

VARIOUS ASPECTS OF THE MATING SYSTEM IN MUCORALES

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Several aspects of the sexuality in Mucorales are discussed. It is stated that neither heterothallism nor homothallism are absolute conditions and that a continuum exists between zygosporous and azygosporous. Mating type switching as known in ascomycetous yeasts would explain several up to now inexplicable phenomena.

The occurrence of zygosporous or azygosporous in interspecific contrasts in Mucorales (Stalpers & Schipper, 1980) together with other encountered aberrations of the generally accepted pattern, revived the interest of the authors in the literature on processes concerning sexual reproduction. Various aspects are treated here, followed by a discussion on the consequences of attributing to the Mucorales the system of mating type switches already known in the ascomycetous yeasts.

HETEROThALLISM VERSUS HOMOTHALLISM

Species belonging to the Mucorales are generally heterothallic. Outside the genus *Zygorhynchus* Vuill. few homothallic species are known. However, neither heterothallism nor homothallism are absolute conditions. Indications of dualism are to be found in both homothallic and heterothallic strains and species. Contrasts of homo- and heterothallic strains may result in imperfect conjugations. Homothallic strains sometimes show a preference for a particular mating type.

Satina & Blakeslee (1930) reported a strong reaction of two strains of the homothallic *Mucor genevensis* Lendn. to both (+) and (-) strains of heterothallic testers. They also found four strains of *Zygorhynchus heterogamus* (Vuill.) Vuill. (homothallic), which predominantly reacted with (-) strains and thus showing a (+) tendency and six strains of *Z. moelleri* Vuill. showing a (-) tendency. Werkman & van den Ende (1974) examined the conversion of sex-hormone precursors in the species mentioned above. Their strain of *M. genevensis* (originating from Blakeslee, but not necessarily used in Satina and Blakeslee's experiments) showed only a (-) reaction. They confirmed the (+) tendency of *Zygorhynchus heterogamus* and the (-) tendency of *Z. moelleri*. In homothallic species (+) and (-) characteristics could not always be demonstrated.

Mass inoculation is customary in mating experiments, and therefore aberrant behaviour of single spores cannot be detected. Blakeslee (1920) made a great number of single vegetative spore isolations from a strain of *M. genevensis* with a (-) tendency. Colonies with an aberrant

appearance were studied in detail with regard to zygospore production and/or (+) or (-) tendency. The following deviations from the parent were observed:

1. Colonies with zygospore production like the parent, but with a (+) tendency toward heterothallic testers;
2. Colonies with hardly any zygospore and a (+) tendency in contrasts;
3. Colonies with hardly any zygospore and a (-) tendency like the parent.

Homothallic species with heterothallic tendencies as well as homothallic strains of typically heterothallic species have been reported. Namyslowski (1906) isolated a homothallic strain of *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind (= *R. nigricans* Ehrenb.). Single vegetative spore isolations produced zygosporic colonies (if grown under favourable conditions). However, the homothallic strain showed many parthenospores and incomplete conjugations. Namyslowski (1910) found another strain of a typically heterothallic species with a homothallic tendency, *Absidia glauca* Hagem var. *paradoxa* Namyslowski (referred to by Blakeslee & al. (1927) as *A. glauca* Hagem no. 920 (-)), which was morphologically identical with *A. glauca*, but showed incomplete conjugations in single culture. When grown with one of Hagem's mating partners of *A. glauca* (Hagem, 1908), numerous zygospores were produced in the marginal area, while no reaction was observed to the other partner. Homothallic strains which are morphologically and physiologically indistinguishable from the heterothallic *Rhizomucor pusillus* (Lindt) Schipper but produce fully developed (mature) zygospores have been reported by Smith (1957), Schipper (1969, 1978), Scholer (1970) and Branner-Jørgensen & Ilum Nielsen (1974).

Homothallic strains were found not to be equally (+) and (-) in their response to heterothallic partners. Blakeslee & al. (1927) never found evidence of sex intergrades in heterothallic strains, though several thousands of intraspecific combinations were made. Recently, however, some indications of the occurrence of mixed (+) and (-) mating types in heterothallic strains have been found. Nottebrock & al. (1974) reported the production of zygospores in matings of certain strains of *Absidia corymbifera* (Cohn in Lichtheim) Sacc. & Trott. with both an accepted (+) and a (-) partner. Unmated strains did not produce zygospores. The present authors tested four of these strains, using the given medium and temperature. A few zygospores were obtained in CBS 429.75 × CBS 269.65; CBS 429.75 × CBS 582.65; and in CBS 269.65 × CBS 582.65.

