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Meiotic Aneuploidy: Its Origins and Induction Following Chemical Treatment in *Sordaria brevicollis*

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A system suitable for the detection of meiotic aneuploidy is described in which various different origins of the aneuploidy can be distinguished. Aneuploid meiotic products are detected as black disomic spores held in asci containing all the products of a single meiosis. Aneuploidy may result from nondisjunction or from a meiosis in which an extra replica of one of the chromosomes has been generated in some other way, e.g., extra replication. By using this system it has been shown that pFPA treatment increase aneuploidy, primarily through an effect on nondisjunction. Preliminary results with trifluralin have indicated that this compound, too, may increase aneuploidy. There is a good possibility that the system can be further developed to permit a more rapid screening using a random plating method; this will allow a more efficient two-part analysis of the effects of compounds under test.

Introduction

It has been estimated that about 15% of recognized human pregnancies abort spontaneously (*1*). Boué et al. (*2*) karyotyped 1500 spontaneous abortions, and showed that 61% had an abnormal chromosome number, and of these 67% were either monosomic or trisomic. In addition, chromosome aneuploidy can result in livebirths with serious defects, e.g., Down's syndrome. Consequently the possibility that some environmental agents might be capable of bringing about an increase in the frequency of meiotic aneuploidy is a real cause for concern.

In an effort to provide a suitable screening method for the detection of such agents several groups of workers (*3-5*) have suggested the use of fungal systems, in which aneuploidy is detected by plating random mitotic or meiotic products. These methods provide a rapid means of assessing the frequency of aneuploidy, but using them it is not possible to deduce how the aneuploid condition originates.

A system has been described (*6*) in which the products of meiosis can be screened while they are still held together in cells, each cell containing the products of a single meiosis. In such a system the various alternative origins of aneuploidy can be distinguished. This paper is concerned with the development of this system and its use in testing chemical compounds.

The Screening System

Sordaria brevicollis is a typical ascomycete fungus; when crossed, the meiotic products are held together within specialized cells (the asci). Each ascus contains the products of a single meiosis. Two spore color mutant alleles of the b_1 (or buff) locus on linkage group II form the basis of the detection system. When the b_1 alleles are crossed, the majority of the asci contain eight color mutant spores. Asci are occasionally formed containing black spores. These spores are disomic for linkage group II, and are black because the b_1 alleles complement.

In this system it can readily be seen that black spores arise through a variety of developmental aberrations. In some cases the black spores are contained within an ascus with an equal number of abortive spores. The most obvious explanation for these asci is that they arise through nondisjunction. Figure 1 illustrates an example of an ascus in which nondisjunction at the first meiotic division has occurred. In other cases, however, the black spores are not found with a balancing number of abortive spores.

