Genet. Res., Camb. (1972), **19**, *pp.* 121–127
Printed in Great Britain

The effect of an inverted chromosome segment on intragenic recombination in another chromosome of *Sordaria brevicollis*

By A. F. AHMAD,* D. J. BOND† and H. L. K. WHITEHOUSE
Botany School, University of Cambridge

(*Received* 15 *November* 1971)

SUMMARY

An inverted segment in one of the chromosomes of *Sordaria brevicollis* has been found to have a marked effect on recombination within a gene in another chromosome. There are two effects on the intragenic recombination pattern, namely, a change in the polarity of recombination and a change in the frequency of parental and recombinant outside markers. The results support the idea that a mechanism exists for cancelling crossovers shortly after they have been formed as a means of controlling their number.

1. INTRODUCTION

According to current ideas (Holliday, 1968; Whitehouse, 1970) crossing-over between homologous chromosomes takes place by breakage and rejoining, and in the rejoining process complementary nucleotide-chains, one derived from each parent, become associated to give regions of heteroduplex or hybrid DNA. The phenomenon of postmeiotic segregation, which is associated with crossing-over, is believed to arise from the presence of the site of a mutation within such hybrid DNA segments. The occurrence of conversion, that is, a 3:1 or a 1:3 ratio of wild-type to mutant allele within the four products of meiosis, is also associated with crossing-over and is attributed to the correction of mispairing in hybrid DNA at the mutant site. This correction of mismatched bases would be brought about by the excision of a segment of one nucleotide-chain and its replacement by a polynucleotide complementary to the other one. Differences between mutants in the frequency of conversion are attributed, in part, to the molecular nature of the mutation on the supposition that the kind of mispairing which it causes in hybrid DNA influences the correction process. Other variations within a gene in the frequency of conversion depend, however, on the position of the mutant site, and these are explained by supposing there are fixed opening-points in the DNA where dissociation of the nucleotide-chains of the parent molecules is initiated, and that the hybrid DNA is of variable extent from these points. The data fit a model where the opening is confined to the ends of genes.

Postmeiotic segregation and conversion, although clearly associated with the

* Present address: Institut für Allgemeine Mikrobiologie, Universität Bern.
† Present address: Department of Genetics, University of Edinburgh.

https://doi.org/10.1017/S0016672300014348 Published online by Cambridge University Press

process of reciprocal exchange both as regards position and chromatids, nevertheless, also occur with quite high frequency in the absence of such an exchange. A possible explanation (Whitehouse, 1967) is that certain of the crossovers are cancelled shortly after they have been formed, as part of a mechanism by which their number and distribution in the chromosomes are controlled. If the cancellation process allowed a segment of hybrid DNA to remain, this would explain the occurrence of postmeiotic segregation and conversion for a mutant in conjunction with a parental arrangement of genes on either side of the mutant site.

Over 50 years ago Sturtevant (1919) discovered that an inverted segment (as he later showed it to be) in linkage group III of *Drosophila melanogaster* increased the frequency of crossing-over in chromosome II. This effect of inversions on crossing-over in other chromosomes is now well known (Steinberg & Fraser, 1944; Ramel, 1962; Suzuki, 1963) though still unexplained. A possible means of testing the crossover-cancellation hypothesis of frequency control is through study of these effects, because they might be operating via such a control mechanism. In other words, when an inversion increases crossing-over in another chromosome, this might be not because the DNA is opened at a larger number of places, but because fewer crossovers than normal are cancelled. The consequence of this would be that postmeiotic segregation and conversion would more often be associated with a crossover configuration of genes on either side and less often with a parental arrangement of these markers. This prediction has now been confirmed from study of recombination at the *buff* spore colour locus in the fungus *Sordaria brevicollis*, and at the locus (*r*) for *rudimentary* wings in *D. melanogaster* (Carlson, 1972).

2. MATERIALS AND METHODS

Mutants of the fungal gene investigated have dull yellow (buff-coloured) spores instead of the normal dark brown. By crossing with a series of spore colour mutants provided by Professor L. S. Olive, it was found that the *buff* gene corresponded in position to the *beige-1* (*b1*) and *grey-3* (*g3*) mutants of Chen (1965), which are situated about 2 units from the centromere in linkage group II. The *buff* mutants were obtained by ultraviolet irradiation, some of them by M. H. V. Cooray. The marker genes used on either side of the *buff* locus were as follows. The proximal marker with respect to the centromere was *mo-1*, a morphological mutant obtained by Cooray using ultraviolet light. This mutant has a sharper outline to the colony than wild-type, and shows about 1·5 % recombination with *buff*. The distal marker was *nic-1*, a nicotinamide-requiring mutant obtained by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) and giving about 1·1 % recombination with *buff*.

