Multispored Asci in Neurospora crassa

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Abstract
Multispored Asci in *Neurospora crassa*

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During the investigation of the effect of temperature on sexual reproduction in Neurospora, the phenomenon of the occurrence of asci with more than eight spores was observed. Such asci will be termed "multi-spored".

The strains used were cr (crisp), re-isolated from strain F945 kindly supplied by Dr. R. W. Barratt, and al-2 (albino), re-isolated from strain 15300 kindly supplied by Professor D. G. Catcheside. Batches of crosses were made by inoculating both strains together on the minimal reproductive medium of Westergaard and Mitchell. Part of each batch was incubated at 25°C, while the rest of the batch was incubated at 30°C. Some of the crosses from each temperature treatment were taken at intervals and the protoperithecia or perithecia fixed, stained and observed cytologically to determine the stage of development reached. Every 24 hours and during meiosis at every 12 hours, some crosses were transferred from incubation at 25°C to 30°C and some of these also were taken at intervals for cytological observation.

It was found that at 25°C protoperithecial growth begins after 24 hours and continues steadily for about a further 48 hours. Development did not take place beyond this stage in crosses incubated continuously at 30°C; the protoperithecia darkened but were ultimately devoid of any ascospores. At 25°C, however, the protoperithecia expand rapidly in the next 48 hours. Meiosis commenced late on the fifth day and the first wave of asci completed the three nuclear divisions late on the sixth day.

Provided protoperithecial growth had commenced at 25°C, crosses transferred from 25°C to 30°C did undergo meiosis and produce ascospores at 30°C. However, a high frequency of aberrant asci was present in all such crosses. As can be seen in Table I, many asci contained more than eight spores. Never more and usually fewer than eight black spores were found in all the multi-spored asci observed. The highest number of spores seen in any one ascus was 22 of which only 3 were black. Asci with fewer than eight spores were also frequent. In addition several asci contained grossly mis-shapen ascospores.

<table>
<thead>
<tr>
<th>Cross* and Treatment</th>
<th>No. of spores per ascus:</th>
<th>Number of asci:</th>
<th>Normal</th>
<th>8-spored</th>
<th>Multi-spored</th>
<th>Less than 8-spored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>8 8.0</td>
<td>23</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>1-14 7.2</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>1-13 7.0</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>2-14 8.1</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>4-15 9.1</td>
<td>5</td>
<td>8</td>
<td>13</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* Samples 1-4. Cross: cr, + x +, al-2
  Sample 5. Cross: +, + o x cr, al-2 o

† Estimated meioses occurred: (1) all at 25°C. (2) at 25°C, during change 25°C to 30°C and at 30°C. (3) during change 25°C to 30°C and at 30°C. (4) all at 30°C. (5) at 25°C, during change 25°C to 30°C and at 30°C.

Samples 1-5 each based on 25 asci selected at random.

The highest frequency of aberrant asci appeared to occur in those crosses in which meiosis was proceeding in some of the asci when the cultures were transferred from incubation at 25°C to 30°C. Cytolo-
gical examination of material from such a cross fixed shortly after transfer to 30° revealed, in one particularly favourable preparation, irregular numbers of chromosomes segregating to the poles and the presence of laggards at late anaphase II. Possibly as a result of temperature shock, chromosome segregation was abnormal; the chromosome tended to become scattered and spores were delimited around the scattered chromosomal material.

Single random ascospore isolates were made from the control cross at 25°; a wild-type recombinant, used as protoperithecial parent, and a double mutant, used a conidial parent, were selected for a further batch of crosses. Transfers from 25° to 30° were made when the first wave of ascal meioses commenced. The results were similar to the previous experiments (Table I).

The phenomena reported above are under further investigation. --Genetics Laboratory, Department of Botany, University of Bristol.

Howe, H.B., Jr. and J. E. Page. Nonconidiation in the new homothallic species, Neurospora terricola. Recently isolated from Wisconsin soil a new eight-spored species of Neurospora which they named Neurospora terricola (WSF 5000). Several interesting features of this new species were described, but true homothallism, previously unknown in Neurospora, is the characteristic having most promise for genetic studies. A number of standard Neurospora techniques would be unusable in future genetic studies with N. terricola, however, because this organism produces no conidia. We have therefore made preliminary attempts, using a transfer of WSF 5000 obtained from the American Type Culture Collection, to promote conidiation with various media and to induce conidiating mutants with UV irradiation. The results to date have indicated marked stability of the nonconidiation trait.

The following four solidified media were tried: Fries; Westergaard-Mitchell; bacto-peptone; and malt, peptone and yeast. The latter two media were developed by Frost (NN 1:11. 1962) specifically to encourage Neurospora conidiation. No conidiation was obtained on any of these media, but bacto-peptone gave heaviest mycelial growth and was therefore used to culture mycelia for irradiation purposes.

Irradiation was employed in two ways. Both methods involved the irradiation of suspensions of mycelial fragments, followed by plating on bacto-peptone to score for conidiation. In the first method, however, the mycelia were always obtained from stock cultures which had not been previously irradiated. The second method differed in that mycelia were irradiated, plated and scored, then harvested from the plates and reirradiated. Various treatment times up to fourteen minutes, just short of 100 per cent kill, were used. Despite several attempts with both methods, growth on the post-irradiation plates was consistently nonconidial. One might surmise that further efforts will yet produce conidiation in N. terricola, however, in view of the occurrence of a number of nonconidiating (fluffy) mutants in the normally conidiating species, N. crassa. Any mutations to ability to produce conidia occurring in our experiments might reasonably have been masked by heterokaryosis. Consequently, the treatment of ascospores instead of mycelial fragments, followed by sorbose plating, might prove to be a more fruitful approach in future trials. (Mr. Page was a member of the Nat. Sci. Foundation's Research Participancy for College Teachers Summer, 1963). --Department of Bacteriology, University of Georgia, Athens, Georgia.

Hsu, K.S. A modifier of the morphological mutant scumbo in Neurospora crassa. The morphological mutant sc (scumbo 5801) was incorporated into the linkage-testers LTIA and LT2a as a centromere marker for linkage group III (Perkins 1959, Genetics). In crosses made for linkage detection where either one of the testers was used as one of the parents, two different phenotypes of sc could be recognized in the progeny. One type grew more vigorously, and was closer to, but easily distinguishable from, the wild-type phenotype. The other gave more restricted growth, which resembled the phenotypic expression of the tester. There was no difficulty in classifying these two types on solid medium, especially if scoring was early. The presence or absence of a modifier linked to the gene pan-l (5531) in group IV seemed to be responsible for the dual expressions of sc, as indicated in Table I.