

Estimation of meiosis and sporulation efficiencies in the fission yeast by ascus analysis*

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SUMMARY

Populations of linear asci are classified according to the number of spores in an ascus. The resultant five numerical classes are further classified into ten spatial classes according to the arrangement of the spores in an ascus and, by inference, into ten historical classes according to the number and origins of failures during the developmental process. An analysis of the observed frequencies of numerical classes allows derivation of the efficiencies of the first meiotic division, the second meiotic division and sporulation in a fission yeast. The analytical method may be useful in locating the site of action of sporulation inhibitors and in identifying meiosis mutants from sporulation mutants.

1. INTRODUCTION

Formation of the meiotic products found in an ascus may be conveniently represented as a three-step developmental process comprising seven individual events (Fig. 1). The diploid nucleus, derived from the fusion of two haploid nuclei, divides during the first meiotic division (I, Fig. 1). The resultant nuclei in turn divide (II, Fig. 1) during the second meiotic division to form four haploid nuclei, which then are individually packaged into spores during sporulation (Yoo, Calleja & Johnson, 1973). The final meiotic products, all confined in an ascus, are visible in the light microscope, and thus the overall efficiency of the developmental process can readily be estimated. However, the immediate products of the first and second meiotic divisions are not easily identified, hence the relative efficiencies of the meiotic and sporulation processes have not readily been estimated. It will by now be obvious that we do not pursue the usual genetical inferences from progeny phenotypes which constitute the highly heuristic, albeit abstract, tetrad analysis (Levine, 1969, for example). Rather, we present an analogous analysis by abstraction, potentially allowing identification and quantitation of the developmental errors to which the meiotic and sporulation processes might be prone, or which might be induced by experimental perturbation. However, we consider its relevance to genetical problems to be obvious.

If the overall efficiency of this developmental process were 100%, there would

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be only one class of asci – all would contain four spores. But up to five numerical classes are to be expected if the efficiency is less: 0-, 1-, 2-, 3- and 4-spore asci. By tabulating the frequencies of the five numerical classes in a population of asci, one cannot only arrive at the overall efficiency of the developmental process, but also determine approximately the efficiencies of the first meiotic division, the second meiotic division and sporulation.

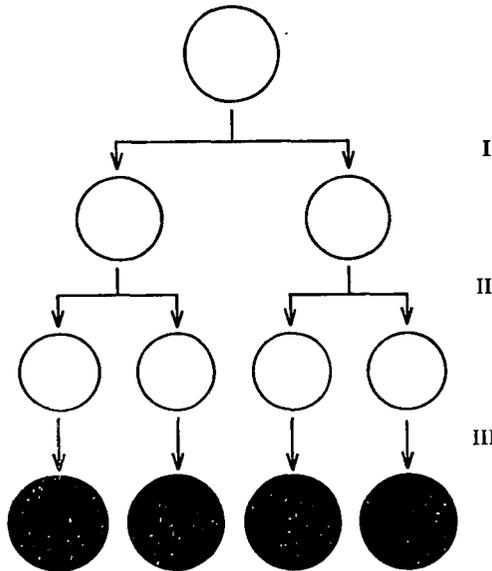


Fig. 1. Meiosis and sporulation in an ordered ascus. I = first meiotic division; II = second meiotic division; III = spore development. Empty circles are nuclei, filled circles are spores.

We have chosen to use the fission yeast, *Schizosaccharomyces pombe*, as a model for meiosis and sporulation. The fission yeast has an advantage over the somewhat more popular model *Saccharomyces* in that its final meiotic products are ordinarily recovered in a linear and ordered ascus. Hence the five numerical classes can be regrouped as ten spatial classes. A third classification scheme according to the individual histories of the different asci may also be formulated. Most of these ten historical classes are not directly recognizable, but their distribution may readily be deduced from the numerical or spatial classes.

