Crossover Interference on NOR-bearing Chromosomes in Arabidopsis

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ABSTRACT

In most eukaryotes, crossovers are not independently distributed along the length of a chromosome. Instead, they appear to avoid close proximity to one another – a phenomenon known as crossover interference. Previously, for three of the five \textit{Arabidopsis} chromosomes, we measured the strength of interference and suggested a model wherein some crossovers experience interference while others do not. Here we show, using the same model, that the fraction of interference-insensitive crossovers is significantly smaller on the remaining two chromosomes. Since these two chromosomes bear the \textit{Arabidopsis} NOR domains, the possibility that these chromosomal regions influence interference is discussed.
Crossover interference, first observed by Sturtevant (1915), governs the genome-wide distribution of recombination events during meiosis. It can be characterized as a quasi-uniform, rather than exponential, distribution of intercrossover map distances. To better understand these processes, we are exploring the regulation of recombination events in *Arabidopsis thaliana* by taking advantage of the *quartet* mutation, which enables the use of tetrad analysis (Preuss et al. 1994). Previously we reported that crossovers on chromosomes 1, 3 and 5 of Arabidopsis display inter-crossover length distributions consistent with a mixture of both interference-sensitive and interference-insensitive recombination events (Copenhaver et al. 2002). The observed data best fit a model in which 20% of the crossovers in Arabidopsis are insensitive to interference. That analysis was not powerful enough to draw conclusions for chromosomes 2 and 4. Chromosomes 2 and 4 are short acrocentric chromosomes that harbor large arrays of ribosomal RNA (rRNA) genes on the distal end of their short arms (Copenhaver et al. 1995; Copenhaver and Pikaard 1996). DNA encoding rRNA (rDNA) is commonly referred to as a nucleolus organizing region (NOR) because of the distinct sub-organellar structure that forms around rRNA genes during interphase (McClintock 1934). Interestingly, NORs have been proposed to function as cis-acting chromosome pairing centers in several species. To examine the influence of both chromosome length and structure on interference, we expanded our previous analysis of Arabidopsis tetrads. Our results indicate that the observed distribution of inter-crossover lengths on chromosomes 2 and 4 is also consistent with a mixture of both interference-sensitive and interference-insensitive crossovers. However, the fraction of interference-insensitive crossovers on the
short, NOR-bearing chromosomes appears to be about seven-fold smaller than it is on the long chromosomes.

**MATERIALS AND METHODS**

**Plant material:** *Arabidopsis thaliana qrt1-1* in the Landsberg background (CS8050) was crossed to *qrt1-2* in the Columbia background (CS8846) to create F1 plants. Individual pollen tetrads from F1 plants were manually crossed onto Landsberg male-sterile1 plants (CS75). Crosses that generated 3 or 4 meiotically related seeds were selected for analysis. Seeds were sown on Pro-mix (Professional Horticulture, Inc.) and stratified for 3-4 days at 4°C. Plants were germinated and grown under long-day conditions (18 hrs. light). All parental strains are available from the Arabidopsis Biological Resource Center at Ohio State University (http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/abrchome.htm).

**DNA preparation and marker analysis:** DNA was extracted from meiotically related progeny plants by grinding single cauline leaves (~ 0.5 – 1 cm) in 200 µl of 50 mM Tris·HCl (pH 8.0), 200 mM NaCl, 0.2 mM EDTA, 0.5% SDS and 100 µg/ml Pronase E (Sigma, St. Louis). After incubation at 37°C for 30 min samples were extracted with phenol and then chloroform. DNA was precipitated by the addition of 1/10th volume of sodium acetate and 2 volumes of 100% ethanol. After centrifugation, DNA pellets were washed twice in 70% ethanol, resuspended in 100 µl TE (10 mM Tris·HCl, pH 8.0, 1 mM EDTA) and stored at 4°C. The PCR-based markers used in this analysis are described at The Arabidopsis Information Resource (http://www.arabidopsis.org/) and include for chromosome 2: RGA, nga1145, m246, mi310, T10J7-t7, THY1B, PLS2,
PLS4, PLS8, nga1126, nga361, m323, nga168, BIO2, ML, GBF3, SGCSNP1098; and for chromosome 4: Tel4N, JV30/31, CIW5, GA1, T5L23.3, nga8, nga1111, DET1, SGCSNP385, CIW6, COP9B, SC5, g4539, AG, CIW7, RPS2, nga1139, JM411, nga1107, DHS1, SGCSNP53. PCR primers were purchased from Research Genetics (Huntsville, AL), Invitrogen Life Technologies (Carlsbad, CA) or MWG (Highpoint, NC). Markers were amplified by PCR using the following parameters: hotstart at 95° 30 sec, denaturing at 94° 15 sec (40 cycles), annealing optimized for each marker between 52° to 57° (10 cycles) followed by 54° to 61° (30 cycles), and extension at 72° (40 cycles) in an MJ Research (Cambridge, MA) DYAD. Markers were visualized with UV light following electrophoresis of PCR products using either 1% agarose gels or 14% native polyacrylamide gels and staining with ethidium bromide. If necessary, polymorphisms were detected by digesting PCR reactions with restriction enzymes prior to electrophoresis (KONIECZNY and AUSUBEL 1993).

