Chapter - I

General Introduction and Historical Review

GENERAL INTRODUCTION AND HISTORICAL REVIEW

Ascomycetes is an extremely heterogeneous group of fungi with very little in common in the extreme groups, except producing their spores in the ascus and with varied pattern and morphology. The mycologist is often confronted with the intricate problem in proper arrangements of the various groups comprising them with phylogenetic relationships Lindau (1887) was the first to attempt the classification of this heterogeneous group of fungi on the basis of gross morphological characters, such as the type of ascocarp, its habitat colour, manner of opening and such other characteristics. He recognized three types of fructifications such as Cleistothecium, Perithecium and Apothecium. The Cleistothecium is an entirely closed fruit-body without an ostiole, with asci scattered and usually spherical in shape and is the characteristic of the series Plectomycetes. Perithecium is a flask shaped fruit-body with a narrow opening the ostiole - and asci arising on a hymenium and is the characteristic of the series Pyrenomycetes. Apothecium is a saucer or cup-shaped fruit-body with a wide opening and the asci lying parallel and is the characteristic of the series Discomycetes. It has been recognized however, through the contributions of Von Hohnel (1907, 1918). Arnaud (1910, 1918, 1921, 1923, 1925, 1930, 1931); Orton (1924); Overton (1906); Petrak (1924, 1925, 1934, 1941, 1947);

Nannfeldt (1932, 1983); Hansford (1937, 1938, 1941, 1944, 1945, 1946-a, 1946-b, 1947, 1948, 1954, 1955-a, 1955-b, 1961, 1963); Wehmeyer (1948, 1952-a, 1952-b); Miller (1949, 1954); Miller and Thompson (1940); Chadefaud (1942, 1943, 1946, 1955); Munk (1953); Luttrell (1940, 1951-a, 1951-b, 1953, 1955, 1964, 1965-a, 1965-b); Von Arx and Muller (1954, 1964); Von Arx (1953, 1970); Holm (1952, 1957, 1958); Martin (1961); Korf (1954, 1957, 1958, 1962, 1963, 1969); Korf and Waruitech (1971); Kamat and Pande (1971); Batista (1951, 1956, 1960); Malloch and Cain (1971); Holz and Butin (1972); Teterevnikova-Babagan and Martirosyan (1972); Martirosyan (1972); Kar and Maity (1972); Parguey-Leduc, Agnes (1972); Kwon-chung (1973); Kamat and Ullasa (1974); Ranalli and Irma (1975); Ellis (1981); Zhena, Chen (1982); Simonyan (1983); Stolk (1983); Barr (1976, 1979, 1983); and many others that the details of development of ascocarp and centrum characters and associated structures are of fundamental importance in establishing and evolving a natural system of classification of Ascomycetes based on true phylogenetic concepts.

The present trend is to recognize only two series Ascolocularíales and Ascohymeniales of Nannfeldt (1932), or Pseudosphaeriales and Perisporiales of Miller (1949) or Bitunicatae and unitunicatae of Luttrell (1951-a and 1965-b), or Prototunicatae and Eutunicatae of Gaumann

(1926, 1927, 1952). Recently Carr and Olive (1958) have suggested the use of chromosome morphology and correlation of ascus development with chromosome events as indicators for phylogenetic relationship, though on a limited scale between different genera like Sordaria and Neurospora.

In current trends of fungal taxonomy the aspects which draw attention in tracing phylogenetic relationship should not go unnoticed such as metabolic patterns (Vogel, 1960, 1961), serology (Coons, 1912; Tempel, 1957; Seeliger, 1960, Tsuchya and Kowatika, 1959), Numerical taxonomy (Sokal and Sneath, 1963; Kendrik and Proctor, 1964, 1969, Ibrahim and Threfal, 1966) and biochemistry (Barnett, 1966, 1968). Recently Hall (1969) and Tyrell (1969) reviewed the molecular approaches to taxonomy of fungi and their biochemical systematics.

