Estimation of Mitotic Stability in Conidial Fungi: A Theoretical Framework

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ABSTRACT

Mitotic stability refers to the probability that genetic elements are transmitted to both daughters during mitosis. This is of practical importance in molecular genetics because autonomous cloning vectors should be transmitted at high frequency during mitosis. In filamentous coenocytic fungi it is difficult to quantify mitotic stability because a fluctuation test is not feasible. We show how to get around this problem by formulating a general model of the transmission of nuclear genetic elements through the course of conidiogenesis. We derive formulas by two different methods for the expected proportion of conidiospores that retain the element as a function of its mitotic stability and the number of generations of spore production. An important by-product yields the exact probability distributions for the number of conidiospores retaining elements at each stage of conidiophore development. We outline, and illustrate through specific numerical examples, how to use these formulas to estimate mitotic stability. Although we use *Aspergillus nidulans* as our biological paradigm, the same general framework can be extended to other fungal species, and possibly to less closely related systems as well.

STUDIES of fungal species have greatly advanced our understanding of many critical molecular genetic concepts and processes. One current application concerns the transmission of autonomous genetic elements, *e.g.*, cloning vectors, and, in particular, their mitotic stability, which is the probability of their transmission to both daughter nuclei during mitosis. Recent work with the fungus, *Aspergillus nidulans*, highlights the pressing need for a theoretical framework from which to estimate mitotic stability in such fungal systems. Colony sectoring methods are available for qualitative detection of mitotic instability. However, due to the coenocytic, filamentous growth form of the organism, classical fluctuation tests (in which mitotic instability is equivalent to mutation) are not practicable, making quantification of mitotic instability impossible by the usual means (CLARKE and CARBON 1980). On the other hand, *A. nidulans* and related fungi produce uninucleate spores (conidia) that, in principle, could be exploited for the purpose of determining mitotic stability. Here we provide a theoretical framework from which to do this, which will prove especially useful in genetic analyses within conidiospore producing fungi where fluctuation tests cannot be performed.

Our analysis applies to the asexual life phase of a haploid, multinucleate fungus that produces conidiospores. In order to understand the underlying model, we begin with a brief review of the relevant developmental features. Further biological details can be found in the recent review of the molecular genetics of *A. nidulans* by TIMBERLAKE (1990). The multinu-

cleated fungus *A. nidulans* is composed of filaments called hyphae that grow vegetatively by elongating and branching. A key part of its asexual cycle involves the production of a conidiophore, which is the complex reproductive apparatus by which spores are produced. The major developmental stages of this process are illustrated in Figure 1. First, a vegetative cell, containing from one to several nuclei, differentiates into a specialized reproductive cell, called a foot cell, from which an aerial stalk arises and forms a globular apical vesicle. During formation of these structures, nuclear divisions occur after which each vesicle typically contains about 64 nuclei (TIMBERLAKE 1990). A layer of buds, called metulae, are produced on the vesicle surface, and the vesicle nuclei undergo a final, roughly synchronous mitotic division, whereupon each metula receives a single nucleus (MIMS, RICHARDSON and TIMBERLAKE 1988). The metulae then undergo one to three divisions to produce sporogenous phialide cells. Finally, the uninucleate phialides each produce a long chain of conidia by undergoing repeated mitoses, in such a way that after each division, one nucleus enters a nascent spore, whereas the other is retained in the phialide. The number of spores in a chain can be determined by direct microscopical examination.

This system provides a convenient way to estimate the mitotic stability of autonomous genetic elements residing in the nucleus. Experimental conditions can be created in which only m^+ nuclei (carrying an autonomous genetic element) migrate from the foot cell into the vesicle to initiate the formation of the con-

FIGURE 1.—Schematic of the fungal conidiophore and the production of conidiospores, where circles within nuclei denote the autonomous genetic elements. For greater detail on these stages refer to MIMS, RICHARDSON and TIMBERLAKE (1988).

idiophore. Specifically, this can be achieved if the cloning vector contains a selectable marker such as *trpC* (or *argB*) and then selecting for tryptophan (arginine) prototrophs in otherwise auxotrophic strains (YELTON, HAMER and TIMBERLAKE 1984; JOHNSTONE, HUGHES and CLUTTERBUCK 1985). At each replication during the subsequent conidiophore development, the element will be passed to either both or just one of the daughter nuclei. At the end of spore production, the overall proportion of spores with the element can be assayed.

