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Some recent contributions to our knowledge of Heterothallism in Fungi

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In 1904 Blakeslee discovered that in certain species of *Mucor* zygospores were produced only when two separate mycelia met. Such species he called "Heterothallic" in contrast to the "Homothallic" ones where zygospores were produced without the requirement of a separate complementary strain. Although he designated the two kinds of mycelia as + and —, he associated the idea of sex with heterothallism as he found only two complementary strains in the group. For some time knowledge of this phenomenon was confined to the Mucorales only. In 1917-18 Mlle. Bensaude and Kniep both independently discovered heterothallism in the Basidiomycetes. Their researches revealed the presence of more than two complementary strains. This, as well as the fact that sex organs are entirely lacking in this group, has led many botanists to dissociate the idea of sex with heterothallism. Following Dodge, heterothallism can be defined as the condition where "monosporous mycelia produce perfect stages only when mated with their reciprocal haplonts". Within the last fifteen years our knowledge of the heterothallism and genetics of the Fungi has advanced with such rapid strides that at the present time this phenomenon is known to exist in at least some species of all the main groups of Fungi. It is the object of the present paper to summarize briefly the more important works done in the various groups in this line.

In the Myxomycetes, which were excluded from the Fungi by DeBary but have been included by many authors in their books on Fungi, Skupienski found that in *Didymium difforme* the plasmodium on fruiting gave rise to spores which were unisexual and produced unisexual myxamoebae that fused only with the myxamoebae of the opposite sex to give rise to a young plasmodium. In the Archimycetes some dioecious forms are known where two separate thalli function as the male and female gametangia, the female being slightly larger than the male. As examples one may cite *Lagenidium Zygorhizidium, Ancylistes closterii* and *Olpidium trifolii.*

Coming to the Phycomycetes proper, we find that in the Oomycetes dioecism was reported in some species of the Saprolegniales by Leitgeb and others but without adequate experimental evidence.

In 1926 Couch first experimentally demonstrated heterothallism (dioecism) in *Dictyuchus*. He found that certain strains when grown separately, invariably remained sexually sterile, but when mated, the male strain produced antheridia and the female oogonia at the points of contact. He also made interspecific crosses between the different strains of *Dictyuchus monosporus, D. magnusii, D. carpophorus* and *D. sterile* and found that the progeny obliterated the specific boundaries. He found a parthenogenetic strain without antheridia and crossed it with a male strain when the antheridia of the latter applied themselves on the oogonia of the parthenogenetic strain. Again, when crossed with a female strain, oogonia were formed both on the female as well as in the parthenogenetic strain but the latter developed its latent faculty of producing antheridia which applied themselves to the oogonia of the female strain and eggs were produced. Couch also made the interesting observation that parts of the germinating mycelium may be male, parts female and parts may be mixed, showing that partial segregation of sex may take place early in the egg-germination. In the other genera of the Saprolegniales heterothallism is not yet known with certainty except in *Achlya bisexualis* reported by Coker and in *Sapromyces reinschii* of the Leptomitaceae reported by Sparrow.

In the Peronosporales, Leonian found that out of his 85 strains of *Phytophthora omnivora* 48 were always heterothallic and the rest were inconstant in their behaviour, being periodically heterothallic and homothallic. Even the heterothallic forms lost their power to produce oogonia in mixed cultures in about 5 generations. An interesting observation was made by Gadd in 1924. *P. Faberi* growing on Cacao and on rubber, separately produced no oospores but when the strains from these two different hosts were mated, abundant oospores resulted. In India Narasimhan in 1930 found that *P. arecae* on *Areca* and *Loranthus* were male strains producing only antheridia while the strain on *Santalum* was female, producing only oogonia. The strains of *P. parasitica* and *P. meadii* in his collection were only female. These observations have given rise to an interesting hypothesis concerning the rarity of oospores in nature which is explained by the possibility of the two sexual strains becoming isolated on the different host plants in nature. The two host plants may grow in sufficiently close proximity to allow for occasional transfer of zoospores of one strain to the other, resulting in the infrequent oospores.

