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Crossover frequencies within paracentric inversions in maize: the implications for homologue pairing models

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SUMMARY

Crossover frequencies within three overlapping heterozygous paracentric inversions of differing length in the long arm of chromosome 1 of maize were estimated from cytological data. These frequencies were found to be close to normal for the regions involved and directly proportional to the respective physical lengths of these inversions. It is suggested that if pairs of centres which are specialized to regulate homologous pairing of the region between them exist in this part of the maize genome, they are most probably numerous and widely distributed.

1. INTRODUCTION

Once matching parts of homologues have approached to within a distance of about 300 nm at meiosis, the synaptonemal complex (SC) seems to mediate close pairing along their length. But information on the prior sequence of events which leads to the establishment of 300 nm spacing is fragmentary, and the simplistic models which have been proposed seem inadequate or actually invoke a high order of underlying complexity (for review see Maguire, 1984). A promising approach for insight on the problem involves the study of the frequency of pairing which has been effective for crossing over in chromosome segments heterozygous for rearrangement. Hawley (1980) reported evidence which suggests that there are four intercalary centres specialized for pairing in the *X* chromosome of Drosophila and that these are separated by an average of 16·5 m.u. They seem to be located at sites of major polytene constrictions and therefore may represent heterochromatic regions. In the material studied, consecutive sites seemed to regulate pairing in the region between them in such a way that crossover frequency was sharply depressed for the entire region when it contained a breakpoint of a heterozygous translocation. This observation implies that there was little or no spreading of pairing which was effective for crossing over, from the putative special sites toward breakpoints (where change of pairing partner would be required for continuation of homologous pairing); if such pairing extension commonly occurred, presumably the sharp declines in crossover frequency would not be found at the boundaries marked by the special sites. Pairs of consecutive sites therefore somehow seemed to act coordinately. A need for two bounding specialized pairing centres to foster homologous pairing, which is effective for crossing over for the region between

them, has also been postulated by Rose, Baillie & Curran (1984) to account for crossover frequencies found in the presence of duplicating fragments in *Caenorhabditis elegans*. It is a question of considerable interest whether evidence for such a pairing mechanism can be found in other widely divergent organisms.

Previously published cytological data are used here to calculate what are believed to be close estimates of the crossover frequencies within regions heterozygous for a series of overlapping inversions of differing length. It is the purpose of this note to point out the striking linearity of the relationship of these estimated crossover frequencies within the inversions to the respective physical length of these inversions, and to consider whether this finding can be reconciled reasonably with predictions of the hypothesis that pairs of specialized pairing centres within homologues somehow interact to mediate the effective pairing at meiosis of the regions between them.

2. MATERIALS AND METHODS

Bridge and fragment frequencies of various classes have been reported in detail for heterozygotes of three inversions which differ substantially in length but share overlapping locations in the distal half of the long arm of chromosome 1 of maize (Maguire, 1982, 1985): In5083 with breakpoints at 1L.70-.87, In4305-25 with breakpoints at 1L.65-.91, and Inld with breakpoints at 1L.55-.92. The data on which calculations presented here are based are listed in Table 1. In the analysis presented here estimation of crossover frequency within each inversion is taken to be equal to the calculated frequency of single crossovers plus twice the calculated frequency of double crossovers. The calculations are based on the assumptions: that single crossovers within an inversion produce a bridge and a fragment at anaphase I, that two-strand doubles within an inversion produce no bridge or fragment, that three-strand doubles within an inversion produce a bridge and a fragment at anaphase I, that four-strand doubles within an inversion produce a double bridge and two fragments at anaphase I, and that three-strand doubles with one crossover within an inversion and one proximal to it produce a fragment at anaphase I and a bridge at anaphase II. It is further assumed that there is no chromatid interference (Rhoades & Dempsey, 1953) and that higher orders of multiple crossovers within the inversions occur with negligible frequency.

