

Distribution of nuclei in conidia

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Abstract

Distribution of nuclei in conidia

In an attempt to hastily review the literature on nuclear distribution in conidio of *Neurospora* for this issue of the NN on spores, I had hoped to be able to find enough data to try to summarize the various environmental and genetic factors which affect nuclear numbers. I have been unable to find any systematic studies of this kind. The initial paper by Huebschman (1952 *Mycologia* 44: 599) is the most widely quoted, but it is nevertheless limited in scope and some of the most widely referred to of his results cannot be repeated in other strains. His data clearly show that the average number of nuclei in conidio is almost doubled if the cultures are grown on complete medium and that casamino acids are particularly effective in this regard. The data show that not only were conidia larger in size (9.7 μ in diameter vs. 7.1 μ) when grown on complete as compared to minimal, but the conidial volume was increased approximately in proportion to the increase in the average nuclear number (2.6 for minimal vs. 6.2 for complete). He also pointed out that the behavior on a given medium was independent of the strain used and that the average nuclear number was independent of age. The distribution of nuclei in conidia on minim.1 and complete was not Poisson in form.

Since it can be cytologically observed that older and larger hyphae tend to have more nuclei per "cell" than younger hyphae, and since cultures grown on complete might be expected to have more nuclei per "cell" than those grown on minimal (as a corollary of Huebschman's observations), one might expect that any environmental condition favoring increased numbers of nuclei per unit volume of the hyphae would increase the number of nuclei per conidium within the limits set by the genome. Conversely, growth-limiting conditions would be expected to result in smaller hyphae and a lower average number of nuclei in conidio. Certain observations, in addition to those above, tend to support these assumptions. For example, Atwood and Mukai (1955 *Genetics* 40: 438) reported that in heterocaryons grown on high concentrations of sorbose the average number of nuclei per conidium was lower than in cultures grown on limiting amounts of sorbose. Strauss (1956 *Rod. Res.* 5: 25) reported a relationship between the amount of phosphate in the medium and the proportion of conidio with more than three nuclei. Likewise, the numerous reports in the literature that cultures grown on minimal and singly-supplemented media have low average numbers of nuclei per conidium support the idea that growth-limiting conditions may affect the numbers of nuclei in the conidia within certain

limits. It is assumed that the low average number of nuclei per conidium is a reflection of the concentration of nuclei in the hyphae prior to conidial formation. K. C. Atwood made an interesting observation some time ago that might bear on this point. Although I do not have the data, he observed that if the average nuclear number was determined on conidia from a slant culture, this number was considerably lower than the average number found in the same culture after water was used to flush out the aerial hyphae and conidia and the culture then allowed to reform conidia in the same slant. Whether this observation is related to the fact that these new conidia were formed largely from older hyphae, with high nuclear numbers, or is related to the increased moisture undoubtedly present in the slant, is unknown. Many of our experiments have suggested that humidity, as an uncontrolled variable, may be an important factor affecting nuclear numbers in conidia, but controlled experiments have not been done.

There are many other observations in the literature which indicate that unspecified variables influence the average nuclear number in genetically similar strains. For example, Weijer (1964 *Con. J. Genet. Cytol.* 6: 383) reported that macroconidia of wild type 74A had an average of 5.6 nuclei per conidium. We have never found an average number this high for many mutants induced in the 74A background or in any other culture. Goodman (1958 *Z. Vererbungslehre* 89: 675) reported that St. Lawrence wild type (presumably 74A) had an average nuclear number of less than two and his graphs showed that there were more than 40% of both uninucleate and binucleate conidia. On the other hand, in over 100 separate determinations we have never found over 30% of the conidia to be uninucleate.

