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VARIATION IN FUNGI

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When techniques of growing fungi in the laboratory in the artificial medium became available, investigations were taken up on characteristics of different fungi relating to biology and physiological requirements. It was, however, observed that fungi, were variable in nature. Considerable variations in cultural characteristics were noted. To eliminate variabiiity and to have "pure cultures" monosporus or monoconidial or hyphal tip cultures were taken recouse to. Inspite of such techniques being adopted rigidly for study of fungi, variations could still be noticed. It was then generally accepted that variations constitute common features in fungi. Hence studies were taken up on effects of different culture media, pH, temperature, exposure to light in relation to growth characters. namely texture, colour, zonation etc. of the mycelium, abundunce of reproductive structures, aim being to find out constancy of characters which may be used for taxnomic purposes. In higher Basidiomycetes purposeful data could be obtained, but in Fungi Imperfecti, mass of data did not lead to any fruitful conclusion. On the other hand variations in cultural characteresties created extreme confusion in some genera from taxonomic stand point particularly in *Fusarium*.

In addition to variations normally noticed in the cultures of fungi in artificial media, some patches distinct from the rest of the mycelium appeared to grow out of the mycelial growth in form of a sector, patch, or fan. These patches were termed as "Saltants" and the phenomenon as "Saltation" a process, which was considered to be akin to mutation. Intensive studies were taken up on occurrence

nature and mechanism of saltation between 1920's and 1940's and large number of publications appeared in scientific journals, propounding theories to explain the phenomenon (Dickinson, 1932).

To explain variations in abundunce of sporulation in *Botrytis cinerea* which produces unicellular multinucleate conidia, Hansen and Smith (1932) and Hansen (1938) propounded a hypothesis named "dual phenomenon" in which they postulated occurrence of two types of nuclei - namely "M" type giving rise to mycelial forms and "C" type - conidial forms. "M" or mycelial types produce abundunt vegetative growth with very little conidial formation and they have "M" type of nuclei and "C" type abundunt conidial formation with very little mycelial growth with "C" type of nuclei. Intergrading forms were supposed to originate from intermingling of "M" and "C" types of nuclei.

Variation in fungi was viewed probably from perspective of Darwinism as a means of struggle for existence. With the advancement of knowledge in cytolcgy and genetics of fungi, this phenomenon began to be investigated on cytological, genetical and biochemical principles.

Fungi have varieties of life cycles with relative importance of haploid and diploid phase. In Phycomycetes, haploid phase is dominant and diploid phase is transitory consisting of resting zygote arising out of male and female nuclei or similar nuclei which cannot be distinguished into male and female. In Ascomycetes true diploid phase is very short and consists of diploid nuclei, in the penultimate cell of ascogenous hyphae, which undergoes immediate reduction division resulting in formation of ascospores. In Basidiomycetes, true diploid stage is also very short but the viable stage is characterised by dikaryotic mycelium with a pair of conjugate nuclei in each somatic cell and basidium. Fungi Imperfecti lacks sexual mechanism altogether and diploid or dikaryotic stage is totolly absent.

Characteristic feature of many fungi is formation of heterokaryons in which genetically different nuclei may coexist and multiply in the same cell or cytoplasmic system. Heterokaryons may originate by mutation in homokaryon or by nuclear migration following anastomoses of hyphae and their fusion. Heterokaryons may be sorted out in the process of sexual reproduction in which new recombinations may give rise to different types of heterokaryons. Besides heterokaryosis, except in Basidiomycetes with monokaryon and dikaryon stages in both of which the number of nuclei is definite one or two as the terminology indicates, and in penultimate cells of ascogenous hyphae in Ascomycetes, hyphal cells are multinucleate. Constancy of number of nuclei are not maintained in these cells. An extreme situation is present in Phycomycetes, where the entire thallus is coenocytic. Asexual reproductive units e.g, conidia, chalamydospores etc. which play an important part in perpetuation, may also be multinucleate and hetero-

karyotic. In a number of cases, multinucleate condition may arise due to division of a single nucleus.