Ascomycetous fungi with richness of their pattern, morphology and highly developed heterogeneous nature comprising pathogens as well as saprogens occuring on different parts of host plants are widely distributed all over the world and are recognized through their chief characters, the asci, which in higher forms are borne in various types of fructifications like cleistothecia, Perithecia, Apothecia, Pseudothecia, Thyriothecia and Hysterothecia etc. Although the large number of species have been enumerated from the

different parts of India in the form of regional lists, diagnostic descriptions are available in comparatively few species. The main contributors have been Blatter (1911); Uppal et al., (1935); Uppal et al., (1949); Sanwall (1953); Ramkrishnan and Sunderan (1953); Ramkrishnan (1956); Butler and Bisby (1932); Tandon and Chandra (1964); Vasudeo (1954, 1962); Tilak (1959, 1960, 1963); Tilak and Rao (1968); Thind et al., (1957, 1958, 1959, 1960, 1966, 1968, 1970); Chona, Munjal and Bajaj (1959); Govindu and Thirumalachar (1961); Hansford and Thirumalachar (1948); Govindu (1963); Bose (1961); Bose and Muller (1964, 1965); Muller and Bose (1959); Kapoor and Gill (1961); Anantanarayanan (1964); Patil and Thirumalachar (1965); Patil (1968); Rangaswami and Seshadri (1970); Seshadri (1967); Muthappa (1967); Chiplonkar (1969); Pande (1969); Ullasa (1970); Kar and Maity (1970, 1971, 1972), Kamat and Ullasa (1974); Thite and Kulkarni (1973, 1975, 1976); Thite and Patil (1978) and many others.

Author has collected some Ascomycetes fungi from Western Ghats near Kolhapur and made detailed study into their diagnostic characters which revealed that some may be referred to be new species, while some are in the nature of new host records and new reports to India, on critical comparative studies and examination of authentic materials.

Our fundamental knowledge of the cytology of the Ascomycetes is mainly derived through the pioneer contributions of Dangeard (1894, 1897, 1904, 1908); Harper (1895-a, 1895-b, 1895-c, 1900, 1905); Maire (1903-a, 1903-b, 1905, 1908); Fraser and Brooks (1909); Fraser and Welford (1909); Clausen (1912); Andrus (1936); Andrus and Harter (1933, 1937); Allen (1905); Kamat and Pande (1971); Thite (1971); Zickler (1973); Jain and Morgan-Jones (1973); Rogers (1972, 1973, 1974, 1975, 1978); Kamat and Ullasa (1974); Huang (1976); Bezerra and Kimbrouch (1976); Fayret and Parguey-Leduc (1976), Hung and Wells (1977); Morgan-Jones and Hulton (1979); Hill (1970); Jones (1978, 1981); Jones and Holcomb (1978); Gottwald and Cameron (1980); Heath (1980). According to these studies it is known that in great majority of Ascomycetes, the nucleus exists in haploid condition through major part of life cycle, while in zygote diploid condition is attained through nuclear fusion. The first division of zygote nucleus being invariably reductional, however, in higher Ascomycetes there is occurance of a unique and intervening stage in life cycle; known as "dikaryophase" where two haploid nuclei are associated in pairs and behave like a physiological unit prior to fusion in "zygote cell". This phase, is however, lacking in lower Ascomycetes, thus showing a progressive and evolutionary line of development in various groups and classes with respect to their nuclear behavior.

The Ascomycetes have furnished a rich field for varied karyological and genetical research. The relatively large size of nucleus in the ascus of many Ascomycetes has facilitated detailed studies in to their structure and behaviour during meiotic division. The importance of this group of fungi in genetical studies is enhanced by the fact that it is frequently possible to recover and analyse all the products of meiotic devisions in the order of their occurence within the ascus.

The contributions made to this field of cytology and nuclear structure by Dangeard (1904, 1907); Drayton (1932, 1934); Martin (1946); Hirsch (1950); McClintock (1945) and many others, marked a new era in the study of Ascomycetes. The outstanding contributions of Dodge (1927, 1928, 1929, 1932, 1936, 1937) on the classical genus Neurospora give renewed impetus to the study of this fundamental aspect of the fungus life cycle which has proved to be of great value in determining the phylogenetic relationships and evolutionary lines of development, in the different groups of this heterogeneous class of fungi. More recent and valuable contributions to this phase are of Olive (1949, 1950, 1953, 1956, 1965); Singleton (1953); Kowalski (1964, 1965-a, 1965-b, 1966, 1968); Rogers (1964, 1965-a, 1965-b, 1966-a, 1966-b, 1968-a, 1968-b, 1968-c,

1969-a, 1969-b, 1970); Uecker (1967); Rudolph and Giesy (1966); Moore (1963, 1964, 1965); Moore and Korf (1963); Mottier (1907); McIntosch (1954); Lindegregic (1934); Lawrence (1952); Guillermound (1904, 1905, 1908, 1911); Gorden (1966, 1968); Gorden and Shaw (1958, 1964); Lespinasse and Salesses (1974); Upadhyay and Ravgi (1973); Harris and Roth (1974); Kimbrough (1974); Sakai, (1975); Efister (1975); O'Donnell, et al., (1976); O'Donnell et al., (1976); Paden and Linton (1976) and many others.

