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The differential maturation of asci and its relevance to recombination studies of *Neurospora, Sordaria* and similar Ascomycetes

By B. C. LAMB*
Genetics Laboratory, Department of Botany, University of Bristol

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1. INTRODUCTION

Between meiosis and the stage at which recombination frequency can be determined is a period of development when post-meiotic phenomena can bias the observed recombination frequency. Thus Lindegren (1935) reported that developmental competition between spores of different genotypes in *Neurospora* asci could bias recombination frequencies and give rise to 'pseudo-linkage'. Houlahan, Beadle & Calhoun (1949) pointed out that selection of complete asci for dissection and spore classification could lead to scoring errors if failure of spores to mature or germinate was associated with particular genotypes or types of segregation. The pitfalls of the spore-classification method of estimating recombination were discussed by Stadler & Towe (1962), who concluded that they are avoided in the segregation-count method when only a single gene-pair is segregating, so that crossover and non-crossover asci both contain two mutant and two wild-type spore pairs. McNelly (1962) noted, however, that significantly higher second-division segregation frequencies for *asco* in *Neurospora crassa* were obtained when asci were mounted for scoring in 1 or 2 M sucrose solutions instead of in water, showing that segregation-count results were subject to experimental bias.

In a series of segregation-count crosses in *N. crassa* and *Sordaria fimicola*, Lamb (1966) showed that asci of the six classes for the segregation of a single pair of alleles could mature at different rates, although all contained two mutant and two wild-type spore pairs. Differential maturation and bursting of the asci caused apparent polarized segregation for the loci and also (as reported briefly by Lamb, 1964) affected the observed frequency of second-division segregation asci. The primary data analysed by Lamb (1966) for polarized segregation are analysed here for changes in the observed frequency of second-division segregation with perithecial maturation; data from further crosses are also considered.

The present paper reports three effects of the differential maturation and bursting of asci: on the observed frequency of second-division segregation; on the observed frequency of recombination where two or more linked markers are segregating, and on the observed ratio of + : mutant progeny in a cross of mutant × wild-type. The aims of these studies were to determine the conditions under which recombination

* Present address: Division of Biology, California Institute of Technology, Pasadena, California, U.S.A. 91109.

results might become biased by the differential bursting of asci and how such biases might be avoided in experimental practice.

2. MATERIALS AND METHODS

The strains of *N. crassa* and *S. fimicola* used have been described by Lamb (1966) except for two *Neurospora* strains: *crisp* (r6L)*A*, (*cr A*), described by Lavigne & Frost (1964), and Emerson wild-type (1535)*a*, (Em *a*). The former paper described the methods used in the segregation-count experiments, where mutants were crossed with wild-types by procedures giving a considerable degree of synchrony in perithecial development; segregation patterns were scored from successive harvests taken at different stages of maturity from the developing crosses. Asci were mounted for scoring in a 2 M sucrose solution (McNelly, 1962). The maturity of a cross was recorded as the time in days from conidiation of protoperithecia (*Neurospora*), or inoculation (*Sordaria*), to harvesting. Details of the crosses and their temperatures of incubation are given in Table 1. Crosses 10 and 11 were made by the same methods as the segregation-count crosses.

Table 1. *Key to crosses**

Neurospora crassa			*Sordaria fimicola*		
Cross No.	Temperature (°C.)	Cross	Cross No.	Temperature (°C.)	Cross
1	17·5	*asco* 35*A* (♀) × A4*a*	7a	12·5	C7*h* × C7+
2	20·0	† *asco* 35*A* (♀) × A4*a*; A4*a* (♀) × *asco* 35*A*	7b	17·5	
			7c	20·0	
3	20·0	A4*a* (♀) × *asco* r4L*A*	7d	25·0	
4	17·5	A4*a* (♀) × *asco* 33*A*	8	25·0	*h* × *h*+
5	21·0	*rib* 4*A* (♀) × *asco* (A256)*a*	9a	12·5	*g* × *g*+
6	25·0	L1*a* (♀) × *ts A*	9b	25·0	
10	21·0	*rib* 4*A* (♀) × Em *a*			
11	25·0	A4*a* (♀) × *cr A*			

* These crosses, except for 10, 11 and 7d(ii), are those considered, with the same numbers, by Lamb (1966).