From analogy with higher plants, it has always been considered that new genetic recombinations can arise only through sexual reproduction. Apart from Fungi Imperfecti, where sexual mechanism is not known, in many other fungi, where sexual reproduction is known to occur, karyogamy and meiosis are not obligatory in perpetuation. To account for variability mutation has been considered to be the only factor. In the opinion of Muller (1947), the concept that such variabilities as noticed in fungi can arise through mutation is not very convincing. Most of the genetic mechanisms, operating in microorganisms namely transformation, transduction, bacterial conjugation, lysogeny, sexduction etc. which have been found in bacteria are not applicable in fungi. The only known alternative mechanism is somatic recombination which can take place in vegetative cells of fungi and lead to the same results as meiotic recombination. This was first observed by Raper (1952) in *Aspergillus nidulans*. Pontecarvo (1954) suggested the term 'parasexuality' to describe this phenomenon which is an alternative and equally effective mechanism to sex in fungi. Stages in parasexual cycle are now well known. The stages consist of (a) formation of heterokaryon by fusion of hyphae having unlike haploid nuclei ; (b) nuclear fusion (frequency 10^{-6} of two haploid unlike nuclei to form a diploid heterozygous nucleus, haploid and diploid nuclei occur in the same heterokaryon and multiply together and later segregation occurs ; (c) mitotic crossing over (10^{-2} per nuclear divison) ; (d) haploidization (10^{-3} per nuclear division) of diploid nucleus. The change in ploidy level does not take place through meiosis (Kafar, 1961). Mitotic recombination and haploidization may take place concurrently.

Parasexuality has been demonstrated in *Aspergillus fumigatus* (Stromnaer and Garber, 1963) ; *A nidulans* (Raper, 1952 ; Pontecarvo and Raper, 1952, Pontecarvo *et al*, 1953, Pontecarvo, 1954) ; *A. niger* (Pontecarvo and Forbes, 1953 ; Kafer, 1961) ; *A. oryzae* and *A. sojae* (Ishitani *et al*., 1956) ; *Cephalosporium mycophyllum* (Tuveson and Coy, 1961) ; *Helminthosporium sativum* (Tinline 1961) ; *Penicillium chrysogenum* (Pontecarvo and Sermanti, 1954). *Fusarium oxysporum f. pisi* (Buxton, 1956 ; Tuveson and Garber, 1959) ; *F. oxysporum f. cubense* (Buxton, 1962) ; *Verticillium albo-atrum* (Hastie, 1962) ; *Coprinus fiematarius* (Gans and Prud' Homme, 1958) and *Ustilago maydis* (Holliday, 1961). Somatic recombinations have been reported to occur in *Schizophyllum commune* and in some species of *Coprinus* in crosses between dikaryons and monokaryons (Buller phenm.non) and genetic mechanism involved may be mitotic (Crowe, 1960).

Investigation of Case and Giles (1958) on certain mutants of *Neurospora crassa* suggest possibility of intragenic recombination. Similar observations have

been made in *Neurospora crassa* by Ishikawa (1962) and *Schizosaccharomyces pombe* (Leupold, 1958).

Questions may now arise on role of mutation in production of new variants. Artificially induced mutants have been produced by action of both physical and chemical mutagenic agents in a number of fungi. While significant morphological changes have not been achieved because of simpler structure of the vegetative body of the fungi, biochemical mutants have been obtained. Among physical mutagens X-ray and ultra-violet rays have been mainly used, while a number of chemical mutagens have been found to be effective. Both X- and UV radiation can cause point and chromosomal mutations. Frequency of mutation increases in a linear fashion upto saturation with incresing doses of X-rays, while with UV radiation, a non linear does effect curve is obtained, which with high doses decreases. Radiation effect can be interpreted by target theory a single hit with X-ray is sufficient to induce mutation, while with UV radiation multiple hits are necessary.