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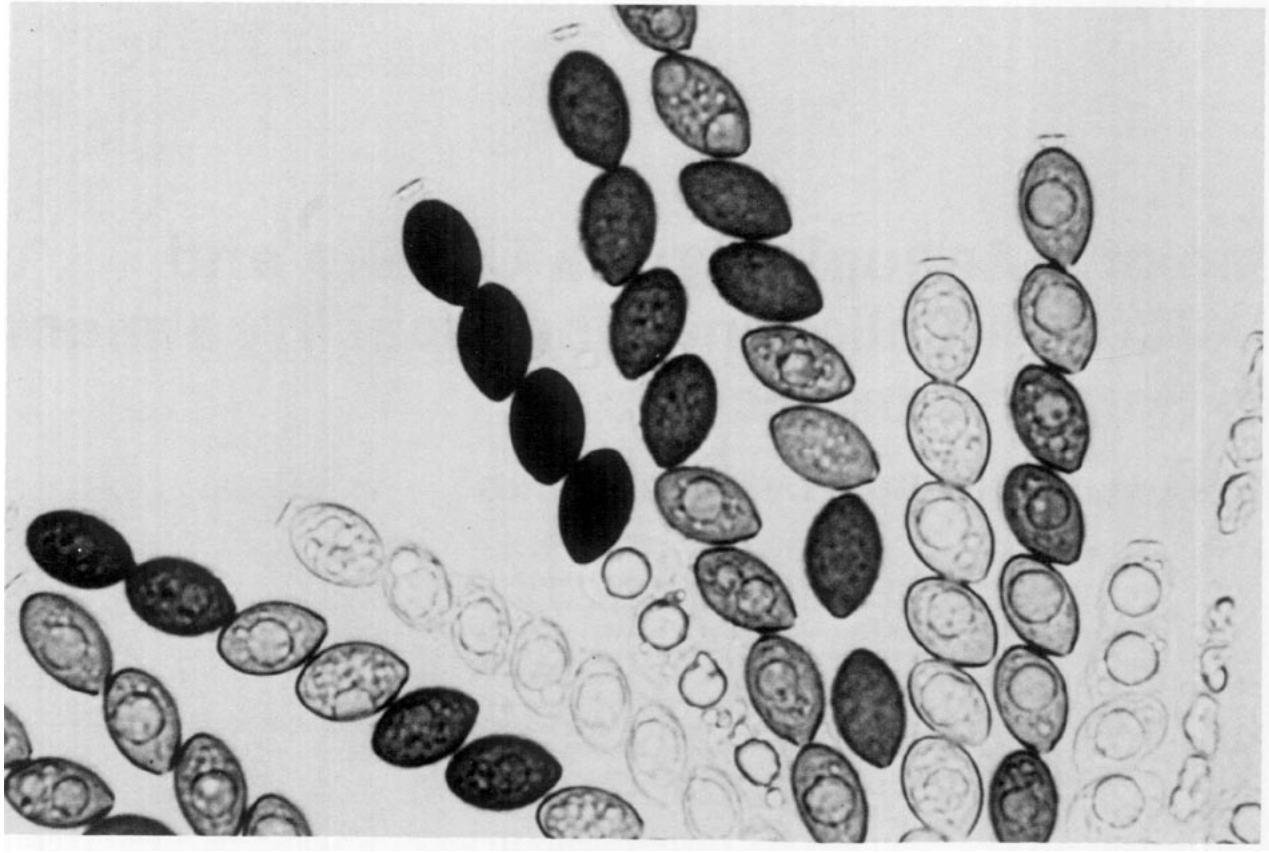

FIGURE 1. An ascus with 4 black : 4 abortive spores arising from nondisjunction at the first division of meiosis.

Meiotic nondisjunction cannot account for these asci. Figure 2 shows an ascus containing four black and four buff spores. In these asci a developmental abnormality has given rise to an extra replica of one or other of the marked homologs. A fuller consideration of the origin of these extrareplication asci can be found in the discussion. In Figure 3 the black spores have arisen through the unusual deposition of the spore wall which has been laid down around several nuclei of complementary genotype, thus forming a heterokaryotic spore. In Figure 4 the ascus containing aneuploid spores contains an odd number of disomic, black spores, and an unequal number of abortive spores. There is no simple explanation for the origin of asci such as these. Examples of this type are, however, rare. Although no case has yet been detected, a chromosome rearrangement resulting in the duplication of the genetically marked interval could mimic nondisjunction by producing black spores.

This system has a clear advantage over systems in which random meiotic products are plated, in that nondisjunction is not confused with other events leading to aneuploidy.

Materials and Methods

Strains

The two strains used in the work reported here had the genotypes: mo^- c70 not 1^+ and mo^+ S6 not 1^-. c70 and S6 are the complementing b_1 alleles, mo^- is a morphological mutant 1 map unit proximal to b_1, and not 1^- is a nicotinamide or tryptophan-requiring auxotroph (formerly designated nic^-) 1.5 map units distal to b_1.

Media

Crosses were routinely carried out on corn meal agar of composition: corn meal agar (Difco), 17 g; sucrose, 3 g; glucose, 2 g; yeast extract, 1 g; distilled water up to 1 liter. When asci were dissected to confirm the disomic nature of the black spores, the spores were germinated in Vogel's N medium (7) of composition: Vogel's salt solution, 20 ml; glucose, 20 g; Difco-Bacto agar, 15 g; sodium acetate, 7 g; distilled water up to 1 liter.