Other indications of dormant ambivalency have been reported: van den Ende & Stegwee (1971) found that staling *Blakeslea trispora* Thaxt. (+) cultures occasionally produce small amounts of sex hormone. Since both (+) and (-) precursors are required for the production of the sex hormone, the complete enzyme system for biosynthesis was probably present. Werkman-Hoogland (1977) found by electrophoretical methods that methyl-dihydrotrispurate dehydrogenase activity is specifically found in *Mucor mucedo* L. ex Fr. (-). Occasionally, however, the same activity was also found in (+) cultures. The interpretation is, that normally this enzyme activity is suppressed in (+) strains, but that under particular circumstances this block is not absolute.

Presence of both (+) and (-) potentialities might also explain Ilum Nielsen's (1978) results using gamma-radiation of *Rhizomucor pusillus* (+) (the CBS tester strain). Single gamma-radiated sporangiospores were subcultured. Three (-) colonies were obtained of which two remained stable and one soon reverted to (+). Homothallic mutants were more numerous and mostly stable. Bu-Lock & Hardy (1979) mated the parent strain, a radiation (+) and a radiation

(-) each with *Mucor mucedo* (+) and (-). Imperfect reactions were obtained in accordance with the mating type. Trisporic acid production was demonstrated in mixed cultures of *Rhizomucor pusillus* (+) and (-), but not in separate cultures.

It can be concluded from the above evidence that neither homothallism nor heterothallism are absolute conditions, and cannot be accepted as sole characters for species distinction.