In order to map the *buff* mutants they were crossed with one another in pairs, one carrying *mo-1* and the other *nic-1*. The crosses were made in Petri dishes of malt agar kept at 25°. At maturity the spores of *S. brevicollis* are forcibly discharged from the asci and appear on the lid of the dish in groups of eight corresponding to individual asci. In the crosses studied about 1 in every 1250 of these octads of

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spores showed recombination at the *buff* locus, indicated by the presence of either one or a pair of dark brown (wild-type) spores. Control crosses homozygous for each mutant in turn indicated that mutation to wild-type was either absent or occurred at a much lower frequency than the wild-type spores observed in crosses between mutants, thus establishing that these were largely or entirely of recombinant origin. The spores in recombinant octads from crosses between different mutants were isolated, germinated and scored for the outside markers. The genotype of the wild-type spores for these markers was used to map the mutants on the assumption that recombination between the alleles and nowhere else in the marked interval happened more often than recombination between the alleles and both proximal and distal to them. On this basis each pair of mutants crossed was orientated with respect to the outside markers.

The occurrence of a paracentric inversion was indicated by abortion of two of the four pairs of spores in about 30 % of the asci in crosses heterozygous for it, by the absence of spore abortion when homozygous, and by anaphase bridges at meiosis (Ahmad, 1970). The inversion (called *ABW-1*) arose in material which had been treated with NG. Crosses were made between the strain carrying the inversion and spore-colour mutants in each of the seven linkage groups in turn. These mutants were provided by Professor Olive. The progeny were tested for the presence of the inversion by crossing them with wild-type and looking for aborted spores. No linkage was found between the occurrence of spore abortion and the mutants in six of the linkage groups, but the inversion showed 7·5 % recombination with *grey-2* (*g2*), a spore colour mutant found by Chen (1965) to be situated 2·8 units from the centromere in linkage group III.

3. RESULTS AND DISCUSSION

The effect of the *ABW-1* inversion on recombination in linkage group II was tested in two experiments. In the first experiment the frequency of crossing-over was studied in a segment of the chromosome marked by the *buff* gene and another spore colour locus, mutations of which give yellow spores. The *yellow-9* locus is situated in the same chromosome arm as *buff* and about eight units from the centromere. The mutants used were S 156 (*buff*) and S 214 (*ylo-9*), both obtained by ultraviolet irradiation, S156 by Cooray. When a *buff* mutant is crossed with a *ylo-9* mutant, a majority of the asci show four buff and four yellow spores, indicating no recombination between *buff* and *ylo-9*. A crossover between the loci gives rise to asci with two buff, two yellow, two dark brown (wild-type) and two colourless (double mutant) spores, and a four-strand double crossover to asci with four dark brown and four colourless spores. The numbers of these three kinds of asci counted in a normal strain and in a cross heterozygous for the inversion are given in Table 1. When the inversion was present, only asci without aborted spores, that is, without crossing-over in the inversion, were counted. It is evident that the frequency of crossing-over between *buff* and *ylo-9* was nearly doubled when the inversion was present in heterozygous form.

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The second experiment was to test the crossover-cancellation hypothesis by studying the frequency of parental combinations of the outside markers when recombination leading to the formation of wild-type spores occurred at the *buff* locus. Two *buff* mutants were crossed, one carrying the proximal marker, *mo-1*, and the other the distal marker, *nic-1*. In one cross *buff* mutants S 156 and S 180 were used and in another S 156 and S 6. The spores in octads containing one or more dark brown spores were isolated, germinated and scored for the outside markers. The results for the wild-type spores are given in Table 2. The data for the two different combinations of alleles are similar and give totals of 28 parental, 29 crossover without the inversion, and 6 parental, 52 crossover in its presence. The frequency

Table 1. *Numbers of asci of* Sordaria brevicollis *with crossovers between the loci for* buff *and* yellow *spore-colour in linkage group II*

