Genet. Res., Camb. (1966), **7**, *pp.* 325–334
With 2 text-figures
Printed in Great Britain

Polarized segregation in Ascomycetes and the differential bursting of asci

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(*Received* 9 *December* 1965)

1. INTRODUCTION

Polarized segregation has been described as: 'the non-random segregation of the members of a pair of homologous chromosomes, or of a pair of daughter chromosomes, with respect to the two poles of a spindle in meiosis' (Lamb, 1964). It was first demonstrated in Ascomycetes by Catcheside (1944) from the data of Zickler (1934) on the segregation of two loci autonomously controlling ascospore pigmentation in *Bombardia lunata*. Segregation at the *rubiginosa* and *lactea* loci appeared to be polarized at the first meiotic division and at the lower spindle of the second division. Mathieson (1956) used the markers *rubiginosa* and *albino* in *Bombardia lunata*, finding a significant bias (the 5 % level of significance is referred to throughout) only at the first division spindle. Nakamura (1961), using *tan spore* in *Neurospora crassa*, found biases similar to those of Zickler's data. McNelly (1962), whose data were analysed for polarized segregation by the present author, and Lamb (1963) studied the segregation of *asco* (37402) in *Neurospora crassa*. Significant biases were found at the first division spindle and at the second division lower spindle in crosses incubated at 15°C. and at 17·5°C. but not over the range 20–30°C. Unlike previous studies, both sets of results showed a significant bias at the second division upper spindle, though only at 15°C.: McNelly's data also showed this bias in one experiment at 27·5°C.

Catcheside (1944) suggested that there was a gradient of some diffusible substance in the ascus to which the chromosomes responded at segregation. Mathieson (1956) thought that the relevant differences in frequency of the ascal segregation patterns might arise post-meiotically but found no evidence for this in *Bombardia*.

In the present study several loci in *Neurospora crassa* and *Sordaria fimicola* were examined for polarized segregation in crosses incubated at various temperatures. It proved possible to score segregation patterns from perithecia at widely differing stages of ascal maturity and to obtain a fairly high degree of synchrony in perithecial development. It was therefore possible to determine whether polarized segregation arises at meiosis or during ascal maturation and bursting. This, the aim of the present investigation, was approached by comparing the frequencies of the

segregation patterns from young perithecia, harvested when ascospore pigmentation was first sufficient for scoring, with those from mature perithecia, harvested after considerable ascal dehiscence had occurred.

2. MATERIALS AND METHODS

The following strains were used:

Neurospora crassa

Wild-types:

Abbott 4*a*, (A4*a*), a re-isolate from the cross Abbott 4*A* × Lindegren 25*a* backcrossed to Abbott 4*A* six times:

Lindegren 1*a*, (L1*a*), a re-isolate from the cross Lindegren 1*A* × Lindegren 25*a* backcrossed to Lindegren 1*A* six times then selfed twice.

Mutant strains:

asco (37402, *lys*-5), requires lysine; spores hyaline to brown, usually inviable. Three isolates were used: *asco* 33*A* and *asco* 35*A*, which were re-isolates from a cross of *asco* to Lindegren 1*a*, and *asco* r4L*A*, a re-isolate of *asco* from the cross Lindegren 1*a* × *asco* 35*A*, backcrossed to Lindegren 1*A* twice. Linkage group VI.

asco (A256, *lys*-5)*a*, similar to *asco* (37402) but induced in Emerson *a* wild-type (1535). Linkage group VI.

tan spore A, (*ts A*) (Fungal genetics stock center number 821), spores tan; reduced germination. Linkage group V.

rib-1 (51602 *t*)*A*, (*rib* 4*A*), requires riboflavine at 30°C., a re-isolate of *rib*-1 from the cross Lindegren 1*A* × *rib*-1*a*, backcrossed to Lindegren 1*A* three times. Linkage group VI.

Sordaria fimicola

Wild-types:

g^+, C7 +, h^+.

Mutant strains:

gray (*g*), spores grey, high germination frequency. *hyaline* (*h*), spores hyaline, very low germination frequency. The two isolates C7*h* and *h* (and their corresponding wild-types, C7 + and h^+) were received from different sources; although these stocks were probably identical originally, the separate designations have been kept as the stocks now differ in some characteristics.