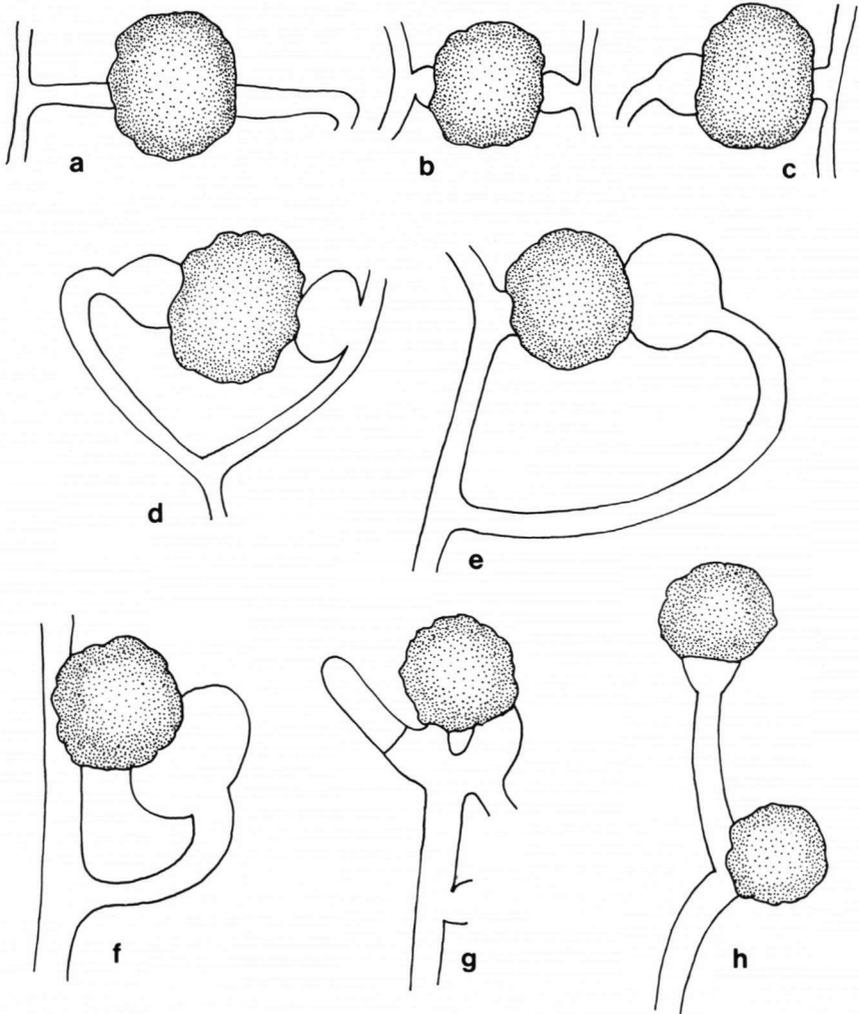


Fig. 1. Zygosporic stages. — a. *Rhizomucor pusillus*, heterothallic; *Rhizomucor pusillus*, homothallic. — b. *Mucor hiemalis*, heterothallic; *Mucor genevensis*, homothallic. — c. *Rhizopus microsporus*, heterothallic; *Rhizopus homothallicus*. — d. *Rhizopus sexualis*, homothallic. — e. *Rhizopus homothallicus*. — f. *Zygorhynchus moelleri*. — g. *Zygorhynchus japonicus*. — h. *Mucor bainieri*, azygosporic. — d., e. Occasional appearances; absence of a septum between suspensors intentional. — f. Young stage—in older stages a septum in the hypha bearing the 'smaller' suspensor is usual. (All schematic, after original drawings.)

FROM ZYGOSPORE TO AZYGOSPORE, A CONTINUUM

In heterothallic species zygospores always result from an interaction between hyphae of two strains of different mating-type, each secreting its own sex-specific substance. In homothallic species zygospores result from an interaction between two hyphae or parts of a hypha of the same strain, each secreting the same sex-specific substance as produced by heterothallic species.

In heterothallic species the two hyphae are not connected prior to copulation. In homothallic species the hyphae are connected though the distance of origin varies considerably (Fig. 1).

In homothallic strains of *Mucor* and *Rhizomucor* the copulating hyphae arise separately and independently from the substrate (Fig. 1a, b). In homothallic strains of *Rhizopus* they occasionally originate as lateral and terminal branches of the same aerial hypha (Fig. 1d, e). In *Zygorhynchus* they are typically formed by the same hypha: a straight lateral branch first recurves toward the terminal part of the main branch, from which it is usually separated by a septum. In *Zygorhynchus japonicus* Kominami copulating hyphae are close to each other and very short (Fig. 1g).

Sometimes there is no septum between conjugating parts, such that these are parts of the same cell. There is no fundamental difference between such a zygospore and an azygospore, when the processes causing zygospore formation are located not at different places in the same cell but closely adjacent, e.g. *Mucor azygosporus* R. K. Benjamin (O'Donnell & al., 1977) and *M. bainieri* Mehrotra & Baijal (Fig. 1h).

The locations of (+) and (–) nuclei, and consequently the sites of production of attracting substance, becomes less defined with decreasing distance and ultimately lose their significance altogether. In *Zygorhynchus moelleri* the terminal part of the main branch acts (–), the lateral (+). However, when the lateral fails to conjugate with the main branch, a second lateral may be formed which conjugates with the first lateral, acting (–) and producing (–) hormones (instead of the terminal branch) (Blakeslee, 1915; Werkman, 1977). Comparable behaviour was observed in *Absidia spinosa* Lendn. (Burgeff, 1924).

In *Zygorhynchus*, zygospores and sporangia (with homothallic sporangiospores) occur on the same hypha. Ling Young (1930) figured a suspensor of *Z. moelleri* proliferating with a sporangium. In a CBS strain of *Z. moelleri*, a conjugating lateral branch was seen to produce the complete apparatus; no septum was observed (Fig. 2). Regeneration of single suspensors in homothallic species was found to produce only homothallic mycelium (Ling Young, 1930; Callen, 1940, inclusive a personal communication by Burgeff).

Lendner (1908) figured a conjugation of two appendages of the same suspensor in a cross mating of the heterothallic *Absidia orchidis* (Vuill.) Hagem.

Conclusion: the above observations imply that the location of (+) and (–) is not fixed.

LOSS OF SEXUAL REPRODUCTION

Strains presumably in the process of losing sexual reproduction are known in *Zygorhynchus moelleri* Vuill. var. *agamus* Namyslowski and *Syzygites megalocarpus* Ehrenb. ex Fr..

(A) *Zygorhynchus moelleri* (syn.: *Z. vuilleminii* Namyslowski, *Z. vuilleminii* race *agamus* Namyslowski, *Mucor saximontensis* Rall).