A large number of chemical substances have been found to be mutagenic, but individual mutagens differ in their potency. Mutation and inactivation rates are not correlated with each other. In generally frequency of mutation among surviving cells increases if length of treatment or concentratation of mutagen is increased. Chemically induced mutants are believed to arise mostly through changes in base sequence of DNA, bases may exchange with other bases, nucleotides may be added or deleted or entire strand of DNA may be shifted or deleted. (Esser and Kuenen, 1967).

Frequency of spontaneous mutation is usually very low. Precise data on mutation rates are available only for reversions of auxotrophy to prototrophy, which are in the order of 10^{-7} to 10^{-9} per nucleus and nuclear division. Spontaneous mutation is believed to involve changes in the DNA base sequence.

In response to exposure to pesticides new strains of fungi resistent to the action of pesticides have arisen in nature presumably due to gene mutation. Strains of *Candida* sp. a dermatophyte showing increased tolerance to amphotericin B and nystatin have decreased sterol content (reason for increased resistance - a condition arising out of slight changes in the gene mechanism responsible for sterol production (Dekker, 1972). It is known that antifungal activity of cycloheximide is due to inhibition of protein synthesis on the ribosomes. Cooper *et al* (1967) observed that in tolerant strains of *Saccharomyces cerivisae* obtained from sensitive strains in the process of gene mutation, composition of ribsome has been slightly changed so that tolerence to antibiotic has been changed without impeding the capacity of ribsome to synthesize proteins. In *Coprinus lagopus* in relations to ethionine - an antimetabolite of methionine, strains have arisen with

resistance to ethionine due to mutation in the structural gene which forms the messanger RNA coding for this enzyme (Lewis, 1963).

Reverse effect has been noticed in *Ustilago maydis* with reference to tolerence to Antimycin A. Normally *U. maydis* can resist action of this antibiotic which interferes with respiration by blocking electron transport between cytochromes *b* and *c* by taking recourse to alternative pathway and another terminal oxidase. Exposure to antibiotic may cause gene mutation, as a result strains may be produced which lack the property of building this alternative pathway and become sensitive to the drug (Georgopoulos and Sisler, 1970).

It has been claimed that such changes are always taking place in nature as fungi are sensitive to mutation. These changes may result in one or two characters without affecting morphology, biology and major physiological processes and may not be perceptible in nature. According to Wolfe (1971), theory of genetic homeostasis indicates that while particular individuals with a particular characteristic change may be selected or produced, whole population does not shift in this direction, since there are many characteristics in the population held in complex balance in the existing environment. It is only when such mutants have a large and continuous selective advantage, population gradually becomes changed and effect becomes perceptible.

Cytological studies carried with a number of fungi have shown existence of polyploidy. Naturally occurring polyploidy has been observed in *Allomyces arbuscula* with chromosome numbers of 8, 16, 24 and 32 in gametophytic generations and of 14, 28, 50 (56) in *Allomyces javanicus*. (Emerson and Wilson, 1949, 1954). Emerson and Wilson (1949) obtained an allopolyploid by crossing polyploid strains of *Allomyces arbuscula* and *A. javanicus*. Chromosome numbers alone provided in these cases clear evidence of polyploidy. In *Cyathus stercoreus*, 12 bivalents are observed in many basidia, but association of four chromosomes (quadrivalants) observed in some suggests polyploidy (Lu, 1964). In *Xylaria curta*, eight to nine bivalents are normally noticed in asci, but in some seven bivalents plus an apparent quadrivalent have been observed (Rodgers, 1968). Spontaneous polyploidy has been observed in *Saccharmyces cerivisiae* (Lindegren and Lindegren, 1951), in *Schizosaccharomyces pombe* (Leupold, 1956), *Penicillium notatum* (Sansome, 1949), *Aspergillus oryzae* and *A. sojae* (Ishitani *et al.* 1956). Polyploidy has also been artificially induced in *Achlya spp.*, *Pythium debaryanum*, *Phytophthora cactorum* (Sansome and Harris, 1963) ; in *Acpergillus oryzae* and *A. sojae* (Ishitani *et al*, 1956) ; in *Penicillium notatum* (Sansome 1946), *Penicillium spp.* (Kostoff, 1946), *Neurospora crassa* (Sansome, 1956), *Podospora anserina* (Franke, 1962), *Aspergillus nidulans* (Pontecorvo and Raper, 1952), *Saccharomyces cerivisae* and other yeasts (Bauch, 1941a, b ; Subramaniam and Ranganathan, 1948). Mutagenic agents employed for the purpose belong to