We show here how these data can be used to estimate formally the probability that the element is passed on to both daughter nuclei in a given mitotic division, which is a measure of its mitotic stability. Our approach hinges on the derivation of an explicit analytic formula for the proportion of m^+ spores that one would expect to see as a function of the probability (p) of a nucleus with an element passing it on to both daughter nuclei and the total number of spore generations (n) assayed. We show how, by numerically calculating this proportion for a wide range of p and n values and comparing this to the observed proportion of m^+ spores, we can obtain a good idea of the mitotic stability p in an experimental system.

For the purposes of this analysis, we have made some simplifying assumptions. First, we assume that a single m^+ nucleus migrates from the foot cell into the vesicle. Second, we require p to be constant throughout the process. Third, we posit that all conidiophores produce 64 metulae. Finally, we assume that each metula produces a single phialide. The effects of

FIGURE 2.—Probabilities of producing m^+ and m^- daughters. (a) The three possible mitoses. Both daughters simultaneously inherit the element with probability p . Otherwise, an m^- type results, which can be either of the daughters, yielding two cases, each with probability $(1 - p)/2$. (b) The two possible mitoses if we are interested in a specific daughter. The probability that a particular daughter cell receives the element is $(1 + p)/2$.

departures from these assumptions are taken up in the discussion section, with the exception of the constancy of p .

The value of this procedure is illustrated through specific numerical examples.

DISTRIBUTION OF m^+ NUCLEI AND CELLS

Our first step is to derive the exact probability distribution of the number of m^+ nuclei and cells over the course of development of the conidiophore.

Preliminary mitoses: The process begins with a single m^+ nucleus (containing an autonomous element carrying a scorable gene), that undergoes six preliminary rounds of mitotic replication. The number of nuclei doubles in each of these mitoses, and thus there will be 64 nuclei at the end of this preliminary stage. When an m^+ nucleus undergoes mitosis, it is assumed to produce two m^+ daughter nuclei with probability p , and one m^+ type and one m^- type, with probability $1 - p$, as indicated in the schematic of Figure 2a. The m^- (minus) types do not have autonomous elements, nor will any of their daughters.

For the first six divisions, time is counted starting from 0 with the original m^+ type cell (see Table 1). Let i_τ be the number of m^+ types after τ rounds of mitosis. It is clear from Figure 2a that the probability distribution of i_1 after one round of mitosis is

$$p(i_1) = \begin{cases} p & \text{if } i_1 = 2 \\ 1 - p & \text{if } i_1 = 1 \\ 0 & \text{otherwise.} \end{cases} \quad (1)$$

The probability distribution for the number of m^+ cells in subsequent generations can be defined recur-

TABLE 1

Summary of the time indices and variables counting the number of m^+ types in the different stages of the fungal life cycle

	Preliminary mitoses						Stem cell lines				
Time	0	1	2	...	6	Restart	0	1	2	...	n
Total nuclei	1	2	4	...	64		64	64	64	...	64
Number of m^+ types	1	i_1	i_2	...	i_6		j_0	j_1	j_2	...	j_n
							Spores				
Total spores							64	128	...	...	64 n
Number of m^+ spores							s_1	s_2	...	...	s_n

