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Chromosome rearrangements resulting in increased aneuploid production in *Sordaria brevicollis*

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SUMMARY

Two spontaneously arising chromosome rearrangements were isolated in *Sordaria brevicollis* from one perithecium. These were detected through increased production of black spores in intercrosses of complementing buff spore colour mutants. One was a reciprocal translocation between linkage groups I and II; the other a reciprocal translocation between linkage groups II and VI. In the former case the translocation resulted in frequent non-disjunction generating black spores which were either tertiary or interchange disomics. The frequency of premature centromere division was also increased. In the case of the translocation involving linkage groups II and VI the black spores were formed as a result of adjacent-1 segregation and were probably duplication/deficiency products.

In both rearrangements the breakpoint in linkage group II was, as far as could be judged, in an identical place. This and the fact that they were isolated from a single fruiting body, suggests that the chromosome breakage event arose as a potential lesion, which replicated before the potential break was either restituted, to restore a normal chromosome, or opened, to form the rearrangements.

1. INTRODUCTION

Over the last few years we have been developing a method for studying meiotically derived aneuploidy using spore colour mutants of the ascomycete fungus *Sordaria brevicollis*. The detection of meiotic aneuploidy relies on the complementation of two closely linked buff spore colour mutations on linkage group II. When, for whatever reason, homologous chromosomes carrying the mutants fail to segregate, so that both end up in one disomic ascospore, complementation restores full pigmentation. The rare disomic products are therefore detected as black ascospores within a population of buff ones. In practice the disomic ascospores are detected whilst they are still retained within the ascus, each ascus containing the products of a single meiosis. Ascus analysis confers the outstanding advantage that different meiotic defects leading to aneuploidy can be

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identified (Bond, 1976, 1983; Bond & McMillan, 1979*a*; Fulton & Bond, 1983). Thus aneuploidy can be distinguished which arises from non-conjunction and non-disjunction at the first division, premature centromere division; non-disjunction at the second division and pre-meiotic errors, such as extra replication of chromosomes or mitotic non-disjunction.

The system has been used to detect the induction of aneuploidy following chemical treatment (Bond & McMillan, 1979*a*, *b*) and is a useful tool for this purpose. Its advantage again derives from the fact that asci are examined which allows the mode of action of any aneuploid inducer to be inferred.

In addition to chemical induction, it is clear that genetic factors can also increase the frequency of aneuploidy. These factors are likely to be of considerable importance in the study of aneuploidy since they can result in large increases in the levels of aneuploidy. This realization has prompted several authors to express concern about the role of such factors in human aneuploidy (Alfi, Chang & Azen, 1980; Hecht, 1982) and has led several groups to initiate a systematic study of meiotic mutants, many of which exhibit increased aneuploidy (for example, Delange & Griffiths, 1980; Herman, Kari & Hartman, 1982). The *Sordaria* system, too, can be used to isolate strains with a genetic predisposition to aneuploidy. The method which we use routinely to examine the asci involves screening rosettes from individual perithecia; each rosette containing up to 300 asci. The spontaneous frequency of aneuploidy is low, so the majority of rosettes contain none or one aneuploid-containing ascus. On very rare occasions rosettes have been detected which contain more than two asci with black spores. This paper reports the analysis of one such rosette which contained several asci each with four black and four abortive spores.

2. MATERIALS AND METHODS

(i) *Media*

Strains were routinely cultured in Vogel's (1956) N medium containing 2% glucose and any necessary growth supplements. 0·7% (w/v) sodium acetate was added to stimulate germination of ascospores. Crosses were carried out on cornmeal agar of composition: 1·7% Difco cornmeal agar, 0·3% sucrose, 0·2% glucose, 0·1% yeast extract.

(ii) *Mutants*

The complementing alleles which form the basis of the system are identified by the symbols *C70* and *S6*. These mutants are alleles of the *buff* (or b_1) locus on linkage group II and are phenotypically distinguishable, the *C70* allele conferring a slightly darker phenotype than *S6*. Flanking marker mutations at the not_1 and met_1 loci (conferring a growth requirement for nicotinamide or tryptophan and methionine respectively) were also present in the *C70* × *S6* crosses.

