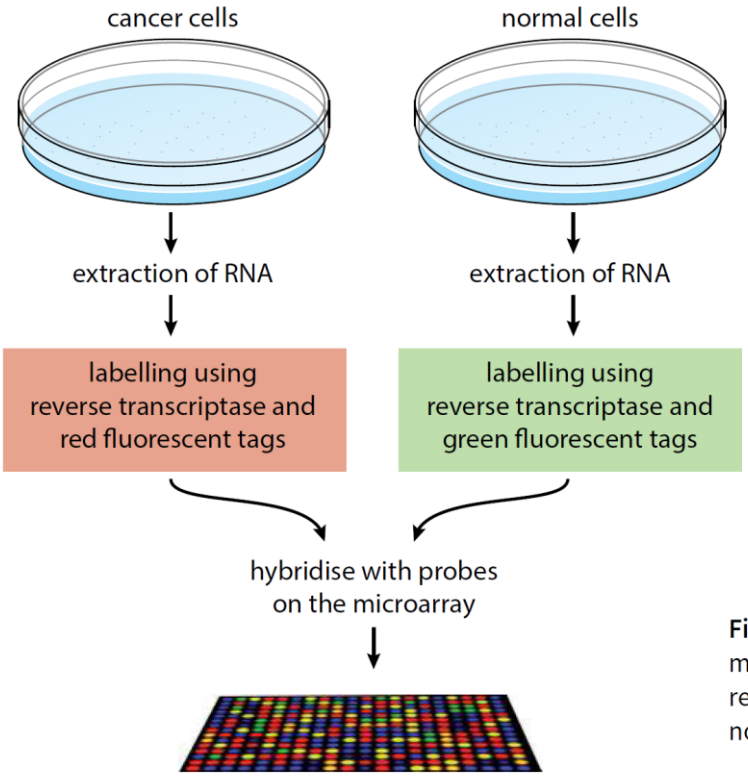


Figure 19.16 How to use a microarray to compare the mRNA molecules present in cancerous and non-cancerous cells. The results identify which genes in the cancerous cells that are not normally expressed are being transcribed.

- **Genome**: all the DNA / genetic material in a person's cell
- **Bioinformatics** is the collection, processing and analysis of biological information and data using computer software from databsases
- Outline how sequencing the genome of Plasmodium and the use of bioinformatics can suggest new targets for anti-malarial drugs:
  - Able to identify and recognise genes
  - Able to predict the primary structure (amino acid sequences) of proteins
  - Predict 3D structure of proteins
  - Able to predict the functions of proteins from the 3D structure
  - Predict which drugs can bind with and block the activity of the proteins in Plasmodium, hence prevents transcription of its gene
- Suggest advantages of using theoretical models in this research, rather than testing possible drugs in the laboratory:
  - cheaper
  - faster/ can try many different drugs in a short period of time
  - can try out changes to, model/ drug structure, to see if more effective
  - no need for, laboratories / equipment
  - (initially) no need for tests on, animals / humans
- Suggest why theoretical modelling cannot completely replace laboratory trials in the search for new drugs:
  - To test that the drug actually works or is effective
  - Safety, its clinical trials and side effects
  - Theoretical modelling will not give information on doses
- **Genetic screening** is the analysis of a person's DNA to check for the presence of a particular allele