sively as follows. The number of m^+ cells in generation τ (i_τ) equals the number of m^+ daughter nuclei produced by the $i_{\tau-1}$ m^+ nuclei present in generation $\tau - 1$. This process is equivalent to $i_{\tau-1}$ independent Bernoulli trials, where a given mitosis is a "success" if two m^+ daughter nuclei are produced (probability p) and a "failure" if only one m^+ daughter nucleus is produced (probability $1 - p$). In general, i_τ equals $i_{\tau-1}$ plus the number of successes, ranging from a minimum of $i_{\tau-1}$ (no successes), to a maximum of $2i_{\tau-1}$ (all successes). It follows that for $\tau = 2, \dots, 6$, the conditional distribution for i_τ given $i_{\tau-1}$ is simply the probability of $i_\tau - i_{\tau-1}$ successes out of $i_{\tau-1}$ independent Bernoulli trials, which is given by the binomial distribution (FELLER 1968, p. 147)

$$p(i_\tau | i_{\tau-1}) = \begin{cases} \binom{i_{\tau-1}}{i_\tau - i_{\tau-1}} p^{i_\tau - i_{\tau-1}} (1 - p)^{2i_{\tau-1} - i_\tau} & \text{if } i_\tau = i_{\tau-1}, \dots, 2i_{\tau-1} \\ 0 & \text{otherwise.} \end{cases} \quad (2)$$

Appealing to well known properties of conditional probabilities (FELLER 1968), we can obtain the probability distribution of $p(i_\tau)$ ($\tau = 2, \dots, 6$) through the decomposition

$$p(i_\tau) = \sum_{i_{\tau-1}=i_{\tau-2}}^{2i_{\tau-2}} p(i_\tau | i_{\tau-1}) p(i_{\tau-1}) \quad (3)$$

for $i_\tau = 1, \dots, 2^\tau$.

(Note that in analogy to i_τ , $i_{\tau-1}$ ranges from $i_{\tau-2}$ to $2i_{\tau-2}$, and that $i_0 = 1$.) Iterating this formula then leads to the exact distribution of the number of m^+ nuclei after the first six mitotic divisions

$$p(i_6) = \sum_{i_1=1}^2 \sum_{i_2=i_1}^{2i_1} \sum_{i_3=i_2}^{2i_2} \sum_{i_4=i_3}^{2i_3} \sum_{i_5=i_4}^{2i_4} p(i_6 | i_5) p(i_5 | i_4) \cdot p(i_4 | i_3) p(i_3 | i_2) p(i_2 | i_1) p(i_1) \quad (4)$$

which reduces after inserting (2) to

$$p(i_6) = \begin{cases} \sum_{i_1=1}^2 \sum_{i_2=i_1}^{2i_1} \sum_{i_3=i_2}^{2i_2} \sum_{i_4=i_3}^{2i_3} \sum_{i_5=i_4}^{2i_4} a p^{i_6-i_1} (1-p)^b p(i_1) & \text{if } i_6 = 1, \dots, 64 \\ 0 & \text{otherwise} \end{cases} \quad (5)$$

where

$$a = \prod_{k=1}^5 \binom{i_k}{i_{k+1} - i_k}$$

$b = i_1 - i_6 + \sum_{k=1}^5 i_k$, the innermost summation index is subject to the additional constraint that $i_6/2 \leq i_5 \leq i_6$, and $p(i_1)$ is defined in (1). Equation 5 gives the probability distribution of m^+ types in the vesicle that will give rise to the 64 metulae (Figure 1), which represent the next major stage of development.

Metulae, phialides and spores: These six preliminary mitoses are followed by two rounds of mitosis in which only one from each pair of daughter nuclei goes on to reproduce, while the other replaces the parental nucleus. Under our simplifying assumptions, there are 64 reproductive cells after each of these two stages. Those formed after the seventh round of mitosis are the metulae, and those after the eighth are the first stem cells (phialides) which will produce the first spores (Figure 1). We now consider the distribution of m^+ stem cells through time because this determines the distribution of m^+ spores.

