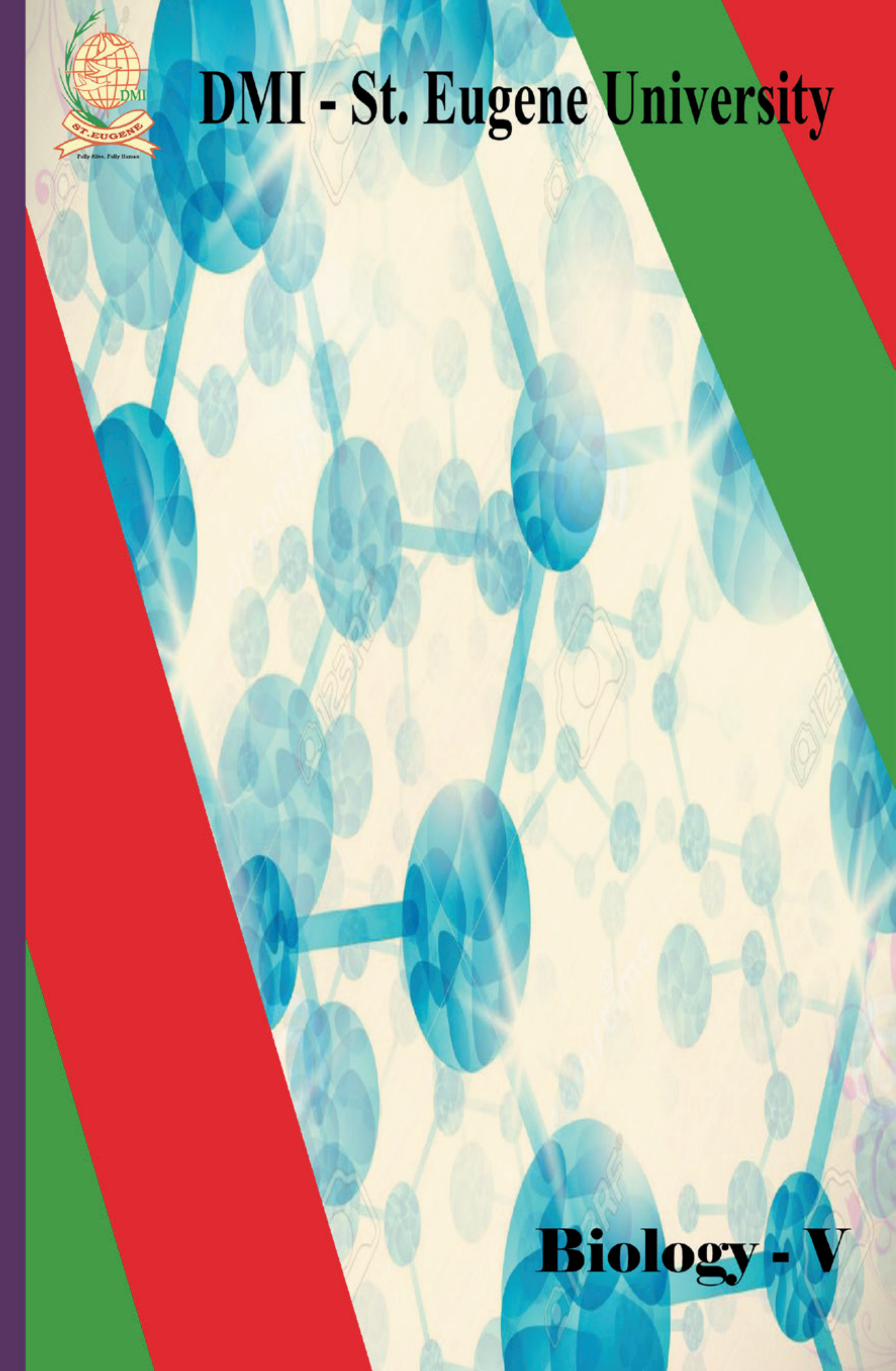
B.Sc. in Secondary Education



DMI - St. Eugene University

(Run by sisters of Daughters of Mary Immaculate and Collaborators)

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From the desk of

Dr. T. X. A. ANANTH, BBA, MSW, MBA, MPhil, PhD

President –University Council

Dear Student Teachers,

Greetings

On behalf of DMI – St. Eugene University, I welcome you to third semester of the fast track teacher training programme, 2015.

At this point, we at DMI – SEU need to thank the Lord Almighty for the strength we have gained to face the second year at IVDL with improved facilities and renewed energy. I am duty bound to express my gratitude for encouragement received from Rev. Fr. Dr. J. E. Arulraj, our Founder-Chancellor of DMI – St. Eugene University, for his moral support, the financial assistance and guidance.

These books form the learning materials for your course in the III Semester and supportive references which will help your teaching career. Each subject is mapped to the syllabus and discussed in detail for easy understanding of the texts. Our dedicated team works to create texts that will be an additional asset in your career as teaching faculty in your respective institutions.

I welcome each of you once again on behalf of our respected Chancellor, members of the faculty and on my own behalf to benefit from the programme and contribute effectively to serve Zambia to reach the level of development envisioned by the country's early freedom fighters and liberators during their years of struggle for independence. Your efforts will be appreciated in harnessing the natural resources to generate the financial wealth that will move Zambia forward in the eyes of the developed world.

It is our endeavour to help you all in building a modern Zambia, a country that is blessed with an abundance of human and natural resources. I wish you all the best there in your second year at IVDL.

Dr. T. X. A. ANANTH

President – University Council

DMI - St. Eugene University Biology - V

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UNIT I

Taxonomy is a branch of science which tries to scientifically classify all the existing living organisms based on certain set of characters for easy of identification and study.

Importance of taxonomy in biology-

Taxonomy was described by Linnaeus and hence he is called as Father of taxonomy. You notice many plant species's scientific names ending with Linn. Taxonomy is the scientific way of classification of all the living creatures on the earth. Even human is called as homosapeian as per taxonomy.

Establishing taxonomy for entire biological species is a very task but these scientists completed it very successfully during their time. Any new plants or animals discovered latter were given name as per the rules of taxonomy established by Linnaeus.

This taxonomy is divided as plant taxonomy, animal or zoological taxonomy, microbial taxonomy etc.

Importance of taxonomy in biology:

1. Taxonomy aims to classify living creature: There are millions of organisms on the Earth of different physical, physiological, regional differences. Taxonomy helps to classify these millions of organisms scientifically into some categories like family, genus, species etc. for ease of study and understanding.

2. Taxonomy helps to ascertain the number of living species on the earth. We have discovered till now some thousands of plants and animal species and are recorded as per taxonomy.

3. Taxonomy helps in getting an idea of what type of characters are present in the plant or animal possess even before seeing or studying them in detail.

Ex: a) In plants: When one hears a plant to be of leguminous family, the characters we can ascertain are that they have nitrogen synthesizing bacteria in their root nodules. They have a seed which can be broken into exact two half etc...

b) In animals: If a living creature is mentioned under mollusc's, it means the animals has some sort of hard shell as a protective factor (like snail). If an animal is called a mammal, it means the creature gives birth to well form babies and also rears them with milk during growth.

4. Taxonomy gives an ideas level of physical development: Taxonomy gives an idea of how far an animal has physical and mental development and its position in the evolution tree of organisms.

Ex: When you hear the word bacteria, you get an idea of single celled organism and fungi as a multi-celled organism yet both or microbes. Physically & evolutionary wise, fungi are advanced than bacteria.

5. Gives an idea of local fauna: Not all plants and animal species are found in all regions of the earth. Example kangaroo is limited to Australia likewise kiwi to New-Zealand etc. Even plants like Neem named as Azadirachta Indica (Indica= India) due its prominent presence in India. Hence taxonomy helps to identify or ascertain the types of plants and animals that can be found in particular region. This helps new scientists to go to the place of existence of the species to collect them in case they need to experiment on them.

How is taxonomy done: Taxonomy is done based on large view to smaller view like

a. Domain: 1. Archea (no nucleus, no organelles in cell), 2. Bacteria No well formed nucleus but has organelles 3. Eukarya: which means well formed nucleus and cell organelles.

b. Kingdom: gives ideas as 1. Animal 2. Plant. 3. Fungi 4. Protista.

c. Phylum: For zoology it gives idea if it is an insect type or worm type etc.

d. Class: Specifies the organism as mammal, bird, reptile etc.

e. Order: If you consider mammal it say whether it is a herbivore or carnivore etc.

f. Family: In botany plants are categorized or leguminous, solanaceae, euphorbeacea etc.families where in the plants in one family have few set of common physical characters.

g. Genus: This keeps the animals more specific ex: Frog as "Rana"

h. Species: This gives even specificity and in the above example of frog it say place of existence or physical character like

Rana tigrina: Indian bull frog, Rana Italica: Italian frog, Rana japonica:Japanese frog.

So taxonomy for Frog is as

Kingdom: Animalia- Means it is "animal type" and not a plant.

Phylum: Chordata- Means has "Spinal cord"

Subphylum: Vertebrata- Has vertebral column dorsally.

Class: Amphibia- Can live both in water and on land (Amphi- two; Bia-living).

Order: Anura – No tail (An= No; Ura = Tail)

Suborder: Neobatrachia- New type of frogs (Neo= new)
Family: Ranidae
Genus: Rana

In Biology importance of Taxonomy is immense for study, research and also seems to be quite interesting.But there are many changes, deviations and modifications of the taxonomy over time due to variations among living organisms. So you find not all animals can have all the sets of taxonomy and some can have extra sets like suborder, sub genera, sub-species etc.

Herbaria

A herbarium is a repository of preserved and labeled plant specimens, arranged to allow easy access and archival storage. The specimens are typically in the form of herbarium sheets: pressed and dried plants that have been glued or sewn to a sheet of heavy paper together with a data label. The label describes useful information including the plant's Latin name, the origin of the collection, the date of collection, and the name of the collector. Herbarium sheets are all of standardized size and arrangement, and are filed in protective storage cabinets. Within these cabinets the specimens are arranged according to the family, genus, and species they represent, as well as the geographic location from which they were collected.

The mission of a herbarium is to preserve and document the diversity of plants - from duckweed to sequoias. This mission to preserve all forms of plant life may be complicated by those that are too large or bulky to press and fit on a standard herbarium sheet. Cacti, palms, and rock-growing lichens present such challenges. In some cases a specimen may be spread out on several separate sheets. Large structures like pine cones may be stored in separate boxes. Delicate organs such as orchid flowers may be pickled and stored in jars. Specimens may also be complemented by photographs added to the sheet.

Other Collections: Many herbaria also preserve specimens of fungi, lichens, and even some bacteria (plant pathogens and cyanobacteria) in their collections. These organisms are not plants, but historically these "plant-like" organisms or structures (such as the fleshy fruiting bodies of fungi) were regarded as plants. Since these specimens may be preserved in much the same way as plants, their place in herbaria has been maintained. However, no herbaria attempt to fully represent the diverse kingdoms of Fungi or Monera.

A Herbarium Specimen: A herbarium specimen is simple in form and low-tech in preparation, yet it preserves a wealth of valuable information. If properly stored, a herbarium specimen will last for centuries without much deterioration. Specimens document the variation in form and geographical

range of species. Specimens preserve short-lived structures like cactus fruits, and rarely produced organs such as bamboo flowers.

Herbaria also document valuable historical collections, such as "type specimens", the original specimens on which a plant's Latin name is based. The data label preserves valuable information about plant habitats. Many herbarium specimens record the existence of plants in habitats now developed and lost.

They may also record the spread of weeds and new introductions like the European Helleborine orchid in Michigan. Some herbaria house the last preserved material of extinct plant species, such as the bizarre Thismia Americana, once found in wet prairies near Chicago.

Research: Herbarium specimens are very useful tools for botanists, particularly taxonomists, who seek to describe plant species, their form, and their habitats. Herbaria located at different institutions regularly loan specimens to each other to aid investigators working on specific groups of plants. Herbarium specimens are also useful for teaching purposes, since live material is not always available.

Other Equipment: In addition to plant specimens, herbaria regularly contain microscopes, plant presses, specimen dryers, and other tools for collecting, preserving and examining plant material. Herbaria often possess a library of books, journals, and maps useful for plant identification and taxonomic study.

Systems of Classification:

Diversity in the living world is also known as biodiversity. No two individuals can be similar in morphology or behavior and this difference is referred to as a Biodiversity. The different kinds of living beings represent a species. In today time there are around 2 million living beings. More of half of them are animals and plants account for nearly half a million. In order to explore the number of new species a project Global Biodiversity Information Facility & Species 2000 has been started. It is quite difficult to detect new

species in dense tropical rain forests and underwater reefs. However, these areas are shrinking due to more of human exploitation.

Fossils are defined as the remains of organisms which are no longer on earth. Microfossils denote to microorganisms. It is quite difficult to track extinct species and keep their respective records.

Systematic is defined as the branch of science that categorizes all living beings (plants, animals and other organisms) according to their names, features. Professionals which are involved in this type of study are known as Taxonomists. Technically, taxonomist and systematic have different meanings.

Different authors have given different definitions of Systematic. According to Simpson (1961) means diversity of organisms. It tells us about the variations occurring in living beings.

Historyof classification:

Different literatures have given different names to animals and plants. There are 740 plants and around one third of animals (250) as compared with plants according to Vedic Literature. Some animals were arranged according to their places of living by Hippocrates and Aristotle. Some used single criteria to classify them. Theophrastus, father of botany classified plants in his book Historia Plantarum. He used multifactor theory for his classification. The first system of artificial classification was given by Pliny the Elder in his book Historia Naturalis. A book named Historia Generalis Plantarum which tells us about 19000 plants was written by John Ray. He introduced the word species and defined it. Carl Linnaeus introduced different books for plants and animals. The former was known as Species Plantarum and later was known as Systema Naturae.

Natural systems of classification come under Classical Taxonomy. Numerical Taxonomy is the modification of this system. It further lead to evolution of phylogenetic (relationship between evolution and genetics)

classification or cladistics. It is also known as New Systematic (History as the basis of their evolution).

Characterization, Identification, Classification (arrangement of organisms according to convenience according to certain features) and Nomenclature (giving names for recognition and differentiation from other organisms) are the basics of Systematic study.

Classical Taxonomy deals with size, shape and form of an individual which defines its perfect expression. The remaining features are considered to be imperfect expressions. It involved many authors such as Plato, Aristotle, Theophrastus and Linnaeus. According to this system, species do not change. They are as important as nucleus of cell. While Modern Taxonomy deals with multiple factors (morphology, physiology, cytology, biochemistry, anatomy, genetics) etc. All factors are considered to be equally important. It involved only single author Julian Huxley. In this system species keep on changing and they are not so important.

Use of Taxonomy:

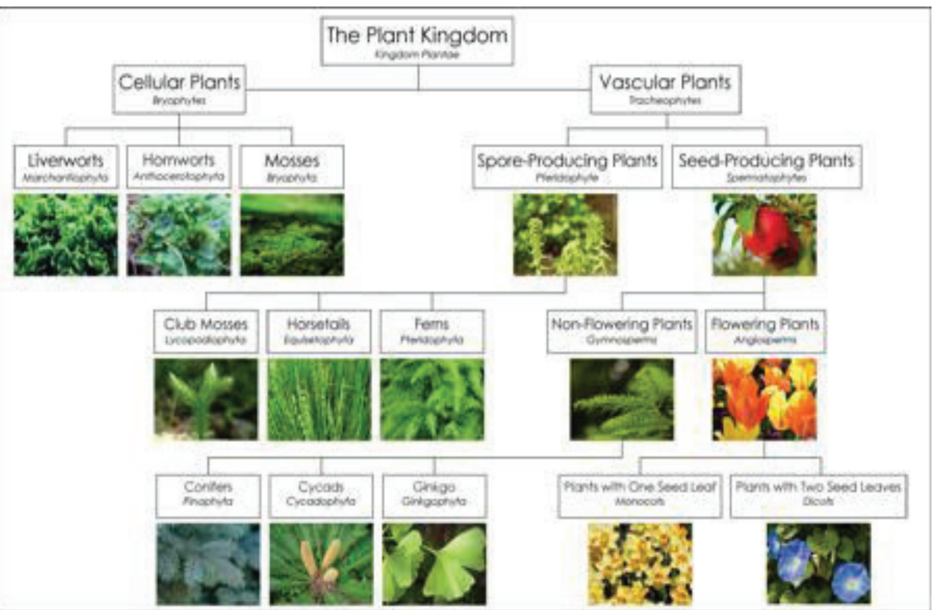
- Evolution & Ecology.
- Study and as indicator of organisms.
- Applied Biology.

Nomenclature (Nomen means name, Calare means call) is divided mainly into two parts: Common and Scientific names. Common Names are given according to the culture, language of specific area. They also rely on certain specific features also. They are also known as Vernacular Names. They are easy to remember but they cannot be used by professionals. Insignificant names, area specifications, and language barriers are the main reason behind it.

Scientific Names are given by professionals and are accepted worldwide. This process of giving scientific names is known as binomial nomenclature. It was given by Carl Linnaeus. It gives specific names and has two words, first means generic (written in capital form) and second responds to specific

(written mostly in small form). E.g. Homo sapiens. Homo is the generic name and sapiens is specific. Some of the scientific names have three words. The third word represents the subspecies (animals) or variety (plants).

There are certain guidelines of binomial nomenclature. These guidelines are provided by certain codes. These codes are updated by the international conferences held every year. Linnaeus made all the rules which are adapted and employed in today time. There are certain features which are quite common in most of species on the basis of which different taxonomic categories can be created are referred to as correlated characters.



Broad outline of Linnaeus

The Linnaean system of classification consists of a hierarchy of graded taxonomic (named) ranks that are called as taxa. Any giventaxon (singular) may contain several lower taxa, which can be usually distinguished based on certain common characteristics. Such lower ranks may in turn be divided into a succession of progressively smaller ranks. The lower the rank of a group, the more similar are the organisms grouped in it. If any two given organisms

can be grouped under the same lower rank or taxon, it implies that the two organisms are structurally, functionally, embryologically similar and that they have had a comparable evolutionary history.

Within the living world as a whole, the biggest taxonomic rank is Kingdom. The next higher rank within a kingdom is the Phylum orDivision. It is customary to use the term phylum for major groups in the animal kingdom and the term division for major groups in the plant kingdom. The phylum or division is a broad grouping of more or less closely related organisms, sharing certain common characteristics.

Each phylum or division has the next taxon called Class. The members of each class exhibit certain distinguishing characters that are unique only to them.

In the same way, using comparable criteria of similarities and relationships, each class can be divided into orders, each order intofamilies, each family into genera and each genus into species. Species is normally the basic or fundamental unit of classification. A species is therefore the narrowest taxonomic category and kingdom is the broadest category in the Linnaean hierarchy.

A typical Linnaean hierarchy has seven taxa represented as follows:

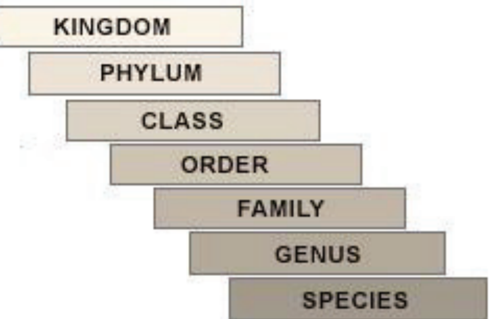
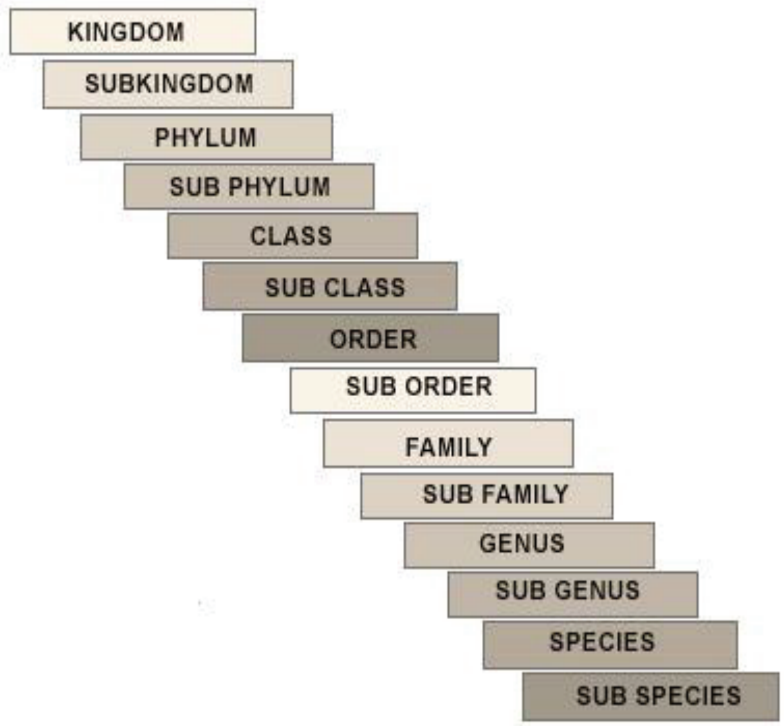


Fig. - Linnaean Hierarchy

Many a times, it may become necessary to make a distinction between two consecutive taxonomic ranks. In such a situation, an additional rank may be

introduced between any two existing ranks. A prefix sub or super is added to such new ranks. For example, between a phylum and a class, there may be subphylum or a superclass. Similarly between a class and an order there may be a subclass or superorder.

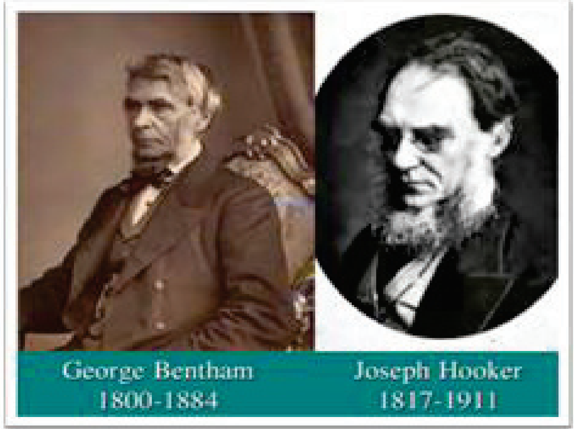
Thus, the extended Linnaean hierarchy may consist of the following taxonomic ranks.



In some cases, it is not universally agreed whether a given group of organisms represent a distinct phylum or sub phylum and so on. In fact, among taxonomists there is a general agreement regarding lower taxonomic ranks, rather than the higher ones.

The higher rank categories are actually being reshuffled more or less constantly. This is probably for the reason that these rankings incorporate most recent knowledge on the evolutionary history of these organisms. As this knowledge improves, the rank categories are rearranged accordingly.

Bentham& Hooker Classification:


George Bentham 1800-1884 Joseph Hooker 1817-1911

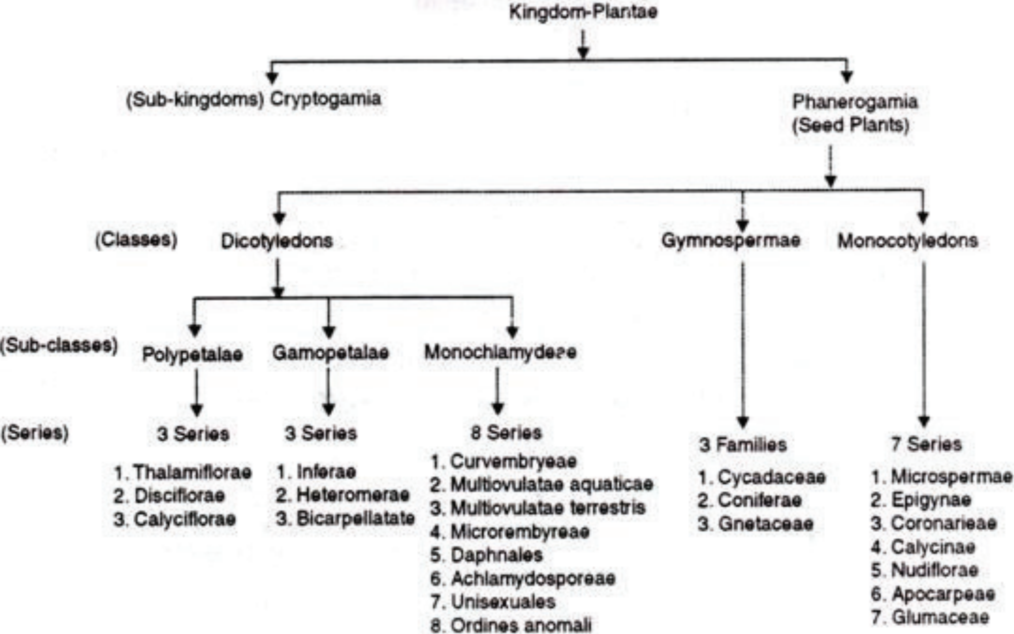
This is one of the most important and the last of the natural systems of classification of seed plants was proposed by two British taxonomists George Bentham (1800-1884), a self trained botanist, and Joseph Dalton Hooker (1817-1911), the first director of the Royal Botanical Garden, Kew (England).

They recorded precise description of most of the plants known at that time. Their monumental work which took about quarter of a century for completion was described in three volumes of Genera Plantarum, published in Latin during July 1862 and April 1883. Bentham and Hooker's system of classification is still used and followed in several herbaria of the world, more particularly in commonwealth countries. It is supposed to be the best system for the students to identify plants in the laboratory.

Salient Features of Bentham and Hooker's system:

1. It is a classification of only the "seed plants" or phanerogams.
2. They described 97,205 species of seed plants belonging to 7,569 genera of202 families starting from Ranunculaceae up to Gramineae.
3. They classified all the seed plants into 3 groups or classes i.e. Dicotyledons (165 families), gymnosperms (3 families) and monocotyledons (34 families).
4. They included disputed orders among Ordines Anomali which they could not place satisfactorily.
5. Monocotyledons were described after the dicotyledones.
6. The dicotyledons were divided into 3 Divisions (Polypetalae, Gamopetalae and Monochlamydeae) and 14 series. Each series again divided into cohorts (modern orders) and cohorts into orders (modern families).

7. The authors did not mention anything about the origin of the angiosperms.
8. Creation of the Disciflorae, a taxon not described by the earlier taxonomists.
9. Among the Monochlamydeae, major taxa, like the series, were divided on the basis of terrestrial and aquatic habits.
10. Polypetalae carries 82 families, 2610 genera & 31,874 species. Gamopetalae carries 45 families 2619 genera & 34,556 species. Monochlamydae includes 36 families, 801 genera & 11,784 species. Similarly Monocotyledons consist 34 families, 1495 genera and 18,576 species.



Merits of Bentham and Hooker's System:

1. Each plant has been described either from the actual specimen or preserved herbarium sheets so that the descriptions are detailed as well as quite accurate.

2. The system is highly practical and is useful to students of systematic botany for easy identification of species.

3. The flora describes geographical distribution of species and genera.

4. The generic descriptions are complete, accurate and based on direct observations.

5. Larger genera have been divided into sub genera, each with specific number of species.

6. Dicots begin with the order Ranales which are now universally considered as to be the most primitive angiosperms.

7. Placing of monocots after the dicot is again a natural one and according to evolutionary trends.

8. The placing of series disciflorae in between thalami florae and calyciflorae is quite natural.

9. The placing of gamopetalae after polypetalae is justified since union of petalsis considered to be an advanced feature over the free condition.

Demerits of Bentham and Hooker's System:

1. Keeping gymnosperms in between dicots and monocots is anomalous.

2. Subclass monochlamydeae is quite artificial.

3. Placing of monochlamydeae after gamopetalae does not seem to be natural.

4. Some of the closely related species are placed distantly while distant species are placed close to each other.

5. Certain families of monochlamydeae are closely related to families in polypetalae, e.g. Chenopodiaceae and Caryophyllaceae.

6. Advanced families, such as Orchiadaceae have been considered primitive in this system by placing them in the beginning. Placing of Orchidaceae in

the beginning of monocotyledons is unnatural as it is one of the most advanced families of monocots. Similarly, Compositae (Asteraceae) has been placed near the beginning of gamopetalae which is quite unnatural.

7. **The following is the detailed version of Bentham &Hooker classification (upto family level):**

1. DICOTYLEDONS – 2 cotyledons, exogenous growth.
1.1. POLYPETALAE – petals separate
1) THALAMIFLORAE – Sepals, Petals and Stamens all attached to receptacle.
a) Ranales – Gynoecium apocarpous.

1. RANUNCULACEAE
2. DILLENIACEAE
3. CALYCANTHACEAE
4. MAGNOLIACEAE
5. ANNONACEAE
6. MENISPERMACEAE
7.BERBERIDACEAE
8. NYMPHACEAE

b) Parietales – Parietal placentation. [NOT Natural. Convergent evolution: Papaveraceae close to Ranales; remainder scattered amongst Rosids]

9. SARRACENIACEAE
10. PAPAVERACEAE
11. CRUCIFERAE
12. CAPPARACEAE
13. RESEDACEAE
14. CISTACEAE
15. VIOLACEAE
16. CANELLACEAE
17. BIXACEAE

c) Polygalineae – Ovary 2-3 septate.

18. PITTOSPORACEAE
19. TREMANDRACEAE
20. POLYGALACEAE

d) Caryophyllineae – Axile placentation.

21. FRANKENIACEAE
22. CARYOPHYLLACEAE
23. PORTULACACEAE
24. TAMARICACEAE

e) Guttiferales – Stamens numerous; Calyx imbricate.

25. ELATINACEAE
26. HYPERICACEAE
27. GUTTIFERAE
28. THEACEAE
29. DIPTEROCARPACEAE
30. SARCOLAENACEAE

f) Malvales – Stamens numerous; Calyx valvate.

31. MALVACEAE;
32. STERCULIACEAE;
33. TILIACEAE.

2) DISCIFLORAE – Ovary superior, immersed in disk of flower.

a) Geraniales – Ovule pendulous, raphae ventral.

34. LINACEAE
35. HUMIRIACEAE
36. MALPIGHIACEAE
37. ZYGOPHYLLACEAE
38. GERANIACEAE
39. RUTACEAE
40. SIMAROUBACEAE
41. OCHNACEAE

42. BURSERACEAE
43. MELIACEAE
44. DICHAPETALACEAE

b) Olacales – Ovule pendulous, raphae dorsal.

45. OLACACEAE
46. AQUIFOLIACEAE

c) Celastrales – Ovule erect raphae ventral.

47. CELASTRACEAE
48. STACKHOUSIACEAE
49. RHAMNACEAE
50. VITACEAE

d) Sapindales – Ovule ascending, raphe ventral to reversed.

51. SAPINDACEAE
52. MELIOSMACEAE (=Sabiaceae)
53. ANACARDIACEAE
54. CORIARIACEAE
55. MORINGACEAE

3) CALYCIFLORAE – Stamens fused to Calyx of flower

a) Rosales – Ovaries separate, rarely united. [NOT Natural: Saxifragales as Eudicots,
Eurosids I, some Asterids]

56. CONNARACEAE
57. LEGUMINOSAE
58. ROSACEAE
59. SAXIFRAGACEAE
60. CRASSULACEAE
61. DROSERACEAE
62. HAMAMELIDACEAE
63. BRUNIACEAE

64. HALORAGACEAE

b) Myrtales – Ovary syncarpous; divided into locules.[still form a natural group at the base of the Rosids]

65. RHIZOPHORACEAE
66. COMBRETACEAE
67. MYRTACEAE
68. MELASTOMATACEAE
69. LYTHRACEAE
70. ONAGRACEAE

c) Passiflorales – Ovary syncarpous; Parietal placentation [more or less natural in the Eurosids I]

71. SAMYDACEAE
72. LOASACEAE
73. TURNERACEAE
74. PASSIFLORACEAE
75. CUCURBITACEAE
76. BIGONIACEAE
77. DATISCACEAE

d) Ficoidales – Ovary syncarous; sub-basal placentation [the basal placentation is critical in placing these families among the Caryophyllids]

78. CACTACEAE
79. AIZOACEAE

e) Umbellales – Ovary syncarpous; 1 ovule per locule. [not wholly natural; these families belong amongst the basal Asterids]

80. UMBELLIFERAE
81. ARALIACEAE
82. CORNACEAE

1.2. GAMOPETALAE – petals fused

1) INFERAE – Ovary inferior; stamen no. = petal no.

a) Rubiales – Stamens epipetalous; locules 2-many; ovules 1-many.

83. CAPRIFOLIACEAE
84. RUBIACEAE

b) Asterales – Stamens epipetalous; locule 1; ovule 1.

85. VALERIANACEAE
86. DIPSACACEAE
87. CALYCERACEAE
88. COMPOSITAE

c) Campanulales – Stamens free; locules 2-6; ovules many.

89. STYLIDACEAE
90. GOODENIACEAE
91. CAMPANULACEAE

2) HETEROMERAE – Ovary superior; stamens opposite petals or double the petal no

a) Ericales – Stamens double petal no.; Ovary 2-many locules.

92. ERICACEAE
93. CLETHRACEAE
95. EPACRIDACEAE (=Ericaceae)
96. DIAPENSIACEAE
97. LENNOACEAE (=Boraginaceae)

b) Primulales – Stamens opposite petals; Ovary 1-locule; axile placentation.

98. PLUMBAGINACEAE
99. PRIMULACEAE
100. MYRSINACEAE

c) Ebenales – Stamens opposite petals; few large seeds.

101. SAPOTACEAE
102. EBENACEAE

103. STYRACACEAE

3) BICARPELLATAE – Ovary superior, with 2 carpels.

a) Gentianales – Corolla regular; leaves opposite.

104. OLEACEAE
105. SALVADORACEAE
106. APOCYNACEAE
107. ASCLEPIADACEAE
108. LOGANIACEAE
109. GENTIANACEAE

b) Polemoniales – Corolla regular; leaves alternate.

110. POLEMONIACEAE
111. HYDROPHYLLACEAE
112. BORAGINACEAE
113. CONVOLVULACEAE
114. SOLANACEAE

c) Personiales – Corolla irregular to oblique; ovules many.

115. SCROPHULARIACEAE
116. GLOBULARIACEAE (=Plantaginaceae)
117. LENTIBULARIACEAE
119. GESNERIACEAE
120. BIGNONIACEAE
121. PEDALIACEAE
122. ACANTHACEAE

d) Lamiales – Corolla irregular to oblique; 1-2 ovules.

123. MYOPORACEAE
125. VERBENACEAE
126. LABIATAE

127. PLANTAGINACEAE

1.3. MONOCHLAMYDEAE – only 1 kind of perianth (not petals + sepals) this division was never intended to be natural – it was merely to help identify these singleperianthed plants. Some are basal families (Aristolochia, Piper) with unusual flower structures, whilst others are highly derived, with sepals or petals lost during evolution. Biochemical methodseased many queries in the placement of many of these families.

1)Curvembryeae: Curved embryos [The curved embryos & seeds make these part of the Caryophyllids]

128. NYCTAGINACEAE
129. ILLECEBRACEAE
130. AMARANTHACEAE
131. CHENOPODIACEAE
133. BATIDACEAE
134. POLYGONACEAE

2) Multiovulatae aquaticae:Aquatics with numerous ovules (Caryophyllids / Palaeoherbs)

135. PODOSTEMACEAE

3) Multiovulatae terrestris: Terrestrial plants with numerousovules

136. NEPENTHACEAE
137. RAFFLESIACEAE

4) Microembryeae:Microscopic seeds (Paleotrees/Palaeoherbs)

138. ARISTOLOCHIACEAE
139. PIPERACEAE
140. CHLORANTHACEAE
141. MYRISTICACEAE
142. MONIMIACEAE

5) Daphnales: Ovary usually with one carpel and single ovule (Paleotrees/Eudicots/Rosids)

143. LAURACEAE
143. HERNANDIACEAE
144. PROTEACEAE
145. THYMELAEACEAE
146. PENAEACEAE
147. ELAEGNACEAE

6) Achlamydosporaeae: Ovary usually inferior, unilocular with one to three ovules. (Eudicots/Asterids)

148. LORANTHACEAE
149. SANTALACEAE
150. BALANOPHORACEAE

7) Unisexulaes: Flowers unisexual (Eudicots/Rosids)

151. EUPHORBIACEAE
152. BALANOPACEAE
153. URTICACEAE
154. PLATANACEAE
155. LEITNERIACEAE
156. JUGLANDACEAE
157. MYRICACEAE
158. CASUARINACEAE
159. BETULACEAE

8) Ordines anomalus: Uncertain relationship (Paleoherbs/Rosids)

160. SALICACEAE
161. LACISTEMCEAE
162. EMPETRACEAE
163. CERATOPHYLLACEAE

2. MONOCOTYLEDONS – 1 cotyledon, Endogenous growth.

1)Microspermae:Ovary inferior and seeds very small

167. HYDROCHARITACEAE
168. BURMANNIACEAE
169. ORCHIDACEAE

2) Epigynae: Ovary usually inferior and seeds large

170. ZINGIBERACEAE
171. BROMELIACEAE
172. HAEMODORACEAE
173. IRIDACEAE
174. AMARYLLIDACEAE
175. VELLOZIACEAE
176. DIOSCOREACEAE

3) Coronarieae: Perianth petaloid and anther superior. Corolla-like flowers

177. STEMONACEAE
178. LILIACEAE
179. PONTEDERIACEAE
180. PHILYDRACEAE
181. XYRIDACEAE
182. MAYACACEAE
183. COMMELINACEAE
184. RAPATEACEAE

4) Calycinae: Perianth sepaloid and ovary superior. Calyx-like flowers

185. FLAGELLARIACEAE
186. JUNCACEAE
187. PALMAE

5) Nudiflorae: Perianth-less flowers and ovary superior

188. PANDANACEAE
189. CYCLANTHACEAE
190. TYPHACEAE
191. ARACEAE

192. LEMNACEAE

6)Apocarpaeae:carpels free

193. TRIURIDACEAE
194. ALISMATACEAE
195. JUNCAGINACEAE

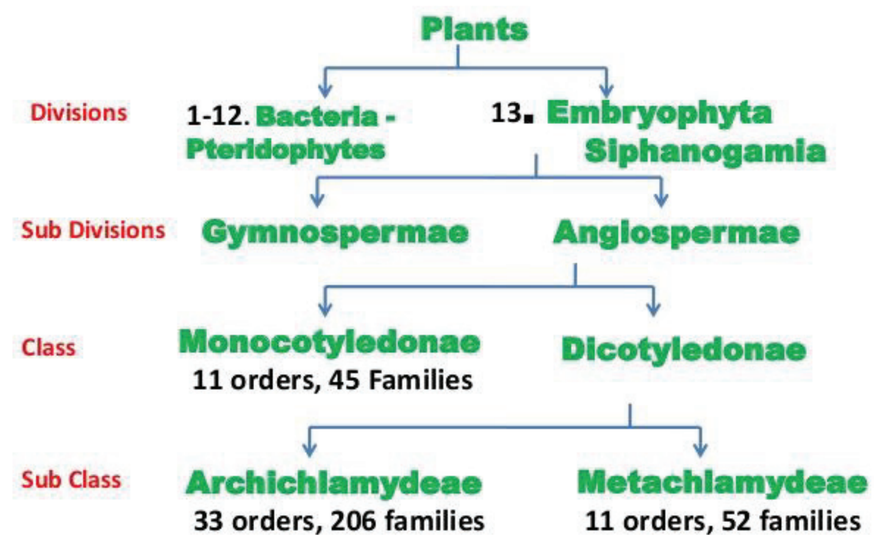
7) Glumaceae: Perianth, small or scale-like chaff

196. ERIOCAULACEAE
197. CENTROLEPIDACEAE
198. RESTIONACEAE
199. CYPERACEAE
200. GRAMINEAE (or POACEAE)

Engler and Prantl's system of Classification:

This is a system of classification of the entire plant kingdom, proposed jointly by two German botanists: Adolf Engler (1844-1930) and Karl A. E. Prantl (1849-1893). It was published in Syllabus der Pflanzenfamilien, and is also known as the Phylogenetic System. This edition is the the Angiospermae part, slightly modified by H. Melchior in 1964, and is also known as the Melchior System.

The plants were sorted by the basis of complexity of floral morphology. Characters like a perianth with one whorl, unisexual flowers and pollination by wind were considered primitive as compared to perianth with two whorls, bisexual flowers and pollination by insects. This was the first major Phylogenetic Classification and that gave a slightly changed August Wilhelm Eichler system. They dealt with the primitive groups as well. It is in line with Adolphe-Théodore Brongniart's 1843 work.



Plant nomenclature:

Nomenclature refers to the naming of things. Botanical nomenclature is (surprise) about naming plants. Bear in mind that plant names refer to abstract entities - the collection of all plants (past, present, and future) that belong to the same group. As you will recall, taxonomy is about grouping. Botanical nomenclature is about applying names to taxonomic groups.

Scientific names of plants reflect the taxonomic group to which the plant belongs. One must first decide on the groups to be recognized; only then does one start to be concerned about assigning an appropriate name to the plant. Common names, at least those that are genuinely common names, usually reflect some conspicuous or valuable characteristic of the plant, not its taxonomic group. The following comments are about scientific names.

Scientific names are never misleading. No matter where you are, every plant has only one correct name. So long as it's taxonomic treatment is not in

dispute. This last is a major reservation, but we can ignore it for now. The universality of scientific names means that even English speaking people can find out what species grow in China or Saudi Arabia by reading a technical flora of these countries. Not only are the names the same, they are always written in the Latin alphabet (which is the same alphabet as these notes).

Pronunciation: There is as little point about worrying over the 'correct' pronunciation of scientific names as there is in worrying over which is the correct pronunciation of English words. It may be difficult to recognize a scientific name if it is spoken by someone from another part of the world BUT one can always recognize it when it is written out. In this, scientific names are no different from other words. Think how hard it can be to understand different versions of English. Nevertheless, it is advantageous to use the same pronunciation as the other people you work with. Just be prepared to modify your pronunciation if you move to another part of the world.

Taxonomy refers to forming groups. Plants that belong to the same group have the same name. The taxonomic decisions concerning how a group is to be treated (what goes in the group, what rank it should be recognized as)**must** be made before it can be assigned a name. It does not matter how you decide what its affinities are (unless, of course, you want others to support and use your treatment), but you must make these decisions before you can decide on an appropriate name for the group. So remember, taxonomy first.
If people are going to communicate around the world, there needs to be an internationally accepted system of nomenclature. Creating such a system was not, and is not, an easy task. It was not until 1930 that agreement was reached on an International Code had become standard around 1753. There were, however, many areas where there was widespread agreement in practice, with some of the practices dating back to before Linnaeus. For reasons that you will learn later, Linnaeus is taken as the starting point for botanical nomenclature. Let's consider for a moment some of the areas of

agreement that existed before there was a formal agreement on an International Code of Botanical Nomenclature.

Towards an International Code

Pre-Linnaean Practices

1) Names were formed like Latin words. The reason is quite straightforward; Latin was the common language among all European peoples - and plant taxonomy as we know it has its origins in Europe.

2) Once a name had been attached to a plant group, it should not be given another name.

3) When commenting on how a name was to be interpreted, one should list the names of others that had used it.

4) It helps to mention some specimens that one has seen.

The first attempt at developing an international agreement was made in Paris in 1867. At this meeting, it was decided that a) the first edition of Linnaeus' Species Plantarum, which was published in 1752, would serve as the starting point of botanical nomenclature and b) if two names had been given to the same plant group, the older name would be the correct name. In addition, various rules were laid down as to what was required to valid publication - a phrase that means "published in such a manner that the name counts". For instance, publication of new names in horticultural catalogs used to be acceptable, but it is not any longer.

Other Codes

In 1892, a group of US botanists held a meeting in Rochester at which they presented some additions and modifications that they considered more

objective (a great phrase in science). Among the changes that they proposed were that a) when publishing a new name one should cite at least one herbarium specimen representing the plant group concerned and b) that, when a species was moved from one genus to another it should, if possible, keeps its specific epithet (it is not possible if that epithet has already been used for another species in the new genus). Some of the new rules conflicted with those proposed in Paris, and the modified version being used at Kew, a major taxonomic center in England.