In *Peronospora parasitica* De Bruyn found that out of 11 strains 8 could be divided into two groups which behaved unisexually, forming oospores only on contact with a strain of the opposite group. One strain was potentially bisexual, reacting with either group, and the rest were homothallic.

In the Zygomycetes the classical observations of Blakeslee have been greatly extended both in his laboratory and elsewhere. It

has been found that though the plants are strictly dimorphic, the sexual differences may show varying degrees of intensity. A strain that is very strongly male will conjugate with strains with all degrees of femaleness but a weakly male strain will not conjugate with one weakly female and *vice versa*. The various attempts at intergeneric and interspecific crosses have mostly resulted in imperfect zygospores. The imperfect hybridizations between the homothallic species and the two strains of the heterothallic ones revealed the presence of two strains of the former — (a) those with a "minus" sexual tendency showing a strong reaction with the "plus" races and weak or no reaction with the 'minus' ones,—examples *Absidia spinosa, Zygorhynchus moelleri,* etc., and (b) those with a 'plus' tendency like *Zygorhynchus heterogamous*. Satina and Blakeslee have shown that plus and minus strains possess quantitative bio-chemical differences. They have obtained a difference between the two sexes in a reaction analogous to the Manilov reaction, in which the female strains showed a greater capacity to reduce N/100 $KMnO_4$ solution. Schopfer found in *Mucor hiemalis* a difference between the + and the — strains in their reaction to toxic substances and in their growth on certain natural media. The + and the — gametangia also showed a difference in their carotin content and a strain of bacteria was found which would attack the + but not the — strain. Schopfer regards these bio-chemical differences to be secondary sexual characters.

Differentiation of the sexes occurs in *Mucor mucedo* in some nuclear division within the zygospore prior to its germination so that the sporangium formed from it contains spores of one sex only. In *Phycomyces nitens* the germ sporangium has spores of both sexes showing that sex-segregation occurs in the sporangium. With a delicate technique "grafting" was accomplished by Burgeff in *Phycomyces nitens* between a + and a — mycelium. The mycelial outgrowths from this operation formed sporangia which contained + spores with nuclei coming directly from the + mycelium, — spores with nuclei from the — mycelium and also neutral spores containing nuclei from both + and — mycelia, the mycelia from the last type of spores forming abortive or imperfect zygospores with either of the two sexes. Burgeff crossed some mutant forms of *Phycomyces blakesleeanus* with the parent and found the forms "pilobolides", "arbusculus' and "pallens" to be recessive to the parent but the form "mucorides" was dominant. In a cross between *P. nitens* and *P. blakesleeanus* the latter was found to be recessive. In the Choanephoraceae Weber and Couch have reported heterothallism in *Blakeslea trispora* and *Choanephora conjuncta* respectively. In *Cunninghamella bertholletiae* Burger could not find any strict sexual dimorphism as seen in the Mucoraceae. Difference of sex was quantitative rather than qualitative and union occurred whenever compatible gametes were present. If one of the strains be

designated as + and all others which reacted with it as —, then there were strains which conjugated with both + and — mycelia.

Lastly there is an interesting hypothesis by Burgeff according to whom the attempts at hybridization have led to parasitism of *Parasitella simplex* on *Absidia glauca*. He found the + strain of *P. simplex* parasitized only the — strain of *A. glauca* and the + mycelium of *A. glauca* was parasitized only by the — strain of *P. simplex*.

Amongst the lower Ascomycetes heterothallism has been reported in *Penicillium luteum* by Derx who found that single spore cultures formed sterile haploid ascocarps, fertile fruit bodies being produced only by the union of the opposite sexual strains. The rarity of ascocarps in this genus may be due, according to him, to the modern methods of purification, *i.e.,* by single spore cultures and it is suggested that strains from different sources should be crossed. Blochwitz, however, thinks that Derx's results are due perhaps to contamination with *Aspergillus glaucus*.

Wieben demonstrated that in *Taphrina epiphylla* and *T. Klebahni* the ascospores or the spores budded off from them are unisexual and uninucleate. Active infection of the host was possible only when the germ-tubes of two ascospores of the opposite sexual tendencies fused and gave rise to a diploid mycelium with each cell containing a dicaryon. In *T. deformans,* however, Fitzpatric observed that though the mycelium carried dicaryons, the germ-tube from a single spore could produce infection. Its nucleus divided into two and thenceforth the two nuclei divided conjugately.

