THE PROPORTIONS OF NUCLEAR TYPES IN NEUROSPORA HETEROCARYONS AS DETERMINED BY PLATING CONIDIA[1]

TIMOTHY PROUT,[2] CHARLES HUEBSCHMAN,[3] HOWARD LEVENE
AND FRANCIS J. RYAN
Department of Zoology, Columbia University, New York

Received April 6, 1953

IN Neurospora the fusion of two strains, each homocaryotic for a different mutant nucleus, results in a heterocaryon in whose continuous cytoplasm the two types of nuclei are intermingled. If the mutations involve requirements for two different nutrients, the heterocaryon will grow without either nutrient, for each mutant nucleus carries the normal allele of the other which enables the heterocaryon to synthesize the nutrients required. During the growth of the heterocaryon on unsupplemented medium, some hyphae, for a variety of reasons, will come to have more favorable proportions of nuclear types, which will allow them to grow faster. These hyphae will, as a consequence, have a greater chance of forming the frontier of the mycelium and will contribute that favorable ratio of nuclear types to the new growth. Thus there will be a constant selection for a ratio of the two types of nuclei that will permit the maximum rate of growth.

If each of the two types of nuclei is capable of supporting the maximum rate of growth when present in low proportions, the nuclear ratios found in established heterocaryons might be expected to be variable; if, on the other hand, each mutant nucleus must be present in higher frequency to permit the maximum growth rate, the ratio of the two types might be expected to be comparatively constant at a value depending upon their relative synthetic efficiencies. For example, if each type of nucleus must form ten percent or more of the total nuclear population in order to permit the maximum rate of growth, any ratio between ten to ninety and ninety to ten will be selected for; if each type of nucleus must form fifty percent or more of the nuclear population, a fifty-fifty ratio will be selected for; finally, if each type of nucleus must be present as sixty percent of the total nuclear population, a fixed ratio somewhere between sixty to forty and forty to sixty will be selected for, but the heterocaryon will grow at less than maximum rate. From considerations such as these BEADLE and COONRADT (1944) have pointed out that studies on the nuclear composi-

---

[1] Supported in part by an American Cancer Society grant recommended by the Committee on Growth of the National Research Council, by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, and by the Office of Naval Research.

[2] A.E.C. Pre-doctoral Fellow.

[3] Deceased, April 25, 1951.

tion of heterocaryons might be made to reveal, among other things, facts about the quantitative aspects of dominance.

Such experimentation has been limited by the inadequacy of the methods thus far employed. Conidia from a heterocaryon may be crossed with another strain, the resulting ascospores isolated and the derived cultures characterized. Among half of the cultures should be found the nuclear types of the heterocaryon. But before the proportion of these types is considered to be equal to the nuclear ratio in the original heterocaryon, it is necessary to establish that neither selective fertilization nor selective spore survival has distorted the proportion. Both of these phenomena are known to occur in Neurospora (BEADLE and COONRADT 1944; SHENG 1951).

Another method for determining nuclear ratios in heterocaryons involves plating out the uninucleate microconidia formed by certain stocks of Neurospora (BARRATT and GARNJOBST 1949). To establish the reliability of this method it is necessary to show, first, that there is random entrance of nuclei into microconidia as they bud from the hyphae, and, second, that there is not differential survival among the plated microconidia. This latter consideration is especially important in view of the extremely small fraction of microconidia which do survive and germinate (RYAN 1951).

Use of the macroconidia of Neurospora also provides a means of separating nuclei from a heterocaryon, but such conidia are usually multinucleate. Nevertheless, some macroconidia contain nuclei of one type only; others, nuclei of the second type only, and the remainder a mixture of the two types. By plating conidia from a heterocaryon, under proper conditions, the proportions of the two homocaryotic classes and the heterocaryotic class can be established. If the hypothesis is adopted that the two types of mutant nuclei are distributed in the mycelium at random with respect to type, and are thus distributed at random among the macroconidia, which are formed by a series of constrictions in the hyphae, then it is possible to predict the relative frequencies of the three conidial classes or combinations of classes in terms of certain parameters. It is also possible to check the hypothesis of randomness against the data.