**Data analysis**: Marker scores for 143 tetrads were recorded and then verified by two individuals to avoid clerical errors. The tetrad data were analyzed using the methods described in the appendix of HOUSWORTH AND STAHL (2003). The details of the analysis differ from those given in COPENHAVER et al. (2002), but the results of the two methods for the two chromosomes studied in this paper are statistically equivalent. The methods of HOUSWORTH AND STAHL (2003) are appropriate here because the chromosomes in this paper are so well marked that the position of a crossover on a chromosome is known within an error never exceeding 4 cM. The methods of HOUSWORTH AND STAHL (2003) are also computationally faster and facilitate simulations required for assessing significance and providing confidence intervals for the data studied in this paper.
RESULTS

**Fundamental recombination parameters:** We examined the frequency and distribution of crossover events in 143 Arabidopsis tetrad by scoring 17 molecular markers on chromosome 2 and 21 markers on chromosome 4. These markers span 98% and 97% of chromosomes 2 and 4 respectively. This coverage represents a 19% improvement for chromosome 2 and 9% improvement for chromosome 4 over tetrad analysis data reported for *Arabidopsis* previously (COPENHAVER *et al.* 1998). Adjacent markers on both chromosomes are separated by less than 10 cM. Pollination with single pollen tetrads sometimes results in fewer than four progeny plants. Both four and three-member tetrads are useful for tetrad analysis since for most purposes the fourth member can be inferred from the genotype of the remaining three members. In this study, 32 of the 143 tetrads examined contained only 3 members. In addition, due to limited DNA stocks, there were seven cases for chromosome 2 and fourteen cases for chromosome 4 where scores were not recorded for at least one marker in one member of an otherwise four-member tetrad.

A summary of the observed crossover events for each chromosome is presented in Table 1. The average number of crossovers per chromosome tetrad (bivalent) was similar, with chromosome 2 experiencing 1.7 per meiosis and chromosome 4 experiencing 1.5. Importantly, these bivalents experienced multiple crossovers in sufficient meioses to enable an examination of interference parameters (Figure 1). On both chromosomes the majority of crossovers occurred on the longer arm of these short, acrocentric chromosomes. In only one case, on chromosome 2, did we observe a meiosis that
apparently lacked a crossover. This could be due to the occurrence of two closely spaced 2-chromatid crossovers not separated by an intervening marker. However, the observed ratio of 2:3:4-chromatid double crossovers argues that our marker density is sufficient to detect nearly all double crossovers. Alternatively, the single meiosis apparently lacking a crossover on chromosome 2 could be explained by an undetected crossover on one or the other extreme terminus of the chromosomes. Such events have been observed in Arabidopsis using cytological analysis (SANCHEZ-MORAN et al. 2002; SANCHEZ MORAN et al. 2001). It is also possible that this bivalent did not experience a crossover: the model we used to simulate expected distributions of inter-crossover distances in Arabidopsis predicts about 1% non-exchange bivalents for chromosome 2.

**Interference-sensitive and insensitive crossovers occur on the NOR-bearing chromosomes in Arabidopsis:** Our previous analyses suggest that the distribution of inter-crossover distances on Arabidopsis chromosomes 1, 3 and 5 is consistent with the presence of a fraction of crossovers that are insensitive to interference. The paucity of crossover events on chromosomes 2 and 4 prevented our drawing any statistically relevant conclusion for these short, acrocentric NOR-bearing chromosomes. To remedy this, we scored a denser set of molecular markers that covers a greater proportion of chromosomes 2 and 4 in approximately 3 times as many tetrads. Using a likelihood ratio test, we examined this expanded data set and asked whether a model wherein each crossover is subject to interference ($p = 0$) was more or less likely than a model wherein some fraction of crossovers is resistant to interference ($p > 0$). The results of this analysis are presented in Table 2. Like the results previously shown for chromosomes 1, 3, and 5,
the data fit the two-pathway model substantially better than the interference-only model. Although the likelihood ratio testing we have presented provides a powerful method of comparing models and gives strong evidence that the addition of interference-insensitive crossovers improves the original counting model, goodness of fit assessment would require on the order of ten times the number of tetrads and is beyond the scope of this study. That is, while the two-pathway model is significantly more likely, due to lack of power in goodness-of-fit testing procedures, we have no statistical evidence that either model fails to fit the data.