In addition to the *C70* and *S6* alleles, spore colour mutants at the following loci were used for mapping purposes: y_1 and y_{10} (linkage group I), y_9 (linkage group II), g_2 (linkage group III), g_6 (linkage group IV), y_4 (linkage group V), h_3 (linkage group VI) and g_7 (linkage group VII).

(iii) *Methods*

The crossing and scoring methods have been fully described previously (Bond, 1976; Bond & McMillan, 1979*a*). It is necessary here only to recall the method of classifying asci containing aneuploid spores (Fulton & Bond, 1983). In *C70* × *S6* crosses there are four main types:

Type I asci contain four black and four abortive spores and originate either from non-conjunction or non-disjunction at the first division.

Type II asci contain 2 black:2 abortive:4 buff spores in a sequence (either 2 buff:4 aneuploid:2 buff; or 2 aneuploid:4 buff:2 aneuploid) which suggests that the aneuploidy arose through precocious centromere division;

Type III asci also contain 2 black:2 abortive:4 buff spores but in this case the spore sequence (2 buff:2 aneuploid:2 buff:2 aneuploid, or 4 aneuploid:4 buff) does not allow an unambiguous origin to be deduced. This type can arise either from precocious centromere division or from non-disjunction at the second division.

Type IV asci contain black spores but no abortive ones. The production of disomic spores without concomitant production of nullisomics can come about either through mitotic non-disjunction or extra-replication of the marked chromosome immediately prior to meiosis.

(iv) *Terminology used for chromosome rearrangements*

In the account that follows the terminology developed by Perkins and his co-workers to describe rearrangements in Neurospora has been used (Perkins & Barry, 1977). Genetic linkage groups are designated by Roman numerals. Following an abbreviation to indicate the type of rearrangement (T for translocations, I for inversions, etc.) the linkage groups involved are indicated in parentheses. An identification number then follows to complete the symbolic description. For reciprocal translocation the linkage groups are separated by a semi-colon; thus T(I; II) symbolizes a reciprocal translocation between the first and second linkage groups. For insertional translocations or quasi-terminal translocations, the linkage groups are separated by an arrow which indicates which is the donor and which the recipient chromosome. Thus T(II → VI) symbolizes a translocation in which genetic material has been transferred to linkage group VI without there being any detectable reciprocal transfer.

3. RESULTS AND DISCUSSION

(i) *Preliminary characterization*

In a control cross, which had not been chemically treated in any way, a rosette of asci was detected which contained several asci each with aneuploid spores. Normal asci containing eight buff ascospores were dissected from this rosette. Unusually, germination of both spore colour mutant alleles was very poor and only four spores, one from each of four separate asci, germinated. Judging initially by the darker buff colour of the spores, all four spores had inherited the *C70* allele, and each was therefore crossed to *S6*. The aneuploid frequency in one of these

crosses was normal (about 8×10^{-4}) but the three others (crosses G101, G102 and G192) exhibited a greatly increased frequency of aneuploidy. The results of these crosses are presented in Table 1, where it can be seen that there were two distinct types. In crosses G102 and G192 about 8% of the asci contained aneuploid spores mainly of type I, whereas G101 was clearly different; about 40% of asci from this cross contained black spores with types II and III being common.

Table 1. *Number (and frequency) of asci in three crosses showing a high frequency of asci containing aneuploid spores*

Spore sequence within ascus*	Ascus type	Cross number		
		G101	G102†	G192†
8 buff spores	Parental Ditype	426 (46·6%)	651 (49·2%)	511 (49·0%)
4bl:4ab and 2bl:2ab:2bl:2ab	I	247 (27·0%)	95 (7·2%)	73 (7·0%)
2bl:4bu:2ab and 2bu:2bl:2ab:2bu	II	67 (7·3%)	1 (0·1%)	1 (0·1%)
2bl:2ab:4bu and 2ab:2bl:4bu and 2bl:2bu:2ab:2bu and 2ab:2bu:2bl:2bu	III	99 (10·8%)	2 (0·2%)	3 (0·3%)
4bl:4bu and 2bl:2bu:2bl:2bu	IV	3 (0·3%)	0 (—)	0 (—)
8 abortive spores		60 (6·6%)	573 (43·3%)	446 (42·8%)
Others		13 (1·3%)	2 (0·2%]	9 (0·9%)

* bl = black; ab = abortive; bu = buff.

