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An investigation into the origins of meiotic aneuploidy using ascus analysis

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SUMMARY

Aneuploidy can result from a variety of defects at meiosis. Results are presented of crosses of *Sordaria brevicollis* in which aneuploid spores are detected through complementation of spore colour mutants at the *buff* or *grey*-6 loci on linkage groups II and IV respectively. By using ascus analysis, the underlying cause of the aneuploidy can be deduced in many cases. Thus non-conjuction (pairing failure) and non-disjunction at the first meiotic division, premature centromere division, non-disjunction at the second division, and pre-meiotic errors such as extra replication of the chromosomes can be distinguished. Both linkage groups were found to give a similar proportion of the different errors. Non-conjunction and first-division non-disjunction formed 60–70 % of detectable cases, whilst premature centromere division and second-division non-disjunction comprised 10 % and 5 % of aneuploids respectively. However, only a small proportion of second-division errors are detected.

It is proposed that the systems described in this paper can form the basis of a valuable screening method for detecting agents which increase aneuploid frequency. The advantages and disadvantages of using lower eukaryotes in this way are discussed.

INTRODUCTION

Man appears to be unusual in that a high proportion of zygotes possess a chromosome abnormality. Because the majority of these abort spontaneously and many may go undetected, estimates vary as to the real frequency of these conceptions. Alberman & Creasy (1977) estimate that 8–10 % of zygotes at conception are chromosomally abnormal, whereas Boué, Boué & Lazar (1975) put the figure as high as 50 %: Whatever the real value two things are generally agreed. Firstly, the frequency is very much higher in Man than in experimental laboratory mammals such as the mouse. Secondly, of the chromosomally abnormal zygotes about 70 % are aneuploid, that is they contain a chromosome number which is not a simple multiple of the haploid set.

Since it is well known that the frequency of aneuploidy can be increased

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experimentally, there has been concern that some environmental pollutants may exist which, as their sole genetic endpoint, cause an increase in this already high level of aneuploidy. Consideration has been given to methods for detecting such agents (see volume 31 of *Environmental Health Perspectives*) but there are severe difficulties in the choice of a suitable system.

These difficulties arise from the fact that methods using experimental mammals are time-consuming and tedious. They are very expensive and it is therefore difficult to see how they could be used in routine screening of the many compounds which need to be tested. Attention has therefore fallen on methods using lower eukaryotes which are rapid, cheap and very convenient, but here too there are problems which arise from their taxonomic remoteness from Man. Consideration of some of the specific difficulties which arise from this can be found in the Discussion to this paper. One point however needs to be made here. Lower-eukaryote systems which simply monitor the presence or absence of induction will not form the basis of a very satisfactory screening method, because extrapolation of the findings from lower to higher eukaryotes is beset with difficulties.

In this paper we describe a system for detecting aneuploidy using the fungus *Sordaria brevicollis*, which can be used to record rather more than simple increases in frequency. The method, which has previously been described in outline (Bond, 1976; Bond & McMillan, 1979), has several outstanding advantages. The most important of these is that the underlying cause of the aneuploidy can be inferred. The method involves examination of intact asci; those which contain aneuploid disomic products can be readily detected. The existence of different types of asci containing different proportions of aneuploid spores reflects the fact that there is more than one way in which aneuploidy arises at meiosis.

MATERIALS AND METHODS

Mutant strains

Aneuploidy can be detected separately for linkage groups II and IV using buff and grey spore colour mutants.

The buff system

C70 and S6 are complementing alleles of the *buff* or b_1 locus, and form the basis of the method for detecting aneuploidy for linkage group II. Flanking markers were used in the experiments to verify the disomic nature of the aneuploids and as an aid to identifying the alleles. The loci used for this pupose were *met-1* and *not-1* (prevously called *nic*), mutations at which confer growth requirements for methionine and nicotinamide or tryptophan respectively.

The grey system

The spore colour mutations used in these experiments were C31, RW25, YS121, YS18 and B9. They were obtained from Dr H. L. K. Whitehouse and are alleles

of the *grey-6* (g_6) locus, which is 5 map units from the centromere of linkage group IV. Linked marker mutations were also available at the *met-2*, *ura*-1 and *pdx-1* loci which confer requirements for methionine, uracil and pyridoxine respectively. These loci are proximal to *grey*-6 and, as for the buff system, can be used to identify the complementing alleles when recovered from disomic spores.

Media

The strains were routinely cultured, and ascospores dissected, into Vogel's (1956) N medium with 2% glucose, 1·5% Difco-Bacto agar and appropriate supplementation to satisfy any growth requirements. Sodium acetate (0·7%) was added to stimulate germination of ascospores. Crosses were carried out on cornmeal agar of composition: Difco Cornmeal Agar 1·7%; sucrose 0·3%; glucose 0·2%, yeast extract 0·1% w/v.

Crossing and scoring methods

Full details of the crossing and scoring methods can be found in Bond (1976, 1982) and Bond & McMillan (1979). Briefly, crosses were incubated at 25 °C. Female parents were fertilized, four days after inoculation, with a microconidial suspension and incubated for a further six days, after which time the crosses were harvested. Perithecia were crushed open in sucrose solution and rosettes of asci transferred to the surface of a 4% agar slab which had been previously flooded with sucrose solution. A coverslip was placed over the rosettes and pressed down to flatten the asci into one plane. The sucrose solution prevented the agar slab drying out unevenly during the transfer of rosettes to its surface, and also facilitated the removal of the coverslip to dissect asci if this was necessary.

The rosettes of asci were examined at 100× magnification using a compound microscope. The number of asci in each rosette was estimated to the nearest ten and any asci containing aneuploid black spores were recorded.