Indian contributions to this field are comparatively less. Tare (1955); Tilak (1959); Kalani (1965); Anantanarayanan (1964); Kulkarni (1963); Patwardhan (1966); Seshadri (1967); Muthappa (1967, 1969, 1970); Jagatap (1967, 1975, 1978); Chiplonkar (1969); Pande (1969); Anahosur (1969); Ranga Rao and Mukarji (1970); Ullasa (1970); Tendulkar (1971); Thite (1972, 1974, 1982); Rai and Chowdhary (1974); Ullasa and Pande illus, 1974 (recd. 1975); Kamat and Ullasa (1974), Patwardhan and Badhe (1979) and Rai and Saxena (1979) have added to this field of Karyology by selecting fungi from different groups such as Erysiphales, Meliolales, Sphaeriales, Hemisphaeriales, Dothidiales, Myriangales, Hysteriales, Clavicipitales and Microthyriales.

However, in comparison with the large number of established and valid species of the Ascomycetes, the reports

on chromosome complements and structure are relatively very meagre. The results obtained in few cases so far studied, appear to show a fairly good correlation between the chromosome complements and the broad taxonomic groups. It has been shown that in general the chromosome complement is larger in members of Ascohymeniales than in Ascolocularriales, varying from 4 to 16 haploid and 8 to 32 deploid in the former, while 2 to 4 haploid and 4 to 8 deploid in latter. According to Olive (1953) the haploid chromosome number in fungi ranges from 2 to 28.

The chromosomes are numbered usually according to their length, the largest chromosomal bivalent being the first and shortest bivalent being the last. The maximum length reported so far for the largest chromosome is 22.4 μ in Neurospora crassa (Singleton, 1953). This largest chromosome may or may not remain attached to the nucleolus, but the second chromosome is attached to it. Same type of nuclear behaviour has been reported by Seshadri (1967). Anahosur (1969) described in Tryblidaria that out of three, two chromosomal pairs were attached to two ends of nucleolus and the third pair remaining free.

Thite (1973) observed that the deploid nucleus in the developing ascus does not loose its homogeneity and can be seen as densely stained mass of chromatin with intact

nuclear membrane corresponding to the condensed nucleus reported by Rogers (1965-a) in Coniochaeta lignaria and Canham (1969) in Hypocrea ceitrina. The nucleolus either disappears early or is masked since it is rarely stained except for what appears to be an eccentric nucleolus. The position of nucleolus is some-times represented by negative image due to non-staining as reported by Finley (1970) in Pellicularia coleroga. During prophase of the first nuclear division two distinct and prominent centrioles emerge out on either pole and they remain attached to the nucleus. The nuclear membrane may be intact or disintigrated. The centrioles may remain very close or more apart from the nucleolus. Many a times one of the centrioles move apart and the other is still remaining attached. Half-moon shaped poler caps, one on each side, of prophase I nucleus were observed in Neurospora terricola by Raju (1978). Centriolar plague and spindle microtubules in the young asci of Sordaria humana were studied by Sakai (1975). Two dispositions of centriolar plague were observed, one entirely continuous to the nuclear envelope in a meiotic division and the other partially joined to the envelope in a mitotic divisions following meiosis. The spindle was formed inside the nuclear envelope and spindle microtubules terminated at the poler protrusion seem to connect directly with the centriolar plague passing through perforations of the nuclear

envelope. At metaphase four bivalents were clearly observed arranged in linear fashion in Meliola osyridicola (Thite, 1973). Sometimes one of the chromosomes get separated early and moves towards the centriole. Many times the orientation of the bivalents is not linear but may be perpendicular or slightly oblique. During anaphase chromosomes move to each pole along with centrioles. Lagging chromosomes were seen during telophase. The spindle orientation is either parallel or slightly oblique to the main axis. Due to oblique spindle orientation it is probable that the crossing-over might takes place as reported by Furtado (1970) in Sordaria brevicola. During this division four chromosomes were 'V' shaped, such type of chromosomes were also reported by Guillermond (1904) in Humaria (= Peziza) rutilans; and Thite (1973) in M.osyridicola. During the third nuclear division mitotic crossing-over of sister nuclei of univalents was noted. The same ascus may show the other type of crossing-over as occured during the second division of the meiosis between the two sister nuclei. Such type of rare meiotic spindle overlapping and crossing-over of nuclei during third division has been shown by Emmerson (1948) in Neurospora crassa.