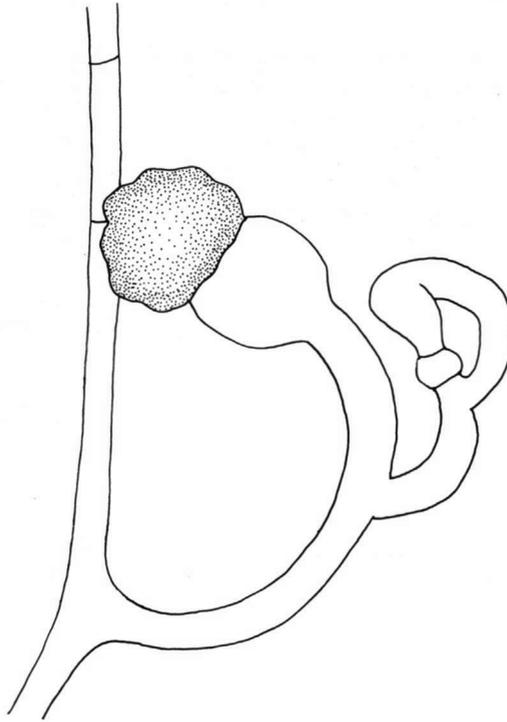


Fig. 2. Zygospore formation in *Zygorhynchus moelleri* CBS 501.66 (original drawing).

In *Zygorhynchus* all species are homothallic. However, after prolonged cultivation zygospore production may be reduced or even cease. Occasionally agamic strains of *Z. moelleri* have been isolated, e.g. *Z. vuilleminii* race *agamus* (Namyslowski, 1910) and *Mucor saximontensis* (Rall, 1965; Schipper, 1971). Though zygospores were absent, or very infrequent, in single cultures of the agamic strains, they had not disappeared entirely.

Blakeslee (1915), Satina & Blakeslee (1930), found that their strains of both *Z. vuilleminii* and *Z. vuilleminii* race *agamus* each showed a (–) tendency; the small, terminal progametangium which reacted with (+) strains of *Mucor*, *Rhizopus* and *Absidia* spp., while occasionally azygospores were formed. Cutter (1942) noted that in *Z. vuilleminii* race *agamus* zygospores with two true suspensors were rare, the majority of zygospores developed without the presence of the smaller (–) gametangium. In the single progametangium, which later produced an azygospore, nuclei in the expanded form were present all through the development, but nuclear fusions were not encountered. He concluded that the strain was practically impotent. Schipper (1971) obtained certain apparently normal zygospores with 2 suspensors, in Namyslowski's strain, through induction by (+) strains of *Mucor circinelloides* Tiegh., *M. hiemalis* Wehmer and *Backusella lamprospora* (Lendn.) Benny & R. K. Benjamin, and in '*Mucor*' *saximontensis* through induction

by (–) strains of *Mucor subtilissimus* Oudem., *M. hiemalis*, and *Backusella lamprospora*. Under favourable conditions contrasted cultures of *Zygorhynchus vuilleminii* race *agamus* and *Mucor saximontensis* showed a wide line of zygospores where the colonies met.

In her investigation of sex-specific enzymes in Mucorales Werkman (1976) found, that in a strain of *Z. moelleri*, the terminal copulating hyphae were the apparent major site of activity of (–) enzymes.

Werkman, Smits & v. d. Ende, in Werkman-Hoogland (1977) could not detect any production of sex-hormones in the agamic '*Mucor saximontensis*', though the fungus was able to convert hormones added from the heterothallic *Blakeslea trispora*. In this manner, zygospores could be induced, though never in large numbers.

(B) *Syzygites megalocarpus* (Syn. *Sporodinia grandis* Link).

Ling Young (1930) observed repeated mitosis in the gametangia from their initiation. At the time of lysis of the fusion wall numerous nuclei were present in both gametangia, and plasma streaming was observed 'to help mix the nuclei'. Azygospores were not uncommon in Ling Young's strain. He illustrated the difficulty in determining (+) and (–) in homothallic strains, by figuring a young zygospore of *Syzygites megalocarpus* borne between suspensors originating from one hypha and in open connection with one another.

Cutter (1942) reviewed the literature on the nuclear cytology of *Syzygites*. Some rather controversial data were discussed. Cutter in his own studies never encountered any indication of nuclear fusion or meiosis. He speculated as to whether the stimulus of plasmogamy, instead of karyogamy, could induce 'zygospore' development (pseudosexual reproduction).

Zygospore production in this species is largely dependent on environmental factors. Werkman & v. d. Ende (1974) were able to induce abundant zygospores under 'naturally' unfavourable conditions, by the application of trisporic acids or the (+) and (–) precursors derived from *Mucor mucedo*. Both (+) and (–) precursors were shown to be transformed to trisporic acids.