The interrelationships among the three different classification schemes are as follows (Table 1). The five numerical classes are designated N_0 to N_4 , corresponding to the number of spores in an ascus. The ten spatial classes are differentiated by the positions of spores found in an ascus. A small zero stands for a spore, an x for the absence of a spore in one of four possible locations. Although there are in theory 16 spatial classes, six pairs of them are mirror images. For example, xxox is not separately identifiable from xoxx. The ten historical classes are each represented by a three-digit convention. Each digit records the number of failures in one of the three steps in the developmental process. The first digit represents the

single event of the first meiotic division, the second digit represents a maximum of two events of the second meiotic division, the third digit a maximum of four events of the sporulation step. An ascus of the historical class 100, for instance, is derived from a failure of the only event in the first meiotic division and is observed as an xxxx ascus. The same numerical and spatial class, however, may be classified as a 020, indicating the failure of both events in the second meiotic division; or a 012, indicating one failure in the second meiotic division, followed by two failures in the sporulation step; or a rare 004, which is an ascus that successfully accomplished its second meiotic division, but suffered four failures during sporulation.

Table 1. *Classes of asci according to number, spatial arrangement and history of spores*

N, numerical (5)	S, spatial (10)	H, historically (10)
N_0	(a) xxxx	100 020 012 004
N_1	(b) x0xx, xx0x } (c) 0xxx, xxx0 }	{ 011 003
N_2	(d) 0xx0 (e) x00x (f) 0x0x, x0x0 (g) 00xx, xx00	— — — 010
N_3	(h) 00x0, 0x00 (i) 000x, x000	} 001
N_4	(j) 0000	000

Note that only the N_4 ascus can be spatially and historically classified immediately and unambiguously. Whereas an N_3 ascus can be historically classified immediately and unambiguously also, it can belong to any of two spatial classes. The N_0 ascus, immediately and unambiguously identified as an xxxx, is the most ambiguous historically, being derivable from any of four historical classes. The N_1 ascus may be ordered spatially in two alternative arrangements, either of which is the result of one of two historical possibilities: 011, one failure in the second meiotic division, followed by one failure during sporulation; or 003, complete success in the second meiotic division, followed by three failures later. An N_2 ascus may be arranged in any of four possible spatial alternatives. The spatial class 00xx may be a 010, one failure in the second meiotic division, or a 002, two failures during sporulation. The other possible spatial classes found in N_2 can belong only to the historical class 002.

2. ANALYSIS

The entire developmental process can be regarded mathematically as a simple kind of branching process. Let q_1 , q_2 and q_3 be the efficiencies of the first meiotic division, the second meiotic division and sporulation, respectively. These numbers can be thought of as the probabilities of the successful completion of meiosis at the first two steps or sporulation at the final step. The numbers

$$p_1 = 1 - q_1, \quad p_2 = 1 - q_2, \quad p_3 = 1 - q_3,$$

represent the corresponding failure probabilities. With the assumption that the events at stages II and III are independent, we can easily derive the probabilities