† Reciprocal cross data pooled as samples were small.

Cross 5, *rib* 4*A* × *asco* (A256)*a*, was used to study effects of differential bursting on recombination between linked markers as well as on the second-division segregation frequency of *asco*. *asco* is in the left arm of linkage group VI, with *rib*-1 in the opposite arm, very close to the centromere, and so map distance can be checked by scoring prototrophs among germinated ascospores (Stadler & Towe, 1962). Spores from dehiscing asci were collected by replacing the lids of the Petri dishes containing the crosses with inverted Petri dish bottoms containing 4% agar. Collections were timed to correspond with the segregation-count harvests. Collected spores were allowed to mature further in a moist atmosphere as spore germination from the first collection rose from 16% after 7 days' storage to 38% after 14 days' storage

(maximum germination 50% as *asco* spores are inviable). Matured spores were heat-shocked 14–24 days after the first collection and 14–41 days after the second collection. After storage, spores were washed with dilute sodium hypochlorite solution (Frost, 1961), then with two changes of water. They were transferred, irrespective of their colour, to separate $3 \times \frac{3}{8}$ in. tubes containing glucose minimal medium (Lamb, 1966) supplemented with 5 mg./l. riboflavine and were heat-shocked at 60°C. for 35 min. Progeny were tested for prototrophy at 30°C. on sorbose minimal medium. 100 auxotrophs from each harvest were also tested at 30°C. on sorbose minimal medium supplemented with riboflavine: all grew well, confirming that they did not carry the *asco* allele, which results in lysine-requirement.

Cross 10, *rib* 4*A* × Em *a*, was made as a control for cross 5 to determine whether the expected 1 : 1 ratio for *rib* : + progeny was found when *asco* was not segregating. To check the effect of riboflavine in the germination medium, half the tubes for collection one of cross 10 contained no riboflavine, the other half 5 mg./l.; for collection two, levels of 5 and 20 mg./l. were used.

In cross 11, A4*a* × *cr A*, the ratio of + : *cr* progeny was followed during the period of ascal dehiscence both from dispersed spores and from perithecial contents. Dispersed spores were collected on 4% agar as in the previous experiment. On harvesting, perithecia were transferred to plates of wetted 4% agar where they were crushed to distribute their contents in the water-film. 482 dispersed spores and 138 spores from crushed perithecia gave less than 30% germination when heat-shocked within 4 days of collection, with 75% and 95% + progeny respectively, so spores were heat-shocked after 15–46 days' maturation at 25°C. Matured spores were washed as described earlier and were heat-shocked at 60°C. for 30 min. in aqueous suspension. 2 ml. aliquots of suspension were pipetted on to plates of Olive's (1956) minimal medium after adjusting the concentration to 20–50 spores/ml. Plates were incubated at 27·5°C. for about 19 hours before scoring by direct microscopic examination as described by Lavigne & Frost (1964). In this and the previous experiment, newly developing perithecia were removed from the spore-maturation plates.

In cross 11, three of the six replicate plates were incubated inverted, with the spores being dehisced downwards. The collecting lids were changed at intervals on four of the replicate plates; the other two were used for continuous spore collection. At least four replicates of each cross were used in the other experiments.

3. RESULTS AND DISCUSSION

(i) *The effect of differential bursting on the observed frequencies of second-division segregation*

The second-division segregation frequencies for the loci at different stages of cross maturation are given in Table 2, and Fig. 1 illustrates some of the more extensive *Neurospora* data for *asco*. Replicate crosses were generally homogeneous for this frequency: the few exceptions are noted in Table 2.