NOTATION

Consider a heterocaryon containing two different types of nuclei, type A, and type B, which cause requirements for substances A and B. This will produce macroconidia which are homocaryotic A, homocaryotic B, and heterocaryotic, in proportions a_{tr}, b_{tr} and h_{tr}, with $a_{tr} + b_{tr} + h_{tr} = 1$.

The frequencies of the three conidial classes can be determined experimentally by plating conidia from a suspension on four types of media. The first plate contains minimal medium, where only heterocaryons can grow, the second is supplemented with A and supports heterocaryons and type A homocaryons, the third is supplemented with B and supports heterocaryons and type B homocaryons, and the fourth is complete, or doubly supplemented, and supports all three types.

Let C_{tr} equal the true average number of conidia per plate, i.e., the average number that would be observed in a large number of replications. Then

$$\begin{aligned} M_{tr} &= C_{tr}h_{tr} && = \text{expected number of conidia growing on minimal,} \\ A_{tr} &= C_{tr}(h_{tr}+a_{tr}) && = \text{expected number of conidia growing on minimal} + A, \\ B_{tr} &= C_{tr}(h_{tr}+b_{tr}) && = \text{expected number of conidia growing on minimal} + B, \\ C_{tr} &= C_{tr}(h_{tr}+a_{tr}+b_{tr}) && = \text{expected number of conidia growing on complete.} \end{aligned} \quad (1)$$

Let M_{ob}, A_{ob}, B_{ob} and C_{ob}, be the observed number of conidia growing on the four media, M_{pl}, A_{pl}, etc., be the estimates of M_{tr}, A_{tr}, etc., based on random mixture and plating out of conidia with no differential mortality, and M_{rd}, A_{rd}, etc., be the estimates of M_{tr}, etc., based on these assumptions plus the assumption of random distribution of nuclei during the formation of conidia. From M_{pl}, etc., we obtain estimates a_{pl}, etc., of the frequencies of conidial types, and from M_{rd}, etc., estimates a_{rd}, etc., on the assumption of random distribution. Also let

$$T_{ob} = M_{ob} + A_{ob} + B_{ob} + C_{ob}. \quad (2)$$

Finally, let

$$\begin{aligned} p &= \text{frequency of type A nuclei in the parent hyphae,} \\ q = 1 - p &= \text{“ “ “ B “ “ “ “ “ }. \end{aligned}$$

THEORY

Random distribution of nuclei

Assuming random distribution of nuclei in all conidia with exactly n nuclei (regardless of type), the frequency of conidia with any specified number of type A nuclei is given by the appropriate term in the binomial expansion

$$(p+q)^n. \quad (3)$$

It follows that

$$\begin{aligned} a_{tr} &= p^n \\ b_{tr} &= q^n \\ h_{tr} &= 1 - p^n - q^n. \end{aligned} \quad (4)$$

All conidia do not contain, however, the same total number of nuclei. There is in fact an array of conidial numbers which can be denoted by $\phi(n)$ (the frequency of conidia with n nuclei). This distribution, $\phi(n)$, must be analyzed with respect to its contributions to each conidial class. The homocaryotic class, a_{tr}, for instance, is contributed to by p of the uninucleate conidia, of which there are $\phi(1)$, and by p^2 of the binucleate conidia, of which there are $\phi(2)$, and by p^3 of the trinucleate conidia, of which there are $\phi(3)$, etc. The equations for the classes denoted above by a_{tr}, b_{tr} and h_{tr}, therefore become the following:

$$
\begin{aligned}
a_{tr} &= p\phi(1) + p^2\phi(2) + p^3\phi(3)\ldots + p^k\phi(k) = \sum_{n=1}^{k} p^n\phi(n), \\
b_{tr} &= q\phi(1) + q^2\phi(2) + q^3\phi(3)\ldots + q^k\phi(k) = \sum_{n=1}^{k} q^n\phi(n), \\
h_{tr} &= 1 - (a_{tr} + b_{tr}) = 1 - \left\{ \sum_{n=1}^{k} p^n\phi(n) + \sum_{n=1}^{k} q^n\phi(n) \right\}.
\end{aligned}
\tag{5}
$$

Figure 1 shows a_{tr}, b_{tr} and h_{tr} as functions of p for the observed distribution $\phi(n)$ of table 1.