Strains for *Neurospora* crosses were grown from fresh conidial inocula for 7 days on slopes of glucose minimal medium (as for the minimal reproductive medium of Westergaard & Mitchell, 1947, but with 2 % glucose, no sucrose and pH not adjusted). Conidiating parents were grown in the light at 25°C.; protoperithecial parents and crosses were incubated in the dark at the temperature being studied. Media were supplemented with lysine at 0·5 mg./ml. for *asco* strains. Crosses were made in Petri dishes containing minimal reproductive medium (Westergaard & Mitchell, 1947); plates of medium were dried for 6 days at 30°C. before inoculation. 1 ml. of a

conidial suspension (about 5×10^5 conidia/ml. distilled water) of the protoperithecial parent was spread evenly on each plate. After 7 days, 3 ml. of an equally dense suspension from the conidiating parent were spread evenly on each protoperithecial culture.

In *Sordaria*, crosses of C7h × C7+ were made by inoculating these strains in opposite quadrants of plates of minimal medium (Olive, 1956) supplemented with 2 mg./l. thiamine. Stocks were grown for 7 days on plates of minimal medium at the

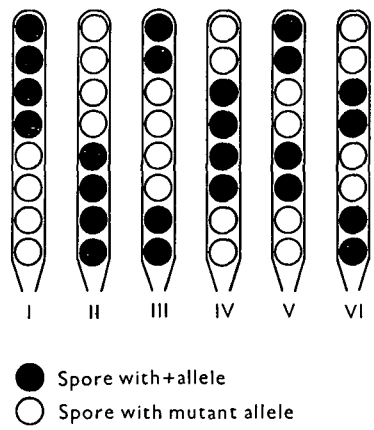

Fig. 1. The six ascal classes for the segregation of a single pair of alleles, numbered according to Whitehouse & Haldane, 1946.

required crossing temperature to provide these inocula. Perithecia which formed along the line where the two strains met were fairly well synchronized. For crosses of $g \times g^+$ and of $h \times h^+$, the two strains were inoculated together in tubes of liquid minimal medium. After 7 days at the crossing temperature, subcultures were made: after a further 7 days' growth in liquid minimal medium, the subcultures were mixed with abrasive powder and placed in a vibratory shaker until the mycelium was broken into short fragments. 1 ml. of this suspension was spread evenly on each plate of minimal medium; the crop of perithecia obtained was quite well synchronized for each of the three types of perithecium formed.

Perithecia were immediately transferred on harvesting to a 2 M sucrose solution (a technique devised by McNelly, 1962) and were opened carefully to minimize ascal breakage in preparation. Clusters of asci were divided into smaller groups and displayed so as to minimize overlapping of asci. Catcheside (1944), Mathieson (1956) and Lamb (1963) have discussed possible errors which might arise during scoring: these were kept to a minimum by scoring only asci in which all eight spores were visible and in which the base was distinguishable from the apex. Groups of overlapping asci were not scored.

The numbering of the six segregation patterns used here, shown in Fig. 1, is that of Whitehouse & Haldane (1946): where different, the numberings of other authors have been altered accordingly when quoted. Spindle bias, the usual measure of

polarized segregation, is given by the percentage excess of +-bearing chromosomes which reach the upper spindle pole (Catcheside, 1944). If an excess of +-bearing chromosomes reaches the lower spindle pole then the bias figure becomes negative.

As a high degree of synchrony in the development of perithecia within plates and between replicate plates was obtained, the age of the crosses could be used as a simple index of perithecial maturity. It is expressed in days from conidiation of protoperithecia (*Neurospora*), or from inoculation (*Sordaria*), to harvesting. It is not a general maturity index and is only for comparisons under similar conditions within a given cross. Details of the crosses made are given in Table 1. At least four replicates were used in each cross. Where a single late harvest was used, it was taken when the Petri dish lids were abundantly covered with dispersed spores. In crosses 1, 2 and 3, series of collections were made until most of the asci had dehisced from the cultures.

Table 1. *Key to crosses*

Neurospora crassa			*Sordaria fimicola*		
Cross number	Temperature °C.	Cross	Cross number	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	

† Reciprocal cross data not heterogeneous at $P = 0{\cdot}05$, pooled as samples were small.