Agreement, at last In 1930, taxonomists finally agreed on a single International Code of Botanical Nomenclature. This Code is revised every 6 years, but the goals of all the revisions are always to achieve stability in scientific nomenclature and or to clarify problems. The revisions are published in Taxon, the journal of the International Society of Plant Taxonomists, and then voted on at a meeting that is held immediately prior to an International Botanical Congress. The last edition of the Code was published in 2000. There is a copy in the herbarium.

Limitations of the Code

Before considering what the Code says, it is important to know what it does, and does not, attempt to do.

It DOES state what to do when you wish to assign a new name to a plant group, how the names of plant groups are to be informed, how to inform people about new names, and how to choose between two (or more) names that have been given to the same plant group.

It DOES NOT provide any information on how to decide whether a group of plants should be given a scientific name or what rank a group should have. These activities are taxonomic, not nomenclatural.

Remember: Taxonomy comes before nomenclature.

The International Code of Botanical Nomenclature

Becoming an expert on botanical nomenclature requires several years of study beyond graduate school, plus access to old and often rare, literature. Knowledge of Latin is also essential because many earlier works are in Latin. What follows is a distillation of some of the keys points of the Code, points that you should endeavor to understand. Some are presented in rather simplified form; be sure to consult the Code itself, plus a nomenclatural expert, before starting a serious argument or proposing a new name.

Principles of Botanical Nomenclature

There are six principles that guide decisions concerning the Code.

Uniqueness Principle (Principle I). The uniqueness principle states that there is only one correct name for a particular taxonomic group within a given taxonomic treatment. It is the central principle upon which all the remainder of the code is based. If people disagree on the taxonomic treatment, they will consider different names to be correct but, within any treatment, each taxonomic group has only one correct name.

Type Principle (Principle II). The type principle states, "The application of names of taxonomic groups is determined by means of nomenclatural types". For vascular plants such as grasses, a nomenclatural type is a herbarium specimen that has been deposited in a herbarium. A nomenclatural type anchors the meaning of a name. If there is an argument as to what kind of plant the author of a name meant by a particular name, one examines the type specimen. No matter what taxonomic treatment is followed, the name must be used in a sense that includes its type specimen.

If, as occasionally happens, the author of a new name provides a description that does not match the type specimen, it is the type specimen, not the

description, that determines what kind of plant is called by the name in question.
Adherence to the type principle did not become mandatory until 1958. Prior to that time, when taxonomists published a new name they frequently simply listed several different specimens that exemplified what they meant by the name, without identifying any particular specimen as the 'top dog' among the examples. All the designated specimens, including their duplicates, are referred to as syntypes: nomenclatural types of a single name, all of which were equally important. This became a problem if later taxonomists decided that there are two or more taxa among the specimens listed. When this happens, it became necessary to determine which of the specimens listed belongs with the original name.

To prevent such situations arising, the rules for designating a type specimen were made more explicit. Since 1990 it has been necessary to identify the exact specimen that is to be the nomenclatural type of the taxon, and the herbarium in which the specimen is located. Between 1958 and 1990 it was enough to specify who collected the specimen, where it was collected, the date on which it was collected, and the collection number it was given, if any. The problem was that, if the collector made several duplicate specimens, each of the duplicates is a syntype. In most instances this is not a problem, but occasionally the supposed duplicates turn out to belong to different species. Requiring that an author state exactly which of the specimens is to be regarded as the nomenclatural type helps prevent even this kind of problem. If possible, the accession number of the type should be specified as well as the name of the herbarium in which it is located, but many older herbaria do not give their specimens accession numbers.

There are several different kinds of type specimen, but the most important are holotypes, lectotypes, neotypes, and epitypes. The next most important are isotypes, syntypes, and paratypes. The first four kinds of type refer to specimens that are, unequivocally, the nomenclatural type of a name. A holotype is a specimen that has been designated the nomenclatural type of a

name by the person creating the name. If the person who originally published a particular name did not designate a holotype, a later taxonomist may select a specimen to serve as the nomenclatural type. This specimen then becomes what is called the lectotype of the name. If the holotype or lectotype is destroyed or lost, a new type specimen can be selected. Such replacement types are called neotypes.

An epitype is a specimen selected to be the nomenclatural type of name for which there is a holotype, lectotype, or neotype available. Why would it be necessary to select another specimen as a nomenclatural type? Sometimes the holotype, lectotype, or neotype simply does not show the features that are needed to determine, unequivocally, to which of two taxa it belongs. In such a case, it cannot be used to fix the meaning of a name. In such situations, another specimen can be selected as the 'anchoring' specimen; it is this specimen that is the epitype.

Priority Principle (Principle III). This principle states, in essence, that if a taxonomic group has been given two or more names, the correct name is the first name that meets the Code's standards for publication. Basically, this means that the priority of a name dates from the time that it was first published and made known to other botanists. Writing the name in a letter (or Email) to a colleague does not count, nor do notes made on herbarium sheets.

Taxonomic groups may end up with two or more names for several reasons. The most common reason is taxonomic disagreement, about which the Code says nothing. Sometimes, the person publishing a later name is simply unaware that the group has already been named. In other cases, two (or more) names were given to different looking specimens of what was later treated as a single group. Whatever the reason, the priority principle states that only the first name validly and legitimately published for a particular taxonomic group is correct.

In determining priority, the date that matters is the date on which the material was actually mailed to other institutions; this is not always the same as the year on the cover of a book or journal.

Retroactivity principle (principle IV). This principle states, "The Rules of nomenclature are retroactive unless expressly limited". The Retroactivity Principle means that anyone proposing a change in the Code needs to consider the effect that the proposed change will have on names published in a wide range of literature and over a considerable period of time. This is an intimidating requirement.

It is why all proposed changes to the Code undergo committee scrutiny before being voted on. If the committee has a problem with a proposed change, one of its members will get in touch with the person proposing the change. The committee member may point out unforeseen consequences of the proposed change. Alternatively, he or she may suggest examples that will make a stronger case for the change, or suggest modifications that will avoid some undesirable consequences.

All proposals to change the Code are published in Taxon, but they remain proposals until they are voted on at the next International Botanical Congress.

Other key provisions of the code

1. Any changes in the Code should be designed to increase the stability of plant nomenclature. No one likes name changes, not even the taxonomists that propose them.

2. Every plant belongs to a species, every species to a genus, every genus to a family, every family to an order, every order to a class, every class to a division (also called a phylum nowadays - a concession to the greater number of zoologists in the world). This is the taxonomic hierarchy. Note that the Code assumes the existence of species. It does NOT state what constitutes a species, let alone discuss whether species are real. The Code also requires

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that plant diversity be summarized in a hierarchical structure. Again, it is not a question of whether such a structure really exists. The fact that the Code assumes the existence of species and a hierarchical structure does not mean that that the assumptions are correct, merely that, in naming plants (and the zoological code is similar in this regard), one must act as if species are real and nature is hierarchical. Many people object to this, but no one has provided a persuasive argument for dropping the system.

Publishing scientific names. Before a name, even a name that has a Latin form can be accepted as a scientific name, it must satisfy several criteria. Some of these have to do with its form, others with how its existence and meaning are made known to others.

Form. Principle V states that a scientific name must be treated as if it were Latin, but the Articles 16-28 of the Code also specify what form the name must take. I have summarized them in the table below.

Rank	Ending	Examples
Division (Phylum)	-phyta	Pinophyta, Magnoliophyta
Class	-opsida	Pinopsida, Liliopsida, Magnoliopsida
Order	-ales	Pinales, Liliales, Magnoliales
Family	-aceae	Pinaceae, Liliaceae, Magnoliaceae
Tribe	-eae	Pineae, Lilieae, Magnolieae
Genus	A noun	Pinus, Lilium, Magnolia
Species	Depends	Pinus flexilis,Lilium grandiflorum, Magnolia grandiflora

Variety Depends Pinus flexilis var. humilus
Form Depends

Family names must be formed by combining a generic name with the suffix –aceae, but there are eight exceptions to this rule. Each of the eight exceptional names was almost universally used, and used in the same sense, throughout the world when the first edition of the Code was prepared and so, in accordance with the overriding goal of achieving nomenclatural stability, it was agreed that they would continue to be used.

The eight names are Gramineae (Grass Family, alternative Poaceae) Palmae (Palm Family, alternatively Arecaceae), Cruciferae (Mustard Family, alternatively Brassicaceae), Leguminosae (Pea family, alternatively Fabaceae), Guttiferae (St. John’s Wort Family, alternatively Clusiaceae), Umbelliferae (Carrot Family, alternatively Apiaceae), Labiatae (Mint Family, alternatively Lamiaceae), and Compositae (Daisy Family, alternatively Asteraceae).

The name of a species is ALWAYS a binomial. 'Grandiflora' is not the name of a species. It has to be combined with a generic name to form the name of a species, as in Magnolia grandiflora. The word 'grandiflora' is what we call the specific epithet. It states which species of Magnolia is under discussion. Specific epithets are often adjectives that describe some attribute of the plant (it helps to learn a little Latin - 'grandiflora' means large flowered), but may refer to the habitat of a species (pratensis -of fields, lacustris - of lakes, saxicola - of rocky places), the place where the species occurs (chinensis, europaea, canadensis), or a person that is somehow connected to the species (the connection may be remote) - wrightii (referring a single, male person named Wright), wrightiae (referring to a single female person named Wright), wrightorum (refering to 2 or more people, one of whom - and possibly only 1 out of a 100 - was male) or wrightarum (referring to 2 or more people with not even one male among them - the Romans were sexist).

Technically speaking, subspecies is a higher rank than variety. A subspecies may include several varieties. In practice, most taxonomists nowadays use one rank or the other, but not both. Europeans tend to use subspecies and expect subspecies to occupy somewhat different areas whereas Americans use variety to denote plants that are different from the plants first put in the species. In practice, the two ranks are used almost interchangeably.

There are several optional ranks that are not listed above. For more information, consult the Code.

Writing Scientific Names

In North America it is customary to write names at the rank of genus and below in italics or some other font that sets them apart from the rest of the text. The most recent edition of the Code recommends that all scientific names, no matter what their rank, be in a different font from the rest of the text. Either practice makes it easy to scan for taxonomic information.

The names of all ranks from subgenus up MUST be capitalized. In most instances, the specific epithet - and epithets for lower rankings, must NOT be capitalized.

There are some exceptions to this rule, cases where it is permissible, but not required, to capitalize the specific or varietal epithet, but you need to be careful

Authorities

You will notice that scientific names are often followed by a word or a capital letter and a period, or one or more unintelligible (to the uninitiated) sets of letters. To join the initiated, read on.

The letters and/or words that follow a scientific name (sometimes they may be within a name - more on that later) are a shorthand reference to the name of the person or person that first gave a name to the entity involved and, in some instances, to the person of persons who first treated it at the rank being used. This is probably easier to understand through some examples.

Consider Oryzopsis exigua Thurber

Note that only the first two words are italicized. This means you are looking at the name of a species. 'Thurber' is the last name of the person who first gave a name to this species - and the name he gave to it is the one shown.

Consider "Oryzopsis asperifolia Michx."

Again, you are looking at the name of a species in the genus Oryzopsis. This species was first named by a fellow whose name is abbreviated to Michx. The period tells you that his name has been abbreviated. His full name was Michaux.

To whom do you think "L." refers to in "Triticum aestivum L."?

"Dichanthelium lanuginosum (Elliott) Gould"

The name is Dichanthelium lanuginosum. As you immediately recognize (because the name is a binomial), the entity being named is being treated as a species. The first person to give a name to this species was a chap whose last name was Elliott, but he named it Panicum lanuginsoum. An inner circle of initiates could tell you that Elliott refers to Walter Elliott, who lived from 1803 to 1887, in eastern North America (There is a book called Authors of Plant Names that provides such insight).

"Gould" stands for Frank W. Gould came along later and decided that, although Elliott was right in describing the species, he should have put it in a

different genus, the genus Dichanthelium. Elliott's name is in parentheses to show that he was the first person to say "Aha, these plants are different"' Gould's name is outside the parentheses because he said, yes, Elliott was right - these plants are different - but they should be included in the genus Dichanthelium, not Panicum

Consider "Distichlis spicata (L.) Greene

Linnaeus [L. stands for Linnaeus] first described the entity, but as Uniola spicata, not Distichlis spicata. Greene was the first person to say no, these plants should be in Distichlis and then publish the combination "Distichlis spicata". Linnaeus gets credit for being the first person to describe the entity, Green for being the person to give it the name shown.

Most journals, and consequently many professors, ask that you cite the authorities for a name when it is first used. It is a rather meaningless exercise. It is meant to say "I am using this name in the sense that it was used by Greene (in the last example)", but really you are probably using it in the sense that it is used in some flora - or based on what your boss told you. The 1999 Congress encouraged editors to be more rational about when it was useful to cite authorities and when not, but I suspect that most journals will continue to require them for some time to come.

Proposing a new name or new combination

If you have to publish a new name or combination, the Code requires that you follow certain rules (which it calls articles). The key requirements are that:

1. The new name or combination be published in a normal botanical outlet (not the Herald Journal or Statesman), copies of which are sent to at least two botanical institutions.
2. If the name is for a new taxon, the distinguishing characteristics of the taxon, and preferably a full description, must be given in Latin and a holotype specified.

3. If the name is simply a new combination, perhaps reflecting the transfer of a species from one genus to another or its demotion to a subspecies, there must be a clear and complete reference to the place where the original name was first published.

Why do scientific names get changed?

1. Discovery of an older name for the taxon that has been overlooked. In the last decade, it has become possible to conserve the name actually being used if one can show that the earlier name has never become established. This is a nomenclatural, not taxonomic reason, for changing a name.
2. Discovery that the name being used for a particular taxon had been applied earlier to some other taxon. This is a nomenclatural, not taxonomic reason, for changing the name.
3. A decision that a species belongs in a different genus, or that a taxon needs to be split, or that the rank of the taxon needs to be changed. These are all taxonomic decisions.

Most name changes reflect taxonomic decisions, but people that do not agree with the decision may continue to use the existing name. This is what non-taxonomists find frustrating, if not infuriating. Such people often become even more frustrated when told that there is no set of criteria or any governing board that determines at what rank a taxon should be recognized at, or what its boundaries should be. There are stronger and weaker arguments, but there is not even complete agreement on which strong arguments are and which are weak. Taxonomy is not a field for those that require certainty in their life.

Other Names

Plants are often known by many different names. The names Convolvulus arvensis; Bindweed, Field bindweed, Common bindweed, Small bindweed, Morning glory, and Liseron des champs all refer to the same species. The scientific name is Convolvulus arvensis. The other names are what are called

common names or vernacular names. I prefer the phrase 'vernacular name' because many so-called common names are simply names constructed to satisfy the demand for a name in a familiar language - they are not names in common use.

Although many people like 'common names', there are many problems associated with them. For instance, Indian ricegrass (Achnatherum hymenoides) is not a close relative of either rice or wild rice (two very different species), but it was used for food by Native Americans and looks something like short grain rice. I regard it as a genuine common name - among English speaking people. But the species was an important component of the diet of the Native Americans in Utah and the west. I rather doubt that it is called Indian Ricegrass in any Native American language.

Sometimes, the common name is the same, or partly the same as the scientific name. Many of you probably have no problem understanding Penstemon and Delphinium, but both of these are scientific names. If you grew up in England or Australia, you would also be familiar with Capsicum as a common name, but in North America the commonly encountered species of Capsicum are called bell peppers or chili peppers. Despite their American names, species of bell peppers and chili peppers are more closely related to potatoes, eggplant, and nightshades than the kind of pepper that we use in pepper grinders.

Problems arise when vernacular names have been created based on scientific names, but the meaning of the scientific name changes. For instance, all species in the genus Agropyron were given common names that incorporated the word 'wheatgrass'.

The trouble is that most of these species have since been kicked out of Agropyron. It is not a huge problem, but it does point up how artificial so many 'common names' are.

Another problem with common names is that they may apply to more than one species. Corn used to the name for the grain most used for flour. In England, corn meant wheat; in Scotland, it meant rye or barley; in these two countries what Americans call corn was known as maize. With the increasing dominance of American English, corn is now generally interpreted as meaning Zea mays - otherwise known as maize. Similarly 'Bluebell' forms part, or all, of the name of many different plants. I learned of it as referring to monocots that are sold here as Wood hyacinths. In Scotland, it applies to what I would call a Harebell. But the northern Utah flora refers to as Arctic bellflower. This same work gives Bluebell as the common name for Mertensia, a third genus and a third family. The USDA PLANTS database lists 18 different species as having Bluebell as part of their common name.
Common names have local value; scientific names have universal value.

Official Names

In some countries, one or more government agencies create plant names in the country's native or official language which they require their employees and contract employees to use. Some of these names are what I would refer to as the truly common names, but many are just extensions of a true common name to other species, often by translating the specific epithet. Official names can be useful in talking to non-botanists, but the result is often a parallel system of nomenclature.

The U.S.A. is one such country. Indian ricegrass appears to be a genuine common name, that is, one that ordinary people coined and used, for the species that used to be known as Oryzopsis hymenoides. Unfortunately, the USDA decided that all species of the genus Oryzopsis should be called ricegrasses so the official name of O. kingii became King Ricegrass and O. asperifolia became Roughleaved ricegrass although neither of these species has ever been used as a source food for humans. The problem with this approach to creating official names (which are generally called common names) is that taxonomic study shows that neither Oryzopsis hymenoides nor O. kingii belongs in Oryzopsis. Oryzopsis hymenoides is now placed in

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either Achnatherum or Stipa (there is taxonomic disagreement) while O. kingii is placed in the genus Ptilagrostis.

Rank of Taxa:

The International Code of Botanical Nomenclature, which determines how plants are named, lists several different ranks, or levels of grouping for plants. Bear in mind that the Code was designed by humans, for humans. It was also developed at a time when the concept of a 'testable hypothesis' had not been developed and when there were fewer people involved in taxonomy.

Principal ranks: The Code states that every plant belongs to one, and only one, species and that every species belongs in a genus, every genus in a family, every family, in an order, every order in a class, every class in a division (which may be referred to as a phylum), and every division in a kingdom. These are the principal ranks, the ranks to which every species belongs.

Secondary ranks: There are also several secondary ranks. These are ranks that may be used, but need not be. The secondary ranks are generally used to subdivide large groups. Thus, a large family may be divided into tribes, a large genus into sections, large sections in series, large species into varieties, and large varieties into forms.

"Large" is used rather loosely here. So long as there are two elements in the group, one can recognize a subgroup. For instance, one could put the two genera of a single family into two tribes, each tribe having one genus, but this is not usually done.

If that is not enough ranks, one can always create additional ranks immediately below any or all of the principal or secondary ranks by adding the prefix "sub-" to the rank concerned. For instance, subspecies is a rank immediately below a species but above a variety. Subfamily is a rank immediately below a family but above a tribe. Similarly, one can insert ranks above any of the recognized ranks, e.g., a superorder or super-division.

The manner in which names are to be formed are summarized in the table below, which shows the ranks in descending order, from the highest, most inclusive, group to the lowest, most exclusive, group.

Limits of the Code: What the Code does not do is state how one is to determine what the appropriate rank for a group is or how to determine whether a group of plants merits formal recognition as a taxonomic group. The Code does not, in other words, provide any guidance in making taxonomic decisions. It merely states how to decide the name of a group that you have decided warrants formal taxonomic recognition. Taxonomy comes first; nomenclature second.

Names

In the table that follows, Red indicates the principal ranks. The italicized name in the first column is the formal, Latin, name for the rank. The second column gives a quick summary of how names at that rank are to be formed. The third column gives some examples. In keeping with the practice adopted in the most recent issue of the Code, all scientific names are italicized. Only names above the rank of species begin with an upper case letter.

You absolutely must learn to recognize when a name is a family name, when it is probably a generic name, and when it is a species name (this last is easy - the name of a species is ALWAYS a binomial). You should also learn the endings of the principal ranks if you are wishing to impress me.

None of the nomenclatural codes recognize the rank of Domain. It is a rather recent rank, created after it was realized that bacteria fall into at least two very distinct groups.

Ophioglossidae

Ordo (Order) Either a distinctive character of the taxon or the name of an included genus followed by –alesMagnoliales.

DMI - St. Eugene University Biology - V

Suborder (suborder) Either a distinctive character of the order or the name of an included genus followed by -ineae
Malvineae

Familia (Family) basically, a generic name plus -aceae (but see eight exceptions) Magnoliaceae

Subfamilia (Subfamily) Similar to family names, but suffix is –oide Panicoideae, Rosoideae

Tribus (Tribe) Similar to family names, but suffix is –eaePaniceae, Roseae

Subtribus (Subtribe) Similar to family names, but suffix is –inaeAndropogoninae, Rosinae

Genus (Genus) A single word; it must be capitalized.Rosa, Ulva

Subgenus (Subgenus) A bit complex, but you must state the name of the genus, the name of the rank, and capitalize the subgeneric epithet. Only the generic name and the epithet are capitalized. Costus subg. Metacostus

Sectio (Section) as for subgenus
Ricinocarpus sect. Anomodiscus

Subsectio (Subsection) as for subgenus
Sapinum subsect. Patentinervia

Series (Series) as for subgenus
Arenaria ser. Anomalae

Subseries (Subseries)as for subgenus

Species (Species)

A binary combination consisting of a generic name plus a single specific epithet (which can be hyphenated). The specific epithet can, under certain circumstances, be capitalized, but it is safer not to do so. Poa pratensis, Scandix pecten-veneris

Subspecies (Subspecies)Name of the species followed by the name of the rank and the epithet for that rankStipa nelsonii subsp. dorei

Varietas (Variety)as for subspecies Lycopodium inundatum var. bigelovii

Subvarietas (Subvariety) as for subspecies

Forma (Form) as for subspecies

Subforma (Subforma) as for subspecies

Alternative Family Names

There are eight family names that have been used for so long that they are accepted as correct, despite the fact that they do not follow the rules. They are listed in the table below, together with the name that the family has if one insists on using family names that are based on a generic name.

PalmaeArecaceaePalm Family

Traditional Name Alternative English name

Gramineae Poaceae Grass family

PalmaeArecaceae Palm Family

CruciferaeBrassicaceae Mustard family

Leguminosae
Fabaceae (be careful) Bean family (Peas, beans, Palo Verde, Mimosa)

Guttiferae Clusiaceae (be careful) St. Johnswort Family

Umbelliferae Apiaceae Umbels (Parsley, Lomatium, Dill)

Compositae Asteraceae Daisy family (Dandelions, daisies)

Labiatae Lamiaceae Mint Family

Warning: Fabaceae is sometimes interpreted to mean just the pea/bean portion of the Leguminosae; the meaning of Leguminosae always includes

the mimosoids and caesalpinoids as well as the pea and bean taxa. Similarly, Guttiferae always includes both the Clusiaceae and Hypericaceae (possibly with some other taxa as well).

Authors of plant names, also known as authorities

The meaning of a plant name is determined by the person(s) who first publishes it. This person(or these people) are known as the author(s) for the name. For some reason, the practice used to be to call these people the authority.

The practice of citing an author with a scientific name started early on, long before the International Code was adapted. To give credit to the person involved, and also help other people understand what was meant by a name, taxonomists adopted the practice of writing the name of the person whose meaning they were following after the plant name, sometimes with a note as to where he (in the early days, always a 'he') described it. For instance, Linnaeus noted that he included in what we would call Solanum tuberosum the species that Bauhin (1623) called Solanum pomiferum.

Origin of binomials

Consider another species, one that we would now call Solanum tuberosum, better known to you as potato. In Species Plantarum, Linnaeus called this species Solanum caule inermi herbaceo, foliis pinnatis integerrimus. Freely translated, this means the Solanum with herbaceous, unarmed stems, pinnately compound leaves with entire margins. Even Linnaeus found this a trifle long, so he wrote in the margin 'tuberosum'. He considered 'Solanum tuberosum' to be the trivial name of the plant, the equivalent of what we would now call a common name, but a common name understood by anyone in the world with grade school education (Latin was taught in grade school). For another example, see the discussion of Zea in Species plantarum.

Taxonomists before Linnaeus had suggested using binomial names for species; Linnaeus did not agree, but by providing a marginal name for all the many species he described, he provided the impetus that led to widespread

agreement that binomials are better than long phrases. When the International Code of Botanical Nomenclature was first proposed, it was formally agreed that vascular plant nomenclature would start with Linnaeus' trivial names. (Later it was decided that the starting point for some groups, notably fungi and algae, would be other works, but that does not concern us).

Effective naming:

The Rule 23a suggests that, each taxon above species, up to and including order, with a given circumscription, position, and rank can bear only one correct name, that is, the earliest that is in accordance with the Rules of this Code.

The name of a species is a binary combination of a generic name and specific epithet. In a given **position,** a species can bear only one correct epithet, that is, the earliest that is in accordance with the Rules of this Code.

Example: The species *Haemophilus pertussis* bears this name in the genus *Haemophilus*. If placed in the genus *Bordetella,* it bears the name *Bordetella pertussis*.

Note 1. In the case of a species, Rule 23a must be applied independently to the generic name and the specific epithet. The specific epithet remains the same on transfer of a species from one genus to another unless the specific epithet has been previously used in the name of another species or subspecies in the genus to which the species is to be transferred

Note 2. The name of a subspecies is a ternary combination of a generic name, a specific epithet, and a subspecific epithet. In a given position a subspecies can bear only one correct subspecific epithet, that is, the earliest that is in accordance with the Rules of this Code. In the case of a subspecies, Rule 23a must be applied independently to the specific and subspecific epithets. The subspecific epithet remains the same on transfer of a subspecies from one species to another, unless the subspecific epithet has been previously used in the name of another species or subspecies in the genus to which the subspecies is to be transferred.

Note 3. The date from which all priorities were determined under the previous editions of the Code was 1 May 1753. After 1 January 1980, under Rule 24a all priorities date from 1 January 1980.

Note 4. The Judicial Commission may make exceptions to Rule 23a by the addition of names to the list of **conserved names** *(nomina conservandd)* or to the list **of rejected names** *(nomina rejicienda*). The Judicial Commission may correct the Approved Lists.

i. By conserved name (nomen conservandum) is meant a name which must be used instead of all earlier synonyms and homonyms. By rejected name (nomen rejiciendum) is meant a name which must not be used to designate any taxon. Only the Judicial Commission can conserve or reject names (see also Rule 56a, b).

ii. **Opinions** on the conservation or rejection of names, issued by the Judicial Commission, are published with other Opinions in the IJSB. Opinions are now numbered serially.

Note 5. Names and epithets may be:

Legitimate—in accordance with the Rules;

Illegitimate—contrary to the Rules;

Effectively published—in printed matter made generally available to the scientific community.

Validly published—effectively published and accompanied by a description of the taxon or a reference to a description and certain other requirements.

Correct—the name which must be adopted for a taxon under the Rules.

Author Citation:
An author should indicate that a name is being proposed for a new taxon by the addition of the appropriate abbreviation for the category to which the taxon belongs.

Note 1.
Appropriate abbreviations are: "ord. Nov." for ordo novus, "gen. Nov." for genus novum, "sp. nov." for species nova, "comb, nov." for combinatio nova. Similar abbreviations may be formed as required.

Note 2.
Although words or abbreviations in Latin are usually printed in italics, such abbreviations as the above are frequently printed in Roman or boldface type when they follow a Latin scientific name in order to differentiate them from the name and draw attention to the abbreviation.

Examples: Order, Actinomycetales ord. Nov.; family, Actinomycetaceae fam. Nov.; genus, Actinomyces gen. nov.; species, Actinomyces bovis sp. nov.

The citation of the name of a taxon that has been previously proposed should include both the name of the author(s) who first published the name and the year of publication. If there are more than two authors of the name, the citation includes only the first author followed by "et al." and the year.

Example: Actinomyces bovis Harz 1877.

Note 1. Correct citation of a name enables the date of publication to be verified, the original description to be found, and the use of the name by different authors for different organisms to be distinguished.

Example: Mycobacterium terrae Wayne 1966, not Mycobacterium terrae Tsukamura 1966.

Note 2. Full citation of the publication should include reference to the page number(s) in the main text of the scientific work in which the name was proposed, not to the summary or abstract of that text even if proposal of the name is mentioned in that summary or abstract.

Example: Bacillus subtilis (Ehrenberg 1835) Cohn 1872, 174. The page number "174" is the page in Cohn's publication (Untersuchungen über Bacterien. Beitr. Biol. Pfl. Heft 2 1:127–224) on which the proposal of the new combination occurs.

Note 3. The citation of a name which is included in an Approved List can include the name of the original author and date of publication followed by the words "Approved Lists 1980" in parentheses.

Example: Bacillus cereus Frankland and Frankland 1888 (Approved Lists 1980); Bacillus subtilis (Ehrenberg 1835) Cohn 1872 (Approved Lists 1980).
Alternatively, a name which is included in an Approved List may be cited simply by the addition of the words "Approved Lists 1980" in parentheses.
Examples: Bacillus cereus (Approved Lists 1980); Bacillus subtilis (Approved Lists 1980).
If indication is given that a name is included in an Approved List without specification of that list, the abbreviation "nom. approb." (Nomen approbatum) may be appended to the name of the taxon.
Example: Bacillus subtilis nom. approb.

If a name or epithet which was published prior to 1 January 1980 but not included in an Approved List is proposed by an author for a different or for the same taxon, the name or epithet must be attributed to the author of the proposal, and the citation should be made accordingly.

Note 1.
If a name or epithet is revived for the same taxon (in the author's opinion), the author may indicate the fact by addition of the abbreviation "nom. rev." (Nomen revictum) after the correct abbreviation (Rule 33a) for the category concerned.

Example: Bacillus palustris sp. Nov. nom. rev.

Note 2. If an author wishes to indicate the names of the original authors of a revived name, he may do so by citation of the name of the taxon, followed by the word "ex" and the name of the original author and the year of publication, in parentheses, followed by the abbreviation "nom. rev."

Example: Bacillus palustris (ex Sickles and Shaw 1934) nom. rev. A subsequent author citing this revived name would use the citation Bacillus palustris Brown 1982, or Bacillus palustris (ex Sickles and Shaw 1934) Brown 1982.
Note 3. If an author wishes to indicate that a reused name has been used for a different taxon, indication is made by citation of the name and the author and year of publication followed by the word "non" (or "not") and the name and year of the publication of the author who first used the name.

If a name is revived under Rule 33c it may be revived in a new combination; that is, the revived species may be transferred to another genus, or the revived subspecies may be transferred to another species, at the time the name is revived. It is not necessary first to revive the name in the original combination.

Example: Bacillus palustris may be revived by Brown as a species of the genus Pseudomonas as Pseudomonas palustris (ex Sickles and Shaw 1934) nom. rev., comb. Nov. A subsequent author could cite it as Pseudomonas palustris (ex Sickles and Shaw 1934) Brown 1982.

Proposal and Subsequent Citation of a New Combination

When an author transfers a species to another genus or a subspecies to another species, then the author who makes the transfer should indicate the formation of the new combination by the addition to the citation of the abbreviation "comb, nov." (Combinatio nova).

This form of citation should be used when the author retains the original specific epithet in the new combination; however, if an author is obliged to substitute a new specific epithet as a result of homonymy, the abbreviation "nom. nov." (Nomen novum) should be used. The original name is referred to as the basonym.

Example: Actinomyces exfoliatus Waksman and Curtis 1916; Streptomyces exfoliatus (Waksman and Curtis 1916) comb. nov. (It was correctly cited this way by Waksman and Henrici in Bergey's Manual of Determinative Bacteriology, 6th ed., The Williams & Wilkins Co., Baltimore, 1948.)

Note 1. If an author transfers a species which has been included in the Approved Lists to another genus, the proposal of the new combination should be made by the addition of the abbreviation "comb, nov." (Combinatio nova) followed in parentheses by the name under which it appeared in the Approved Lists.

Example: If Bordetella parapertussis appears in the Approved Lists 1980 and is transferred by Smith in 1983 to the genus Moraxella, the citation by Smith may be as follows: Moraxella parapertussis (Eldering and Kendrick 1938) comb. nov. (Bordetella parapertussis Approved Lists 1980). Another author citing this proposal would then use the citation: Moraxella parapertussis (Eldering and Kendrick 1938) Smith 1983 (Bordetella parapertussis Approved Lists 1980).

The citation of a new combination which has been previously proposed should include the name of the original author in parentheses followed by the name of the author(s) who proposed the new combination and the year of publication of the new combination.

Example: Bacillus polymyxa (Prazmowski) Macé 1889 or Bacillus polymyxa (Prazmowski 1880) Macé 1889.

Note 1. The inclusion of the date of the publication of the original author of the name is to be preferred, although it is sometimes omitted since the date can be expected to be found in the publication of the author(s) who proposed the new combination.

Example: Bacillus polymyxa (Prazmowski 1880) Macé 1889 is to be preferred to Bacillus polymyxa (Prazmowski) Macé 1889.

Note 2. When, however, the author who formed the new combination was obliged to substitute a new specific epithet to avoid homonomy, the name of the author of the original specific epithet is omitted.

Example: Streptamyces aurioscleroticus Pridham 1970 is correct, not Streptomyces aurioscleroticus (Thirumalachar el al. 1966) Pridham 1970 [see Example to Rule 41a (l) for explanation].

When a taxon from subspecies to genus is altered in rank but retains its name or epithet, the original author(s) must be cited in parentheses followed by the name of the author(s) who effected the alteration and the year of publication.

Example: Actinomyces exfoliatus Waksman and Curtis 1916 to Actinomyces chromogenes subsp. exfoliatus (Waksman and Curtis 1916) Krasil'nikov 1941.

Citation of the Name of a Taxon who's Circumscription Has Been Emended

If an alteration of the diagnostic characters or of the circumscription of a taxon modifies the nature of the taxon, the author responsible may be indicated by the addition to the author citation of the abbreviation "emend." (Emendavit) followed by the name of the author responsible for the change.

Example: Rhodopseudomonas Czurda and Maresch 1937 emend, van Niel 1944 (see Opinion 49).

Citation of a Name Conserved so as to Exclude the Type

A name conserved so as to exclude the type is not to be ascribed to the original author, but the author whose concept of the name is conserved must be cited as authority.

Example: Aeromonas liquefaciens, the original type species of the genus Aeromonas, has been excluded from Aeromonas.

The generic name Aeromonas is now attributed to Stanier 1943, not to Kluyver and van Niel 1936, and with a new type species, A. hydrophila.

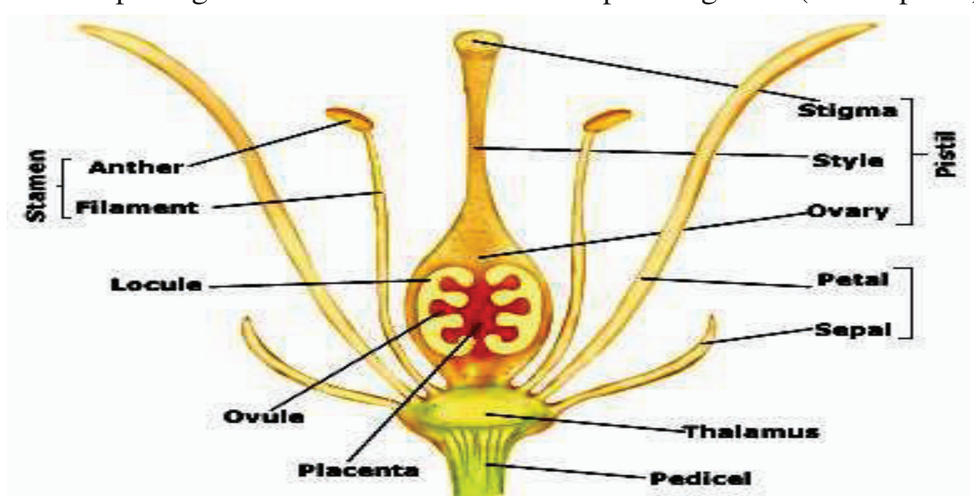
UNIT II

TAXONOMY: STUDY OF FLORAL CHARACTERISTICS

Morphologically, the flower is considered as a compressed shoot in which sepals, petals, stamens and carpels are successive lateral organs.There is considerable variation in size, shape, colour of flowers and the arrangement of floral parts within the flower, but all flowers have the same basic plan. A typical flower has four sets of appendages, the outer two sets are known as accessory organs and the inner two sets as essential organs

The accessory organs are sepals, which together form the calyx; and petals, which make up the corolla.in many plants, particularly monocotyledons, calyx and corolla are undifferentiated and then the term perianth is used collectively for them and the individual members are called tepals.Sepals are usually green leaf-like structures which protect the inner floral appendages. Petals are generally large,elaborate, brightly coloured structures, which help in attracting insects for pollination.

The essential organs are stamens (microsporophylls), which make up the androecium and carpels (megasporophylls), which together form the gynoecium. The androecium consists of one or more stamens, the male reproductive structures of the flower. The stamen is typically a slender organ and consists of two more or less distinct parts,a proximal sterile part, the filament, and a distal fertile part, the anther. The anther usually consists of four microsporangia which contain numerous pollen grains (microspores).



The strip of tissue that lies between a pair of sporangia is known as connectiveThe gynoecium, made up of one or more carpels, is the female reproductive organ of the flower. The carpel is divided into a proximal ovule bearing parts, the ovary; a distal pollen receptive part, the stigma and a median sterile part, the style. The ovary has one or more chambers known locules. The area where the ovules are attached is usually more or less enlarged and is said to be the placenta. The pattern of ovule arrangement in the ovary is called placentation. There are various types of placentation.

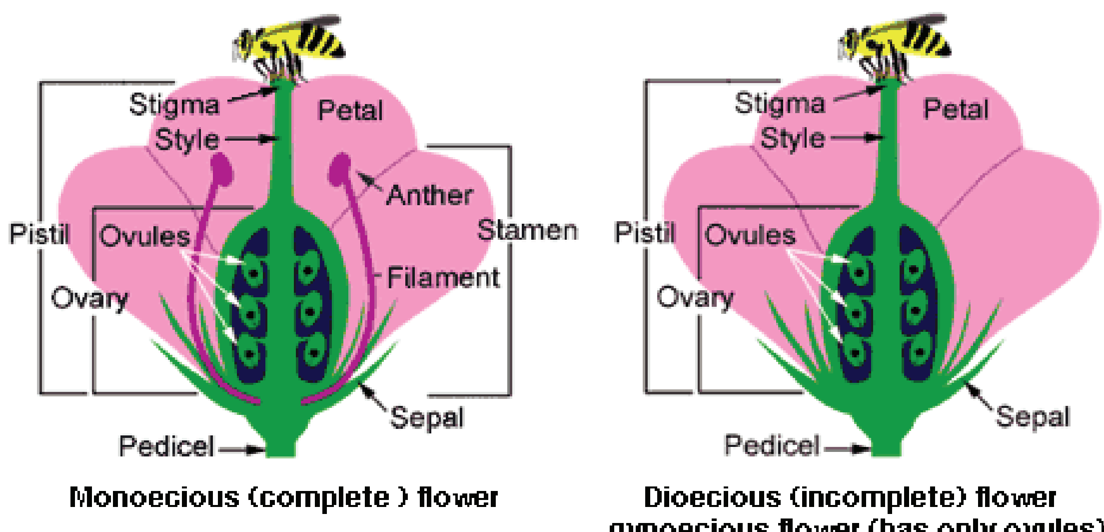
SOME IMPORTANT TECHNICAL TERMS

Complete and incomplete flowers

A flower having all four whorls (i.e calyx, corolla, androecium and gynoecium) is called complete and if any of the whorls is missing then it is said to be incomplete

Perfect and imperfect flowers

Flowers are said to be bisexual or perfect when they have both androecium and gynoecium and unisexual or imperfect when they have only one of these organs. Unisexual flowers having only stamens are called staminate (male flowers) and only carpels as pistillate (female flowers). When both staminate and pistillate flowers are born on the same plant, the condition is known as monoecius, and when staminate and pistillate flowers are present on different plants, it is said to be dioecious. When both bisexual and unisexual flowers are present on a plant, the condition is known as polygamous.



Monoecious (complete) flower

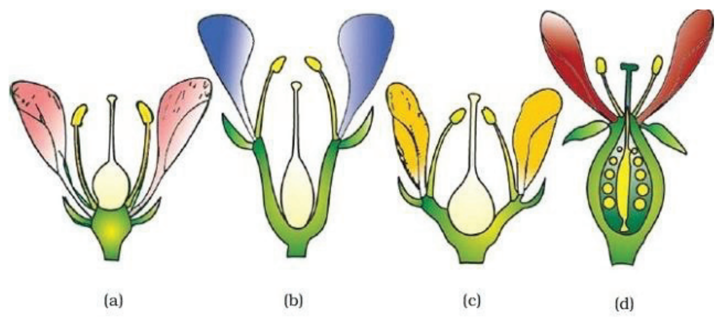
Dioecious (incomplete) flower gynoecious flower (has only ovules)

Relative position of floral parts on the Thalamus

On the basis of relative position of floral parts on the thalamus flower are of three kinds: hypogenous, perigynous or epigynous.

The flower is said to be hypogynous if sepals, petals and stamens are inserted below the ovary. In such flowers the ovary is superior and rest of the floral organs inferior, eg. Brassica (mustard), Lycopersicum esculentum (tomato)

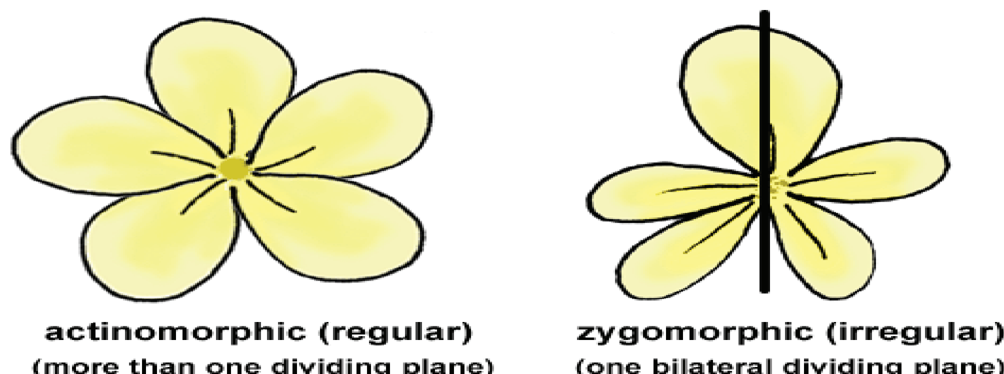
In rose, strawberry, peach etc. The thalamus forms a cup shaped structure around the ovary and sepal, petals and stamens appears to be inserted on the rim of the cup. Such flowers are known as perigynous and the ovary as semi –inferior .when the thalamus completely encloses the ovary and fuses with the ovary wall and sepals ,petals and stemen seem arise above the ovary ,the flower is said to be epigynous.in such flowers the ovary is inferior and other flraral organs as superior e.g . Cucumber, apple, etc.



Position of floral parts on thalamus : (a) Hypogynous (b) and (c) Perigynous (d) Epigynous

Symmetry:

When a flower can be divided into two exactly equal halves by a vertical section passing through the centre in any plane it is said to be symmetrical,



Regular or actinomorphic,e.g. Mustard (brassica), Tomato (lycopersicum), Dianthus.

When a flower can be divided into two similar halves by a vertical section in one plane only, it is said to be Zygomorphic, or bilaterally symmetrical e.g Pisum (pea), Bauhinia, Cassia, Antirrhinum (Snapdragon) etc

Types of aestivation in corolla : (a) Valvate (b) Twisted (c) Imbricate (d) Vexillary

Aestivation:

The mode of arrangement of sepals/petals in a floral bud is said to be aestivation. The following types of aestivationhave been recognized.