Table 2. *Second-division segregation frequencies at different stages of cross maturation**

Cross No.‡	Maturity (days)	Total asci	2nd div. asci (%)	D.F.	$\chi^2_{2\times n}$	Total class 1 asci / Total 2nd div. asci	Total class 2 asci / Total 2nd div. asci
Neurospora crassa							
1	12	287	33·5	4	52·5***	0·9	1·1
	14	1398	20·9			2·1	1·7
	19	661	18·8			2·5	1·8
	27	909	18·3			2·5	1·9
	33	1598	15·8			3·1	2·2
2	8	1053	23·8	6	59·7***	1·7	1·5
	10	2219	19·8			2·1	1·9
	13	2245	17·8			2·4	2·2
	16	1495	16·1			2·7	2·5
	19	2302	15·4†			2·9	2·6
	22	2514	16·9			2·7	2·2
	27	1493	14·1			3·2	2·9
3	9	2496	28·7†	5	226·9***	1·2	1·2
	10	2421	27·7			1·4	1·3
	11	2467	26·3			1·5	1·3
	12	2386	22·9			1·8	1·6
	20	3331	18·6			2·4	2·0
	37	2303	14·2			3·3	2·7
4	15	2034	35·8	1	13·8***	0·9	0·9
	15w	2096	30·4			1·3	1·0
	21	2058	27·2	1	1·7	1·6	1·1
	21w	2045	25·4			1·7	1·2
5	12	1295	30·1	1	14·8***	1·2	1·1
	23	1385	23·5			1·8	1·5
6	7	2001	43·6	1	0·1	0·64	0·65
	13	2070	43·3			0·82	0·49
Sordaria fimicola							
7a	19	2044	53·2	1	3·4	0·40	0·48
	27	2433	55·9			0·44	0·35
7b	10	2971	47·5	1	4·4**	0·55	0·56
	10w	2569	50·3			0·53	0·46
	15	2190	53·0	1	0·7	0·48	0·41
	15w	2254	51·8			0·57	0·36
7c	7	3257	45·1	1	33·1***	0·62	0·59
	18	2086	53·2			0·54	0·34
7d(i)	5	2464	58·1	1	1·3	0·36	0·36
	15	2742	59·7			0·40	0·28

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Table 2—*continued*

Cross No.‡	Maturity (days)	Total asci	2nd div. asci (%)	D.F.	$\chi^2_{2\times n}$	Total class 1 asci / Total 2nd div. asci	Total class 2 asci / Total 2nd div. asci
7d(ii)	6	2092	57·6	1	3·3	0·36	0·37
	6w	2268	60·4			0·39	0·27
8	4	2703	56·3	1	10·0***	0·38	0·40
	10	2764	60·6			0·36	0·30
9a	16	2184	64·2	1	4·7**	0·27	0·29
	23	2252	67·3			0·27	0·21
9b	5	2463	63·3	1	2·5	0·30	0·28
	9	2003	65·6			0·29	0·23

* The corresponding polarized segregation data for these crosses were given by Lamb (1966), except for cross 7d(ii).

‡ Cross details in Table 1, maturity calculated as given in Methods.

w Asci mounted in water: all others in 2 M sucrose solution.

** Significant at $P = 0{\cdot}05$.

*** Significant at $P = 0{\cdot}01$.

† Replicates heterogeneous at $P = 0{\cdot}05$.

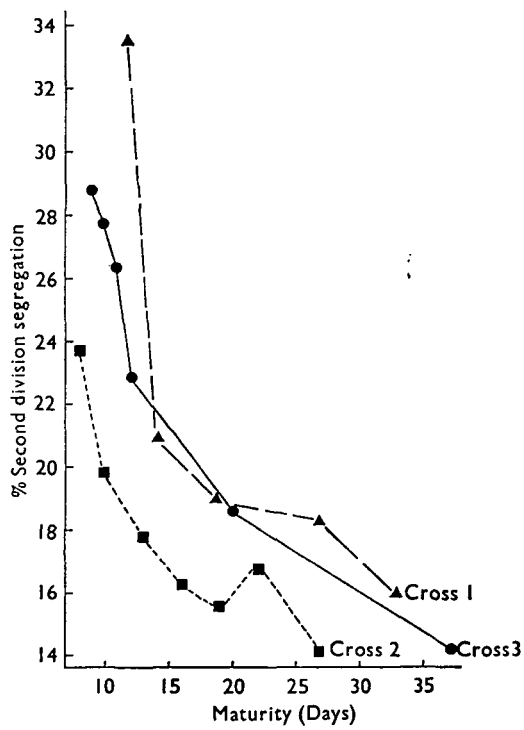

Fig. 1. The relation between the observed frequency of second-division segregation for *asco* and the maturity of three *Neurospora* crosses.