† In these crosses a small number of asci with 4 buff:4 abortive spores were not counted. In later crosses such asci occurred with a frequency of about 5%.

The differences were consistent and were inherited. Two lines were established from these crosses, *C70* spores isolated from G102 and G192 all produced aneuploids, when crossed to *S6*, with a characteristic 7–10% frequency whereas *C70* spores from G101 gave 40% aneuploid-containing asci.

(ii) *The G102 and G192 lines carry a reciprocal translocation between linkage groups I and II*

The high frequency of asci with eight abortive spores was the first indication that the G102 line carried a chromosome rearrangement. Random spore isolates indicated that the genetic factor inducing aneuploidy was very closely linked to the *C70* allele on linkage group II and also to mating type on linkage group I. The simplest hypothesis to explain this is that the G102 and G192 lines both carry a

reciprocal translocation between linkage groups I and II. This hypothesis was further supported by the results of crosses to various spore colour mutants on linkage group I (Table 2). It can be seen that the spore colour mutants and *C70* are linked when the *C70* is derived from the G102 line, but unlinked when the *C70* is derived from any other source.

Table 2. *Ascus analysis of crosses between* C70 *and spore colour mutants on linkage group* 1*:* B5 *and* B6 *are allelles at the* y_1 *locus;* C33 *is a mutant at the* y_{10} *locus*

(*a*) *C70* isolated derived from G101 or G192

No. of abortive spores	Ascus type*	Cross *C70 T(I:II) G192 × B6*	*C70 T(I:II) G102 × B5*	*C70 T(I:II) G192 × C33*
	Parental Ditype	529 (45·8 %)	147 (52·3 %)	233 (41·0 %)
0	Tetratype	7 (0·6 %)	1 (0·4 %)	29 (5·1 %)
	Non-parental Ditype	0	0	0
	4bl:4ab	16 (1·4 %)	5 (1·8 %)	23 (4·9 %)
4	4m:4ab	29 (2·5 %)	11 (3·9 %)	35 (6·2 %)
	2m:2bl:4ab	6 (0·5 %)	0	5 (0·9 %)
8	8 abortive	568 (49·2 %)	117 (41·6 %)	243 (42·8 %)
	Total asci	1155	281	568

(*b*) Control crosses (*C70* isolates derived from stock crosses)

	C70 × B6	*C70 × B5*	*C70 × C33*
Parental Ditype	125 (43·1 %)	81 (47·4 %)	135 (42·1 %)
Tetratype	36 (12·4 %)	11 (6·4 %)	75 (23·4 %)
Non-parental Ditype	129 (44·5 %)	79 (46·2 %)	111 (34·6 %)

* bl = black; ab = abortive; m = spore colour mutant (*C70* and other markers not distinguished).

Mapping experiments were carried out to locate the breakpoint on linkage group II by crossing the reciprocal translocation (marked with *C70*) to *S187* (an allele at *yellow-9* locus) which maps 11 map units distal to *buff*. Recombination in the *buff-yellow* interval was normal and dissection of tetratypes located the breakpoint proximal to the buff locus.

When the *C70 T(I:II) G102* isolate was crossed to wild type the frequency of asci with a 2:4:2 sequence of buff and black spores, which arise from crossing over in the *buff*-centromere interval, was reduced from the normal 4 % to 1 %. Dissection of the few 2:4:2 asci which were formed confirmed that the breakpoint was proximal to *buff* and allowed the translocation to be recombined away from the *C70* allele. The reciprocal translocation could therefore be combined with the other spore colour mutants and crosses which were homozygous for the reciprocal translocation could be set up.

The results of these crosses can be seen in Table 3. As expected, the spore abortion, characteristic of crosses which are heterozygous for a rearrangement, disappeared in the homozygous crosses. The results clearly show that the *C70* and

C33 mutants are closely linked in these crosses, which confirms that either the *buff* locus has been translocated to the centromere segment of linkage group I or the y_{10} locus has been translocated so that it is physically linked to the centromere of linkage group II.