It is possible, therefore, to predict the relative frequency of the three conidial classes in terms of $\phi(n)$, the distribution of nuclear numbers, and p, the frequency of one of the two types of nuclei in the mycelium. By combining these expressions one can find the frequency of conidia growing on each of the four media; thus on minimal plus A we have the frequency

$$F_A = h_{tr} + a_{tr} = 1 - b_{tr}, \tag{6}$$

giving the true average number of conidia growing as

$$A_{tr} = C_{tr}F_A = C_{tr}\left(1 - \sum_{n=1}^{k} q^n\phi(n)\right). \tag{7}$$

It should be mentioned at this point that there is an alternative method for predicting the relative frequencies of the conidial classes. This method requires that an additional hypothesis be made, namely that $\phi(n)$ is a truncated Poisson distribution (the class of conidia with zero nuclei is missing). Although this would greatly simplify the statistical analysis to be described below, the method cannot be used because $\phi(n)$ has been found not to be a Poisson distribution (HUEBSCHMAN 1952). Hence, the experimentally determined distribution (see below) must be used.

Minimum chi-square estimates

The data from plating on the four media give a series of observed values; the theory of random distribution gives a corresponding series of expected values. An experiment of such a design, therefore, lends itself to a chi-square analysis. For the discussion of chi-square, we will simplify the notation and write

$$\chi^2 = \sum_{i=1}^{4} \frac{(O_i - CF_i)^2}{CF_i}, \tag{8}$$

where the O_i are the observed numbers on the four media, the F_i are the expected frequencies under the assumption of random distribution, and $C = C_{tr}$. It will be noted that χ^2 depends on two unknown parameters which must be estimated from the data: p, the proportion of A nuclei, and C_{tr}, the average number of conidia per plate. Since the method of maximum likelihood leads to equations which are difficult to solve, and since the values of χ^2 are wanted in any case, the method of minimum χ^2 was used. Both minimum χ^2 and maximum likelihood are asymptotically efficient and are equivalent in large samples (NEYMAN 1949) (in this context a large sample means a large value of C_{tr}). The problem thus is to find, for a given set of observed values, a pair of numbers, one for C and one for p, for which the expression (8) is a minimum.

The partial derivative of χ^2 with respect to C is

$$\frac{\partial \chi^2}{\partial C} = \Sigma F_i - \frac{1}{C^2} \sum \left(\frac{O_i^2}{F_i} \right) \tag{9}$$

and minimizing by equating to zero and solving for C yields

$$C = \sqrt{\frac{\sum \left(\frac{O_i^2}{F_i} \right)}{\Sigma F_i}}. \tag{10}$$

For any given value of p, where C is allowed to vary, equation (10) gives the value of C for which χ^2 is a minimum.

Substituting the expression (10) for C in equation (8) and simplifying gives

$$\chi^2 = 2\left\{ \sqrt{\left(\sum \frac{O_i^2}{F_i} \right) (\Sigma F_i)} - \Sigma O_i \right\} = 2\{\Sigma\, CF_i - \Sigma O_i\}. \tag{11}$$

Now, if p is allowed to vary, the value of p is desired at which χ^2 is a minimum. If R is allowed to denote the quantity under the radical in equation (11),

$$R = \left(\sum \frac{O_i^2}{F_i} \right) (\Sigma F_i), \tag{12}$$

then when R is a minimum χ^2 is a minimum. If R is differentiated with respect to p and set equal to zero, it can be seen that it is impossible to get a solution explicitly in terms of p, just as it is impossible to solve equation (12) for p. The value of p, at which R (and χ^2) is a minimum, must therefore be determined by iteration, and it is easier to perform this iteration on equation (12) than on the more complex equation $\partial R/\partial p = 0$.