RESULTS AND DISCUSSION

The buff system

Typical results of C70 × S6 crosses can be seen in Table 1. The crosses which gave these results were control crosses from various experiments in which possible aneuploid induction was being monitored. In each experiment the asci were examined until 50 containing disomic black spores had been detected. All crosses were of the type *met*$^-$ S6 *not*$^-$ × *met*$^+$ C70 *not*$^+$, but the same isolates were not used in every experiment. Apart from these three linked loci and mating type, however, there were no other known genes segregating in the crosses.

Considering first the overall frequency of meiotic aneuploidy; it can be seen that there was some variation in the frequency of aneuploid containing asci. There was significant heterogeneity between experiments as judged by a G test ($G_H = 21·2$

Table 1. *Number (and frequency* $\times 10^{-4}$*) of asci containing black aneuploid spores in buff* $\times$ *buff crosses*

Ascus type		Spore sequence within ascus	Experiment number 1	2	3	4	5	6	7	8	9	10
I	4 black: 4 abortive	4Bl: 4Ab* and 2Bl: 2Ab: 2Bl: 2Ab	31 (4·5)	28 (4·3)	23 (3·9)	27 (4·0)	30 (5·5)	33 (5·9)	30 (5·8)	29 (3·2)	28 (7·0)	31 (4·4)
II	2 black:† 2 abortive: 4 buff	2Bl: 4Bu: 2Ab and 2Bu: 2Bl: 2Ab: 2Bu	2 (0·3)	3 (0·5)	5 (0·8)	4 (0·6)	1 (0·2)	2 (0·4)	1 (0·4)	4 (0·2)	0 (—)	3 (0·4)
III	2 black:† 2 abortive:	2Bu: 2Bl: 2Bu: 2Ab and 2Bu: 2Ab: 2Bu: 2Bl:	3 (0·5)	0 (0·2)	3 (0·5)	2 (0·3)	2 (0·4)	0 (—)	2 (0·4)	3 (0·3)	0 (—)	1 (0·1)
	4 buff	2Bl: 2Ab: 4Bu: and 2Ab: 2Bl: 4Bn	3 (0·5)	6 (0·8)	5 (0·8)	1 (0·2)	3 (0·6)	4 (0·7)	5 (1·0)	2 (0·2)	5 (1·2)	1 (0·1)
IV	(*a*) 4 black: 4 buff	4Bl: 4Bu and 2Bl: 2Bu: 2Bl: 2Bu	5 (0·7)	5 (0·8)	8 (1·3)	6 (0·9)	11 (2·0)	3 (0·5)	8 (1·5)	4 (0·5)	5 (1·2)	1 (0·1)
	(*b*) 2 black: 6 buff	All sequences	1 (0·2)	5 (0·8)	3 (0·5)	2 (0·3)	0 (—)	2 (0·4)	0 (—)	1 (0·1)	0 (—)	0 (—)
	Miscellaneous‡		1 (0·2)	0 (—)	2 (0·4)	4 (0·6)	0 (—)	5 (0·9)	1 (0·2)	3 (0·3)	7 (1·7)	2 (0·2)
	Unclassified§		4 (0·6)	3 (0·5)	1 (0·2)	4 (0·6)	3 (0·6)	1 (0·2)	3 (0·6)	4 (0·5)	5 (1·2)	10 (1·4)
	Total aneuploids		50 (7·3)	50 (7·6)	50 (8·4)	50 (7·4)	50 (9·2)	50 (9·0)	50 (9·6)	50 (5·6)	50 (12·4)	50 (7·1)
	Total asci screened		68450	65840	59650	679760	54210	55850	52190	89610	40280	70250

* Bl = Black, Ab = abortive, Bu = buff.

† Type II asci have a 2 : 4 : 2 sequence of normal and aneuploid spores; type III asci have either a 2 : 2 : 2 : 2 or a 4 : 4 sequence of normal and aneuploid spores.

‡ Including asci with less than 8 spores and asci with an uneven number of black and abortive spores.

§ Asci for which the full sequence of spores was not determined because the material was too fragile for further analysis. The majority of those had 2 black spores.

with 9 D.F., $P < 0{\cdot}02$). These results are typical; most crosses give an aneuploid frequency of about 8×10^{-4} but crosses which are significantly different from this value are encountered occasionally. The underlying causes of this heterogeneity are not known. In the present data experiments 8 and 9 gave aneuploid frequencies which were lower and higher respectively than the typical average. It is these two crosses which together make the data heterogeneous. This is illustrated diagrammatically below.

8, 10, 1, 4, 2, 3, 6, 5, 7, 9.

The experiments are ranked in increasing order of aneuploid frequency. The bars underline those experiments for which the data is not significantly heterogeneous at the 5% level of probability.

Returning to Table 1, the asci which contained aneuploid spores have been classified into four specified types (which were the most common) and a fifth miscellaneous category. One of the merits of this system for studying aneuploidy is the ease with which the aneuploid spores can be detected. This can be seen in Plates 1 and 2, which show an example of each type of ascus.

Type 1 asci contain 4 black and 4 abortive spores and were the most common type, comprising about 60 % of the asci containing aneuploids. These asci had either a 4 : 4 or an alternate 2 : 2 : 2 : 2 sequence of black and abortive spores. Both these sequences are consistent with the idea that the aneuploidy results from an error at the first division of meiosis, the 2 : 2 : 2 : 2 sequence coming about through spindle overlap. Spindle overlap is common in *Sordaria brevicollis* (Chen & Olive, 1965; Berg, 1966); when it occurs the positions of the two centrally located spore pairs are switched so that 4 : 4 sequences are converted into 2 : 2 : 2 : 2s.