Let j_t denote the number of m^+ stem cells in stem cell generation t . Since it is the number of spore generations that is of interest, it is helpful to restart time at the metulae stage (Table 1) so that j_0 counts the number of m^+ metulae, j_1 the number of m^+ cells in the initial generation of stem cells, and so on. The initial j_1 m^+ stem cells are the only ones whose lineage can produce m^+ stem cells or spores. Because an m^+ stem cell would produce either zero or one m^+ daughter stem cell, the number of m^+ stem cells does not increase over these divisions; hence $0 \leq j_t \leq j_{t-1}$ for $t = 1, \dots, n$. Modulo this difference, the probability distribution for the number of m^+ type cells in each stem cell generation t , is derived analogously to that for $p(i_\tau)$ in (2-3). The distribution at $t = 0$ among the 64 metulae is conditional on the distribution of i_6 , the number of m^+ nuclei in the vesicle, so that

$$p(j_0) = \sum_{i_6=1}^{64} p(j_0 | i_6) p(i_6) \quad \text{for } j_0 = 0, \dots, 64 \quad (6)$$

where the conditional distribution for j_0 is the binomial probability,

$$p(j_0 | i_6) = \begin{cases} \binom{i_6}{j_0} \check{p}^{j_0} (1 - \check{p})^{i_6-j_0} & \text{if } j_0 = 0, \dots, i_6 \\ 0 & \text{otherwise.} \end{cases}$$

Note that here a "success" is the event that an m^+ parent cell transmits the element to its reproductive daughter cell, which has probability,

$$\check{p} = p + \frac{1-p}{2} = \frac{1+p}{2}$$

(i.e., the probability that a given m^+ parent cell transmits the element to a specified daughter nucleus, as shown in Figure 2b).

The same reasoning shows that the distribution of m^+ stem cells for $t \geq 1$ can be defined recursively via the decomposition,

$$p(j_t) = \sum_{j_{t-1}=0}^{j_{t-2}} p(j_t | j_{t-1}) p(j_{t-1}) \quad \text{for } j_t = 0, \dots, 64 \quad (8)$$

where $j_{-1} \equiv i_6$, and the distribution of j_t conditioned on j_{t-1} is

$$p(j_t | j_{t-1}) = \begin{cases} \binom{j_{t-1}}{j_t} \check{p}^{j_t} (1 - \check{p})^{j_{t-1}-j_t} & \text{if } j_t = 0, \dots, j_{t-1} \\ 0 & \text{otherwise.} \end{cases} \quad (9)$$

The probability distribution of j_t for $t \geq 1$ is thus

$$p(j_t) = \begin{cases} \sum_{i_6=1}^{64} \sum_{j_0=0}^{i_6} \dots \sum_{j_{t-1}=0}^{j_{t-2}} c \check{p}^d (1 - \check{p})^{i_6-j} p(i_6) & \text{if } j_t = 0, \dots, 64 \\ 0 & \text{otherwise} \end{cases} \quad (10)$$

where

$$c = \binom{i_6}{j_0} \prod_{k=0}^{t-1} \binom{j_k}{j_{k+1}}$$

$d = \sum_{k=0}^t j_k$, and the innermost summation is subject to the additional constraint that $j_{t-1} \geq j_t$.

With this foundation in place, we are now ready to describe the distribution of m^+ spores in each spore generation $t = 1, 2, \dots, n$. Let s_t be the number of m^+ spores in spore generation t . These spores are produced by the j_t m^+ stem cells, and in analogy to (8–9), their distribution is conditional on j_t , with

$$p(s_t) = \sum_{j_t=0}^{j_{t-1}} p(s_t | j_t) p(j_t) \quad \text{for } s_t = 0, \dots, 64 \quad (11)$$

where

$$p(s_t | j_t) = \begin{cases} \binom{j_t}{s_t} \check{p}^{s_t} (1 - \check{p})^{j_t-s_t} & \text{if } s_t = 0, \dots, j_t \\ 0 & \text{otherwise.} \end{cases}$$

EXPECTED FREQUENCY OF m^+ SPORES

Having established the probability distribution of s_t , the number of m^+ spore cells in each stem cell generation t , we are now ready to compute the overall expected frequency of m^+ spores in the conidiophore.

