Cytological Evidence Opposing the Theory of Brachymeiosis in the Ascomycetes

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Recently a collection of the discomycete, *Patella melaloma,* was obtained by the writer from burnt-over ground in this area. The fungus has been maintained in culture, where it readily produces its fruiting structures or apothecia. The species is of particular interest because it is one of several discomycetes studied by Gwynne-Vaughan (*1*), the most outstanding contemporary proponent of the theory of double fertilization (two nuclear fusions) and brachymeiosis (two reductional divisions) in the ascomycetes.

Probably the majority of geneticists and most cytologists working on the ascomycetes have doubted for some time that these two processes occur. Thus far, however, no convincing evidence has been presented either to prove or to disprove the theory for those species in which these phenomena are said to exist. Most of the species of ascomycetes in which double fertilization and brachymeiosis have been reported to occur have proved unfavorable for genetical study. In those heterothallic species which might be used for genetical study such difficulties are experienced as inability to obtain a satisfactory percentage of ascospore germinations, or failure of the ascospores to occur in an orderly sequence in the ascus, or failure of the fungus to fruit well in culture. All the cytological evidence bearing on this subject has thus far been in the form of drawings and descriptions of sectioned material stained with the use of techniques which are frequently inadequate for revealing the chromosome numbers of the nuclei at various phases in the life cycle.

With the use of the propiono-carmine staining technique, recently employed with excellent results by Wheeler *et al.* (*2*) in a cytological study of ascus development in *Glomerella,* the writer has been able to observe the three successive nuclear divisions in the ascus of *Patella melaloma* and to determine with certainty the number of chromosomes present during each division. One of the most important features of this technique is that it leaves the spindle fibers unstained and that the chromosomes stand out clearly in the nuclear vacuole. Photographs demonstrating the numbers of chromosomes in all three divisions in the ascus have been obtained and will be included in a paper to be published later.

Gwynne-Vaughan (*1*), in her study of this species, reported that the nuclei fused in pairs in the ascogonium to produce diploid nuclei. These diploid nuclei were believed to pair among themselves, migrate into the ascogenous hyphae, and finally fuse in pairs in the young asci. Thus each ascus was believed to contain a tetraploid nucleus. She stated that the tetraploid number of 8 chromosomes (4 pairs) was observed at metaphase of the first division in the ascus. These chromosomes were believed to pass in groups of 4 (the diploid number) to opposite poles of the spindle. In this way the first reductional division was completed. Each daughter nucleus was then observed to divide mitotically, 4 chromosomes passing to opposite spindle poles. Then in the third division, each of the 4 nuclei was believed to undergo a second reductional division with 2 chromosomes (the haploid number) passing to opposite spindle poles. Therefore each of the 8 ascospores was supposed to receive a nucleus with only 2 chromosomes.

The writer is able to confirm Gwynne-Vaughan's report that there are 4 pairs of chromosomes present at the beginning of the first nuclear division in the ascus, and that a complement of 4 passes to each pole as the division is completed. In the second division also, 4 chromosomes appear and divide, a complement of 4 passing into each daughter nucleus. But the third division, instead of being reductional as Gwynne-Vaughan described it, is similar to the second division, and 4 chromosomes, rather than 2, pass into the daughter nuclei. The 8 ascospore nuclei, therefore, contain 4 chromosomes each. It is obvious from this brief description that the chromosome number is reduced only in the first division. The diploid number of chromosomes is 8 and the haploid number is 4. The third division is nonreductional. It is therefore obvious that brachymeiosis does not occur in this fungus. It is equally obvious that there can be no double fertilization in the life cycle.

The writer intends to extend these observations to other species in which double fertilization and brachymeiosis have been reported. The propiono-carmine technique is recommended as an efficient method for obtaining chromosome counts in the ascomycetes.

References

1. GWYNNE-VAUGHAN, H. C. I. *Ann. Bot.,* 1937, **1**, 99.
2. WHEELER, H. E. *et al. Amer. J. Bot.,* 1948, **35**, 722.

Inability of Thymine and Adenine to Substitute for Pteroylglutamic Acid in the Folic Acid-deficient Rat

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Although it has long been recognized that thymine can replace folic acid for growth of microorganisms (*3*), it has not been emphasized sufficiently that both thymine and purine bases are required for this purpose (*5, 6*). If an analogous situation exists in animals, it might be expected that thymine alone would be ineffective in replacing folic acid in a synthetic type of diet which does not supply purine bases, and this has indeed been shown true for both the rat and the chick (*1, 3, 4*). Experi-

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