Type II and type III asci are superficially very similar. Each has 2 black, 2 abortive and 4 buff spores, but they are distinguished by the sequence of spores in the ascus. In type II asci the black and abortive spores are located in opposite halves of the ascus, and this arrangement is not affected by spindle overlap because the sequence of normal (n) and aneuploid (a) is 2n : 4a : 2n or 2a : 4n : 2a. The location of the disomic and nullisomic spores in opposite halves of the ascus implies an origin at the first meiotic division. The same conclusion cannot be drawn about type III asci, which have a spore sequence which is affected by spindle overlap, and as a result the meiotic division at which these originate is uncertain. Random orientation of the spindles at the second division will generate 2n : 2a : 2n : 2a (type III) as often as the 2 : 4 : 2 sequences (type II). Partial spindle overlap can divert a potential 2 : 2 : 2 : 2 sequence into a 4 : 4 sequence. Therefore those apparently arising at the second meiotic division, because the aneuploid spore pairs are located in the same half of the ascus, may well have originated at the first division, if spindle overlap had occurred to disturb the spore sequence.

Type IV asci are characterized by the fact that the asci containing black disomic spores do not also contain abortive nullisomic spores. The most commonly occurring ascus of this type had 4 black and 4 buff spores, but others with 2 black

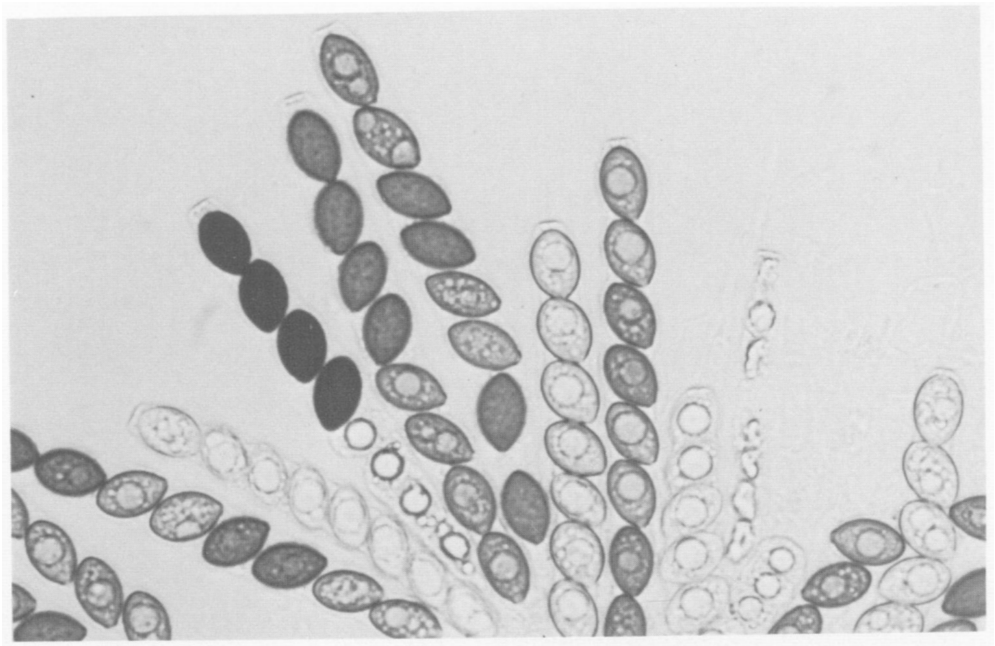
and 6 buff spores were also seen. (Although none was found in the crosses analysed here another type IV ascus, containing 8 disomic spores, is seen occasionally.)

The miscellaneous asci comprise a mixture of types of which the most common contains two black disomics, four abortive and two buff spores. In the crosses reported here this type was not common enough to warrant separate classification.

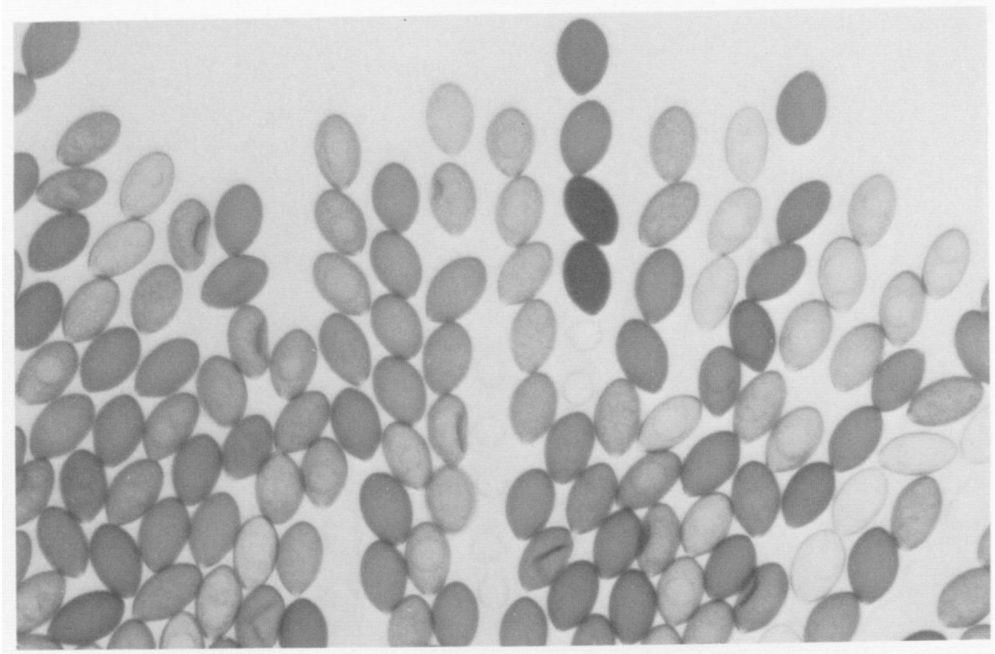
The existence of different types of asci containing aneuploid spores reflects the fact that aneuploidy may arise at meiosis from a variety of defects, many of which are distinguishable using the Sordaria system. These defects are illustrated in Fig. 1, which also shows the ascus types which result from them.