For each value of p tried, four values of F_i must be calculated, as well as their sum, ΣF_i. If there are several sets of plating experiments on which these operations have to be done, then it is convenient to estimate graphically these values of F_i for each p tried. A curve may be computed for each F_i and for ΣF_i using selected values of p, and, of course, the predetermined distribution $\phi(n)$. The resulting curves were used in the calculations for this paper.

Tests of hypotheses

Theoretically, if the hypothesis, H_{pl}, is true that the suspensions of conidia are thoroughly mixed and plating is carefully done, and there are no disturbing factors such as differential mortality on the different media, the observed numbers O_i should have Poisson distributions with expected values $C_{tr}F_i$. If in addition the hypothesis, H_{rd}, of random distribution is true, then we have seen that the F_i are the functions of p given above. In any case, there is no reason to suppose that the deviations from the four Poisson distributions cancel each other and that the sum of the observed values equals the sum of the expected values. This is not the usual situation for the use of χ^2; however, it can be shown that the usual theory still holds and that the expression (8) has approximately a chi-square distribution. The number of degrees of freedom is the number of classes less the number of estimated parameters, or in this case $4-2=2$. The problem could be restated so that the usual theory held, but it would be rather artificial and would be asymptotically equivalent to the method used.

Obviously, p can not be estimated, nor can the hypothesis of random distribution of nuclei, H_{rd}, be tested, unless the hypothesis, H_{pl}, that there is no plating error is true. This hypothesis can be tested as follows. If it is true, then by (1)

$$M_{tr} + C_{tr} = A_{tr} + B_{tr}. \tag{13}$$

Hence, $M_{ob} + C_{ob}$ and $A_{ob} + B_{ob}$ will have Poisson distributions with the same mean and

$$t = \frac{(M_{ob} + C_{ob}) - (A_{ob} + B_{ob})}{\sqrt{(M_{ob} + C_{ob}) + (A_{ob} + B_{ob})}} \tag{14}$$

will be approximately a standard normal deviate, or $\chi^2_{pl} = t^2$ will have a chi-square distribution with one degree of freedom, and can be used to test H_{pl}. Under this hypothesis the maximum likelihood estimates M_{pl} of M_{tr}, etc., can be shown to be

$$\begin{aligned} M_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{M_{ob}}{M_{ob} + C_{ob}}\right), \\ A_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{A_{ob}}{A_{ob} + B_{ob}}\right), \\ B_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{B_{ob}}{A_{ob} + B_{ob}}\right), \\ C_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{C_{ob}}{M_{ob} + C_{ob}}\right). \end{aligned} \tag{15}$$

From these estimates, maximum likelihood estimates

$$\begin{aligned} a_{pl} &= (A_{pl} - M_{pl})/T_{ob} = (C_{pl} - B_{pl})/T_{ob}, \\ b_{pl} &= (B_{pl} - M_{pl})/T_{ob} = (C_{pl} - A_{pl})/T_{ob}, \\ h_{pl} &= M_{pl}/T_{ob}, \end{aligned} \tag{16}$$

of the proportions of the three types of conidia may be obtained. Note that no such estimates are possible without the hypothesis H_{pl}. In passing it may be mentioned that χ^2_{pl} could be calculated from the usual formula, using M_{ob}, etc., as the " observed " values and M_{pl}, etc., as the " expected " values, but it is algebraically identical with t^2 above, and t^2 is much easier to calculate.

The χ^2 with two degrees of freedom described earlier is appropriate for testing the combined hypothesis of no plating error and random distribution of nuclei. Let us call this chi-square χ^2_{tot}. It would be desirable to test the hypothesis of random distribution of nuclei separately on the assumption that the conidia were randomly distributed. NEYMAN (1949) has shown that whenever there are two hypotheses H_1 and H_2, testable by χ^2_1 and χ^2_2 respectively, and H_2 implies the truth of H_1, we can test H_2 under the assumption that H_1 is true by

$$\chi^2_{diff} = \chi^2_2 - \chi^2_1, \tag{17}$$

and χ^2_{diff} will be distributed as chi-square with a number of degrees of freedom equal to the number of degrees of freedom of χ^2_2 minus the number of degrees of freedom of χ^2_1. Applying this in our case we find

$$\chi^2_{rd} = \chi^2_{tot} - \chi^2_{pl} \tag{18}$$

is distributed as chi-square with one degree of freedom and specifically tests the hypothesis H_{rd} when H_{pl} is true.

