THE GENETICS OF A PROBABLE INSERTIONAL TRANSLOCATION IN *SORDARIA BREVICOLLIS*

D. J. BOND

Institute of Animal Genetics, University of Edinburgh, King's Buildings, Edinburgh, Scotland

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ABSTRACT

A chromosome rearrangement has been isolated and characterized in *Sordaria brevicollis*. Crosses to spore color mutants on each of the seven linkage groups have enabled the breakpoints to be mapped. The simplest hypothesis to account for the results is that a piece of linkage group VI has been translocated to linkage group V and inserted 2.7 map units from its centromere. Previous reports of markers on this linkage group with centromere distances greater than 2.7 units make it unlikely that the translocation is quasiterminal.

THE work of PERKINS and his co-workers (*cf.*, PERKINS 1974; PERKINS and BARRY 1977) has rigorously established that various types of chromosome rearrangements can be characterized in ascomycete fungi from the pattern of spore abortion that results when the rearrangement is crossed to a normal strain. This work has also demonstrated the important advantages to be gained in the genetic study of an organism from the availability of a large collection of rearrangements.

HESLOT (1958) pioneered the simultaneous study of chromosome rearrangements and spore color mutants in *Sordaria macrospora*, where a great deal can be concluded from direct microscopic examination of asci, not only about the nature of the rearrangement but also about its linkage relationships. HESLOT confined his analysis to reciprocal translocations and inversions. He did not consider insertional translocations in which "a piece is deleted in one chromosome and inserted into the gap created by a break in a second (nonhomologous) chromosome" (SWANSON 1963). This latter type of rearrangement has been extensively studied in Neurospora (PERKINS 1972; PERKINS and BARRY 1977). It is to be expected that insertional translocations may sometimes be quasiterminal in that the translocated segment will be capped only by the telomere and will have no known genetic marker distal to the translocated segment. Truly terminal translocations, which would result in a telomere becoming interstitial through fusion with a translocated piece, are thought not to occur (SWANSON 1963; WHITE 1973).

The discovery of a rearrangement in Sordaria that is most probably an insertional translocation made it possible to extend the HESLOT analysis concerned

with the simultaneous segregation of chromosome rearrangements and linked spore color mutants. This genetic analysis is presented here.

MATERIALS AND METHODS

Strains: The insertional translocation was discovered while a cross of two complementing b_1 alleles was being examined for nondisjunction. A group of asci from a single perithecium was detected that exhibited a high frequency of abortive spores. Dissection of asci from this cluster verified that this character (the ability to give a high frequency of abortive spores) was inherited in Mendelian fashion, and subsequent crosses to wild type allowed it to be reisolated into a wild-type spore color background—this isolate was given the isolation number B542.

Spore color mutants at the following loci were used for mapping purposes: y_{10} (linkage group I), b_1 (linkage group II), g_2 (linkage group III), y_{13} and g_5 (linkage group IV), y_4 (linkage group V), h_3 and g_1 (linkage group VI), and g_7 (linkage group VII). Details of the location of all loci except y_{10} and y_{13} can be found in CHEN (1965). HELEN SANG provided several mutants at the h_3 locus.

Media and methods: Crossing, culturing and scoring methods have been fully described previously (MACDONALD and BOND 1974). Asci from mature crosses were mounted in sucrose solution and examined at 400× magnification, using a Nikon L-Ke microscope. Ascus dissection was carried out on 4% agar slabs.

RESULTS AND DISCUSSION

When strain B542 was crossed to wild type, the majority of resulting asci contained either eight phenotypically normal spores (8:0ab) or four normal and four abortive spores (4:4ab). Some asci with six normal and two abortive spores (6:2ab) were also generated. The frequencies of these ascus types can be seen in Table 1. Asci in which all eight ascospores are abortive (0:8ab) are difficult to score when the perithecia are crushed open in sucrose solution. In all crosses, a number of asci with poorly developed spores can be seen, but it is often difficult to distinguish immature asci from asci containing eight abortive products. In crosses involving B542, the frequency of asci with eight poorly developed spores was in most cases no greater than in normal × normal crosses. When ascospores were examined as discharged octads on the petri dish lids, groups with all eight spores abortive were very infrequent.

