NUCLEAR DISTRIBUTION IN CONIDIA OF NEUROSPORA HETEROKARYONS[1]

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IN *Neurospora* heterokaryons with two genetically different kinds of nuclei, some of the macroconidia are heterokaryotic and some are homokaryotic for either of the nuclear types. The relative frequencies of these three classes of conidia can be found experimentally if suitable genetic markers are present in the nuclei. It is reasonable to suppose that these frequencies are related both to the number and distribution of nuclei in the conidia and to the frequencies of the two nuclear types in the culture. PROUT *et al.* (1953) have tested the assumption that the different nuclei are distributed randomly, according to their frequencies, into conidia of each given number class. Three different heterokaryons were used, the conidia from a number of cultures of each being plated on differential media. All were assumed to have the same frequency distribution of conidia with different numbers of nuclei per cell. Values for the proportions of nuclear types were found which, when transformed into frequencies of conidial types on the randomness assumption, would give the best fit to the observed conidial frequencies. Even when the best fit was obtained, the observed frequencies of homokaryotic cells were ordinarily in excess of the expected.

Similar experiments on another heterokaryon will be reported here. In these experiments the nuclear ratio and the average number of nuclei per conidium were changed by varying the medium. Deviations from the expected conidial frequencies were observed, always in the same direction as those found by PROUT *et al.* Whether such deviations preclude satisfactory estimation of the nuclear ratio is questionable. A simple approximation for the nuclear ratio from plating data will be given, based on assumptions differing in some ways from those of the randomness hypothesis and suggested by the nature of the observed departure from randomness.

MATERIALS AND METHODS

The nuclear components of the heterokaryon used were arginine (29997A) and methionine-amycelial (4894-422A), the latter a double mutant combining a methionine requirement and a rather drastic morphological modification. These components form a nutritionally nonexacting heterokaryon of normal morphology. The stock was maintained on Neurospora minimal medium and transferred to agar slants having various amounts of added sorbose. Conidia were taken from these cultures, suspended in water, strained through fine glass wool to remove mycelial fragments, appropriately diluted, and added to pour plates of sorbose agar. The plating medium was Neurospora minimal with 1.0 percent sorbose and 0.1 percent sucrose and was supplemented with 50 μg of dl-methionine per ml or 50 μg of l-arginine per ml or both, as indicated. Colonies were counted after 4 days at 33°C. Conidia were stained for nuclear counts, using the technique described by HUEBSCHMAN (1952).

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TABLE 1

Frequency distribution of conidia with different numbers of nuclei

Expt.	Sorbose in minimal medium (mg/cc)	Nuclei per cell							Total cells	Av. no. of nuclei/cell, $\bar{n}$
		1	2	3	4	5	6	>6		
a	0.00	92	364	313	130	51	30	20	1000	2.88
b	0.0003	64	329	316	166	61	25	39	1000	3.16
c	0.009	99	381	322	131	47	13	7	1000	2.71
d	0.039	101	356	308	129	60	29	17	1000	2.87
e	0.16	199	448	223	78	28	13	11	1000	2.39
f	0.62	208	447	224	77	25	10	9	1000	2.34
g	2.5	224	389	212	103	44	13	15	1000	2.48

TABLE 2

Colony counts on different media. Each count is an average of four plates

Expt.	Minimal medium	Methionine		Arginine	Methionine + Arginine		Correction factor for methionine plates
		Normal	Amycelial		Normal	Amycelial	
a	336	268	103	423	368	99	1.25
b	213	177	79	331	256	50	1.20
c	234	193	183	314	254	208	1.21
d	122	84	210	138	98	195	1.45
e	92	67	193	110	103	149	1.37
f	71	63	143	101	64	136	1.13
g	98	53	175	119	76	210	1.84

RESULTS

On the basis of preliminary observations, it was suspected that the nuclear ratio and number of nuclei per conidium for this heterokaryon would both be changed by addition of sorbose to the medium. This proved correct and afforded a means of varying these parameters over a suitable range. Seven different cultures were studied. Table 1 shows the distributions of numbers of nuclei in conidia from these cultures.