There are two likely errors at the first meiotic division which will give rise to type I asci. Either failure of separation of synapsed homologues (non-disjunction in its strict sense) or pairing failure (non-conjunction, Sturtevant & Beadle, 1939) followed by random segregation of the resulting univalents could be responsible. Although these defects have a similar outcome they can, theoretically, be distinguished because the aneuploid products resulting from non-conjunction will contain non-recombinant chromosomes. This was the basis of the method used by Merriam & Frost (1964) to distinguish non-conjunction from other meiotic errors in Drosophila. Using multiply marked *X* chromosomes, they concluded that non-conjunction was a possible source of some, but not all, meiotic aneuploidy. Whether the disomic spores in 4 black : 4 abortive asci in Sordaria contain a disproportionate number of non-recombinant chromosomes cannot be determined using the buff system, since linkage group II is not sufficiently well marked. However, for induced aneuploidy the question may be partially answered using the grey system to be described below.

Type II asci were observed in Neurospora by Threlkeld & Stoltz (1970). They suggested that this type of ascus arose from the premature division of the centromere at the first meiotic division. As a result, three of the four chromatids comprising a bivalent move to one pole whilst only one moves to the other. Type III asci can also originate in this way but, since the spore sequence in this type is ambiguous with reference to the division at which the aneuploid spores are generated, non-disjunction at the second division is also a possible explanation for them. Premature centromere division will be expected to generate type II and type III asci equally often, non-disjunction at the second division only generates type III, therefore the extent of the latter defect can be judged by the greater frequency of type III compared to type II. The small numbers do not justify comparing individual crosses, but it can be seen that, taking all ten control crosses, there were 25 type IIs and 51 type IIIs, which probably indicates that some non-disjunction at the second division occurred in these control crosses. If premature centromere division is equally likely to lead to a type II and a type III ascus, then it would appear that half of the type IIIs originated through second-division non-disjunction. Provided that spindle overlap is equally likely to occur in meioses with either defect, then type III asci in which the aneuploid spore pairs are adjacent and those in which the pairs are alternate should be found with equal frequency. It can be seen however that this is not so. Thirty-five asci possessed adjacent aneuploid



(*a*)



(*b*)

PLATE 1

Aneuploid containing asci from c70 × S6 crosses. (*a*) Type I ascus containing 4 black : 4 abortive spores. Origin : non-conjunction or non-disjunction. (*b*) Type II ascus containing 2 black : 2 abortive : 4 buff spores. The sequence is important. The aneuploid spores are in opposites halves of the ascus, and partial spindle overlap (which switches the position of the two central spore pairs) does not alter this arrangement. Origin: premature centromere division.

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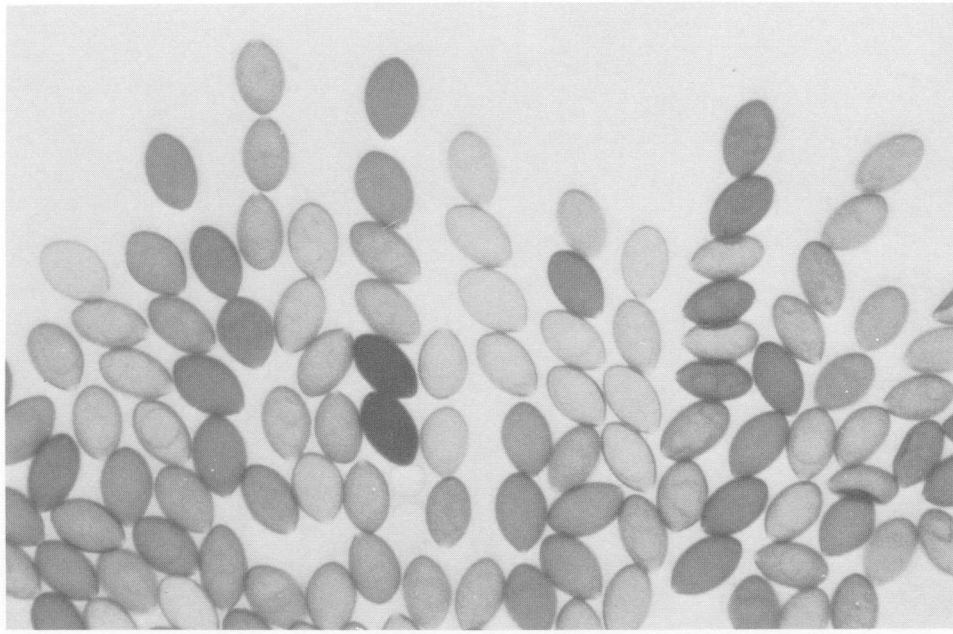

(*c*)