These observations led to the hypothesis that the spore abortion resulted from the presence of a chromosome rearrangement in which a piece of one chromosome had become translocated to a nonhomologous chromosome. When such trans-

TABLE 1

Results of ascus analysis from the cross B542 × wild type

8+0 abortive	No. (and %) of asci with 4+:4 abortive			6+:2 abortive
	4:4 sequence	2:2:2:2 sequence	2:4:2 sequence	
204(44.7)	102(22.4)	72(16.0)	1(0.2)	76(16.7)
		175(38.6)		

locations are crossed to a strain with a normal sequence, ascospore abortion occurs 25% of the time because the donor chromosome assorts independently of the balancing inserted chromosome (see Figure 1, PERKINS 1972). Asci with two abortive spores (and additionally containing two normal, two translocation and two duplication-bearing ascospores) are formed whenever the breakpoints on either the donor or inserted chromosome segregate at the second division. Thus, the frequency of these asci depends on the centromere distance of the breakpoints and varies from one translocation to another. In this particular case, both breakpoints are near to their respective centromeres, as judged from the low frequency of asci with six normal and two defective spores, and therefore the translocated segment is probably inserted into a nonhomologous chromosome near its centromere. However, as will be discussed later, it is not possible to be absolutely certain of this conclusion.

In an attempt to locate the breakpoints, the insertional translocation was crossed to spore color mutants on each of the seven linkage groups. The genotype of the abortive spores at the color locus can be inferred from the color of the nonabortive spores in the ascus. Abortive spores always contain the donor chromosome without the balancing inserted chromosome. If a spore color mutant marks a third uninvolved chromosome, then obviously the abortive spores and spore color will assort independently.

The results of these crosses are presented in Table 2 and show that these expectations were fulfilled for the crosses to markers other than those on linkage groups V and VI. When asci without abortive spores were dissected from each of these crosses, confirmation was obtained that the rearrangement assorted independently of spore color; recombinants of the translocation and color were readily obtained.

In some cases, rather large deviations from expectations were observed. For example, in the cross $y_{10} \times$ B542 there was an excess of 4m:4ab asci compared to 4+:4ab asci, and in the cross $y_{13} \times$ B542 the reverse situation was found. When crosses involving spore color mutants are scored, factors such as the immaturity of wild-type spores and, particularly in the case of gray mutants, the darkening of mutant spores, can lead to the misclassification of asci. In addition, other factors such as the preferential discharge of particular ascus types or sequences (LAMB 1966; MACDONALD and BOND 1974) also give rise to deviations from theoretical expectation. These factors probably account for some of the deviations observed.

Figure 1 illustrates the expected outcome if a color locus marks either the inserted chromosome (column 1), the donor chromosome (column 2), or the translocated segment (column 3). The results of the crosses $y_4 \times$ B542, $h_3 \times$ B542 and $g_1 \times$ B542 were consistent with the hypothesis that these loci respectively marked these three positions.

Considering first $y_4 \times$ B542, the situation is simplified by the fact that the y_4 locus shows complete centromere linkage (CHEN 1965). In this cross, in asci with four abortive spores, it was almost always the spore color mutants that aborted because the wild-type allele marked the inserted chromosome and therefore never