- Advantages of screening for faulty alleles of BRCA2 (genes causing breast cancer) before any symptoms occur:
  - If present, it enables lifestyle changes and early treatments
  - If it is not present, removes worry
  - Preventative treatment may be cheaper than treating the disease itself
- Disadvantages of screening for faulty alleles of BRCA2 before any symptoms occur:
  - If present, it may cause worry
  - Even if present, person may not develop cancer
  - The test is expensive
  - May have implications for life insurance
  - May decide not to have children
- Cystic fibrosis (CF) is a genetic disease caused by an autosomal recessive allele, causing the build up in thick mucus produced in the lungs and other parts of the body – prone to bacterial infections; gene therapy has been attempted to treat CF since 1993, hence outline the basic principles of gene therapy for the treatment of CF:
  - CF caused by mutation of the CFTR gene
  - CFTR transporter protein becomes defective
  - Hence insert a normal dominant CFTR allele into the DNA in cells of respiratory system using a vector; e.g. liposomes taken as spray or a harmless virus, however, not all cells take up virus and may cause unpleasant side-effects
  - The effects only lasts for a short period of time, and the treatment needs repeating
- Huntington's disease cannot be treated with gene therapy:
  - Allele is dominant, hence will still be expressed even when normal recessive allele is present
  - Gene therapy only used to treat recessive allele disorders, as it cannot remove dominant allele
  - Dominant allele affects tissues in many parts of the body
- **Embryo biopsy**: removing a cell from an embryo for testing
- The social and ethical considerations of using gene testing of Huntington allele, using IVF to have a child:
  - Embryos might be destroyed
  - It is considered wrong for parents to choose, as it is contrary to believes and values
  - Less chance of Huntington allele being passed on / decrease in frequency of Huntington allele
  - People with the faulty allele who otherwise would not have children can now do so
- Genetic engineering is improving the quality (nutrition) and yield of crop plants and livestock, therefore is solving the demand for food in the world
- Describe how the vitamin A content of rice can be enhanced by genetic modification:
  - Vitamin A found in aleurone layer of rice seeds
  - White rice does not contain aleurone layer which contains vitamin A, carotenoids and β carotene, as it is removed
  - Genes coding for vitamin A production extracted from bacteria (*Pantoea ananatis*) and daffodils inserted into plasmids and promoters added
  - Plasmids put into *Agrobacterium tumefaciens*
  - *Agrobacterium tumefaciens* mixed with rice embryos
  - Some embryos take up bacteria and vitamin A gene and grow into adult plants, which produce seeds with vitamin A/carotene in endosperm
  - The genetically modified rice is called Golden Rice^{TM}