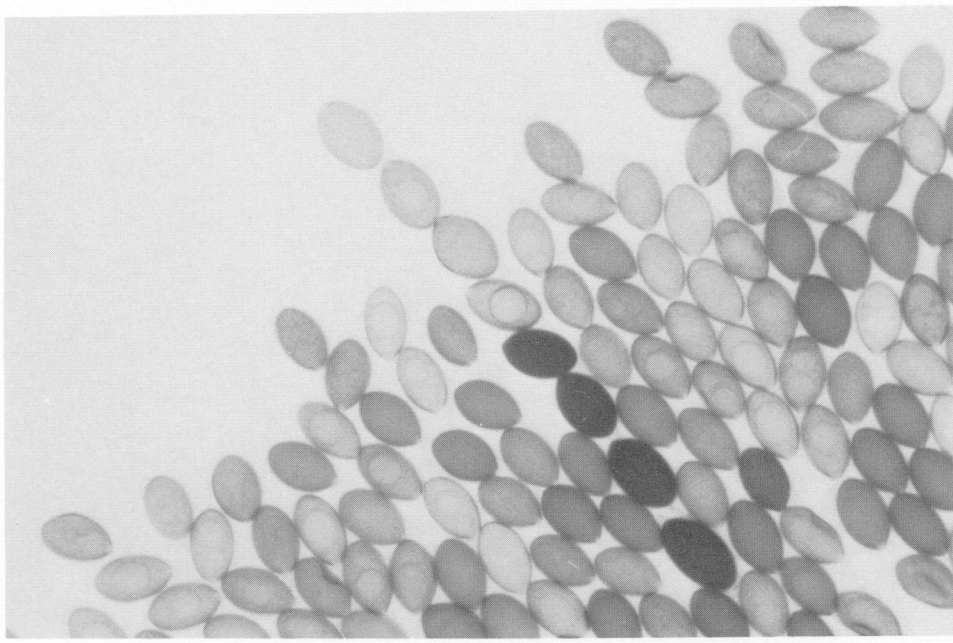

PLATE 2

(*c*) Type III ascus containing 2 black : 2 abortive and 4 buff spores. Partial spindle overlap will affect the position of the aneuploid spores. Origin: premature centromere division or second-division non-disjunction. (*d*) Type IV ascus containing black disomic spores but no nullisomics. Origin: pre-meiotic error.

A. M. FULTON AND D. J. BOND

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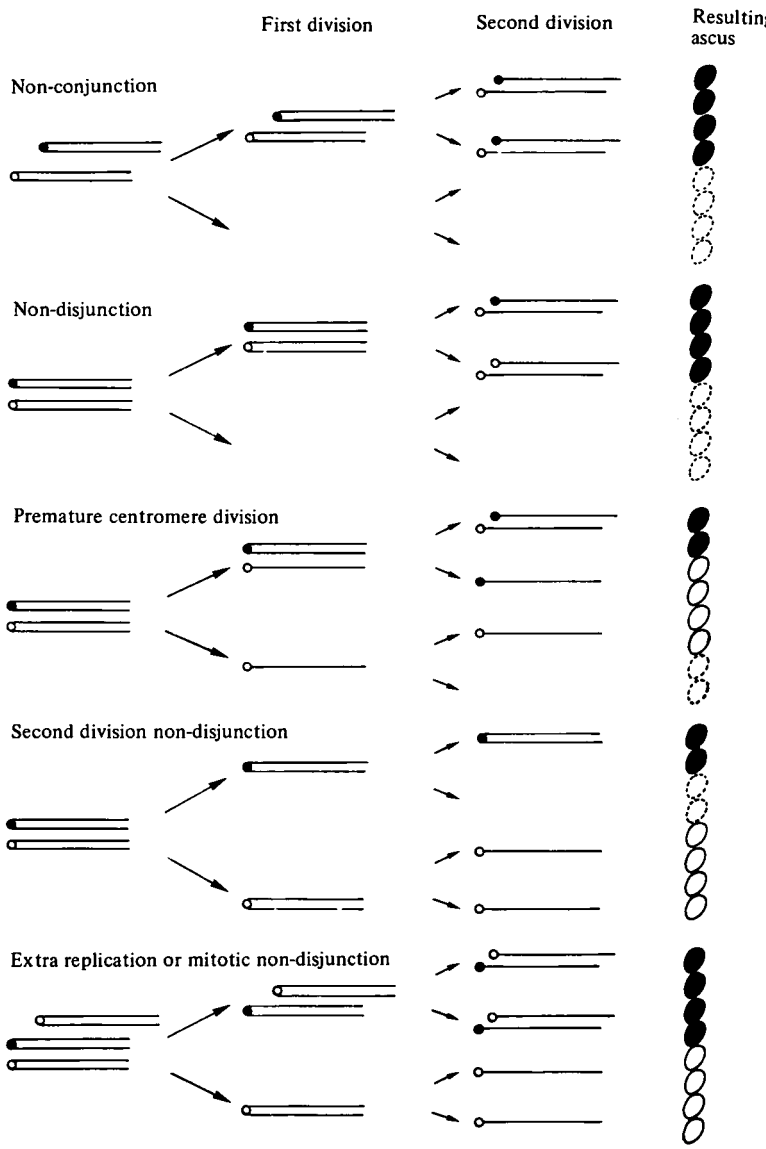

Fig. 1. Schematic representation of the errors leading to aneuploidy and the resulting ascus types.

spore pairs, only sixteen had an alternate arrangement. One possible explanation for this is that spindle overlap is very infrequent in asci with second-division non-disjunction but occurs with normal frequency in asci with premature centromere division. Since second-division non-disjunction could arise from a spindle defect this is quite possible, and experiments to test it are being planned.