TABLE 2

No. (and frequency) of asci with:—

CROSS		0 abortive spores			4 abortive spores			2 abortive spores	
	No. and type of spores		4+:4m		4+:4ab	4m:4ab	2+:2m:4ab	4+:2m:2ab	2+:4m:2ab
	Sequence of spores	4:4	2:2:2:2	2:4:2	All sequences	All sequences	All sequences	All sequences	All sequences
	Linkage group involved								
y_{10} × B542	I	91(15.6)	92(15.7)	44(7.5)	69(11.8)	127(21.7)	52(8.9)	47(8.0)	63(10.8)
b_1 × B542	II	129(19.1)	145(21.4)	8(1.2)	128(18.9)	122(18.0)	14(2.1)	59(8.7)	71(10.5)
g_2 × B542	III	93(18.3)	88(17.4)	13(2.6)	91(17.9)	81(16.0)	21(4.0)	60(11.8)	60(11.8)
y_{13} × B542	IV	297(31.8)	97(10.3)	16(1.7)	253(27.1)	164(17.5)	17(1.8)	49(5.3)	40(4.3)
g_5 × B542	IV	271(22.7)	195(16.3)	43(3.6)	245(20.5)	357(29.8)	25(2.1)	22(1.8)	39(3.3)
y_4 × B542	V	179(17.2)	264(25.4)	4(0.4)	391(37.7)	1(0.1)	0(—)	175(16.8)	25(2.4)
h_3 × B542	VI	309(28.3)	229(21.0)	3(0.3)	2(0.2)	368(33.8)	5(0.5)	11(1.0)	164(15.0)
g_1 × B542	VI	218(25.8)	189(22.4)	2(0.2)	346(41.0)	1(0.1)	2(0.2)	82(9.7)	4(0.5)
g_7 × B542	VII	103(17.9)	106(18.4)	12(2.1)	118(20.5)	82(14.2)	19(3.3)	64(11.2)	72(12.5)

Results of crosses of B542 to representative spore color mutants on each of the seven linkage groups.

ended up in an ascospore that was deficient for any chromosome material (Figure 1, column 1, line 2). A crossover that recombines the inserted segment and the color locus generates an ascus with two abortive spores, because now one of the breakpoints will segregate at the second division. There are two types of asci with two abortive spores (4+:2m:2ab and 2+:4m:2ab), and these occurred with unequal frequency. The reason for this can be readily seen by reference to column 1, lines 3 through 8, in Figure 1. A crossover in the inserted chromosome will recombine the inserted segment and the color marker, and therefore 4+:2m:2ab and 2+:4m:2ab are equally probable (lines 3 and 4). A crossover in the donor chromosome, on the other hand, gives only 4+:2m:2ab asci (lines 5 through 8). Therefore, the extent of the inequality is a reflection of the relative frequencies of crossing over in the two interstitial regions.

The insertional translocation hypothesis will also explain the result of the $h_3 \times$ B542 and $g_1 \times$ B542 crosses, if it is proposed that the breakpoint on the donor chromosome (linkage group VI) is located between the h_3 and g_1 loci and that the latter locus has been translocated to linkage group V. Figure 1 also illustrates the expectations in these two cases (columns 2 and 3). In the case of $g_1 \times$ B542, only three ascus classes are expected, but seven unexpected asci were observed in a total of 844. These may have been the result of a misclassification of spore phenotypes. The absence of suitable markers on linkage group VI makes it impossible to obtain any genetic evidence on the length of the translocated segment. No evidence is available either on the frequency with which the translocated segment pairs with its homologous nontranslocated portion. If such pairing is frequent, then crossing over could also generate some of these exceptional asci.

Estimation of the position of the breakpoints: The above results are compatible with the view that the breakpoints are located near the centromeres of linkage groups V and VI. To obtain the best estimates of the location of the breakpoints, the data were subjected to maximum likelihood analysis, using standard numerical maximizing procedures. The results were analysed on the assumption that crossovers had a Poisson distribution. The best estimates obtained of the various distances were: centromere distance of the linkage group V breakpoint = 2.7 map units, the centromere distance of the linkage group VI breakpoint = 7.7 map units, and the h_3 locus 5.7 map units from its centromere.