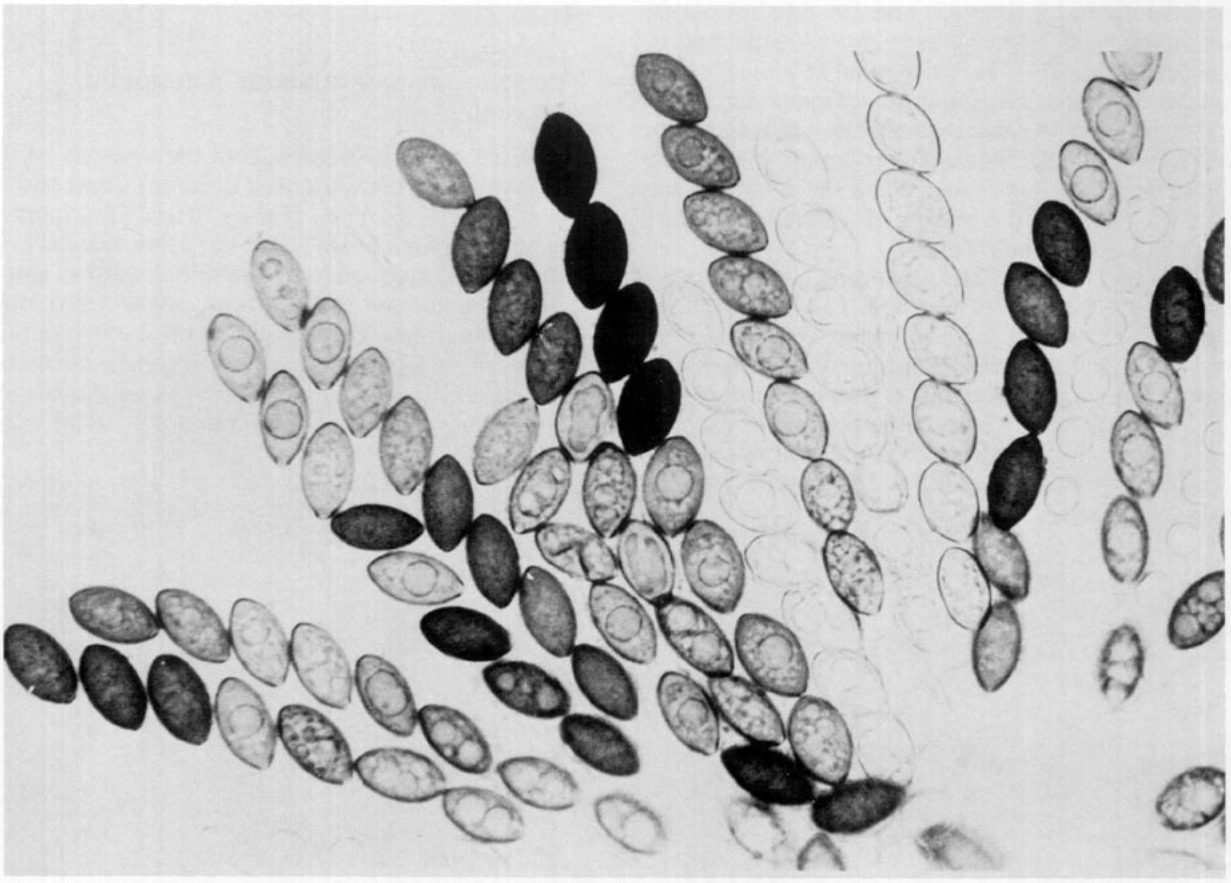

FIGURE 2. An ascus with 4 black : 4 buff spores. In asci of this type an extra replica of one of the homologs has arisen during development.

Crossing Method and Method of Treatment

The strains were inoculated onto separate plates of corn meal agar, and incubated for 4 days at 25°C. The cultures were then fertilized by harvesting microconidia from one of the plates in a small volume of sterile distilled water (approximately 10 ml). The microconidia were dislodged from the mycelium by scraping the surface of the culture with a sterilized glass rod. The resulting suspension was used to fertilize two or three plates of the female parent. Either strain can be used as the female, but the mo^+ S6 not 1^- culture was usually selected because this culture gave more fertile crosses. The female culture was then incubated for a further 5 or 6 days, after which time ascospore discharge indicated that the cross was mature.