Some of the Ascomycetes parasitic on insects are dioecious and the motility of their hosts probably overcomes the difficulties of fertilization. In *Pericystis apis* and *P. alvei* Claussen observed that the male plants were larger than the female. In Laboulbeniales there are also dioecious forms such as *Amorphomyces falagriae* where there are paired spores of different sizes, the smaller giving rise to the male and the bigger to the larger female.

In the Discomycetes Dodge reported heterothallism in *Ascobolus magnificus* which is structurally monoecious bearing both antheridia and oogonia, but functionally dioecious. The antheridia of strain A will fertilize only the oogonia of strain B and the antheridia of strain B will unite only with oogonia of strain A. The fungus was, therefore, self-sterile. A similar situation has been observed by Betts in *A. carbonarius*. *A. strobilinus,* however, is homothallic. In *A. stercorarius* Dowding observed that the oidia produced by this fungus are unisexual and are transported by mites or flies and possibly wind, to the mycelium of the opposite sex where apothecia are produced. A similar function for the oidia or microconidia has been discovered in *Sclerotinia gladioli* by Drayton. As early as 1861 Tulasne described them as spermatia and prophesised that "sometime or other it would be demonstrated that there

resided in them a certain force or nature like that of pollen". Drayton found that the mycelium produced microconidia like *Botrytis,* sclerotia like *Sclerotium* and receptive hyphae. Apothecia were produced only when microconidia from a compatible strain were placed on the receptive hyphae. Fruit bodies did not develop in pairings of vegetative hyphae or if microconidia were placed on the vegetative hyphae. The fungus was thus found to be self-sterile but it is inter-fertile as the strains on *Crocus* and *Gladiolus* were found to be compatible strains and could fertilize mutually. *Sclerotinia sclerotiorum* and *S. Ricini,* however, formed apothecia in monoascosporic cultures and were probably self-fertile. The condition in *Ascobolus* and *Sclerotinia,* therefore, is not sex-heterothallism or the condition of maleness and femaleness of the two strains such as occur in the Mucorales, but physiological hetero-thallism based on one sterility or compatibility factor which segregates in a 1 : 1 ratio in ascospore formation. It is comparable to the self sterility to their own pollen in such flowering plants as *Primula* where there are also only two interfertile but intra-sterile types. The condition in *Humaria granulata* is interesting. It is an apogamous form and the oogonia fuse with hyphae only from a complementary strain. Gwynne-Vaughan who worked out this species, holds that this is not sex-heterothallism as the male organs are entirely lacking. She has, therefore, advanced the theory of "Nutritive heterothallism" on the assumption of a hereditary ability of the different strains to extract different nutritive substances. Suppose one of the mycelia can extract only food substance A from the substratum and the complementary mycelium can absorb only the food substance B present in the same substratum. When both the strains are present the substances A as well as B, both of which are necessary for ascocarp formation, will be absorbed and fruit-bodies will result. This theory has been tested by Gregor in the pyrenomycete *Ceratostomella paradoxa* by using various sub-stances to induce the formation of fruit bodies in single-spore cultures but negative results were obtained and Gwynne-Vaughan and Williamson themselves stated that they have not yet been able to justify the term. Among the Hysteriales in *Lophodermium pinastri,* the cause of Pine blight, Jones observed that apothecia arise only from inter-mingling of mycelia after multiple infection.

In the Pyrenomycetes heterothallism is known in a number of forms but the work of Dodge and others on *Neurospora* has thrown a flood of light on the genetics and heterothallism of the Ascomycetes. *N. sitophila, N. crassa,* and *N. tetrasperma* are heterothallic but *N. erythraea* is homothallic. Moreau believes that actual contact between the complementary strains is not necessary for the production of perithecia but these are due to the diffusion of certain substances analogous to hormones. In a U tube with a constricted middle and the two strains placed at the two ends, perithecia appeared only on one end, and the narrow part when

