A Cytotaxonomic Study of Three Species of Gelasinospora

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Introduction

Gelasinospora, a genus of pyrenomycetes closely allied to Neurospora and Fimetaria, was erected by Dowding (1933) on the basis of her studies on G. cerealis and G. tetrasperma, to include those two species. Since that time four additional species have been described: G. retispora and G. adjuncta by Cain (1950), G. calospora by the Moreaus (1949), and G. autosteira by Alexopoulos and Sun (1950). Interestingly enough they were all described at about the same time from widely separated localities in the world, independently by three investigators of teams, none of whom had knowledge of the other's work. Even more interesting is the fact that the morphology of the last three of the above species is so similar as to suggest strongly that they may be one and the same. This similarity is brought out in Table 1, which summarizes the taxonomic characters of all six species of Gelasinospora.

In 1951, Moreau and Moreau published a short paper in which, on the basis of published descriptions alone, they reduced G. adjuncta, G. autosteira, and G. tetrasperma to synonymy under G. calospora. Although on the basis of perithecial, ascal, and ascosporic measurements this combination species appears to be justified, it seemed advisable to undertake a somewhat more extensive study of the situation before accepting the conclusions of these authors. A clarification of the relationship of G. calospora and G. autosteira was of particular interest to us since these two organisms are currently employed for physiological and genetic studies in our laboratory. Because G. adjuncta is being investigated by Cain and his students in Canada, it was

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not included in these studies, although cultures of G. adjuncta and G. autosteira have been exchanged by our two laboratories. G. cerealis was included in these studies as a contrasting form.

Table 1. Taxonomic characters of six species of Gelasinospora

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>tetrasperma</th>
<th>cerealis</th>
<th>calospora</th>
<th>adjuncta</th>
<th>retispora</th>
<th>autosteira</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of spores to the ascus</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Size of perithecia in microns</td>
<td>600-700</td>
<td>600-700</td>
<td>550-750</td>
<td>700-1000</td>
<td>700-1000</td>
<td>650-710</td>
</tr>
<tr>
<td></td>
<td>300-400</td>
<td>350-600</td>
<td>460-600</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Size of asci in microns</td>
<td>150-180</td>
<td>214-260</td>
<td>160-250</td>
<td>200-260</td>
<td>250-300</td>
<td>218-244</td>
</tr>
<tr>
<td></td>
<td>×</td>
<td>30-33</td>
<td>×</td>
<td>18-21</td>
<td>20-24</td>
<td>×</td>
</tr>
<tr>
<td>Size of ascospores in microns</td>
<td>20-28</td>
<td>26-32</td>
<td>23-28</td>
<td>22-27</td>
<td>28-33</td>
<td>16-26</td>
</tr>
<tr>
<td></td>
<td>×</td>
<td>18-16</td>
<td>×</td>
<td>12-15</td>
<td>14-17</td>
<td>×</td>
</tr>
<tr>
<td>Shape of ascospores</td>
<td>Ellipsoid, rounded at the ends.</td>
<td>Ellipsoid, narrow at the ends.</td>
<td>Homothallic</td>
<td>Heterothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
</tr>
<tr>
<td>Sexual compatibility</td>
<td>Secondary Homothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
<td>Heterothallic</td>
<td>Homothallic</td>
<td>Homothallic</td>
</tr>
<tr>
<td>Ascospore germination</td>
<td>One vesicle</td>
<td>Two vesicles</td>
<td>Mostly two vesicles</td>
<td></td>
<td></td>
<td>One vesicle</td>
</tr>
</tbody>
</table>

The object of this investigation, then, was to attempt to throw some light through cytological evidence on the relationship between G. calospora and G. autosteira, two morphologically similar species, the former homothallic, the latter heterothallic, and to compare and contrast the cytology of these two forms with that of G. cerealis, a species distinctly different morphologically.

Materials and methods

Cultures. A culture of G. calospora was kindly furnished by Dr. Claude Moreau, and one of G. cerealis by Dr. E. Silver Keeping. The cultures of G. autosteira employed were our own isolations from Spanish moss (Tillandsia usneoides). All cultures were single spored. G. calospora and G. cerealis, both homothallic, grow and fruit well without difficulty. All cultures of these organisms used, therefore, were made by mycelial transfers and were direct descendants of the single spore originally selected. The situation with G. autosteira was very different. Not only is this organism heterothallic, requiring the pairing of two compatible strains for fruiting, but it presents additional difficulties in that, after several transfers, paired
cultures lose their ability to form perithecia. Consequently, it was necessary to make continuous isolations of single ascospores throughout the progress of this work in order to induce perithecial formation.