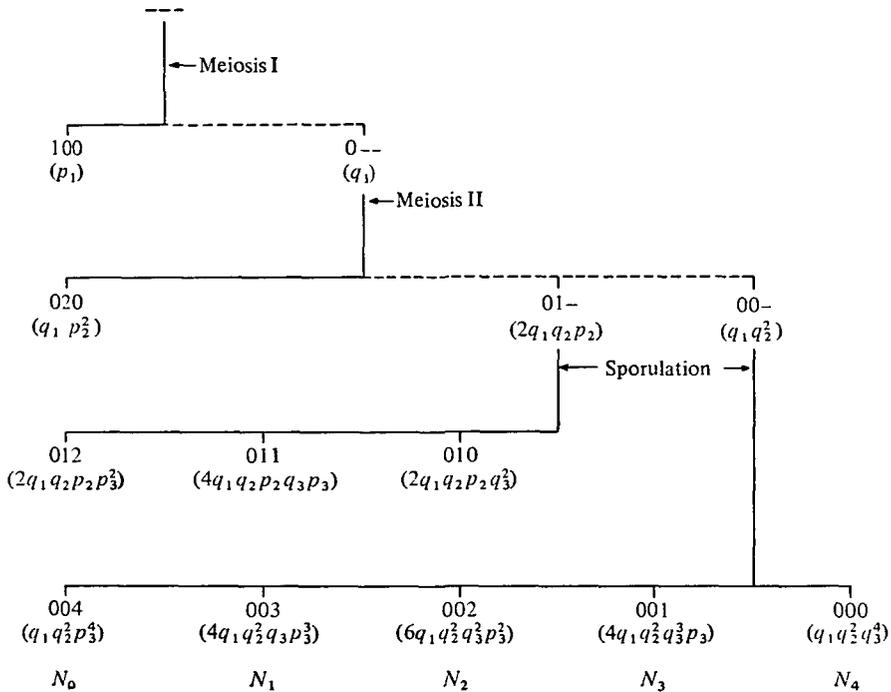


Fig. 2. Mathematical evolution of the ten historical classes of asci. A '—' refers to an event that has not yet occurred, the numerals 0, 1, etc. describe the number of 'failed' events. The expression with p 's and q 's under each triplet is the probability of that event.

of the numerical classes, the spatial classes and the historical classes. The evolution of each historical class is shown in Fig. 2. The resultant 'tree' lists all historical classes and their antecedents with corresponding probabilities. If a given historical class produces more than one spatial class, then all of these spatial classes (counting mirror-image pairs such as oxox and xoxo as distinct) are equiprobable. Because each historical class leads unambiguously to a single numerical class, the numerical class probabilities are easy to compute. Hence, given the values of q_1 , q_2 and q_3 , the expected frequencies of all the historical, spatial and numerical classes can be determined.

It is interesting to note that the numerical class probabilities can be derived in a completely different way using the notion of a probability-generating function borrowed from the theory of branching processes. Let

$$f_1(s) = p_1 + q_1s^2,$$

$$f_2(s) = p_2 + q_2s^2$$

and

$$f_3(s) = p_3 + q_3s.$$

Then

$$f_1(f_2(f_3(s))) = \sum_{i=0}^4 P_i s^i,$$

where P_i is the probability of the numerical class N_i .

The problem is to determine q_1 , q_2 and q_3 so that the resulting frequencies of either the numerical or spatial classes might match the observed as closely as possible. A preliminary analysis of the observed data showed a difficulty in accomplishing this with the spatial data. Thus first we group the observed data into numerical classes and try to fit the model on this basis. Given any values for q_1 , q_2 and q_3 , let P_i be the probability that an ascus will be in numerical class i , $0 \leq i \leq 4$, and let n_i be the observed number of asci in class i . Then we can define a function, known as the likelihood function, by

$$L(q_1, q_2, q_3) = \prod_{i=0}^4 P_i^{n_i}.$$

The values of q_1 , q_2 and q_3 which maximize this function are called the maximum likelihood estimates of these probabilities. We can also define a goodness of fit function

$$\chi^2(q_1, q_2, q_3) = \sum_{i=0}^4 \frac{(n_i - NP_i)^2}{n_i},$$

where $N = \sum_{i=0}^4 n_i$, and estimate q_1 , q_2 and q_3 , by minimizing χ^2 . Both L and χ^2 were used to estimate q_1 , q_2 and q_3 and both produced the same results. The expected frequencies of the numerical, spatial and historical classes were computed on the basis of these estimates (Tables 2–5). The total number of expected spores is $4Nq_1q_2q_3$, and the overall efficiency of the entire process is $q_1q_2q_3$.