Finally, it should be noted that the estimate of p is only valid when H_{rd} is true, and hence the estimate is suspect if either χ^2_{pl} or χ^2_{rd} is significant.

RESULTS

Table 1 shows the distribution of numbers of nuclei among macroconidia from three strains of *Neurospora crassa*. The cytological procedures used in counting the nuclei are described elsewhere (HUEBSCHMAN 1952). Only one

TABLE 1

The distribution of different numbers of nuclei among macroconidia.

Number of nuclei	Numbers of conidia			Frequency of conidia
	Expt. 1	Expt. 2	Expt. 3	
1	71	83	318	0.141
2	214	284	869	0.410
3	139	186	555	0.264
4	53	74	216	0.103
5	26	38	92	0.047
6	7	6	41	0.016
7	3	3	17	0.007
8	0	3	15	0.005
>8	2	2	15	0.006
Total conidia	515	679	2138	0.999

Expt. 1: heterocaryon between 5531-A and 4545-A on minimal medium at 25°C, 7 days old.

Expt. 2: strain 1-A on minimal medium at 25°C, 4 days old.

Expt. 3: heterocaryon between 1633-422-A and 5531-4637-A on minimal medium at 25°C (ATWOOD and NORMAN 1949).

of the strains studied was a heterocaryon whose nuclear ratio was analyzed; ideally, the cytological analysis should be performed on the same culture as is plated. The distributions are, however, a reproducible function of the conditions of growth employed in producing the conidia (HUEBSCHMAN 1952), and the three distributions shown here are homogeneous by χ^2 test ($\chi^2 = 16.47$, d.f. = 16, $P = 0.42$). The frequencies of conidia containing different numbers of nuclei that are shown in this table were used to calculate the curves in figure 1.

The construction of the heterocaryons and the determination of the proportions of homo- and heterocaryotic conidia were carried out by usual techniques (RYAN 1951). Strain 4545-A is lysineless and requires that amino acid for growth; 5531-A is panthothenicless; 1633-A is para-aminobenzoicless; 1-A is wild type and will grow on minimal medium, as will heterocaryons between the mutant strains. Since some of the conidia are homocaryotic and others heterocaryotic, when a sample of conidia from a heterocaryon of, say, 4545-A and 5531-A is plated on minimal medium, only heterocaryotic conidia form colonies. On agar medium supplemented with lysine, conidia homocaryotic for lysineless nuclei and the heterocaryotic conidia grow, while, conversely, on medium supplemented with pantothenate, the heterocaryotic conidia and those homocaryotic for pantothenicless nuclei grow. On medium supplemented with pantothenate and lysine all types of conidia form colonies. Thus are obtained the four classes of data, which have previously been discussed in general terms.

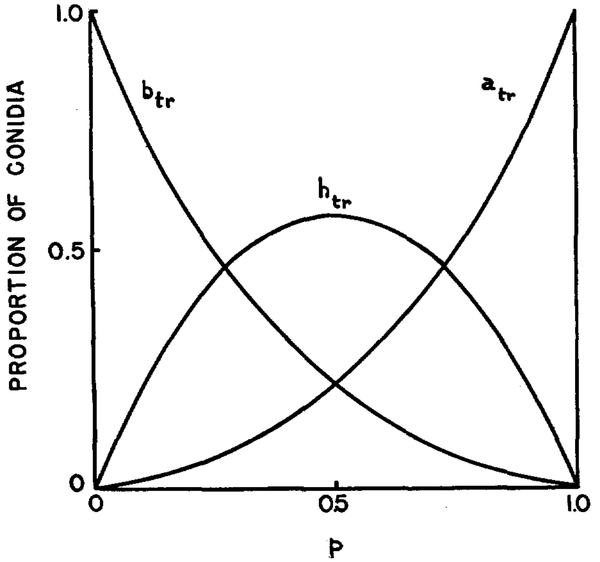

FIGURE 1.—Proportions of conidia, homocaryotic for A nuclei (a_{tr}), homocaryotic for B (b_{tr}) and heterocaryotic (h_{tr}), as functions of the frequency of type A nuclei (p).