Treatment of the crosses was carried out by using the method described by Griffiths and Delange (*5*), where the fertilized culture was flooded with a small volume of a solution of the test compound, either immediately after fertilization or after a delay of some hours.

Harvesting and Scoring Methods

When mature, the crosses were harvested by scraping the perithecia (mature fruiting bodies) off the surface of the mycelium. They were then crushed open, on a slide in 10% sucrose solution, using a coverslip. At this stage the harvested asci were often left, in which case the groups of asci released from the perithecia became trapped in crystals of sugar as the water evaporated. Whenever necessary, the sugar crystals were redissolved, thus releasing the asci.

To score a cross the groups of asci were transferred, using tungsten needles, to the surface of a 4% agar slab placed on a slide. The slab was flooded with sucrose solution, while this transfer was being carried out, to facilitate the removal of the asci from the needles, and to prevent the 4% agar slab drying out unevenly. When the solution had almost evaporated, a coverslip was lowered over the preparation and pressed down gently to flatten the asci. The preparation was then screened at 100X magnification by use of a Nikon L-Ke microscope. The number of asci in

each clump was estimated, and the number of asci containing black spores was recorded. This method has been shown (*6*) to be a reliable and accurate method of determining aneuploid frequency.

The agar slab was necessary only as an aid for the subsequent removal of the coverslip prior to dissection of an interesting ascus. When the coverslip was lifted off the agar, the groups of asci almost invariably stuck to the agar slab.

With this method of screening asci, a rapid analysis of crosses is possible. The most time-consuming activity is preparing the slides for screening. A slide of 10,000 asci may take 15-20 min to prepare, while screening it takes a further 5-10 min. Consequently, a very large number of asci can be examined in a working day, and a frequency of nondisjunction as low as 0.5×10^{-4} can be detected relatively easily.

Results

Variation in Spontaneous Aneuploid Frequency

Table 1 contains results from the analysis of untreated crosses carried out at different times and by use of different isolates of the c70 and S6 strains. It can be seen that considerable variation existed both in the overall frequency of aneuploid spores, and in the proportion of these which arose from nondisjunction. Replicate crosses set up at any one time did not show this variation, but formed a set within which both the aneuploid frequency and the proportion of nondisjunctive asci were statistically homogenous.

The underlying basis of this variation is currently being investigated, since for a system to have