3. RESULTS

Table 2 gives the extent of polarized segregation at the three spindles at different stages of maturity. Replicates were generally homogeneous for degree of polarized segregation and the few exceptions are noted in Table 2. The reciprocal cross data have been pooled in cross 2 since they showed no heterogeneity for polarized segregation. In no cross was there a significant positive bias at any spindle when the young asci were first scoreable except where asci were mounted in water instead of in a 2 M sucrose solution: this is discussed later. As the asci dehisced, significant positive biases were observed for the first division and second division lower spindles in nearly all crosses and at the second division upper spindle in two crosses.

The absence of polarized segregation from the young perithecia when no, or few, asci had dehisced indicates that polarized segregation did not arise at meiosis but

Footnotes to Table 2.

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

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

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

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

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

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Table 2. *The extent of polarized segregation at different stages of maturation*

Cross No.‡	Maturity (days)	1st div. asci	Bias % 1st div. spindle	2nd div. asci	Bias % 2nd div. spindles		III+IV −V−VI as % 2nd div. asci
					Lower	Upper	
1	12	191	−7·9	96	+2·1	−2·1	0·0
	14	1106	+10·5**	292	+2·1	−1·4	+2·1
	19	537	+15·8**†	124	+30·7**	+0·9	−11·3
	27	743	+13·3**	166	+28·9**	+2·4	+3·6
	33	1345	+17·9**	253	+18·6**	+6·7	+3·6
2	8	802	+3·7	251	+1·2	−2·8	−0·4
	10	1779	+5·5*	440	+2·7	−2·7	+6·4
	13	1845	+5·7*	400	+3·5	+8·5	+2·0
	16	1254	+3·8	241	+6·2	+1·2	−5·4
	19	1947	+6·8**	355	+11·0*	+9·3	+3·1
	22	2090	+9·9**	424	+11·3*	+4·3	−2·4
	27	1283	+6·2*	210	+16·2*	+1·9	+9·5
3	9	1779	−0·1	717	−3·8	+2·1	+2·9
	10	1751	+3·7	670	−2·7	+3·3	+1·5
	11	1819	+6·9**	648	+13·0**	+3·1	+4·9
	12	1840	+4·8*	546	+11·4**	+7·3	−1·5
	20	2710	+7·8**	621	+15·0**	+4·4	−0·5
	37	1975	+11·0**	328	+10·4	+2·4	+1·8
4	15	1305	+0·5	729	+1·5	+3·4	+1·8
	15 w	1459	+11·6**	637	+7·1	+5·5	+7·1
	21	1498	+15·9**	560	+21·1**	+3·9	0·0
	21 w	1525	+17·8**	520	+26·5**	+5·0	+4·6
5	12	905	+4·8	390	−1·5	−6·2	+4·1
	23	1059	+9·5**	326	+8·0	+6·1	+3·7
6	7	1129	−0·6	872	−6·7*	+5·0	+2·1
	13	1174	+24·5**	896	+18·5**	+11·8**	+0·4
7a	19	957	−8·7**	1087	−8·2**	−5·1	−3·4
	27	1072	+11·4**	1361	+11·4**	−4·2	+6·1*
7b	10	1560	−0·9	1411	−1·2	−2·3	−3·6
	10 w	1276	+6·9*	1293	+7·7**	+0·2	+2·1
	15	1029	+8·1**	1161	+7·7**	−2·3	+3·7
	15 w	1087	+22·2**	1167	+10·4**	+0·9	−2·1
7c	7	1788	+2·6	1469	+1·2	+4·8	+0·1
	18	977	+23·0**†	1109	+8·6**	+4·6	−4·1
7d	5	1032	−1·0	1432	+1·0	+2·7	−2·0
	15	1105	+17·5**†	1637	+8·5**	+6·3*	+1·2
8	4	1180	−2·2	1523	−0·6	−0·1	−2·2
	10	1090	+9·2**	1674	+7·5**	+0·8	+1·8
9a	16	782	−4·1	1402	−1·1	+2·1	−1·7
	23	737	+12·3**	1515	+5·2*	−0·6	+2·7
9b	5	903	+4·8	1560	+1·2	−3·9	+1·0
	9	689	+11·2**	1314	+4·1	+0·9	0·0