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In all the *asco* crosses the observed second-division segregation frequency was at a maximum when no, or only few, asci had dehisced, but decreased significantly as dehiscence proceeded, sometimes to less than half the initial value. No significant change in this frequency with maturation was observed for *tan spore*. In contrast, the observed second-division segregation frequencies of *gray* and *hyaline* in *Sordaria* rose with increasing perithecial maturity.

To determine whether these observed changes in second-division segregation frequency with perithecial maturation resulted from changes in crossover frequency during successive meioses or from a differential maturation and bursting of asci, some asci from crosses 4, 7b and 7d(ii) were mounted as usual in 2 M sucrose solution, which prevents further ascal dehiscence, while others were mounted in water and were scored at least 1 hour later. In water, the more mature asci dehisce after water uptake on account of their high osmotic pressure, simulating the dehiscence which normally occurs with further maturation (Lamb, 1966). Table 2, cross 4, shows that young *Neurospora* asci mounted in water had a highly significant reduction in second-division segregation frequency for *asco* when compared with sucrose-mounted asci: the change was less marked, but in the same direction, with asci from more mature perithecia. Other data on the selective bursting in water of asci showing second-division segregation for *asco* have been given by McNelly (1962) and McNelly-Ingle, Lamb & Frost (1966). In contrast, the *Sordaria* data from crosses 7b and 7d(ii) show higher second-division segregation frequencies in water-mounted young asci than in sucrose-mounted ones, but no significant change with the more mature asci.

The changes in second-division segregation frequency resulting from differential bursting on mounting asci in water were thus of a similar nature to those occurring with increasing perithecial maturity. It is therefore likely that the observed changes in this frequency with increasing perithecial maturity were generally spurious and due to a differential maturation and bursting of asci, rather than to true changes in crossover frequency during successive meioses in perithecia. Further information was obtained from study of changes in the frequencies of the various classes of asci.

Six classes of asci can be recognized when one character-difference is segregating, depending on the arrangement of the pairs of spores. Counting from the apex of the ascus, class 1 has the sequence $+, +, m, m$; class 2 $m, m, +, +$; class 3 $+, m, m, +$; class 4 $m, +, +, m$; class 5 $+, m, +, m$; and class 6 $m, +, m, +$, where $+$ stands for a wild-type spore-pair and m for a mutant pair (illustrated in Fig. 1, Lamb, 1966).

The order of ascal maturation and bursting which I found (Lamb, 1966) for these *Neurospora* and *Sordaria* crosses was: class 2 before class 1, and, among the second-division segregation classes, class 6 first, then classes 3, 4 and 5 in that order. This sequence was attributed to impaired metabolism in the vicinity of mutant-bearing nuclei in the developing asci, so asci with $+$-bearing nuclei in the basal regions would be able to translocate nutrients more rapidly into the ascus than those with mutant-bearing nuclei there. If the greatest proximal location of

+-bearing nuclei resulted in the most rapid ascal maturation, the complete order of maturation would be class 2 first, then 6, 3, 4, 5 and 1 in that order. With increasing perithecial maturity, the ratio of class 2 asci to the second-division segregation total would fall and the ratio of class 1 asci to the second-division segregation total would rise: the observed changes in second-division segregation frequency would depend on the relative fall and rise of these two ratios.

In cross 6, involving *tan spore* in *Neurospora*, the fall and rise of these two ratios (given in Table 2) were almost equal and so the change in second-division segregation for *ts* with maturity was negligible; a similar effect occurred when mature *Sordaria* asci were mounted in water (cross 7b).

In *Sordaria* (crosses 7, 8, 9) there were marked decreases in all crosses in the ratio of class 2 asci to the second-division segregation total with increasing maturity, without correspondingly large increases in the ratio of class 1 to second-division segregation asci: increases in second-division segregation frequency resulted. Crosses 7c and 8 provided some evidence of a true rise in crossover frequency in successive meioses, since the ratio of class 1 asci to the second-division segregation total (which, as already explained, was expected to rise with maturity as found in crosses 7a and 7d) actually decreased. Changes in crossover frequency with maternal age are known in *Drosophila* (Bridges, 1927). Strickland & Thorpe (1963) reported a decrease in crossover frequency in successively produced asci of a single *Neurospora* perithecium but their procedure would not have distinguished this from effects of a differential bursting of asci.