Cross	Numbers of asci: Without crossing-over	With a single crossover	With a four-strand double crossover	Total	Total no. of crossovers	Crossover spores (%) and standard error
Without inversion	1294	183	7	1484	197	6·64 ± 0·46
Heterozygous for inversion in linkage group III	1237	351	22	1610	395	12·27 ± 0·57

of asci with wild-type spores was of the same order of magnitude in the presence of the inversion as in its absence. It is evident that the crosses that were heterozygous for the *ABW-1* inversion show a significant reduction ($\chi^2 = 20{\cdot}8$, $n = 1$, $P < 0{\cdot}01$) in the frequency of parental outside marker configurations, as predicted by the hypothesis. Moreover, the magnitude of this reduction matches the increase in crossover frequency observed for the *buff-yellow* interval: in both experiments the crossover frequency was increased by about 80%. Thus it is possible that the increase in crossing-over in linkage group II could be entirely accounted for by a reduction in the frequency with which crossovers are cancelled. It seems likely, however, for reasons discussed below, that the inversion is also affecting the initial opening of the DNA for crossing-over. Study of the genotype of the buff spores in recombinant asci in the presence and absence of the inversion will provide information about the mechanism of crossover cancellation: two current models (Holliday, 1964; Whitehouse & Hastings, 1965) differ in their explanation of parental outside markers when an inner marker shows conversion. The frequency of asci showing reciprocal recombination of alleles will be of particular interest. Such study has been hindered hitherto by poor germination.

The evidence that the *ABW-1* inversion is influencing the initial opening of the DNA for crossing-over is as follows. It was found that in the presence of the inver-

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Table 2. *Frequencies of various configurations of outside markers in wild-type spores from asci derived from crosses between two* buff *spore-colour mutants of* Sordaria brevicollis *in the presence and in the absence of the* ABW-1 *inversion in linkage group III. Only one wild-type spore has been counted from each ascus. When two were present they both showed the same outside marker combination*

Inversion in linkage group III	Alleles crossed	Non-crossover for outside markers		Crossover for outside markers		Total no. of asci	Crossover (%) and standard error
		Recombination between alleles and proximal to them	Recombination between alleles and distal to them	Recombination between alleles only	Recombination between alleles and both proximal and distal to them		
Absent	S156 × S180	5	9	15	0	29	51·7 ± 9·3
	S156 × S6	5	9	14	0	28	50·0 ± 9·4
	Total	10	18	29	0	57	50·9 ± 6·6
Present in heterozygous form	S156 × S180	1	2	27	0	30	90·0 ± 5·5
	S156 × S6	3	0	25	0	28	89·3 ± 5·8
	Total	4	2	52	0	58	89·6 ± 4·0

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sion only 1 out of 58 or 1·7 % of the recombinant asci had single wild-type spores compared with 16 out of 57 or 28 % in its absence, the difference being significant ($\chi^2 = 15{\cdot}1$, $n = 1$, $P < 0{\cdot}01$). A possible explanation is that the inversion is affecting the activity of the enzymes presumed to be responsible for the correction of mispairing. It seems more likely, however, that the change in the relative frequency of postmeiotic segregation and conversion is an indirect result of an influence of the inversion on the frequency with which recombination is initiated from one end of the gene rather than the other, for the following reason.

When recombination of a pair of alleles is associated with a parental arrangement of flanking markers, additional recombination must have taken place either on the proximal or distal side of the alleles. In crosses lacking the *ABW-1* inversion, the wild-type spores which occurred singly and which had a parental arrangement of the flanking markers almost always had this additional recombination on the distal side. On the other hand, the wild-type spores which occurred in pairs did not show this polarity, the additional recombination being proximal or distal about equally often. This difference between asci showing postmeiotic segregation and those showing conversion was found in each of six different crosses of *buff* alleles. The total data amounted to two proximal, 37 distal for the single spores and 32 proximal, 36 distal for the pairs (Bond, 1969). The individual spores of a pair were alike as regards outside marker genotype and have been counted as one. The difference between the singles and pairs is significant ($\chi^2 = 19{\cdot}3$, $n = 1$, $P < 0{\cdot}01$). The relationship between the side of the alleles which showed the additional recombination, and the frequency of postmeiotic segregation, might be due to the distal mutants happening to be of a kind such that the mispairing they cause in hybrid DNA is often not recognized by the correcting enzyme, while the proximal mutants happen to be ones that are readily recognized. This cannot be the explanation, however, because in crosses between each mutant and wild-type, all of them showed postmeiotic segregation about equally often. It seems likely, therefore, that the postmeiotic segregation is largely restricted to the hybrid DNA of distal origin, and that the source of the hybrid DNA, that is, whether from a proximal or distal direction, influences the frequency of correction of mispairing. This means that postmeiotic segregation is not determined merely by the nature of the mutation. It thus seems probable that, as well as allowing fewer crossovers to be cancelled, the inversion may influence the initial opening of the DNA for crossing-over at certain places such as the distal end of *buff*. Specific effects such as this are needed to explain why each inversion studied in *Drosophila melanogaster* has usually been found to affect crossing-over in the other chromosomes in a distinctive way (Steinberg & Fraser, 1944; Ramel, 1962; Suzuki, 1963), and indeed evidence is presented by Carlson (1972) that in *Drosophila*, as in *Sordaria*, the polarity of intragenic recombination is affected by an inversion in another chromosome.