Interference-insensitive crossovers occur less frequently on the NOR-bearing chromosomes: Our previous analysis of crossover interference on chromosomes 1, 3 and 5 in Arabidopsis yielded an estimate of the fraction of crossovers insensitive to interference, \( p \), of 0.2 for each chromosome (COPENHAVER et al. 2002). The current analysis reveals that chromosome 2 and 4 have much smaller values of \( p \), approximately 0.03 and 0.05, respectively, with 95% confidence intervals of (0.003, 0.059) and (0.023, 0.097), respectively. Thus, the distributions of crossovers on small, acrocentric, NOR-bearing chromosomes of Arabidopsis conform more closely to the simple counting model (FOSS et al. 1993) than do those on the remaining chromosomes, which are longer, metacentric and lack NOR regions.

DISCUSSION

Pairing centers and interference: Pairing is an essential step in organizing and properly distributing homologous chromosomes during meiosis (McKee 2004). Genetic
and cytological analyses indicate that pairing is not dependent on the formation of
double-strand breaks (DSBs) but that DSB repair by crossing-over likely plays a role in
stabilizing pairing (CHA et al. 2000; ZICKLER and KLECKNER 1999). Conversely,
homologous meiotic recombination is operationally dependent on some juxtaposition of
homologous chromosomes. Homologous chromosome pairing can also be stabilized via
specialized pairing centers. In Caenorhabditis elegans each chromosome has a pairing
site (MCKIM et al. 1988), and pairing centers have been observed in plants as well
(MAGUIRE 1986). In Drosophila the NOR is a well-characterized pairing site (MCKEE
and KARPEN 1990). Indeed, it has been determined that only a few copies of the
intergenic spacer regions of the Drosophila rDNA are necessary to mediate chromosome
pairing (MCKEE et al. 1992). Similar NOR-driven chromosome pairing has been
observed in mammals (STITOU et al. 1997). Given the necessity of homolog pairing, the
relationship between pairing and recombination, the ability of NORs to serve as pairing
centers, and the interference data presented in this paper, it is pertinent to ask if there is a
relationship between the presence of an NOR on a chromosome and the frequency of
non-interfering crossovers.

In Arabidopsis, homologous chromosome association appears to involve
telomeres. FISH analysis of Arabidopsis chromosomes shows that telomeres associate
with the NOR during pre-meiotic interphase. That association presumably assists in
pairing and is later lost during leptotene and replaced with a loose bouquet formation in
zygotene (ARMSTRONG et al. 2001). Whether loose zygotene telomere association is
comparable to the strong “classical bouquet” seen in other organisms remains unclear. In
asyl, an Arabidopsis mutant that abolishes synapsis, the pre-meiotic NOR-associated
telomere clustering is maintained. However, because *asyl* mutants fail to synapse the chromosome pairs eventually disjoin yielding univalents, including the NOR-bearing chromosomes (ARMSTRONG et al. 2001). Thus, the NOR regions may be implicated in assisting chromosome organization during meiosis, but more analysis needs to be done to determine any specific role in pairing.

The shortage of non-interfering crossovers on the NOR-bearing chromosomes of Arabidopsis can be rationalized in the framework presented in COPENHAVER et al. (2002) and expanded in STAHL et al. (2004). By several criteria, Arabidopsis is a “Group II” organism, whose chromosome synapsis depends on recombination functions (GRELON et al. 2001). We postulate that the crossovers resulting from these presynaptic events are interference-free. Events initiated post-synaptically, on the other hand, give rise to crossovers that are subject to interference (among each other), presumably according to the counting rules of FOSS et al. (1993). Within this framework, the presence of NORs acting as pairing centers on the two short chromosomes reduces the need for the noninterfering crossovers. It is possible that the differentiation from non-interfering to interfering crossovers is controlled by the establishment of synapsis.