The proposed explanation of the order of ascal bursting appears inadequate to account completely for the *asco* data from *Neurospora:* here the ratios of class 1 and of class 2 asci to the second-division segregation totals both rose with increasing maturity. An additional 'distribution effect' is therefore proposed: the more even distribution of +-bearing nuclei (with unimpaired metabolism in their vicinity) throughout second-division segregation asci could result in faster maturation than in first-division segregation asci, where +-bearing nuclei are confined to half the ascus. This effect might have influenced the *ts* results; it does not appear to have operated appreciably in the *Sordaria* crosses, where the smaller size of the asci may reduce its importance.

This additional postulate does not affect the earlier conclusion (Lamb, 1966) that a differential maturation and bursting of asci causes apparent polarized segregation, nor does it affect the proposed explanation of the differences in maturation rate within the two separate categories: first- and second-division segregation asci. Such a 'distribution effect' would not affect the rate of maturation of class 1 asci compared with that of class 2 asci, or of classes 3 and 5 compared with classes 4 and 6, or of classes 3 and 6 compared with classes 4 and 5, these three comparisons being the basis of polarized segregation studies in Ascomycetes.

Another possible explanation of the *asco* data is that the more rapid development of second-division segregation asci might arise from the binucleate ascal stage, when such asci would have two nuclei containing the *asco*$^+$ allele, in contrast to one such nucleus in the corresponding first-division segregation asci: further

information on the synthetic activity of chromosomes at this stage would assist the evaluation of this idea.

These *Neurospora* and *Sordaria* experiments showed that second-division segregation frequencies obtained from segregation-count data could be biased considerably by a differential maturation and bursting of asci: both under-estimates (for *asco* in *Neurospora*) and over-estimates (for *gray* and *hyaline* in *Sordaria*) of this frequency were obtained by scoring harvests taken after appreciable ascal dehiscence. Such a bias could also affect results obtained using other mutants and other, similar, fungi.

(ii) *The effect of differential bursting on the observed frequency of recombination between* asco *and* rib

In cross 5, the observed recombination (prototroph) frequency between *asco* and *rib* decreased significantly between the first and second collections (Table 3). Results from spores matured for different periods were homogeneous ($2 \times 5\chi^2 = 3{\cdot}7$, $P = 0{\cdot}3$–$0{\cdot}5$ for first collection, $2 \times 9\chi^2 = 7{\cdot}8$, $P = 0{\cdot}3$–$0{\cdot}5$ for second collection). As *rib*-1 is auxotrophic only at 30°C. and above, it was not expected to bring about differential maturation effects of its own in the cross at 25°C. In the control, cross 10, the expected 1 : 1 ratio for *rib* : + progeny was obtained in dispersed spores from young and from mature perithecia. No significant differences in this ratio were observed between progeny from tubes containing different concentrations of riboflavine ($2 \times 2\chi^2$ gave $P = 0{\cdot}2$–$0{\cdot}3$ for first collection, and $P = 0{\cdot}5$–$0{\cdot}7$ for second collection).

Table 3. *Changes in recombination measure from dispersed spores and ordered tetrads with time from conidiation to harvesting*

	Dispersed spores					Ordered tetrads		
Time (days)	Spores tested	Total progeny	Germination (%)	+Progeny (%)*	$\chi^2_{2\times2}$	Total asci	2nd div. asci (%)	$\chi^2_{2\times2}$
Cross 5: *rib* 4*A* × *asco* (A256)*a*								
12–13	1820	759	41·7†	18·7	15·6‡	1295	30·1	14·8‡
23–24	1935	819	42·3†	11·6		1385	23·5	
Cross 10: *rib* 4*A* × Em *a*								
12–13	430	357	83·0	47·6	1·3			
23–24	590	495	83·9	51·5				

* % + progeny = % recombination between *asco* and *rib* in cross 5.
† *asco* spores inviable, so value for *asco*$^+$ spores is about double this.
‡ Significant at $P = 0{\cdot}01$.