The total number of m^+ spores after n spore generations is

$$S(p, n) = \sum_{t=1}^n s_t \quad (12)$$

while the overall proportion of m^+ spores after n generations is

$$f(p, n) = \frac{S(p, n)}{64n}. \quad (13)$$

The average observed fraction of m^+ spores over spore generations 1 to n corresponds to the expected value of $f(p, n)$,

$$ef(p, n) = \frac{1}{64n} \mathcal{E}S(p, n) = \frac{1}{64n} \sum_{t=1}^n \mathcal{E}s_t \quad (14)$$

where

$$\mathcal{E}s_t = \sum_{s_t=0}^{64} s_t p(s_t). \quad (15)$$

The expected proportion can be calculated numerically for specified values of p and n after substituting Equations 1, 5, and 10–11 into (14–15).

Alternatively, a closed-form solution for the proportion of m^+ spores can be obtained more simply, based solely on the expected number of m^+ spores in each generation, without recourse to the exact probability distribution. This approach hinges on the set of indicator variables x_{kt} defined as

$$x_{kt} = \begin{cases} 1 & \text{if spore } k \text{ in generation } t \text{ is } m^+ \\ 0 & \text{otherwise} \end{cases}$$

where in any spore generation $t = 1, 2, \dots, n$, we arbitrarily number the spores $k = 1, 2, \dots, 64$. These indicator variables can be thought of as either random variables or the actual observed states of the spores, and provide a useful decomposition of the total and expected number of spores in each generation t via the relations,

$$s_t = \sum_{k=1}^{64} x_{kt} \quad (16)$$

and

$$\mathcal{E}s_t = \sum_{k=1}^{64} \mathcal{E}x_{kt}. \quad (17)$$

The expected value of each random variable x_{kt} can be calculated easily for any time t by thinking of an m^+ spore as coming from an unbroken chain of mitoses starting from the initial m^+ nucleus. Spore k in generation t is m^+ if and only if the element was passed through the first seven mitoses to produce the metula, then through t stem cell generations, and one final mitosis to produce the spore. Consequently, the probability that a spore in spore generation t is m^+ is $\check{p}^7 \check{p}^t \check{p} = \check{p}^{8+t}$, and the probability that it is m^- is $1 - \check{p}^{8+t}$. It

FIGURE 3.—The expected proportion of m^+ spores as a function of the mitotic stability p , for four representative values of the number of spore generations n .

follows that,

$$ex_{kt} = 1 \cdot \check{p}^{8+t} + 0 \cdot (1 - \check{p}^{8+t}) = \check{p}^{8+t} \quad (18)$$

which shows in conjunction with Equations 14 and 17 that

$$\begin{aligned} ef(p, n) &= \frac{1}{64n} \sum_{t=1}^n \sum_{k=1}^{64} ex_{kt} \\ &= \frac{1}{64n} 64 \sum_{t=1}^n \check{p}^{8+t} = \frac{1}{n} \check{p}^9 \sum_{t=0}^{n-1} \check{p}^t. \end{aligned} \quad (19)$$

Recognizing that the final summation represents the partial sum of a geometric series, we find that the overall proportion of m^+ spores across all 64 stem cell lineages over generations 1 through n has the simple, explicit formula

$$ef(p, n) = \frac{\check{p}^9(1 - \check{p}^n)}{n(1 - \check{p})}. \quad (20)$$

Extensive numerical work confirms that Equations 14–15 and 20 yield identical results.

(As a footnote, Equations 17 and 18 also yield the expected proportion of m^+ spores in each generation t ,

$$e \frac{s_t}{64} = \frac{1}{64} es_t = \frac{1}{64} \sum_{k=1}^{64} ex_{kt} = \frac{1}{64} 64 ex_{kt} = \check{p}^{8+t} \quad (21)$$

which is simply the probability that a given spore in generation t is m^+ . This quantity, however, is not presently observed; what is observed is the proportion of m^+ spores combined over the n spore generations, that is, spores are not counted either within a stem cell line or a single generation.)