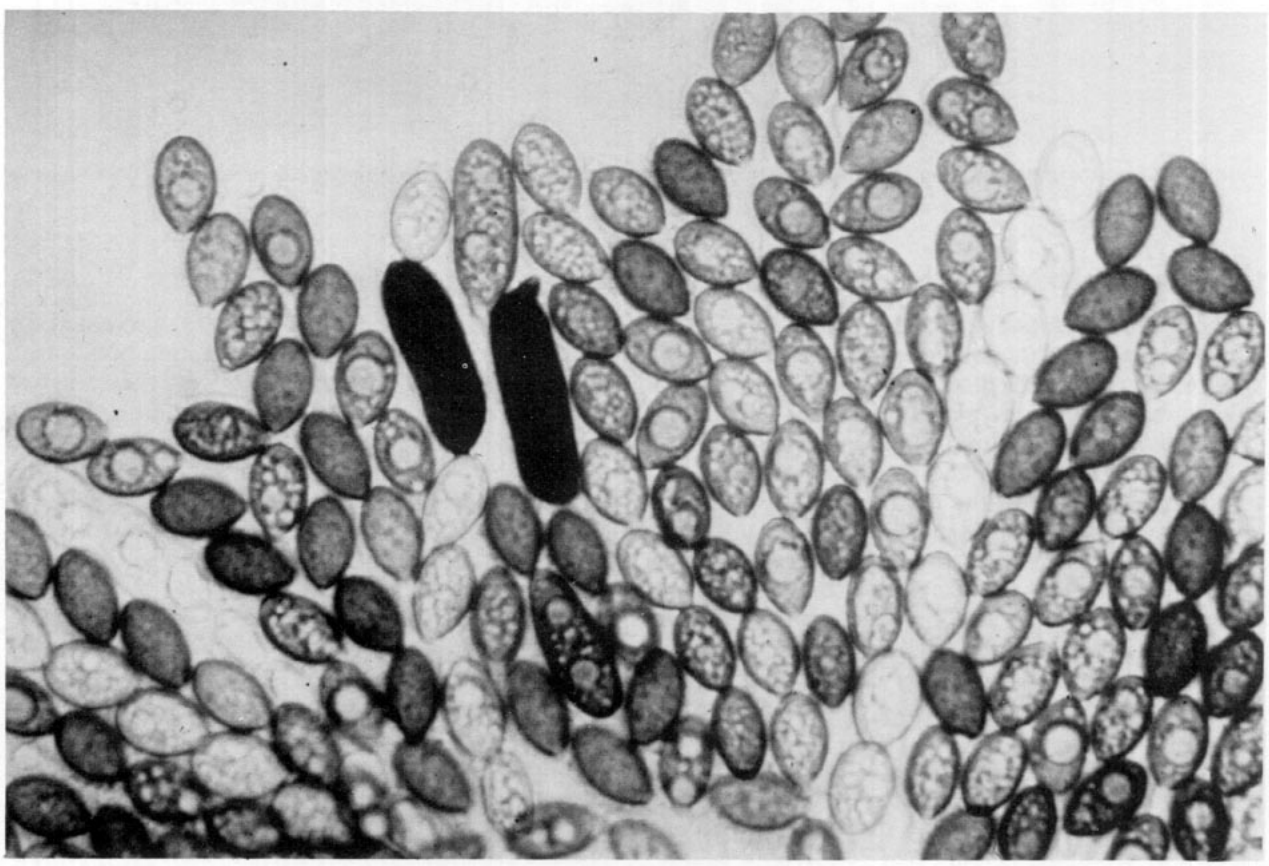

FIGURE 3. Two asci, each containing a large heterokaryotic spore in which the spore wall has been deposited around nuclei of complementary genotype. Large homokaryotic spores, containing identical genotypes, are also present.