incubated gave rise to no mycelial growth. Dodge and Aronescu, on the other hand, found that perithecia arose only by contact of the two strains even in the middle of the U tube if sufficient oxygen is present. In *N. sitophila* Wilcox isolated all the 8 ascospores of a row and found that either the + and — spores alternated in pairs or 4 spores of one sex lay between the two terminal pairs of the opposite sex. They never alternated singly, thus showing that segregation of sex took place at the 2nd division in the ascus. Lindegreen found that in *N. crassa* sex-segregation took place more frequently in the first than in the 2nd division so that more often the 4 consecutive spores were of one sex. *N. sitophila* formed orange coloured monilioid conidia but one of its mutant forms produced albinistic conidia of *Botrytis* or *Sclerotinia* type. On crossing the albinistic strain with the coloured one, the factors for sex and colour behaved as independent unit characters and segregated separately. Of the 8 ascospores, four gave rise to albinistic strains of which two were + and two — and the other four gave rise to two + and two — normal coloured strains. Based on their position in the ascus, it was found that both the characters may segregate at the first division or both may segregate at the second or one at the first and another at the second division in the ascus. The progeny of a cross between albinistic *N. sitophila* and normal *N. crassa* consisted mostly of *N. crassa* type, a few were albinistic and some showed combination of characters being albinistic intermediates. *N. tetrasperma* normally has 4 spores in the ascus each of which is binucleate and bi-sexual, giving rise to perithecia. Often, however, the number of spores is increased to 5 to 6 by the division of one or two of the larger binucleate spores into two smaller uninucleate ones which are then unisexual and require fusion with a complementary strain to produce perithecia. It was found that treatment with X-ray affected the nuclei of one sex only in a binucleate bisexual ascospore so that they behaved as unisexual spores. Some strains were found with aborted indurated asci. The lethal for ascus abortion was heritable being carried in the nucleus and each binucleate spore had one normal and one lethal nucleus. The F_1 hybrid of this and the normal strain showed some aborted asci. The small uninucleate ascospores that happened to get the lethal nucleus died soon after germination while others without it produced normal mycelium. In a cross between *N. sitophila* and *N. tetrasperma* the progeny resembled the former more closely but there was much spore abortion. A condition similar to *N. tetrasperma* has been observed in *Pleurage anserina* by Dowding and Ames. The ascus has three kinds of spores, (a) normally four binucleate, bisexual spores, (b) two giant 4-nucleate bisexual spores and (c) occasionally a few dwarf uninucleate unisexual spores. The mycelia from uninucleate spores were self-sterile but reciprocally fertile and those from normal and giant spores self-fertile but isolations from young hyphal tips were self-sterile. The

mycelium from normal ascospores is, therefore, considered to comprise of two hermaphroditic cultures growing intermingled as one, each giving rise to male and female organs, which are self-sterile but reciprocally fertile. It is therefore held to be no longer justifiable to conclude that a culture derived from a single spore is simply bisexual and self-fertile because it produces fruiting bodies when grown alone. In *Diaporthe perniciosa,* Cayley found three forms of heterothallism—(1) true sex-heterothallism, (2) heterothallism based on one or two self-sterility factors, these forms showing intra-perithecial aversion and (3) heterothallism based on inter-racial sterility but self-fertility factors—these showed inter-racial aversion, *i.e.,* a sterility between biologic races. This negative sterility factor causing aversion is segregated independently of sex. Edgerton found that in *Glomerella cingulata* the two complementary strains, which had formerly been regarded as different species, each separately gives rise to a few perithecia with small or immature asci but at their point of contact, abundant perithecia with large well-developed asci are produced. Shear and Wood think this to be a case of hybridization but Edgerton does not regard it to be so. *Ceratostomella pluriannulata, C. ulmi* and *C. paradoxa* are heterothallic forms, the last in single spore cultures giving rise only to the conidial form *Thielaviopsis paradoxa.*