Samples of the same conidia were plated on minimal, singly supplemented, and doubly supplemented media. Heterokaryotic conidia can initiate growth on any of the media, but those homokaryotic for methionine-amycelial or arginine can grow only where the respective supplements have been added. Since the methionine-amycelial homokaryons are morphologically distinct, they were scored separately where methionine was supplied. The results are shown in table 2. There is a deficit in the colony count wherever methionine is present in the medium. The number of normal colonies on methionine medium should equal the number on minimal, but it is less in every case. Similarly, there are fewer normal colonies on doubly supplemented medium than on arginine medium, where equal numbers are expected. This anomaly was manifest in the appearance of methionine plates; the colonies, both normal and amycelial, were variable in size, ranging to the limit of visibility. Further incubation did not change this picture, but it was found in subsequent experiments that the use of l-methionine (instead of dl) and reduction of the concentration to 25 μg per ml would obviate the effect. On minimal and arginine medium the colony size

TABLE 3

Observed and expected frequencies of conidial types, with estimates of $\hat{p}$ *by the iterative method, first approach*

Expt.	Conidial types						T (Total)	Estimated p or $\hat{p}$	χ^2	Degrees of freedom	Signif. level
	Ta Obs.	$T\alpha$ Expected	Tb Obs.	$T\beta$ Expected	Tr Obs.	$T\rho$ Expected					
a	129	129	87	81.1	336	341.9	552	0.555	0.531	1	0.47
b	95	61.3	118	81.6	213	283.1	426	0.468	52.12	2	<0.01
c	222	186.8	80	51.1	234	298.1	536	0.654	36.76	2	<0.01
d	305	298.9	16	9.2	122	134.9	443	0.886	6.38	2	0.02–0.05
e	265	265	18	14.2	92	95.8	375	0.858	1.17	1	0.27
f	161	149.7	30	18.8	71	93.5	262	0.775	12.94	2	<0.01
g	324		21		98		443	0.876	2.54	1	0.11

was uniform. It cannot be ascertained from the counts whether the methionine inhibition applies equally to methionine-amycelial homokaryons and other conidial types, but one would suppose this to be the case from the appearance of the plates. The assumption was made that methionine inhibition did not affect the ratio of normal to amycelial colonies. It appeared also that the severity of the effect was variable from plate to plate. The data were therefore corrected in each case by setting the count of normal colonies on methionine medium equal to the count on minimal, and computing a new value for the count of methionine-amycelial colonies according to the ratio of normal to amycelial colonies on the methionine plate. The numbers of arginine homokaryons were estimated by subtracting the counts on minimal from those on arginine medium. The corrected data are given in table 3 as the observed numbers of the three cell types.

According to the random distribution hypothesis, the proportion, p, of nuclei of the first type and the proportions $C_1, C_2, C_3, \ldots$, etc. of conidia, having 1, 2, 3, . . . , etc. nuclei, are related to the proportion, α, of homokaryotic conidia of the first type; β, of the second type, and ρ, of heterokaryotic conidia by the equations

$$\alpha = C_1p + C_2p^2 + C_3p^3 + \ldots + C_np^n, \tag{1}$$

$$\beta = C(1 - p) + C_2(1 - p)^2 + C_3(1 - p)^3 + \ldots + C_n(1 - p)^n, \tag{2}$$

and

$$\rho = 1 - (\alpha + \beta). \tag{3}$$

To test the hypothesis, by using a (the observed values of α), C_1, etc., p was first estimated from equation (1) by successive approximation. This estimate of p and the total number of conidia, T, were then used to compute expected values, β and ρ in equations (2) and (3). These expected values were compared with the observed values, b and r, by the usual χ^2 test with one degree of freedom. Because of the manner in which p was estimated and because some of the observed values were obtained by subtracting plate counts, the computed statistic does not have a χ^2 distribution, even asymptotically. However, it was simple to compute and served as a sort of preliminary test of significance. The χ^2 computed in this manner will always be greater than