If the observed excess of type III asci does reflect a second division origin, then

the real frequency of this defect may be quite high, but it is difficult to estimate its true extent for the following reasons. If the aneuploid spores detected in this system have to carry both a C70 and S6 allele before they are black, non-disjunction at the second-division can only be detected when the non-disjoining chromosome carries different alleles on its two chromatids. This comes about only when there has been a crossover in the centromere interval. Since the *buff* locus is closely linked to its centromere (approximately 4 map units away) only about 8% of second-division non-disjunction will be detected. There is however a complicating factor. Homoallelic C70 × C70 crosses (but not S6 × S6) possess a small number of asci which have spores which are noticeably darker than normal. These are usually located in asci with abortive spores, and by marking the C70 chromosomes in these crosses it has been shown that these darker spores are disomic. The darker phenotype in these spores presumably arises from a dosage effect where two copies of the C70 mutation nearly restore a wild-type phenotype. The extent of this pigmentation varies considerably from cross to cross, and in some cases the C70/C70 disomic spores are very dark and could easily be scored as aneuploids should they arise, through second-division non-disjunction, in a C70 × S6 cross. This would mean that type III asci arising from non-disjunction would not have to have a crossover in the centromere interval.

In an attempt to see what proportion of type II asci have such a crossover and to see if any of the aneuploid spores are C70/C70 disomics, ascus dissection experiments have been started in order to analyse the genotype of the two homologues present in type II and type III asci. Preliminary data indicate that the type III asci all contain C70/S6 aneuploids, but it is too soon to draw any conclusion on the proportion of the type III asci which are recombinant in the centromere interval.

Considering type IV asci, it seems evident that these originate from an ascus initial which is not diploid, as in the normal case, but trisomic. This trisomy could arise from a pre-meiotic error such as mitotic non-disjunction or an extra replication event involving one chromosome in the genome. Both these possibilities lead to the expectation that type IV asci should be clustered. If the pre-meiotic error occurs early on in the development of the perithecium, then, as a result of mitotic division, several aneuploids should be generated from the one initial event. In fact, no clustering of these asci has ever been observed. This can be explained. It is known that aneuploid nuclei in both Sordaria and Neurospora are unstable. Any extra chromosomes are rapidly lost, so that each nucleus soon regains the haploid number of chromosomes. It might be therefore that the only disomic nuclei arising before meiosis which contribute to aneuploidy in ascospores are those arising immediately before the fusion of nuclei to form the ascus initial. The type IV asci with only two black spores have a similar origin, the explanation for there being only two, and not four, black spores being that there has been a crossover in the centromere–buff interval. This results in the formation of one pair of disomic spores which are homoallelic and not black (Bond, 1976). Ascus dissection experiments have shown that these asci do have a crossover in the centromere interval as expected.

Table 2. *Number (and frequency* $\times 10^{-4}$*) of asci containing black aneuploid spores in grey* $\times$ *grey crosses*

	Ascus type	Spore sequence within ascus	Grey-6 Alleles crossed and experiment number: RW25 × C31 1	RW25 × C31 2	C31 × YS121 3	RW25 × YS121 4
I	4 Black: 4 abortive	4 Bl: 4Ab and 2 Bl: 2Ab: 2Bl: 2Ab	31 (4·5)	36 (5·6)	33 (9·4)	18 (1·3)
II	2 Black* 2 abortive 4 grey	2Bl: 4Gy: 2Ab and 2Gy: 2Bl: 2Ab: 2Gy	5 (0·7)	9 (1·4)	4 (1·1)	1 (0·07)
III	2 Black* 2 abortive 4 grey	2Gy: 2Bl: 2Gy: 2Ab and 2Gy: 2Ab: 2Gy: 2Bl	2 (0·3)	3 (0·5)	5 (1·4)	2 (0·15)
		2Bl: 2Ab: 4Gy and 2Ab: 2Bl: 4Gy	9 (1·3)	2 (0·3)	5 (1·4)	0 (—)
IV	(*a*) 4 Black 4 grey	4Bl: 4Gy and 2Bl: 2Gy; 2Bl: 2Gy	See Table 3	See Table 3	See Table 3	See Table 3
	(*b*) 2 black 6 grey	All sequences				
	Miscellaneous	—	3 (0·4)	0 (—)	3 (0·9)	5 (0·4)
	Unclassified	—	0 (—)	0 (—)	0 (—)	0 (—)
	Total aneuploids	—	50 (7·2)	50 (7·8)	50 (14·3)	26 (1·9)
	Total asci screened	—	69640	63790	35020	135790

* Type II asci have a 2 : 4 : 2 sequence of normal and aneuploid spores, type III asci have either a 2 : 2 : 2 : 2 or a 4 : 4 sequence of normal and aneuploid spores.

The grey system

All the *grey-6* alleles complemented each other, disomic spores being noticeably darker than either parental phenotype. However, only crosses involving the alleles C31, RW25 and YS121 were regarded as useful for aneuploid detection because the YS18 allele was too dark, and complementation in crosses involving B9 was too weak, to allow aneuploid spores to be detected easily using the rosette

Table 3. *Number (and frequency %) of asci with 2 black : 6 grey and 4 black : 4 grey spores in grey × grey crosses*

Alleles cross	Asci screened	2 black : 6 grey	4 black : 4 grey
RW25 × C31	69640	1207 (1·7)	7
	63790	1181 (1·8)	6*
	9960	211 (2·1)	1
	9770	239 (2·4)	1
	9460	102 (1·1)	2
	7370	112 (1·5)	0
	8630	103 (1·2)	2†
	6110	120 (1·9)	0
	19040	137 (0·7)	3
C31 × YS121	35020	419 (1·2)	1
	6980	59 (0·8)	0
	32500	258 (0·8)	5
RW25 × YS121	135790	79 (0·06)	2
	8390	8 (0·10)	1
	6360	5 (0·08)	0
	9800	5 (0·05)	4†

* Two asci with large black spores.
† One ascus with large black spores.

examination method. In addition, disomic spores in homoallelic crosses of RW25 × RW25 and YS121 × YS121 were also dark, this reflecting a dosage effect similar to that found in C70 × C70 crosses.