NUMERICAL RESULTS AND DISCUSSION

Although it is not possible to solve Equations 14–15 or 20 for closed-form solutions for the mitotic stability (p) as a function of the observed proportion of m^+ spores, these equations allow us to gain numerical insight into the overall process, including the

FIGURE 4.—The expected proportion of m^+ spores for specified values of the mitotic stability p as a function of the number of spore cell generations n .

values of p and n that could yield observed results. First, as a direct application, they allow calculation of the expected proportion of m^+ spores for various values of p , after a fixed number (n) of spore generations. Representative values are shown in Figure 3 after $n = 25, 50, 75$ and 100 spore generations for p values ranging from 0.9 to 1.0. From this we can see, for instance, that if the mitotic stability is 0.98, about 74% of the spores will retain the element after 50 spore generations, while if the mitotic stability is 0.9, only about 22% of spores will retain the element after 50 such generations.

These formulas also provide useful insight into how quickly the proportion of m^+ spores declines as the number of spore generations (n) increases. The basic nature of this effect is suggested by a comparison of the four curves in Figure 3. The effect of n is perhaps best illustrated, however, by Figure 4 in which the proportion of m^+ spores, $ef(p, n)$ is graphed as a function of n for various p values. Note that for $n \geq 5$, these curves are almost linear and tend to diverge in later generations, with the rate at which $ef(p, n)$ declines with n decreasing only slowly with increases of p .

Most importantly, these explicit formulas can be used to numerically estimate mitotic stability from the observed proportion of m^+ spores in a sample. Suppose, for instance, that after 50 spore generations 75% of the total spores assayed have the element. As we saw in Figure 3, this would be consistent with a p value of slightly over 0.98. In general, a mitotic stability estimate can be obtained by calculating $ef(p, 50)$ for a finer scale of p values flanking the area of the putative true p value, as shown in Table 2. From this we see that a p value of 0.982 would yield an observed frequency of 75% m^+ spores. Other sample mitotic stability estimates are given in the second column of Table 3.

One potential complication for such applications arises because spores can be assayed after various

TABLE 2

Expected proportion of m^+ spores after $n = 50$ and $n = 75$ spore generations for a finer scale of p values

p	$ef(p, 50)$	$ef(p, 75)$
0.980	0.7217	0.6448
0.981	0.7332	0.6585
0.982	0.7450	0.6725
0.983	0.7570	0.6868
0.984	0.7692	0.7016
0.985	0.7817	0.7167
0.986	0.7944	0.7323
0.987	0.8073	0.7482
0.988	0.8205	0.7646
0.989	0.8339	0.7815
0.990	0.8476	0.7988

numbers of spore generations, and often the experimenter may not know the precise generation n in which spores have been harvested. Fortunately, the estimate of the mitotic stability, p , does not appear to be overly sensitive to the exact generation in which the spores are collected. In particular, p estimates will not be significantly different within ± 5 spore generations. If there is greater uncertainty in the n value, then this analysis can be used to provide bounds on the true mitotic stability value. For instance, if in the example above, the number of spore generations assayed is only known to be between 50 and 75, the p value will be between 0.982 and 0.988 if 75% of the spores possess the element (see Table 2).

Finally, we should recall that our biological paradigm is idealized, in that it is based on four simplifying assumptions. We now examine each of these in turn (with the exception of the constancy of p) and show that they should make little difference in practical applications. We first consider the consequences when two, three or four nuclei (rather than just one) migrate from the foot cell into the vesicle (Figure 1). We still assume that the total number of stem cells is 64; the only difference is that there will be fewer preliminary mitoses to reach this stage. For example, with a two nuclei start, only five preliminary mitoses will be required to attain the 64-cell stage, while only four are required if four cells migrate. Mathematically, this can be taken into account by simple modifications of the distributions of i_1 and i_2 and Equation 20. We assume that if multiple nuclei migrate from the foot cell into the vesicle, then they are all m^+ (or all m^-). This is based on the work of CLUTTERBUCK and SPATHAS (1984), who analyzed conidiophores that developed from heterokaryotic hyphae (formed from the fusion of compatible but distinct individuals) and concluded that, although foot cells are probably multinucleate, the nuclei are almost always of the same type. Thus, if two nuclei migrate initially, then the random process effectively begins after time $t = 1$, when the distribu-

