Pittenger, T. H. Distribution of nuclei in conidio.

In on attempt to hastily review the literature on nuclear distribution in conidio of Neurospora for this issue of the NN on spores, I hod 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 poper 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 ore grown on complete medium ond that casamino acids ore particularly effective in this regard. The data show that not only were conidia larger in sire (9.7 μ in diometer 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 overage 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 ond that the average nuclear number was independent of age. The distribution of nuclei in conidia on minim.1 ond complete was not Poisson in form.

Since it can be cytologically observed that older ond larger hyphae tend to hove more nuclei per "cell" than younger hyphoe, and since cultures grown on complete might be expected to hove more nuclei per “cell” than those grown on minimal (os a corollary of Huebschman's observations), one might expect that ony 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 ond 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 thot 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 phosphote in the medium ond the proportion of conidio with more than three nuclei. Likewise, the numerous reports in the literature thot cultures grown on minimal and singly-supplemented media hove low average numbers of nuclei per conidium support the ideo thot growth-limiting conditions may affect the numbers of nuclei in the conidia within certain limits. It is assumed thot 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 mode on interesting observation some time ogo that might bear on this point. Although I do not hove the data, he observed that if the average nuclear number was determined on conidio 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 some slant. Whether this observation is related to the fact that there new conidio 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 hove suggested that humidity, as an uncontrolled variable, moy be an important factor affecting nuclear numbers in conidio, but controlled experiments hove 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 hod an overage of 5.6 nuclei per conidium. We hove never found on 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) hod 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 some 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 mocroconidio. 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 hove had the same parents in common), all responded to changer 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 ond I hove examined the nuclear distribution in a large number of heterocaryons and homocaryons involving the morkerr 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 on overage nuclear number of 4.2 and 4. 18 in two separate determinations and heterocaryons in which this strain was in the majority usually hod a high overage nuclear number as well. However, in another experiment, when the some 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 moy vary considerably in the same strain grown at different times, it is clear that environmental factors hove on effect on nuclear numbers. However, since there environmental factors hove 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 on 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 con 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
		52 cultures with average nuclear number of 2.26 (Range 1.87-2.59)									
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						
		2, cultures with average nuclear number of 2.77 (Range 2.62-2.941									
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					
		30 cultures with average nuclear number of 3.29 (Range 3.0-4.2)									
Ave.	8.0	28.4	28.9	lb.9	a.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 pre- rented in NN#7. The data ore bared on nuclear distribution in 109 separate determinations, but many strains were used several timer. Nuclei in 400-500 conidio 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 soy that, because 52 of the 109 cultures hod an average nuclear number of 2.26, this average nuclear number is the most commonly encountered. The standard errors for each nuclear number class hove not been determined, but the range in values within each class is quite large. - - - Department of Agronomy, Kansas State University, Manhattan, Kansas 66504.