Some complicating factors are resolved as follows. The presence of a fragment is apparently masked some of the time by its having been carried poleward, and in the process obscured by overlapping poleward dyads, but the presence of a bridge (either at anaphase I or anaphase II, depending on the crossover class) is taken as a reliable indicator of the occurrence of a crossover event. Also, since two-strand doubles within an inversion give the same appearance as no crossovers within an inversion, and three-strand doubles within an inversion give the same appearance as single crossovers within an inversion, the overall estimates of frequencies of singles and doubles within each inversion must include compensation for these sources of error. In addition, as a partial check of accuracy, estimates are calculated in two differing ways and the results compared and averaged. Both methods depend upon the expectation of ratio of 2-strand:3-strand:4-strand

doubles = 1:2:1. Method 1 is based on the observed frequency of anaphase I cells with a double bridge and two fragments (derived from 4-strand doubles) so that the total frequency of double crossovers within the inversion is taken to be equal to 4 × this value. In this case, one half of this calculated total frequency of double crossovers within the inversion must be subtracted from the frequency of anaphase I cells with a single bridge, so that these are not counted as single crossovers instead of as the three-strand doubles they are here presumed to represent. Method 2 makes

Table 1. *Observed frequencies of cells in various categories at anaphase I and anaphase II for the three inversions*

	Anaphase I single bridge without attached fragment		Anaphase I single bridge with attached fragment		Anaphase I double bridge		Anaphase II bridge	
Inversion	No.	%	No.	%	No.	%	No.	%
In5083	90/535	16·8	5/535	0·9	1/535	0·2	52/580	9·0
In4305-25	49/273	18·0	17/273	6·3	5/273	1·8	62/626	9·9
Inld	157/545	28·8	36/545	6·6	20/545	3·7	85/786	10·8

Table 2. *Estimates* (%) *of single, double and total crossover frequencies in each of the three inversions by two methods of calculation* (*described in the text*)

	Estimated percent cells with single crossover within		Estimated percent cells with double crossovers within		Estimated crossover frequency within	
Inversion	Method I	Method 2	Method 1	Method 2	Method 1	Method 2
In5083	35·3	34·8	0·8	1·3	36·9	37·4
					Av. = 37·15	
In4305-25	40·6	37·9	7·2	9·9	55·0	57·7
					Av. = 56·35	
Inld	49·4	50·4	15·2	14·2	79·8	78·8
					Av. = 79·30	

use of the assumption that where 3-strand doubles have occurred, the fragment is usually found to be associated with the bridge at anaphase I (Maguire 1982, 1985). In this case the total frequency of double crossovers within the inversion is taken to be equal to the sum of cells presumed to have had 3-strand doubles on this basis, plus twice the frequency of anaphase I cells with a double bridge and two fragments. Also, of those cells with a single bridge, only those to which a fragment was not physically associated are counted as having had a single crossover within the inversion. In addition to these calculations, in both methods the frequency of cells with three-strand doubles with one crossover within the inversion and one proximal to it is taken to be most accurately estimated as equal to twice the frequency of cells with a bridge at anaphase II. This value is included in the estimation of frequency of cells with a single cross-over within the inversion. Other classes of double crossovers, with one within the inversion and one outside

it, produce a single bridge and fragment at anaphase I, and therefore do not impose the necessity of further correction. The two methods of calculation give similar values (differences not significant). Results of calculations are listed in Table 2. In each case the frequency of singles within the inversion was significantly elevated beyond Poisson distribution prediction (at the expense of frequency of cells of zero crossover and double crossover rank), as expected in the presence of positive chiasma interference.

All samples studied were from plants grown in a growth chamber under the same controlled environmental conditions. All stocks had similar genetic background (KYS inbred). There was no conspicuous heterochromatic region in the entire arm of chromosome 1 in this material.

3. RESULTS AND DISCUSSION

The relationship of calculated crossover frequencies within the three inversions to their respective physical lengths is illustrated graphically in Fig. 1. This relationship is clearly linear, and most simply suggests the existence of a fine-grained distribution of capability for pairing which is effective for crossing over along most of the distal half of the long arm of chromosome 1, but other possibilities are by no means ruled out. The estimated crossover frequencies of 79%, 56% and 37% respectively for inversions Inld, In4305-25 and In5083 are consistent with map unit contents of at least 40, 28 and 19 map units. These genetic map extents probably represent underestimates, since some crossover suppression would be expected to accompany heterozygosity for the rearrangements. The average numbers of estimated map units per unit physical length are 0·85, 0·86 and 0·86 for Inld, In4305-25 and In5083 respectively, compared to about 0·79 for the entire long arm of chromosome 1 (Neuffer, Jones & Zuber, 1968). Somewhat higher than average crossover frequency per unit physical length is normally expected for the distal part of a chromosome arm; it seems reasonable to suggest that the estimated crossover frequencies within the heterozygous inversions are not greatly different from normal for these regions.