TABLE 3

Estimates of p for a given proportion of m^+ spores at $n = 50$

Percent m^+ spores	Initial nuclei		
	1	2	4
1	0.5636	0.5302	0.4901
5	0.7544	0.7389	0.7208
10	0.8257	0.8164	0.8056
20	0.8877	0.8827	0.8771
30	0.9197	0.9164	0.9129
40	0.9407	0.9384	0.9360
50	0.9561	0.9545	0.9528
60	0.9682	0.9671	0.9659
70	0.9781	0.9774	0.9766
75	0.9824	0.9818	0.9812
80	0.9864	0.9860	0.9855
90	0.9937	0.9935	0.9932
99	0.9994	0.9994	0.9994

tion of m^+ nuclei is fixed at

$$p(i_1) = \begin{cases} 1 & \text{if } i_1 = 2 \\ 0 & \text{otherwise.} \end{cases} \quad (22)$$

Our previous development accordingly applies with $ef(p, n)$ in Equation 20 increased by the factor $\check{p}^{-1}$. Similarly, if four nuclei migrate, then the random process effectively starts at time 2, when the distribution of m^+ nuclei is fixed at

$$p(i_2) = \begin{cases} 1 & \text{if } i_2 = 4 \\ 0 & \text{otherwise.} \end{cases} \quad (23)$$

This requires that $ef(p, n)$ in (20) be increased by the factor $\check{p}^{-2}$, while, since time $t = 1$ is now irrelevant, Equation 5 becomes

$$p(i_6) = \begin{cases} \sum_{i_3=4}^8 \sum_{i_4=i_3}^{2i_3} \sum_{i_5=i_4}^{2i_4} a' p^{i_6-i_2} (1-p)^{b'} & \text{if } i_6 = 1, \dots, 64 \\ 0 & \text{otherwise} \end{cases} \quad (24)$$

where

$$a' = \prod_{k=2}^5 \binom{i_k}{i_{k+1} - i_k}$$

and $b' = i_2 - i_6 + \sum_{k=2}^5 i_k$.

The ultimate effect of a multinucleate start is that the $ef(p, n)$ curves in Figures 3 and 4 will be shifted upward (Figure 5). Practically speaking, this means that the same mitotic stability p will result in a higher expected proportion of m^+ spores, and hence, that a given proportion of m^+ spores will yield a somewhat lower estimate for the mitotic stability from that obtained under the single nucleus treatment (see Table 3). In general, $ef(p, n)$ will be a weighted average of the three cases, with lower and upper bounds determined by the one and four nuclei cases, respectively. Estimates and/or bounds for the mitotic stability can be obtained from this composite $ef(p, n)$ function fol-

TABLE 4

Estimates of p for a given proportion of m^+ spores at $n = 50$ assuming all metulae have one, two or three phialide branches

Percent m^+ spores	Phialide branches		
	1	2	3
1	0.5636	0.5774	0.5899
5	0.7544	0.7611	0.7672
10	0.8257	0.8299	0.8338
20	0.8877	0.8900	0.8922
30	0.9197	0.9212	0.9226
40	0.9407	0.9417	0.9427
50	0.9561	0.9568	0.9575
60	0.9682	0.9687	0.9692
70	0.9781	0.9784	0.9787
75	0.9824	0.9827	0.9830
80	0.9864	0.9866	0.9868
90	0.9937	0.9938	0.9938
99	0.9994	0.9994	0.9994

lowing the same basic procedure developed above for a single nucleus start.

In practice, however, estimates based on the simpler, single nucleus treatment should usually suffice in the two cases most likely encountered. First, as shown by the sample values in Table 3, a high p estimate (*i.e.*, ≥ 0.91), such as that expected when a centromere is present, would be virtually unchanged by taking multinucleate starts into account. Conversely, if a sufficiently low p estimate, consistent with the lack of a centromere, were obtained from (20), it should not be necessary to invoke the more complicated general formulation as this would yield a still lower p estimate.

