INHERITANCE OF MATING TYPE AND A LETAL FACTOR IN COSMARium BOTRYTIS VAR. SUBTUMIDUM WISTR.

BY RICHARD C. STARR

DEPARTMENT OF BOTANY, INDIANA UNIVERSITY, BLOOMINGTON, INDIANA

Communicated by R. E. Cleland, September 28, 1954

The wide diversity of cell shapes, ornamentations, and chloroplastids in the desmids, their unusual method of semicell regeneration in vegetative multiplication, and their rather large size have been of interest to investigators in many fields of experimental biology. Investigations have not been as fruitful as anticipated, perhaps because these organisms could not be induced to reproduce sexually in culture. The phenomenon of sexual reproduction is rarely observed in natural populations of desmids, and, although they have been cultivated successfully for many years, it was not until recently that strains were isolated which undergo sexual union in culture. The sexual strains of Cosmarium botrytis var. subtumidum have provided the first material for genetic investigations in the desmids.

The cells of C. botrytis var. subtumidum are haploid, and, inasmuch as the clonal cultures of the sexual strains arose through multiplication from single cells, it may be assumed that the population in each culture is genetically pure. The strains multiply rapidly by vegetative multiplication in Pringsheim’s soil-water medium and can be maintained for months without transfer. Sexual reproduction is effected by mixing cells of the heterothallic strains in the small depressions of Pyrex spot plates or in shallow watch glasses. These are placed in deep Petri dishes containing water to prevent excessive evaporation from the depressions. Special gametes are not produced, the vegetative cells acting as such directly. Pairing of the gametes occurs within 24–48 hours after the strains have been mixed, and fusion of the gametic protoplasts is accomplished within another 24–48 hours. Actual fusion requires only four to seven minutes, but events leading up to fusion may occupy a much longer period of time. Details of this process have been reported more fully elsewhere. Preliminary experiments have revealed that light is necessary for the initial pairing of the gametes, but the relation of the later phases of conjugation to light has not been ascertained.

Germination of the zygospore has been studied by DeBary, Klebahn, and more recently by the writer. At the beginning of germination, the protoplast escapes through a rupture in the walls of the zygospore. Nuclear and cytoplasmic divisions occur in the freed protoplast. Four nuclei are produced, but two disintegrate, resulting in the production of two rather than four gones by each germinating zygospore.
The two gones formed by a germinating zygospore were isolated, using fine glass pipettes, and were placed in separate tubes of soil-water culture medium. After sufficient vegetative multiplication had occurred, cells from each gonal culture were mixed separately with cells of each parental strain in order to ascertain their respective sexual potentialities. It had been suspected at the time of isolation of the original strains that determination of mating type might be due to more than a single factor, inasmuch as several strains of the same species from the same population had been found to be intersterile. It was of interest, therefore, to analyze the sister-gones from a single zygospore in order to ascertain whether a phenomenon such as the tetrapolar sexuality of certain fungi might exist and account for the apparently sterile strains in the natural population.

In matings of the heterothallic parental strains, occasional zygotes were observed which appeared to have died shortly after their formation, prior to the production of the spiny wall characteristic of the zygospores of the species. However, the abundance of normal zygospores produced at the same time indicated that such aborted zygotes probably resulted from some unfavorable situation of the environment. On the other hand, in certain backcrosses of gonal cultures with the \textit{minus} parental strain, viable zygotes were never formed. Gametic pairing and fusion occurred as in those matings resulting in viable zygotes and zygospores, but soon after fusion, within 30 minutes to an hour when observed under the microscope, the zygotes lysed, and their contents escaped through small holes or ruptures in the membrane. The remains of the aborted zygote were in evidence for a long time as an accumulation of starch grains imbedded in the mucilaginous sheath which had been secreted earlier by the paired gametes. The empty walls of the gametes could be seen on each side of the starch mass.

Gonal cultures which were backcrossed with the \textit{plus} parental strain never resulted in the production of aborted zygotes in numbers larger than could be accounted for by environmental effects; however, when these same cultures were crossed with other gonal cultures, a number of them failed to form viable zygotes. Table 1 summarizes the results of the various backcrosses.

Although it is generally assumed that meiosis occurs in the germination of the zygospores of all placoderm desmids, it is only in \textit{Hyalotheca dissiliens} (Sm.) Breb. that the process has been studied critically. In the zygospores of this alga, Pothoff\textsuperscript{7} found that, as a result of two divisions, four haploid nuclei were formed. Three of these degenerated soon after division, the remaining one serving as the nucleus of the single gone produced by the germinating zygospore. In the genera \textit{Closterium} and \textit{Cosmarium}, whose zygospores commonly produce two gones, Klebahn\textsuperscript{4} has shown that two nuclear divisions occur in the protoplast of the germinating zygospore, but he was not aware of the meiotic nature of the process. His figures clearly indicate, however, that, when each of the two nuclei formed by the first division undergoes a second division, a normal nucleus and a degenerative nucleus are the result. Preliminary cytological observations of the germinating zygospores of \textit{Cosmarium botrytis} var. \textit{subtumidum} indicate that the division of the nucleus is meiotic, approximately 30 bivalents being present at diakinesis. The writer's preparations confirm the observations of Klebahn that the two nuclei which survive are non–sister-nuclei of the four formed as a result of the two divisions.
In Table 1, the ratio of 56 to 49 for mating types is in close agreement with the expected ratio of 1 to 1, if mating type is determined by a single factor. In 22 of 23