These breakpoint positions make it likely that the rearrangement is an insertional translocation in which the transposed segment is interstitial. The breakpoint positions make it unlikely that the rearrangement is either a quasiterminal reciprocal translocation (in which only a nonessential tip of linkage group V has been translocated to linkage group VI) or a quasiterminal insertional translocation. CHEN (1965) and FIELDS (1970) have both reported mutants on linkage group V with centromere distances greater than 2.7 map units. Unfortunately, these mutants were not available for use in the work reported here, and consequently it was not possible to demonstrate unequivocally the existence of genetic markers distal to the proposed insertion. PERKINS (1974) has pointed out the difficulties of distinguishing an insertional translocation from a reciprocal trans-

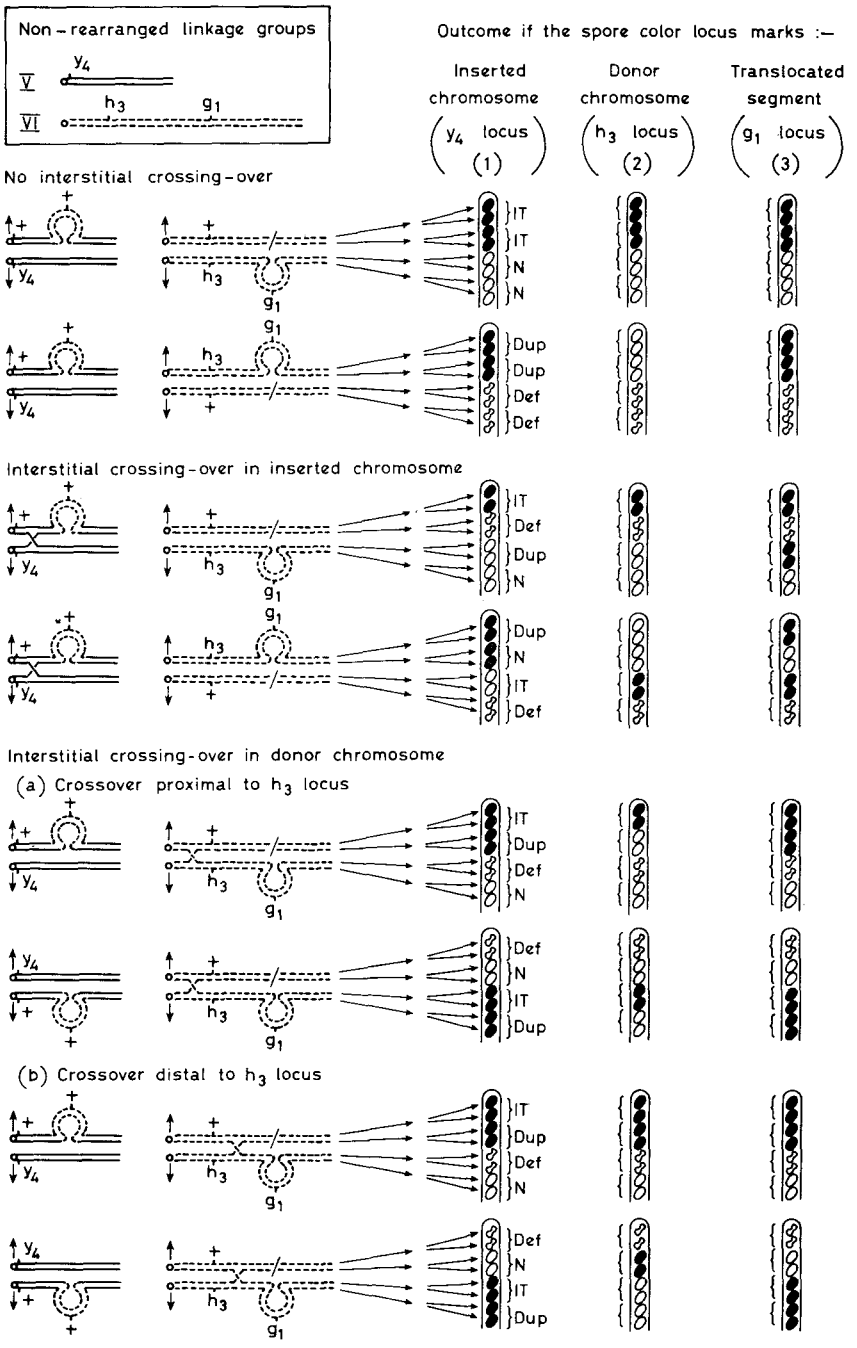

FIGURE 1.—Illustrated outcome of an insertional translocation crossed to a spore color locus that marks: (1) the inserted chromosome, (2) the donor chromosome, and (3) the translocated segment. Linkage groups V and VI are represented by solid and dashed lines, respectively. Multiple crossovers and simultaneous crossovers are not illustrated. The sequence of spores

location in which one of the translocated segments is a nonessential terminal piece. The difficulties are very pronounced in an organism like *Sordaria brevicollis* where there is a comparative paucity of genetic markers. It is indeed fortuitous that the only three markers on linkage groups V and VI available in this study clearly marked the donor chromosome, the recipient chromosome, and the translocated segment.

It is not possible with the markers available to distinguish rigorously between three alternative possibilities. The rearrangement could be (1) an insertional translocation, (2) a quasiterminal insertional translocation, or (3) a quasiterminal reciprocal translocation. Even in a genetically well-marked organism like Neurospora, there is no genetic test that will distinguish (2) and (3). These differ only in the origin of the telomere [which in (2) has not been translocated] and the telomere cannot be genetically marked. The close proximity of the breakpoints to the centromeres and the previous reports of markers with larger centromere distances makes (1) the most likely.