Table 2 contains a comparison of the observed numbers of colonies on the different agar media and those expected on the basis of no plating error (EXP. PL.) and on the basis of random distribution of nuclei (EXP. R.D.). In each case the rarer component has been labeled A, the other B, as indicated by the

TABLE 2

The data secured by plating conidia from heterocaryons in four different media and the results of analyzing the same.

	Medium					Estimates		Chi square		
	M	A	B	C	T	$\hat{p}$	$\frac{A_{ob}}{A_{ob}+B_{ob}}$	χ^2_{tot}	χ^2_{pl}	χ^2_{rd}
4545-A + 5531-A (lysineless (A) + pantothenicless (B))										
Heterocaryon 1										
Obs.	54.	77.	198.	215.	544.	0.16	0.27	1.1	0.07	1.0
Exp. pl.	54.6	76.2	195.8	217.4	544.					
Exp. r.d.	65.2	73.5	200.1	207.	545.8					
Heterocaryon 2										
Obs.	49.	50.	143.	178.	420.	0.16	0.26	3.78	2.75	1.03
Exp. pl.	45.3	54.4	155.6	164.7	420.					
Exp. r.d.	51.2	56.7	154.3	159.8	422.					
Heterocaryon 3										
Obs.	58.	94.	218.	231.	601.	0.16	0.30	5.28	0.88	4.40
Exp. pl.	60.3	90.5	210.	240.2	601.					
Exp. r.d.	73.2	81.1	220.8	228.6	603.7					
Heterocaryon 4										
Obs.	60.	95.	171.	204.	530.	0.20	0.35	5.17	0.01	5.16*
Exp. pl.	60.2	94.7	170.3	204.8	530.					
Exp. r.d.	70.8	82.5	185.3	194.	532.6					
Heterocaryon 5										
Obs.	50.	75.	151.	183.	459.	0.18	0.33	3.80	0.11	3.69
Exp. pl.	49.3	76.1	153.4	180.2	459.					
Exp. r.d.	57.9	66.8	164.8	171.5	461.					
Heterocaryon 6										
Obs.	37.	43.	105.	105.	290.	0.18	0.29	0.14	0.12	0.02
Exp. pl.	37.8	42.1	102.9	107.2	290.					
Exp. r.d.	38.2	42.4	103.7	107.9	292.1					
4545-A + 1633-A (lysineless (A) + para-aminobenzoicless (B))										
Heterocaryon 1										
Obs.	56.	81.	137.	182.	456.	0.21	0.37	5.6	0.88	4.7*
Exp. pl.	53.6	84.7	143.3	174.4	456.					
Exp. r.d.	62.9	72.8	157.7	165.6	459.					
Heterocaryon 2										
Obs.	53.	126.	136.	215.	530.	0.30	0.48	30.**	0.07	30.**
Exp. pl.	52.4	127.5	137.5	212.6	530.					
Exp. r.d.	89.8	105.3	167.2	183.2	545.5					
Heterocaryon 3										
Obs.	74.	194.	158.	216.	642.	0.70	0.55	18.**	5.99*	12.**
Exp. pl.	81.9	176.9	144.1	239.1	642.					
Exp. r.d.	107.2	199.6	125.8	218.8	651.4					

* = significant χ^2 ($P < 0.05$).