Murray (unpublished results) has investigated the effect of heterozygosity for a structural change in *Neurospora crassa* on intragenic recombination in another chromosome. The rearrangement used was an insertional translocation symbolized

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T (*IR* $\overset{\text{In}}{\rightarrow}$ *VR*) S1325, which has part of the right arm of linkage group I inserted in inverted sequence into the right arm of linkage group V (see Murray, 1968). The structural change is associated with niacin requirement (*nic-2*). Recombination was studied in the *me-7* (methionine-7) gene in linkage group VII using the mutants *thi-3* (thiamine-3) and *wc* (white collar) as flanking markers. The *me-7* mutants used were NM 56 and NM 73 (see Murray, 1969). Strains of genotypes *me-7* (NM56) *thi-1 A* and *me-7* (NM 73) *wc a* were crossed and methionine-independent progeny selected and scored for the flanking markers. The experiment was repeated with the second parental strain carrying the inverted translocation. No marked effect of the aberration was found on the frequency of parental and recombinant outside marker configurations. This result is contrary to that found with the inversion in *Sordaria*, but this is not surprising because studies (Steinberg & Fraser, 1944; Ramel, 1962; Suzuki, 1963) with *Drosophila* have shown that there is much variation between one structural change and another in their effects on crossing-over in other chromosomes.

We thank Professor L. S. Olive for mutants at spore-colour loci of *Sordaria brevicollis* and Mr M. H. V. Cooray for further mutants. We are grateful to Dr Noreen Murray for permission to refer to her unpublished work. D. J.B. thanks the Science Research Council and latterly the Medical Research Council for financial support.

REFERENCES

Ahmad, A. F. (1970). Cytogenetic studies in *Sordaria brevicollis*. Ph.D. thesis, Cambridge University.

Bond, D. J. (1969). Genetic recombination in *Sordaria brevicollis*. Ph.D. thesis, Cambridge University.

Carlson, P. S. (1972). The effects of inversions and the *C(3)G* mutation on intragenic recombination in *Drosophila*. *Genetical Research* **19**, 129–132.

Chen, K. C. (1965). The genetics of *Sordaria brevicollis*. I. Determination of seven linkage groups. *Genetics* **51**, 509–517.

Holliday, R. (1964). A mechanism for gene conversion in fungi. *Genetical Research* **5**, 282–304.

Holliday, R. (1968). Genetic recombination in Fungi, in *Replication and Recombination of Genetic Material*, eds. Peacock, W. J. & Brock, R. D., Australian Academy of Science (Canberra).

Murray, N. E. (1968). Polarized intragenic recombination in chromosome rearrangements of *Neurospora*. *Genetics* **58**, 181–191.

Murray, N. E. (1969). Reversal of polarized recombination of alleles in Neurospora as a function of their position. *Genetics* **61**, 67–77.

Ramel, C. (1962). Interchromosomal effects of inversions in *Drosophila melanogaster*. I. Crossing over. *Hereditas* **48**, 1–58.

Steinberg, A. G. & Fraser, F. C. (1944). Studies on the effect of *X* chromosome inversions on crossing over in the third chromosome of *Drosophila melanogaster*. *Genetics* **29**, 83–103.

Sturtevant, A. H. (1919). Inherited linkage variations in the second chromosome. *Carnegie Institute of Washington Publication* **278**, 305–341.

Suzuki, D. T. (1963). Interchromosomal effects on crossing over in *Drosophila melanogaster*. II. A re-examination of X-chromosome inversion effects. *Genetics* **48**, 1605–1617.

Whitehouse, H. L. K. (1967). Secondary crossing over. *Nature, Lond.* **215**, 1352–1359.

Whitehouse, H. L. K. (1970). The mechanism of genetic recombination. *Biological Reviews* **45**, 265–315.

Whitehouse, H. L. K. & Hastings, P. J. (1965). The analysis of genetic recombination on the polaron hybrid DNA model. *Genetical Research* **6**, 27–92.

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