If centres specialized for pairing exist in the distal half of maize chromosome 1 with only about the same frequency per map unit as those thought to exist in the *X* chromosome of Drosophila, these inversions might contain about one to four such centres, and at least one probably would need to be fortuitously located near each inversion breakpoint.

It is possible that pairing which is effective for crossing over within regions heterozygous for the maize inversions studied is governed by the coordinate action of pairs of flanking centres specialized for pairing, but if so it seems probable that either there are numerous such centres dispersed along the chromosome regions in question, or inversion breakpoints tend to be located or perceived to be located at such centres. There are, in fact, some striking similarities of breakpoint locations of existing maize inversions, including the distal breakpoints of the two longer inversions utilized here (Longley, 1961). Also, note that maize inversion breakpoints are identified by measuring the locations of loop configurations at pachytene, where homologues reverse the orientation of their pairing with respect to the remainder

of the bivalent; thus it is conceivable that the position of tight loop configurations more accurately reflects the location of pairing centres than breakpoints, a condition which could escape detection in maize.

On the other hand, somatic pairing of polytene chromosomes in Drosophila seems to display accurate homologous matching in the presence of rearrangements of short regions, and it has been suggested that matching single bands may pair autonomously in the presence of adjacent evolutionary divergence of DNA sequences (Riede & Renz, 1983). There is also evidence that homologous pairing

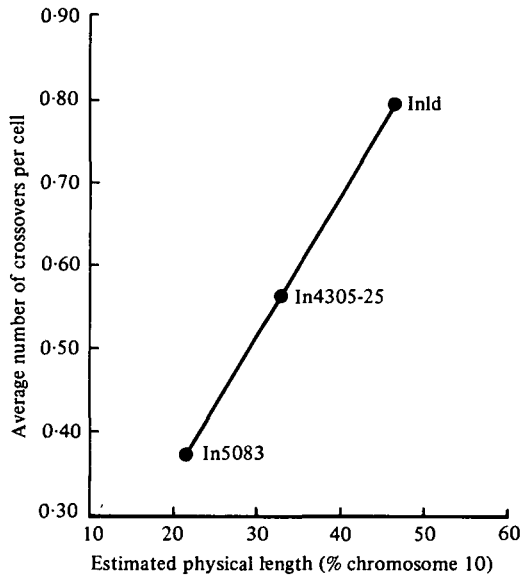

Fig. 1. The relationship of estimated crossover frequency within the three inversions to their respective physical lengths. Estimates of crossover frequency represent the average of two methods of calculation, as described in the text. The extrapolation of the line passes near the origin.

occurs with substantial frequency at meiosis in Drosophila within a region heterozygous for a short paracentric inversion. Chovnick (1973) reported frequency of conversion at the rosy locus to be reduced, but of the same order of magnitude within heterozygous inversion IN $(3R)P_{18}$ as that found for the same region in homozygous normal sequence material, although reciprocal recombination frequency was strongly depressed (presumably because recombinant chromatids were eliminated by bridge formation). Reciprocal recombination frequency within a Drosophila heterozygous medium-sized inversion has been estimated to be approximately normally distributed, except for slight relative depression near the breakpoints, but reduced overall to about 25% of normal sequence expectation (Novitski & Braver, 1953). This study relied upon use of specially constructed stocks in which some of the single crossover products are recoverable. Crossover frequency in heterozygous paracentric inversions in Drosophila is usually estimated from double recombinant frequency, and doubles are strongly inhibited by

interference in short regions. The cytological location of inversion breakpoints can be accurately determined, however, with respect to polytene chromosome bands in Drosophila, so that the potential errors inherent with maize breakpoint determination do not exist.

Questions which call for resolution in future study are the following.

1. Do heterozygous inversion breakpoints and heterozygous translocation breakpoints have fundamentally different effects on pairing which is effective for crossing over? Although homologous pairing within heterozygous inversions requires a change of pairing partner only to another portion of the same chromosome rather than to a different chromosome (as is the case for homologous pairing for heterozygous translocations), it seems unlikely that simple zipping up to the breakpoints markedly facilitates the effective pairing switch. Crossover suppression is most commonly noted in the immediate vicinity of breakpoints in inversion heterozygotes, suggesting that independent events of pairing initiation may be required for changes of pairing partner.

2. Are there artifacts in either or both cases which tend to lead to erroneous conclusions?

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