** = highly significant χ^2 ($P < 0.001$).

TABLE 2 (*continued*)

	Medium					Estimates		Chi square		
	M	A	B	C	T	$\hat{p}$	$\frac{A_{ob}}{A_{ob}+B_{ob}}$	χ^2_{tot}	χ^2_{pl}	χ^2_{rd}
Heterocaryon 4										
Obs.	72.	186.	231.	288.	777.	0.33	0.45	34.5**	4.18*	30.3**
Exp. pl.	77.7	173.3	215.2	310.8	777.					
Exp. r.d.	135.4	161.7	235.4	263.	795.5					
Heterocaryon 5										
Obs..	85.	149.	171.	218.	623.	0.36	0.47	7.7*	0.46	7.2*
Exp. pl.	87.4	145.	166.5	224.1	623.					
Exp. r.d.	107.5	134.1	180.2	204.8	626.6					
Heterocaryon 6										
Obs.	33.	73.	85.	124.	315.	0.29	0.46	13.1*	0.003	13.1**
Exp. pl.	33.1	72.8	84.7	124.4	315.					
Exp. r.d.	52.6	61.2	99.4	108.4	321.6					
1633-A + 5531-A (para-aminobenzoicless (A) + pantothenicless (B))										
Heterocaryon 1										
Obs.	96.	117.	245.	302.	760.	0.21	0.32	5.8	1.71	4.1*
Exp. pl.	91.7	122.8	257.2	288.3	760.					
Exp. r.d.	104.5	121.	262.	275.1	762.6					
Heterocaryon 2										
Obs.	89.	128.	192.	226.	635.	0.28	0.40	3.0	0.04	3.0
Exp. pl.	89.7	127.	190.5	227.8	635.					
Exp. r.d.	100.6	117.4	201.1	217.4	636.5					
Heterocaryon 3										
Ops.	97.	203.	236.	348.	884.	0.33	0.46	33.2**	0.04	33.2**
Exp. pl.	96.4	204.4	237.6	345.6	884.					
Exp. r.d.	153.4	183.2	266.6	297.9	901.1					
Heterocaryon 4										
Obs.	117.	137.	302.	379.	935.	0.16	0.32	5.4	3.48	1.9
Exp. pl.	110.2	145.9	321.6	357.3	935.					
Exp. r.d.	114.2	126.4	344.3	356.5	941.4					

table. The column headed $\hat{p}$ gives the minimum chi-square estimate of the proportion of the A type of nucleus in the heterocaryon. The χ^2_{tot}, with two degrees of freedom, and the two components, χ^2_{pl}, for plating with one degree of freedom, and χ^2_{rd}, for random distribution with one degree of freedom, are calculated according to the method above. Significant values ($P < 0.05$) are denoted by an asterisk and highly significant values ($P < 0.001$) are denoted by two asterisks.

Each of the separate heterocaryons of a particular combination listed in table 2 was secured by isolating a hyphal tip from a common original heterocaryotic culture and was allowed to grow and form conidia on minimal medium independently. This procedure would be expected to allow the operation of whatever selective forces determine nuclear ratios in heterocaryons.

Before discussing the results it is necessary to consider the magnitude of the error introduced by the calculation of $\hat{p}$ by the graphical method rather than directly from the nuclear distribution. This was checked in the instance of heterocaryon No. 5 between para-aminobenzoicless and lysineless which gave, by graphical analysis, a $\hat{p}$ of 0.36 and a χ^2 of 7.7. Direct calculation yielded a $\hat{p}$ of 0.39 and a χ^2 of 8.3. Where a more precise value of p is desired, direct calculation of p should be employed.

DISCUSSION

There seems to be no evidence of non-random plating or differential mortality. Two of the 16 χ^2_{pl} values are slightly significant ($p = 0.014$ and $p = 0.041$), but this could happen by chance. The sum of the 16 χ^2_{pl} values is 20.79, giving $P = 0.23$. We can thus go on to consider the hypothesis of random distribution of nuclei, assuming H_{pl} to be true.

The nature of the distribution of nuclei seems to depend on the heterocaryon involved. Thus for lysineless-pantothenicless only one value out of six of χ^2_{rd} is significant ($P = 0.023$) and this could easily happen by chance. On the other hand, for lysineless-para-aminobenzoicless all six χ^2_{rd} values are significant, four of them highly so. In this case, where the evidence for non-random distribution of the nuclei is strong, it is not surprising that the estimate of p, based upon the assumption of randomness, can show considerable variation. The third heterocaryon is more erratic, with one extremely large χ^2_{rd} and one barely significant one out of four samples.