More than 60% of the Basidiomycetes are supposed to be heterothallic. In Ustilaginales, Kniep showed that in *Ustilago violacea* and other forms, the sporidia belonged to two groups which fused with each other. Dickinson observed that single sporidial cultures of *U. levis* (*U. Kolleri*) and *U. hordei* could not infect the host which was only possible after fusion with sporidia of the opposite sex. He also found that the four spores from the same basidium showed differences in vegetative characters such as colour of the mycelium, form of the colony, etc., and these characters together with sex segregate independently either at the 1st or at the 2nd division in the promycelium. Hüttig has shown that temperature has a considerable effect on the proportion of segregation at the two divisions in *U. avenae.* In *U. Zeae* Hanna found that monosporidial cultures could infect tender parts of the host but chlamydospores were not formed. He also showed that there were 4 strains with two alleomorphic pair of genes-AaBb. If disjunction occurs in the 1st division then sporidia of one promycelium will be of 2 kinds, either AB and ab or aB and Ab, but if disjunction occurs at the 2nd division, then all the four kinds of sporidia will be borne on the same mycelium. The same condition was found in *Sorosporium reilianum* and Bauch also obtained similar results with *U. longissima.* Hanna crossed *U. avenae* which forms loose smut with echinulate spores and *U. levis* forming covered smut with smooth spores and found that the appearance of the heads produced by the hybrids was variable, being more of the loose type and that echinulate was dominant over smooth.

Crossing *Tilletia laevis* with *T. tritici* he found *T. laevis* to be dominant and Rodenhiser found *Sphacelotheca cruenta* to be dominant over *S. sorghi.*

In 1927 Craigie discovered heterothallism in the Rusts and found that monosporidial mycelia of *Puccinia graminis* and *P. helianthi* produced both spermogonia and sterile aecial fundaments, but if spermatia only from the complementary strain were placed on it, fertile aecia resulted. The spermatia, which were long held to be of doubtful function, bring about the diplodization of the haploid monosporidial mycelium in two ways—(1) the spermatial hyphae grow down into the aecial beds and fuse there with the basal cells forming the "two-legged" cells which cut off aecidiospores as observed by Blackman and Christman or (2) spermatia fuse with the receptive hyphae which come out of the stomata, or between the epidermal cells or from the ostiole of the spermagonium, and the spermatial nuclei pass through the mycelium diplodizing every cell, the "two-legged" cells originating by the downward proliferation of the basal cells. The first process according to Hanna takes 48 hours in *P. graminis* and Allen observed that by the second process at least 60% of the cells in *P. sorghi* become diploid only 24 hours after spermatization. According to Brown diplodization can be accomplished even by a mycelium carrying a dicaryon like that forming the uredinia. Newton and Johnson found that a hybrid resembles, in pathogenic characters, the aecial parent more than the spermatial parent. This is explained as cytoplasmic inheritance as the aecial parent contributes the major part of the protoplasm of the hybrid. Craigie, however, observed that the pathogenic behaviour of the hybrid, in *P. graminis* to be intermediate between the parental forms. A cross between forms with orange and greyish-brown uredinia gave rise to hybrid with normal red pustules which in the F_2 generation formed 4 types of uredinia — normal red, orange, greyish-brown and almost colourless. That hybridization occurs in nature is shown by the dissociation, in pure cultures, of the heterozygous strains into its constituent forms and this probably explains the reported break down in the resistance of the host. Heterothallism has been reported in a number of genera of the Uredinales such as *Puccinia, Uromyces, Gymnosporangium, Melampsora, Endophyllum,* etc., and an investigation into the other genera will certainly prove fruitful.

In some species of the Autobasidiomycetes heterothalism has been intensively studied. Kniep found in *Schizophyllum commune* that the mycelium from a single spore can give rise to the fruit bodies but spores formed from them are of the same sex and clamp connections are entirely absent though the hyphae exhibit anastomoses. Thus it was found that the clamp-connections aer not solely nutritive as was thought before. He also found that there are four different strains whose genetic constitution is control-