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arose later as a product of differential rates of maturation and bursting of asci of the various segregation classes. If 'polarized segregation' is post-meiotic, analysis of bias in terms of the three meiotic spindles must be replaced by analysis in terms of individual ascal classes. The data in Table 2 indicate that asci with the class I

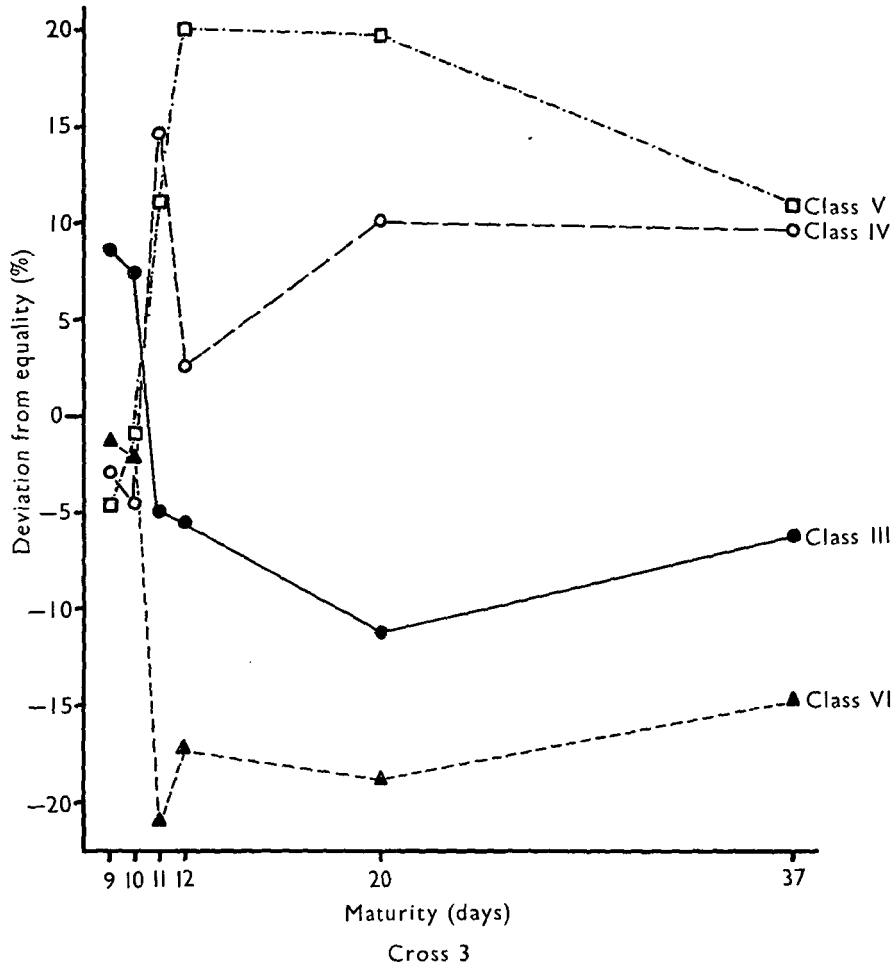

Fig. 2. The relation between maturity and the deviation from equality amongst the four ascal classes showing second division segregation.

arrangement of spores tend to mature to dehiscence point more slowly than class II asci. In the absence of polarized segregation and other biasing phenomena, such as spindle overlap or nuclear passing, the four second division classes are expected to be equally frequent. The relationship between maturity and the deviation from equality amongst these classes is shown for cross 3 in Fig. 2. It can be inferred from this that class VI asci tend to mature and burst first, then class III, class IV and finally class V. Similar trends were shown in the other crosses. These results agree well with those of McNelly (1962) and Lamb (1963), where the trend in those results

with significant polarized segregation was class I > II and class V > IV > III > VI. The order is not quite as above in Zickler's or Mathieson's data but is found if these data are combined.

If those kinds of ascus which tend to burst earlier also tended to become pigmented earlier, biases of negative sign would be expected in perithecia from which no dehiscence had occurred. This was difficult to test as in most crosses some ascal dehiscence began almost as soon as, or even before, pigmentation was sufficiently intense for scoring. The best test was in cross 7a, where at 12·5°C. many asci became pigmented before dehiscence commenced: the significant negative biases obtained confirm the above expectation. Figure 2 (cross 3) and cross 6 provide similar but less complete evidence.