Although the evidence is not conclusive we favour the hypothesis that the *buff* locus has been translocated to linkage group I for the following reasons. Firstly, the reduced recombination frequency in the *buff*-centromere interval in crosses heterozygous for the translocation is consistent with the breakpoint being in this region. Secondly, when crosses homozygous for the translocation but segregating at the *buff* locus were set up, the *buff* locus had a different centromere distance (1·2 units) from its normal value of 4 map units.

Table 3. *Results of crosses between* C70 *and* C33, *alleles of the* b_1 *and* y_{10} *loci respectively, which were homozygous for the G102-derived chromosome rearrangement*

	Cross	
Ascus type	*C70 C33 T(I:II) G102* × $+^{C70}+^{C33}$*T(I:II) G102*	*C70* $+^{C33}$*T(I:II) G102* × $+^{C70}$*C33 T(I:II) G102*
Parental ditype	1065 (89·8%)	649 (87·6%)
Tetratype	120 (10·1%)	89 (12·0%)
Non-parental ditype	1 (0·1%)	3 (0·4%)

The relationship of the various loci used to analyse the reciprocal translocation and the position of the breakpoints are illustrated in Fig. 1. The breakpoints in both chromosomes are near their respective centromeres. In *C70 T(I:II)* × *S6* crosses, crossing over in either centromere-breakpoint interval will be expected to generate asci with 4 buff:4 abortive spores in a 2:4:2 sequence. Asci of this type and with this sequence are very rare although 4 buff:4 abortive asci in other sequences do arise from non-disjunction (see below). Further evidence for the centromere linkage of the breakpoints can be obtained from the data presented in Table 2. The frequency of tetratype asci in the two point crosses was low and this confirms that the breakpoints are near the spore colour loci, which are themselves closely linked to their centromeres.

(iii) *The black spores in* S6 × C70 T(I:II) *crosses are tertiary disomics or interchange disomics*

In normal *S6* × *C70* crosses the nuclei in black disomic spores are unstable and, following germination, rapidly lose one or other copy of the second linkage group and become haploid. Since the loss of the extra chromosome is random a culture established from a disomic spore becomes heterokaryotic, about half the nuclei being marked with the *S6* allele and the other half being marked with the *C70* allele.

The black spores from the G102 and G192 crosses did not behave like this; cultures established from germinated black spores were homokaryotic, all nuclei being marked with one or other *buff* allele. Black spores were of two sorts; following