<p>| TABLE 1 |
|--------------------------|------------------|
| <strong>ANALYSIS FOR THE FACTORS OF MATING TYPE AND LETHALITY IN 105 GONES RESULTING FROM A CROSS BETWEEN THE PLUS AND MINUS PARENTAL STRAINS OF COSMARUM BOTRYTIS VAR. SUBTUMIDUM</strong> |</p>
<table>
<thead>
<tr>
<th><strong>NO. OF GONES</strong></th>
<th><strong>Total analyzed</strong></th>
<th><strong>Having plus mating type</strong></th>
<th><strong>Having minus mating type, resulting in viable zygotes when mated with plus parent</strong></th>
<th><strong>Having minus mating type, resulting in aborted zygotes when mated with plus parent</strong></th>
<th><strong>Having plus mating type, resulting in viable zygotes when mated with minus parent</strong></th>
<th><strong>Having minus mating type, resulting in aborted zygotes when mated with minus parent</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NO. OF GONES</strong></td>
<td>105</td>
<td>49</td>
<td>56</td>
<td>0</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td><strong>Having minus mating type, resulting in viable zygotes when mated with minus parent</strong></td>
<td>36</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

zygospores in which both gones were analyzed, the two gones were found to be of different mating types; in one instance, both gones were of the same mating type. Inasmuch as cytological preparations have indicated that the nuclei of the two gones are non-sister-nuclei of the second division, it is evident that the mating-type locus segregates, as a rule, at the first division. The one exception in which both gones were of the same mating type can best be explained by a crossover prior to the first division involving the mating-type locus and its centromere, with the chance survival of those two non-sister-nuclei which carried the same mating type. The probability of the survival of gones with the same mating type following such crossovers is 50 per cent. As in the ascus of Neurospora where the linear arrangement of the ascospores formed following meiosis forms an ordered tetrad indicating with great clarity the pattern of gene segregation, the disintegration of two non-sister-nuclei in the germinating zygospore of Cosmarium results in the production of two gones which may be analyzed in a similar manner. The loss of two nuclei is obviously a disadvantage in other respects.

In many haploid organisms, lethality is expressed by the inability to synthesize some organic substance necessary for a life-process, and, unless this substance is absorbed from an outside source, death results. These same lethals will usually express themselves in a homozygous dikaryon or diploid stage, but when they are associated with normal alleles they are usually masked by the action of the latter.

In *Cosmarium botrytis* var. *subtumidum* a lethal factor was present in a haploid clone (the minus parental strain) isolated from a natural population, but it did not produce any detectable effects in the haploid condition. It was not until after sexual fusion with a normal strain (the plus parental strain) and the resulting segregation and recombination at meiosis in the germinating zygospores that gones were produced which, when backcrossed with the lethal-containing parental strain, resulted in the combination of two lethal factors in the zygote and in its subsequent death. That the lethal factor does not express itself in the haploid cell seems to indicate that its action or lack of action probably is involved in biosyntheses essential only to the zygote and its maturation. Cytological preparations show that the fusion of the two gametic nuclei does not occur until long after formation of the resistant spiny walls of the zygospore. Therefore, it would appear that the lethal fac-
tor is expressed while the zygote is in a dikaryon stage, inasmuch as abortion occurs prior to the formation of the spines.

Analysis of the two gones from single zygospores indicated that, as a rule, one gone contains a lethal factor, while the other does not. In 21 of 23 zygospores analyzed this was found to be true. Of the remaining two, one zygospore produced two gones both of which carried lethals; the other produced two gones both of which carried the normal allele. These results indicate that the lethal factor is a single factor which segregates, as a rule, at the first nuclear division in the zygospore.

In Table 1 it can be seen that, of the 49 gones having the plus mating type, approximately 50 per cent show a recombination of the plus-mating-type locus with the lethal gene which was contributed by the minus parental strain. This is evident from the backcrossoves which resulted in aborted zygotes. Such a percentage is to be expected if mating type and the lethal factors are determined by single genes located on different chromosomes. It should be noted that of the 56 gones having the minus mating type, only 20 possess lethal factors; 36 gones then represent recombinations of the minus mating type with the normal allele. This high percentage of recombination might be indicative of the greater viability of clones with the minus mating type and the normal allele in contrast to that of clones with the minus mating type and the lethal factor; but the relatively small number of progeny analyzed is hardly adequate for such conclusions at this time. It is very probable, however, that the genes for mating type and the lethal factor are unlinked.

Summary.—The heterothallic strains of Cosmarium botrytis var. subtumidum Wittr. provide the first material for genetic investigations in the desmids. As a result of the survival of only two non-sister-nuclei following the second division in meiosis, two gones rather than four are produced by a germinating zygospore.

The mating type of sexual strains is shown to be determined by a single gene, which segregates in the normal Mendelian fashion. The two mating types segregate, as a rule, in the first division of the meiotic process; therefore, the two gones from a single zygospore are usually of different mating types.

The abortion of all zygotes resulting from certain backcrossoves of gones with the minus parental strain is due to a single factor which is undetectable in the haploid vegetative cell or in the heterozygous condition in the zygote. Zygotes formed by the fusion of two gametes, each carrying the lethal factor, always lyse shortly after their formation. The lethal factor is inherited as a single gene, segregating, as a rule, at the first division in meiosis. There appears to be no linkage between the genes for mating type and lethality.


3 Sexual strains used in this work are the writer's isolations Nos. 3 and 4. Inasmuch as there is no detectable difference in their behavior during conjugation, they are designated as plus and minus parental strains, respectively.


5 A. DeBary, Untersuchungen über die Familie der Conjugaten (Leipzig, 1858).