A tendency for the spores in each ascus to become pigmented in a particular order would affect the observed frequencies of the ascal classes in incompletely mature perithecia (Mathieson, 1956). In these *Neurospora* crosses there was no clear trend for one ascal end to mature or become pigmented before the other. Singleton (1953) found in his *Neurospora* crosses that asci matured from the base upwards, while Mathieson (1956) found maturation from the apex downwards in *Bombardia lunata*. As similar polarized segregation trends occur in these two fungi, this phenomenon cannot be the general cause of their origin: further evidence is provided below.

In young perithecia the composition of the population of scoreable asci is affected by immature asci becoming pigmented as well as by dehiscence of mature asci; the rise in ascal osmotic pressure during maturation enables these two effects to be distinguished. Some asci in crosses 4 and 7b were mounted as usual in 2 M sucrose solution, which prevents further dehiscence: others were mounted in water and were scored about 1 hour after mounting. In water the more mature asci dehisce after water uptake on account of their high osmotic pressure, simulating the dehiscence that normally occurs with the passage of time but without any further pigmentation of immature asci. From Table 2 (crosses 4 and 7b) it can be seen that the sucrose-mounted asci, as in other experiments, showed no polarized segregation in young perithecia but had highly significant biases in the more mature perithecia. In contrast, water-mounted asci showed significant polarized segregation even in young perithecia and still greater bias in the more mature perithecia than the sucrose-mounted ones. The high degree of bias shown by young asci mounted in water and the lack of it in sucrose-mounted ones show that bursting alone is sufficiently differential with respect to the various ascal classes to bring about the apparent polarized segregation observed in this series of experiments. These results eliminate an alternative hypothesis to explain the increase in polarized segregation with maturity of the perithecia—namely that bursting is non-differential but successively maturing asci in a perithecium show a progressive increase in the degree of a meiotically-produced polarized segregation.

Mathieson (1956) noted that where segregation appeared polarized in *Bombardia* and *Chromocrea* (Mathieson, 1952) the mutants affected the viability or rate of maturing of the spores. In addition to the effects on pigmentation and germination listed earlier, the mutants used in this study also retarded ascal maturation:

asco × *asco* and *ts* × *ts* crosses were infertile, maturing very slowly, and $h \times h$ and $g \times g$ crosses matured more slowly than $+ \times +$. Thus the mutants which show apparent polarized segregation result in the slower, and often only partial, completion of the full sexual cycle in comparison to the corresponding wild-types. This indicates that metabolism in the cytoplasm associated with nuclei carrying these mutations is impaired, resulting in slower maturation. The slower maturation of *mutant* (♀) × + and of + (♀) × *mutant* than of + × + shows that this still applies when +-bearing nuclei are present in the cytoplasm.

The relative frequencies of the asymmetrical (V and VI) and symmetrical (III and IV) second division segregation classes (Table 2, last column) showed no clear evidence of spindle overlap or nuclear passing (Whitehouse & Haldane, 1946; Howe, 1956) at any of the loci studied in *Neurospora crassa* or *Sordaria fimicola*.

4. DISCUSSION

In all known examples of polarized segregation in Ascomycetes, the direction in which the +-bearing chromosome appears to segregate more frequently than expected is to the upper pole of the spindle: this suggests there is a common mechanism, probably involving some property of the mutant genes or loci themselves rather than other genes on the same chromosomes. The following hypothesis has been developed to explain how differences in the order of spore-pair nuclei could bring about differences in maturation and bursting between the ascal classes. Translocation into and up the ascus of nutrients for development and maturation is probably mediated largely by active transport mechanisms. The eventual maturation of any region of the ascus would therefore depend on the metabolism of all regions of the ascal cytoplasm proximal to that region. Class II asci would then be better supplied with nutrients than class I asci, which have mutant-bearing nuclei (with impaired metabolism in their vicinity, as discussed earlier) in the proximal region. Class II asci are thus expected to mature faster and dehisce earlier than class I asci, as was found, giving rise to apparent polarized segregation at the first division spindle. Asci of classes VI and III have +-bearing nuclei in the important basal region (adjoining the ascogenous hypha and proximal to all other regions) and are expected to mature faster than class IV and V asci, accounting for apparent bias at the second division lower spindle. The order of nuclei in the distal half would be less important but class VI would be expected to burst before class III, and class IV before V, giving rise to apparent bias at the second division upper spindle. This bias is expected to be less prominent than those at the other two spindles, as found.