TABLE 4

Observed and expected frequencies of conidial types, iterative method of Prout et al

Expt.	Conidial types						$\hat{p}$	χ^2	Signif. level
	Ta Obs.	Ta Expected	$T(b+r)$ Obs.	$T(\beta+\rho)$ Expected	Tr Obs.	$T\rho$ Expected			
a	129	128.5	423	419.7	336	339.8	0.557	0.070	0.79
b	95	90.5	331	299.4	213	254.3	0.578	10.264	0.0014
c	222	220.1	314	293.3	234	258.6	0.715	3.819	0.051
d	305	304.4	130	134.5	122	126.2	0.875	0.235	0.63
e	265	264.7	110	108.1	92	94.3	0.860	0.088	0.76
f	161	160.7	101	93.7	71	79.4	0.813	1.454	0.23
g	324	323.8	119	116.0	98	101.3	0.878	0.183	0.67

TABLE 5

Comparisons of p *estimated by different means*

Expt.	Iterative method first approach	Approximation eq. (4)	Iterative method of PROUT *et al.*
a	0.555	0.556	0.557
b	0.468	0.463	0.578
c	0.654	0.659	0.715
d	0.886	0.864	0.875
e	0.858	0.846	0.860
f	0.775	0.763	0.813
g	0.876	0.861	0.878

the minimum χ^2 so that if the hypothesis is supported by the preliminary test, a second computation is not necessary. If the significance level for the preliminary test was below 10 percent, p was re-estimated by the more laborious method of minimum χ^2, by use of all three observed values simultaneously. It is felt that the sample sizes were large enough to minimize the effect of the error introduced by subtracting plate counts; i.e., the values of χ^2 which were obtained may be taken as fairly representative of those which would have been found if it had been possible to observe the numbers of the three cell types directly. As shown in table 3, the deviation from randomness was significant in four out of the seven cases.

As a second approach, the method of PROUT *et al.* was applied. The methionine plate was corrected as previously described and the number on the arginine plate was used directly. The χ^2 computed from classes Ta, $T(b+r)$, and Tr was minimized with respect to p and T. These results, summarized in table 4, confirm the conclusion from table 3; namely, that heterokaryotic cells are less frequent than expected and homokaryotic cells more frequent. The estimates of p by the two procedures are not greatly different.

DISCUSSION

Whereas it is clear that the randomness hypothesis often fails to explain the data, it is by no means evident that estimates of p by methods based on random distribu-

tion are correspondingly erroneous. A deficit of heterokaryotic conidia, with roughly proportional increases in both classes of homokaryotic cells, could lead to enormous deviations from random nuclear distribution with negligible change in the minimum χ^2 estimate, $\hat{p}$. The relation of p to the plating results might be hopelessly obscured if the assortment of nuclei were preferential, according to genotype, but this does not appear to be the case for any of the material which has so far been studied. The experimental findings suggest that the distribution of genetically marked nuclei departs from random in only one respect, a tendency for like nuclei to occur together. This tendency is not unexpected in view of the circumstances of conidiation. Conidia are formed at the terminal branches of the aerial hyphae, and the daughter nuclei arising by mitosis in these locations have little opportunity to become mixed in a random nuclear pool. Consequently, the multinucleate conidia will tend preferentially to include isogenic nuclei. Moreover, as PROUT *et al.* have pointed out, experimentally isolated hyphal tips are sometimes found to be homokaryotic. Perhaps the culture, at the time of conidiation, should be regarded as partly heterokaryotic and partly a mosaic of homokaryotic regions. Such a model leads without further assumptions to the observed deviations from the random distribution.