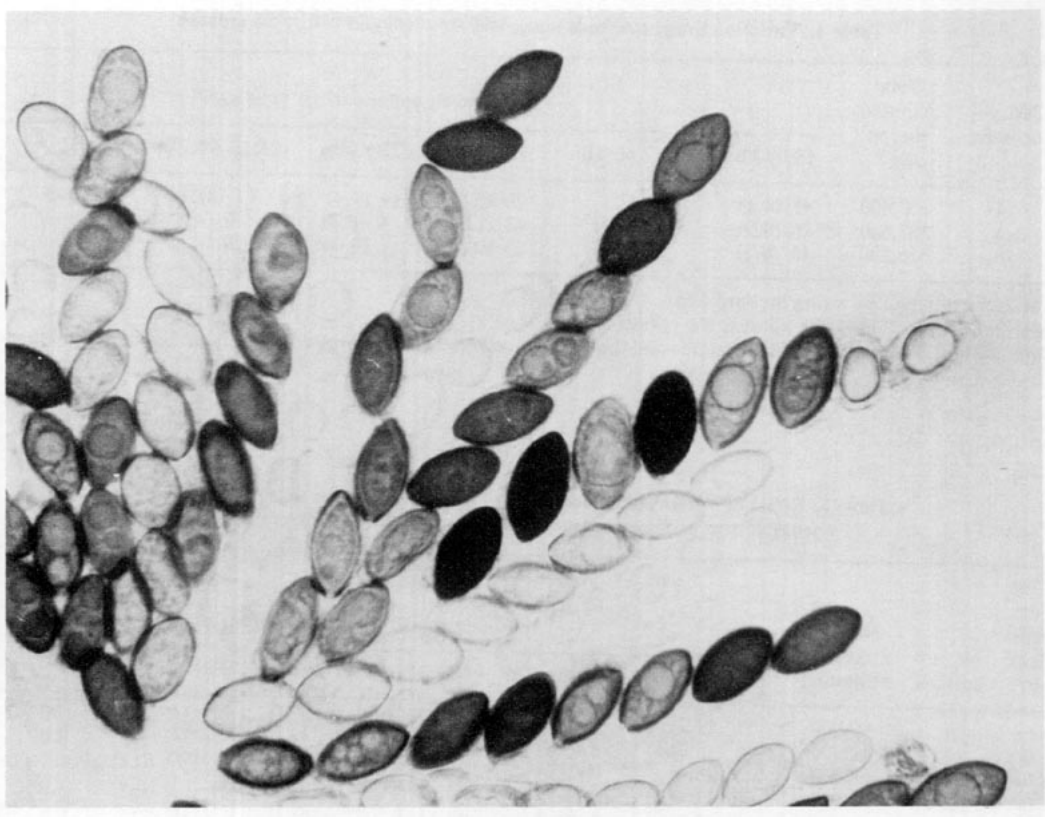

FIGURE 4. An ascus of more complex origin, in which the black, disomic, spores are not balanced by an equal number of abortive spores.

maximum sensitivity the factors influencing the spontaneous rate must be understood.

Induction of Nondisjunction with *p*-Fluorophenylalanine (pFPA)

Table 2 contains the results of experiments in which developing crosses were treated with pFPA solutions. The results show clearly that pFPA is a very effective agent in inducing meiotic aneuploidy. Within the range of concentrations tested there was a clear dose response: the higher the pFPA concentration, the greater the increase in aneuploidy.

It can be seen that the increase was largely accounted for by an increase in nondisjunction frequency. Both first division nondisjunction (resulting in 4+:4 abortive asci) and second division nondisjunction (giving 2+:2 abortive:4buff asci) were increased by the treatment.

If division I and division II nondisjunction occur with equal frequency, then, because the b_1 alleles are near the centromere, nondisjunction at the first division will be detected six times more often than nondisjunction at the second division (*6*). This is so because the latter event will result in a black, disomic spore pair only when the complementing alleles segregate at the second division. There was some evidence from the data that the ratio of division I to division II nondisjunction was altered after pFPA treatment, with a disproportionate increase in the secondary types. If real, this effect could have come about either through an effect of pFPA treatment on nondisjunction at the second division or through an effect on second division segregation frequency of the b_1 gene, or both. Unpublished work by MacDonald, however, revealed no effect of pFPA on the second division segregation frequency of several spore color genes.

pFPA treatment had no consistent effect on other sources of aneuploidy. Extrareplication types (4+:4 buff and 2+:6 buff) did not show a dose-dependent increase, although the frequency of 4+:4 buff asci was increased in some crosses. There was no repeatability between experiments. The increase detected in some cases probably reflects some general disturbance of fungal metabolism.

Table 1. Variation in spontaneous aneuploid frequency in c70 × S6 crosses.

Expt. no.	No. of crosses scored	Total parental ditype asci[a]	No. (and frequency × 10^{-4}) of asci[b]						
			4B1:4Ab	2B1:2Ab:4Bu	4B1:4Bu	2B1:6Bu	2B1:4Ab:2Bu	8B1	Others[c]
1	4	100,400	41 (4.1)	7 (0.7)	20 (2.0)	14 (1.4)	1 (0.1)	1 (0.1)	8 (0.8)
2	4	287,600	26 (0.9)	2 (0.07)	43 (1.5)	8 (0.3)	0 (-)	0 (-)	4 (0.14)
3	18	316,200	15 (0.5)	1 (0.03)	15 (0.5)	2 (0.06)	0 (-)	0 (-)	4 (0.1)

[a]Number of asci estimated by using method 1 (*6*).
[b]B1 = black spores; Ab = abortive spores; Bu = buff spores.
[c]Includes asci with an odd number of black spores and those with abnormal giant spores which contain both parental nuclear types.