Another factor possibly influencing bias at the second division upper spindle is the nature of the apical spore-pair, which might affect dehiscence of the apical pore. There is evidence of such an effect at 12·5°C. in *Sordaria* (crosses 7a and 9a) where bias at this spindle is negative in mature perithecia although positive at the other two spindles. The excess of class IV asci over class V in mature perithecia in these two crosses might be related to the presence of mutant-bearing nuclei adjacent to the apical pore in class IV asci, which could retard dehiscence at this low temperature.

This apart, the frequencies of the ascal classes from mature perithecia were generally in excellent agreement with the order I > II, V > IV > III > VI predicted by the hypothesis.

The relative class frequencies in the *Bombardia* studies are similar to those of the present *Neurospora* and *Sordaria* data but Mathieson (1956) found no correlation between the degree of bias in a perithecium and its 'maturity index'. Her findings would, however, be compatible with the present results if apparent bias in the younger *Bombardia* perithecia resulted from the earlier pigmentation she observed of the distal spores in each ascus and if bias in the maturer perithecia originated by differential bursting. A combination of bias produced in these two ways at different stages of ascal maturity would account for the apparent lack of correlation between bias and perithecial maturity in *Bombardia*. The absence of polarized segregation at the *g* locus of *Sordaria fimicola* reported by Olive (1956) is also compatible with the present data if young perithecia had been scored. In Berg's (1966) study of polarized segregation in *Sordaria brevicollis*, differential bursting of asci after crushing perithecia in water provides an alternative or additional explanation of the bias observed to the given one of faster maturation of distal spores.

If a mutant gene giving rise to differential bursting is segregating in a cross, linked mutations not having this effect themselves could also show apparent polarized segregation, especially if closely linked. The presence of two unlinked mutants in a cross, both resulting in differential bursting, could—with some experimental procedures—give rise to apparent cases of 'affinity' in Ascomycetes.

In conclusion, differential bursting of asci in these experiments affected the relative frequencies of the ascal classes in such a way as to cause apparent polarized segregation for certain mutants. What is perhaps more important to genetical analysis, it also affected the relative frequencies of asci showing first or second division segregation (Lamb, 1964). Scoring a harvest for apparent polarized segregation, as well as for second division segregation frequency, can give a good indication whether differential bursting and the possible biasing of this frequency have occurred. Analysis of this aspect of the present data is nearing completion.

SUMMARY

Polarized segregation was studied at the *asco* and *tan spore* loci of *Neurospora crassa* and at the *gray* and *hyaline* loci of *Sordaria fimicola*. Different alleles, reisolates and temperatures of incubation of crosses were studied with the *asco* locus and different temperatures of incubation with the *h* and *g* loci. The mutants were crossed with the corresponding wild-types by methods giving a considerable degree of synchrony in the development of perithecia; segregation patterns were scored from perithecia at widely differing stages of ascal maturity. Significant polarized segregation was not generally observed when little or no dehiscence had occurred from a cross but usually became readily apparent as dehiscence proceeded. This indicated that the differences in frequency between the ascal classes that had previously been interpreted as showing polarized segregation at meiosis were actually

due to a post-meiotic differential bursting of asci. This bursting was studied separately from the usually concurrent pigmentation of immature asci by comparing results from asci mounted in water with those from asci in the 2 M sucrose solution which was used throughout these experiments: the differential bursting produced by mounting asci in water gave similar polarized segregation results to those from natural dehiscence. A hypothesis to account for differential bursting in physiological terms was advanced and its predictions discussed. It seems likely that cases of apparent polarized segregation described in other Ascomycetes are also due to post-meiotic phenomena.

I am indebted to Professor D. G. Catcheside, Professor L. S. Olive, Dr B. S. Cox, Dr L. C. Frost and the Fungal Genetics Stock Center for kindly providing strains used in this work and to my supervisor, Dr L. C. Frost, for his valuable criticism and assistance in preparation of the manuscript. I wish to thank the S.R.C. for the award of a research studentship during the course of this work.

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