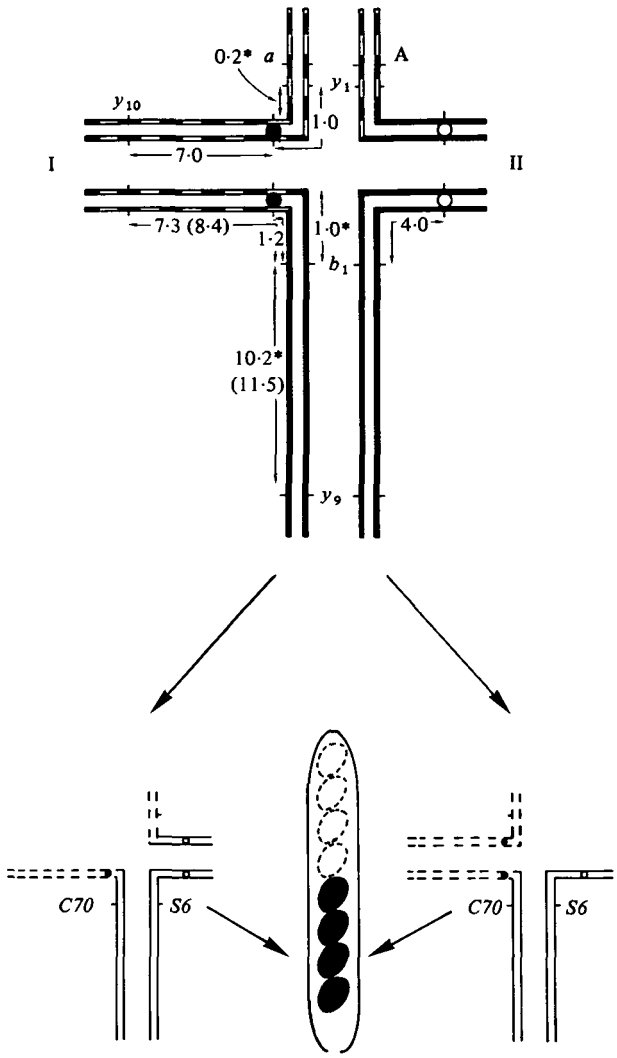

Fig. 1. Upper figure: linkage map of the reciprocal translocation involving linkage groups I and II. Map distances marked with an asterisk are derived from crosses which were heterozygous for the rearrangement; those in brackets are estimates from crosses in which some double crossovers could be detected and other undetected doubles could be allowed for by assuming no chromatid interference. Lower figure: proposed origin of type I asci in *S6* × *C70 T(I:II)* crosses. Black spores are either tertiary disomics (right-hand figure) or interchange disomics (left-hand figure).

germination a black spore either gave rise to a *C70 A* culture which also carried the reciprocal translocation or gave rise to a *S6 a* culture which did not have the rearrangement.

This result is exactly that expected if the black spores were tertiary disomics or interchange disomics. These will be formed following the non-disjunction of one pair of centromeres and the normal disjunction of the other. As can be seen by reference to Fig. 1, this 3:1 segregation will result in two different types of disomic spore depending on which pair of centromeres fail to disjoin. Non-disjunction of

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centromere I will result in black disomic spores if the non-disjoining pair assorts together with the non-rearranged second chromosome. The resultant tertiary disomic spore will contain a structurally normal first and second chromosome together with a rearranged chromosome carrying the *C70* allele. After germination the only viable haploid nuclei will be those which have lost the rearranged chromosome. Non-disjunction of centromere II will be expected to give a different result. Black disomic spores, in this case, will be interchange disomics and will contain both reciprocal translocation products and a structurally normal second chromosome. Following germination viable haploid nuclei will have lost the normal second chromosome. The relative frequency of these two types can therefore be used as a measure of the relative non-disjunction frequency of the two centromere pairs.

Dissection of 31 type I asci gave the result that 8 gave rise to *S6 a* cultures, i.e. arose from non-disjunction of II, whilst 23 gave *C70 A T(I:II)* cultures which therefore arose from non-disjunction of centromere I.

The hypothesis that the black spores in *C70* × *S6* crosses established from either G102 and G192 were the result of non-disjunction gained support from experiments in which crosses were treated with *p*-fluorophenylalanine (pFPA), which is potent inducer of non-disjuction in fungi (Griffiths & Delange, 1977). Following treatment of *S6 a* × *C70 A T(I:II)* crosses aneuploidy frequency was consistently increased. For example, in one cross, using the G192-derived rearrangement, aneuploidy was increased from 7·0 % to 22·2 % following exposure of the crosses to 2·5 ml of a 20 mg/l solution of pFPA. Non-disjunction will also be expected to give rise to asci with 4 buff:4 abortive spores if the non-disjunction event does not result in the inclusion of both complementing alleles within a spore. Typically such asci occur with a frequency of about 5 %.

(iv) *The G101 line carries a translocation between linkage groups II and VI*

Following the discovery that derivatives from G102 and G192 carried a reciprocal translocation, the G101 cross was examined carefully to see if there was any evidence that it, too, carried a chromosome rearrangement. Cross G101 showed close linkage of the aneuploid inducing factor to the *C70* allele but, unlike *C70* spores from G102 and G192, there was no linkage between the *buff* and mating type loci.

Crosses of *C70* isolates derived from G101 to spore colour mutants on each of the six remaining linkage groups showed that there was linkage between the *buff* locus and the *hyaline-3* locus on linkage group VI. The results are presented in Table 4, the clear linkage of the *buff* and *hyaline* loci is indicated by the large excess of parental ditype asci. Asci with abortive spores and characteristic of the rearrangement were, of course, present in these crosses. However, in one of the crosses the frequency of asci with two abortive spores was significantly higher than normal. In the course of analysing several crosses involving the G101-derived rearrangement significant heterogeneity has been observed in the frequency of asci with two abortive spores. The majority of crosses gave between 15 % and 22 % of these asci but values outside this range were not uncommon. The frequency of 41·4 % observed

in the *hya C70 T(II: VI)* × wild-type cross was the highest value observed. As will be seen below, asci with two abortive spores are thought to arise from meioses in which there has been second division segregation for one of the chromosome breakpoints. The observed heterogeneity presumably reflects variation in crossover frequency resulting in second division segregation, but we have not investigated the underlying basis for it.