ZYGOSPORE GERMINATION

Within the bounds of our knowledge, homothallically produced zygospores give homothallic mycelia on germination, the zygospores of heterothallic species produce germ sporangia containing either (+) or (–) spores; with the exception of *Phycomyces* Kunze ex Fr., which produces (+), (–) and (+/–) spores in a single germ sporangium—the (+/–) condition being only temporarily (cf. Blakeslee, 1906; Burgeff, 1915, 1928; Cutter, 1942a, 1942b; Gauger, 1965). Gauger (1965, 1966, 1975) germinated zygospores of *Mucor hiemalis* and obtained a few azygosporic strains which reverted to (+), (–) and azygosporic conditions. In the azygosporic strains sporangiospores proved to be large, predominantly uninucleate with large nuclei, compared to the relatively smaller, multinucleate spores with smaller nuclei of the heterothallic tester strains. The azygosporic strains are considered to be diploids, heterozygous for mating type. Gauger (1977) germinated immature zygospores of *Rhizopus stolonifer* and obtained bisexual strains which segregated into (+) and (–) strains. Paired suspensor isolates did not always give rise to the respective genotypes of the parents. An explanation for the exchange of features could not be given 'unless nuclear movement can occur, following meiosis, from immature zygospores into the suspensor cells'.

As mentioned earlier, Ling Young, Burgeff, and Callen found only homothallic mycelia after regeneration of single suspensors in homothallic species.

Burgeff (1928) and Eslava & al. (1975) stated that germ sporangia in *Phycomyces* generally result from only one meiosis, while Cerda-Olmeda (1975) reported two meioses in each zygospore.

Olive (in Ainsworth & Sussman, 1965) deduced from the results obtained by Burgeff (1915, 1928) on germinating *Phycomyces blakesleeanus* Burgeff and *P. nitens* Kunze, by Köhler (1935) on *Mucor mucedo*, by Sjöwall (1946) on *Rhizopus stolonifer*, that only one member of the tetrad from meiosis survived.

NUCLEAR BEHAVIOUR

Hyphae in the Mucorales are coenocytic, as are progametangia, gametangia, and the resulting zygospores. Data in literature on the number of nuclei in sporangiospores are at variance, e.g. according to Cutter (1942) *Mucor hiemalis* resembles *M. genevensis*, the initials of sporangiospores in the latter being described as multinucleate blocks. Robinow (1957) recorded that most of the spores in *Mucor hiemalis* (+) and (-) had one nucleus, a few two. Gauger (1975) counted a high percentage of multinucleate sporangiospores (approx. 40–80%) in 'normal' strains of *Mucor hiemalis*, while in azygosporic strains the majority of sporangiospores were uninucleate. Storck & Morill (1977) reported in *Mucor hiemalis*, and also in some other *Mucor* species, predominantly uninucleate sporangiospores.

Active nuclei have changing forms and sizes (Cutter, 1942; Robinow, 1957; Laane, 1974); chromosomes have never been actually seen. In actively growing parts, such as contacting progametangia, the number of nuclei increases, due to migrating nuclei with a strong cytoplasmic flow and due to repeated nuclear division. In purely heterothallic strains, with one type of nucleus, the parts of the mycelium which become sexually active react as (+) or (-) after the mating type of the strain. In homothallic strains the nuclei seem to segregate after mating type: part of the mycelium becomes (+) and another part becomes (-), thus accomplishing a complete sexual cycle within the organism. The location of the (+) and (-) groups of nuclei, i.e. their distance, determines the type of zygospore as discussed earlier. After the breakdown of the fusion wall, the contents of the gametangia mix and the (+) and (-) nuclei generally fuse. However, Callen (1940) observed in *Rhizopus sexualis* (G. Smith) Callen an association of nuclei in pairs, but no fusion. This association of nuclei was also observed at points far removed from the partially dissolved fusion wall. Cutter (1942) was unable to determine the parental origin of such paired nuclei in several other species. Sassen (1965) observed paired nuclei in the progametangia of *Phycomyces blakesleeanus*, with a transmission electron microscope. Fusion of (+) and (-) nuclei could not be established.

Young zygospores contain numerous nuclei, old zygospores considerably less. The intermediate stage is uncertain as the dark zygospore wall prevents observation. The wall can be peeled off from the mature spore. No morphological evidence of meiotic division was found during zygospore formation and its dormancy (Laane, 1974, on *Absidia glauca*).