If the data on spatial classes had been unambiguous, we could have established q_1 , q_2 and q_3 by maximizing the redefined likelihood function

$$L'(q_1, q_2, q_3) = \prod_{i=1}^{10} P_i^{n_i},$$

where n_i is the number of observed asci in spatial class i , and p_i is the probability that an ascus will be in the spatial class. The χ^2 approach can be similarly modified and might have been useful not only in estimating the q_i 's, but also in assessing how well the model fits the observations.

3. EXPERIMENTAL METHODS

Cells of the homothallic strain 360-2, a derivative of *Schizosaccharomyces pombe* NCYC 132 (ATCC 26192), were grown and induced to sporulate as described before (Calleja, Yoo & Johnson, 1977). They were harvested at 48 h after the start of induction and treated with Pronase to disperse all remaining flocs. Asci were scored in a phase-contrast microscope. A total of 55 095 asci, collected from about 50 similar but separate experiments over a period of several months, were counted. They were scored for both spore number and spore position, except for 2860 asci of the numerical classes N_1 , N_2 and N_3 , whose spatial arrangements of spores were deemed ambiguous. These spatially unclassifiable asci, representing a little over 5% of the total population, were nevertheless included in the tabulation of numerical classes. Clearly, 4-spore asci with aborted spores (shriveled, with drastically altered refractility, and therefore not counted as spores) were unambiguously assignable to spatial classes of the numerical classes N_3 , N_2 and N_1 . But N_3 asci where no fourth spore seemed ever to have been initiated (or N_2 asci without third or fourth, etc.) led to doubtful spatial assignment if one of the counted spores was sited in an unusual position in the linear ascus, i.e. a spore was observed to straddle between two possible idealized locations.

4. RESULTS

The observed distribution of numerical classes is compared with the expected distribution derived both from a maximization of the likelihood function and from a minimization of χ^2 (Table 2). As mentioned above, both methods of analysis give the same results. Analyses were performed three times on the data as they were being accumulated. We estimated the q_i 's on the basis of 14 331, 49 871 and 55 095 asci, obtaining the same results in all cases. This indicates sufficient homogeneity over the different experiments.

The probabilities of the first meiotic division, the second meiotic division and sporulation are 0.899, 0.847 and 0.796, respectively. They represent the efficiencies of the three steps in the developmental process. The overall efficiency is simply the product of the three probabilities: $q_1 q_2 q_3 = 0.606$ or 60.6%.

The observed distribution of spatial classes is also compared with the expected (Table 3). The expected frequencies of the spatial classes that belong to numerical classes N_0 and N_4 are directly derived from Table 2. Those of the spatial classes that belong to the other numerical classes had to be calculated.

From the observed distribution of numerical classes, we derived the expected distribution of historical classes (Table 4). The distribution of spatial classes within a historical class is also presented to give the reader an idea as to what percentage of asci in a given historical class is expected to belong to a spatial class. Failure in the first meiotic division, represented by historical class 100, accounts for about 10%, whereas failures in the second meiotic division, represented by historical classes 010, 011, 012 and 020, account for about 25%. Sporulation

failures, represented by historical classes 001, 002, 003 and 004, as well as 011 and 012, account for over 47 %. The perfect asci, 000, account for a little over 25 %, as seen already in Tables 2 and 3.

Table 2. *Observed and expected distribution of numerical classes*

N	Observed		Expected		(Difference) ² Observed
	Frequency	%	Frequency	%	
N ₀	7298	13.25	7299	13.25	0.00
N ₁	5260	9.55	5132	9.31	3.11
N ₂	13599	24.68	13762	24.98	1.95
N ₃	14596	26.49	14630	26.55	0.08
N ₄	14342	26.03	14272	25.90	0.34
	55095	100	55095	100	—