*Media.* Difco corn meal agar was used to grow *G. calospora* and *G. cerealis*. Spanish moss agar was used for *G. autosteira* because it hastened perithecial formation and was particularly favorable for studies of perithecial development which were undertaken in conjunction with the cytological studies, but which are not reported in this paper. Spanish moss agar was prepared by steaming 15 g. of air dry Spanish moss in 500 ml. distilled water, filtering through filter paper, adding 1.5 per cent Difco agar, and autoclaving at 15 lb. for 20 min.

*Staining procedure.* For cytological studies of the developing asci a modification of the iron-propiono-carmin smear technique (Wheeler *et al.*, 1948) was used. Just after the perithecial beaks were formed a block of agar 10 × 20 mm., bearing perithecia was cut out and fixed in 3:1 absolute alcohol-propionic acid 24–48 hr. The material was then passed through a series of alcohols to distilled water and was then mordanted with 4 per cent iron alum for 10 min. After washing with several changes of distilled water the agar blocks were stored in slightly acidified water overnight. A wide field dissecting microscope was used for dissecting the perithecia and for subsequently mounting the asci under a cover glass in propiono-carmin. The slides thus prepared were heated gently by passing through an alcohol flame several times, excess stain was drained off, and the mounts were finally sealed with glycerine jelly. After the mounts had aged for at least 48 hr. they were studied using an Ortholux microscope equipped with a 2 mm. apochromatic objective and 15 × periplan oculars.

*Spore treatment.* Since ascospores of *G. autosteira* do not germinate readily, the heat treatment described by Dodge (1912) was employed to induce germination. The method consists of placing the spores on agar in a Petri dish and heating slowly in an oven up to 65–68°C., taking 30 min. to reach that temperature, cutting off the heat, and permitting the oven to cool down to 35°C. before removing the spores.

Spore germination

The heat treatment described above increased the percentage of spore germination in all three species of *Gelasinospora* under study, but *G. calospora* and *G. autosteira* responded to lower temperatures than did *G. cerealis* as shown in Table 2.

Characteristic differences were found in the method of spore germination in the three species. Spores of *G. autosteira* germinate with the formation of a vesicle on one end of the spore. No exception to this method was observed. In *G. calospora* about 82 per cent of the spores germinate with
the formation of two vesicles, one at each end of the spore, and about 18 per cent germinate from one end only. The spores of *G. cerealis* observed

germinated from both ends with about 3 per cent producing a third germ tube. Dowding (1933) has reported that some spores of *G. cerealis* produce but one vesicle; this was not observed in any of our cultures.

### Cytology of ascus development

The developmental stages of the ascus were essentially the same for all three species under study and the following discussion will refer to all unless otherwise noted. The major cytological differences observed were in the number of chromosomes and in the behavior of the nucleolus.

**Stages in ascus development.** The formation and development of the crozier in *Gelasinospora* is similar to that described for many of the Ascomycetes. It begins with the formation of a hook from a binucleate cell (Fig. 1C); one of the two nuclei migrates to the hook and the other remains near the base of the cell; a conjugate division takes place (Figs. 2, 10) and thus a crozier with four nuclei is formed. Six univalent chromosomes were counted in the croziers of *G. autosteira* (Figs. 2, 10), and seven in *G. cerealis* (Figs. 3, 11). In *G. calospora* the mitotic figures found were never clear enough to permit accurate counting of chromosomes and, consequently, the chromosome number prior to nuclear fusion could not be determined in this species.

Reorganization of the four nuclei resulting from conjugate division is followed by septation, as a result of which a uninucleate hook cell, a binucleate penultimate cell, and a uninucleate basal cell are produced (Figs. 1A, B). The development of the ascus proceeds from the penultimate ascus

<table>
<thead>
<tr>
<th>Species</th>
<th>50°C</th>
<th>65-68°C</th>
<th>70°C</th>
<th>72-75°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. calospora</em></td>
<td>80</td>
<td>36</td>
<td>94</td>
<td>50</td>
</tr>
<tr>
<td><em>G. autosteira</em></td>
<td>18</td>
<td>0</td>
<td>86</td>
<td>0-75c</td>
</tr>
<tr>
<td><em>G. cerealis</em></td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>20</td>
</tr>
</tbody>
</table>

* Spores sown in mass.
* Spores sown singly.
* In *G. autosteira* ascospore germination varies with the parental crossings.
mother cell. It elongates and its two nuclei fuse (Fig. 4). Young asci, each with a large fusion nucleus, still attached to the croziers were frequently found. The hook cell often fuses with the basal cell of the crozier and forms a binucleate cell. From this newly-formed binucleate cell another
crozier is formed (Fig. 4). This process continues so that a nearly mature perithecium exhibits different stages of ascus formation upon dissection.