➢ **Valvate**

When the members of a whorl are in contact with each other by their margins, without overlapping or turning. E.g. Brassica (mustard)

➢ **Twisted** (contorted)

When the members of a whorl rolled up in such a way that on margin of a member covers a margin of the next member and other is covered by the one before it in a cross section, they appear as curved spokes in a wheel, e.g. Hibiscus (China rose)

➢ **Imbricate**

In imbricate aestivation one member of the whorl is exterior, one interior and the rest three having one margin exterior and the other interior e.g.Cassia, Caesalphinia

➢ **Vexillary**

Of the five petals, the posterior one is the largest and nearly covers the two lateral petals, which in turn overlap the two anterior petals. This type of aestivation is a characteristic feature of the pea family

Corolla shape:

I.Cruciform

It consists of four free petals, each differential into a claw and limb; arranged in the form of a cross, e.g. mustard

II.Caryophyllaceous

It consists of five petals with comparatively long claws and with limbs placed at right angles to the claws, e.g.dianthus

III.Rosaceous

This form of corolla consists of petals not distinguishable into limbs and claws, and spreading regularly outwards, e.g. rose

IV.Campanulate

The shape of the corolla looks like a bell e.g. gooseberry

V.Tubular

Corolla is cylindrical or tube-like; more or less equally expanded from the base to the apex e.g. disc florets of sunflower

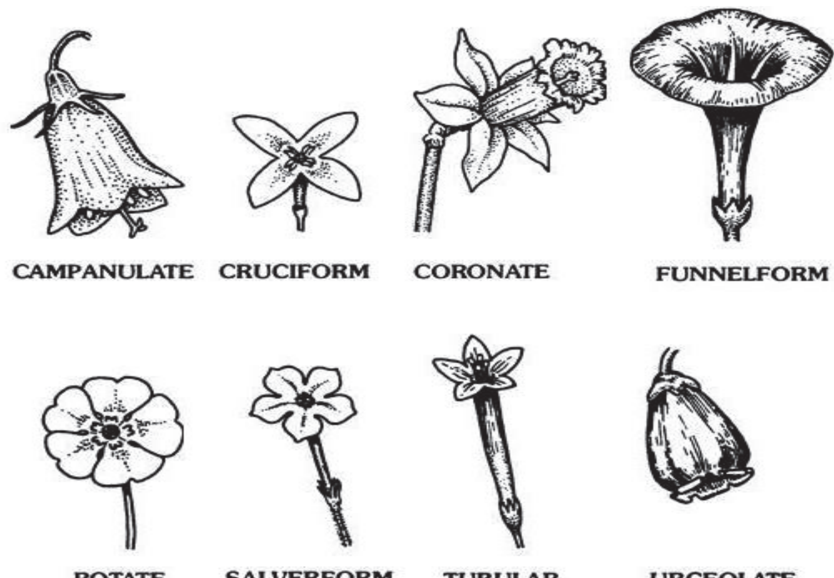
VI.Infundibuliform

Funnel-shaped; with tube gradually widening upwards and passing intensibly into a limb, e.g. petunia

VII.Rotate

Wheel-shaped; circular and flat, apllied to a gamopetalous corolla with a short tube, e.g. vinca

VIII.Papilionaceous



Butterfly like; a zygomorphic corolla consisting of five petals. One large posterior standard (or vexillum), two lateral wings (or alae) and two inner most fused petals

IX.Bilabiate

Two-lipped, zygomorphic corolla, e.g. ocimum

X.Personate

Bilabiate corolla having a prominent palate, e.g. snapdragon

XI.Ligulate

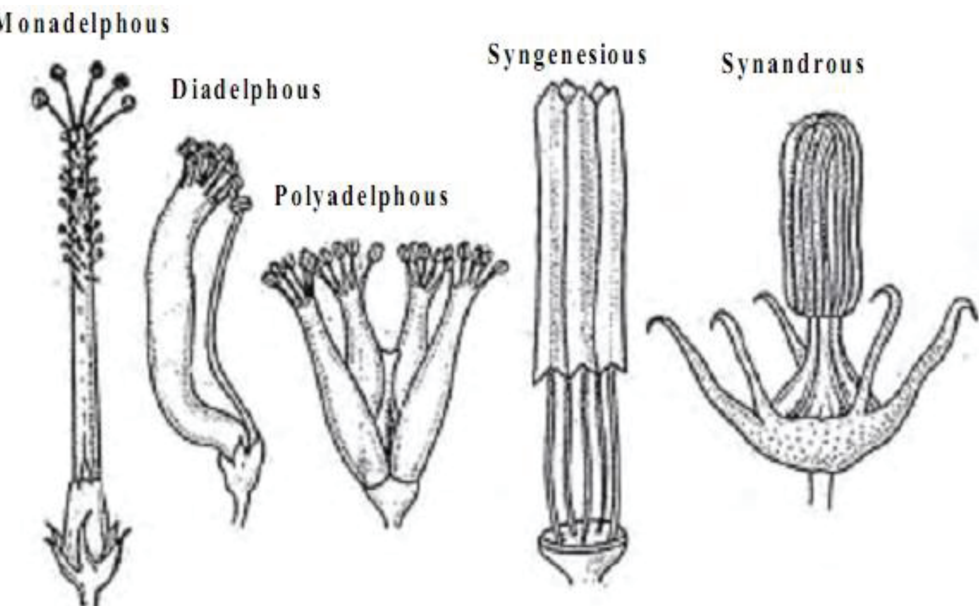
Strap-shaped; corolla forms a short narrow tube below but flattened above like a strap. E.g. ray florets of sunflower

XII.Urceolate

Urn-shaped; pitcher-like, hollow and contracted at the mouth

Cohesion of stamens:

In an androecium stamens may be free from one another (polyandrous condition) or they show different degree of cohesion



I.Monadelphous

Stamens are united in one group by their filaments only but the anthers remain free. E.g. china rose (hibiscus)

II.Diadelphous

The filaments are united into two bundles but the anthers remain free, e.g. pea (Pisum), where there are ten stamens of which the filaments of nine stamens are united into one bundle and the tenth posterior stamen stands apart.

III.Polydelphous

The filaments are united into several groups, the anthers remaining free. E.g. Bombax

IV.Syngenesious

The anthers are united to form a cylinder around the style; filaments remain free. E.g. Sunflower (Helianthus)

V.Synandrous

All stamens united throughout their whole length, involving both the filaments and the anthers. e.g.Cucurbita.

Length of Stamens

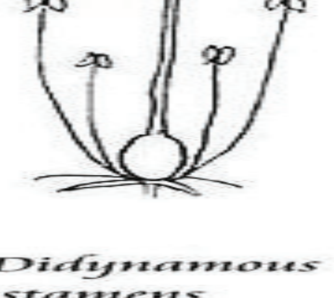
All stamens in a flower may be of the same length or they are of different lengths. The latter condition may represent the following types.

I.Didynamous

An androecium of four stamens, two short and two long e. g., Ocimum.

II.Tetradynamous

An androecium of six stamens, with inner four long and outer two short , e. g. Brassica.



Didynamous stamens

Tetradynamous stamens

Placentation

The arrangement of ovules within the ovary is known as placentation. The following types of placentation have been recognised.

I.Marginal

In this type, the gynoecium is monocarpellary with unilocular ovary. The placenta is borne on the fused margins of the same carpet. The ovules are present along the Ventral suture of the carpet. This condition is found in legumes.

II.Axile

In this type of placentation the gynoecium is multicarpellary and syncarpous and the ovary is multilocular. The placenta is borne on fused margins of the same carpet. The Ovules are borne on confluent margins which meet on the central axis. This type of placentation is found in tomato, citrus, China rose, etc.

III.Parietal

In this type, the gynoecium is multicarpellary and syncarpous and the ovary is unilocular. The placenta is borne on fused margins of the same carpel. This type occurs in water melon and other cucurbits.

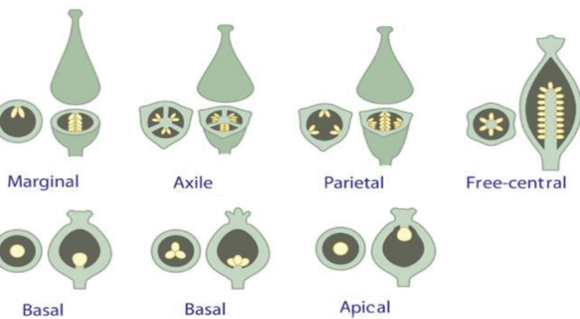
Types of Ovules

IV.Free-central

In this type, the gynoecium is multicarpellary and syncarpous, the ovary is unilocular and the ovules appear to arise from the central column this type of placentation is found in the Dianthus, Primula, etc

V.Basal

In this type, the ovary is unilocular and the solitary ovule appears to arise from the base of the ovary as in sunflower (Helianthus)



VI.Superficial

In this type of placentation the gynoecium is multicarpellary and syncarpous and most of the internal surface of the ovary wall is covered with ovules, as in water lily (Nymphaea)

Types of Ovules

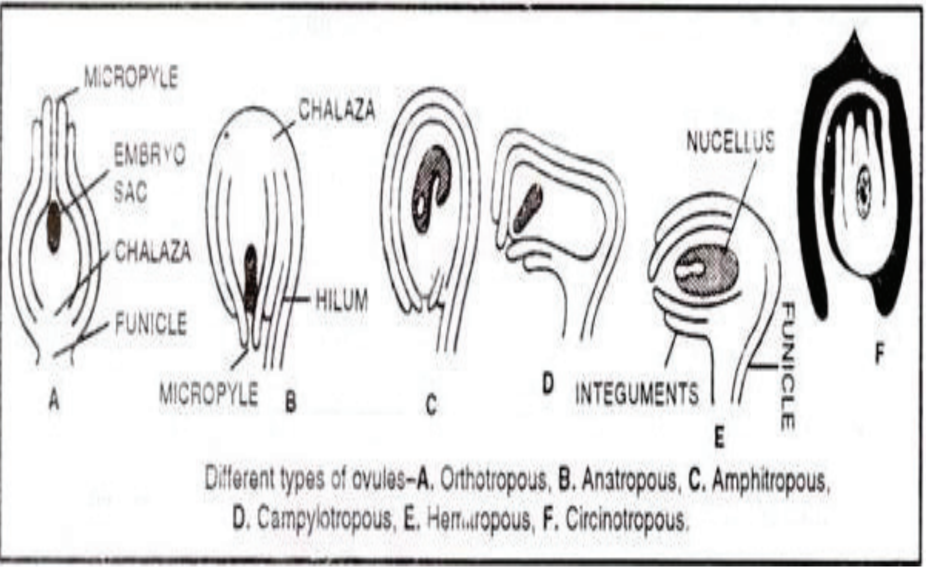
Depending upon the relative position of micropyle and chalaza at maturity, the following types of ovules have been recognized.

I.Orthotropous (Atropous)

The ovule is upright with micropyle, chalaza and funicle falling in a straight line. e. g. Polygonum

II.Anatropous

In this type, due to unilateral growth of the funicle, the whole body of the ovule is inverted through 180 ° and as such the micropyle comes close to the base of the funicle. The nucellus remains straight. Thus micropyle and chalaza lie in one line and the funicle lies parallel to it. It is the most common type of ovule in the flowering plants.



Different types of ovules-A. Orthotropous, B. Anatropous, C. Amphitropous, D. Campylotropous, E. Hemi-tropous, F. Circinotropous.

III.Campylotropous

This type of ovule has a curved body but its curvature is less than that of the anatropous ovule. The micropyle and chalaza as such are not in a straight line and the funicle lies at right angles to the chalaza, e. g. Chenopodium

IV.Hemitropous

In this type, the body of the ovule is turned through 90 ° i.e It is horizontally placed on the funicle. Hence the micropyle and chalaza are in a horizontal line and the funicle lies at right angles to it, e. g. Primula.

V.Amphitropous

In this type, there is also a pronounced curved body like that of anatropous ovule and the embryo sac bends and becomes horse-shoe shaped, e. g. Atisma.

VI.Circinotropous

In this type, the funicle is very long and forms a complete circle around the body of the ovule, e. g. Opuntia, Plumbago.

Inflorescence

A flower is solitary, when occurring singly. The solitary flower may occupy terminal or axillary on a stem or branch. Flowers borne in cluster, together with the stem and bracts associated with them form inflorescences. The aggregation of flowers in an inflorescence makes them more conspicuous to attract insects for pollination.

I.Racemose inflorescence

Floral axis shows indeterminate growth producing flowers in an acropetal order. The various forms of racemose inflorescences are as follows.

1.Raceme. e. g. Brassica.

2. Panicle. e. g. Melia.

3. Spike. e. g. Justicia.

4. Spikelet. It is a very small spike with one or few flowers (florets). It is the characteristic inflorescence of grasses.

5.Catkin. e. g. Morasalba (Mulberry).

6.Spadix. E.g. musa (Banana)

7.Corymb. E.g. iberis (Candytuft)

8.Umbel. It is a characteristic inflorescence of the family Umbelliferae (Apiaceae)

9.Head or capitulum. The head inflorescence is characteristic of the family Compositae (Asteraceae)

II.Cymose inflorescence

Growth of the main axis determinate. They are three types.

1.Uniparous (monochasial) cyme, e.g. Solanum, Heliotropium

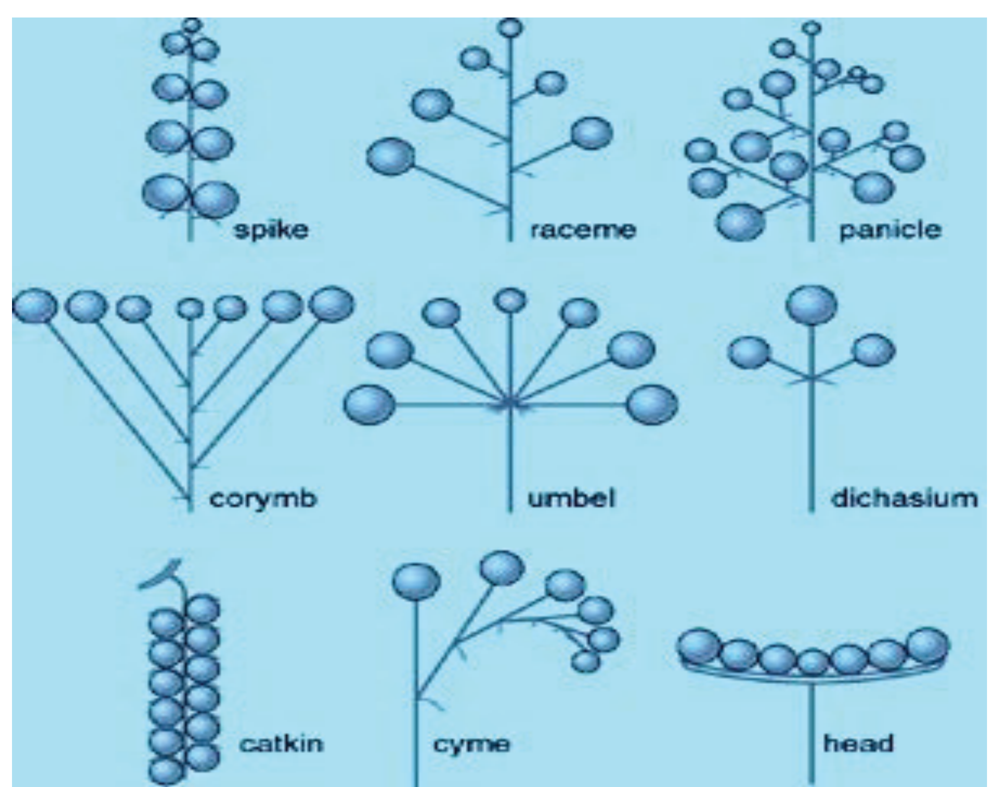
2.Biparous(dichasial) cyme e.g. Dianthus (pink), Jasminum (jasmine)

3.Multiparous (multichasial) cyme. E.g. Hamelia

III.Special types of inflorescences

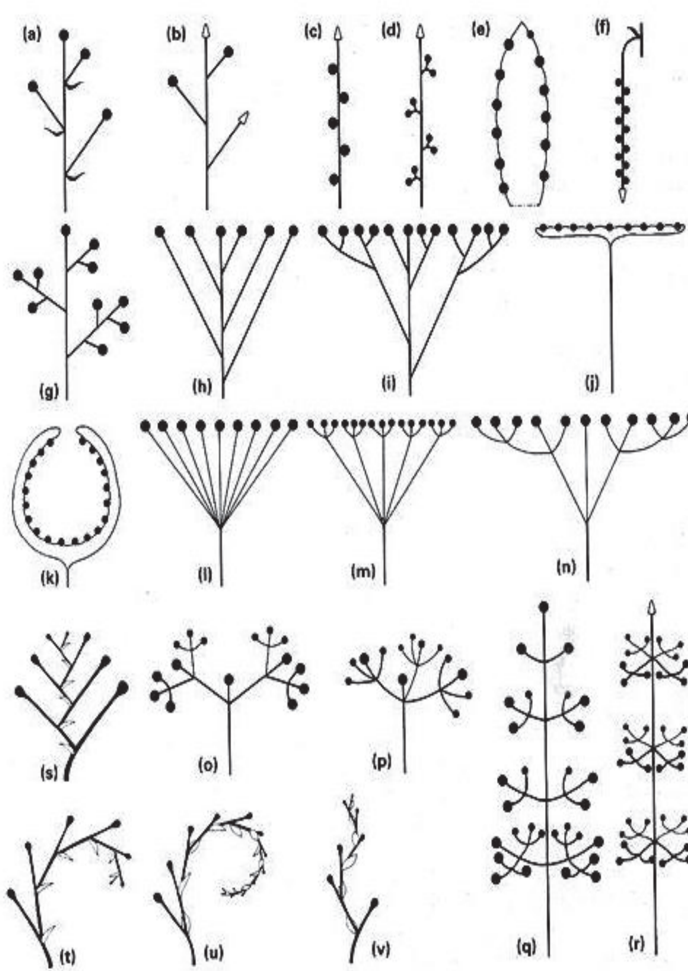
1.Cyathium. Characteristic of the genus Euphorbia.

2.Verticillaster. Characteristic of the members of the family Lamiaceae.



3.Hypanthodium. Characteristic of Ficus species

Patterns of Inflorescence:



Diagrammatic representation of inflorescence types. a, b) raceme, c, d) spike, e) spadix, f) catkin, g) panicle, h, i) corymb, j) capitulum, k) hypanthodium, l-n) umbel, o) dichasial chyme, p) pleiochasial cyme, q) thryse, r) verticillaster, s-v) monochasial cymes (s, rhipidium; t, drepanium; u, cincinnus; v, bostryx).

Variation in flower structure taken for observation while doing a floral diagram and the formula:

Flowers of different species may vary in the number, arrangement and degree of fusion of the component parts. Examine them carefully and observe the main variations. Note the variations in a sequential order as listed below

I.Inflorescence

1.Flowers solitary or in clusters (florescence).

2.If solitary, whether they are in terminal or in axillary position on a stem or branch.

3.If inflorescence is present, then racemose/cymose/special types.

a) If racemose, then then raceme/spikelet/catkin/spadix/corymb/umbel/head (or capitulum).

b) If cymose, then monochasial (scorpoid or helicoid)/dichasial/multichasial cyme.

c) If special type, then Cyathium/Verticillaster/ hypanthodium.

II.Flower

1.Bracts. Ebracteate/bracteate.

2.Sessile/pedicellate.

3.Presence of floral whorls. Complete/incomplete.

4.Symmetry. Actinomorphic/zygomorphic.

5.Presence of reproductive parts. Hermaphrodite (bisexual) unisexual; if unisexual whether staminate or pistillate.

6.Number of floral parts in a whorl. Dimerous/trimerous/tetramerous/ pentamerous.

7.Position of floral parts on thalamus. Hypogynous/perigynous/epigynous.

8.Colour.

9.Any other special feature (if present).

III.Calyx

1.Number of sepals

2.Cohesion (degree of fusion). Polysepalous/gamosepalous.

3.Aestivation. Valvate/twisted/ Umbricate/quincuncial/vexillary.

4.Colour. Green and leaf-like/coloured and petal-like (petaloid).

5.Any other special feature (if present).

IV.Corolla

1.Number of petals.
2.Cohesion. Polypetalous/gamopetalous;if gamopetalous, whether forming corolla tube.
3.Aestivation. Valvate/twisted/imbricate/quincuncial/vexillary.
4.Shape. Cruciform/caryophyllaceous/rosaceous /bell-shaped/tubular/funnelshaped/rotate/papiiionaceous/bilabiate/personate/ligula e.
5.Colour.
6.Any other special feature (if present), like spur, nectary, corona, etc.

V.Perianth

When sepals and petals are indistinguishable from one another, then they are collectively called as parianth. If parianth is present, note the following features

1.Number of tepals
2.Number of wborls
3.Cohesion. Polytepalous/gamotepalous.
4.Aestivation. Valvate/twisted/imbricate/quincuncial.
5.Colour.
6.Any other special feature (if present).

VI.Androecium

1.Number of stamens.fixed or indefinite and in how many whorls.
2.Cohesion of stamens. Free (polyandrous)/fused; if fused whether monadelphous/diadelphous/polyadelphous/syngenesious/syndrous (fused with gynoecium) free
3. Adhesion of stamens. Epipetalous (base of filaments attached to petals)/epitepalous (base of filaments attached to tepals)/gynandrous (fused with Gynoecium) free.
4.Length of filament. Whether all filaments are equal or not; whether didynamous/tetradynamous condition present.

5.Insertion. Inserted/exserted.
6.Attachment of filament to anther. Adnate/basifixed/dorsifixed/versatile.
7.Number of theca.Dithecous/monothecous.
8.Anther dehiscence. Introrse/extrorse.
9.Any other special feature (if present).

VII.Gynoecium

1.Number of carpels. Monocarpellary/bicarpellary/tricarpellary/pentacarpellary/multicarpellary.
2.Cohesion of carpels. Apocarpous (free)/syncarpous (fused).
3.Position of ovary. Superior (ovary stand proud upon the receptacle) inferior (sunk down into the receptacle)/semi-inferior.
4.Number of locules in the ovary. Unilocular/bilocular/trilocular/tetralocular/ pentalocular/multilocular.
5.Number of ovules in each locule.
6.Placentation. Marginal/axile/parietal/free-central/basal/superficial.
7.Style. Number of styles and their length; whether terminal/lateral/gynobasic.
8.Stigma. Number and shape of stigma-capitate/plumose/discoid dumb-bell shaped/bifid/sticky.
9.Any other special feature (if present), like presence of nectariferous disc around the ovary.

Recording floral Structure

With so many variations in flower structure in various species of plant, we use the following three ways of recording flower structure.

i.Drawing the half-flower

This provides an elevation of the flower. The flower should be cut along the median plan in such a way that you get two equal halves. This would give an idea of the relative position of floral parts on the receptacle, Draw a half flower and/or construct a simple diagram of the cut surface,i e. a vertical section

ii.Floral diagram

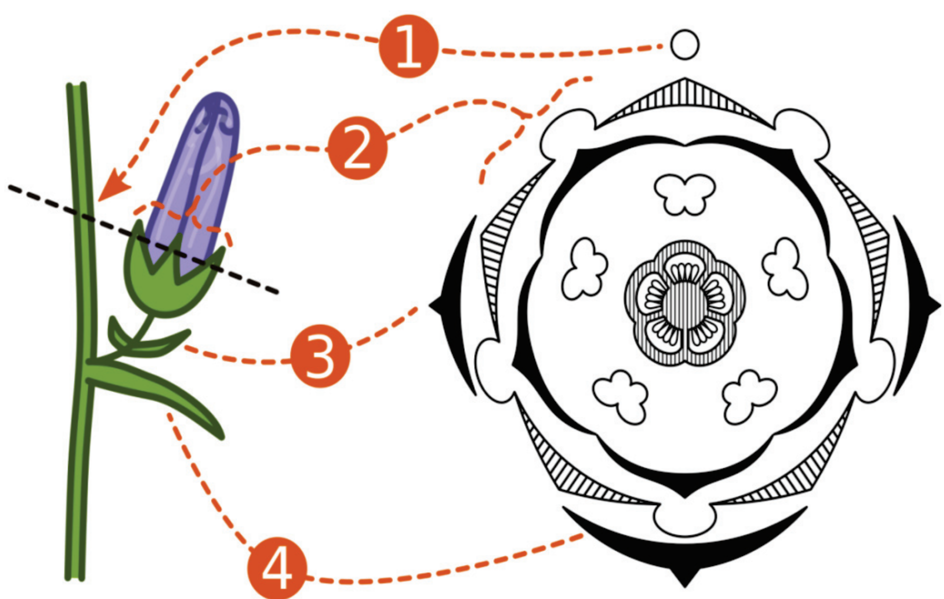
The number of parts of flower, their arrangement, aestivation, adhesion, cohesion and position with respect to the mother axis may be represented by a diagram known as the floral diagram. It provides a groundplan of the flower as viewed from above, and looks like a diagrammatic transverse section.

In constructing a floral diagram hold the flower so the bract, if present, faces you and the axis is furthest away.

In the floral diagram, the floral whorls (i.e. calyx, corolla, androecium, gynoecium), are represented on concentric circles:

- The calyx is outermost, the corolla internal to the calyx, the next is the androecium, and the gynoecium in the center as it appears in a cross section.
- The arrangement of floral parts in whorls or spirals is shown accordingly.
- Adhesion and cohesion of members of floral whorls are shown by connecting the respective parts with lines. If the sepals, petals or stamens are joined, link them with simple brackets;
- If the stamens arise from the petals, link them with radial line. As for example, if a flower shows five sepals that are free, five stamens which show adhesion with petals.
- A black circle on the top represents the position of the mother axis.
- The side of the flower nearest the axis is the posterior side, and the side away from the axis, the anterior side.
- Mother axis is, however, not drawn in case of terminal flowers.
- If a bract is present, a section of it is drawn below the diagram, and if bracteoles are present, they are drawn on the sides.
- If a sepal or petal has a spur, this is shown by drawing a loop at the back of the organ. Look carefully whether the stamens are in one or more whorls.
- Draw them accordingly.
- Introrse anthers are drawn facing towards the gynoecium and the extrorse anthers towards the petals.
- Staminodes are represented either by a cross or astrick. The gynoecium is represented by a transversesection of the ovary.

Floral formula: Floral formula is written in coded form and it enables to work out the structure of the flower.



In floral formula the different whorls are represented by capital letters as follows:

K for calyx; C for corolla;

A for androecium and

G for gynoecium.

If the sepal's andpetals are represented by a perianth, the symbol used is P.

Each letter is followed by a figure denoting the number of units in the whorl. If the number is large and variable it is expressed as infinity.

For example, k5 means that there are five free sepals in a single whorl. Bracket round the figures indicates the fused nature of the parts, e. g. k5. If more than one whorl of the same set of units is present, they are shown

separately; for instance, if stamens are in two whorls of five each, it is coded as A5 + 5. If some units are fused but others free, only the former are put in brackets, e.g. a (4) +2. If one whorl is united with another, their symbols are tied by an arc above them, e.g. C5. A5 indicates the epipetalous stamens.

For bracts and bracteoeles, symbol Br and Brl are used respectively. Actinomorphy is designated by the sign + at the beginning of the formula, whereas zygomorphy is represented by % or @

If the flower is hermaphrodite (bisexual) it is designated by the symbol showing both male and female structures as → and + if staminate (male) by … and if pistillate (female) +.

A superior ovary is designated by putting a bar below the gynoecium number. E.g. G5; an inferior ovary is designated by putting a bar above the gynoecium number e.g. G5. ___

The two floral formulae given below may illustrate the point.

1.Br Brl.........
2.Ebr………….

The first floral formula illustrates that the flower is bracteate, bracteolate, actinomorphic, hermaphrodite, sepals five and free, petals five and free, stamens five and free and carpels two, fused and the ovary is superior.

The second floral formula illustrates that the flower is ebracteate, actinomorphic, staminate, sepals five and fused, petals five and fused, stamens five and free, epipetalous and carpels absent.

Characteristics of Various Flowers

Examine the flowers of different plants. Describe them in semi-technical language. In each case sketch the half-flower and floral diagram and give the floral formula. Start with a simple actinomorphic flower which lacks any special complications. Then examine other flowers, both actinomorphic and zygomorphic, displaying the normal rangeof variations. In each case consider the possible functional significance of the floral pattern.

For examining, first take a flowering twig of the plant. Observe whether the floweris solitary or in cluster. Then take a flower and observe whether it has pedicel. Then with the help of forceps remove the sepals; count the number of sepals. Find out if they are united or free. Then take out petals, and observe like that of sepals. Then remove the petals and look for stamens.

Then remove the stamens, and study the gynoecium. With the help of a sharp blade, cut a thin transverse section of the ovary and examine it under the dissecting microscope. Count the number of carpels *, locules, ovules per locule, and type of placentation. Arrange all dissected floral parts sequentially and observe its floral characteristics and that of the family.

<u>Distinguishing Characters of Families of:</u>

<u>DICOTYLEDONES - A few samples for easy understanding</u>

1. Venation reticulate

2. Flowers pentamerous

Dicotyledones is sub-divided into three divisions, viz. Polypetalae, Gamopetalae and Monochlamydeae. Of these, only first two are considered here.

Division 1. Polypetalae

1. Petals distinct and free.

Polypetalae has been divided into three series, viz. Thalamiflorae, Discifloraeand Calyciforae. Of these only Thalamiflorae and Calyciflorae are considered here.

Series 1. Thalamiflorae

1. Thalamus somewhat dome-shaped.

2. Flowers hypogynous and ovary superior.

Order 1. Malvales

1. Stamens numerous, monadelphous; anthers monothecous.

2. Ovary 3-many locular with axile placentation.

Family Malvaceae

1. Leaves stipulate.

2. Flowers solitary axillary or terminal, actinomorphic, pentamerous and hypogynous.

3. Petals 5, twisted.

4. Stamens numerous, monadelphous; anthers monothecous.

5. Gynoecium multicarpellary, syncarpous and multilocular.

Series 2. Calyciflorae

1. Thalamus cup-shaped.

2. Flowers perigynous.

Order 1. Rosales

1. Leaves alternate and stipulate.

2. Gynoecium of one or more carpets.

Family Leguminosae

The family has been divided into three sub-families, viz. Papilionate, caesalpinioideae and mimosoideae.

Sub-family papilonatea

1.Flowers zygomorphic
2.Corolla papilionaceous with descending imbricate aestivation.
3.Stamens usually 10, diadelphous.
4.Gynoecium monocarpellary; ovary unilocular with marginal placentation.
5.Fruit usually a pod

Division 2. Gamopetalae

1.Petals fused
Division Gamopetalae includes 3 series, viz. Inferae, Heteromerae and Bicarpellatea. Of these, only two series, viz. Inferae and Bicarpellatea are described here

Series 1.Inferae

1.Stamens usually as many as corolla lobes
2.Ovary inferior.

Order 1. Asterales

1.Stamens epipetalous
2.Ovary unilocular with single ovule

Family Asteraceae (composite)

1.Inflorescence head or capitulum.
2.Calyx usually modified into pappus
3.Stamens epipetalous and syngenesious
4.Ovary inferior, unilocular with a solitary basal ovule.
5.Fruit cypsela

Series 2. Bicarpellatae

1.Stamens as many or fewer than corolla lobes
2.Carpels usually two; ovary superior

Order 1.Polemoniales

1.Leaves generally alternate and exstipulate.
2.Flowers actinomorphic.
3.Stamens as many as the corolla lobes

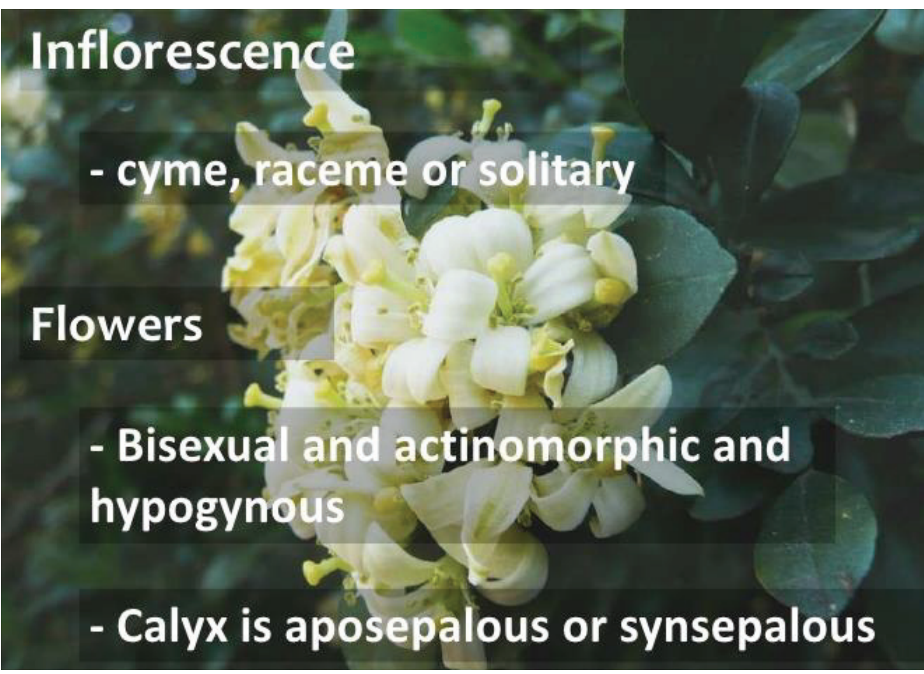
Family Solanaceae

1.Flowers solitary or in cymes
2.Anthers conniving

3.Ovary bilocular with many ovules in each locule
4.Placentae swollen and oblique septum.

Specific understanding offew families of dicotyledons

i) Family Rutaceae:



Habit:

These are shrubs and trees, (rarely herbs).

Leaves:

The leaves are simple or compound, alternate or rarely opposite, and gland-dotted.

Flowers:

These are regular, bisexual and hypogynous. The disc below the ovary is prominent, and a ring or cap-like.

Calyx:

There are 4 or 5 sepals free or connate below and imbricate.

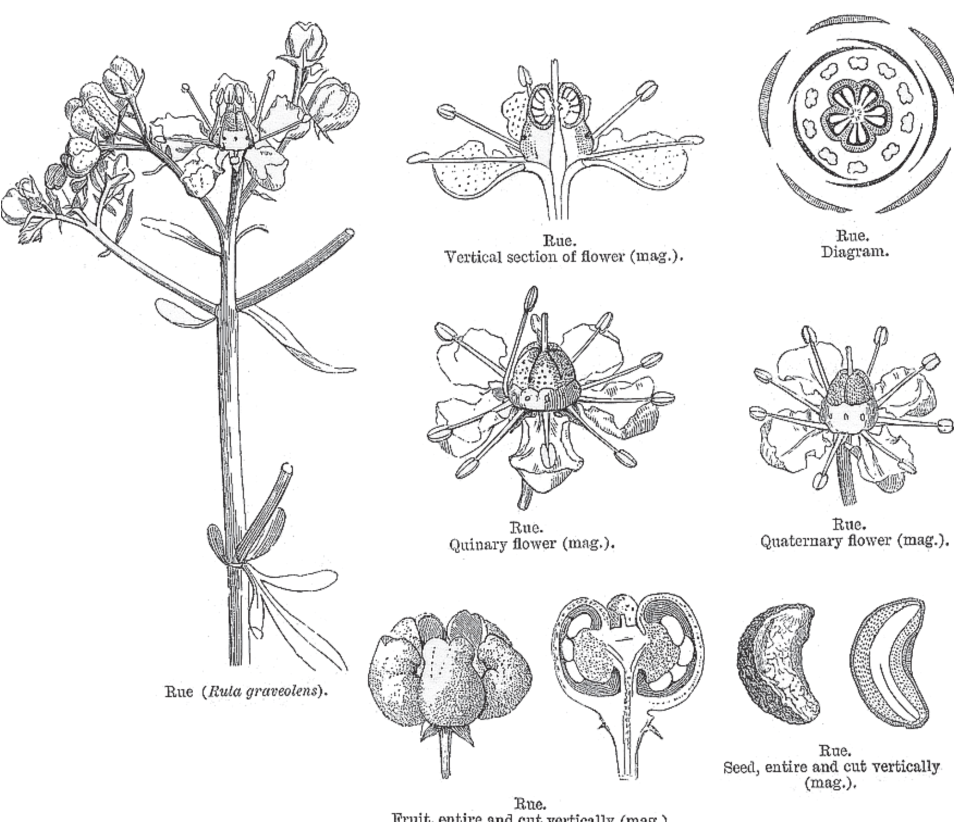
Corollo:

Petals 4 or 5, free, imbricate.

Androecium:

The number of stamens varies, they can be as many, or more often twice as many, as the petals (obdiplostemonous), or numerous, as in *Citrus* and *Aegle*. They are free or united in irregular bundles (polyadelphous), and inserted on the disc.

Sour lime-Citrus aurntifolia- complete floral pattern of the dwarf twig with flowers

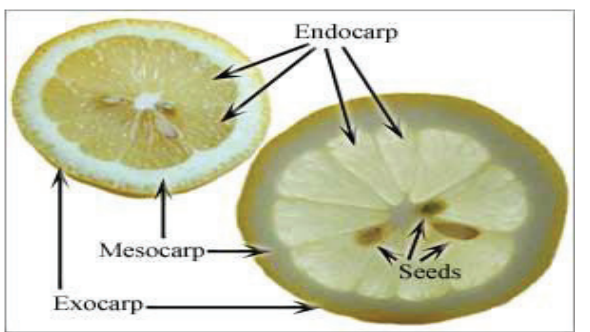


Gynoecium:

There are generally 4 to 5 carpels, or several as in citrus. They are syncarpousor free at the base and united above, and either sessile or seated on the disc. The ovary is generally 4 or 5- locular, or multilocular as in citrus, with axileplacentation(parietal in Limonia only). There are usually 2 to several (rarely 1) ovules in each loculus, arranged in two rows.

Fruit:

This is a berry, capsule or hesperidium.



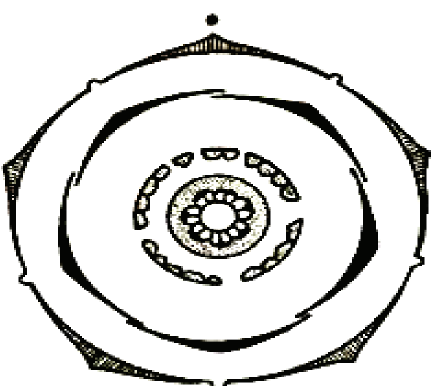
Modified berry: Hesperidium Fruit from a compound pistil of the citrus family(Rutaceae). Exocarp: an aromatic rind. Mesocarp: white pith. Endocarp: fleshy juicy sacs. Seeds: covered by seed coat. Examples: orange, lemon, grapefruit.

Seeds:

The seeds may or may not have an endosperm. Polyembryony is frequent in citrus, e.g. lemon and orange (but not pummelo and citron)

Floral formula:

Floral formula: $\oplus$, ⚥, $K_{(5)}$, $C_{4\text{-}5}$, A_{∞}, (polyadelphous) $\underline{G_{\infty}}$



Floral diagram of *Citrus aurantium*

Rutaceae is similar to Meliaceae by virus of its obdiplostemonous stamens (i.e. stamens in 2 whorls, the outer opposite to the petals), the presence of the disc, and often 5 carpels, the anatropous ovule with ventral raphae, and the types of fruit. Hutchinson has separated the above two families from Geraniales and placed them under two separate orders-Rutales and Meliales, respectively. Rutales is also related to spindales(Sapindaceae, Anacardiaceae. etc), but in the later order; the leaves are not gland-dotted**.**

Examples:

The larger genera are: Fagara (over 200 sp), Rutar (60 sp), and Evodia (45 sp).

Useful plants: Citrus (e.g. lime, lemon, orange, citron and pummelo), Wood-apple (Aeglemarmelos). The pulp containing ***marmelosin*** is an effective remedy for chronic dysentery and constipation, Elephant-apple (Limonia acidissima), Chinese box (Murrayapaniculata) - wood hard and useful, curry leaf plant

(M. koenigii) leaves used for flavouring curries, Micromelumpubescens-an evergreen tree, etc.

Other common plants:

Rue (rutagraveolens) a strong smelling small herb, Glycosmis arborea, Clausenaheptaphylla, c.pentaphylla, Luvungascandens. A large thorny climbing shrub, Toddalia aculeate , large thorny climbing shrub, Zanthoxylumbudrunga, a prickly tree, Z. alatum ,a small aromatic tree, branches used as toothbrushes and fruit as a condiment like coriander, Z piperia, pungent fruit yield Japanese pepper, Evodiaroxburbhiana, a tree, etc.

ii) <u>**FAMILY ROSACEAE**</u>

Habit:

These are herbs, shrubs, trees and climbers.

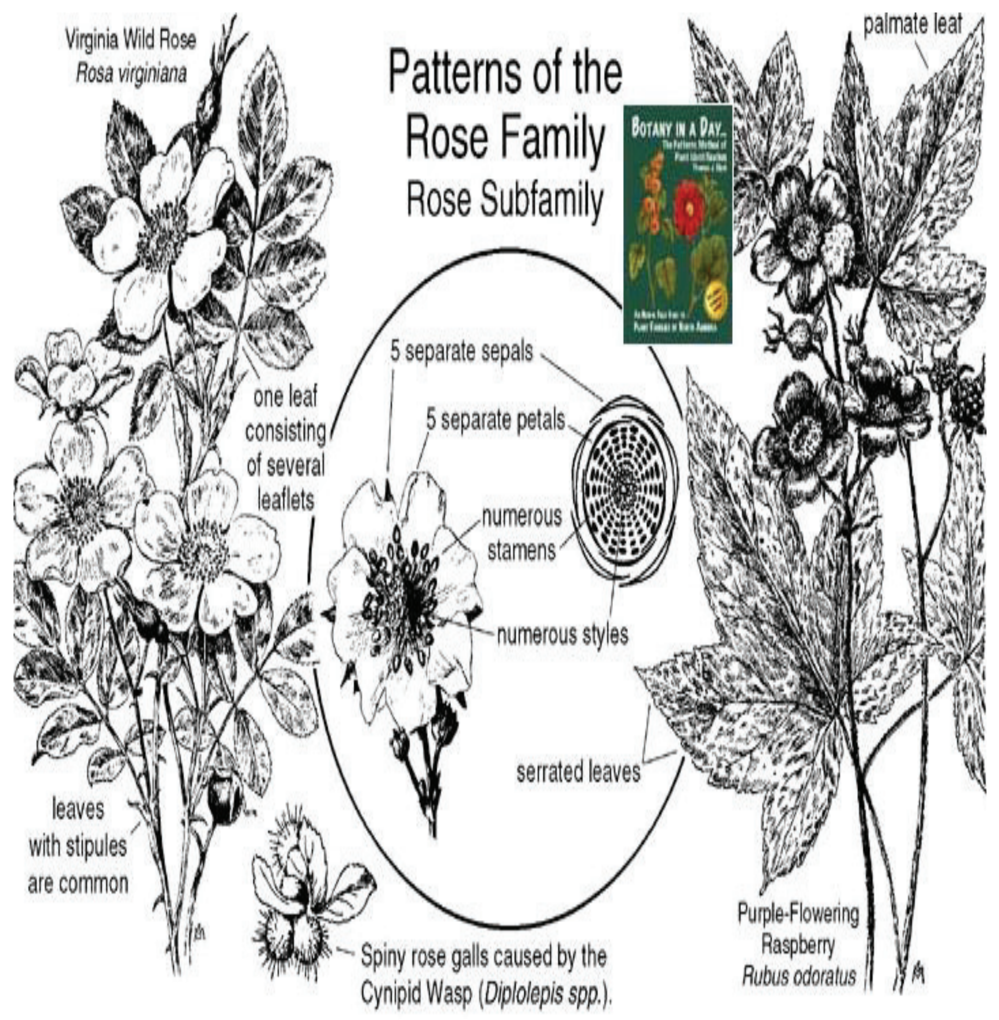
Leaves:

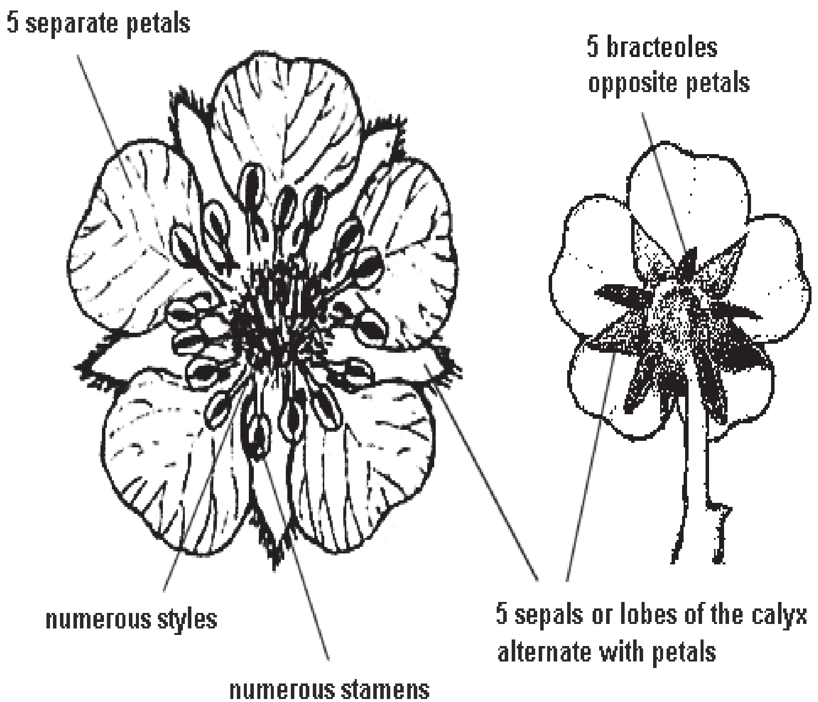
The leaves are simple or compound and alternate .there are 2 stipules, often adnate to the petiole.