Next we examine our assumption that each conidiophore produces exactly 64 metulae. The number of metulae on the conidiophore is thought to be limited by the surface area of the vesicle (TIMBERLAKE 1990), and under standard experimental conditions, the vesicles are large enough to accommodate about 64 metulae. Local variation in nutrient content means that the number of metulae, l , may be slightly lower, say $32 < l < 64$. If we still assume that there were six mitotic divisions in the vesicle to produce the nuclei that will migrate into the l nascent metulae, then we simply need to replace the number 64 with the variable l in Equations 13–17, 19 and 21. Since the l 's cancel in the expectations, slight variation in the number of metulae should have no effect on the final proportion of m^+ conidia.

Finally, we address our assumption that each metula produces only one phialide. Metulae produce a primary phialide, but will often have secondary and tertiary phialides budding off their sides. Since these phialides are produced sequentially, a spore that came from the second (or third) branch on a given metula would have a slightly lower probability of being m^+ , because it would have gone through one (or two)

FIGURE 5.—The expected proportion of m^+ spores as a function of the mitotic stability p , when starting from one, two or four m^+ nuclei. Proportions are calculated after $n = 50$ spore generations.

extra mitosis. The effect of additional phialides, then, is ultimately analogous to that of having multinucleate starts: it simply alters the number of divisions leading to a mature spore. This complication can be taken into account by noting that, in general, there is a distribution of metulae that produce one, two or three phialides, and we specify their proportions by b_1 , b_2 and b_3 respectively. The expected proportion of m^+ spores across spore generations 1 through n is a simple modification of (20),

$$ef(p, n) = \left[\frac{\check{p}^9(1 - \check{p}^n)}{n(1 - \check{p})} \right] (c_1 + c_2\check{p} + c_3\check{p}^2) \quad (25)$$

where the coefficients $c_1 = b_1 + b_2/2 + b_3/3$, $c_2 = b_2/2 + b_3/3$, and $c_3 = b_3/3$, correspond to the fraction of spore lines derived from primary, secondary and tertiary phialides, respectively. For any given mitotic stability p , there will be a slight decrease in the expected proportion of m^+ spores from that calculated via (20). The greatest deviation would result when all metulae produce three phialides (*i.e.*, $b_1 = b_2 = 0$ and $b_3 = 1$). This deviation is about as large as that obtained from our analysis when starting from 4 nuclei, although in this case the curves are shifted downward. In Table 4 we compare the estimates for p at $n = 50$ for the three extreme cases, namely all metulae produce a single phialide ($b_1 = 1.0$), two phialides ($b_2 = 1.0$) or three ($b_3 = 1.0$). As with the other possible departures from our simplifying assumptions, the deviations are all very small (*i.e.*, $< 1\%$).

The mechanisms whereby different fungal species produce conidiospores are quite numerous (ALEXOPOULOS and MIMS 1979). Although those that produce multinucleate conidia (for example, *Neurospora*) are not amenable to our treatment, many others can be treated with simple modifications of our model. Our biological paradigm, *A. nidulans*, has one of the more complex mechanisms for the production of conidiospores, as do the other members of the family Eurotiaceae. For example, *Penicillium chrysogenum* has

roughly the same structures, albeit with some extra branching of the conidiophore. This extra branching compartmentalizes the nuclei but does not severely alter the mathematical framework. Simpler fungi, such as *Ceratocystis fimbriata* or *Sphaerotheca castagnei*, produce clonal strings of conidia directly off a simple stalk, lacking the metulae and phialides, and, thus, they could be analyzed by a straightforward simplification of our model (namely by eliminating the preliminary mitoses and thinking of the conidiophore as a phialide). Although it is impractical to enumerate explicitly all the modifications to our model that would treat the various fungal species, our treatment provides a general method to estimate mitotic stability in a wide variety of filamentous fungi. It is possible that the same theoretical construct might be applicable to still other, less closely related systems. One such potential application is to the fate of unstable genetic elements through the divisions accompanying embryogenesis in plants and animals.

A program to calculate the mitotic stability estimates is available upon request.

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