Table 4. *Ascus analysis of crosses heterozygous for the G101 derived rearrangement and segregating at the* buff *and* hyaline *loci on linkage groups II and VI respectively*

		Cross					
		C70 T(II: VI) G101 × *hya*			*hya C70 T(II: VI) G101* × wild type		
No. of abortive spores	Ascus type	No.	(%)		No.	(%)	
	Parental Ditype	262	(43·4)		163	(26·8)	
0	Tetratype	15	(2·5)		37	(5·9)	
	Non-parental Ditype	2	(0·3)		18	(2·8)	
	2bl:4m:2ab*	29	(4·8)		77	(12·2)	
2	2m:4bl:2ab	50	(8·3)	21·9 %	89	(14·1)	41·4 %
	6m:2ab	53	(8·8)		96	(15·2)	
	4bl:4ab	117	(19·4)		14	(2·2)	
4	4m:4ab	60	(10·0)	31·8 %	99	(15·7)	24·1 %
	2m:2bl:4ab	15	(2·5)		39	(6·2)	

* Bl = black; ab = abortive; m = spore colour mutant (hyaline and buff not distinguished in this table).

These results are indicative of a translocation between linkage groups II and VI, and this rearrangement is designated *T(II: VI) G101*. Its precise nature will be discussed more fully below.

Crosses to *S187*, an allele of the *yellow-9* locus, located one of the breakpoints in the region proximal to the *buff* locus (data not shown). In crosses of *C70 T(II: VI) G101* to wild type there was a reduced 2:4:2 frequency similar to that observed in the G102/G192 lines. Dissection of the few 2:4:2's which were detected allowed the translocation to be isolated free from the *C70* allele and thus various crosses homozygous for the translocation but segregating for spore colour could be set up.

As expected, the spore abortion characteristic of the heterozygous crosses disappeared in these homozygous crosses. The results are presented in Table 5, where it can be seen that the *C70* and *hya* mutations are still linked, indicating that the *buff* locus has been translocated to the *hya* linkage group. This conclusion is supported by the result from the *C70 T(II: VI) G101* × $+^{C70}$*T(II: VI) G101*, which shows an altered centromere distance for the *C70* allele. In the homozygous cross about 11 % of asci had a 2:4:2 sequence of buff and black spores, indicative of crossing over in the centromere interval (compared to 4 % in normal crosses).

Superficially both the spectrum of ascus types and the sequences of spores within

the asci are consistent with either an insertional translocation or a quasiterminal reciprocal translocation in the G101 line. A quasiterminal reciprocal translocation is one in which one of the breakpoints is effectively terminal. In such a case it is assumed, without any direct evidence, that the tip breakpoint is subterminal rather than terminal, because it is thought that a stable chromosome rearrangement will

Table 5. *Results of crosses between C70 and hya which were homozygous for the G101 derived rearrangements*

	Cross
Ascus type	*hya C70 T(II: VI) G101* × $+^{hya}+^{C70}$*T(II: VI) G101*
Parental ditype	232
Tetratype	93
Non-parental ditype	19

not be formed unless a chromosome break is capped by a telomere (Perkins & Barry, 1977). When one of the breakpoints is in such a subterminal position then adjacent-1 segregation will generate a duplication/deficiency product which is expected to be viable, because it will be deficient only for a dispensable chromosome tip. Adjacent-1 segregation will, therefore, result in the abortion of half the products of a meiotic division rather than all of them and this would appear to fit the observations on the G101-derived rearrangement. However, the apparently normal black spores from the asci with 4 black:4 abortive spores are inviable (see below) and the rearrangement cannot be a quasiterminal translocation unless the inviability is due to the lethal consequences of duplicating particular loci. For this reason, Fig. 2 illustrates a reciprocal translocation in which one of the breakpoints is near the tip of linkage group VI.