It is obvious that number of nuclei in the ascospore provided a valuable character on the familial or genetic level. Berthet (1964-a, 1964-b) found the number of nuclei in the ascospore to be a leading character in Pezizales, where,

most of the genera have uninucleate ascospores, whereas there are four nuclei in Sacroscyphaceae and Halvellaceae, while in Morchellaceae there are thirty nuclei. Binucleate ascospores were rarely observed as an abnormality in Cryptomyces mullerii Ullasa by Ullasa and Pande (1975). Ascospores generally have a single nucleus, but some times become binucleate due to the mitotic division of their nuclei in Achaetomiella meguspora, Rai and Saxena (1979).

Patwardhan (1966) showed that the cytological studies and chromosome complements are suggestive of relationships and evolutionary trends in the genus Phyllactinia (Erysiphaceae) and that such studies may be useful in determining and delimiting the species. The chromosome complements in different species of Phyllactinia e.g. Phyllactinia-yarwoodi n = 5, (Patwardhan, 1966); Phyllactinia corylea n - 10, (Colson, 1938); Phyllactinia gmelinae n = 10, (Patwardhan, 1966) and Phyllactinia species n = 8 (Harper, 1905) show no definite correlation between different species in respect of chromosome counts. From these reports possibly it appears that either 4 is the basic number for this genus and the other forms with 5, 8 and 10 complements in haploid phase are polyploid forms in evolutionary line of development, or there are two basic numbers 4 and 5 (haploid) for this genus and 8 and 10 (haploid) are derived from them.

Contributions of different workers to this field of research show that occasionally there is uniformity of chromosomal events and chromosome counts in different species of the same genus e.g. different species of the genus Neurospora (N.crassa, N.tetrasperma, N.sitifolia) have haploid complements of 7 chromosomes. Cordiceps agariciformis and Cordiceps militaris have n = 2, Glomerella cingulata and Glomerella phyllanthi have n = 4. Different species of Patella show uniformly 4 chromosomes in haploid and 8 in diploid (Olive, 1950; Raymond, 1934). Similar type of uniformity is also exhibited by two species of Myriangium where the haploid chromosome complement is 4. Hirsch (1949) has reported similarity in cytological events in Hypocreaceae studied by her. Anahosur (1969) observed remarkable uniformity in chromosome complements in the two members of the order Hysteriales viz. Tryblidaria sp. and Lecanidium sp. Similar observations have been made by Muthappa (1967-a, 1970) in Tryblidariella sp.

From these reports and from the clue suggested by Carr and Olive (1958) it appears that chromosomal counts are suggestive of phylogenetic relationships between different genera of the group. This mode of approach in determining relationship in the Ascomycetes based on cytology and chrosome complements merits consideration but will have to be preceded by carefully planned cytological investigations

of different species of different taxonomic groups. The position appears encouraging and significant in this respect having regard to the occurrence of remarkable correlation between chromosome complements and the two braad series of Ascomycetes as indicated by Kamat and Pande (1971) such studies could eventually provide sufficient data for formulating a more natural system of classification based on nuclear structures instead of pure external morphology.

The genus Meliola was selected for cytological and developmental studies, the results of which are represented in the following Chapter.

Materials and Method (General) :

The material for these studies was collected at various stages of development from castle-Rock, Amboli, Ratnagiri, Panhala and Kandar-doh (Peth-lond). The infected leaves were collected, pressed, dried and preserved for taxonomical studies from which semipermanent slides were prepared. For the cytological and developmental studies the infected leaves were fixed on the spot and in the laboratory in Formol - Acetic-Alcohol fixative. The material after fixation was passed through various grades of alcohol to paraffin. Microtome sections of thickness of 5 to 7 μ were cut and stained in Haematoxylin and were mounted in Canada - Balsum.

Fixation : Following fixative with following formula was used.

(1) Formol - Acetic - Alcohol (F.A.A.) :

Formaldehyde 40 %	..	5 ml.
Glacial acetic acid	..	5 ml.
Ethyl Alcohol 50% or 70%	..	100 ml.

Staining : Following stains were used :

(1) Cotton blue in Lactophenol :

Semipermanent preparations were made by mounting the sections either in pure Lactophenol or in Cotton blue in Lactophenol.

(2) Heidenhein's Haematoxylin :

It has the following composition -

Haematoxylin	..	0.5 gm.
Ethyl Alcohol (Absolute)	..	5 ml.
Distilled water	..	100 ml.

The Haematoxylin powder was first dissolved in Absolute alcohol to which distilled water was added. It was then ripened either by exposing to the atmosphere for few days or by passing air in it for few hours.

Heidenhein's Haematoxylin followed by counter staining in orange G proved to be the best and gave satisfactory results with sharp pictures of the various structures.