Inflorescence:

The flowers are solitary or in terminal cymes or racemes.

Flowers: These are regular bisexual, rosaceous, and usually perigynous. The receptacle is hollowed and cup-shaped. They are rarely epygynious (as in apple and pear). A disc is often present in the form of a ring.





Calyx:

There are 5 sepals, adnate to the receptacle. The lobes are free, the epicalyx is often present.

Corolla:

There are 5 petals (many in cultivated roses). They are free, usually imbricate, and alternate with the sepals. The petals are usually whit or pink.

Androecium:

There are many stamens incurved in the bud, and arranged in cyclic order.

Gynoecium:

There are many carpels usually. They are free (as in rose) or sometimes (5), united (as in apple and pear). On occasion there is only 1 (as in plum and peach) carpel. The ovary is unilocular or 5-locular in the syncarpous pistil, with usually 2 ovules in each loculus. The ovules are anatropous and pendulous.

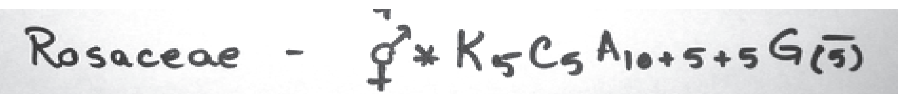
Fruit:

The fruit varies in forms, drupe, follicle, berry, achene or pome.

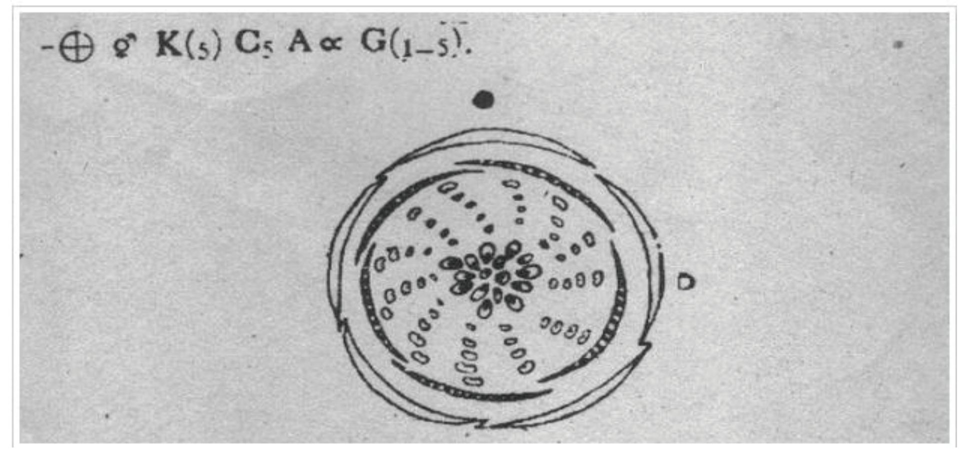
Seeds:

The seeds are ex-albuminous.

Floral formula:



Floral formula and floral diagram (Rose plant)



There are wide variations in the floral structure of Rosaceae. The flowers may be bisexual or unisexual. There are usually many stamens and sometimes 10. There are many carpels, sometimes 5-10 or even 1 (as in

Prunus). They are apocarpus or syncarpous. The ovary is superior or inferior and the fruit varying. The family is related to Leguminosae by perigyny, and sometimes monadelphy, zygomorphy and simple pistil in some of its members. Rosaceae is also related to Myrtaceae through Pyrus, having (2-5) carpels.

Economically this is an important family. Otto of-rose is mostly obtained from Rosa damascene and R. centifolia. Bulgaria, most famous for its roses, is the world's biggest center for the distillation of this essential oil. There are many fleshy edible fruits, e.g. plum, peach, prune, apricot, strawberry, apple, pear, etc. several varieties of rose are ornamental garden plants, as also are many species of Spiraea (grown in hill stations).

Examples:

The large genera are Potentilla (over 300 sp.), Rubus (250 sp.), Rosa (over 200 sp.), Prunus (150 sp.), Spiraea (100 sp.), and Pyrus (50 sp).

Some examples are Dog rose (R canina), Wild rose (R. gigatea), Damask or Bussora rose (R. damascena and R. centifolia), Musk rose (R. moschata), R.indica, R.alba, and several hybrids (Spireaecantoniensis) has white flowers, in clusters (grown as a hedge plant inshillong), loquat (Eriobotrya japonica), plum (Prunus domestica), Prune is the dried plum, peach (P. persica), Apricot (P.armeniaca), Almond (P. amygdalus), Cherry (P. avium), Quince (Cydonia oblonga), Strawberry (Fragariavesca), Apple (Malus sylvestris), pear (Pyrus communis and P.pyrifolia), Silver weed (Potentilla fulgens), common in the hills, Raspberry (rubus ideaus), Wild raspberry (R. moluccanus) and many other wild species in the hills.

Economic Importance

This family has a great economic importance for mankind. It has great importance in temperate (cold) region. This family is ranked third in the flowering families for commercial importance in thetemperate zone.

1- Fruit:

Many fruits are obtained from the plants of this family. Some important fruits are Apple, Pear, Almond Peach, Apricot , and Strawberry etc.

2- Ornamental Plants:

A large number of plants of this family are ornamental. They are grown in gardens for their beautiful and scented flowers. The genus Rosa is widely cultivated for decorative purpose. It has been growing since ancient times. Its cultivated names are in thousands. Many other genera are also grown in gardens and parks for beautiful flowers.

3- Wood:

The branches of *Crataegus* are used as walking sticks and wood. The wood of*pints pactia* is used for making tobacco pipes.

Commercial and medicinal uses of Petuls

The petals of some common roses are called **gulabs** in many Asian countries like Pakistan. The gbiabs have following uses: e These petals are used for making **gulkand**

Petals of rose are used tor extraction of **rose oil.** This oil is used in perfumes. The petals give **Ark-Gulab** on distillation with water. This Ark-Gulab is used for curing eye disease and for many other purposes.

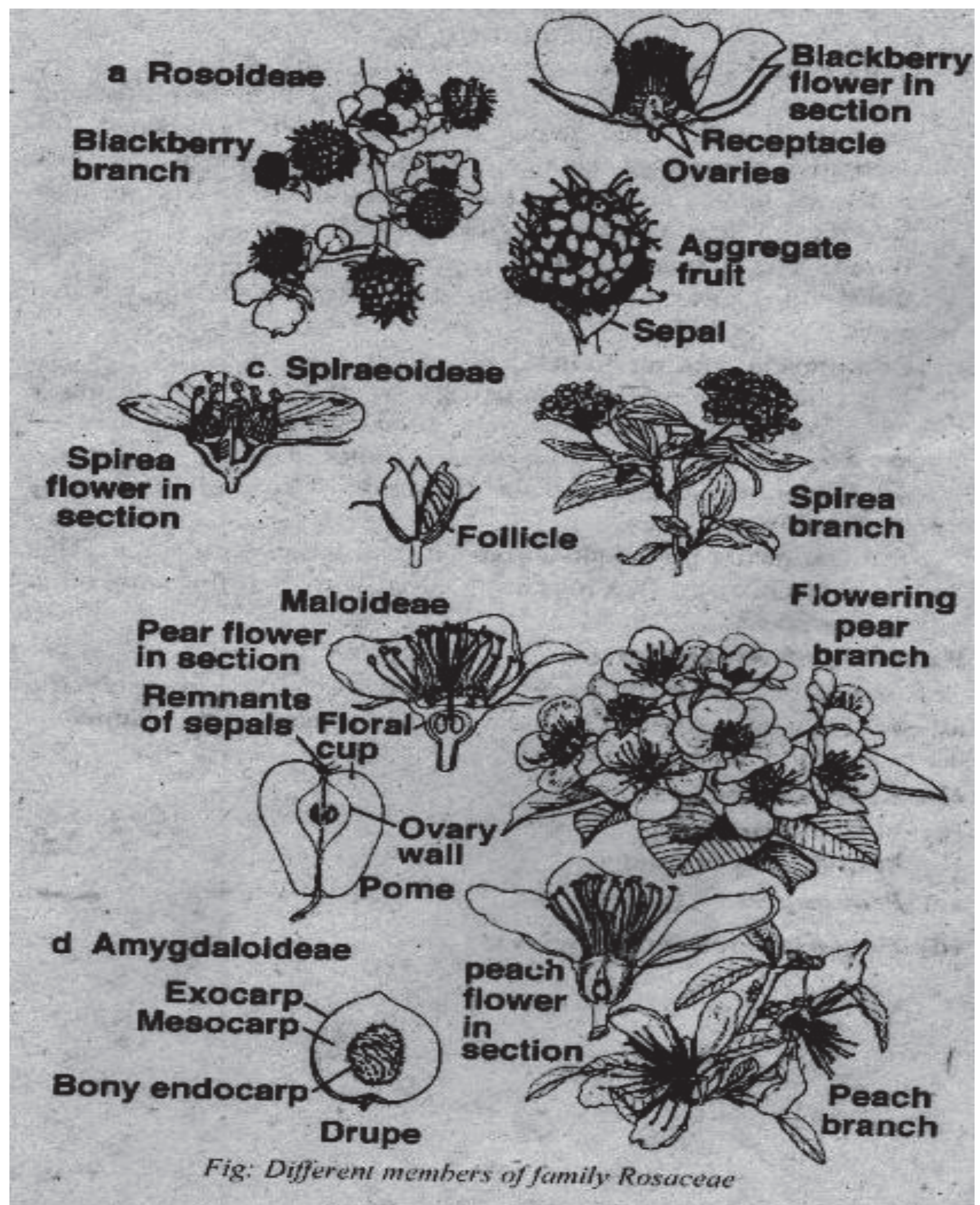


Fig: Different members of family Rosaceae

iii) Family Caesalpiniaceae:

Caesalpinia, after Andrea Cesalpini (1519-1603) an Italian botanist (Professor at Padua) and physician to Pope Clement VIII. In Australia the legume families are usually treated separately. The family in the broad sense is referred to as the Fabaceae or by the old and alternative name of Leguminosae.

The Caesalpiniaceae or Caesalpinioideae are trees or shrubs with alternate, compound leaves; extra-floral nectaries or glands are common on the rachis. The family/subfamily is distinguished by the posterior petal being overlapped by the lateral petals. Fertile stamens 3-10, filament often short, staminodes often present, anthers often opening by pores. Fruit is a pod.

Type of plant: Shrubsand trees rarely climbers or herbs.

Leaves: Unipinnate or bipinnate, rarely simple, stipules absent.

Inflorescence, floral, fruit and seed morphology: Flowers solitary, or aggregated in 'inflorescences'; when aggregated, in racemes, or in corymbs, or in fascicles, or in panicles.

The ultimate inflorescence unit is racemose.

Inflorescences terminal, or axillary.

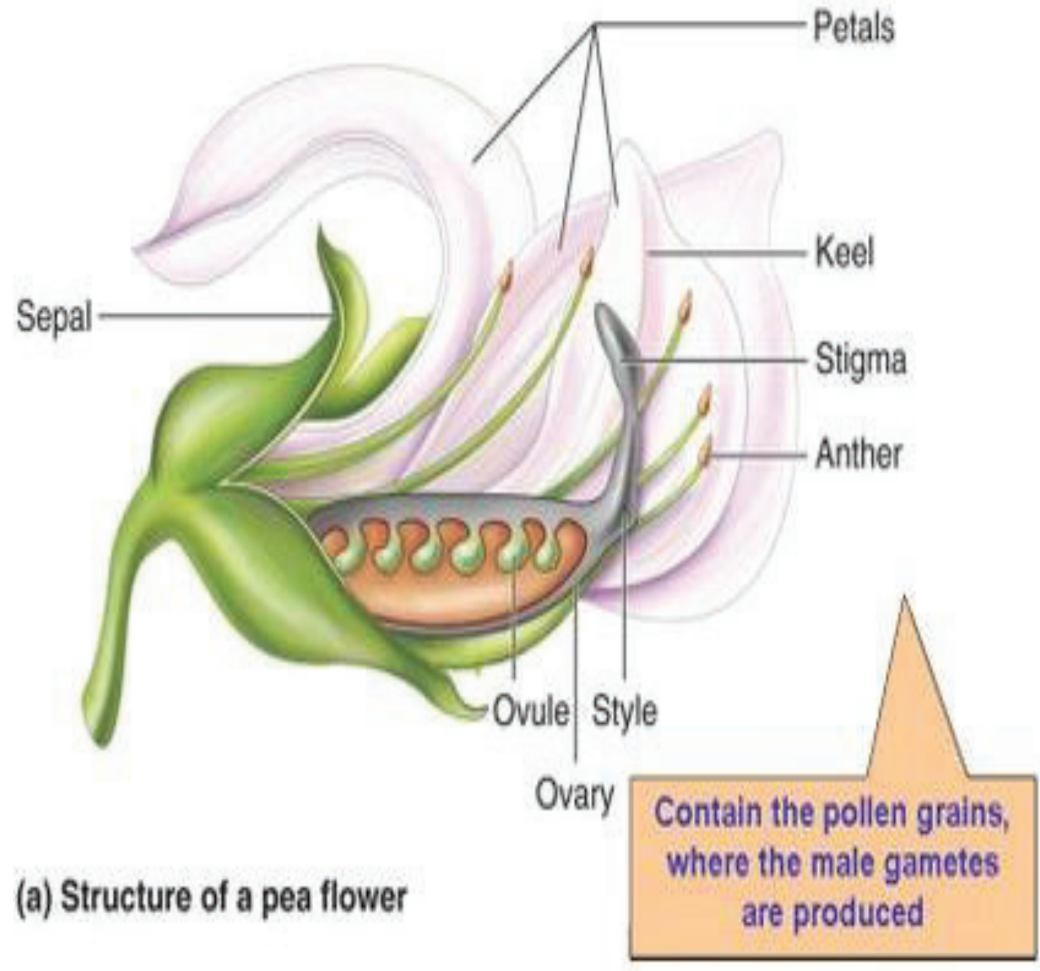
Flowers minute to large; somewhat irregular to very irregular (mostly), or regular (not infrequently); commonly more or less zygomorphic.

The floral irregularity involving the perianth and involving the androecium.

Flowers 'pseudo-papilionaceous' (usually, the corolla 'ascending'), or papilionaceous (corolla 'descending' in some Cassieae), or neither papilionaceous nor pseudo-papilionaceous pentamerous or not; tricyclic, or tetracyclic (usually), or pentacyclic to polycyclic.

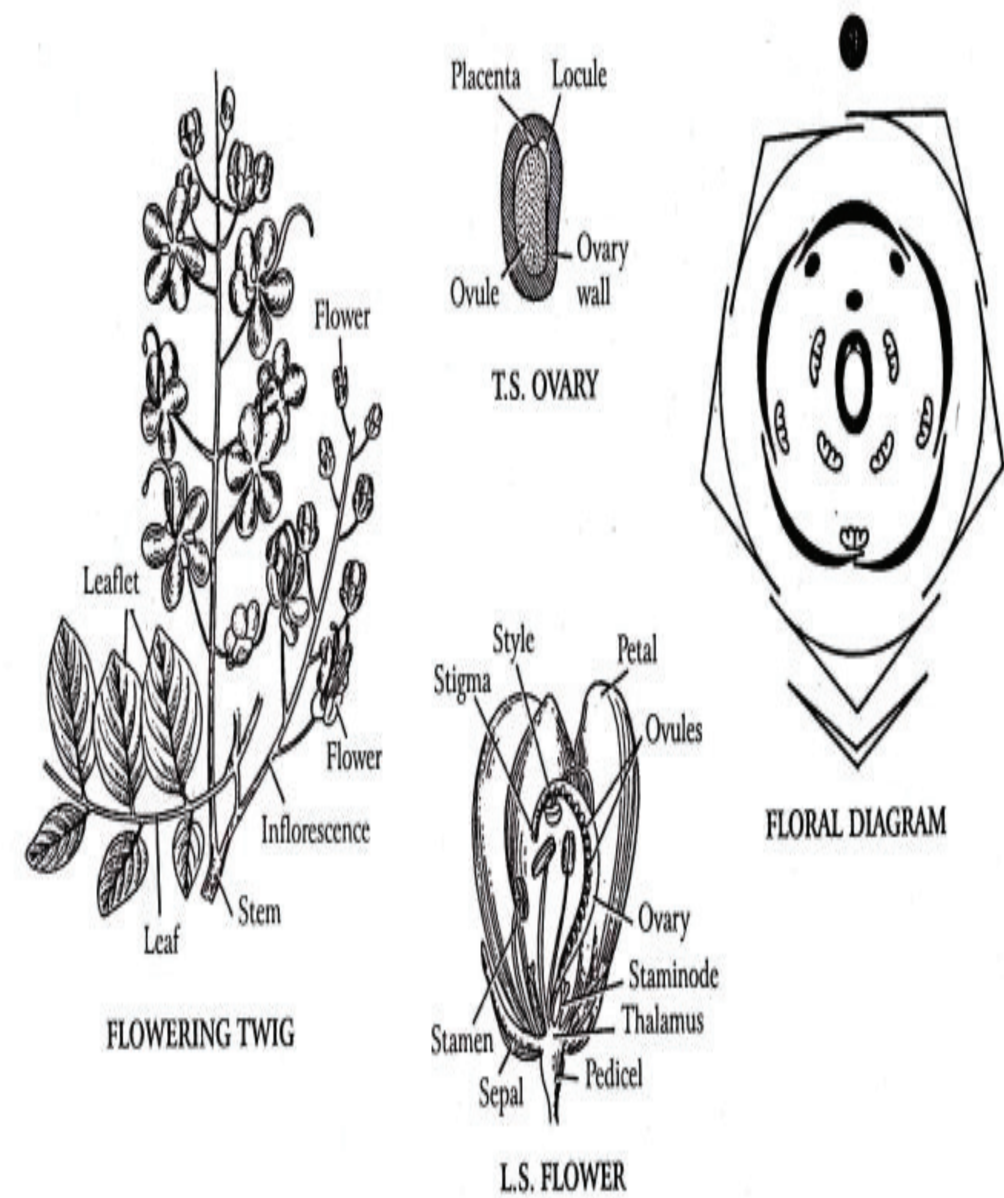
Floral receptacle developing a gynophore or with neither androphore nor gynophore.

Free hypanthium present or absen**floral formula:**



(a) Structure of a pea flower

Floral Formula: $\dagger$, ⚥ , $K_{(5)}$, C_5 , A_3 stamenoid , +4+3 , $\underline{G}_1$



Summary of the family Caesalpinieae:

Floral Characters:

Inflorescence:	Racemose, raceme or panicle.
Flower:	Pedicellate, complete, bisexual, zygomorphic, hypogynous or slightly perigy-nous, bracteate.
Calyx:	5 sepals, polysepalous, aestivation-imbricate, odd sepal is at anterior side, inferior.
Corolla:	5 petals, polypetalous, aestivation is **ascending imbricate**, posterior petal is inner most, inferior.
Androecium:	10 stamens, polyandrous, unequal size, the lowest three stamens are long with curved filaments, 4 lateral stamens are shorter and upper most three are very reduced in size, called **stamenoids**, dithecous, dorsifixed, introse.
Gynoecium:	Monocarpellary, ovary superior, unilocular, ovules along the margin of ovary, style short, stigma terminal and hairy.
Placentation:	Marginal
Fruit:	Legume, internally divided into one-seeded chambers.
Seed:	Albuminous

Examples of Caesalpniaceae:

The larger genera are cassia (450 sp), bauhinia (250 sp) and caesalpinia (100 sp).

Useful plants:

Tamarind (Tamarindusindica) fruit widely used for sour preparations, heartwood very hard and durable, flowers golden yellow, etc. Medicinal:

indiansenna (Cassia angustifilia; Saracaindica, fever nut (caesalpinia crista=C. bonducellor), etc.

Dyes:

Sappan or brazil wood -Caesalpinia sappan-wood yields a valuable red dye used extensively for dyeing silk and wool, starch colored with this dye and pods yield a high percentage of tannin, logwood (haematoxylon), an American plant. Wood yields the dye haematoxylin.

Ornamental:

Camel's foot tree (bauhinia purpurea and B. variegate), Golmohur (Delonixregia), dwarf gol mohur or peacock flower (Poinciana pulcherrima), Jerusalem thorn (parkinsonia aculeate) and Peltophorumferrugineam.

iv) Family Rubiaceae

Habit:

These are herbs (erect or prostrate), shrubs, trees, and climbers, sometimes thorny.

Leaves:

The leaves are simple, entire, opposite, (discussate) or whorled, with interpetiolar (sometimes intrapetiolar) stipules.

Inflorescence:

The inflorescence is typically cymose, frequently dichasial and branched, sometimes in globose heads.

Flowers:

The flowers are regular, bisexual, epigynous, sometimes dimorphic, as in some species of Randia and Oldenlandia.

Calyx:

There are usually (4) sometimes (5). It is gamosepalous. The calyx-tube adnates to the ovary.

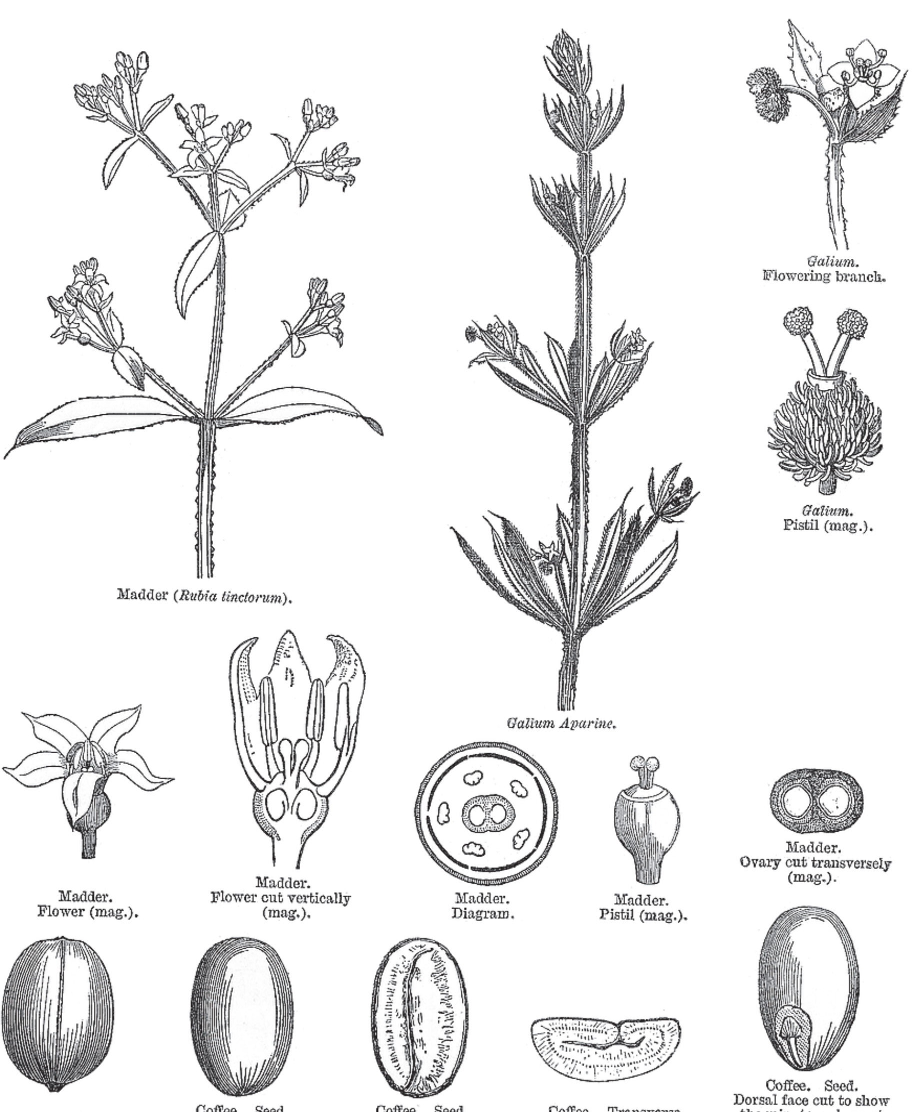
Corolla:

There are usually (4) sometimes (5). It is gamopetalous, generally rotate. The aestivation is valvate, imbricate or twisted.

Floral taxonomic understanding of Rubiaceae:

Androecium:

The stamens are as epipetalous, inserted within or at the mouth of the corolla-



tube, alternating with the corolla-lobes.

Gynoecium:

The carpels are (2), syncarpous. The ovary is inferior, commonly 2-locular, with 1- ovule in each. The disc is usually anullar at the base of the style.

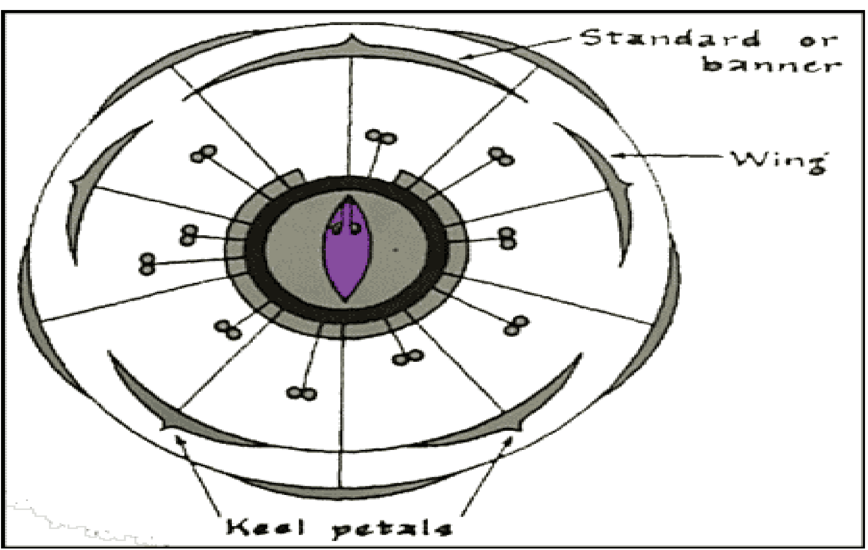
Fruit:

The fruit is a berry, drupe or capsule.

Seed:

The seed has fleshy or horny endosperm.

Floral formula: Rubiceae



Rubiceae is related to Caprifloliaceae, but in the latter the interpetiolar stipules are wanting and the carpels are (5-3). Rubiaceae is also distantly related to *Compositae* by virtue of the head or capitulum, as in Anthocephalus, Uncaria, Nauclea, Adina, etc.

Examples: The larger genera are: Psychotria (600 sp), Ixora (over 300 sp.) Pavetta (over 300 sp.), Galium (300 sp.), gardenia (250 sp.),Gardenia(250 sp), Randia(0ver 200 sp.), Oldenlandia (over 200 sp.), Mussaenda (200 sp).

Useful plants

Medicinal:

Cinchona yields quinine which is extracted from root and stem, the bark of the ipecac (Psychotriaipecacuanha=Cephaelis ipeccacuanha) yields emetine, Paederia foetida- Leaves are good stomachic, Oldenlandia corymboosa- entire plant used as a remedy for jaundice, liver disorders and remittent fever etc.

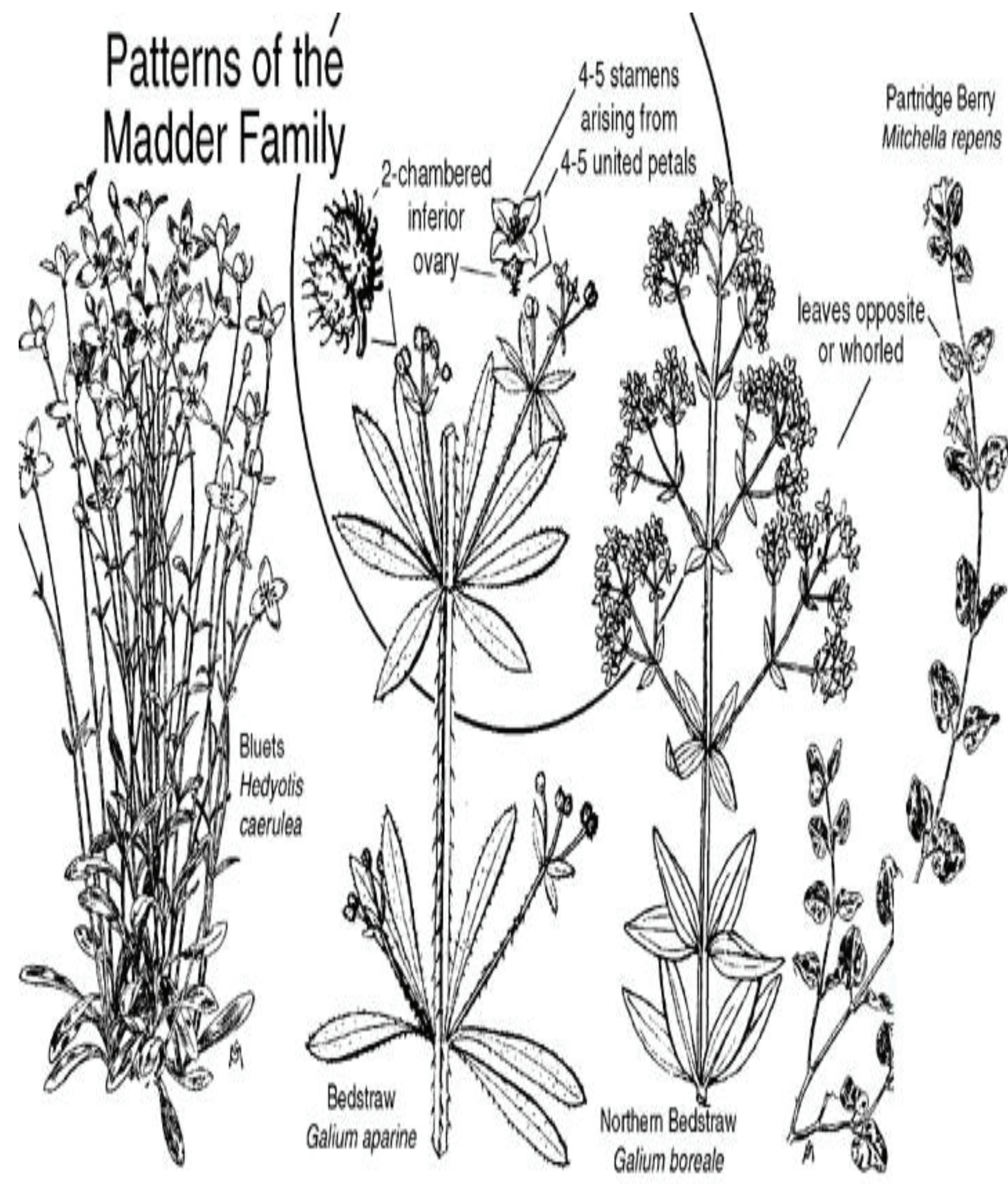
Ornamental:

Ixoracoccina, I. parviflora, pavettaindica, Gardenia jasminoides (=G.florida), Anthocephalusindicus,Adinacordiforlia, Cephalanthusoccidentails, Randia fasculata, Hamelia patens. Mussaenda- Flowers usually yellow or orange and one of the sepals much enlarged, the latter being white in M. frondosa, cream and yellow in M.incana and bright scarlet in M. erythrophylla.

Dyes:

Madder (Rubia cordifolia) and Morindatinctoria are rich sources of dyes used in commercial market.

Beverage: Coffee (coffee Arabica and C. robusta)- Seeds are the source of coffee powder.



v) FAMILY SOLANACEAE.

Habit:

These are herbs and shrubs; bi-collateral bundles or internal phloem are often present.

Leaves:

These are simple, sometimes pinnate, as in tomato, and alternate

Flowers:

These are regular, seldom Zygomorphic, bisexual and hypogynous.

Calyx:

The sepals are (5), united and persistence.

Corolla:

The petals are (5) and united. It is usually funnel or cup-shaped, 5-lobed. The lobes are valvate or twisted in the bud.

Androecium:

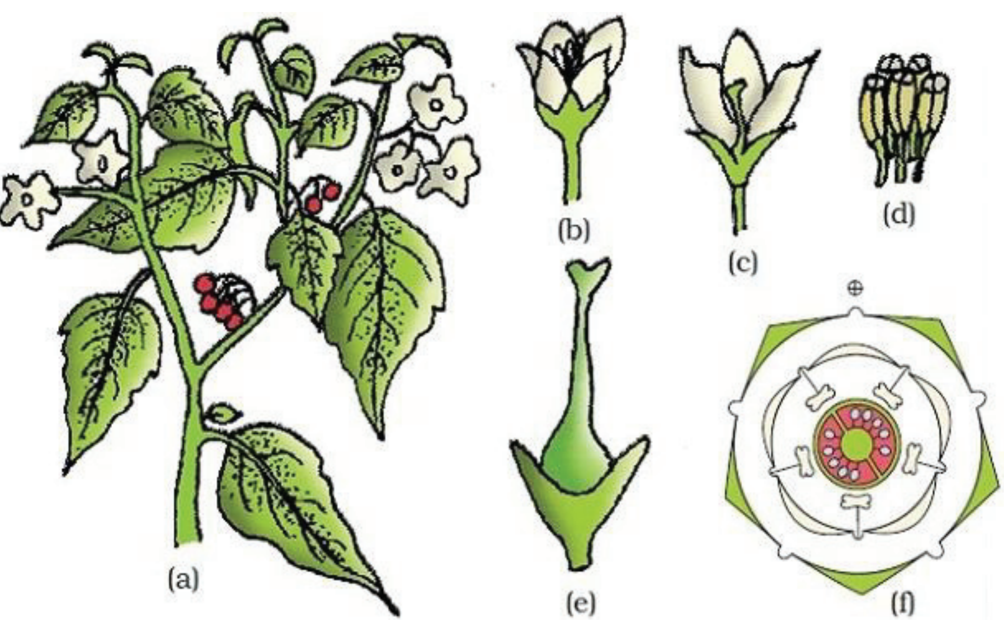
The stamens are (5), epipetalous and alternate with corolla lobes. The anthers are apparently connate and often open by means of pores.

Gynoecium:

The carpels are (2) and syncarpous. The ovary is superior and oblique placed. It is 2-celled or 4-celled, owing to the development of a false septum, as in tomato and thorn-apple. There are many ovules in each chamber. The placentation is axile.

Fruit:

The fruit is a berry or capsule with many needs



Solanum nigrum (makoi) plant : (a) Flowering twig (b) Flower (c) L.S. of flower (d) Stamens (e) Carpel (f) Floral diagram

Floral formula:

Solanceae is related to Convolvulaceae. It is closely related to Scrophulariaceae through Brunfelsia and Schinzanthus, which have a zygomorphic corolla and 4 or 2 stamens. Solananceae is however, generally distinguished from the latter family by its regular corolla, twisted aestivation, five stamens, obliquely placed carpels, often bicollateral vascular bundles in the stem, etc.

Examples:

The larger genera are olanum (1,500 sp.), Cestrum (250 sp., mostly American), Physalis (100 sp.), Nicotiana (100 sp.), and Capsicum (50 sp.).

Useful plants

Vegetables:

Potato (Solanumtuberosum), brinjal (S.melongena), chilli or red paper (Capsicum annuum), fruits pungent, mainly used as a condiment, bell-pepper (C. grossum)-fruits not pungent used as a vegetable, and tomato (lycopersicum esculentum)-fruits are succulent and edible.

Medicinal:

Deadly nightshade (Atropa belladonna), thorn-apple (Datura stramonium) and Daturametel (=D.fastuosa). Seeds narcotic and very poisonous, Henbane (Hyoscyamus niger), Bittersweet (solanum dulcamara,S. surattense (=S. xanthocarpum and Withaniasomnifera- used as medicine

Narcotic:

Tobacco (Nicotiana tabacum)-Commercial tobacco and also a source of nicotine (an insecticide).

Fruit:

Gooseberry (Physalisperuviana)

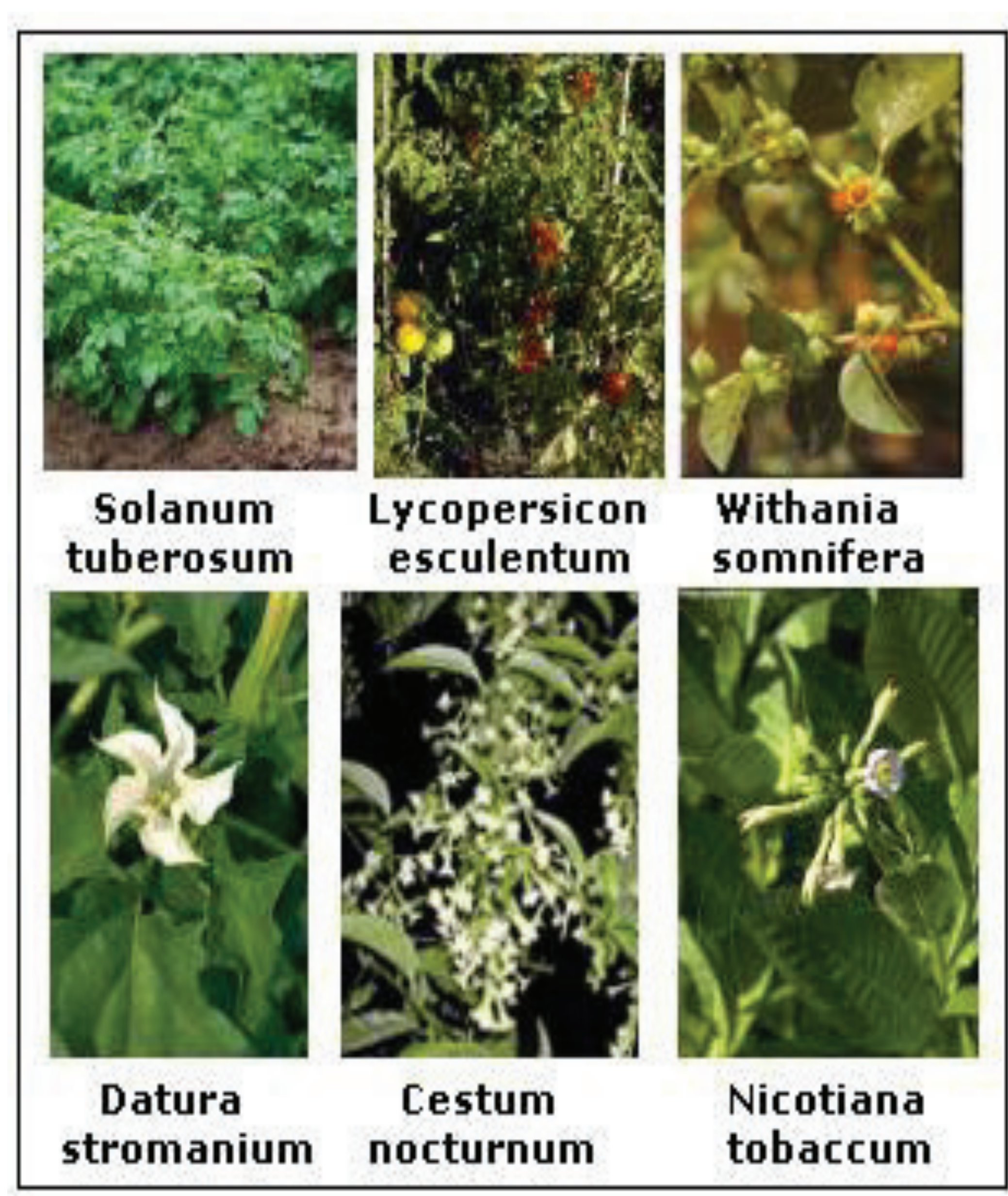
Ornamental:

Petunia hybryda, queen of the night (Cestrum nocturnum and C. parqui- Both sweet scented, Brunfelsia hopeana (=Franciscea bicolor)- Flowers white changing to blue, sweet-scented, Schizanthus pinnatus- a beautiful garden herb..

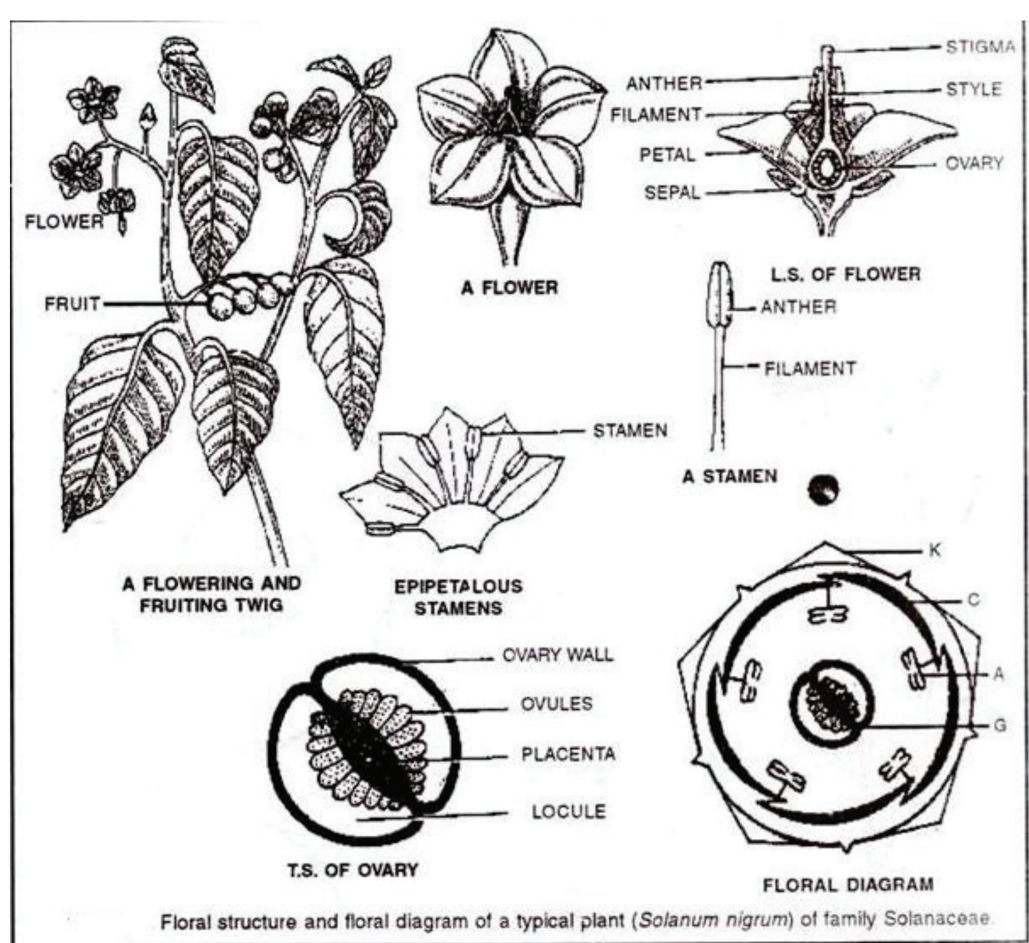
Other common plants:

Black nightshade (Solanum nigrum), Egg plant (S. ferox), Wild gooseberry (Physalis minima) and Wild tobacco (Nicotianaplumbaginifolia).