$$q_1 = 0.899; q_2 = 0.847; q_3 = 0.796; q_1q_2q_3 = 0.606.$$

Table 3. *Observed and expected distribution of spatial classes.*

N	S	Observed		Expected	
		Frequency	%	Frequency	%
N ₀	(a) xxxx	7298	13.25	7299	13.25
N ₁	(b) xoxx, xxox	2476	4.49	2566	4.66
	(c) oxxx, xxxo	2226	4.04	2566	4.66
	[o]	558	1.01	—	—
N ₂	(d) oxxo	963	1.75	937	1.70
	(e) xoox	1258	2.28	937	1.70
	(f) oxox, xoxo	2259	4.10	1874	3.40
	(g) ooxx, xxoo	8132	14.76	10012	18.17
	[oo]	987	1.79	—	—
N ₃	(h) ooxo, oxoo	6272	11.38	7315	13.28
	(i) ooox, xooo	7009	12.72	7315	13.28
	[ooo]	1315	2.39	—	—
N ₄	(j) oooo	14342	26.03	14272	25.90

[o], [oo], [ooo] = 1-, 2- and 3-spore classes of doubtful spatial class, respectively.

Finally, we tabulate for the sake of clarity the expected relative frequency of historical classes derivable from numerical and spatial classes that can occur in more than one way (Table 5). For instance, asci of numerical class N₀ can arise four different ways, but more than 75 % of them are expected to belong to historical class 100, and only less than 1 % is due to 004, or four sporulation failures.

Table 4. *Expected distributions of historical classes and of their consequent numerical and spatial classes*

H	N	S	Frequency (%)	
000	N ₄	oooo	—	25.90
001	N ₃	ooox	13.28	} 26.56
		ooxo	13.28	
002	N ₂	oxxo	1.70	} 10.20
		xoox	1.70	
		oxox	3.40	
		oxxx	3.40	
003	N ₁	xxxo	0.87	} 1.74
		xxox	0.87	
004	N ₀	xxxx	—	0.11
010	N ₂	xxoo	—	14.77
011	N ₁	xxxo	3.79	} 7.58
		xxox	3.79	
012	N ₀	xxxx	—	0.97
020	N ₀	xxxx	—	2.11
100	N ₀	xxxx	—	10.06

Table 5. *Expected relative frequencies of historical classes of numerical or spatial classes that can occur in more than one way*

N	S	H	Relative frequency (%)
N ₀	xxxx	100	75.93
		020	15.93
		012	7.32
		004	0.83
N ₁	Both	011	81.29
		003	18.71
N ₂	All four	010	59.15
		002	40.85
N ₂	xxoo only	010	81.29
		002	18.71

5. DISCUSSION

Neurospora geneticists (Davis & de Serres, 1970) presume that aborted spores relate to 'chromosomal aberrations which exist in heterozygous conditions in a cross'. Indeed, their presumption is probably correct, if limited, for their system. However, the *S. pombe* system under discussion is homothallic, and such heterozygosity of chromosomal aberrations should not pertain. This should justify our operating hypothesis that the failures reported are physiological rather than genetical *per se*.

The analysis of distribution of numerical classes has allowed estimation of the efficiencies of the first and second meiotic divisions and of the sporulation process (the overall efficiency is, as mentioned above, easily estimated). The analysis has also allowed prediction of the spatial classes, which can be experimentally observed,

and of historical classes, which can only be derived. The distribution of historical classes, in turn, allows prediction that the majority of the sporeless asci were derived from the historical class 100, a failure in the first meiotic division, and that most of the N_1 asci belong to the historical class 011, one failure in the second meiotic division, followed by one failure during sporulation (Tables 4, 5). Fewer than 20% could have been derived from the historical class 003, which had three failures during sporulation. Indeed, the same ratio between the more probable and the less probable historical class is obtained from an analysis of spatial class xxoo: the more probable 010 occurred greater than 80% of the time and the less probable 002 less than 20%. The less probable historical class 002, however, accounted for about 40% of all members of N_2 , and consequently, the more probable class 010 can occur about 60% of the time.