The phenotype of the duplication-bearing strains: Ascus dissection from the three crosses involving markers on linkage groups V and VI confirmed the linkage. From these crosses, all black spores from asci without spore abortion carried the translocation; none of the spore color mutants did so. Ascus dissection of asci with four abortive spores permitted the analysis of the duplication strains. The nonabortive spores from these asci germinated fairly readily. Growth was originally sparse and the resulting colony was irregular in outline. When crossed, the duplications formed perithecia fairly readily, but the majority of these were empty. A few perithecia contained poorly developed asci, and only a small proportion of these contained any pigmented ascospores.

The behavior of Sordaria duplications appears to be similar to that described for Neurospora (PERKINS 1972). In Neurospora, the duplication strains can be unstable (PERKINS 1972; NEWMEYER and GALEAZZI 1977) and loss of one or other of the duplicated regions can occur (TURNER 1977). In Aspergillus, duplication-bearing strains are also mitotically unstable (ROPER 1973), mitotic loss of the extra chromosome segment resulting in a colony with visible sectors. In Sordaria, no sectors with a different growth rate or morphology have been seen during growth of duplication strains.

In an attempt to detect the loss of the inserted segment in Sordaria, duplications from $g_1 \times$ B542 were crossed to wild type. Duplications from this cross carry both the mutant and wild-type alleles at the g_1 locus, the duplication-bearing spores being black because of the dominance of the wild-type allele. Segregation of the spore color mutant would be expected to occur in all asci within a perithecium if it arose through fertilization by a nucleus in which loss of the inserted segment

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drawn is correct for the chromosome orientation illustrated, but is not the only possible sequence. Some sequencies, however, are not expected to occur, for example a 2:4:2 sequence of black and mutant spores. The abbreviation (IT) indicates a spore carrying an insertional translocation, (N) a spore with a normal chromosome complement, (Def) a spore deficient for the translocated segment, and (Dup) a spore duplicated for the translocated segment.

had occurred. Using this method of screening, a large number of perithecia can be examined both for the segregation of the spore color marker within and for the restoration of normal fertility to a perithecium. This method provides a quite powerful means for detecting duplication loss. Screening of crosses through the inspection of discharged ascospores revealed no examples of such perithecia. The duplications generated by this insertional translocation are therefore not particularly unstable.

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