A few plants belonging to family Solanaceae:



Floral diagram:



Floral structure and floral diagram of a typical plant (*Solanum nigrum*) of family Solanaceae

In this unit we have understood the principles and practice of flowering plant taxonomy. Emphasis is placed on acquiring the facility to use appropriate terminology in order to identify plants, as well as understanding the historical context and investigative procedures of taxonomists in designing a classification. This lesson has been of particular importance is gaining an understanding of the philosophical bases in taxonomy and the relevance of this field to other areas of biology.

UNIT III

History of Cell Biology

The cell theory, or cell doctrine, states that all organisms are composed of similar units of organization, called cells. The concept was formally articulated in 1839 by Schleiden & Schwann and has remained as the foundation of modern biology. The idea predates other great paradigms of biology including Darwin's theory of evolution (1859), Mendel's laws of inheritance (1865), and the establishment of comparative biochemistry (1940).

First Cells Seen in Cork

While the invention of the telescope made the Cosmos accessible to human observation, the microsope opened up smaller worlds, showing what living forms were composed of. The cell was first discovered and named by Robert Hooke in 1665. He remarked that it looked strangely similar to cellula or small rooms which monks inhabited, thus deriving the name. However what Hooke actually saw was the dead cell walls of plant cells (cork) as it appeared under the microscope. Hooke's description of these cells was published in Micrographia. The cell walls observed by Hooke gave no indication of the nucleus and other organelles found in most living cells. The first man to witness a live cell under a microscope was Anton van Leeuwenhoek, who in 1674 described the algae Spirogyra. Van Leeuwenhoek probably also saw bacteria.

Formulation of the Cell Theory

In 1838, Theodor Schwann and Matthias Schleiden were enjoying after-dinner coffee and talking about their studies on cells. It has been suggested that when Schwann heard Schleiden describe plant cells with nuclei, he was struck by the similarity of these plant cells to cells he had observed in animal tissues. The two scientists went immediately to Schwann's lab to look at his slides. Schwann published his book on animal and plant cells (Schwann

1839) the next year, a treatise devoid of acknowledgments of anyone else's contribution, including that of Schleiden (1838). He summarized his observations into three conclusions about cells:

The cell is the unit of structure, physiology, and organization in living things.

The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.

Cells form by free-cell formation, similar to the formation of crystals (spontaneous generation).

We know today that the first two tenets are correct, but the third is clearly wrong. The correct interpretation of cell formation by division was finally promoted by others and formally enunciated in Rudolph Virchow's powerful dictum, Omnis cellula e cellula,: "All cells only arise from pre-existing cells".

Modern Cell Theory

All known living things are made up of cells.

The cell is structural & functional unit of all living things.

All cells come from pre-existing cells by division. (Spontaneous Generation does not occur).

Cells contain hereditary information which is passed from cell to cell during cell division.

All cells are basically the same in chemical composition.

All energy flow (metabolism & biochemistry) of life occurs within cells.

As with the rapid growth of molecular biology in the mid-20th century, cell biology research exploded in the 1950's. It became possible to maintain, grow, and manipulate cells outside of living organisms. The first continuous cell line to be so cultured was in 1951 by George Otto Gey and coworkers,

derived from cervical cancer cells taken from Henrietta Lacks, who died from her cancer in 1951. The cell line, which was eventually referred to as HeLa cells, have been the watershed in studying cell biology in the way that the structure of DNA was the significant breakthrough of molecular biology.

In an avalanche of progress in the study of cells, the coming decade included the characterization of the minimal media requirements for cells and development of sterile cell culture techniques. It was also aided by the prior advances in electron microscopy, and later advances such as development of transfection methods, discovery of green fluorescent protein in jellyfish, and discovery of small interfering RNA (siRNA), among others.

A Timeline

1595 – Jansen credited with 1st compound microscope

1655 – Hooke described 'cells' in cork.

1674 – Leeuwenhoek discovered protozoa. He saw bacteria some 9 years later.

1833 – Brown descibed the cell nucleus in cells of the orchid.

1838 – Schleiden and Schwann proposed cell theory.

1840 – Albrecht von Roelliker realized that sperm cells and egg cells are also cells.

1856 – N. Pringsheim observed how a sperm cell penetrated an egg cell.

1858 – Rudolf Virchow (physician, pathologist and anthropologist) expounds his famous conclusion: omnis cellula e cellula that is cells develop only from existing cells [cells come from preexisting cells]

1857 – Kolliker described mitochondria.

1879 – Flemming described chromosome behavior during mitosis.

1883 – Germ cells are haploid, chromosome theory of heredity.

1898 – Golgi described the Golgi apparatus.

1938 – Behrens used differential centrifugation to separate nuclei from cytoplasm.

1939 – Siemens produced the first commercial transmission electron microscope.

1952 – Gey and coworkers established a continuous human cell line.

1955 – Eagle systematically defined the nutritional needs of animal cells in culture.

1957 – Meselson, Stahl and Vinograd developed density gradient centrifugation in cesium chloride solutions for separating nucleic acids.

1965 – Ham introduced a defined serum-free medium. Cambridge Instruments produced the first commercial scanning electron microscope.

1976 – Sato and colleagues publish papers showing that different cell lines require different mixtures of hormones and growth factors in serum-free media.

1981 – Transgenic mice and fruit flies are produced. Mouse embryonic stem cell line established.

1995 – Tsien identifies mutant of GFP with enhanced spectral properties

1998 – Mice are cloned from somatic cells.

1999 – Hamilton and Baulcombe discover siRNA as part of post-transcriptional gene silencing (PTGS) in plants

History of Molecular Biology

Despite its prominence in the contemporary life sciences, molecular biology is a relatively young discipline, originating in the 1930s and 1940s, and

becoming institutionalized in the 1950s and 1960s. It should not be surprising, then, that many of the philosophical issues in molecular biology are closely intertwined with this recent history. This section sketches four facets of molecular biology's development: its origins, its classical period, its subsequent migration into other biological domains, and its more recent turn to genomics and post-genomics. The rich historiography of molecular biology can only be briefly utilized in this shortened history.

Origins

The field of molecular biology arose from the convergence of work by geneticists, physicists, and structural chemists on a common problem: the nature of inheritance. In the early twentieth century, although the nascent field of genetics was guided by Mendel's laws of segregation and independent assortment, the actual mechanisms of gene reproduction, mutation and expression remained unknown. Thomas Hunt Morgan and his colleagues utilized the fruit fly, Drosophila melanogaster, as a model organism to study the relationship between the gene and the chromosomes in the hereditary process (Morgan 1926; discussed in Darden 1991; Darden and Maull 1977; Kohler 1994; Roll-Hanson 1978; Wimsatt 1992). A former student of Morgan's, Hermann J. Muller, recognized the "gene as a basis of life", and so set out to investigate its structure (Muller 1926). Muller discovered the mutagenic effect of x-rays on Drosophila, and utilized this phenomenon as a tool to explore the size and nature of the gene.

Muller's request did not go unanswered. The next decade saw several famous physicists turn their attention to the nature of inheritance (Keller 1990; Kendrew 1967). In what is Life, the physicist Erwin proposed ways in which the principles of quantum physics might account for the stability, yet mutability, of the gene for a reinterpretation see Kay 2000). Max Delbrueck also became interested in the physical basis of heredity after hearing a lecture by his teacher, quantum physicist to investigate the self-reproductive characteristic of life, Delbrueck used bacteriophage, viruses that infect bacteria and then multiply very rapidly.

The establishment of “The Phage Group” in the early 1940s by Delbrueck and another physicist-turned-biologist Salvador Luria marked a critical point in the rise of molecular biology Delbrueck’s colleague at Cal Tech, Linus Pauling, utilized his knowledge of structural chemistry to study macromolecular structure. Pauling contributed both theoretical work on the nature of chemical bonds and experimental work using x-ray crystallography to discover the physical structure of macromolecular compounds.

As suggested in the brief history above, experimentation figured prominently in the rise of molecular biology (see also the entry on experimentation in biology). X-ray crystallography allowed molecular biologists to investigate the structure of macromolecules (see Celebrating Crystallography in Other Internet Resources). Alfred Hershey and Martha Chase (1952) used phage viruses to confirm that the genetic material transmitted from generation to generation was DNA and not proteins (see Hershey-Chase Experiment in Other Internet Resources). Muller (1927) used x-rays to intervene on and alter gene function, thus revealing the application of methods from physics to a biological domain (see Elof Carlson on Muller’s Research in Other Internet Resources).

Recognizing quite early the importance of these new physical and structural chemical approaches to biology, Warren Weaver, then the director of the Natural Sciences section of the Rockefeller Foundation, introduced the term “molecular biology” in a 1938 report to the Foundation. Weaver wrote,

And gradually there is coming into being a new branch of science—molecular biology—which is beginning to uncover many secrets concerning the ultimate units of the living cell….in which delicate modern techniques are being used to investigate ever more minute details of certain life processes (quoted in Olby 1994: 442).

But perhaps a more telling account of the term’s origin came from Francis Crick’s explanation for why he began calling himself a molecular biologist:

This brief recapitulation of the origins of molecular biology reflects themes addressed by philosophers, such as reduction (see Section 3.1), the concept of

the gene (see Section 2.3), and experimentation (see Section 3.4). For Schroedinger, biology was to be reduced to the more fundamental principles of physics, while Delbrueck instead resisted such a reduction and sought what made biology unique. Muller’s shift from Mendelian genetics to the study of gene structure raises the question of the relation between the genes concepts found in those separate fields of genetics. And the import of experimental methods from physics to biology raised the question of the relation between those disciplines.

Classical Period

Molecular biology’s classical period began in 1953, with James Watson and Francis Crick’s discovery of the double helical structure of DNA (Watson and Crick 1953a,b). Watson and Crick’s scientific relationship unified the various disciplinary approaches discussed above: Watson, a student of Luria and the phage group, recognized the need to utilize crystallography to elucidate the structure of DNA; Crick, a physicist enticed by Schroedinger’s what is Life? To turn to biology, became trained in, and contributed to the theory of, x-ray crystallography. At Cambridge University, Watson and Crick found that they shared an interest in genes and the structure of DNA.

Watson and Crick collaborated to build a model of the double helical structure of DNA, with its two helical strands held together by hydrogen-bonded base pairs (Olby 1994). They made extensive use of data from x-ray crystallography work on DNA by Maurice Wilkins and Rosalind Franklin at King’s College, London (Maddox 2002), Crick’s theoretical work on crystallography (Crick 1988), and the model building techniques pioneered by Pauling.

With the structure of DNA in hand, molecular biology shifted its focus to how the double helical structure aided elucidation of the mechanisms of genetic replication and function, the keys to understanding the role of genes in heredity. This subsequent research was guided by the notion that the gene was an informational molecule.

"Information" replaced earlier talk of biological "specificity". Watson and Crick's second paper of 1953, which discussed the genetical implications of their recently discovered (Watson and Crick 1953a) double-helical structure of DNA, used both "code" and "information":

…it therefore seems likely that the precise sequence of the bases is the code which carries the genetical information…. (Watson and Crick 1953b: 244, emphasis added)

In 1958, Francis Crick used and characterized the concept of information in the context of stating the "central dogma" of molecular biology. Crick characterized the central dogma as follows:

This states that once "information" has passed into protein it cannot get out again. In more detail, the transfer of information from nucleic acid to nucleic acid or from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible. Information means here the precise determination of sequence, either of bases in the nucleic acid or of amino acid residues in the protein.

It is important not to confuse the genetic code and genetic information. The genetic code refers to the relation between three bases of DNA, called a "codon", and one amino acid. Tables available in molecular biology textbooks (e.g., Watson et al. 1988: frontispiece) show the relation between 64 codons and 20 amino acids. For example, CAC codes for histidine. Only a few exceptions for these coding relations have been found, in a few anomalous cases (see the list in a small table in Alberts et al. 2002: 814). In contrast, genetic information refers to the linear sequence of codons along the DNA, which (in the simplest case) are transcribed to messenger RNA, which are translated to linearly order the amino acids in a protein.

With the genetic code elucidated and the relationship between genes and their molecular products traced, it seemed in the late 1960s that the concept of the gene was secure in its connection between gene structure and gene function. The machinery of protein synthesis translated the coded

information in the linear order of nucleic acid bases into the linear order of amino acids in a protein.

However, such "colinear" simplicity did not persist. In the late 1970s, a series of discoveries by molecular biologists complicated the straightforward relationship between a single, continuous DNA sequence and its protein product. Overlapping genes were discovered (Barrell et al. 1976); such genes were considered "overlapping" because two different amino acid chains might be read from the same stretch of nucleic acids by starting from different points on the DNA sequence. And split genes were found.

In contrast to the colinearity hypothesis that a continuous nucleic acid sequence generated an amino acid chain, it became apparent that stretches of DNA were often split between coding regions (exons) and non-coding regions (introns). Moreover, the exons might be separated by vast portions of this non-coding, supposedly "junk DNA". The distinction between exons and introns became even more complicated when alternative splicing was discovered the following year.

A series of exons could be spliced together in a variety of ways, thus generating a variety of molecular products. Discoveries such as overlapping genes, split genes, and alternative splicing forced molecular biologists to rethink their understanding of what actually made a gene…a gene. These developments in molecular biology have received philosophical scrutiny. Molecular biologists sought to discover mechanisms, drawing the attention of philosophers to this concept.

Also, conceptualizing DNA as an informational molecule was a move that philosophers have subjected to critical scrutiny. Finally, the concept of the gene (see Section 2.3) itself has intrigued philosophers. Complex molecular mechanisms, such as alternative splicing, have obligated philosophers to consider to what the term "gene" actually refers. Experimentation also figured prominently in the classical period Matthew Meselson and Frank Stahl utilized bacteria grown with different weights combined with

centrifugation to determine how DNA, as modeled by Watson and Crick, was replicated.

Going Molecular

In a 1963 letter to Max Perutz, molecular biologist Sydney Brenner foreshadowed what would be molecular biology's next intellectual migration:

It is now widely realized that nearly all the "classical" problems of molecular biology have either been solved or will be solved in the next decade…. Because of this, I have long felt that the future of molecular biology lies in the extension of research to other fields of biology, notably development and the nervous system.

Along with Brenner, in the late 1960s and early 1970s, many of the leading molecular biologists from the classical period redirected their research agendas, utilizing the newly developed molecular techniques to investigate unsolved problems in other fields. Francois Jacob, Jacques Monod and their colleagues used the bacteria Escherichia coli to investigate how environmental conditions impact gene expression and regulation.

The study of behavior and the nervous system also lured some molecular biologists. Finding appropriate model organisms that could be subjected to molecular genetic analyses proved challenging. Returning to the fruit flies used in Mendelian genetics, Seymour Benzer induced behavioral mutations in Drosophila as a "genetic scalpel" to investigate the pathways from genes to behavior. And at Cambridge, Sydney Brenner developed the nematode worm, Caenorhabditis elegans, to study the nervous system, as well as the genetics of behavior. In subsequent decades, the study of cells was transformed from descriptive cytology into molecular cell biology. Molecular evolution developed as a phylogenetic method for the comparison of DNA sequences and whole genomes; molecular systematics sought to research the evolution of the genetic code as well as the rates of that evolutionary process by comparing similarities and differences between molecules. The immunological relationship between antibodies and antigens was

recharacterized at the molecular level. And the study of oncogenes in cancer research was just one example of molecular medicine.

This process of "going molecular" thus generally amounted to using experimental methods from molecular biology to examine complex phenomena (be it gene regulation, behavior, or evolution) at the molecular level. The molecularization of many fields introduced a range of issues of interest to philosophers. Inferences made about research on model organisms such as worms and flies raised questions about extrapolation. And the reductive techniques of molecular biology raised questions about whether scientific investigations should always strive to reduce to lower and lower levels.

Going Genomic and Post-Genomic

In the 1970s, as many of the leading molecular biologists were migrating into other fields, molecular biology itself was going genomic. The genome is a collection of nucleic acid base pairs within an organism's cells (adenine (A) pairs with thymine (T) and cytosine (C) with guanine (G)). The number of base pairs varies widely among species. For example, the flu-causing Haemophilus influenzae (the first bacterial genome to be sequenced) has roughly 1.9 million base pairs in its genome, while the flu-catching Homo sapiens carry more than 3 billion base pairs in its genome. The history of genomics is the history of the development and use of new experimental and computational methods for producing, storing, and interpreting such sequence data.

Frederick Sanger played a seminal role in initiating such developments, creating influential DNA sequencing techniques in the 1950s and 1960s. Equally important was Edwin Southern's development of a method to detect specific sequences of DNA in DNA samples. The Southern Blot, as it came to be known, starts by digesting a strand of DNA into many small DNA fragments; those fragments are then separated (in a process called gel electrophoresis) based on size, placed on filter paper which "blots" the DNA fragments on to a new medium, and then chemically labeled with DNA

probes; the probes then allow for identification and visualization of the DNA fragments.

The human genome project received most of the public attention; hundreds of genomes have been sequenced to date, including the cat, the mouse, rice and a flock of bird genomes. One of the most shocking results of those sequencing projects was the total number of genes found in the genomes. The human genome contains 20,000 to 25,000 genes; the cat contains 20,285 genes, the mouse 24,174, and rice 32,000 to 50,000. So in contrast to early assumptions stemming from the classical period of molecular biology about how genes produced proteins which in turn produced organisms, it turned out that neither organismal complexity nor even position on the food chain was predictive of gene-number.

The increased attention to sequencing genomes encouraged a number of disciplines to "go genomic", including behavioral genetics, developmental biology, cell biology, and evolutionary biology. What's more, genomics has been institutionalized with textbooks and journals, such as Genomics and Genome Research. And the human genome project itself has turned its attention from a standardized human genome to variation between genomes in the form of the Human Genome Diversity Initiative.

But just as a number of disciplines "went molecular" while molecular biology itself was wrestling with the complexities posed by split genes and overlapping genes, so too are fields going genomic while genomics itself is wrestling with the complexities posed by how a mere 20,000 genes can construct a human while a grain of rice requires 50,000 genes. A related challenge was making sense of the genetic similarity claims. For example, how to interpret the finding that human and pumpkin genomes are 75% similar? Does this finding tell us anything substantive about our overall similarity to pumpkins? To help answer such questions, genomics is now supplemented by post-genomics. There is ongoing debate about what actually constitutes post-genomics, but the general trend is a focus beyond the mere sequence of As, Cs, Ts, and Gs and instead on the complex, cellular mechanisms involved in generating such a variety of protein products from a

relatively small number of protein-coding regions in the genome. Post-genomics utilizes the sequence information provided by genomics but then situates it in an analysis of all the other entities and activities involved in the mechanisms of transcription (transcriptomics), regulation (regulomics), metabolism (metabolomics), and expression (proteomics).

Developments in genomics and post-genomics have sparked a number of philosophical questions about molecular biology. Since the genome requires a vast array of other mechanisms to facilitate the generation of a protein product, can DNA really be causally prioritized Similarly, in the face of such interdependent mechanisms involved in transcription, regulation, and expression, can DNA alone be privileged as the bearer of hereditary information, or is information distributed across all such entities and activities And is it appropriate to extrapolate from information about other species' genomes to how the human genome operates.

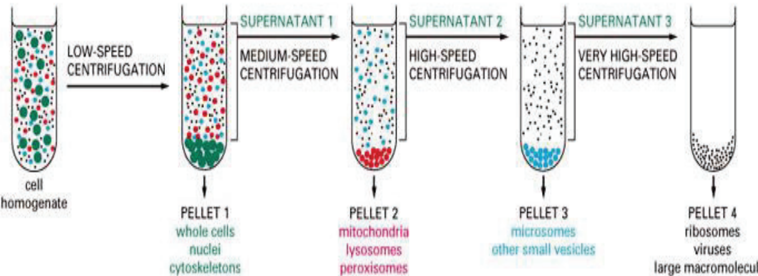
Cytological Techniques of Cell Fractionation

Homogenization

This means separating different parts and organelles of a cell, so that they can be studied in detail. All the processes of cell metabolism have been studied in this way. The most common method of fractionating cells is to use differential centrifugation. This begins with breaking open of the cells-homogenisation

The resulting 'soup' is then centrifuged at progressively higher speeds and for longer periods of time. Note the order in which the organelles separate:

N.B: Chloroplasts separate just ahead of mitochondria (being larger and heavier).



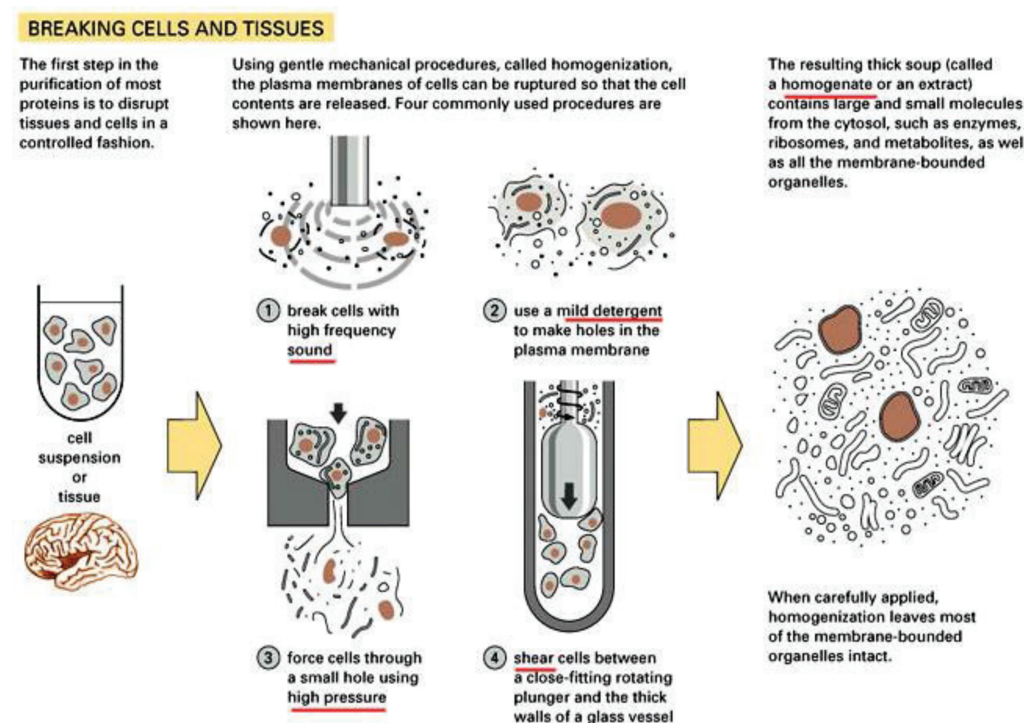
The basic principle for all microscopes is that the cell is composed of smaller physical units, the organelles. Definition of the organelles is possible with microscopy, but the function of individual organelles is often beyond the ability of observations through a microscope. We are able to increase our chemical knowledge of organelle function by isolating organelles into reasonably pure fractions.

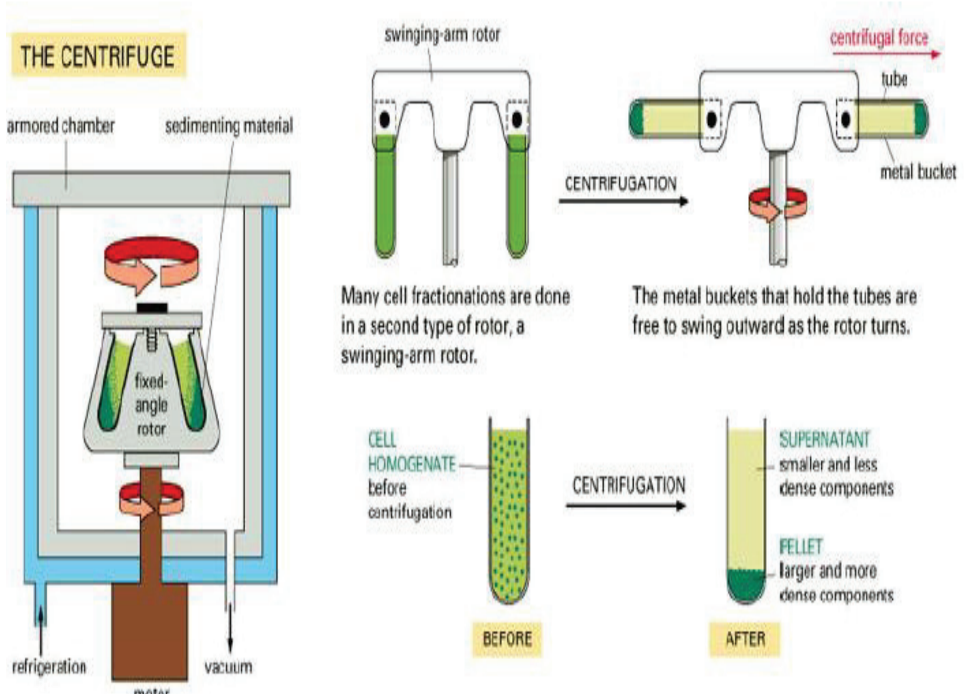
A host of fractionation procedures are employed by cell biologists. Each organelle has characteristics (size, shape and density for example) which make it different from other organelles within the same cell. If the cell is broken open in a gentle manner, each of its organelles can be subsequently isolated. The process of breaking open cells is **homogenization** and the subsequent isolation of organelles is**fractionation**. Isolating the organelles requires the use of physical chemistry techniques, and those techniques can range from the use of simple sieves, gravity sedimentation or differential precipitation, to ultracentrifugation of fluorescent labeled organelles in computer generated density gradients.

Homogenization

Often, the first step in the preparation of isolated organelles is to obtain a "pure" sample for further analysis. Cells which are not attached to others (such as blood or suspension tissue cultures) can be separated if they have distinct shapes, densities or characteristics which can be marked (such as charge, antigen or enzyme presence). Cells which are part of a more solid tissue (such as liver or kidney) will first need to be separated from all connections with other cells. In some cases this can be performed by simply chelating the environment (removing Ca^{++} and/or Mg^{++}), but in most instances the cells will need to be enzymatically or mechanically disaggregated. This often results in subtle changes to the cells, and at a minimum will disrupt such cell-cell communications as **DESMOSOMES** and **TIGHT JUNCTIONS**.

Homogenization techniques can be divided into those brought about by





Centrifugation is the most widely used procedure to separate the homogenate into different parts, or fractions. The homogenate is placed in test tubes and rotated at high speed in a centrifuge (sometimes called an ultracentrifuge). Present-day ultracentrifuges rotate at speeds up to 100,000 revolutions per minute and produce enormous forces, as high as 600,000 times gravity.

At such speeds, centrifuge chambers must be refrigerated and evacuated so that friction does not heat up the homogenate. The centrifuge is surrounded by thick armor plating, since an unbalanced rotor can shatter with an explosive release of energy. A fixed-angle rotor can hold larger volumes than a swinging-arm rotor, but the pellet forms less evenly.

osmotic alteration of the media which cells are found in, or those which require physical force to disrupt cell structure. The physical

Means encompass use of mortars and pestles, blenders, compression and/or expansion, or ultrasonification.

Osmotic alterations

Many organelles are easier to separate if the cells are slightly swollen. The inbibition of water into a cell will cause osmotic swelling of the cell and/or organelle, which can often assist in the rupture of the cell and subsequent organelle separation. The use of a hypo-osmotic buffer can be very

beneficial, for example, in the isolation of mitochondria and in the isolation of mitotic chromosomes.

Mortars, Pestles

Perhaps the most common procedures are done with glass mortar and pestle arrangements with manufactured, controlled bore sizes. The addition of a motor driven Teflon pestle creates the Potter- homogenizer. Ultrasonification is a useful adjunct to this procedure, but is often sufficient by itself.

To obtain pure organelles, the cells must be ruptured, so that the cell membrane is broken, but the organelle to be studied is not. The process of rupturing a cell is known as **homogenization** of the cell. It also varies from simple mortar/pestle grinding (with the aid of sand or glass beads) for many plant materials, to repeated high velocity compression and expansion in what is known as a "French Press." The French Press is very powerful and can disrupt bacteria and viral particles as well. It is favored for use when **molecular dissociation** is required, such as in the separation of DNA from the nematode worm *C. elegans*. Often, cell rupture is aided by rapid freezing (in liquid nitrogen) and subsequent application of mechanical forces.

With all forms of homogenization, the shear force must be carefully controlled. Too little and the organelles will not be separated, too much and even the molecules can be broken.

Blenders

For molecular separations, mechanical blenders are often used, varying in sophistication from household blenders to high speed blenders with specially designed blades and chambers (e.g. a Tissue Homogenizer). The mechanical procedures are augmented by various organic solvents (for phase separations) and/or detergents to assist the denaturation and separation of molecules (e.g. DNA from histones). When specific molecules are sought, care must be taken to inhibit powerful degradation enzymes (such as RNase when

extracting RNA). This can be accomplished by subjecting the specimen to cold temperature, or by adding specific organic inhibitors (Diethylpyrocarbonate for RNase), or both.

Compression/Expansion

For cellular material which is difficult to shear by the above mentioned techniques (plant cells and bacteria), a device known as a "French Press" is ocassionally used. This device forces slurry of the cells through an orifice (opening) at very high pressures. The rapid expansion of the pressure from within literally "blows" the cells apart. While this technique is not often required, it is the only way to break open some materials. The units have capacities from 1 to 40 ml and can reach pressures of 20,000-40,000 pounds per square inch (psi).

Ultrasonification

Ultrasonicators have been used with increasing popularity to separate organelles from cells, particularly from tissue culture cells. Light use of an ultrasonic wave can readily remove cells from a tissue culture substrate (such as the culture flask). It can also be adjusted to merely separate cells, or to break open the plasma membrane and leave the internal organelles intact.

Isolation and Fractionation of Subcellular Organelles

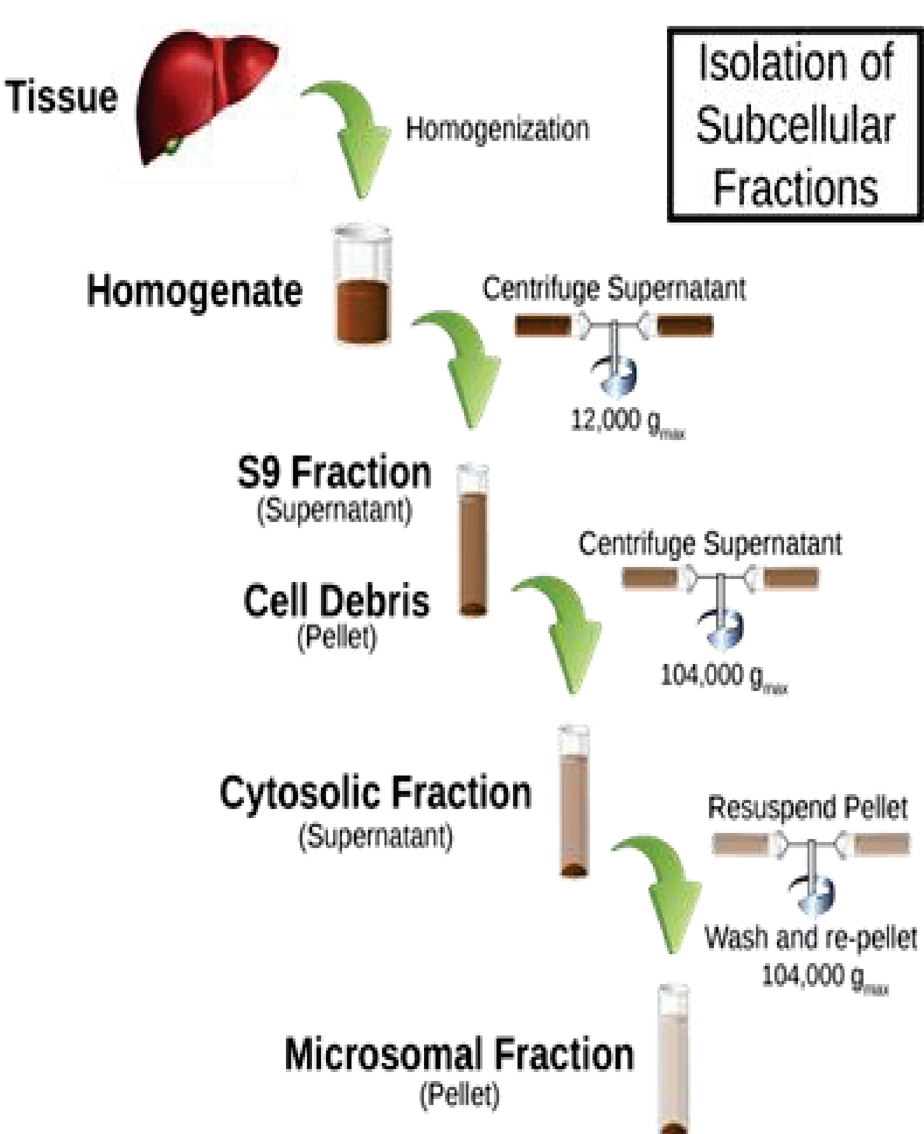
An important aspect of the study of the Biochemistry and Cell Biology of eukaryotes is the study of the function of cell organelles. This often means that organelles have to be purified so that the are free of other cell components with the minimum damage to the structure and function of the organelle. Various methods can be used for the disruption of the tissue depending on the size of the cells, the collagen content of the tissue (or in the case of plant cells, the thickness of the cell walls). Liver is perhaps the most easily fractionated of all tissues with relatively large cells (10 -20 μm) which are readily broken.

The homogeniser used is a power -driven Teflon homogeniser (Potter-Elvehjem) rotating in a closely-fitting glass tube. The size of the gap between the pestle and the glass is such that the rotating pestle applies a shearing force which breaks open the cell releasing the contents without breaking the organelles. The degree of homogenisation of the tissue depends on (a), the speed of rotation of the pestle, (b), the clearance between the pestle and the glass container, (c), the number of strokes of the pestle and (d), the thrust force applied. If the pestle is too tight or too many strokes are applied the organelles will be damaged. The development of the best procedure for the homogenisation of any tissue for the preparation of intact organelles is a matter of the application of scientific principles plus trial and error based on experimentation.

Th other important factor for the isolation of intact organelles is the composition of the homogenisation medium, particularly the osmotic strength of the medium. As with intact cells, a hypo-osmolar medium causes organelle swelling and breakage. Usually the organelles are more susceptible to osmotic damage than the cells from which they are derived.

- The homogenisation should therefore be performed in an iso-osmotic medium containing an inert substance, for example 0.25 M sucrose (or mannitol). Sometimes iso-osmotic 0.1M KCl is used, reflecting the cytoplasmic concentration of the salt in most cells.
- Usually a dilute buffer is also added to prevent large fluctuations in pH which may damage organelles. The maintenance of a pH gradient is especially important in the function of some organelles.

Chelators of divalent metal ions such as EDTA or EGTA are often added because such metal ions may damage organelles.



Centrifugation of subcellular organelles

Once the cell-free homogenate has been prepared the next step is the use of differential centrifugation to separate out the various organelles on the basis of their size and density (see Table1).

Table 1. Size and Density of Some Sub-cellular Organelles from Liver

	Size □m	Density (g/cm^3)(in sucrose medium)
Nuclei	3 - 12	>1.30
Mitochondria	0.5 - 2.0	1.17 - 1.21
Lysosomes	0.2 - 0.4	1.20 - 1.22
Peroxisomes	0.2 - 0.5	1.23
Endoplasmic reticulum Vesicles (microsomes)	0.05 - 0.30	1.15 (smooth ER)
		1.22 (rough ER)
Golgi stacks	~ 1.0	1.10 - 1.13
Golgi vesicles	~0.05	
Plasma membrane sheets	20	1.15 - 1.19
Plasma membrane vesicles	0.05	< 1.17

Differential Centrifugation

Particles may be separated on the basis of their size and density by differential centrifugation.

Relative Centrifugal Field (RCF)

This is the force applied to a particle which is in a centrifuge rotor, which is rotating about a central pivot at a given speed.

$$RCF(g) = 1.12 \times r \times (rpm/1000)^2$$

Where r = distance of particle from the centre of rotation and rpm is the speed of rotation in revolutions per minute. It follows that the *g* force is greater at the bottom of the tube than at the top and therefore particles near the bottom of the tube sediment faster than those at the top. This is one reason why some centrifuge rotors are designed to hold the centrifuge tube at a steep angle, to reduce the difference in *g* force at the top and bottom of the tube.

The rate of sedimentation of a particle (v) is given by the following equation:

Where r_p and ρ_p are, respectively, the radius and density of the particle, ρ_m the density of the medium, g is the centrifugal force and η is the viscosity of the liquid.

$$v = \frac{2 r_p^2 (\rho_p - \rho_m) g}{9\eta}$$

The rate at which a particle is sedimented is therefore dependent on:

(a) (The radius of the particle)^2 and is therefore related to the cross-sectional are of the organelle.

(b) The difference between the density of the organelle and the density of the medium in which the organelle is suspended. When the two densities are equal then the organelle will not sediment regardless of the g force applied and, indeed if the density of the medium is greater than that of the particle, then it will tend to float towards the top of the tube.

(c) The g force applied ~ proportional to $(rpm/1000)^2$.

(d) The sedimentation rate is inversely proportional to the viscosity of the medium. The higher the viscosity, the slower the rate of sedimentation.

Isolated organelles sediment at different rates related to their size and density (See Table 2)

Table 2. Organelles sedimented by centrifugation at increasing speeds

[1] 1,000 x g (10 min)
Unbroken cells, nuclei, plasma membrane sheets, heavy

mitochondria plus smaller, trapped particles.

[2] 3,000 x g (10 min)
Heavy mitochondria, plasma membrane fragments plus
smaller, trapped particles.

[3] 10,000 x g (20 min)
Mitochondria, lysosomes, peroxisome, some Golgi

membranes and rough endoplasmic reticulum.

[4] 100,000 x g (60 min)
Membrane vesicles derived from smooth and rough

endoplasmic reticulum, Golgi vesicles and plasma

membrane vesicles.

[5] 100,000 x g

Supernatant
Cytoplasmic components plus any soluble organelle

components released during homogenisation and

fractionation.

Characterisation of Organelles in Fractions

Organelles may be identified by their characteristic appearance under the electron microscope (see Molecular Cell Biology, Chapt. 5 Lodish *et al*.2000, 2004) or with fluorescent-tagged antibodies raised against specific organelle components or by measuring specific marker enzymes.

Marker Enzymes

Each organelle has a specific role to play in cellular function and it therefore follows that certain components (including proteins and enzymes) are only found associated with one organelle. This is not the case for all proteins as there is an increasing body of evidence that some proteins are translocated from one cellular compartment to another as part of the normal cell function. However there are certain enzymes that are easy to measure which are located primarily in a single type of organelle or cell compartment. These are known as marker enzymes. The genes for these marker enzymes also code for a leader or signal peptide which directs the particular protein into a specific cellular location. The assay of marker enzymes can be used to track the fate of a particular organelle during a fractionation procedure.

Let us examine how marker enzymes may be used to identify organelles, starting with a structure which is not an organelle as such but that does have very specific and important functions. i.e. the plasma membrane.

Plasma membrane

The plasma membrane has many essential functions, including transporting nutrients into the cell and removing waste products, preventing unwanted materials from entering the cell and preventing the loss of essential

metabolites as well as maintaining the intracellular ions, pH and osmotic pressure of the cytoplasm.

A good marker for the hepatic plasma membrane is 5’ nucleotidase:

$$AMP \rightarrow adenosine + Pi$$

This activity is located on the extracellular surface of the plasma membrane of the liver – its function here is not entirely clear.

Plasma membrane fractions are often prepared by homogenisation of tissue in dilute (1mM) $NaHCO_3$. depending on the degree of homogenisation of a tissue the marker enzyme tends to be located in large sheets of membrane fragments which tend to sediment at low speed (1,000 x g) along with nuclei, whole cells and large mitochondria. However this fraction may be sub-fractionated on a discontinouous sucrose gradient where the fraction suspended in 0.25M sucrose which is overlaid on 37% sucrose which, in turn, is overlaid on 57% sucrose and centrifuged at 75,000 x g for 16 h. The nuclei and whole cells sediment to the bottom of the tube, mitochondria accumulate at the interface of the 37% and 57% sucrose, while the plasma membrane accumulates at the interface of the 0.25 M sucrose and the 37% sucrose (Fig 1a). Much smaller, plasma membrane vesicles can also form on homogenisation which appear in the ‘microsomal’ or 100,000 x g pellet along with the endoplasmic reticulum. This fraction can also be sub-fractionated using the discontinous gradient described above with the plasma membrane vesicles accumulating at the 0.25 M sucrose / 37% sucrose interface.

Fig: Subfractionation of ‘nuclear’ pellet, containing plasma membrane fragments

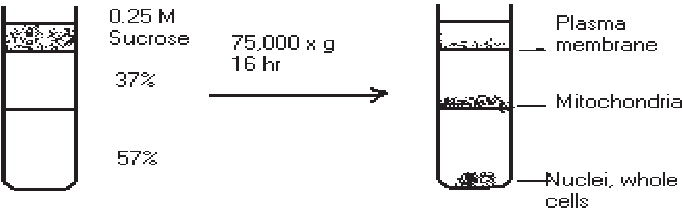
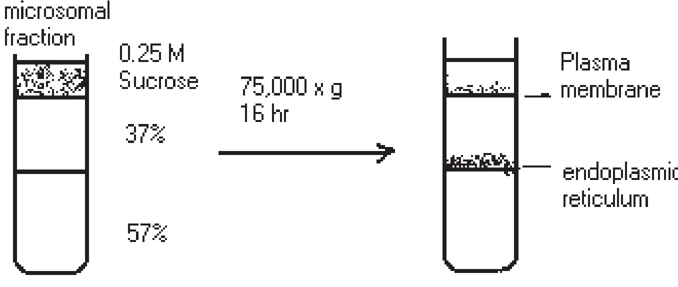


Fig: Subfractionation of microsomal pellet, containing plasma membrane vesicles



Why might you need to isolate pure plasma membrane preparations?

To study various aspects of plasma membrane function for example:

Hormone receptors: adrenergic, glucagon insulin receptors etc

Signal transduction components G-proteins, adenylate cyclase

Transport proteins Glut-2, Glut-4, Na^+K^+ATPase, Ion channel proteins

Can you think of any other functional components of plasma membranes?