spindle poison group (colchicine, camphor. acanaphthene), growth promoting substances like indole, napthalene acetic acid or carcinogenic substances like benzopyrene and methyl cholantherene (Esser and Kuenen, 1967). Polyploid nuclei showed greater volume and cells containing polyploid nuclei was 1.6 to 2.5 times greater than average cells and DNA content nearly double of that of haploid nuclei. Polyploid strains, however, regularly undergo reduction to haploid number after a time even in the vegetative phase and nuclear migration may result in a heterokaryotic condition with nuclei of different chromosome numbers. Somatic reduction was first noticed by Pontecorvo *et al* (1953) in *Aspergillus nidulans* and later in *Neurospora crassa* by Sansome (1956) and later by other workers. This reduction may take place unusually rapidly in *Podospora anserina* (Franke, 1962). It is ratner unfortunate that investigation on polyploidy in fungi have not received adequate attention particularly in relation to pathogenicity. Multinucleate condition in fungi is considered to be a means of adaptation, so polyploidy may have a special significance.

Semipermanent changes or modifications "daurmodification" due to changes in cytoplasm was reported earlier. Recently Jinks (1954, 1956, 1957, 1958) have shown involvement of cytoplasm in producing continuous variation in *Aspergillus glaucus*. It has been observed that variation in characters like germination of spores, growth rate, pigmentation and perithecial density are under control of cytoplasm during differentiation and ageing. Hyphal tip culture from *Aspergillus glaucus* and *A nidulans* showed greater spontaneous variations which are of cytoplasmic origin. Correlated responses between certain characters namely lower germinability and slower growth rate and greater germinability and faster growth rate may be due to binding of all determinant to one cytoplasmic element or that a single pleiotropic demand is involved. Cultures from hyphal tips have been found to show greater variations while those from conidia much less and single ascospore practically nil. These differential behaviours of the cultures from three different sources namely hyphal tip, conidia and ascospore are attributed to different cytoplasmic contents. It has also been suggested that for the formation of different organs, hyphae, conidia, perithecia etc. requirements of cytoplasm may be different. Ability of the fungus to form perithecia often lost due to continuous vegetative propagation may be explained on the basis of unbalancing of cytoplasm variations noted in respect of conidial formation and 'dual phenomenon' may be explained in terms of cytoplasmic variation.

Discontinuous variation in the form of sectors ('Saltant patches') have been noticed in a number of fungi. Small or 'petite' cells in yeasts which are considered to be vegetative mutants are examples of extrachromosal inheritance. Studies by Ephrussi and his coworkers (1951, 1954, 1956) have shown that the 'petite'

cells result from a slower division rate of the cells which compose 'petite' colony. Decrease in growth rate is due to the fact that cells cannot undergo respiration, but only fermentation. This respiratory defect results from a change in the complement of respiratory enzymes - vegetative mutants lacking in atleast four enzymes connected with respiratory system. Formation of respiratory enzymes has been found to be controlled by an interplay of chromosomal and extrachromosomal factors, which are probably mitochondria. Similar observations have been made in respect of "poky" mutants in *Neurospora crassa*. ',Poky" mutants which show slower vegetative growth have an altered complement of cytochromes and respiratory enzymes' (Mitchell *et al* 1953, Hardesty and Mitchell, 1963). Exact nature of genome-plasmone relationship in this case is not known (Silage, 1963) but extrachromosomal inheritance through mitochondria appears to be involed (Esser ano Kuenen, 1967).