This type of departure from randomness has an important consequence from the standpoint of the estimation of p; namely, that the average nuclear numbers among the different cell types become more alike. If the average nuclear number were the same in the two types of homokaryotic cells, then the ratio of the types in a rather large segment of the nuclear population would be known. When random distribution is the rule, the nuclear composition of conidia of each number class follows the binomial distribution with the proportions of homokaryotic cells represented by only the first and last terms. This regime would lead to marked differences in average nuclear number among the three cell types. In general, the heterokaryotic cells would have the highest number of nuclei, and the more frequent homokaryotic type would have a higher average number than the less frequent. However, insofar as like nuclei occur together in excess of chance, there will be an increased frequency of homokaryotic cells with higher nuclear numbers. This will tend to equalize the nuclear numbers in the three classes, particularly in the two homokaryotic classes. The small variance of the typical nuclear distribution works in favor of similarity of numbers of nuclei among the classes of cells, although the presence of uninucleate cells, which must be homokaryotic, will create some difference in nuclear number between homokaryotic and heterokaryotic cells.

In view of the trend toward equality of nuclear number in the two homokaryotic types, the following approximation for p is developed. First, it is recognized that each heterokaryotic cell has at least one nucleus of each type; thus two nuclei per heterokaryotic cell are of known type. Second, the ratio of types among nuclei in excess of those of known type is assumed for the sake of approximation to be the same as the ratio of the cell types among homokaryotic cells, i.e., a/b. Let us consider a cell population of size N. The size of the corresponding nuclear population is $N\bar{n}$, where $\bar{n}$ is the average number of nuclei per cell. Among the heterokaryotic cells there are at least Nr nuclei of each type. The remaining number of nuclei in the whole population is $N\bar{n} - 2Nr$, and if we assume that in this group the proportion of nuclei

of the first type is $a/1 - r$, then the total number of nuclei of the first type would be $Nr + [N(\bar{n} - 2r)a/1 - r]$. Thus,

$$p \approx \frac{r(1 - r) + a(n - 2r)}{\bar{n}(1 - r)}$$

and also

$$1 - p \approx \frac{r(1 - r) + b(\bar{n} - 2r)}{\bar{n}(1 - r)}.$$

This approximation has the interesting property of becoming more nearly correct in principle as the departure from random distribution increases. At the same time, roughly proportional increases in both homokaryotic cell types will have relatively small effects on the minimum χ^2 estimate, $\hat{p}$, in comparison to the effects on χ^2 itself. It is therefore to be expected that approximation (4) and the minimum χ^2 estimates should be in fair agreement even when χ^2 is large. Rather close agreement is observed as shown in table 5. It has been pointed out by PROUT *et al.* that the quantity $a + r/1 + r$, which represents the ratio of the number of colonies on the first supplement to the sum of those on the first and second supplements, will also approach p as r decreases. From the uniform direction of the deviations from random distribution, it can be inferred that the true value of p lies somewhere between this ratio and $\hat{p}$. From similar considerations it can also be inferred that approximation (4), with its assumption of equality of average nuclear number per cell in the different homokaryons, should ordinarily yield estimates deviating slightly from $\hat{p}$ and from the true p in the direction of 0.5, but not so far in this direction as $a + r/1 + r$. Therefore, the frequently excellent agreement of approximation (4) with results determined by the random distribution hypothesis, particularly by the first approach, lends strength to the notion that p can be quite satisfactorily estimated by either procedure. The simplicity of the approximation makes it preferable for ordinary purposes.

SUMMARY

The distribution of genetically marked nuclei in macroconidia formed by heterokaryotic cultures of Neurospora is frequently nonrandom. The departure from randomness takes the form of an increased tendency for like nuclei to occur together in the same cell. This type of nonrandom behavior is not a serious obstacle to the determination, with fair accuracy, of the relative frequency of a given nuclear type. A simple approximation is developed by which such frequencies can be estimated from plating data and nuclear counts.

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