Since both microconidial (largely uninucleate, but see Pittenger NN#7) and macroconidial strains are known, as well as strains which produce both types, it is clear that genetic factors affect nuclear numbers. Grigg (1965 NN#7: 12) has also reported that it is possible to induce either microconidiation or macroconidiation in the same strain. Certain evidence suggests that the genotype may determine to what extent a strain can respond to its external environment in regard to changes in the average nuclear number in macroconidia. For example, Huebschman (*loc. cit.*) has clearly shown that for the strains he used (from the isolation numbers they would appear to be genetically similar or at least to have had the same parents in common), all responded to change in the media in the sense that the average nuclear number could be increased by growth on complete medium. On the other hand, Kihara (1962 NN#2: 8) and Pittenger (1965 NN#7: 4) showed that complete medium had no effect on increasing the average nuclear number in the strains they used. The simplest explanation of such differences is simply that all strains are not capable of responding to complete medium.

Since the last note I included in NN#7 on this subject, M. Grindle and I have examined the nuclear distribution in a large number of heterocaryons and homocaryons involving the markers ad-4 (F4), pan-2 (B3)a, al-1, al-2 and cot. A pan-2; cot strain grown on Vogel's medium at 25°C had an average nuclear number of 4.2 and 4.18 in two separate determinations and heterocaryons in which this strain was in the majority usually had a high average nuclear number as well. However, in another experiment, when the same strain was grown on the medium of Westergaard and Mitchell, the average nuclear number was only 2.9. Strains with cot, alone or in combination with other markers, often have high average nuclear numbers, but there are enough exceptions that it is not possible to conclude that cot alone is responsible for these increases.

Since the average nuclear number may vary considerably in the same strain grown at different times, it is clear that environmental factors have an effect on nuclear numbers. However, since these environmental factors have not yet been well defined, it is less clear in many cases to what extent various genetic factors affect nuclear numbers in macroconidia. From the fact that many strains do not show an increase in nuclear numbers on complete medium, it would appear that the residual genotype may set some upper limit on this value. Experiments with a variety of wild type strains grown under controlled conditions are now needed to more clearly define the effect of genetic and environmental factors on nuclear numbers. In the meantime, all that can be done with the existing data is to combine it in some way so that the investigator has some notion of the nuclear distribution that can be expected in cultures with certain average nuclear numbers.

Table 1. Number of Nuclei per Conidium

	1	2	3	4	5	6	7	8	9	10	11
<u>52 cultures with average nuclear number of 2.26 (Range 1.87-2.59)</u>											
Ave.	17.3	48.6	23.0	6.4	2.8	0.7	.2				
High	28.9	57.8	31.9	13.2	7.1	1.6	2.1				
Low	3.8	38.2	9.9	2.6	0.4						
<u>2. cultures with average nuclear number of 2.77 (Range 2.62-2.941)</u>											
Ave.	12.0	39.6	29.5	10.6	5.2	2.2	1.0	0.5			
High	17.5	42.4	34.9	16.3	8.6	3.8	5.5	1.1			
Low	7.9	28.6	25.2	8.4	2.6	0.9					
<u>30 cultures with average nuclear number of 3.29 (Range 3.0-4.2)</u>											
Ave.	8.0	28.4	28.9	16.9	4.4	3.8	2.2	1.5	0.7	0.6	0.5
High	20.0	34.5	36.4	19.9	14.8	13.5	5.3	5.6	2.0	2.0	5.5
Low	2.5	12.2	18.6	10.7	4.6	2.2	0.9	0.3			

In the foregoing table I have combined some of the recent data obtained by Grindle and myself with the data that I presented in NN#7. The data are based on nuclear distribution in 109 separate determinations, but many strains were used several times. Nuclei in 400-500 conidia were usually scored and the data include both homocaryons and heterocaryons. Sufficiently different genetic backgrounds and auxotrophic markers were used to make the data fairly representative of the average nuclear numbers one will routinely encounter. It should be pointed out that because of the way these data were collected one cannot say that, because 52 of the 109 cultures had an average nuclear number of 2.26, this average nuclear number is the most commonly encountered. The standard errors for each nuclear number class have not been determined, but the range in values within each class is quite large. • • • Department of Agronomy, Kansas State University, Manhattan, Kansas 66504.