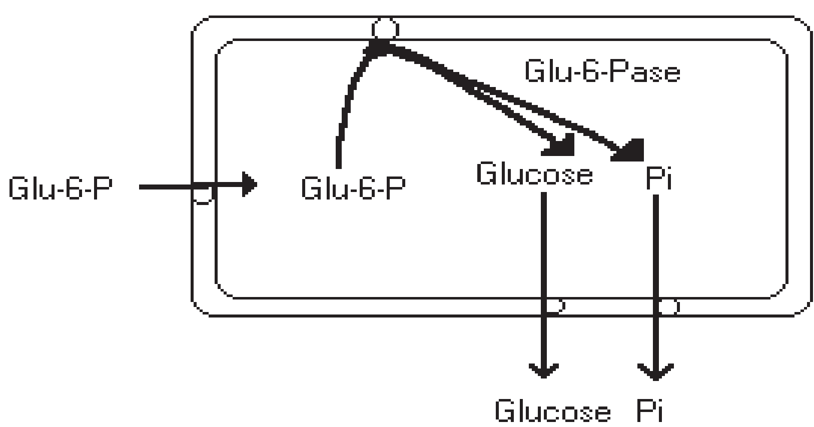
To show the plasma membrane localisation of these proteins you would need to show that they co-fractionate with the 5'nucleotidase. Having established that a protein is associated with a particuar fraction, it is possible to use this as a first step in a purification procedure. The next step is often the solubilization of the membrane-associated protein with a detergent.

Endoplasmic Reticulum

The ER is the network of channels for proteins targeted for specific modifications rather than cytosolic proteins. There are two types of ER visible under the electron microscope; the rough ER has ribosomes attached and is the site of protein synthesis while the smooth ER does not have ribosomes but has enzymes involved in lipid synthesis and steroid metabolism. A marker enzyme for liver ER is glucose-6-phosphatase which catalyses the following reaction:

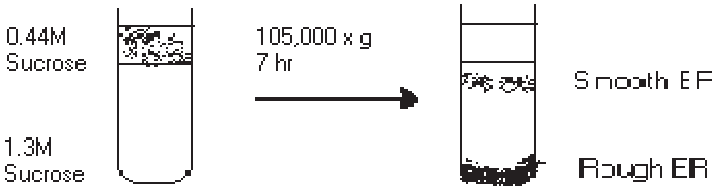
Glu-6-P →
Glucose + Pi

This enzyme plays a key role in the regulation of glucose output by liver and thus in control of blood glucose. It is the final enzyme of the gluconeogenic pathway and also in the mobilisation of glycogen in the liver. If the enzyme activity is determined in a microsomal fraction the activity is rather low unless a detergent is added. This is because the active site of the enzyme is on the inside of the microsomal vesicles. Glucose-6-phosphate uptake by the vesicles is rate limiting; there is a specific Glu-6-P transporter which is essential for the activity because otherwise the ER membrane is impermeable to the phosphorylated derivatives. The activity of Glu-6-phosphatase appears much greater when detergent is added to permeabilise the membrane. This phenomenon is known as LATENCY and this can also been shown for enzymes located in other organelles (e.g. lysosomes) when exposed to hydrophilic substrates.



Separation of Rough and Smooth Endoplasmic Reticulum

This is relatively easily done, a 10,000 x g, post-mitochondrial supernatant is prepared in 0.44 M sucrose and layered on to 1.3 M sucrose (44%, □ = 1.20) then centrifuged at 105,000 x g for 7 h. The rough microsomes sediment to the bottom of the tube while the smooth ER remains suspended at the top of the 1.3 M sucrose



B) Biochemical Techniques:

Origin of Chromotography

Chromatography was invented by the Russian botanist, Mikhail Semyonovich Tsvet, in the year 1901. Several procedures of chromatography havebecome popular since then.Chromatography is a multi-step, laboratory

process and is used to separate mixtures of various chemicals into their individual components. Its governing principle is that different chemicals in a mixture have different degrees of dissolving in a liquid or sticking to a solid surface. In simple words, it can identify a chemical and separate it from a dense mixture of other chemicals and depict it on a surface. The process through which constituents of a mixture are segregated and analyzed by physical means is broadly classified as adsorption and partition chromatography. In these processes, a stream of liquid, that is in mobile phase, is made to flow through a tube known as a column, and is packed with porous solid material, which is in the stationary phase. The sample of the mixture that is to be analyzed is then sent through the mobile phase, and as the mixture proceeds in the tube, the compounds are separated. This process is preferred over many other techniques, as it doesn't cause any molecular changes in the composition of the chemicals involved.

Applications

- Chromatography has evolved to be one of the most widely used chemical techniques to separate particles and contaminates in chemical plants. For example, in the chemical industries, pesticides and insecticides like DDT in groundwater, and PCBs (Polychlorinated biphenyls) are removed with the help of thin layer chromatography.
- In the field of organic chemistry and pharmacy, chiral compounds are very close to each other in terms of atomic or molecular weight, element composition, and the physical properties. However, they exist in two different forms, called the enantiomers and optical isomers. Both these compounds may appear to be same, but they have very different chemical properties. So, in pharmacy, chromatography becomes crucial to analyze the exact chiral compound so that correct medicines can be manufactured. For instance, a compound called thalidomide has two optical isomers, and one of the isomers can cause birth defect if a pregnant woman consumes it in the early stages of pregnancy. So, it is important to carefully separate these isomers.

- The chromatography technique can also be used to separate any amount of quantities ranging from micrograms in laboratories to tons in chemical plants.

- As a major testing tool, liquid chromatography is used by government agencies to separate toxic materials from drinking water and also to monitor air quality.

- It is also used by pharmaceutical companies to prepare large amounts of pure materials, that are further required in making medicines.

- Also, it is used to check the presence of any contamination in manufactured compounds and to detect harmful polychlorinated biphenyls (PCBs) that are present in fish.

- Paper chromatography is used as a technique to separate the additives, vitamins, preservatives, proteins, and amino acids.

- Gas and paper chromatography is also very popular in forensic science and is used in fiber analysis and DNA and RNA fingerprinting. It is also used to detect residue or chemicals that were present in case of fire or explosion, which in turn helps in solving different cases.

- Gas chromatography is also used to detect the presence of alcohol in blood and drugs or medications in urine.

- It is also used by scientists to study the relation of various mixtures with one another to purify different components or calculate the amount of mixture present in each specimen.

These were some of the various uses of chromatography. This technology has gained immense industrial popularity in the past few decades, as it can separate chemicals efficiently, separate chemicals that differ in their atomic orientations in space, and is cost-effective aswell.

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Electrophoresis

Electrophoresis may be the main technique for molecular separation in today's cell biology laboratory. Because it is such a powerful technique, and yet reasonably easy and inexpensive, it has become commonplace. In spite of the many physical arrangments for the apparatus, and regardless of the medium through which molecules are allowed to migrate, all electrophoretic separations depend upon the charge distribution of the molecules being separated.

Electrophoresis can be one dimensional (i.e. one plane of separation) or two dimensional. One dimensional electrophoresis is used for most routine protein and nucleic acid separations. Two dimensional separation of proteins is used for finger printing , and when properly constructed can be extremely accurate in resolving all of the proteins present within a cell (greater than 1,500).

The support medium for electrophoresis can be formed into a gel within a tube or it can be layered into flat sheets. The tubes are used for easy one dimensional separations (nearly anyone can make their own apparatus from inexpensive materials found in any lab), while the sheets have a larger surface area and are better for two- dimensional separations.

When the detergent SDS (sodium dodecyl sulfate) is used with proteins, all of the proteins become negatively charged by their attachment to the SDS anions. When separated on a polyacrylamide gel, the procedure is abbreviated as SDS--PAGE (for Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis). The technique has become a standard means for molecular weight determination.

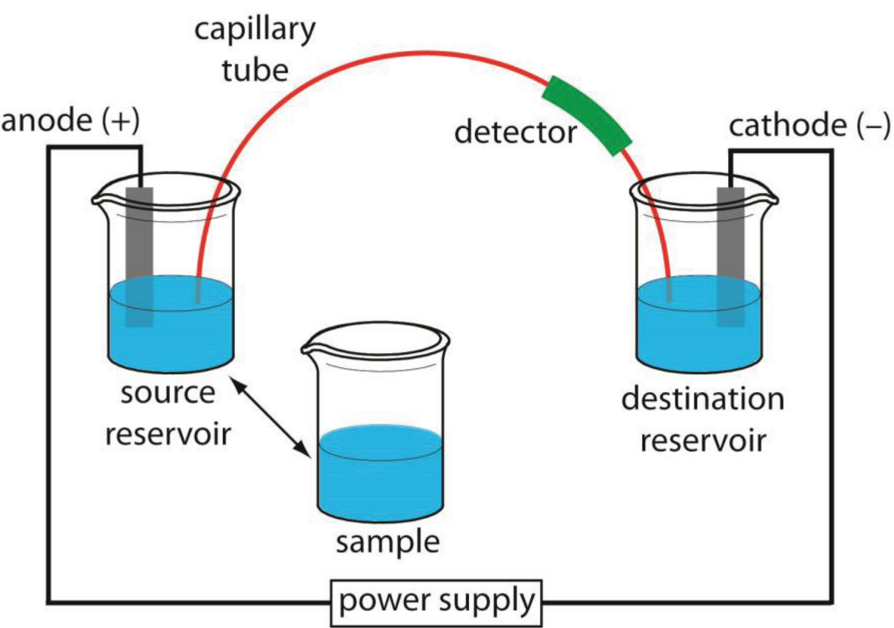
Polyacrylamide gels are formed from the polymerization of two compounds, acrylamide and N,N_1-methylene- bis-acrylamide (Bis, for short). Bis is a cross-linking agent for the gels. The polymerization is initiated by the addition of ammonium persulfate along with either β-dimethyl amino-propionitrile (DMAP) or N,N,N_1,N_1,- tetramethylethylenediamine (TEMED). The gels are neutral, hydrophillic, three-dimensional networks of long hydrocarbons crosslinked by methylene groups.

The separation of molecules within a gel is determined by the relative size of the pores formed within the gel. The pore size of a gel is determined by two factors, the total amount of acrylamide present (designated as %T) and the amount of cross-linker (%C). As the total amount of acrylamide increases, the pore size decreases. With cross- linking, 5%C gives the smallest pore size. Any increase or decrease in %C increases the pore size. Gels are designated as percent solutions and will have two necessary parameters. The total acrylamide is given as a % (w/v) of the acrylamide plus the bis-acrylamide. Thus, a 7 1/2 %T would indicate that there is a total of 7.5 gms of acrylamide and bis per 100 ml of gel. A gel designated as 7.5%T:5%C would have a total of 7.5% (w/v) acrylamide + bis, and the bis would be 5% of the total (with pure acrylamide composing the remaining 2.5%).

Proteins with molecular weights ranging from 10,000 to 1,000,000 may be separated with 7 1/2% acrylamide gels, while proteins with higher molecular weights require lower acrylamide gel concentrations. Conversely, gels up to 30% have been used to separate small polypeptides. The higher the gel concentration, the smaller the pore size of the gel and the better it will be able to separate smaller molecules. The percent gel to use depends on the molecular weight of the protein to be separated. Use 5% gels for proteins ranging from 60,000 to 200,000 daltons, 10% gels for a range of 16,000 to 70,000 daltons and 15% gels for a range of 12,000 to 45,000 daltons.

Cationic vs anionic systems

In electrophoresis, proteins are separated on the basis of charge, and the charge of a protein can be either + or -- , depending upon the pH of the buffer. In normal operation, a column of gel is partitioned into three sections, known as the Separating or Running Gel, the Stacking Gel and the Sample Gel. The sample gel may be eliminated and the sample introduced via a dense non-convective medium such as sucrose. Electrodes are attached to the ends of the column and an electric current passed through the partitioned gels. If the electrodes are arranged in such a way that the upper bath is -- (cathode), while the lower bath is + (anode), and -- anions are allowed to flow toward the anode, the system is known as an anionic system. Flow in the opposite direction, with + cations flowing to the cathode are a cationic system.



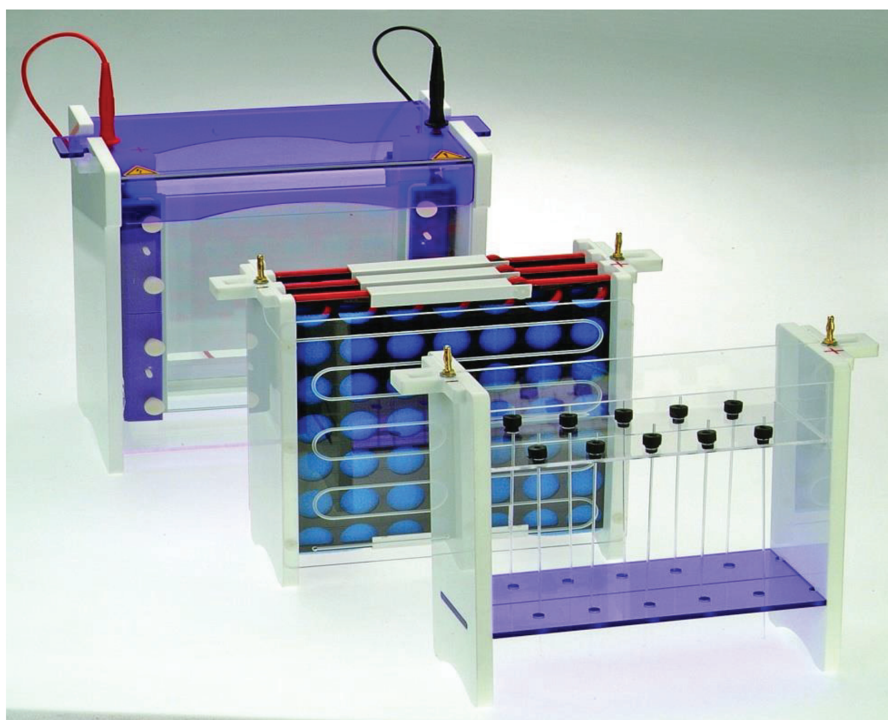
Tube vs Slab Systems

Two basic approaches have been used in the design of electrophoresis protocols. One, column electrophoresis, uses tubular gels formed in glass tubes, while the other, slab gel electrophoresis, uses flat gels formed between two plates of glass. Tube gels have an advantage in that the movement of molecules through the gels is less prone to lateral movement and thus there is a slightly improved resolution of the bands, particularly for proteins. It is also more economical, since it is relatively easy to construct homemade systems from materials on hand. However, slab gels have the advantage of allowing for two dimensional analyses, and of running multiple samples simultaneously in the same gel.

Slab gels are designed with multiple lanes set up such that samples run in parallel. The size and number of the lanes can be varied and, since the samples run in the same medium, there is less likelihood of sample variation due to minor changes in the gel structure. Slab gels are unquestionably the the technique of choice for any blot analyses and for autoradiographic

analysis. Consequently, for laboratories performing routine nucleic acid analyses, and those employing antigenic controls, slab gels have become standard. The availability of reasonably priced commercial slab gel units has increased the use of slab gel systems, and the use of tube gels is becoming rare.

The theory and operation of slab gel electrophoresis is identical to tube gel electrophoresis. Which system is used depends more on the experience of the investigator than on any other factor, and the availability of equipment.



Continuous vs discontinuous gel systems

The original use of gels as separating media involved using a single gel with a uniform pH throughout. Molecules were separated on the basis of their mobility through a single gel matrix. This system has only occasional use in today's laboratory. It has been replaced with discontinous, multiple gel systems. In multiple gel systems, a separating gel is augmented with a stacking gel and an optional sample gel. These gels can have different

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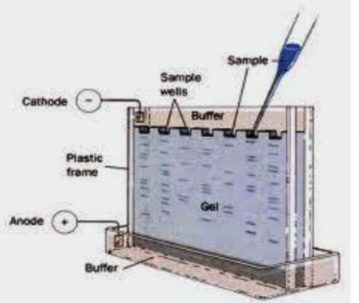
concentrations of the same support media, or may be completely different agents. The key difference is how the molecules separate when they enter the separating gel. The proteins in the sample gel will concentrate into a small zone in the stacking gel before entering the separating gel. The zone within the stacking gel can range in thickness from a few microns to a full millimeter. As the proteins are stacked in concentrated bands, they continue to migrate into the separating gel in concentrated narrow bands. The bands then are separated from each other on a discontinuous (i.e. disc) pH gel.

Once the protein bands enter the separating gel, separation of the bands is enhanced by ions passing through the gel column in pairs. Each ioin in the pair has the same charge polarity as the protein (usually negative), but differ in charge magnitude. One ion will have a much greater charge magnitude than the proteins, while the other has a lesser charge magnitude than the proteins. The ion having a greater charge will move faster and is thus the leading ion, while the ion with the lesser charge will be the trailing ion. When an anionic system is employed, the Cl^- and glycinate (glycine as its acid derivative) ions are derived from the reservoir buffer (Tris-Glycine). The leading ion is usually Cl^- glycinate is the trailing ion. Chloride ions enter the separating gel first and rapidly move down the gel, followed by the proteins and then the glycinate ions. The glycinate ions overtake the proteins and ultimately establish a uniform linear voltage gradient within the gel. The proteins then sort themselves within this gradient according to their charge

Continuous - discontinuous gel systems



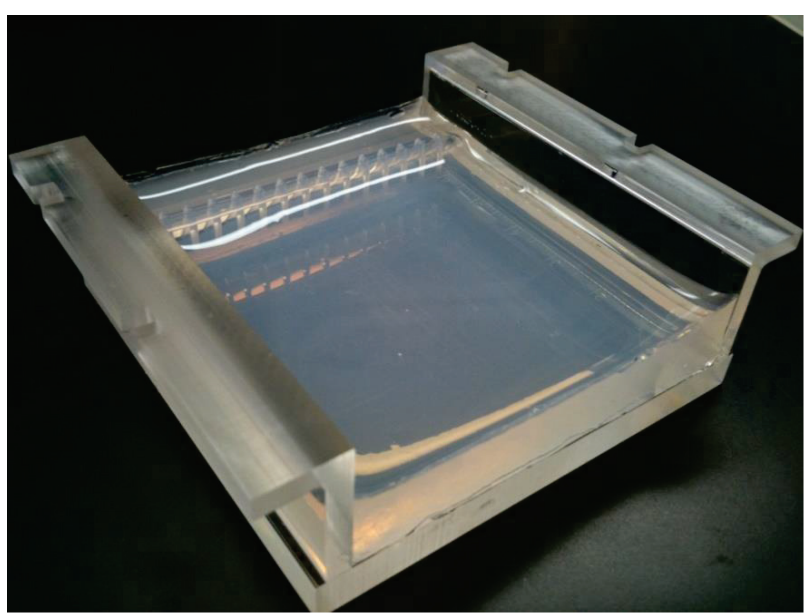
- **Continuous system**--gel and tank buffers are the same, single phase gel; examples are PAGE, agarose, and starch gels.
- **Discontinuous system**--gel and tank buffers are different, two phase gel (stacking gel); example is PAGE.

and size.

Agarose Gels

While acrylamide gels have become the standard for protein analysis, they are less suitable for extremely high molecular weight nucleic acids (above 200,000 daltons). In order to properly separate these large molecules, the acrylamide concentration needs to be reduced to a level where it remains liquid.

The gels can be formed, however, by the addition of agarose, a naturally linear polysaccharide, to the low concentration of acrylamide. With the addition of agarose, acrylamide concentrations of 0.5% can be used and molecular weights of up to 3.5 x 10_6 daltons can be separated. This is particularly useful for the separation of large sequences of DNA. Consequently, agarose-acrylamide gels are used extensively in today's genetic laboratories for the determination of gene maps.



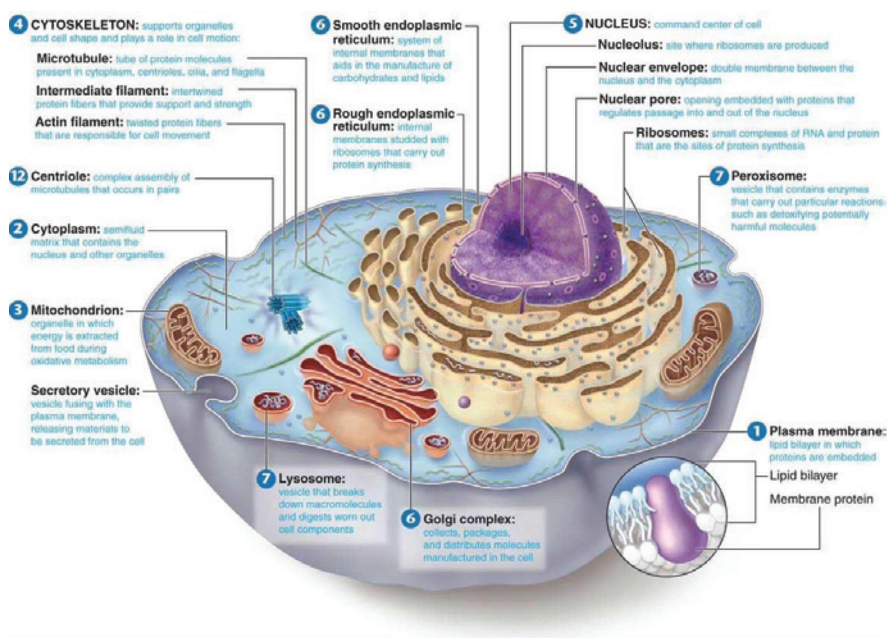
UNIT IV

CELL ULTRASTRUCTURE

Animal cell

Introduction

Cell is a structural and functional unit of all living organisms, except viruses. Cells show a large variation. But all the cells belonging to a single tissue are generally similar in size and shape. Cells may be spherical, cylindrical, rod-shaped, hexagonal cylindrical or of irregular shape. Red blood corpuscles (RBCs) have a depressed central area and resemble a 'balushahi' in shape. The size of cells ranges from 0.2 to 5.0u (um, microns or micrometers) in bacteria to up to 75 mm in the case of ostrich eggs (without shell). But most of the cells are cylinders of 15-30u in diameter.



The number of cells in an individual may range from one (bacteria, protozoa etc.) through $6*10^{12}$ (human beings) to even a larger number in animals like whales and elephants. The following structures are visible in a

cell with the aid of light microscope: cell wall, cytoplasm, nucleus, chloroplast and mitochondria. However, studies with electron microscope have revealed many more structures within a eukaryotic cell. The various structures seen in plant cells are

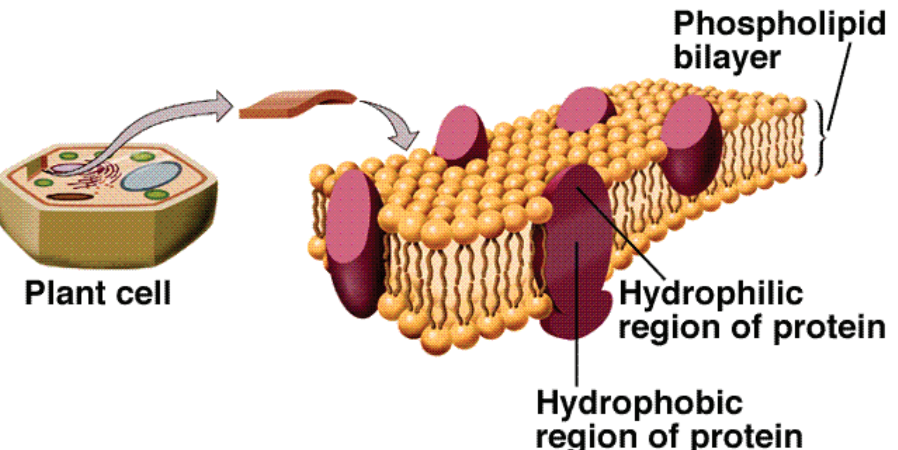
1. Cell wall
2. Plasma lemma
3. Endoplasmic reticulum (ER)
4. Ribosomes
5. Golgi bodies
6. Lysosomes
7. Spherosomes
8. Chloroplasts
9. Mitochondria etc. and
10. Nucleus

Animal cells lack cell wall and chloroplasts, while centrioles are not found in plant cells. Further, RBCs in animals are devoid of nuclei.

Plasma lemma/Plasma membrane:

The membrane in closing cytoplasm of a cell is known as **plasma lemma or plasma** membrane. Plasma lemma is composed of lipids and proteins. The average composition of plasma lemma is as follows: protein 40%, and lipids 60%, the major potion of lipids consists of phospholipids e.g lecithin, cephalin, sphingomyelin etc. in addition it contains some other types of lipids such as cholesterol, cerebroside and ganglioside, as well as some polysaccharides. The remaining potion of the lipid component is made up of triglycerides or the common fats.

Three distinct layers are seen in the electron micrographs of plasma lemma of tissues fixed with osmium tetra-oxides. Two of these layers are relatively dense and osmiophinic in nature; each of them is about 20 A thick. The two osmiophinic layers are separated by a relatively light osmiophobic layer of about 35 A thicknesses. These three (two osmiophilic and one osmiophobic) layers together are known as a **unit membrane.**This term was coined by Robertson. The average thickness of a unit membrane is about 75 A.



It is probable that the plasma lemma may contain pores of about 10 A diameters, or at least it functions as if it has pores of 10 A diameters. The structure of other membranes found in a cell is similar to that of plasma lemma.

The arrangements and orientations of lipid and protein molecules in the unit membrane are not clearly understood. In 1935, davson and danieli proposed the **lipid-protein bilayer** model of membrane. According to this model, two layers of lipid molecules are arranged in the center unit of a unit membrane, each layer being being only one molecule thick.

The polar ends of all lipid molecules in a layer are oriented towards the outside, while their non-polar ends are situated towards the center of the membrane. On the outside of each of the two layers of lipid molecules, a layer of protein molecules of one molecule thickness is located. This model agrees well with the unit membrane structure seen in electron micrographs. However, many properties of plasma lemma and other biological membranes are difficult to explain according to this simple model.

A more flexible model, referred to as fluid-mosaic model, has been proposed to account for the various properties of biological membranes. According to this model, membranes are made up of two layers of lipid molecules (lipid bilayer), each layer being only one molecule thick.

The lipid molecules are arranged in the same manner as in the davson-danieli or lipid-protein bilayer model, that is, the polar ends of lipid molecules are oriented towards the outside of the membrane, while the non-polar ends face inside. The protein molecules are embedded either fully or partially in the lipid bilayer (instead of being arranged on the outside of the lipid bilayer, as proposed in the davson-danieli model.

The chief function of plasma lemma is to regulate the movement of various molecules into and out of the cytoplasm. Smaller molecules, like water, pass through the plasma lemma more easily than relatively larger molecules. Similarly, lipid soluble molecules pass through the plasma lemma more readily than lipid insoluble ones. Solid particles and some solutes enter the cytoplasm through phagocytosis and pinocytosis, respectively.

Cellular wastes are excreted out of the cell by the process in which the sequence of events is essentially the opposite of that in pinocytosis and phagocytosis. Vesicles containing waste materials migrate to the plasma lemma, and their membranes fuse with plasma lemma at the point of contact. Eventually, the fused potion of the two membranes is disrupted as a result of which the vesicles become integrated into the plasma lemma.

In addition to the passive movement of molecules across plasma lemma, some ions are transported across plasma lemma by means of active transport. Active transport of ions is dependent on the energy provided by ATP (adenosine triphosphate). $Ca2+$ and $Mg2+$ ions are transported into the cytoplasm by means of active transport; as a result, these ions can be accumulated in the cytoplasm in much higher concentrations than those in the outside medium in which the cell is present.

Cytoplasm

The substance, except nucleus, surrounded by the plasma lemma is known as **cytoplasm.** Electron micrographs reveal a number of membranous and other structures in the cytoplasm. The cytoplasm minus these structures is known as **hyaloplasm**. The cytoplasm may contain the following structures: (1) endoplasmic reticulum (ER), (2) ribosomes, (3)golgi bodies, (4) lysososmes, (5) spherosomes, (6) vacuoles, (7) centrioles (in animals only), (8) microtubules, (9) mitochondria, and (10) plastids (in green plants only). Of these, mitochondria and plastids contain DNA (the genetic material).

Endoplasmic Reticulum

The cytoplasm contains an extensive network of membrane-enclosed spaces: these spaces along with the membranes enclosing them are known as **endoplasmic reticulum (ER)**. ER consists of three types of elements:

1) vesicles of 25-500 mu diameter
2) tubules of 50-100 mu diameter and
3) 40-50 mu thick cisterns of variable length and width

The tubules may or may not be extensively branched, and the cisterns may or may not be connected with each other. ER is generally present in differentiated cells; it is not found in RBCs, eggs, undifferentiated cells and prokaryotic cells. ER is connected with plasma lemma as well as with nuclear envelope. It is believed that ER originates from the outer membrane of nuclear envelope. The ultrastructure of ER membranes is the same as that of a unit membrane, that is, it has two osmiophilic layers separated by an osmiophobic layer.

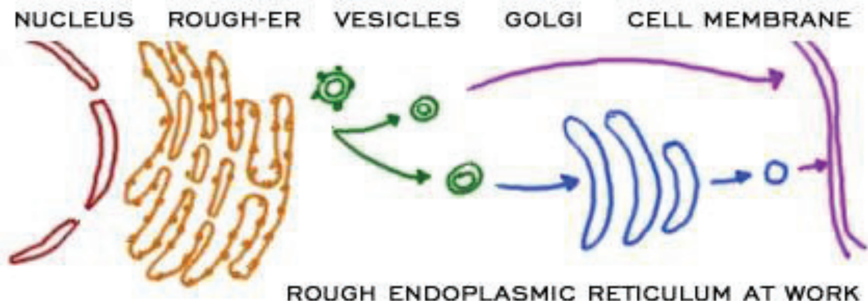
ER is grouped into the following two categories:

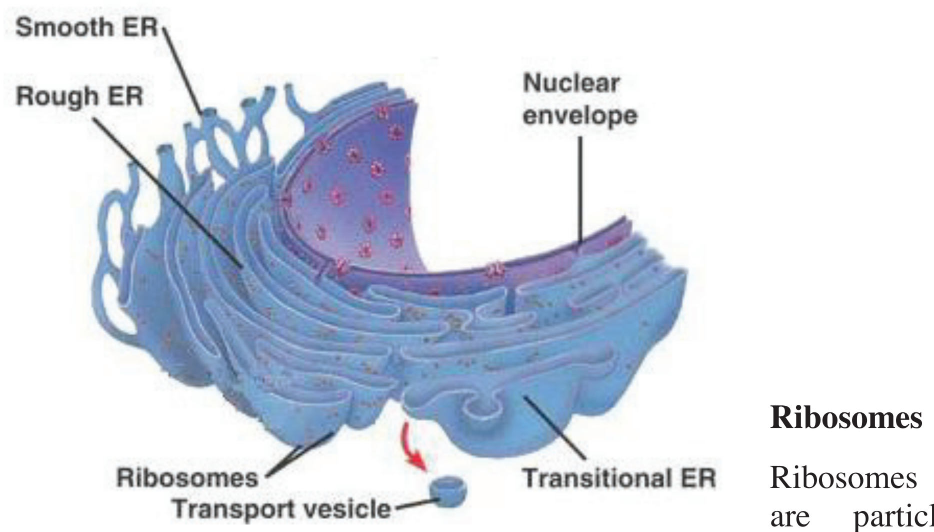
1) Smooth ER and
2) Rough ER.

In the **smooth ER** elements, both outer and inner surfaces are regular and smooth. In those cells where little or no protein synthesis takes places, only smooth ER is found. In **rough ER** elements, the outer surfaces of membranes have a rough appearance due to the attachments of ribosomes. Rough ER is mainly composed of cisterns (membrane enclosed plate-like elements) and is found in cells actively involved in protein synthesis. Smooth and rough ER change into each other as per the needs of a cell.

The main functions of ER are as follows: (l) it provides the structural base for protein (rough ER), lipid and phospholipid (both rough and smooth ER) syntheses; with (2) it provides the channel for the transport of materials synthesized in association with ER to the various parts of a cell and even outside the cell; (3) it provides a controlled passage for the export of mRNA (messenger RNA) molecules from nucleus to rough ER, and (4) several

enzyme molecules, e.g., glucose-6-phosphatase , ATPase etc., are embedded in the membranes of ER.





Ribosomes

Ribosomes are particles of about 200 A in diameter; they are composed of RNA and protein. Generally ribosomes are attached to the outer faces of ER membranes; this converts smooth ER elements into rough ER. But some ribosomes are present in the hyaloplasm unattached to any membrane. On the basis of the sedimentation rate, ribosomes are classified into the following three groups:

(1) 80 S size animal ribosomes,

(2) 80 S plant ribosomes, and

(3) 70 S eukaryotic organelle (mitochondria and plastid) and prokaryotic ribosomes.

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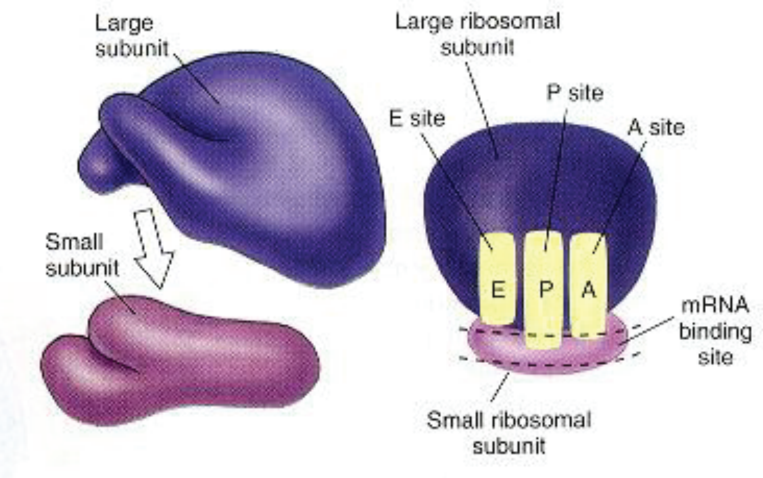


Ribosome Subunits

The smaller subunit fits into a depression on the surface of the larger one. The A, P, and E sites on the ribosome play key roles in protein synthesis.

In the conditions of low Mg^{2+} concentration, each 70 S prokaryotic ribosome dissociates into 50 S and 30 S subunits. The smaller subunit of ribosome is placed onto the larger subunit much like a cap-on-the-head (Fig. 2.6). These ribosomes have 37% protein and 63% RNA.

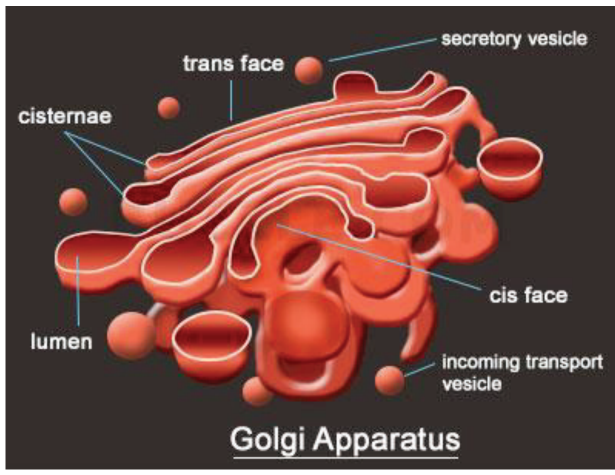
In E. coli, each 50 S larger subunit has one molecule each of a 23 S and a 5 S (about 120 nucleotides) RNA, while the 30 S smaller subunit has one molecule of 16 S RNA (about 1,600 nucleotides). The larger subunit has 34 different proteins (L_1, to L_{34}), while the smaller subunit has 21 different proteins (S_1, to S_{21}). Detailed three dimensional models of ribosomes have been proposed but their description is beyond the scope of this book.

Ribosomes are synthesized and assembled in nucleolus (within the nucleus) from where they migrate into the cytoplasm. Ribosomes are essential for protein synthesis as mRNAs can support protein synthesis only when they are attached to ribosomes.

Golgi bodies

It consists of 2-7 flat cisternae stacked close to each other. A network of 300-500 /3.Tubules emerges from around the margins of the cisternae (Fig. 2.7). In addition, vesicles of 200-800 A diameters are also present on the

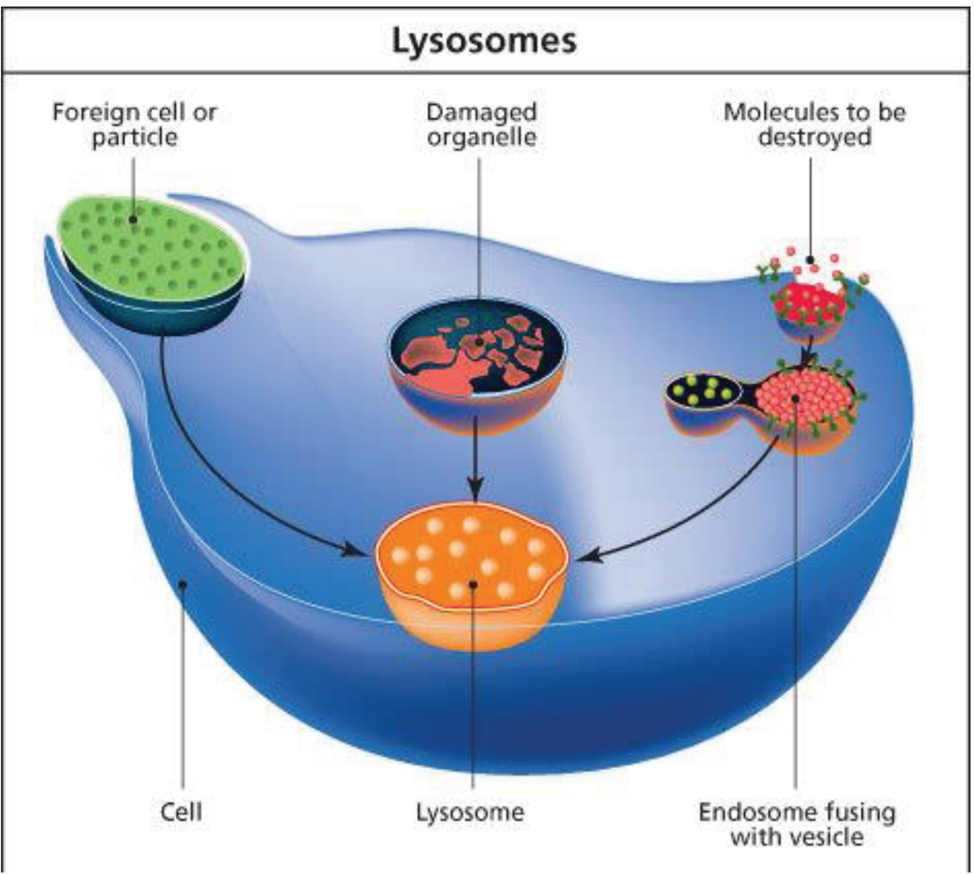
margins of the cisternae. The number of Golgi bodies in a cell depends on the synthetic activity of the cell. In a synthetically active cell, several well differentiated and developed Golgi bodies are present, while synthetically inactive cells have few poorly developed Golgi bodies. Golgi bodies originate from ER elements.



It is believed that materials synthesized in association with ER (proteins, lipids, phospholipids, etc.) are transported to Golgi bodies, where they are packed into vesicles cut off from them Thus Golgi bodies function as packaging plants of the cell.

Lysosomes

Lysosomes are vesicles of 400-800 mus and contain several hydrolytic enzymes. The main enzyme present in lysosomes is acid phosphatase; other enzymes are Acid DNAase, acid RNAase, B-galactosidase, etc. The membrane surrounding a lysosome has a unit membrane organization



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Lysosomes are of the following two types:

1) primary and
2) Secondary lysosomes.

Primary lysosomes

Primary lysosomes are produced by Golgi bodies and contain hydrolytic enzymes only. They fuse with food vacuoles produced through phagocytosis and pinocytosis to generate secondary lysosomes.

Secondary lysosomes

Secondary lysosomes contain both hydrolytic enzymes as well as food materials. The food particles are ultimately digested by the hydrolytic enzymes and absorbed into the hyaloplasm, while the undigested portion of

food materials remains in the secondary lysosomes; such lysosomes are known as residual bodies.

The enzymes present in the lysosomes are capable of digesting any living organism. When a white blood cell (WBC) ingests (by phagocytosis) a bacterium or some l some other organism, all the lysosomes of the WBC fuse with the food vacuole thus produced. As a result, the bacterium as well as the WBC itself is lysed. In some situations, the enzymes present in lysosomes are released into the cytoplasm, which leads to the lysis of concerned cell **(autolysis).** Thus the function of lysosomes is digestion (lysis) of food particles and microorganisms ingested by a cell and also to cause autolysis of the cell, if required.

Spherosome

Spherosome are vesicles of 0.5-1u diameter and contain, upto 98%, lipid; they. Also contain some acid phosphatase. Spherosomes are found in plant cells only; they do not occur in animal and prokaryotic cells. Spherosomes are surrounded by a single unit membrane. According to some scientists, spherosomes function in lipid storage, but some others do not agree with this view.

Vacuole

Plant cells have one or more vacuoles of variable size. In mature and differentiated cells, the major part of cytoplasm is occupied by a large vacuole, and the cytoplasm is pushed to the periphery of cells.

The material contained in the vacuoles is referred to as **cell sap**. Cell sap is relatively less dense than the surrounding cytoplasm. Cell sap contains sugars, salts, proteins, phenols etc. as well as some specific pigments, e. g, anthocyanin. Generally, vacuoles are surrounded by u unit membrane; this membrane is referred to as **tonoplast**. Tonoplasts exhibit differences in permeability as compared to the plasma lemma.

Centriole

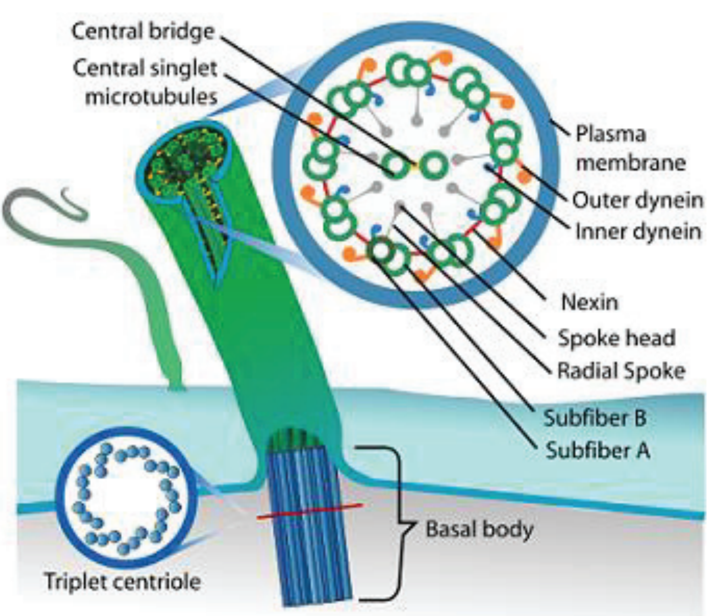
Centrioles are cylindrical structure of about 1200-1500 A in diameter and of about 3.000-5,000 A in length. They are always present in pairs, one centriole being oriented vertical to the other (Fig. 2.8). Centrioles are confined to animal cells, and are not found in plant cells. However, basal

bodies found at the base of flagella of plant cells are identical with centrioles in their ultrastructure. Generally, new centrioles arise in association with preexisting centrioles; they organize at an interval from and at right angles to the two preexisting centrioles in a cell.

But at least in some cases, preexisting centrioles are not essential for the organization of new centrioles. Electron micrographs of transverse sections of centrioles reveal nine fibrils arranged at the periphery of a centriole.

Each fibril is composed of three microfibris arranged in a single plane so that each tibril has it that topography. The fibrils of a centriole are oriented at an angle on the periphery. The inner microfibril of each of the nine fibrils is connected with the outer microfibril of a neighboring fibril; at the same time, it is also connected with the microfibril located in the center of the centriole. Thus the transverse ultrastructure of a centriole is somewhat similar to the structure of the wheel of a bullock-cart.

In animal cells, centrioles are involved in the organization of spindle apparatus. In fact, a pair of centrioles lies at each of the two poles in a cell from which spindle fibres radiate toward the equatorial plate. In both plant and animal cells, centrioles serve as the basal bodies of flagella.