Even less is known of the nuclear behaviour during germination of the zygospore. Burgeff (1928) observed that the germ tubes in *Phycomyces* contained or received large 'presumably diploid' nuclei. Cutter (1942) studied nuclear behaviour in 15 zygosporic Mucorales. From the

number and volume of nuclei present, he—tentatively—determined time and site of 'meiosis'. He found four general patterns of development in this group of Mucorales. In zygospores of *Zygorhynchus moelleri*, *Mucor genevensis*, *M. hiemalis*, *Absidia spinosa*, *Parasitella parasitica* (Bainier) Syd., and *Blakeslea trispora* he observed fused nuclei only in the young stage (1–6 days), after that period all nuclei were unfused. In *Rhizopus stolonifer* and *Absidia glauca*, the germinating zygospore contained both fused and unfused nuclei. However, fused nuclei were not observed in the germ-tube, or in the germ sporangium. In *Phycomyces*, both 'fused' and 'unfused' nuclei occurred in germ tube and germ sporangium.

In *Zygorhynchus moelleri* var. *agamus* and *Syzygites megalocarpus*, both presumably in the process of losing 'sexual' reproduction as they lack true zygospores, fused nuclei were not encountered in any stage of the development.

TRANSPOSABLE GENES

Recently new data became available on mating type switches in ascomycetous yeasts (Herskowitz & *al.* in Loomis & Leighton, 1979; Hicks & *al.*, 1979; Leupold, 1980). The phenomenon of transposable genes was described and a hypothesis developed, known as the 'cassette model'. This hypothesis allows switching of the opposite mating types a and α (homologous with + and -), which are both present on the same chromosome, though only one is expressed. The HO-gene (for homothallism) permits frequent switching, while the ho-gene normally prevents this.

In yeasts every nuclear division is expressed in an individual; homothallic strains thus consist of approx. 50% a and 50% α individuals. In the Mucorales, however, the situation is different. Supposing, that transposition of mating type also occurs in the Mucorales, then we have a coenocytic mycelium of which every cell contains a great number of nuclei. In a homothallic strain the number of the two types of nuclei will be about equal, but in heterothallic strains various models may occur. The most simple, and probably by far the most common case is the presence of only + or only - nuclei. However, although the ho-gene suppresses the transposition of the mating type, it does not prevent it in every case; this may result in an organism in which most of the nuclei have one mating type, but a minority have the other.

This hypothesis offers an explanation for some of the aberrations mentioned earlier. —

(1) It is in agreement with the statement that homothallism and heterothallism are not absolute conditions. Although it favours either a fifty-fifty distribution or the occurrence of only one type of nucleus, a different distribution is permitted.

(2) The results of Blakeslee (1920) are easily explained with the supposition that his original strain of *Mucor genevensis* contained a minority of nuclei with the opposite mating type.

(3) Homothallic strains of *Rhizomucor pusillus*, typically a heterothallic species, are found in nature (Schipper, 1969), indicating that the ho-gene is relatively easily inactivated. It was therefore a suitable object for the experiments conducted by Ilum Nielsen (1978), in which the same effect was obtained using gamma-radiation.

(4) Homothallic species with heterothallic tendencies and heterothallic species with homothallic tendencies can be explained if the ratio of the mating type nuclei is not 1 or 0.

CONCLUSIONS

- (a) Mating activity always starts in actively growing hyphae.
- (b) Nutrition is very important in zygospore production.
- (c) In intra-specific matings the sexual process is initiated by a strong plasmas and nuclear flow, and nuclear division.
- (d) There is no proof that paired nuclei in zygospore initiation are necessarily of a mixed (+) and (-) nature; nuclei might even pair in the progametangium.
- (e) The size of the nucleus is no certain indication towards diploidy or haploidy.
- (f) Both (+) and (-) potentialities are probably present in each nucleus, though only one is expressed. Latent potentialities may become expressed by, for example, a strong impulse toward sexual reproduction when the mating partners are physiologically incompatible, thus resulting in the production of parthenospores. The interaction of fully active (+) and (-) factors is missing in parthenospore production; parthenospores develop later than true zygospores and are rarely fully developed.
- (g) Neither homo- nor heterothallism are absolute conditions.
- (h) From zygospore to azygospore is a continuum.
- (i) Location of (+) and (-) in homothallic species is less fixed with decreasing distance.
- (j) The theory of mating type switches offers an explanation for several 'aberrations'.

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