Table 6. *Misclassification of spatial classes due to relocation of spores (arrows show directions of net displacements)*

Spatial class	Consequence
(b) xxx	Overestimate
↑	
(c) oxxx	Underestimate
(d) oxxo	No consequence
(e) xoox	Overestimate
↑	
(f) oxox	Overestimate
↑	
(g) oxxx	Underestimate
(h) ooxo	Underestimate
↓	
(i) ooox	Overestimate

Certain predictions of the analysis were not fulfilled regarding the spatial classes. For instance, we expected $b = c$, $d = e$, $d + e = f$ and $h = i$ (Table 3). Of these, only the prediction $d + e = f$ was observed at an acceptable level of significance. We rationalize this anomaly to misclassification due to displacement of spores (Table 6). The observed distribution suggests a net displacement of polar spores toward the middle of the ascus, leading to the misclassification of a polar spore as a central spore. Thus, we overestimate xxx (b) and oox (i), when the doubtfuls are apportioned proportionately, and underestimate oxxx (c) and ooxo (h). However, for rationalizing the observed distribution of spatial classes among the N_2 asci, something else must be invoked. We suggest that there was also a net displacement of the central spore toward the other half of an ascus when that other half was devoid of spores. Thus, most of the misclassification among N_2 asci is due to the misclassification of oxxx (g) as oxox (f) and of oxox (f) as xoox (e). Because oxxo (d) represents only a small fraction of all N_2 asci, the relocation of spores in this particular class is of little consequence.

Alternatively, the anomaly among spatial classes may be attributed to spindle

overlap (nuclear slippage) during meiosis II (Whitehouse, 1942; Hawthorne & Mortimer, 1960; Shaw, 1962; Chen & Olive, 1965). For example, if an error occurs after meiosis I (historically, 010) such that one of the two nuclei is marked for failure, a partial spindle overlap could generate an oxox (*f*) from a prospective ooxx (*g*), leading to the observed overestimate of oxox (*f*) and the observed underestimate of ooxx (*g*). However, a partial spindle overlap cannot generate an xoox (*e*) from a prospective ooxx (*g*); and a complete overlap should generate an xoox (*e*) or an oxox (*d*) with equal probability. Either instance fails to account for the observed plurality of xoox (*e*) over oxox (*d*). Whatever the explanation for the anomaly, the very occurrence of asci of ambiguous spatial classes suggests movement of spores from their usual locations.

The collected data plus their analysis imply that spores can relocate in an ascus when a putative spore position is left open through abortion. Note that even if that implication be invalidated eventually, the analysis of historical classes will not be affected, for it did not depend upon the observed distribution of spatial classes. To the best of our knowledge, similar migration has been previously discussed only with respect to spores of the genus *Bacillus* (Hitchens, 1975, 1976) and then somewhat anomalously. For example, spores in *B. megaterium* were found to shift in about 10% of cases (Hitchens, 1975), but then doubts were expressed about relocation of spores in *B. cereus* (Hitchens, 1976) with those doubts reinforced by (incorrect) reference to an older paper on *B. megaterium* (Bayne-Jones & Petrilli, 1933).

It surprises us that analysis of spore locations, relocations and its similar application to the study of meiosis and sporulation as physiological processes rather than as merely genetic events, has not happened with respect to the genus *Neurospora*, with its linear 8-spore ascus. There is more than a coincidental resemblance between our discernment of a meiosis II physiological error and the geneticist's second division segregation. Perhaps genetical knowledge about heterozygosity of aberrant chromosomes leading to abortion has made such analysis seem trivial. However, it is clear that ascus analysis, as we call our approach, should be useful in locating the temporal and physiological sites of action of inhibitors and stimulators of sporulation. By simply taking into consideration the distribution of numerical classes of asci in the absence and in the presence of drugs, one can arrive at the changes in the efficiencies of the first meiotic division, the second meiotic division and spore development. The method may also be applied as a screening technique for meiotic mutants isolated as sporulation defectives.

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