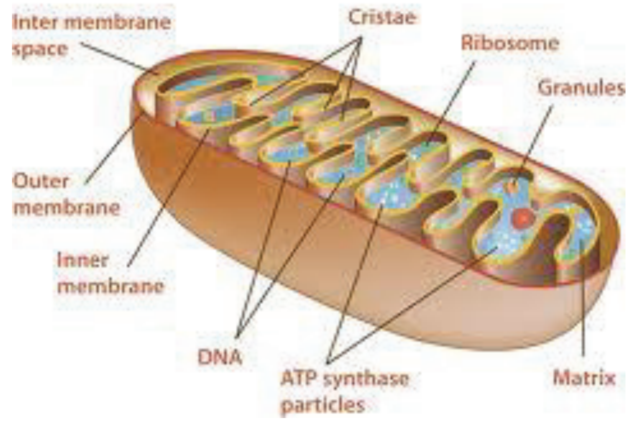
Microtubules

Microtubules are tubules of 150-160 A in diameter their wall is about 45-70 A in thickness. In plants, the walls of microtubules are composed of 13 fibres made up of protein molecules. It is likely that lipid molecules provide stability to the structure of microtubule walls. In centrioles and flagella, each microfibril is essentially a microtubule. Microtubules also constitute the fibres of spindle apparatus.

Microtubules are primarily found in dividing cells in the form of spindle fibres, and are responsible for chromosome movement during cell division. But they are also found it non-dividing cells, and provide a degree of stability to the various structures present in the cytoplasm.

Mitochondria

The term mitochondria (mitos = thread + chondrion = granule) was first used by Benda in 1897, but they were first seen about 20 years earlier. Mitochondria are cylindrical bodies with an average diameter of 0.2 to l u and ordinarily 3-10 u in length. An average cell may have 200 to 800 mitochondria. But in some protozoa, e.g., Chaos chaos, there may be as many as 500,000 mitochondria in a cell. The average composition of mitochondria is as follows: protein, 70%; lipids 25-30%; RNA, approximately 1%; and DNA, less than l%.



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Mitochondria are surrounded by two concentric unit membranes. The outer membrane is about 60 A thick and regular in outline. The inner membrane is situated about 20-60 A away from the outer one, is about 60 A thick, and is infolded at several places. The in-folds of inner membrane are known as cristae. Each Crista is about 140-180 A in thickness. The space outside the cristae, i.e., on the inside of the inner membrane, is known as matrix (Fig. 2.1). In electron micrographs, mitochondrial membranes may show one of the following three ultrastructures: (1) typical unit membrane organization, (2) cross-bar ultrastructure, and (3) fur-coated structure. **The unitmembrane ultrastructure** consists of two typical layers separated by an osmiophilic layer (Fig. 2.9b). In the cross-bar ultrastructure, numerous Osmiophobic spheres of 40-50 A diametre are embeded in an osmophiliclayes of 75- 80 A thickness. This gives the impression as if the two osmiophobic layers of a unit membrane are connected with each other by several osmiophilic cross-bars; this structure resembles a metal or bamboo ladder. The **fur-coated ultrastructure** is obtained by negative staining of crista membranes with agents like phosphotungstic acid. A large number of particles of 75-100 A in diameter are attached to the outer surface of cirsta membranes by means of 45-50 A long stalks. It is not clear which of the three organizations is of common occurrence.

The outer membrane is considerably more permeable than the inner membrane. The inner membrane is composed of numerous identical units referred to as oxysomes. Each oxysome is capable of complete electron transport to O_2 as well as coupled oxidative phosphorylation. Clearly, the components of electron transport and coupled phosphorylation are integral parts of the inner membrane.

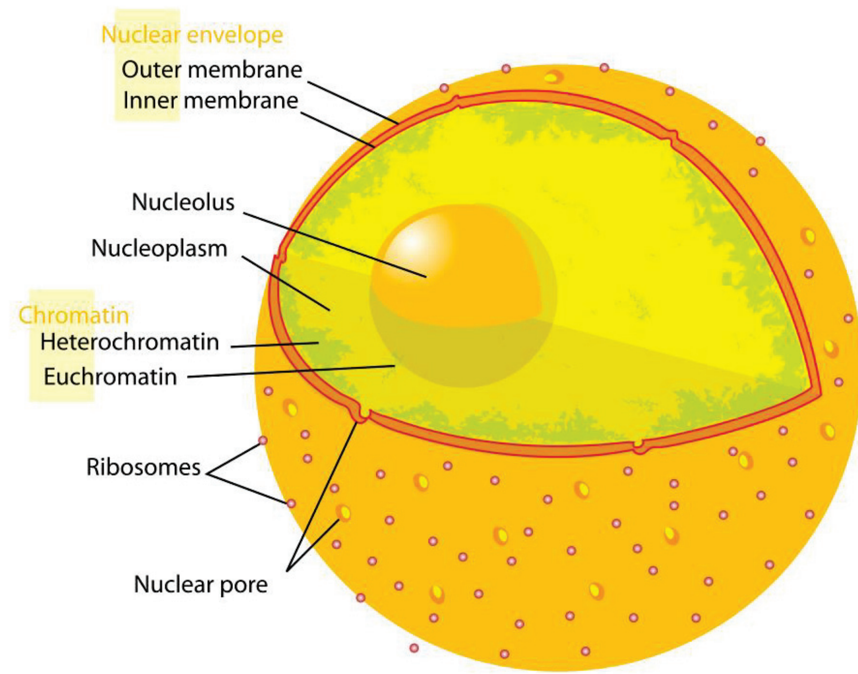
Mitochondria arise by fission of preexisting mitochondria. Growth and fission of mitochondria has been filmed by time-lapse photography of living cells. On the other hand, two or more smaller mitochondria may fuse together to produce a larger one.

Each mitochondrion contains several copies of ring-shaped DNA molecule. Mitochondria contain ribosomes, which are distinct from those present in the cytoplasm; they along with ribosomes of chloroplasts appear to constitute a distinct category of ribosomes (organelle ribosomes).

Mitochondrial matrix contains enzymes for the oxidation of amino acids and fatty acids, and the enzymes of Kreb's cycle. The enzymes of electron transport and oxidative phosphorylation are located in the inner membrane. Thus the main function of mitochondria is the oxidation of carbohydrates, amino acids and fatty acids. They produce ATP by utilizing the energy obtained from these oxidations. This ATP provides the necessary energy for various biochemical reactions of cells. Since mitochondria contain DNA, they also contribute to heredity by way of cytoplasmic inheritance.

Nucleus

Nuclei take up relatively deep stain with basic dyes, and are generally spherical in shape. But in differentiated tissues, they may exhibit considerable variation in shape. The size of nuclei varies with the physiological state and the degree of differentiation of the cells. In physiologically active and undifferentiated cells, nuclei are relatively larger in size, while in physiologically inactive and differentiated cells they are relatively smaller.



Nuclear size is also affected by the chromosome number (or the amount of DNA) of a species, as well as by the ploidy level of a cell or individual. In general, a cell contains a single nucleus, but in some tissues, e. g., early stages of endosperm development, and in some organisms, e. g. some protozoa, some fungi etc., two or more nuclei are present within a single cell. In contrast, some specialized cells, e. g., RBCs and companion cells of phloem, do not contain any nucleus.

Nucleus is the store-house of almost all the genetic information needed for the functioning of a cell/organism. It produces ribosomes, tRNA (transfer RNA), mRNA (messenger RNA) and certain other RNAs, which produce the various structural and enzymatic proteins. In essence, nucleus governs the development of almost all the traits of an organism by providing the information necessary for the synthesis of various structural enzymatic and regulator proteins.

The significance of nuclei in development was first demonstrated by a German scientist, Hammerling in 1934, through transplantation studies in the unicellular alga Acetabularia. He clearly demonstrated that the development of characters in this alga was governed by the nucleus and not by the cytoplasm

Pea nuclei contain 14% DNA, 12% RNA and 74% protein on dry weight basis. In electron micrographs, the following three distinct types of structures are discernible in interphase nuclei: (1) nuclear envelope, (2) nucleolus, and (3) chromatin fibers.

Nuclear Envelope

Nucleus is enclosed by two concentric membranes, each being 70-80 A in thickness. There is a space of about 200-300 A between the two membranes; this space is known as the **perinuclearspace**. The two membranes, together with the perinuclear space, are known as **nuclearenvelope**. In the nuclear envelope, **nuclearpores** of 200-400 A in diameter are arranged in a hexagonal pattern at an interval of 800 A from each other. In fact, **nuclearpores** or **annuli** are not real pores in that the membrane is continuous throughout the area of an annulus.

The two membranes of nuclear envelope are fused with each other at the peripheri of each annulus. At the peripheri of an annulus, eight annular granules are embedded in each of the two nuclear membranes. In the center of the annulus, another annular granule is located. The annuli provide the main channel for transport of materials from and into the nucleus.

Nucleolus

Interphase nuclei contain relatively dense sphericle bodies called **Nucleous**. Generally, nucleoli disappear during cell division, in the late prophase of mitosis and meiosis, and the nucleolar material is distributed onto the chromosomes. At the end of cell division, the nucleolar materials dissociate from chromosomes and fuse together and become organized into a new nucleolus.

Nucleolus is ordinarily attached to a specific region (**nucleolus organizer region**) of a specific chromosome (**nucleolus organizer chromosome**). At mitotic metaphase, the nucleolus organizer region generally appears as an additional constriction or even as a gap; this region therefore is known as **secondary constriction**. The part of chromosome beyond the secondary constriction appears as a satellite; such chromosomes, therefore, are often referred to as **satellite chromosomes or sat-chromosomes.**

Ordinarily, two nucleoli are present in an interphase nucleus, but during meiotic prophase a Single nucleolus can be seen (since the two homologous nucleolus organizer chromosomes pair to form a single bivalent). But in certain special situations, one nucleus may contain more than two nucleoli.

The average chemical composition of nucleoli is as follows: protein, 70%; RNA, 20%; and a small amount of DNA. The ultrastructure of nucleoli reveals the following four regions: (1) amorphous region, (2) granular region, (3) fibrillary region, and (4) chromatin fibers. The central region of a nucleolus is amorphous and is composed mainly of proteins. The amorphous region is surrounded by nucleolonema; this consists of a *granular portion* mostly containing ribosomes of 150-200 A diameters, and a **fibrillar region** primarily containing RNA fibers of 50-150 A diameters. In addition, chromatin fibers from the nucleolarorganiser region also pass through nucleoli.

The main function of nucleoli is the production and organization of ribosomes. Ribosomal RNA (rRNA) is produced by genes located in the nucleolar organizer regions of sat-chromosomes.

Chromatin fibers

In transverse sections, transversely and obliquely-cut ends of chromatin fibers are seen throughout the interphase nucleus, except in the major part of nucleolus, the average diameter of chromatin fibers is about 300 13. In interphase nuclei, chromatin fibers are relatively much more condensed in heterochromatin than those in euchromatin. As a result, heterochromatic regions appear much denser than euchromatic regions.

During interphase, one end (telomore) of each chromosome is attached at the peripheri of an annulus. The rest of the chromosome dangles free in the nucleus in a highly, but loosely, folded state (Fig. 2. 12B). Thus interphase nuclei are filled with a mass of folded chromatin fibers. Chromatin fibers contain about 40% DNA, 55% protein and 4-5% RNA. They are the basic units of chromosome structure. They are also the fundamental basis of inheritance since they contain the genetic material, *viz*., DNA.

UNIT V

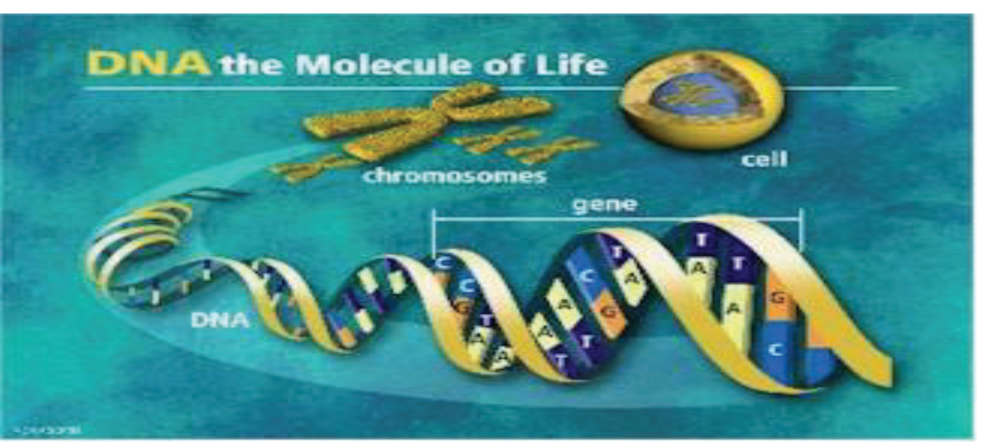
DNA STRUCTURE

Each species of living organism has a unique set of inherited characteristics that makes it different from other species. Each species has its own developmental plan—often described as a sort of "blueprint" for building the organism— which is encoded in the DNA molecules present in its cells. This developmental plan determines the characteristics that are inherited.

Because organisms in the same species share the same developmental plan, organisms that are members of the same species usually resemble one another, although some notable exceptions usually are differences between males and females. For example, it is easy to distinguish a human being from a chimpanzee or a gorilla. A human being habitually stands upright and has long legs, relatively little body hair, a large brain, and a flat face with a prominent nose jutting chin, distinct lips, and small teeth.

All of these traits are inherited—part of our developmental plan—and help set us apart as Homo sapiens.

Given the advances of *molecular,* or modern, genetics, it is possible to study differences between species through the comparison and analysis of the DNA itself. There is no fundamental distinction between classical and molecular genetics. They are different and complementary ways of studying the same thing: the function of the genetic material. In this book we include many examples showing how molecular and classical genetics can be used in combination to enhance the power of genetic analysis.



KEY NOTES

Nucleotides

DNA is a polymer containing chains of nucleotide monomers. Nucleotide contains a sugar, a base and a phosphate group. The sugar is 2'-deoxyribose which has five carbons named 1' (prime) 2' etc. There are four types of base: adenine and guanine have two carbon-nitrogen rings and are purines while thymine and cytosine have a single ring and are pyrimidines. The bases are attached to the 1' carbon of the deoxyribose. A sugar plus a base is termed a nucleoside. A nucleotide has one, two or three phosphate groups attached to the 5' carbon of the sugar. Nucleotides occur as individual molecules or polymerized as DNA or RNA.

DNA Polynucleotides

Nucleotide triphosphates of the four bases are joined to form DNA polynucleotide chains. Two phosphates are lost duringpolymerization and the nucleotides are joined by the remaining phosphate. A phosphodiester bond forms between the 5' phosphate of one nucleotide and the 3' hydroxyl of the next nucleotide. The polynucleotide has a free 5' phosphate at one end (5' end) and a free 3'OH (3' end) at the other end. The sequence of bases encodes the generic information. It can be read 5'-3' or 3' -5'.

Polynucleotides are extremely long. It is possible to have 4n different sequences.

The double helix

DNA molecules are composed of two polynucleotide strands wrapped around each other to form a double helix. The sugar- phosphate part of the molecule forms a backbone. The bases face inwards and are stack on top of each other. The two polynucleotide chains run in opposite directions. The double helix is right-handed and executives a turn every 10 bases. The helix has a major groove which mediates interactions with proteins. Variant DNA structures have been identified including Z DNA which has a left- handed helix.

Complementary base pairing

Hydrogen bonds between bases on the two DNA strands stabilize the double helix. The available space between the strands restricts the bases that can interact such that a purine always interacts with a pyrimidine. Thus, A interacts only with T and G only with C. This is called complementary base pairing. The restriction on base pairing means that the sequences of bases on the two strands are related to each other, such that the sequence of one determines the other. This allows genetic information to be preserved during replication of the DNA and expression of the genes. Disruption of the hydrogen bonds between the bases by heat or chemicals or by the action of enzymes causes the strands of the double helix to separate.

RNA structure

In RNA thymine is replaced by uracil and 2-deoxyribose buy ribose. RNA normally exists as a single polynucleotide strand however, short stretches of base pairing may occur between complementary sequences.

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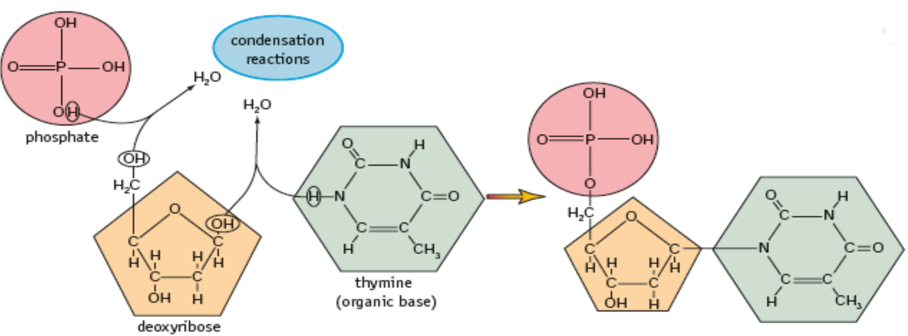
Lesson in detail:

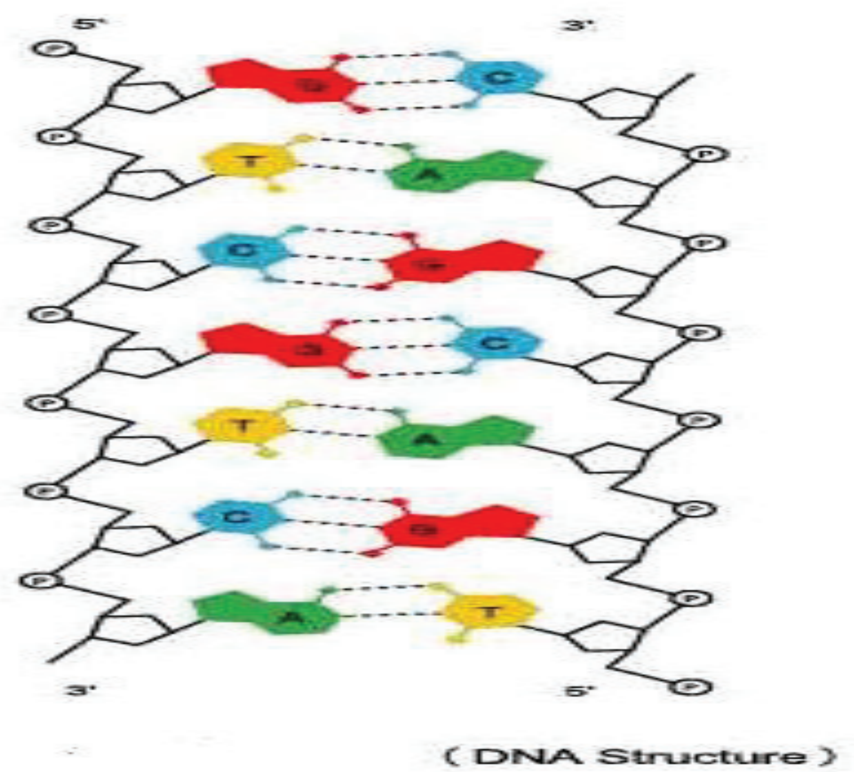
Nucleotides

The ability to carry the genetic information required by a cell to reproduce itself is closely related to the structure of DNA molecules. DNA molecule is a polymer and consists of a long chain of monomers called **nucleotides**. The DNA molecule is said to be a polynucleotide. Each nucleotide has three parts: a sugar, a nitrogen containing ring-structure called a **base,** and a phosphate group. The sugar present in DNA is a five carbon pentose called 2'-deoxyribose in which the _OH group on carbon 2 of ribose is replaced by hydrogen. The carbon atoms in the sugar are numbered 1-5. The numbers are given a das (') referred to as **prime** to distinguish them from the numbers of the atoms the base. The numbering is important because it indicates where other components of the nucleotide are attached to the sugar.



Nucleotides contain one of four bases: **adenine, guanine, thymine or cytosine**. These are complex molecules containing carbon and nitrogen ring structures. Adenine and guanine contain two carbon-nitrogen rings and are known as **purines.** Cytosine and thymine contain a single ring and are called **pyrimidines.** The bases are attached to the sugar by a bond between the 1' carbon of the sugar and nitrogen at position 9 of the purines or position 1 of the pyrimidines. A sugar plus a base is called a nucleoside

(fig.3a). Nucleotides contain phosphate groups (PO4) attached to the 5' carbon of the sugar (fig.3b). A nucleoside is called a nucleotide when a phosphate group attached, the attachment can consist of one, two or three phosphate groups joined together.the phosphate groups are a,b and y, with a directly attached to the sugar. Nucleotides may exist in cells as individual molecules (nucleotide) triphosphate play an important role in cells as the carriers of energy used power enzymatic reactions) or polymerized as nucleic acids (DNA or RNA).



(DNA Structure)

DNA Polynucleotides

Nucleotide triphosphates are joined together to give polynucleotides. There are four used to synthesize DNA polynucleotides, 2'-deoxyadenosine 5'-triphosphate (dATP), 2'-deoxythymidine 5'triphosphate (dTTPorT), 2'-deoxycytosine 5'-triphosphate (dCTPorC) and 2-deoxyguanosine 5'-triphosphate (dGTPorG). The B and Y phosphates are lost during

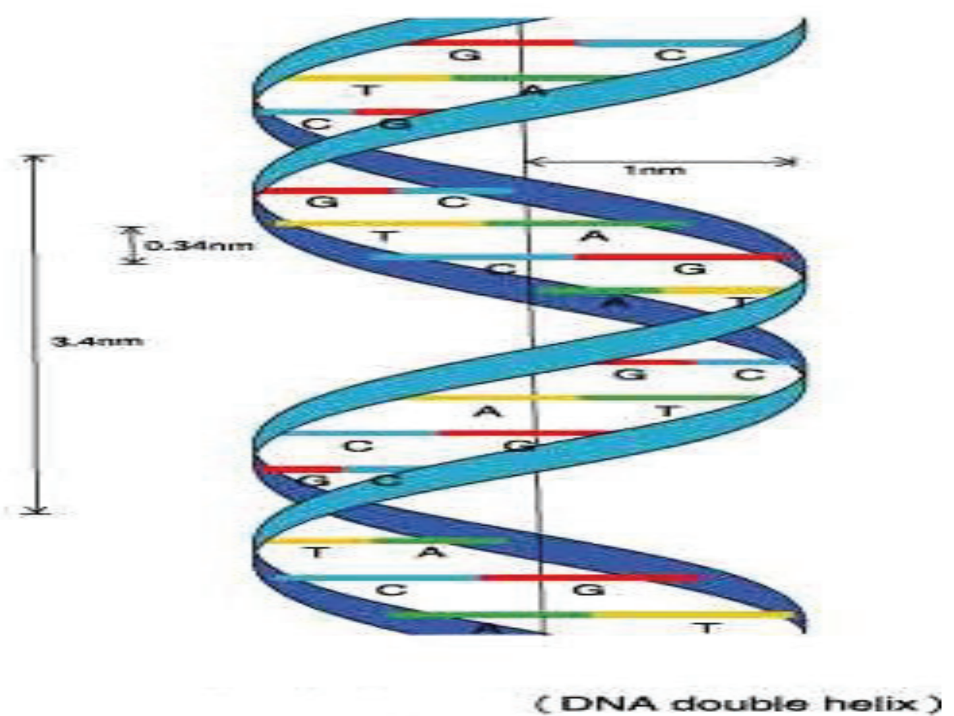
polymerization and the nucleotide units are joined together by the remaining phosphate. The 5' phosphate of one nucleotide forms a bond with the 3' carbon of the next nucleotide eliminates the –OH group on the 3' carbon during the reaction. The bond is called a **3'-5' phosphodiester bond** (C-O-P). The polynucleotide chain has a free 5' triphosphate at one end known as the 5' end and a free 3' hydroxyl group at the other end called the 3' end. This distinction gives the DNA polynucleotide polarity so that a DNA molecule can be described as running 5'-3' or 3'-5'.

It is the sequence of the bases in the DNA polynucleotide that encodes the genetic information. This sequence is always written in the 5'-3' direction (polymerase enzymes copy DNA molecules in this direction). Polynucleotides can be extremely long with no apparent limit to the number of nuclotides and no restrictions on the sequence of nucleotides. The maximum number of possible base sequence for a polynucleotide is 4n, where n is the number of nucleotides. This is an enormous number. For example, a polynucleotide containing just six bases could be arranged as 4(6)=4096 different sequences.

The double helix

DNA molecules have a very distinct and characteristic three-dimensional structure known as double helix.

The structure of DNA was discovered in 1953 by Watson and Crick working in Cambridge using X-ray diffraction pictures taken by Franklin and Wilkins. DNA exists as two polynucleotide chains wrapped around each other to form the double helix. The sugar-phosphate part of the molecule forms a spine or backbone which is on the outside of the helix. The bases, which are flat molecules, face inwards towards the center of the helix and are stacked on top of each other like a pile of plates.



(DNA double helix)

X-ray diffraction pictures of the double helix show repeated patterns of bands that reflect the regularity of the structure of the DNA. The double helix executes a turn every 10 base pairs. The pitch of the helix is 34A so the spacing between bases is 3.4A. The diameter of the helix is 20A. The double helix is said to be 3 **anti-parallels**. Only anti-parallel polynucleotides form a stable helix. The double helix is not absolutely regular and when viewed from the outside a **major groove** and a **minor groove** can be seen. These are important for interaction with proteins, for replication of the DNA and for expression of the genetic information. The double helix is right-handed. This means that if the double helix were a spiral staircase and you were climbing up, the sugar-phosphate backbone would be on your right.

A number of variant forms of DNA occur when crystals of the molecule are formed under different conditions. The form present in cells is called the **B form.** Another form called the A form has a slightly more compact structure. Other forms that exist are C, D, E and Z, which is striking because it exists as

a left-handed helix. Regions in chromosomes containing non-standard structures such as Z-DNA have recently been identified.

Complementary base pairing

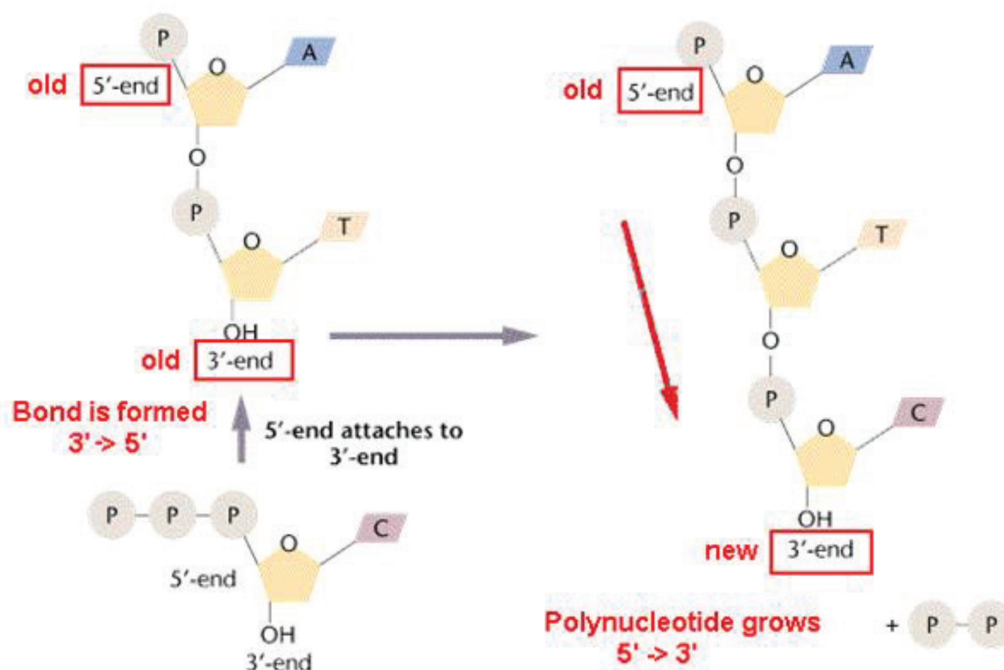
The bases of the two polynucleotide chains interact with each other. The space between the polynucleotides is such that a two-ring purine interacts with a single-ring pyrimidine. Thus, thymine always interacts with adenine and guanine with cytosine. Hydrogen bonds form between A and T and three between G and C. thus, G-C bonds are stronger than A-T bonds. The way in which the bases form pairs between the two DNA strands is known as **complementary base pairing** and is of fundamental importance.

Combinations other than G-C and A-T do not work because they are too large or too small to fit inside the helix or they do not align correctly to allow hydrogen bond formation. Because G must always bond to C and A to T the sequences of the two strands are related to each other and are said to be complementary with the sequence of one strand predicting and determining the sequence of the other. This means that one strand can be used to replicate the other. This is a vital mechanism for retaining genetic information and passing it on to other cells following cell division. Complementary base pairing is also essential for the expression of genetic information and is central to the way DNA sequences are transcribed into mRNAand translated into protein.

The double helix is stabilized by hydrogen bonds between the base pairs. These can be disrupted by heat and some chemicals. This result in separation of the double helix into two strands and the molecule is said to be denatured. In cells enzymes can separate the strands of the double helix for the purposes of copying the DNA and for expression of the genetic information.

DNA Replication:

• each nucleotide contains a deoxyribose molecule with 5 carbons, with a nitrogenous base attached to carbon 1 and a phosphate attached to carbon 5
• a DNA strand is built with a repeating deoxyribose - phosphate - deoxyribose - phosphate backbone
• thus, one end of the DNA molecule has a free 5' phosphate and the other has a free 3' carbon; replication only adds DNA nucleotides at the 3' end



Enzymes involved in DNA replication:

• Helicase:
○ controls unwinding of coiled DNA
○ separates complementary strands of DNA, producing a replication fork

• **RNA Primase:**
○ DNA polymerase III is only able to add DNA nucleotides to a free 3' end on an existing DNA strand
○ therefore, RNA primase uses the DNA template to synthesize a short 10 RNA nucleotide sequence known as an *RNA primer*

• **DNA polymerase III**:
○ DNA polymerase III uses a single parent strand of DNA as a template
○ adding free *dexoyribonucleoside triphosphates* from solution to the parent/template strand
○ according to the complementary base pairing rules (A=T, G=C)
○ DNA polymerase III can only add *deoxyribonucleoside triphosphates* to a free 3' end of an existing nucleotide strand
○ thus, on only one of the two strands of DNA can DNA polymerase III synthesize continuously in the direction toward the replication fork: this is known as the*leading strand*

• **DNA polymerase I:**
○ DNA polymerase I is a proofreading enzyme
○ removes the RNA nucleotides of the RNA primer
○ replacing them with DNA nucleotides

• **DNA ligase:**
○ forms covalent bonds linking together Okazaki fragments
○ completing DNA synthesis along the lagging strand

Okazaki fragments:

• DNA polymerase III is unable to work directly on the *lagging strand* because it lacks a free 3' end on an existing DNA strand

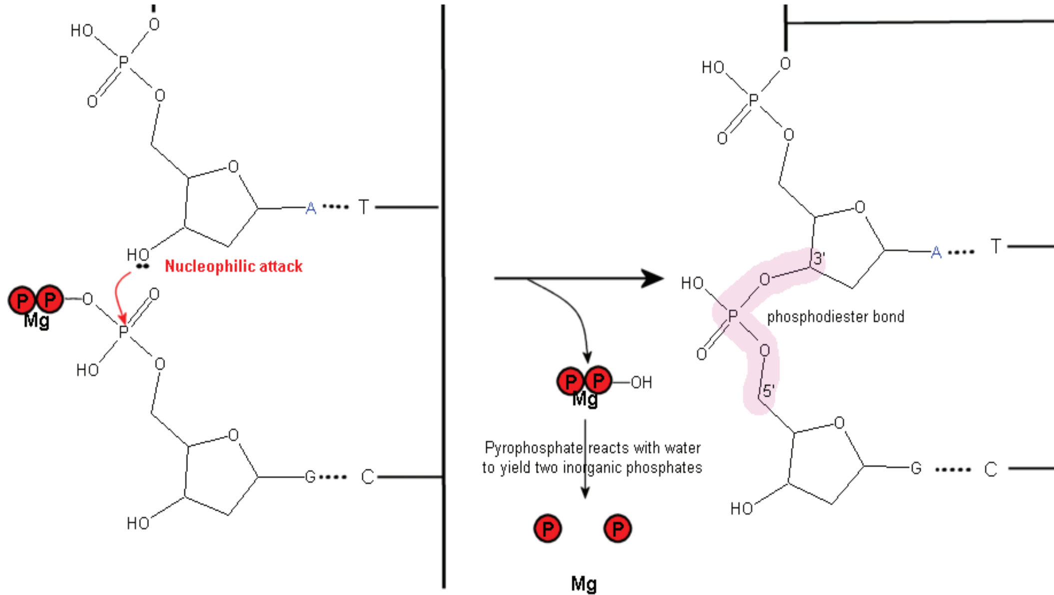
• therefore, lagging strand synthesis begins when *RNA primase* uses the DNA template to synthesize a short 10 RNA nucleotide sequence known as an *RNA primer*

• DNA polymerase III then uses the free 3' end of the RNA primer to synthesize longer sequences of DNA, about 100 DNA nucleotides in length, known as *Okazaki fragments*

Dexoyribonucleoside triphosphates:

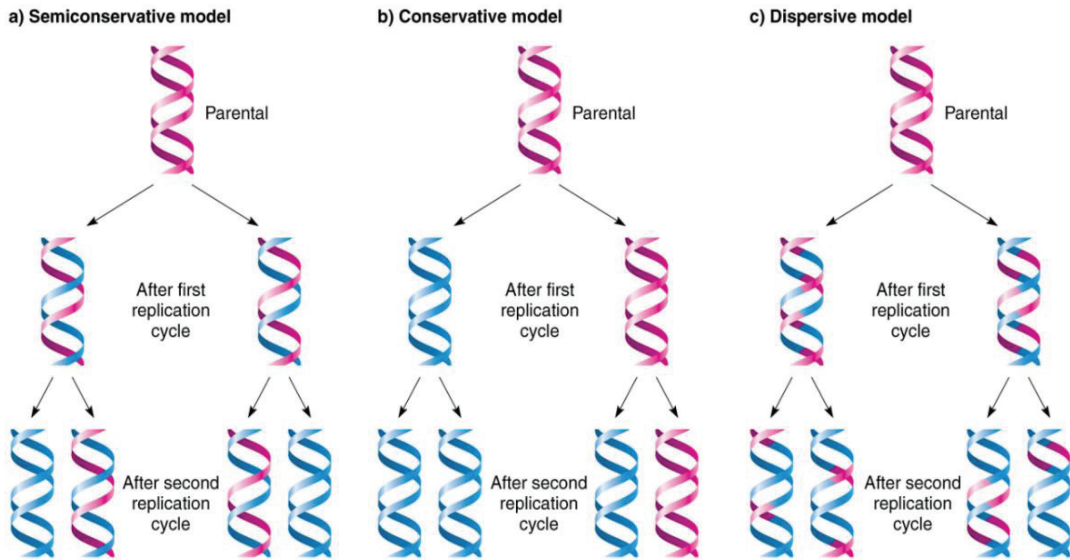
• Hydrolysis of phosphate groups provides the energy to form phosphodiester covalent bonds between nucleotides.

Mechanism and Enzymology:



Hydrolysis of DNA strand

DNA Replication:



Brief note on replication of DNA and the types taken for study in general:

In the **semi-conservative** model, the two parental strands separate and each makes a copy of itself. After one round of replication, the two daughter molecules each comprise one old and one new strand. Note that after two rounds, two of the **DNA** molecules consist only of new material, while the other two contain one old and one new strand.

In the **conservative** model, the parental molecule directs synthesis of an entirely new double-stranded molecule, such that after one round of replication, one molecule is conserved as two old strands. This is repeated in the second round.

In the **dispersive** model, material in the two parental strands is distributed more or less randomly between two daughter molecules. In the model shown here, old material is distributed symmetrically between the two daughter's molecules. Other distributions are possible.

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The semi-conservative model is the intuitively appealing model, because separation of the two strands provides two templates, each of which carries all the information of the original molecule. It also turns out to be the correct one (Meselson & Stahl 1958).

In detail on Semi Conservative Replication of DN

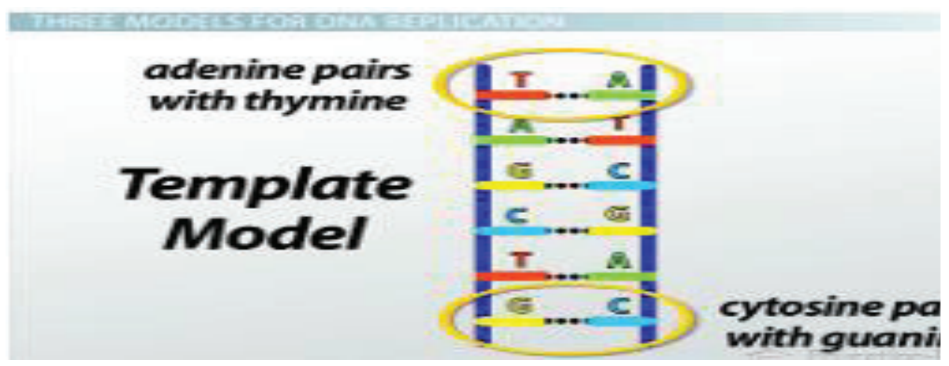
Significance of DNA and its functions: DNA is the genetic material, and genes are made of DNA. DNA therefore has two essential functions: replication and expression.

Replication means that the DNA, with all its genes, must be copied every time a cell divides. Expression means that the genes on DNA must control characteristics. A gene is a section of DNA that codes for particular protein. Characteristics are controlled by genes through the proteins they code for and thus responsible for heredity and controlling all the metabolic activities in the living cells. Expression can be split into two parts: transcription (making RNA) and translation (making proteins).

The Double-Helix Model of DNA

The model that Watson and Crick proposed in 1953 to describe the molecular structure of DNA was a landmark discovery. But at the time, many scientists weren't convinced that the model was right. Along with their structural model of DNA, Watson and Crick also proposed a model to describe how DNA is copied inside a cell. Many scientists thought their model of DNA production didn't make sense, and it led some people to doubt whether they were even right about the double helix. Scientists had known for a very long time thatorganisms make copies of their DNA. Making extra copies of the instructions in DNA allows an organism to grow and reproduce. The scientific word for 'copy' is 'replication.' So when we talk about DNA making copies of it, we call it **DNA replication**.

A DNA chain is composed of smaller components called nucleotides. Each nucleotide is composed of a sugar, a phosphate and a nitrogenous base. The nucleotides are arranged into two strands that link together like rungs on a ladder, and the ladder is twisted into a shape we call a double helix. Watson and Crick first proposed this structural model, and further scientific study has shown that they were basically correct.



In the template model, a DNA strand serves as the template for a ne

Three Different Models for DNA Replication

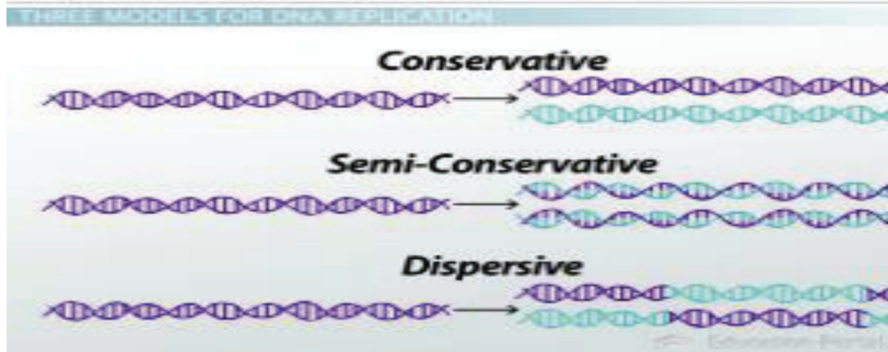
Watson and Crick had proposed that in order to copy itself, DNA would have to open down the center, sort of like a zipper coming apart, so that a new DNA strand could be built on top of the exposed strands. Following the rules of complimentary base pairing, adenine would pair with thymine, and cytosine would pair with guanine. Thisidea was called a template model, since one DNA strand serves as the template for a new one.

Watson and Crick figured that this model would result in two new double strands of DNA, each one with one strand of parent (or template) DNA and one strand of daughter (or newly-synthesized) DNA. They called this the **semi-conservative** model, because half of the parent DNA was conserved in each new DNA molecule.

Scientists looked at the double helix of DNA and wondered how in the world it could possibly open itself up without getting tangled or torn apart. So they thought up some other ideas about how DNA replication works. One hypothesis, called the **dispersive model**, suggested that DNA only copied

itself for short chunks at a time, producing new strands that alternated parent and daughter DNA. Another idea, called the**conservative model**, argued that DNA didn't split open at all, but somehow kept the parent strands intact while creating an entirely new and separate copy.

Nobody knew for sure how DNA replication really worked until two scientists named Matthew Meselson and Franklin Stahl devised an ingenious experiment in 1958. They realized that they could test all three models at once by keeping track of what happens to one parental DNA strand as it generates a series of copies.



The three proposed models of DNA replication

Each model predicts a different distribution of parent DNA following a round of DNA replication. If Meselson and Stahl were able to keep track of parent versus new DNA, they could either support or refute the predictions of the three different models.

The Meselson-Stahl Experiment

Meselson and Stahl decided the best way to tag the parent DNA would be to change one of the atoms in the parent DNA molecule. Remember that nitrogen is found in the nitrogenous bases of each nucleotide. So they

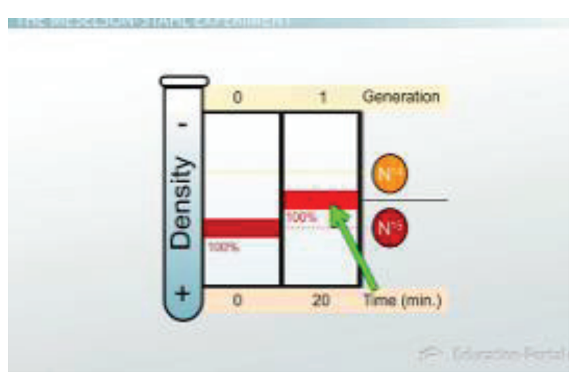
decided to use an isotope of nitrogen to distinguish between parent and newly-copied DNA. The isotope of nitrogen had an extra neutron in the nucleus, which made it heavier.

You can see from any periodic table that most nitrogen atoms have an atomic weight of 14. We call these atoms N-14. But an isotope with an extra neutron has a weight of 15, so we call it N-15. The scientists decided to start with parent DNA molecules that only contained N-15. If only N-14 nucleotides were available during DNA replication, they would be able to tell which parts had come from the original double strand and which parts had been created during the replication process.

In order to make DNA go through many rounds of replication, Meselson and Stahl harnessed the reproductive powers of the common bacteria E. coli. They made sure that the first batch of bacteria contained only N-15 DNA. Then, they put the bacteria into a medium that only contained N-14 atoms. That way, whenever the bacteria reproduced, they would be forced to incorporate the N-14 into their new DNA. The scientists sat back and let the bacteria go to work.

With each new generation of bacteria, Meselson and Stahl took a sample so that they could see how the N-15 DNA was being distributed in the daughter molecules. Now, you may be wondering, how could they tell the difference between N-15 and N-14 DNA? It's not like you can actually see an isotope of nitrogen. How did the scientists know how much N-15 was inside each molecule?

The answer is the atomic weight. Because N-15 has one extra neutron, it's slightly heavier than N-14 and therefore makes the DNA molecule denser. We can separate DNA molecules based on the differences in their densities. To do this, we use a centrifuge, a device that spins a test tube at very high speeds. When a test tube is spun inside a centrifuge, all the contents are pushed toward the bottom. The substances that are heaviest sink farther down the tube, and the lighter substances float. So if you apply a centrifugal force to a mixture of two types of DNA, the heavier N-15 DNA sinks to a lower level than the N-14 molecules.