As *rib* is very close to the centromere, nearly all asci with second-division segregation for *asco* also show recombination between *asco* and *rib*. The change in recombination frequency in this interval should therefore parallel closely the change in second-division segregation frequency for *asco*, as was found (Table 3). The

change in the second-division segregation frequency of *asco* resembled that in crosses 1, 2, 3 and 4, in which *rib* was not segregating, so the large apparent change in recombination frequency with time of collection of dispersed spores was most likely an artifact resulting from the differential maturation and bursting of asci showing different divisions of segregation for *asco*. In this experiment, the recombination frequency obtained from those spores collected first was probably an over-estimate of the actual value, as spores from asci with second-division segregation for *asco* would have been disproportionately frequent in the early collection; conversely, the recombination frequency obtained from the later spore collection was probably an under-estimate.

The present results indicate that a bias having its origin in the arrangement of spore-pair nuclei in ordered asci may affect recombination results obtained from dispersed spores. Such a bias (arising from a differential maturation of asci) in recombination data obtained from dispersed spores could occur when any mutant having a similar effect on ascal maturation to that of *asco* was segregating in a cross. The bias is most simply avoided by collecting all the spores dispersed and the spores remaining in the perithecia.

(iii) *The ratio of crisp to wild-type amongst progeny from dispersed spores and spores from crushed perithecia*

The results for cross 11 are given in Table 4. Changes in the frequency of +- progeny were rather variable with respect to cross maturity and also between results from inverted and normally-orientated perithecia. Instead of the expected 1 : 1 ratio for *cr* : +, there was a clear trend, often significant, in each collection for a

Table 4. *The relation of the frequency of wild-type progeny to the time from conidiation to spore collection amongst dispersed and non-dispersed spores*

Dispersed spores					Spores from perithecia				
Time (days)	Plate position	Colonies scored	% +	Germination (%)	Time (days)	Plate position	Colonies scored	% +	Germination (%)
7–8	Normal	1147	48·0	91	9	Normal	897	57·3**	87
7–8	Inverted	553	48·1	90	9	Inverted	1425	61·5**†	77
15–17	Normal	558	50·2	74	17	Normal	1142	52·8	77
15–17	Inverted	1701	46·6**	80	17	Inverted	1358	53·6**	80
23–24	Normal	2163	46·3**	85	27	Normal	1855	54·7**	79
23–24	Inverted	490	47·1	94	27	Inverted	992	53·6*	78
7–27	Normal	909	42·9**	76					
7–27	Inverted	942	47·2	73					

* Deviation from 1 : 1 ratio for *cr* : + significant at $P = 0{\cdot}05$.
** Deviation from 1 : 1 ratio for *cr* : + significant at $P = 0{\cdot}01$.
† Replicates heterogeneous at $P = 0{\cdot}01$.

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deficiency of +-progeny from the dispersed spores and an excess of +-progeny from spores from crushed perithecia. Germination differences seem an unlikely explanation of these deviations. Mitchell (1958) studied the ratio of + : mutant progeny in dispersed spores from a number of *Neurospora* crosses between auxotrophic mutants and wild-type strains, finding marked deficiencies of +-progeny in several of the crosses. McNelly (1962) reported significant excesses in *Neurospora* of either mutant or non-mutant progeny in spore samples from the walls of the culture tubes, but not in samples taken both from walls and perithecia. Mitchell considered that deviations from the expected 1 : 1 ratio might originate at meiosis but McNelly's report and the results of cross 11 indicate that these deviations originate at spore dispersal.

When ordered tetrads from crosses of mutant × + are dissected and classified, 4 : 4 segregations of mutant : + are regularly observed. If eight spores from each ascus were dehisced and collected, only germination differences could influence the expected 1 : 1 ratio. Examination of the collection plates in cross 11 revealed that groups of eight spores were infrequent, most groups containing three to five spores only. Mature perithecia, carefully opened in 2 M sucrose solution, showed many dehisced asci containing some unexpelled spores, which must originally have occupied the basal positions in the asci. Incomplete spore-expulsion in combination with a differential maturation of asci could explain the results of cross 11 and Mitchell's data.