Table 2. Effect of *p*-fluorophenylalanine (pFPA) treatment on the frequency of aneuploid spores from crosses of two complementing buff mutants.

pFPA treatment, mg/l.	Time of treatment, hr	No. of crosses	No. of asci examined	Number (and frequency × 10^{-4}) of asci[a]						
				4B1:4Ab	2B1:2Ab:4Bu	4B1:4Bu	2B1:6Bu	2B1:4Ab:2Bu	8B1	Others
0	-	4	89,900	11 (1.2)	2 (0.2)	4 (0.4)	1 (0.1)	0 -	0 -	1 (0.1)
1	0	4	112,700	36 (3.2)	4 (0.4)	2 (0.2)	0 -	0 -	0 -	0 -
4	0	4	68,700	25 (3.6)	4 (0.6)	15 (2.2)	4 (0.6)	3 (0.4)	1 (0.1)	2 (0.3)
10	0	3	44,900	50 (11.4)	14 (3.1)	5 (1.1)	2 (0.4)	4 (0.9)	0 -	2 (0.4)
1	5	4	67,800	15 (2.2)	3 (0.4)	12 (1.8)	1 (0.1)	0 -	1 (0.1)	3 (0.4)
4	5	4	119,200	36 (3.0)	11 (0.9)	23 (1.9)	2 (0.2)	4 (0.3)	0 -	2 (0.2)
10	5	4	68,200	46 (6.7)	13 (1.9)	9 (1.3)	1 (0.1)	2 (0.3)	0 -	2 (0.3)
0	-	14	365,000	39 (1.1)	12 (0.3)	14 (0.4)	8 (0.2)	3 (0.1)	2 (0.05)	6 (0.2)
4	0	8	388,100	216 (5.6)	79 (2.0)	16 (0.4)	3 (0.1)	6 (0.2)	0 -	4 (0.1)
10	0	6	204,300	209 (10.2)	78 (3.8)	0 -	3 (0.1)	14 (0.7)	0 -	12 (0.6)

[a]As in Table 1.

Aneuploid Frequency after Treatment with *Trifluralin* (*α*, *α*, *α*-*Trifluoro-2,6-dinitro-N, N*-dipropyl-*p*-toluidine)

Table 3 contains the results of preliminary experiments with trifluralin. In all the experiments performed to date, trifluralin consistently increased the frequency of aneuploid spores, particularly those arising in the extrareplication asci (4+:4 buff). There was, however, no dose-related increase over the range of trifluralin concentrations tested. In addition, the control frequency of aneuploids in one of the experiments was abnormally low, and consequently judgement must be reserved on whether or not trifluralin acts as an inducer of aneuploidy in *Sordaria*.

Other Treatments

Ramel and Magnusson (*8*) reported that organic mercury compounds, for example phenylmercuric acetate, increased the frequency of XXY exceptionals in Drosophila without there being any detectable increase in XO types. Among the explanations which they considered for this result was the possibility that the treatment had resulted in an extra replication of the chromosomes, or that an equational division of the centromere had occurred at the first meiotic division. Phenylmercuric acetate has been tested in Sordaria to see whether it specifically increased extrareplication asci, but the results have proved negative. Phenylmercuric acetate is fungicidal, and it may well be that the Sordaria system is therefore unsuitable for testing compounds of this type.

Discussion

Development of a Random Spore Plating Method

The results presented in the previous section show that detection of aneuploid inducing agents using

Sordaria is a practicable proposition. The method in which groups of asci are screened provides a fairly rapid, if somewhat laborious, means of estimating aneuploid frequency. The system has the outstanding merit that, because intact asci are examined, the origin of the aneuploid condition can be determined. In comparison with random spore plating methods however the screening process is time consuming, and the optimum system would probably be one combining the merits of both methods.

We have been attempting to achieve this end by marking linkage group II with mutations conferring growth requirements. Results to date are encouraging, and we are in the process of constructing c70 and S6 strains of the following genotypes:

	nic⁻	+met¹	S6	not⁻	+ilv
			o		
and					
	+nic	met 1⁻	c70	+not	ilv⁻
			o		

where nic⁻, met⁻, not⁻, and ilv⁻ are recessive mutations conferring nicotinamide, methionine, nicotinamide or tryptophan, and isoleucine/valine requirements respectively. From a preliminary investigation of the genetic distances separating the markers the triple crossover necessary to generate a prototrophic spore by recombination is expected to occur with a frequency not greater than 1×10^{-4}, assuming no interference between crossovers. It is possible, therefore, that these strains will be suitable for the detection of aneuploidy by random spore plating. Many independent mutations at the met 1 locus have also been isolated, and some of these might well be complementing alleles. Another possibility would then be to incorporate these alleles into the strains used for the detection of aneuploidy.