After one replication, the DNA was converted to a of N-15 and N-14 DNA

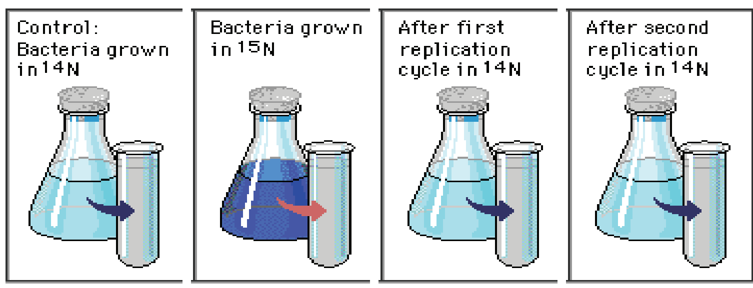
Every time Meselson and Stahl wanted to take a sample of the bacterial DNA, they had to chop up the tiny organisms and empty all the contents into a test tube. They mixed in a salt solution and then spun the test tube for many hours to make the substances separate out. Then they used special techniques to see how far the DNA molecules sank inside the tube.

When they sampled their first group of bacteria, Meselson and Stahl saw a darkened band in the test tube where the N-15 DNA had sunkand gathered in one spot. But after they let the bacteria reproduce, they got much different results in their samples. The DNA still sank down in the tube, but not nearly as far as the first generation. It was a lighter form of DNA, meaning that it wasn't completely made with the N-15 isotope. After one replication, the entire DNA had been converted to a hybrid of N-15 and N-14 DNA.

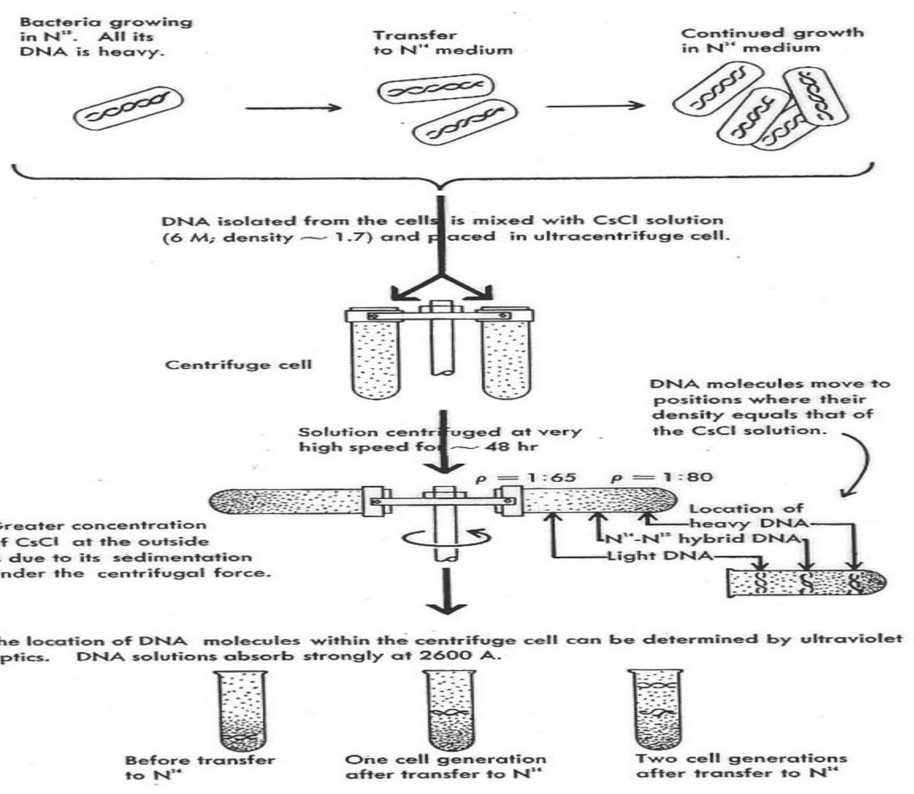
Results of the Experiment: Meselson and Stahl tested the hypothesis of DNA replication. They cultured bacteria in a 15N medium. 15N is a heavy isotope of nitrogen so the DNA synthesized is of heavy density. They then shifted the bacteria to a 14N medium, DNA was isolated at different times corresponding to replication cycles 0, 1, and 2. After one replication cycle, the DNA was all of intermediate density. This rules out the conservative replication model, which predicts that both heavy density DNA and light density DNA will be present, but none of intermediate density will be present. This result is consistent with the semiconservative replication model,

which predicts that all DNA molecules will consist of one 15N-labeled DNA strand and one 14N-labeled DNA strand. The result does not rule out the dispersive replication model, which also predicts that all DNA will be of intermediate density, consisting of interspersed double-stranded 15N-labeled and 14N-labeled segments.

After two replication cycles, two bands of DNA were seen, one of intermediate density and one of light density. This result is exactly what the semi-conservative model predicts: half should be 15N-14N intermediate density DNA and half should be 14N-14N light density DNA. This result rules out the dispersive replication model, which predicts that after replication cycle 1, the DNA density of all DNA molecules will gradually become lower, so no intermediate density DNA should remain at replication cycle 2. The semi-conservative model is correct.



The processes involved:



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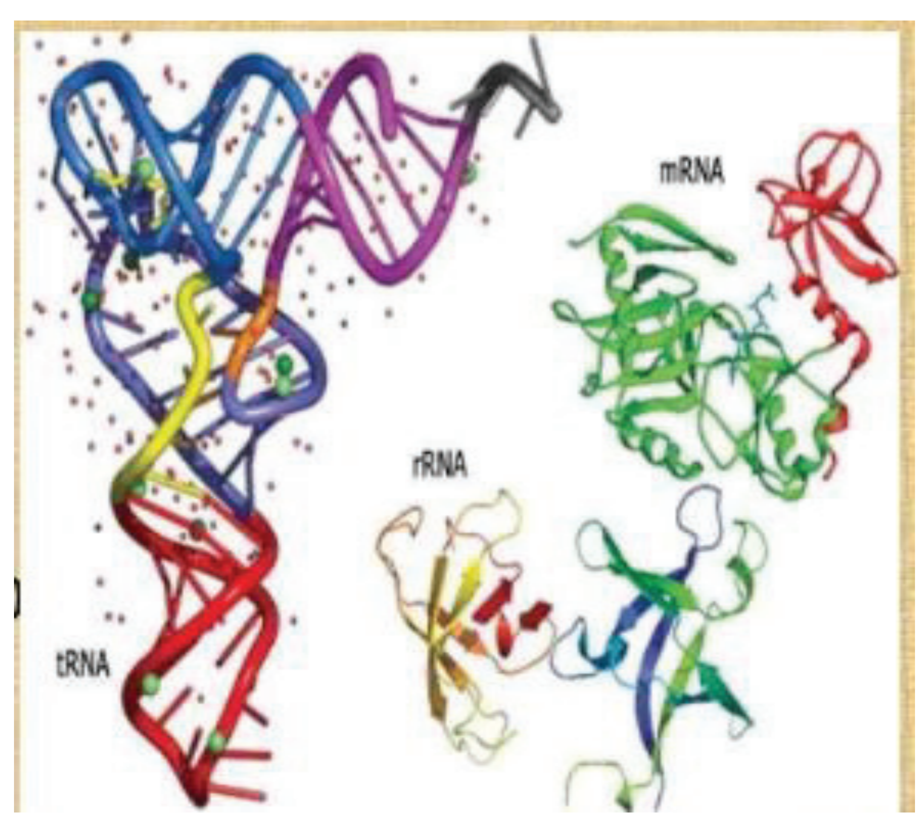
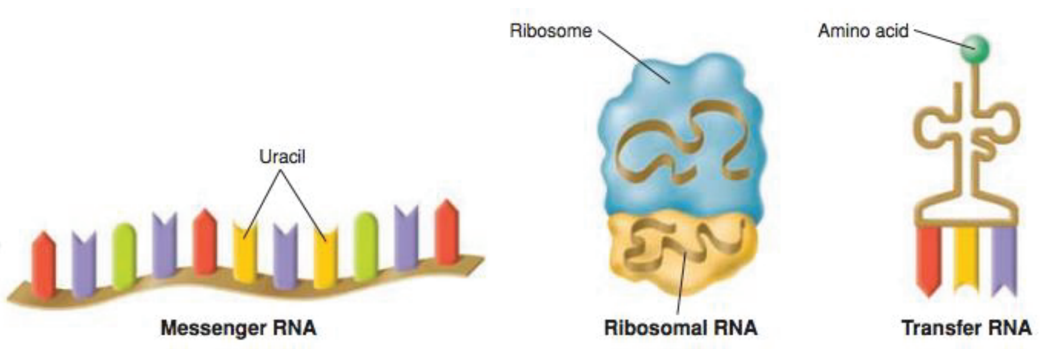
RNA structure

The structure of RNA is similar to that of DNA but a number of important different exist. In RNA ribose replaces 2'-deoxyribose and the base thymine is replaced by another base, Uracil, which can also base pair with adenine. In addition, RNA molecules normally exist as a single polynucleotide strand and do not form a double helix. However, it is possible for base pairing to occur between complementary parts of the same RNA strand resulting in short double-stranded regions.

Types of RNA: Basic three types of RNA are **mRNA**, **tRNA** and **rRNA**





Types of RNA The three main types of RNA are messenger RNA, ribosomal RNA, and transfer RNA. Ribosomal RNA is combined with proteins to form ribosomes.

Type 1: TRANSFER RNA

Key notes

Transfer RNAs (tRNAs) are small RNA molecules containing 75 to 95 nucleotides. Cells contain many different tRNA molecules. Most of the tRNAs function as carriers of amino acids and participate in protein synthesis. However, tRNAs also take part in reactions not related to ribosome-dependent translation.

The first nucleic acid to be completely sequenced was the tRNA that binds the amino acid alanine ($tRNA^{Ala}$). This pioneering work was performed by Holley and co-workers long before the invention of today's rapid sequencing techniques. For his work, Holley received the 1968 Nobel Prize in Physiology or Medicine.

Role in translation

Transfer RNAs (tRNAs) are small molecules that bring amino acids together for protein synthesis in an order specified by a messenger RNA (mRNA) sequence. Cells contain a number of different tRNAs each of which binds a specific ammo acid. Each tRNA also binds a specific codon in the RNA allowing it to place its amino acid in the correct position.

Structure

Base-pairing of tRNA molecules produces a cloverleaf structure composed of stem-loops called arms. These include: the acceptor arm which is the point of attachment of the amino acid; the anticodon arm which recognizes codons in the mRNA sequence, the DHU arm which contains dihyrouracil; the optional arm; and the TC arm which contains pseudouracil. Transfer RNAs have conserved nucleotides that correspond to base paired regions. The tertiary structure predicts similar base pairing to the 2D cloverleaf representation and shows the acceptor and anticodon arms at opposite ends of the molecule.

Synthesis and processing

Transfer RNA genes occur as multiple copies each of which encodes several tRNAs. They are transcribed by RNA l po ymerase III as pre-tRNAs which are processed by ribonucleases to release mature tRNAs. RNAseP (**well known as a catalytic ribonucleoprotein that processes the 5′ leader sequence of precursor tRNA.)**‘Contains ribozyme activity. RNase P is also essential for the transcription of other small noncoding RNA genes. The sequence CCA is added to eukaryotic tRNAs after transcription.

Modification of nucleotides

The nucleotides in tRNA molecules are modified following transcription. Modifications include: methylation, base rearrangements, double bond saturation, deamination, sulfation and addition of larger groups. The function of the modifications is uncertain.

Lessson in detail:

Role in translation

Cells contain three types of RNA - transfer, ribosomal and messenger -which are produced by transcription from DNA. Transfer and ribosomal RNAs form part of the machinery of protein synthesis and messenger RNAs act as the template for the synthesis of proteins during translation.

Transfer RNAs (tRNAs) are small molecules that act as adapters during protein synthesis; they link the nucleotide sequence of the messenger RNA (mRNA) to the amino acid sequence of the polypeptide. Cells contain a number of tRNAs each of which can bind a specific amino acid. Each tRNA recognizes a codon in the mRNA allowing it to place its amino acid in the correct position in the growing polypeptide chain as determined by the sequence of the mRNA.

Structure

Transfer RNA molecules contain between 74 and 95 nucleotides. Base-airing takes place between complementary parts of the nucleotide sequence resulting in a structure known as the **cloverleaf** which is characteristic of tRNA molecules (fig. 1). The cloverleaf is compased of a series of **semi-loop** structures known as arms. These include;

• The acceptor arm which is formed by base-pairing between nucleotides at the 5'and 3' ends of the trna. The sequence CCA, which occurs at the 3' terminus, is notbase-paired and is the point of attachment for amino acids.

• The **D** or **DHU arm** is a stem-loop structure containing dihydrouracil, an unusual pyridine nucleotide.

• The **anticodon arm** is responsible for recosnizing and binding codons in the m'RNA.

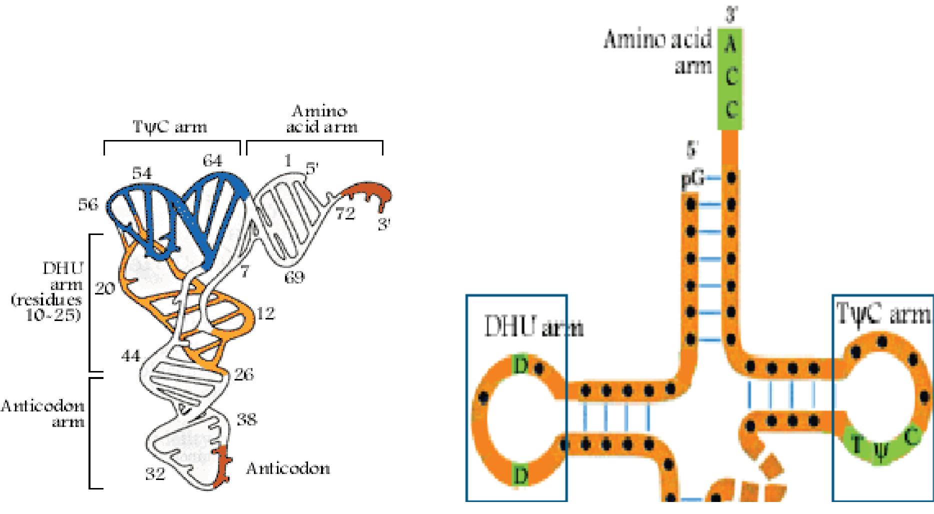
• The **extra, optional or variable arm** occurs only in some t'RNAs. It may be small containing only 2-3 nucleotides (class I t'RNAs) or larger containing 13-21 nucleotides with up to five base pairs in a stem (class II t RNAs).

• The TC arm contains the sequence TC,is a modified nucleotide called pseudouracil.

Comparison of different tRNAs shows that parts of the nucleotide sequence are conserved. At certain positions the nucleotide present is invariant and is the same in all tRNAs. At other positions, the nucleotide is always a purine

or a pyrimidine and is said to be semi-invariant and at other points the sequence is not conserved and the nucleotide present varies.

The cloverleaf structure is a two-dimensional description of the tRNA molecule. A more accurate representation is obtained from its three-dimensional (3D) structure which has been determined by X-ray diffraction (Fig. 2). The base paired nucleotides described in the cloverleaf structure are still present but some nucleotides which appear far apart 1n the cloverleaf are base-paired in the 3D Structure. Many of the nucleotides that are base-paired are invariant or semi-invariant. In the 3D structure the acceptor arm and the anticodon loop are at opposite ends of the molecule consistent with their role in translation.



Synthesis and processing

Transfer RNAs are synthesized by transcription of tRNA genes by the RNA polymerase III enzyme (see Topic A4). The genes exist as multiple copies, especially in eukaryotic cells, reflecting the large requirement of cells for tRNA. The tRNAs are produced as precursor RNA molecules called **pre-tRNAs** which are processed to give mature tRNAs (Fig. 3). Several tRNA genes may be transcribed together as a single pre-tRNA which is then

processed by ribonucleases that cleave at the 5' and 3' ends of each tRNA sequence. In prokaryotes, processing is carried out in an ordered series of steps by the ribonucleases, RNaseD and P.

RNaseP, which is found in prokaryotes and eukaryotes, is unusual in that it has an RNA component with catalytic activity known as a ribozyme. Eukaryotic tRNAs differ from their prokaryotic counterparts in that many of them are transcribed containing a short intron which is removed during processing. The sequence CCA is present at the 3' terminus of all tRNAs and is the point of attachment for amino acids. In eukaryotes, CCA is not present-in the DNA of the tRNA gene but is added later by a tRNA nucleotidyl transferase. In prokaryotes, the CCA is present in the coding sequence but is sometimes removed by RNaseD and then replaced by a prokaryotic nucleotidyl transferase.

Modification of nucleotides

Transfer RNAs contain unusual nucleotides produced after transcription by chemical modification. The most common modifications are:

• **Methylation** of the ribose sugar of the nucleotide. For example, guanosine is methylated to 7-methylguanosine. .

• **Base rearrangements**. These involve interchanging of the positions of atoms in a purine or pyrimidine ring. An example is the conversion of uridine to pseudouridine.

• **Double-bond saturation**. An example is the conversion of uridine to dihyrouridine.

• **Deamination.** This involves removal of amino groups from bases. Forexample, guanosine is deaminated to produce inosine.

• **Sulfur substitution.** For example, the oxygen atom of uridine is replaced bysulfur to give 4-thiouridine.

• **Addition of larger groups.** An example is the conversation of gaunosine to queosine.

Over 50 types of modification have been described, each carried out by a different t RNA modifying enzyme. The reasons for most of the

modifications are unknown but in some cases roles have been assigned for modified nucleotides within the anticodon loop.

Type 2: RIBOSOMAL RNA
Key notes

Ribosomes

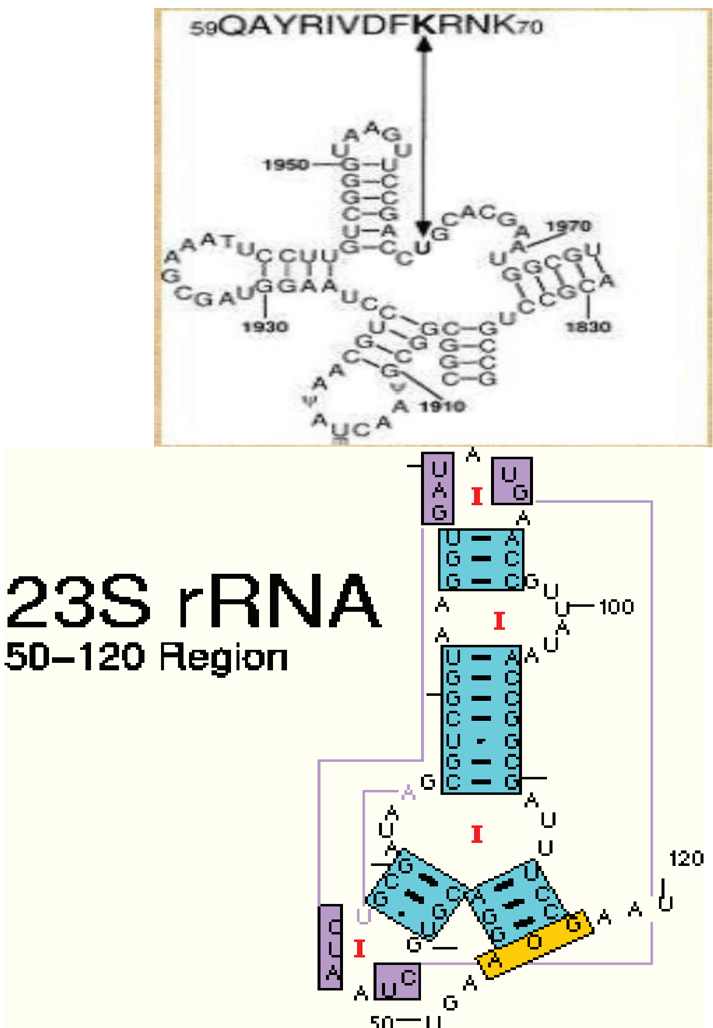
Ribosomes are macromolecular structures composed of ribosomal RNA (rRNA) and protein. They occur in large numbers in the cytoplasm where they translate messenger RNA into protein. Ribosomes have large and small subunits with characteristic sizes, described in terms of sedimentation (5) values. Prokaryotic ribosomes are 70S with 50S and 30S subunits. They contain three rRNAs (23S, 16S and 5S). Eukaryotic ribosomes are 80S and have 60S and 40S subunits. They contain four rRNAs (28S, 18S, 5.8S and 5S).

Transcription and processing of r RNA genes in prokaryotes

The three rRNAs in E. coli are transcribed from a single gene. Seven copies orthe gene occur in the genome. A single transcript is produced which isprocessed to give mature rRNAs. Processing involves folding of the RNA,attachment of ribosomal proteins, methylation of bases and cleavage byribonucleases.

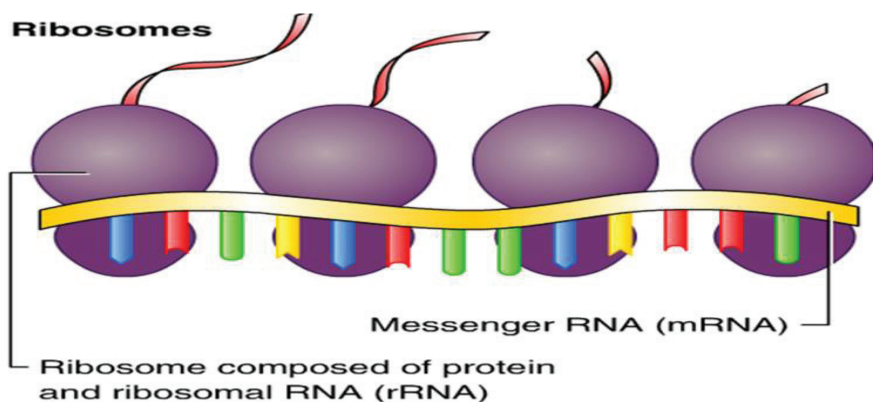
Eukaryotic rRNAs are transcribed from a single gene present in multiple copies arranged as a series of clusters. The genes are transcribed in the nucleolus by RNA polymerase I. A single pre-rRNA is synthesized which is processed to give mature 28S, 18S and 5.8S rRNAs. Processing involves folding of the rRNA, attachment of ribosomal proteins, methylation of ribose sugars by small nuclear ribonucleoproteins (snRNPs) and removal of spacer sequences between the rRNAs by ribonucleases. The 5S rRNA is transcribed separately from unlinked genes by RNA polymerase III.

Ribosomes



Ribosomes are macromolecular structures composed of ribosomal RNA RNA (rRNA)bound to protein. They occur in the cell cytoplasm where they bind to messenger RNA and translate it to produce proteins. Large numbers of ribosomes are required to fulfill the cell's requirement for protein. A typical bacterium contains 200 000 ribosomes which account for 80% of its RNA and 10% of its protein. Because ribosomes are very large, estimates of their molecular weight are difficult to obtain. The size of a ribosome is

measured by its **S value** (Svedberg units) which is related to the rate at which it passes through a dense solution such as sucrose when centrifuged at high speed. The **S** value is determined by the size, shape and the macromolecular structure of the ribosome.



Each ribosome is composed of two parts called the **large and small subunits** (fig 1), in prokaryotes such as E. coli, the ribosome is 70S and is made up of 50 and 30S subunits (S values are not additive). The 50S subunit contains two rRNAs (23S and 5S) complexes with 31 polypeptides. The 30S subunit contains single rRNA (16S) and 21 polypeptides. In eukaryotes, the ribosome is 80S and is composed of 60S and 40S subunits. The 60S subunit contains three rRNAs(28S, 5.8S and 5S) and about 49 polypeptides; the 40S Subunit contains one rRNA (18S) and about 33 proteins.

In ribosomes the rRNA molecules adopt a characteristic three-dimensional structure which is stabilized by complementary base-pairing both within and between RNA molecules. The RNA molecules are believed to from a frame-work to which the proteins, which provide most of the functional activity of the ribosome, are attached. Some of the RNA molecules also have enzymatic activity. These are known as **ribozymes** and they may contribute to the functioning of the ribosome.

Cells contain large numbers of ribosomes which must be replicated when the cell divides. As a result, cells have a huge requirement for rRNA which is

produced by transcription of rRNA genes by RNA polymerase. To ensure that correct numbers of each of the different rRNAs are produced, they are transcribed together from a single gene present m the genome as multiple copies.

In prokaryotes such as E. coli RNA Seven r genes occur scattered throughout the genome. Each gene contains one copy each of the 16S, 23S and 5S rRNA sequences arrange consecutively. In addition, between one and four transfer RNA (tRNA) sequences are present in each gene. The gene is transcribed to produce a single RNA molecule called **pre-rRNA** (30S) which is processed to produce individual rRNAs and tRNAs.

Processing involves a series of defined steps. Following transcription, the RNA molecule folds and complementary parts of the sequence base-pair to give a series of stem-loop structures. The ribosomal proteins then bind to the folded RNA. At this stage some of the bases in the RNA are modified by the addition of methy groups. Finally, the RNA is cleaved at specific points by the ribonucleose, RNAs III, to release the 5S , 23S AND 16S r RNAs. Further trimming at the 5’ and 3’ ends by other ribonucleases called M5, M16 and M23 then yields mature rRNAs.

In eukaryotes, the sequences of the 28S, 18S and 5.8S rRNAs are present in a single gene which exists as multiple copies separated from each other by short nontranscribed regions. In humans, there are about 200 genes arranged as a series of five clusters of about 40 genes on separate chromosomes. The genes are transcribed by **RNA polymerase** I in the cell nucleus in a region known as the **nucleolus.** In humans, a single pre-rRNA (45S) is synthesized which is processed to give individual 28S, 185 and 5.8S rRNAs. The 5S rRNA is transcribed separately by the RNA polymerase III enzyme from unlinked genes as a short 121 base transcript which does not undergo processing.

The eukaryotic pre-rRNA is processed in a similar way to its prokaryotic counterpart. Following transcription, the pre-rRNA folds and ribosomal proteins bind to it. Some of the bases are then modified by methylation of the

ribose sugar. This reaction is catalyzed by molecules composed of RNA and protein called small nuclear ribonucleo proteins (snRNPs, pronounced gnu-rps).

Mature 28S, 18S and 5.8S rRNAs are then produced by a series of steps in which the pre-rRNA is cleaved by ribonucleases. Initial cleavage of the 45S pre-rRNA occurs in regions known as the external transcribed spacers (ETSs). This is followed by cleavage in regions known as the **internal transcribesd spacers (ITS)** to produce 20s precursor r RNAs. Further cleaveage produces mature 28S, 18S and 5.8S r RNAs. Further clavage produces mature 28S, 18S and 5.8S r RNAs. In the final processing step the 5.8S r RNA base pairs with the 28S r RNA.

Type 3: MESSENGERS RNA

Key notes

Synthesis and processing

Messenger RNA produced in the (mRNA) acts as a template for protein synthesis; It is produced in the nucleus by transcription of protein coding genes by RNA polymerase II. The m RNA is initially transcribed as a transcribed as a precursor called pre-mRNA which contains noncoding intron sequences that are subsequently removed splicing. The 5’ end of the mRNA is modified by capping and the 3’end is modified by polyadenylation. RNA transcribed by RNA polymerase II exists in the nucleus as a population of molecules known as heterogeneous nuclear RNA (hnRNA).

Splicing

This process involves the removal of introns from pre-mRNAs. The sequences GT and AG occur at the ends of lntrons and are part of larger 5' and 3' splicing signal sequences. Another signal sequence called the branchpoint sequence occurs in the intron. Splicing involves cleavage of the 5' end of the intron and its attachment to the branch-point sequence to form a tailed loop. The intron is then released by being cleaved at its 3' end and the exons are brought together and joined. Splicing is catalyzed by small nuclear

ribonucleoproteins (snRNPs)-U1 binds to the 5' splice site and U2 to the branch-point sequence; U5 and U4/ 6 then form a complex with U1 and U2 called the spliceosome which holds the mRNA in the correct orientation for splicing and provides the enzymatic activities required for excision of the intron and joining of the exons.

Capping

Eukarryotic mRNAs are modified at the 5' end by the addition of the modified nucleotide, 7- methylguanosine, in an unusual 5'->5' triphosphate linkage to first nucleotide of the mRNA. This modification is known as capping and protects the mRNA from degradation by 5' exonucleases.

Polyadenylation

Most eukaryotic mRNAs are modified at their 3' ends by the addition of a poly a tail (Polyadenylation). The pre-mRNA is cleaved about 20 bases downstream of the polyadenylation signal sequence, 5' AAUAAA 3', and poly a polymerase adds a run of adenine residues. Polyadenylation is thought to protect the 3' end from degradation by exonucleases.

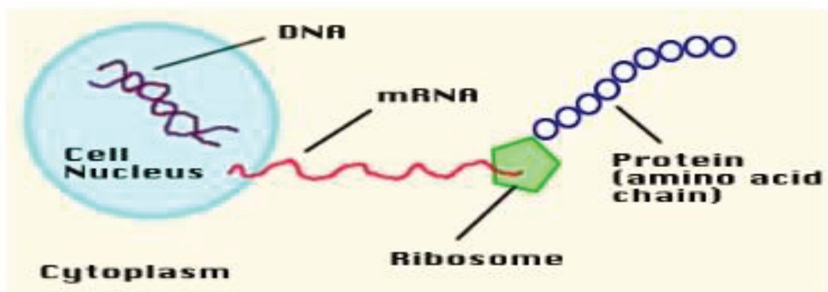
mRNAs stability

mRNA is relatively unstable compared with ribosomal and transfer RNA. This allows cells to regulate protein levels by altering the rate of gene transcription. Prokaryotic mRNAs have much shorter half-lives than eukaryotic mRNAs.

Alternative processing of m RNA.

Variations in splicing patterns produce mRNAs with different sequences from a Single pre-mRNA allowing production of variant proteins. Splicing patterns can vary to include or exclude one or more exons. Use of alternative Polyadenylation signals also produces variant mRNAs. mRNA sequences can be altered by RNA editing which involves changing sequences by the insertion, deletion or substitution of individual bases.

Messenger RNA (m-RNA)



<u>**Lesson in detail**</u>**:**

Synthesis and processing

In eukaryotes, messenger RNA (mRNA) produced by transcription of protein coding genes by the RNA polymerase II enzyme acts as a template for protein synthesis during translation. The coding information in eukaryotic genes is discontinuous and is arranged as a series of exons separated by noncoding introns. mRNAissynthesized as a precursor known as pre-mRNA by transcription of the exon and intron sequences. Before acting as a template for protein synthesis, the pre-mRNA undergoes a series of processing events to produce mature mRNA. Noncoding intron sequences are removed by a process called **splicing** which makes the coding sequences continuous and ensures that the mRNA is an accurate template for protein synthesis.

In addition, the 5’ end of the RNA is altered by the addition of a modified nucleotide in a process known as capping and the 3' end is modified by the addition of a tail of up to 250 adenines in a process called polyadenylation. RNA transcribed by RNA polymerase II exists in the nucleus as a population of molecules of different the nucleus as a population of molecules of different lengths (reflecting variations in gene size) and at different stages of processing and is known collectively as **heterogeneous nuclear RNA (hnRNA).**

In prokaryotes, mRNAis not processed and translation of the message begins even before transcription is complete. Prokaryotic genes do not normally contain introns and so splicing is unnecessary.

Translation :
messenger RNA (mRNA)
ribosome binding signal
initiation codon
AUG
CAU
AGGAGGU
start of transcript
UUUUU
hairpin loop
end of message

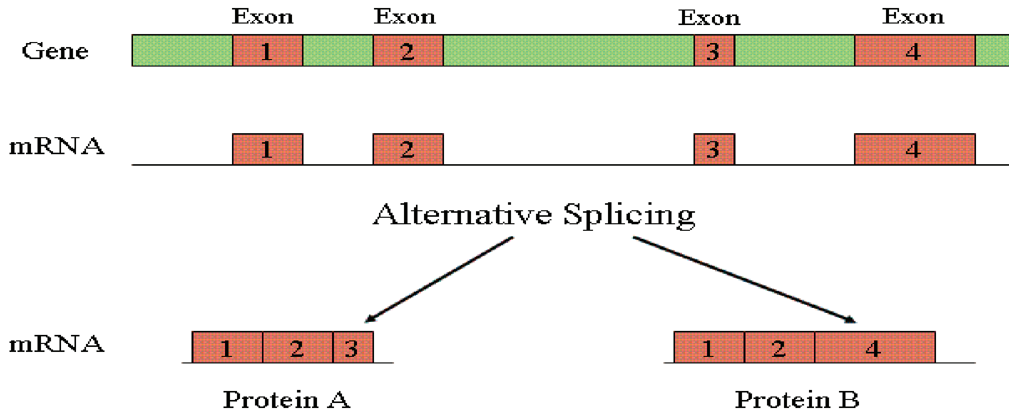
Splicing

This process takes place in the nucleus and involves the removal of noncoding, intron sequences from pre-mRNAs to produce mature mRNAs in which the coding sequences, corresponding to the exons, are continuous. The mature spliced mRNA is then exported to the cytoplasm where it acts as a template for protein synthesis.

Splicing depends on the presence of signal sequences in the pre-mRNA. In almost all genes the first two nucleotides at the 5' end of an intron are GT and the last two at the 3' end are AG. These are part of larger signal sequences present at the 5' and 3' ends of the introns. The complete 5' signal sequence is **5' AGGTAAGT 3'** and the 3' sequence is **5' YYYYYYNCAG 3'** (Y = pyrimi-dine; N = any nucleotide). In addition, in vertebrates the sequence, 5'

CURAY 3' (R = purine), which is called the **branchpoint sequence**, is present in the intron 10-40 bases upstream of the 3' signal sequence.

A more specific sequence, **5' UACUAAC 3'**, occurs in introns of yeast.



Splicing occurs in two steps (Fig. 1). In the first step the 2' hydroxyl group of the adenine of the branch- point sequence attacks the phosphodiester bond 5' to the G of the GT (**5' splice site**). The bond is broken releasing the 5' end of the intron and attaching it to the branchpoint sequence. The intron now forms a tailed loop structure called a **lariat**. In the second step the 3' end of the intron is cleaved after the G of the AG (**3' splice site**), the intron is released and the two exon sequences are joined together.

Splicing is catalyzed by a group of molecules called **small nuclear ribonucleoproteins** (snRNPs, pronounced *snurps*). These are composed of small RNA molecules rich in uracil called U RNAs or small nuclear RNAs (snRNAS) that exist complexed with proteins. Many different snRNPs exist but the most abundant are U1, U2, U4, U5, and U6 which catalyze the splicing reaction. Each snRNP contains a single U RNA molecule except U4 and U6 which exist base- paired to each other in the same snRNP. The RNA components of the snRNPs interact by base-pairing with the splicing signal sequences.

The U1 snRNP binds to the 5' splice site and the U2 snRNP binds to the branchpoint sequence. The remaining snRNPs, U5 and U4/U6, then form a

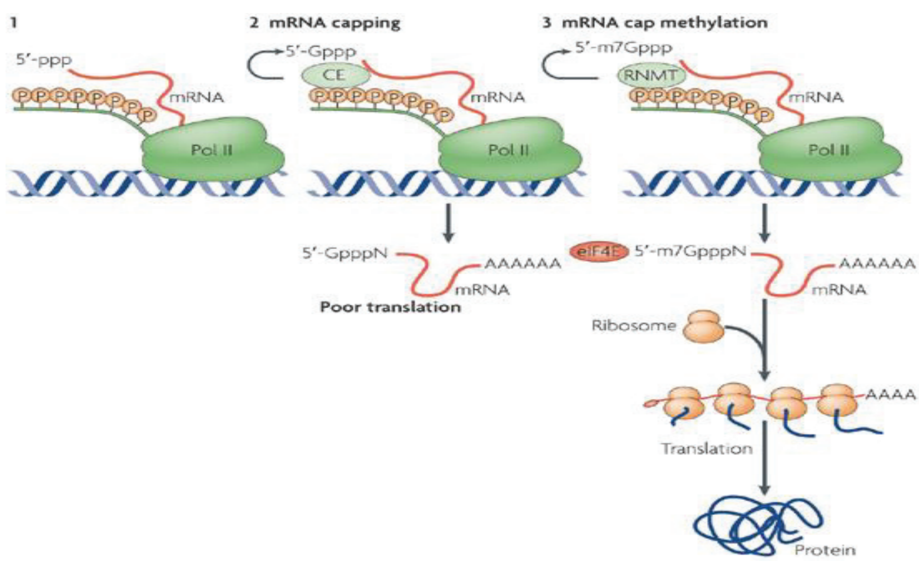
complex with U1 and U2 causing the intron to loop out and the exons to be brought together. The combination of the pre-mRNA and the snRNPs is called the **spliceosome** and this is responsible for folding the pre-mRNA into the correct conformation for splicing (Fig. 2). The spliceosome also catalyzes the cutting and joining reactions that excise the intron and ligate the exons. Once splicing is completed the spliceosome dissociates. The functioning of the spliceosome is not fully understood and the components responsible for all of its enzymatic activities have not been identified.

Although almost all introns are spliced by a spliceosome, there are some examples of intron splicing which occur by different mechanisms. Introns in ribosomal RNA genes in some unicellular organisms can adopt a three-dimensional shape by base-pairing; this then acts as an RNA cutting enzyme, known as a **ribozyme** that contributes to its own splicing. Introns also occur in transfer RNA genes which are removed by the action of ribonucleases in a similar way to the processing of transfer RNA molecules.

Capping

Eukaryotic mRNAs are altered at their 5' end by a modification known as capping which involves addition of the modified nucleotide, **7-methylguanosine**.

The cap is added by the enzyme guanyltransferase which joins GTP by an unusual 5'->5' triphosphate linkage to the first nucleotide of the mRNA. Methyl transferase enzymes then add a -CH, group to the 7-nitrogen of the guanine ring and, usually, to the 2' hydroxyl group on the ribose sugar of the next two nucleotides. Capping protects the mRNA from being degraded from the 5' end by exonucleases in the cytoplasm and is also a signal allowing the ribosome to recognize the start of mRNA molecule.



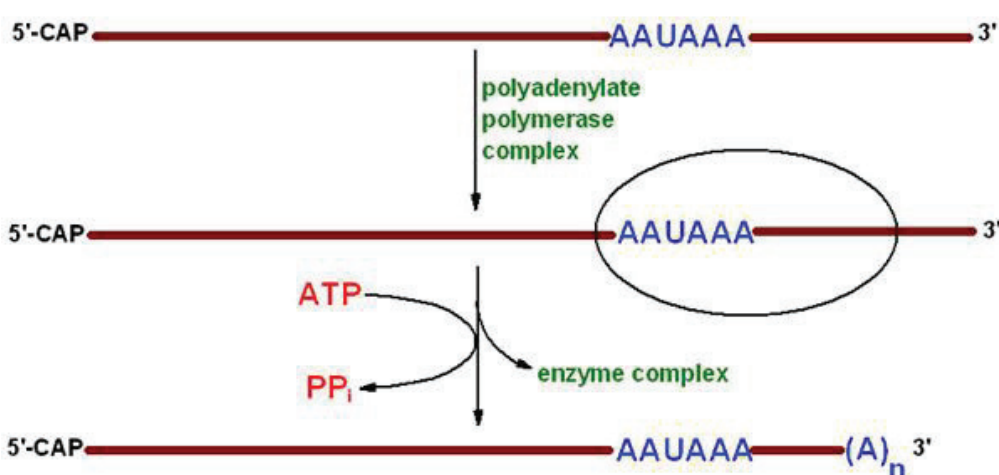
Capping as seen in mRNA:

Polyadenylation

Most eukaryotic pre-mRNAs are modified at their 3' ends bv the addition of a Sequence of up to 250 adenines known as a poly A tail, This modification is called polyadenylation and requires the presence of signal sequences in the pre-mRNA. These consist of the **polyadenylation signal sequence,** 5' AAUAAA 3’ which occurs near the 3' end of the pre-mRNA. The sequence YA (Y = pyrimidine) occurs in the next 11-20 bases and a GU rich sequence is often present further downstream.

A number of specific proteins recognize and bind these signal sequences forming a complex which cleaves the mRNA about 20 nucleotides downstream of the 5' AAUAAA 3' sequence.

The enzyme **poly(A)polymerase** then adds adenines to the 3' end of the molecule. The purpose of the poly A tail is uncertain but it may serve to protect the mRNAs from degradation of the coding sequence at the 3' end by exonucleaseases. However, some mRNAs, notably those encoding histone proteins, have no poly A tail.



mRNAs stability

Unlike ribosomal and transfer RNAs which are stable within cells, mRNA is relatively short lived. This is because cells regulate protein levels in the cytoplasm primarily by changing the rate of gene transcription. Because mRNAs are short-lived, changes in the rate of transcription of genes are reflected by changes in the amount of mRNA available for protein synthesis. In bacterial cells the half-life for a mRNA is just a few min. In eukaryotic cells a typical half-life might be as much as 6 h, although some mRNAs, such as those encoding the globin polypeptides that make up hemoglobin, are very long-lasting.

Alternative processing of mRNAs

Variations can occur in the way pre-mRNAs are processed which generates different mRNAs and hence different proteins from a single gene sequence. This occurs by **alternative splicing** of the pre-mRNA in which cells vary the splice sites they use such that particular exons`may be removed or retained during splicing. In addition, **alternative polyadenylation** signals present in the pre-mRNA can lead to the production of mRNAs with different sequences at the 3' end (Fig. 3). For example, the use of an upstream version of alternative polyadenylation sites may exclude exons downstream of it producing an mRNA encoding a truncated protein.

The same pre-mRNA may be alternatively processed within a single cell type, between different cell types and in the same cell type at different stages of development. The proteins produced following alternative processing of a pre-m RNA are related to each other but may have some different functions or characteristics. For example, alternative processing of immunoglobulin pre-mRNAs leads to the synthesis of proteins that may or may not contain

Hydrophobic amino acid sequences that allow them bind to cell membranes this leads to the production of alternative membrane-bound and secreted forms of immunoglobulin proteins.

Pre-mRNAs may also undergo alternative processing by **RNA editing.** In this process the sequence of the Pre-mRNA is altered by the insertion, deletion or substitution of bases. RNA editing was first identified in association with some parasitic protozoa in which the transcripts of many of

► **Comparing DNA (Deoxyribo Nucleic Acid) and RNA (Ribo Nucleic Acid)**

DNA	RNA
Polynucleotide composed of two strands(Double helix)	Polynucleotide composed of single strand (Single helix
Pentose sugar in nucleotide is a deoxyribose sugar.	Pentose sugar in nucleotides is a ribose sugar.
Nitrogen bases of pyrimidin are cytosine and Thymine (Uracil not present)	Nitrogen base of pyrimidin is cytosine and Uracil (Thymine is not present.
Long molecule composed of millions of base pairs.	Only a short length of DNA, represents a gene.
Molecular mass is high	Molecular mass is less
Occurs as one type and found in nucleus of the cells	Occurs as three structures(mRNA,tRNA and rRNA), made in nucleus and found in cell cytoplasm

the mitochondrial genes were found to be extensively modified by the insertion of uracil residues. Examples of RNA editing have also been identified in vertebrates, although the modifications found were much less extensive. In humans, the pre-mRNA* Of the apolipoprotein B gene is edited in intestinal cells by a C to U substitution that produces a stop codon; this leads to the synthesis of a shortened form of the protein. In liver cells, where editing does not occur, a full-length version of the protein is produced.

Roles of RNA in Translation

All three major forms of RNA—mRNA, tRNA, and rRNA—are involved in the process of translation.

Messenger RNA
Carries instructions for polypeptide synthesis from nucleus to ribosomes in the cytoplasm.

Transfer RNA
Carries amino acids to the ribosome and matches them to the coded mRNA message.

Ribosomal RNA
Forms an important part of both subunits of the ribosome.

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