led by two pairs of allelomorphic characters AaBb so that the four kinds are AB, Ab, aB and ab. Union occurs between mycelia without a common factor, for example, between AB and ab but not between AB and Ab. The constitution of the basidiospores in a basidium will be determined by the division in which segregation of these two factors occurs, in the same manner as found by Hanna in *Ustilago Zeae.* On this basis Kniep held that segregation took place in the 1st division in *Aleurodiscus polygonius* as he found only two types of basidiospores in a basidium, but according to Hanna it takes place in the second division in *Coprinus lagopus* as all the four kinds of spores are present in a basidium. Many species of *Hypholoma, Collybia velutipes, Trametes suaveolens,* etc., are known to be quadripolar but not all the forms are such. There are many bipolar species having only two forms controlled by a single pair of allelomorphic characters Aa, such as *Coprinus rostrupianus, C. radians, Fomes roseus, Panaeolus complanatus,* etc. Bose found *Polyporus ostreiformis* and *Polystictus hirsutus* to be strictly bisexual and that aversion showed by them is not related to sex. *Polyporus borealis,* however is tetrapolar. There are many homothallic forms also such as *Coprinus sterquilinus, C. ephemeroides, Polyporus resinosus,* etc.

Sass observed in *Coprinus ephemerus* and other forms that in addition to the 4-spored basidia, some of the basidia bore only two spores which were binucleate and gave rise to homothallic mycelium but the hyphal tips were uninucleate and fused with uninucleate hyphae carrying the nucleus of the opposite sex. The condition was similar to that described by Ames in *Pleurage anserina.*

Oort was able to induce "illegitimate unions" between two mycelia of *C. fimetarius* which were genetically alike for one factor such as between AB and Ab, and found that the clamp connections were abnormal and the basidia were 2-spored.

Vandendreis observed that 27 cultures of *C. radians* after some time passed spontaneously from the haploid to the diploid condition, showing clamp connections. He holds the view that all the species are "hetero-homothallic", being at the beginning heterothallic. The homothallic forms change from haploid to diploid very soon but in the species regarded as heterothallic this change takes a long time if allowed to occur spontaneously. The work of Buller shows that in addition to the mating of mycelia, diplodization may be brought about by the oidia produced by the haploid mycelia and transported to the mycelium of the opposite sex by mites, flies and wind. The nucleus of a single oidium may diplodize the whole mycelium as, though united in a dicaryon, it may divide independently and the daughter nucleus may go to the next cell by forming a clamp. Buller found that the oidial nucleus, in this manner, travelled at the rate of about 1·5 mm. per hour.

Crossing the strains from distant geographical places Vandendries noted inter-racial sterility in *C. micaceus* but complete inter-racial fertility was observed by Hanna in *C. lagopus* and by Kniep in *Schizophyllum commune*. The mycelia from each of the six fruit bodies from different places studied in *C. lagopus* separated into 4 groups, as usual, but all of them were fertile with all the strains derived from other fruit bodies. There were, therefore, not 4 but 24 distinct groups.

Such conditions in these forms have led people to theorize about their nature. It has been assumed that there are only two sexes and the interactions between them according to Kniep, are controlled by positive sex factors which may undergo frequent mutations giving rise to new sexual strains. Thus factors AaBb may mutate to give rise to A'a' B'b' showing complete fertility with the parent. To obviate the necessity of the assumption of the infinite number of allelomorphs possible according to Kniep's theory, Brunswik suggested the simpler conception of one or more negative sterility factors. All the strains that do not possess such factors will unite with each other. Cayley has put a corollary to the last hypothesis by suggesting that the mycelia are potentially bisexual but do not fruit due to the presence of self-sterility factors. Hartmann has advanced the theory of relative sexuality according to which they are potentially bisexual but behave unisexually, the sex being determined by internal factors such as the activity of the protoplasm and not by chromosomes as postulated by Kniep and Brunswik. In the presence of a strain with similar but stronger sexual tendency it will behave as if of opposite sex.

From what has been said in the preceding pages it is clear that heterothallism exists from the lowest to the highest groups of Fungi. The recent genetical studies have revealed results that can to some extent parallel those obtained in the flowering plants. It has been seen that heterothallism can be present even in those forms which bear both the male and the female organs. Gwynne-Vaughan thinks that this condition arose in connection with their invasion of land. It is surmised that "in a changing environment the limited variability associated with constant self-fertilization became harmful and since an evolutionary step can apparently never be retraced, survival lay with those forms which by a fresh device, ensured the combination of characters from different individuals". The study of heterothallism has explained the sterility as also the production of abundant fruit bodies at one time and their scarcity at another of several forms and, where environment is not the controlling factor in the production of perfect stages, it has given us the key to handle the problem in many a species of Fungi.

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