(v) *The black spores in S6 × C70 T(II: VI) crosses are probably duplication/deficiency products*

Regardless of the exact nature of the rearrangement in the G101 line it is clear that the spores produced in the *S6 × C70 T(II: VI) G101* crosses are different both from normal disomics and from the tertiary disomics in the G102 and G192 lines. Either of the rearrangement hypotheses outlined above simultaneously affords an explanation for the frequent occurrence of black spores in the *S6 × C70 T(II: VI) G101* crosses and for the spectrum of ascus types containing black spores. On the reciprocal translocation hypothesis the black spores are duplication/deficiency products which have a normal appearance because they do not lack any genes necessary for normal spore pigmentation and development but are inviable because they are deficient for some essential genetic material. The asci with four black spores arise from adjacent-1 segregation, those with two black spores from second division segregation of either of the breakpoints.

Unfortunately it has not been possible to obtain direct confirmation that the black spores are duplication/deficiency products because the spores do not

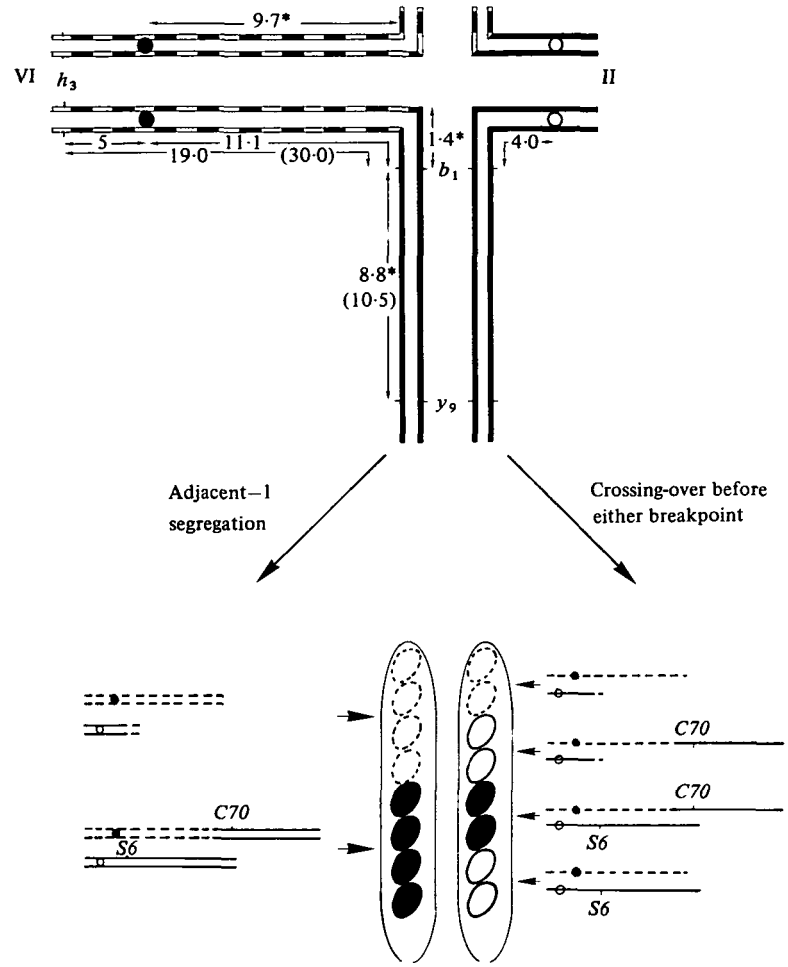

Fig. 2. Upper figure: linkage map of the reciprocal translocation involving linkage groups II and VI. Asterisks and bracketed figures as for Fig. 1. Lower figure: proposed origin of type I (left-hand figure) and type II and III (right-hand figure) asci. In *S6* × *C70 T(II: VI) G101* crosses black spores are all duplication/deficiency products.

germinate. Several dozen asci have been dissected but none of the black spores has ever germinated. This finding provides the basis for the conclusion that the black spores are different from normal disomics, but obviously precludes further analysis. The germination failure can be explained if the black spores are deficient for genetic material necessary for growth, but is not so easily understood if the black spores are simply duplication products (such as would be formed if the rearrangement was a quasiterminal or insertional translocation) because other lines exist in Sordaria in which black spores arise from duplication of linkage group II and these do germinate.

pFPA treatment of *S6* × *C70 T(II: VI) G101* crosses did not result in an increase in the frequency of asci with black spores. This is to be expected if the black spores

arise from adjacent-1 segregation and provides further evidence that the black spores in this case arise in a different way from those in the G102 and G192 lines.