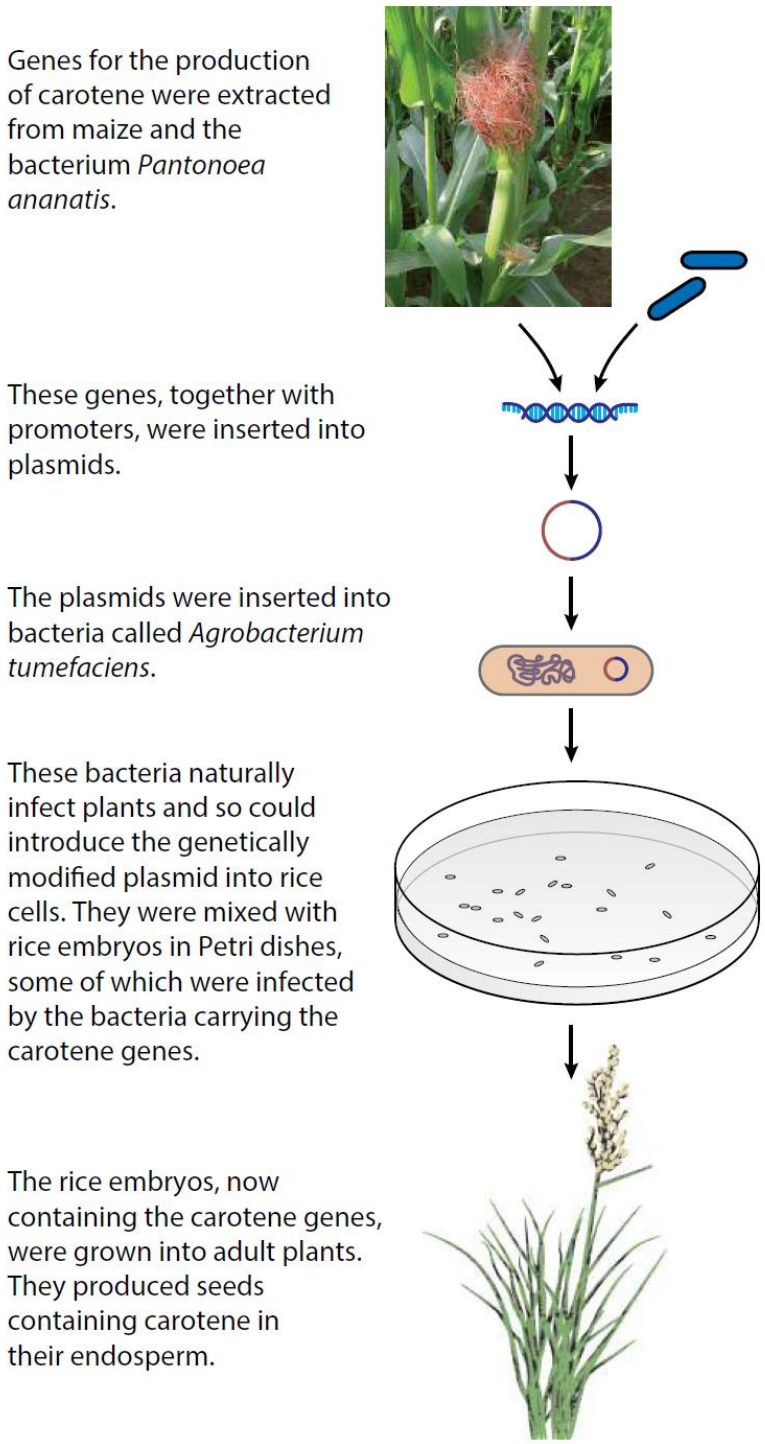


Figure 19.22 Pro-vitamin A enhanced rice was engineered using genes from the maize and a bacterium.

- Herbicide-resistant crops:
  - ❖ Oil seed rape is grown in many parts of the world as a source of vegetable oil, used in biodiesel fuel, lubricant and food
    - ➢ Gene technology has been used to produce herbicide-resistant strains
    - ➢ Growing a herbicide-resistant crop allows fields to be sprayed with herbicide after the crop has germinated, killing any weeds that would otherwise compete with the crop for space, light, water or ions, hence increases the yield of the crop
    - ➢ Oil seed rape that is resistant to the herbicide glyphosate and glufosinate
    - ➢ Vector used: *Agrobacterium tumefaciens*
  - ❖ Tobacco made resistant to two herbicides: sulfonylurea and dinitroaniline

- Insect-resistant crops causing an increase in yield:

❖ Bt maize protected against corn borer
  - A gene for a toxin, Bt toxin, is lethal to insects that eat it but harmless to other animals, has been taken from a bacterium, *Bacillus thuringiensis*
  - Crop plants that contain the Bt toxin gene from *B. thuringiensis* produce their own insecticides, however, insect populations can evolve resistance to toxins
  - Large numbers of crop plants containing the genes for Bt toxin may accelerate the evolution of resistance to it
  - Bt resistance in corn borers happens to be a recessive allele

- Outline the disadvantages of using plants that have been genetically modified:
  - GM seed could be difficult for farmers in developing countries to obtain
  - High cost of (buying) GM seed might reduce efforts to relieve poverty
  - May not grow well in all conditions as other traits not selected for
  - Possibility of allergic reactions in humans / toxicity of more herbicide left after use / adverse effects on the immune system
  - Under-developed countries becoming more dependent on other countries
  - Cross-pollination with, wild plants/organic crops, resulting to new more resistant weeds/"superweeds"
  - Loss of traditional varieties
  - Loss of genetic diversity
  - Harm to other species, e.g. effect on rest of food chain
- GM salmon
  - A growth-hormone regulating gene from a Pacific Chinook salmon and a promoter from an ocean pout were injected into a fertilised egg of an Atlantic salmon
  - By producing growth hormone throughout the year, the salmon are able to grow all year, instead of just in spring and summer, hence fishes reach market size in about eighteen months, compared with the three years needed by an unmodified fish.