The results of the crosses RW25 × C31, C31 × YS121 and RW25 × YS121 are presented in Table 2. The RW25 × YS121 data are the combined results from two crosses neither of which yielded enough material to give a total of 50 aneuploids. It can be seen that overall aneuploid frequency varied from cross to cross, but it is not yet possible to say whether this variation is similar to that observed in *buff* × *buff* crosses, or whether the chromosome associated with each *grey-6* allele has a characteristic non-disjunction frequency. More replicate crosses are required to establish this point. It can also be seen that, with the exception of type IVs the same ascus types were detected in these crosses as in *buff* × *buff* crosses.

The major differences between the buff and grey systems is that in each of the grey crosses, asci with two black and six grey spores were seen frequently. Analysis of these asci revealed that the black spores were recombinant rather than

aneuploid. As might be expected, the frequency of recombination was a characteristic of the alleles crossed. This can be seen in Table 3, which contains data from some crosses that were scored for recombination frequency only. Asci with four black and four grey spores were also occasionally seen. Sometimes the black spores in these asci were noticeably larger than normal, and when analysed these large spores turned out to be aneuploid. More often however the black spores were of normal size, and we do not know what fraction of these are recombinant and what fraction are aneuploid.

The grey system has no advantage over the buff system, but potentialy it is a useful addition to it. The fact that the *grey-6* alleles recombine with an appreciable frequency means that grey crosses can be used to test whether an inducing agent increases aneuploidy through affecting chromosome pairing and/or recombination. Should an experimental treatment result in a significant increase in aneuploidy by increasing type I asci, the effect of the treatment on the recombination of the *grey-6* alleles can be easily monitored in the same crosses. Thus if the increase arises from non-conjunction, a reduction in recombination frequency might be expected.

The origins of aneuploidy

Although the data from both the buff and grey crosses were heterogeneous when the total aneuploid frequency was considered, the proportions of the different types of asci did not show any large differences between crosses. In order to compare the frequencies with which the different meiotic errors were detected the data were combined separately for the buff and grey crosses, and the result are presented in Fig. 2, together with the Neurospora data of Threlkeld & Stoltz (1970). It was assumed that in Sordaria the excess of type 3 asci compared to type 2 could be attributed to non-disjunction at the second division. The spectra for the buff and grey crosses are quite similar, most cases of detectable aneuploidy arising from non-conjunction or non-disjunction.

The data of Threlkeld & Stoltz (1970) obtained in Neurospora make an interesting comparison. They only analysed asci which contained both disomic and abortive spores, i.e. pre-meiotic errors were not reported. Forty-five out of 76 asci arose from premature centromere division; in six of these the premature centromere division occurred in both homologues. In Neurospora therefore the proportion of aneuploidy arising from this defect was greater then in Sordaria. In both organisms second-division errors constituted a minority of detected cases. In Neurospora only 3 out of the 76 asci originated in this way, but since the *pan* locus used to detect the aneuploidy is only 2 map units from its centromere, the detected cases comprise only 4% of the total. Therefore, it is likely that second-division errors do occur with a frequency similar to that of first-division errors. The complications of estimating the true frequency of second-division errors in Sordaria were discussed earlier. However, it is likely that a similar conclusion also holds for Sordaria, where only 8–10% of second-division errors are detected.

It is quite clear that meiotic aneuploidy in Sordaria arises from a number of

meiotic defects. This is not a peculiarity of fungal meiosis, there is no doubt that similar defects occur in other organisms, including Man.

The fact that pairing failure can lead to increased aneuploidy is clearly established in both plants and animals (See Golubovskaya, 1979 for a review). However, in both mouse and Man extensive pairing failure in spermatogenesis