Indications of cytoplasmic inheritance have been noticed in *Blastocladiella emersonii* (Cantino and Harnestein, 1954). Role of cytoplasmic inheritance in variations in Hymenomycetes was first shown by Harder (1927) in *Pholiota mutabilis*. Cytoplasmic varitions of continuous nature was also recorded in *Collybia velutipes*. (Aschan, 1952). The position has been reviewed by Papazian (1958).

In Hymenomycetes, a dikaryon is usually produced by migration of nuclei from one homokaryon say A to another homokaryon say B. When nuclear migration from A to B takes place, cytoplasmic contribution of A is virtually nil and cytoplasm of B is involved in expression of characters like mycelial growth. Reciprocally constituted dikaryon in which migration from B to A takes place and cytoplasm of A is involved in mycelial growth in the dikaryotic phase. Investigations of Day (1959) on *Coprinus lagopus* on reciprocal crosses using a mutant with *pale gills* showed that tetrad formation in that agaric is controlled by cytoplasm.

Slmilarly mutual antagonism between mycelia of two strains of the same fungus and formation of a line of separation when two strains growing in the same agar plate have been ascribed to incompatibility of different cytoplasm, though the process is determined by genes. (Esser ann Kuenen, 1967).

Formation of 'saltant' patches or sectors differing form the original mycelium in growth form of the hyphae, failure to form conidia, density of conidiophores, number of fruit bodies or other morphological macroscopic features has been noticed in many fungi. It is now considered that formation of sectors is due to mutations in the cytoplasum which alter the habit of fungal thallus frequently, as studied in *Aspergillus glaucus* (Sharpe, 1958), *Pestalozzia annulata* (Chevaugeon and Leport, 1960), *Curvullariu pallescens* (Cuzin, 1964). Formation of sectors is not

dependent on the genome and this type of mycelial modification persists through asexual reproduction. This mycelial modification in the mutant can be transferred to normal mycellium through hyphal fusions.

These variation which are due to changes in cytoplasm have been ascribed to the existence of cytoplasmic determinants or particles which are self replicating. Role of cytoplasm in many fungi has not been limited only to determination of specific steps in differentiation in morphogeneses, as controlled or directed by the genetic information in the nucleus. Cytoplasm has been ascribed to have an independent function in control of developmental processes or may be considered as autonomous determinants af genetic information. Genetic role of mitochondria appears to be involved in the process. Enzymes and pigments deficient in colonies arising due to cytoplasmic variations are all associated with mitochondrial changes. exact mechanism of which is not know, though structural changes in mitochondrial DNA have been detected

In respect of fungi inciting deseases in plants considerable variation in pathogenecity is noticed within the same species. A species, represents not only a totality of individuals with a morphological resemblance amongst themselves and their progeny, but essentially similar physiological and parasitic abilities. Recognition of these facts has led to the discovery of parasitic specialization. Parasitic abilities are however genetically controlled. In obligate parasites, virulence and avirulence have been found to be controlled by one or more genes. Variations arise mainly due to hybridization and mutation. However there are evidences of involvement of extrachromosomal factors in such cases. In crosses between different races of *Puccinia graminis tritici*, Johnson (1946) observed reciprocal differences in pathogenecity. These characters were inherited unaltered through a number of generations. Analysis of crosses showed that genome differences were not responsible for changes in pathogenicity.

We are gradually beginning to understand the various mechanisms involved in inducing variation in fungi. It may, however, be pointed out that work has been carried with a very limited number of fungi more amenable for genetical study. Further work is needed to elucidate the phenomenon in diverse groups

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