

Pittenger, T. H. The distribution of nuclei in conidia of cultures having different average numbers of nuclei.

Not a great deal is known concerning the various factors which affect the number of nuclei present in conidia of *Neurospora*. Huebschman (1952 *Mycologia* 44:599) reported that conidia

grown on complete media had a much higher average nuclear number than those grown on minimal medium. Weijer (1964 *Canad. J. Genet. Cytol.* 6:383) reported that wild type 74A had an average nuclear number of 5.6 when grown on minimal medium and Kihara (1962 *NN*#2:8), working with wild type strain 3.1a, a strain derived from crosses involving 74A, showed that the average nuclear number was apparently less than two. Furthermore, she was unable to demonstrate that growth on complete media increased the average nuclear number. Both cultural conditions and genetic background might be expected to influence nuclear number, but at present little is known of the relative effects of such factors. Since not only the average number of nuclei in conidia, but also the frequency distribution of the various nuclear classes, are important in both radiation experiments and in heterokaryon studies, additional data of this type may be useful to others and a summary of some rather extensive data is presented below.

In some of the early work on heterokaryons by K. C. Atwood and myself, the average number of nuclei in over 33,000 conidia from a variety of cultures was determined. A detailed listing of the 64 homokaryotic and heterokaryotic cultures examined would be more useful, but because of space limitations only a summary of our results is given. I have simply taken the various cultures, divided them into three groups having different average numbers of nuclei, and summarized the frequencies of the various classes in order to illustrate how the distribution of the numbers of nuclei per conidium changes as the average nuclear number increases. The average number of nuclei in group A (28 cultures) ranged from 2.01 to 2.48; Group B (18 cultures) averages ranged from 2.50 to 2.88; and Group C (18 cultures) averages ranged from 3.0 to 3.88.

Frequency of number of nuclei per conidium.

	1	2	3	4	5	6	7	8	9	10	11
A	17.50	50.12	22.30	5.70	2.75	0.73	0.61	0.24			
B	11.44	41.83	28.98	9.87	4.21	1.87	0.72	0.64	0.42		
C	7.95	30.43	29.78	16.32	7.01	3.12	2.12	1.27	0.89	0.64	0.43

The data summarized above include the data from 18 homokaryotic cultures and 46 heterokaryotic cultures; an average of over 500 conidia were cytologically examined per culture. The 46 heterokaryons (eight different combinations of strains were examined) were all grown on minimal Westergaard's medium and the average nuclear number was 2.56. The 18 separate homokaryotic cultures, including mutant strains with requirements for pantothenate, nicotinic acid, lysine, arginine and p-aminobenzoic acid, had an average of 2.76 nuclei per conidium. Nine of the 18 homokaryons had an average of 3.0 or greater, while only nine of the 46 heterokaryons had averages of 3.0 or greater. Three of the 18 homokaryons were grown on complete medium (0.5% yeast extract and 0.5% casamino acids); the remainder were grown on the supplements required by the individual mutants. There was no effect of the complete medium in increasing the number of nuclei per conidium as reported by Heubschman.

Of the 64 cultures examined, the lowest average number of nuclei per conidium was 2.01 in an al-1, pan-1 + nic-2; al-2 heterokaryon, and the highest was 3.88 found in the same nuclear combination prepared at a different time. The overall average of the 64 cultures was 2.62.

The lowest average number I have encountered (not included above) was in a pseudo-wild type culture with an average of 1.25. It was phenotypically a microconidial culture and one of the parents was pe, fl. Over 2,500 conidia were examined and 77% were uninucleate, 20% had two nuclei and 2.1% had three nuclei.

In the above experiments the general procedure followed for staining was that proposed by Huebschman, with a few exceptions. Conidia were first dusted onto coverslips covered with a very thin layer of albumin

and then patted firmly. The entire staining procedure was carried out in Columbia jars rather than in the larger Coplin jars. In most of our experiments, rather than using 50 ml of 2% aqueous Azure A + 3 ml of 10% NaHSO_4 + 3 ml of 1 N HCl for staining, we simply placed 10 ml of a 1% aqueous solution of Azure A in a Columbia jar and, approximately half an hour before the stain was used, added 2 or 3 drops of thionyl chloride. - - - Department of Agronomy, Kansas State University, Manhattan, Kansas.