After the fusion of the two nuclei the penultimate cell of the crozier may be regarded as the young ascus. At this stage the young ascus has a fairly large nucleolus and a group of chromosomes so entangled that the individual chromosome strands cannot be traced. The nuclei appear to remain in this phase for a long time, but further development, once on its way, is quite rapid as indicated by the fact that, in most preparations, the two most prevalent stages are the uninucleate young asci and the fully formed asci with the ascospores already differentiated. The meiotic and mitotic nuclear divisions appear, therefore, to proceed rapidly.

Following the stage described above, in which the chromosomes are entangled, the chromosomes become shorter and thicker. Synapsis now occurs as evidenced by the fact that the chromosomes appear to be double. It is in diakinesis (Figs. 5, 12), first metaphase (Figs. 6, 13; 7, 14; 8, 15), and first anaphase, that the individual chromosomes can best be recognized and the number counted. From diplotene to metaphase I, six bivalents were counted in G. calospora and G. autosteira, and seven bivalents in G. cerealis (Figs. 8, 15). In anaphase I, 12 chromosomes (6-6) are recognizable in G.
calospora and G. autosteira, and 14 chromosomes (7-7) in G. cerealis. The spindle apparatus was always found to be parallel to the long axis of the ascus (Figs. 7, 14). The nucleolus persists in the first division. In most preparations a single nucleolus was evident, but two and even three nucleoli were also found (Figs. 6, 13). In G. calospora and G. autosteira the nucleolus is generally situated far away from the group of chromosomes (Figs. 5, 12); in G. cerealis, on the contrary, the nucleolus is always found attached to one chromosome which, presumably, contains the nucleolus organizing region (Figs. 8, 15).

After the first division is completed the two daughter nuclei reorganize and immediately go into the second division. The chromosomes now appear smaller, but are still visible, and in metaphase II and anaphase II they may be easily counted. The counts here agree in the three species with those made in preparations of first division figures. Actual spindles of the second division have not been stained, but from the arrangement of the daughter nuclei it is evident that the orientation of the spindles may be either parallel or oblique to the long axis of the ascus. The nucleolus was found in a number of second division preparations particularly at the metaphase.

Prophase III appears to be of short duration. It was only found once. At third metaphase and anaphase, the chromosomes are extremely thin rendering them difficult to photograph. The spindles now lie either perpendicular or oblique to the long axis of the ascus (Figs. 9, 16). No evidence of a nucleolus was ever observed during the third division. In the young ascospores, however, the nucleolus reappears in the one-nucleate stage.

When the eight nuclei of the ascus reorganize after the third division, delimitation of the ascospores begins. The young spores are hyaline and each contains a single nucleus. Mitotic division takes place in the young ascospore while it is still hyaline and its contents are homogeneous. Soon the ascospores become highly vacuolated and filled with oil. The two organized nuclei are still evident at this stage. Later the ascospores change to yellow and eventually to dark brown. The pits in the walls, characteristic of the genus, can best be seen just before the ascospores mature.

The frequent appearance of a rod-like structure during karyokinesis is of interest. It has been observed in all three species under study and in all stages of division in the asci. It has never been seen in the croziers and must, therefore, develop after that stage is completed. Although frequently seen, the rod was not always present. In general the rod appears to be beaded and more or less stiff, and does not stain as deeply as the chromosomes. Its position with reference to the chromosomes varies, and its behavior, as will be brought out in the discussion that follows is not that of a chromosome.
Discussion

The ascal cytology of the three species under investigation is very much the same in its general aspects and closely resembles that of Neurospora and other pyrenomycetes which have been investigated. Some of the interesting points which have been brought out in this work concern the number of chromosomes, the behavior of the nucleolus, and the peculiar rod-like structure.