Since χ^2_{rd} has only one degree of freedom, there is essentially only one discrepancy between the values expected under random distribution and those expected under random plating alone. All other discrepancies can be predicted, given one of them. Study of table 2 shows the discrepancy can be best expressed as a deficiency of heterocaryons as compared with that expected under random distribution. Only one heterocaryon shows a slight excess of heterocaryotic conidia, and χ^2_{rd} for this is non-significant.

This suggests that within conidia the nuclei are correlated with respect to type; like types of nuclei tend to occur together. This effect could be produced by the entrance of a single nucleus into the bud of a conidia-producing hypha. This nucleus would have a head start in supplying the growing hypha with nuclei, all of the same type. Subsequently, nuclei from the main stem may invade the hypha, but an excess of homocaryons of one type will nevertheless be produced by this hypha. Sampling conidia from many hyphae, as is actually done, would yield an excess of homocaryons of both types. In fact, homocaryotic hyphal tips have been isolated in these laboratories more frequently than would be expected simply on the assumption of a random distribution of nuclei.

Clusters could also be produced if, when a nucleus divides, the two daughter nuclei take a considerable time to become randomly mixed with the existing nuclear population. This effect would be increased if the daughter nuclei were in the vicinity of each other when they had divided again.

The finding of small χ^2's in the experiment with lysineless-pantothenicless and large χ^2's with lysineless-para-aminobenzoicless strongly suggests that the mechanism by which nuclei enter the conidia is subject to variation and that this variation is influenced at least in part by the type of mutants making up the heterocaryon.

If the hypothesis of clustering were pushed to an extreme, there would be only homocaryotic conidia, and they would appear in the proportions p A's to q B's. Hence a suitable estimate of p would be $A_{ob}/(A_{ob}+B_{ob})$. The fact that many conidia grow on minimal shows that this hypothesis is false, and that $A_{ob}/(A_{ob}+B_{ob})$ is an overestimate of p. On the other hand, the hypothesis of random distribution is probably not quite correct either, though it may be a good approximation for the lysineless-pantothenicless heterocaryon. Hence it can be assumed that the minimum χ^2 estimate of $\hat{p}$ is in general too small and that the true values of p lie somewhere between the values of $\hat{p}$ and $A_{ob}/(A_{ob}+B_{ob})$ in table 2. The values $\hat{p}$ may be good approximations where $\chi^2{}_{rd}$ is consistently low, but are to be looked on with suspicion in general. On the other hand, the difference between $\hat{p}$ and $A_{ob}/(A_{ob}+B_{ob})$ is not very great, and the general order of magnitude of p may be considered well enough established for some types of genetical studies.

SUMMARY

A method is described for determining the ratio of nuclei in Neurospora heterocaryons which uses differential counts of the numbers of homocaryotic and heterocaryotic macroconidia. The method is based upon the assumption of random distribution of nuclei among the conidia and it is shown how this assumption can be tested. This method has been applied to data secured from three heterocaryons. In one case the data were consistent with the assumption, while in the remainder there was frequent evidence for non-randomness. The procedures for determining nuclear ratios and the possible causes of non-randomness are discussed.

LITERATURE CITED

ATWOOD, K. C., and A. NORMAN, 1949 On the interpretation of multi-hit survival curves. Proc. Nat. Acad. Sci. **35**: 696–709.

BARRATT, R. W., and L. GARNJOBST, 1949 Genetics of a colonial microconidiating mutant strain of *Neurospora crassa*. Genetics **34**: 351–369.

BEADLE, G. W., and V. L. COONRADT, 1944 Heterocaryosis in *Neurospora crassa*. Genetics **29**: 291–308.

HUEBSCHMAN, C., 1952 A method for varying the average number of nuclei in the conidia of *Neurospora crassa*. Mycologia **44**: 599–604.

NEYMAN, J., 1949 Contribution to the theory of the χ^2 test. Proceedings, Berkeley Symposium on Mathematical Statistics and Probability: 239–273.

RYAN, F. J., 1951 Selected methods of Neurospora genetics. Meth. Med. Res. **3**: 51–75.

SHENG, T. C., 1951 A gene that causes natural death in *Neurospora crassa*. Genetics **36**: 199–212.