Asci which do not mature sufficiently, or which fail in competition with other asci to reach a favourable position for dehiscence through the ostiole, will not contribute spores to the collection plates, all eight spores remaining within the perithecium. When less than eight spores are collected from a dehiscing class 2 ascus, an excess of *crisp* progeny will be obtained from the dispersed spores and an excess of +-progeny from the perithecium. Class 1 asci would give the reverse excesses in dispersed spores and in perithecia but the results obtained are accounted for if more class 2 asci expelled spores to the collecting plates than class 1 asci. If *crisp* had a similar effect on ascal maturity to that of the spore colour markers previously described, asci of class 2 for *crisp* could receive a more rapid supply of nutrients than those of class 1 and be at a relative advantage in developing and expelling their spores towards the collecting surface. The ostioles often became blocked with spores in older perithecia, again favouring a larger contribution to the collecting plates from class 2 asci if these matured faster than class 1 asci. If asci of classes 3 and 6 matured faster than those of classes 4 and 5, similar, but less marked, excesses would result when five to seven spores were collected from asci with second-division segregation for *crisp*. This explanation would apply equally well to the auxotrophic markers used by Mitchell.

4. SUGGESTIONS CONCERNING EXPERIMENTAL PROCEDURES

Biasing effects from a differential bursting of asci are largely avoided in segregation-counts if perithecia are harvested when first ripe and if asci are mounted in media of high osmotic pressure to minimize subsequent dehiscence. A comparison

of the changes in second-division segregation frequency (Table 2) with the polarized segregation data for the same crosses (Lamb, 1966, Table 2) shows that the onset of apparent polarized segregation can be used to indicate the occurrence of differential bursting. Scoring for apparent polarized segregation enables a check to be made for differential bursting on the results of a single harvest, even from non-synchronized perithecia.

If mutants used in conversion studies affected the rate of ascal maturation, asci with different ratios of + : mutant spores might mature at different rates. Asci with less than four +-spores might mature and dehisce later than those with four or more +-spores. They might also tend to expel less spores than the latter kind: marked bias could then be introduced by scoring groups of expelled spores as conversion frequencies are usually scored only from complete octads. A check on conversion frequencies from intact asci would be a useful safeguard for studies of collected octads.

Bias of mutant : non-mutant ratios and of recombination frequencies is best avoided from random spores by sampling spores from perithecia as well as dispersed spores. Where only dispersed spores are sampled, as in Strickland's (1960) rapid method of obtaining unordered tetrads, comparisons of results from spores dispersed from young and from old perithecia might reveal differential maturation effects. Sequential ascus collection (Strickland & Thorpe, 1963) from single perithecia would be unnecessary for this if crossing methods giving synchrony of perithecial development were used (Lamb, 1966).

SUMMARY

Four ascospore-pigmentation mutants were crossed with their wild-types by methods giving a considerable degree of synchrony in perithecial development; segregation patterns were scored from perithecia at different stages of maturity. The observed second-division segregation frequencies for *asco* in *N. crassa* were at a maximum when little or no ascal dehiscence had occurred but decreased markedly as dehiscence proceeded. No significant change in this frequency with maturity occurred for *tan spore* in *Neurospora* but in *S. fimicola* the observed second-division segregation frequencies for *gray* and *hyaline* rose with increasing perithecial maturity. As similar changes in this frequency were observed when asci were mounted in water instead of the 2 M sucrose solution normally used, it was concluded that changes in this frequency with maturity generally resulted from a differential maturation and bursting of asci with different spore arrangements, rather than from changes in crossover frequency in successive meioses. Some evidence of the latter phenomenon was found in two of the *Sordaria* crosses.

In a cross of *asco* × *rib*-1 in *Neurospora*, the observed frequency of recombination between the two loci was significantly higher in dispersed spores collected from young perithecia than in those from more mature perithecia: this effect was probably an artifact resulting from the differential maturation and bursting of asci with different divisions of segregation for *asco*. In a cross of *crisp* × + in *Neurospora*, an

excess of *crisp* progeny was obtained from dispersed spores and an excess of +-progeny from spores from harvested perithecia. These deviations from the expected 1 : 1 ratio for *cr* : + were thought to result from a differential maturation of asci with different segregation patterns for *crisp* and the failure of many dehiscing asci to expel all eight spores.

Hypotheses to explain the various phenomena observed were discussed. Suggestions were made concerning the avoidance of bias from the differential maturation and bursting of asci in experimental procedures used in the study of recombination and gene conversion. This bias may be responsible for some phenomena previously attributed to events at meiosis.

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