(vi) *Chromosome rearrangements and aneuploid production*

The two rearrangements analysed in this paper reflect two different ways in which black spores, resulting from complementation at the *buff* locus, can arise. The G101-derived aneuploids apparently result from adjacent-1 segregation, whilst in G102 and G192 derived lines the increased aneuploid frequency arises from increased non-disjunction brought about by the presence of a reciprocal translocation.

The 3:1 segregation which generates aneuploidy in the G102 and G192 lines is well known in *Neurospora* (Perkins & Barry, 1977) and has been reported in man (Lindenbaum & Bobrow, 1975). In *Neurospora* the frequency of 3:1 segregations varies from rearrangement to rearrangement and is normally well below 30%. There is no *a priori* expectation for its frequency (Perkins & Barry, 1977) but it is likely that normal disjunction of chromosomes will be most disturbed when a breakpoint is located near a centromere. Burnham (1949) showed adjacent-2 segregation in a reciprocal translocation occurred more frequently when a breakpoint was near a centromere. If the genetic distances presented in Fig. 1 reflect the underlying physical distances then the results presented here support this finding because non-disjunction of linkage group I centromere (giving tertiary disomics) occurs more often than non-disjunction of linkage group II centromere (giving interchange disomics).

The reciprocal translocation also results in an increased frequency of premature centromere division. This is seen as an increased frequency of type II and type III asci. Although the number of such events presented in Table 1 is small, further counts have established that the frequency recorded in the table is typical. Premature centromere division occurs in about 0·5% of those meioses which give rise to viable products. This suggests that normal centromere behaviour at the first division is dependent on structural integrity and/or normal chromosome pairing in the centromere region. When heterozygous, the reciprocal translocation increases both non-disjunction at the first division and premature centromere division. There is no pronounced effect on the frequency of second division non-disjunction, on extra-replication of the chromosomes, or on mitotic non-disjunction.

Considering the chromosome rearrangement present in the G101 line, if the presence of a reciprocal translocation increased the frequency of non-disjunction in *S6* × *C70 T(II : VI)* crosses this would go undetected because the resulting asci with 4 black:4 abortive spores would be confounded with those resulting from adjacent-1 segregation. Such 3:1 segregation events cannot be very frequent, however, because black spores arising in this way would be expected to germinate and have the properties of a tertiary disomic. None have been detected in several dozen ascus dissections and random spore platings.

(vii) *The origin of the rearrangements*

A most interesting feature of the results is that two rearrangements arose spontaneously in the same perithecium and apparently have a breakpoint in a similar place in linkage group II. Admittedly the resolution of the mapping is not particularly fine but, as far as can be judged, the breakpoint in both translocations occurred in the *buff*-centromere interval about 1 map unit from the *buff* gene. Four *C70* isolates were analysed from the one fruiting body, one was normal and three carried very closely linked rearrangements. One explanation for this is that the breaks involved in the rearrangements first arose as potential lesions which replicated before they became manifest as chromosome breaks. The concept of a potential lesion is one which was developed by Auerbach (1951) and Auerbach & Moser (1953) to account for the delayed action of mutagenic treatment in producing chromosome rearrangements. Slizynska (1957) examined formaldehyde-induced lesions in *Drosophila* and reported extensive mosaicism, which would be most easily explained by the suggestion that, following chemical treatment, potential breaks were replicated and the breaks were later either restituted or opened to form rearrangements. The concept of the potential lesion has not been extended to spontaneously occurring mutations.

Following the isolation of these spontaneously occurring rearrangements, we have been analysing all examples where a rosette contains more than one ascus with aneuploid spores. In many cases the co-incident aneuploidy in the rosette has an underlying genetic basis. We plan to report the analysis of these strains in future.

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