Methods of plating random ascospores have already been worked out, and we are reasonably confident that, in the near future, we will be able to subject crosses to a two part analysis. Random spores will be plated to determine the aneuploid frequency, and a sample of asci also screened, from the same cross, to determine the proportions arising from the various developmental errors.

Origin of Aneuploid Ascospores

In those asci in which two or four disomic spores are formed together with an equal number of abortive spores, the simplest explanation of their origin, i.e., nondisjunction, is in all probability the correct one. In other cases where the disomic spores are formed without the coincident formation of any abortives, there is no compelling single explanation. These asci might well arise in a variety of ways. They may arise, for example, through an extrareplication of one or other of the marked homologs or from mitotic nondisjunction immediately before meiosis. In both these cases, when the disomic nucleus originates early in perithecial development, mitotic division of the $n + 1$ nucleus should give rise to clusters of asci, each ascus containing disomic spores. No examples of clusters of this type have ever been detected. If the $n + 1$ nuclei arise in this way, therefore, the absence of clusters suggests that the disomic nucleus is unstable, and the extra chromosome has a high probability of being lost. On this hypothesis, only those disomic nuclei generated immediately before the nuclear fusion to form the diploid ascus initial result in disomic ascospores.

However, this postulated instability of the $n + 1$ nucleus during mitotic divisions is not apparent dur-

Table 3. Effect of trifluralin treatment on the frequency of aneuploid spores from crosses of two complementing buff mutants.

Trifluralin treatment, mg/l.	Time of treatment, hr	No. of crosses	No. of asci examined	Number (and frequency × 10^{-4}) of asci with:[a]						
				4B1:4Ab	2B1:2Ab:4Bu	4B1:4Bu	2B1:6Bu	2B1:4Ab:2Bu	8B1	Others
0	-	18	144,900	18 (1.2)	3 (0.2)	4 (0.3)	2 (0.1)	1 (0.1)	0 -	3 (0.2)
10	0	8	87,900	29 (3.3)	5 (0.6)	11 (1.3)	1 (0.1)	0 -	1 (0.1)	3 (0.3)
20	0	6	46,400	11 (2.4)	2 (0.4)	7 (1.5)	0 -	0 -	0 -	1 (0.2)
30	0	7	58,600	19 (3.2)	2 (0.3)	7 (1.2)	0 -	0 -	0 -	1 (0.2)
50	0	6	54,800	8 (1.5)	0 -	10 (1.8)	0 -	0 -	0 -	1 (0.2)
0	-	11	135,200	0 -	0 -	0 -	1 (0.07)	0 -	0 -	0 -
10	0	4	75,500	13 (1.7)	2 (0.3)	7 (0.9)	3 (0.4)	1 0.1	0 -	2 (0.3)
25	0	3	59,800	15 (2.5)	3 (0.5)	7 (1.1)	1 (0.2)	0 -	0 -	2 (0.2)
50	0	4	43,700	11 (2.5)	5 (1.1)	1 (0.2)	0 -	0 -	0 -	0 -

[a]As in Table 1.

ing ascus development. In cases of asci containing disomic spores, examples where there is an odd number of black spores are comparatively rare. There is apparently a small probability of mitotic loss during ascus development. This contradiction may be more apparent than real. It could be that mitotic chromosome loss from aneuploid nuclei is high in both cases, but in the developing ascospore wall formation includes the "lost" chromosome in a high proportion of cases. In other words, the spore wall is laid down around a comparatively large volume of cytoplasm which in most cases happens to include the extra chromosome copy. In any event, given the complexity of the processes of chromosome replication and division it is unlikely that these asci comprise a homogenous group with a common origin.

In conclusion, the results presented in this paper and the projected development to permit screening by both ascus and random spore analysis make this system an extremely promising one for the detection of agents inducing aneuploidy.

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