As far as could be determined by chromosome counts, before karyogamy in two species and during the three ascal divisions in all three species, the haploid number of chromosomes in G. calospora and G. autosteira, the two morphologically similar forms, is six, and that of G. cerealis is seven. In a number of preparations the chromosomes could be counted clearly and we believe the numbers given to be correct. On the other hand, we are well aware of the disagreement of a number of investigators concerning the chromosome numbers in the much studied Neurospora tetrasperma in which Colson (1934) and Cutter (1946) counted six chromosomes, but Fincham (1949) and McClintock (1945) counted seven, and in N. sitophila in which Wilcox (1928) counted six and McClintock (1945) and Dodge, Singleton, and Rolnick (1950) reported seven. In view of the above we should welcome confirmation of our counts by other investigators. Our counts tend to support the conclusion of the Moreaus (1951) that G. autosteira should not be considered different from G. calospora, and at the same time present cytological evidence for the difference between these two forms on the one hand, and the morphologically different G. cerealis on the other.

In this connection it is interesting to record that no cytological difference was discovered between the homothallic G. calospora and the heterothallic, closely related G. autosteira. This is in contrast to the reports of Hirsch (1947, 1949) for Hopomyces solani in which the homothallic form is reported to have six chromosomes, whereas the heterothallic H. solani f. cucurbitae may have various numbers of chromosomes depending on the sex of the isolate.

The behavior of the nucleolus in the three species of Gelasinospora considered here may offer an additional cytological clue of relationships. In G. cerealis the nucleolus is closely associated with the chromosome which may be interpreted as the nucleolus organizing chromosome; in G. calospora and G. autosteira, on the contrary, the nucleolus is often situated conspicuously far away from the group of chromosomes.

The function of the rod-like structure which often appears in karyokinesis in all three species has not been determined. It has been found in all stages of nuclear division in the asci, but not in all preparations. It has never been seen in the croziers and must, therefore, develop after karyogamy. When the chromosomes synapse the rods remain unpaired (Figs. 6, 13; 7, 14);
when the nuclei are reorganizing the rods remain distinct. The rods cannot, therefore, be interpreted as chromosomes under any circumstances. In some preparations (Figs. 9, 16) the rods are at the poles of the spindle assuming the position of centrosomes, but in others their position is not one that centrosomes would normally assume. Furthermore, in some preparations, centrosome-like bodies were seen at the apexes of the spindle in addition to the rods which were clearly discernible elsewhere in the same preparation (Figs. 7, 14). Structures similar to these rods have been described in other fungi; these have been termed nuclear beaks or spindle horns in Neurospora by Lindegren and Ruman (1938), Cutter (1946), Fincham (1949), and Colson (1934), and in Gelasinospora tetrasperma by Dodge (1937). McClintock (1935) described the centriole in Neurospora crassa as a rod-like structure in the first and second ascus divisions. In the same organism Singleton (1953) found a pair of rods which he believes “represent the division products of the simple centriole present at the pole of the anaphase spindle....” He states later that these rod-like structures are actually triangular plates viewed from one side. It is very possible that the rods we found in the three species of Gelasinospora are comparable to the rods found by McClintock (1945) and Singleton (1953) but their position, as shown in Figs. 7, 14 make their identification as centrosomes doubtful.

**Taxonomy**

In view of the morphological similarity between G. calospora and G. autosteira, and the similarity in chromosome numbers and behavior of the nucleolus as contrasted with those of G. cerealis, a morphologically distinct species, it is concluded that the two forms are not sufficiently different to regard them as distinct species. On the other hand, the fact that one is homothallic and the other heterothallic, and that the spores of the former germinate mostly by two vesicles whereas those of the latter always germinate by one vesicle, is regarded as sufficiently important to recognize G. autosteira as a variety of G. calospora for which we propose the name: Gelasinospora calospora (Mouton) Moreau et Moreau, var. autosteira (Alexopoulos et Sun) Alexopoulos et Sun, comb. et stat. nov. (based on G. autosteira Alexopoulos et Sun, Mycologia 42: 723–734, 1950).

**Summary**

The cytology of the ascus in Gelasinospora calospora, G. autosteira, and G. cerealis has been studied; haploid counts of 6, 6, and 7 chromosomes, respectively, have been made; the nucleolus of the first two species is often situated far away from the chromosome groupings whereas in the last species it is closely associated with the chromosomes; a rod-like structure resembling
the centriole of *Neurospora crassa* as described by McClintock and by Singleton is present in all three species. On the basis of morphological characteristics, spore germination studies, ascus cytology, chromosome numbers, and behavior of nucleolus, *G. autosteira* is reduced to a variety of *G. calospora*.

References


Wilcox, M. W. 1928. The sexuality and arrangement of the spores in the ascus of *Neurospora sitophila*. Mycologia 23: 3-17.