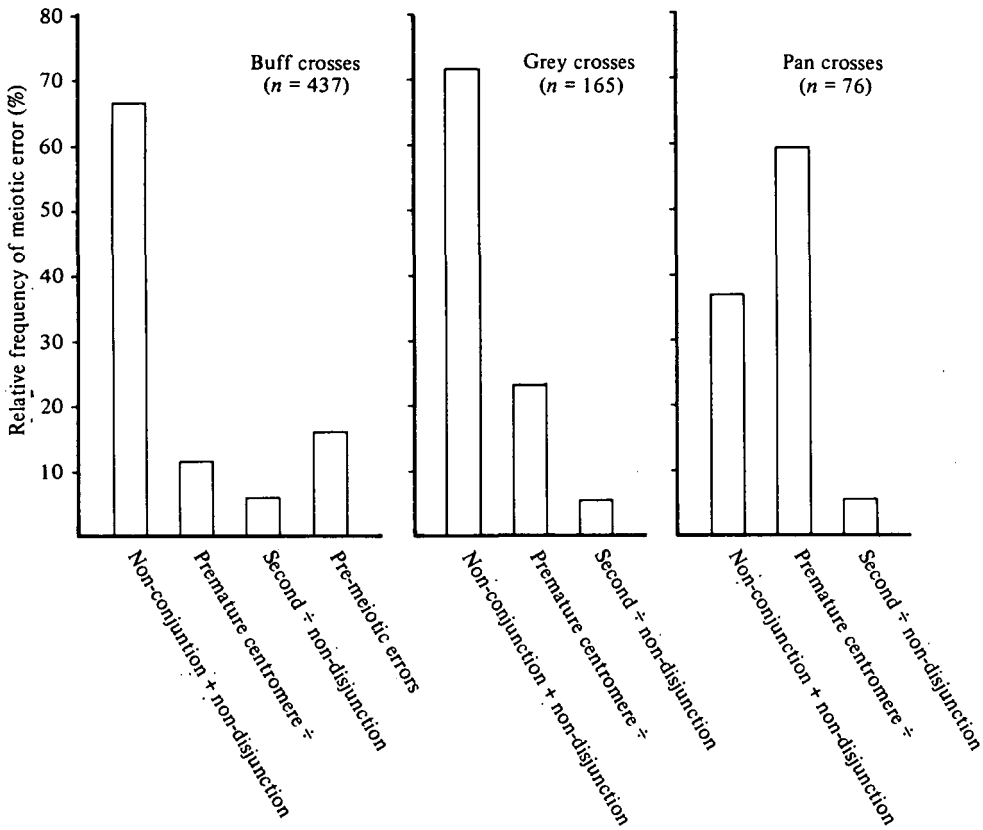

Fig. 2. The relative frequency of errors giving rise to dectectable aneuploidy in Sordaria and Neurospora. In the grey system of Sordaria, pre-meiotic errors are not detectable. The Neurospora data are taken from Threlkeld & Stoltz (1970), where pre-meiotic errors were not recorded.

results in germ-cell arrest (Beechey, 1973; Purnell, 1973; Chandley *et al.* 1976), and it is not clear to what extent this acts as a selective mechanism for eliminating potential aneuploids or to what extent occasional asynapsis can lead to aneuploidy in mammals. Polani & Jagiello (1976) and Speed (1977) were unable to detect increased aneuploidy at metaphase II in mouse oocytes in spite of an increased univalent frequency at metaphase I.

Premature centromere division has often been reported by cytogeneticists. In mice Polani & Jagiello (1976) called the phenomenon giving rise to single

chromatids at the end of the first meiotic division 'pre-division' whilst Hansmann & El-Nahass (1979) called it 'pre-segregation'. In Man, Fitzgerald *et al.* (1975) and Galloway & Buckton (1978) reported the premature centromere division of the *X*-chromosome which led to increased sex chromosome aneuploidy in the cells of older women. German (1979) reported that patients suffering from Roberts' syndrome possessed chromosomes with defective centromeres in which the centromere regions showed a characteristic early separation. This was taken as evidence for a genetically determined disturbance of the normal mechanism of chromosome disjunction.

Non-disjunction at the second-division can be distinguished from first-division errors by the use of centromere markers or chromosome polymorphisms, provided that the polymorphism does not recombine with its centromere. In Drosophila spontaneously occurring non-disjunction at the second-division is very rare (Merriam & Frost, 1964), but mutants in which the frequency is greatly increased have been isolated (Davis, 1971). In mammals including Man, the isolation of *XYY* sex chromosome aneuploids which can only arise at meiosis through a second-division error in spermatogenesis can be regarded as evidence for errors at this stage, but it is possible for these to arise through mitotic error in the early zygote if the resultant mosaicism goes undetected. In Man second-division errors have been detected using appropriate chromosome polymorphisms. The frequency of second-division errors may be overestimated (Langenbeck *et al.* 1976; Jacobs & Morton, 1977) because some reports used only selected data.

The one possible mechanism for aneuploid production for which no evidence exists outside the fungi is extra-replication of chromosomes. Endoreduplication resulting in the formation of diplochromosomes is well documented but this involves the whole chromosome set and generates polyploidy. The one case reported where the phenomenon generated aneuploidy appears to be in yeast, where Moustacchi, Hottinguer de Margerie & Fabre (1967) reported the existence of a mutant in which increased aneuploidy resulted from a tendency for chromosomes to undergo an extra replication event.

The fact that there are several defects of meiosis which result in aneuploidy means that any aneuploidy detection system should be selected with care. Systems, for example, which are used to monitor aneuploid induction at mitosis are likely to miss any substances which interfere with chromosome pairing and result in increased non-conjunction. A considerable advantage of the system described here is that it is based on ascus analysis. Should a treatment specifically affect one part of the cell division process and increase aneuploidy as a result, this can be detected through an increase in one particular type of aneuploid-containing ascus. Thus agents which separately affect chromosome pairing, chromosome centromere division or spindle movement can be distinguished.

Whilst methods based on lower eukaryotes have obvious advantages in terms of cost and experimental convenience, it is known that the spindles of lower eukaryotes differ from those in higher organisms in their tubulin components. Colchicine is a well-known inhibitor of chromosome movement and has been shown

to be a powerful inducer of aneuploidy in Chinese hamster oocytes (Sugawara & Mikamo, 1980). Lower eukaryotes are insensitive to this drug (Sansome & Bannon, 1946; Haber *et al.* 1972), and this is known to reside in a difference in the tubulin component of the microtubules. Colchicine binds to tubulin from higher eukaryotes at a colchicine binding site (Bryan, 1972); the corresponding site on tubulin from lower eukaryotes is insensitive to the drug (Heath, 1979). This difference highlights one considerable weakness of systems based on lower eukaryotes. How large a range of aneuploid inducers hazardous to Man can be detected using them?

Since existing mutagen testing systems are designed to detect an array of chemicals interacting with DNA it is likely that an aneuploidy testing system will be most valuable if it can identify substances, not acting on DNA, which induce aneuploidy as their sole genetic endpoint. Spindle poisons are possible candidates in this respect, but if the spindle of lower eukaryotes is too different from that of higher organisms results cannot be extrapolated with any confidence. However, the spindle is not completely different. Other drugs are known which bind to tubulin from both sources, so it may be that the majority of spindle inhibitors can be detected.

A requirement in the evaluation of lower-eukaryote-based systems is therefore to survey a range of potential aneuploid inducers. If the effects of these selected substances can be shown to be specific and to increase particular types of aneuploid-containing asci in the Sordaria system then greater confidence can be placed in testing systems using lower eukaryotes. A start has been made on this task and it is hoped to report the results shortly.

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