In *D. melanogaster* and *C. elegans*, both of which are Group I organisms lacking synapsis-promoting recombination events (STAHL et al. 2004), deletion of pairing centers, such as NORs, decreases crossingover and deters synapsis (HAWLEY 1980; VILLENEUVE 1994). Examination of pairing partner switches in autotetraploid lines suggests that Arabidopsis chromosomes also harbor multiple autonomous pairing sites (SANTOS et al. 2003). Low frequencies of chromosome 2 and 4 multivalent formation in autotetraploid lines may indicate the existence of a particularly strong pairing site,
perhaps the NOR, that dominates the pairing choice for the length of these chromosomes (SANTOS et al. 2003). It is interesting to note that in these studies chromosome 2 exhibits the lowest multivalent frequencies (and therefore the most persistent bivalent pairing), and in our experiments chromosome 2 exhibits the fewest non-interfering crossovers. These observations suggest that the presence of NOR domains (putative pairing centers) influences the relative frequencies of two distinct classes of crossovers in Arabidopsis. We find this hypothesis particularly interesting since it implies that interference is regulated at a chromosomal level in a manner that reflects chromosome architecture.

**Distribution of interference-sensitive and insensitive crossovers:** The mathematical model that we used to simulate the distribution of inter-crossover distances on the chromosomes of Arabidopsis has two variables: $p$, which is the portion of interference-insensitive crossovers out of the total crossover population, and $m$, which is the obligate number of “failures” between any two interfering crossovers. As an aside, it should be noted that many other intriguing models of interference have been proposed (e.g., BORNER et al. 2004; FUJITANI et al. 2002; KING and MORTIMER 1990). The interference parameters for chromosomes 1, 3 and 5 in our previous study varied from 10 to 17, and all estimates in that range were statistically indistinguishable due to the lack of statistical power of the analysis. The level of interference for chromosomes 2 and 4 estimated in this study is about $m = 9$ (+/- 2). Thus, interference-sensitive crossovers may be subject to the same intensity of interference ($m$) on all the Arabidopsis chromosomes. This is striking given the large difference in the frequencies ($p$) of the interference-insensitive crossovers. We propose that this reflects a fundamental difference in the way
that interference insensitive and sensitive crossovers are apportioned on chromosomes, while the strength of interference ($m$) is consistent for all crossovers subject to interference, regardless of chromosome.

HIGGINS et al. (2004) recently provided genetic support for the two-pathway model of recombination in Arabidopsis by examining mutants in the $MSH4$ gene. In $S. \textit{cerevisiae}$ $msh4$ mutants have reduced crossover frequency and the residual crossovers are free of interference (NOVAK et al. 2001) Chiasmata in $Atmsh4$ mutants are reduced by approximately 85% and the remaining chiasmata are distributed randomly among cells and chromosomes suggesting that they are free of interference. However, the initial analysis of $Atmsh4$ lacked the resolution to measure interference on individual chromosomes. Nonetheless, it is intriguing to note that the observed 85% reduction in chiasmata is consistent with the modeling prediction that approximately 80% of all crossovers on chromosomes 1, 3 and 5 are insensitive to interference (COPENHAVER et al. 2002). It should be noted that HIGGENS et al. (2004) did not report any differences in the level of non-interfering crossovers between the five Arabidopsis chromosomes.

This study examines interference on chromosomes 2 and 4 of Arabidopsis. In addition to harboring large NOR domains these chromosomes are also shorter than the other three Arabidopsis chromosomes. In yeast, short chromosomes have been shown to have a higher crossover density and exhibit weaker interference when compared to longer chromosomes (KABACK et al. 1999). Consistent with these observations, DE LOS SANTOS et al. (2003) report that interference insensitive crossovers occur more frequently on smaller chromosomes in yeast. Taken together these findings suggest that one would expect that chromosomes 2 and 4 should have a relatively higher fraction of interference.
insensitive crossovers – precisely the opposite of what we observe. This discrepancy highlights the need for analysis that disentangles the effects of chromosome stature and chromosome architecture (NOR presence) on crossover interference.

The counting model of FOSS et al. (1993) proposes that $m$ represents non-crossover gene conversion events. We have calculated the predicted number of gene conversions expected on chromosomes 2 and 4 using the assumptions and formulas described in COPENHAVER et al. (2002) for similar calculations on chromosomes 1, 3 and 5. These calculations predict 0.74 and 0.83 gene conversions for chromosomes 2 and 4 respectively in the 143 tetrads assayed. This is consistent with our observed data described here, which did not detect any gene conversions.

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FIGURE 1. Distribution of crossover events on NOR-bearing Arabidopsis chromosomes. Crossover events (black boxes) in 16 intervals on chromosomes 2 and 20 intervals on chromosome 4 (white boxes) were detected by scoring PCR-based molecular markers in 143 Arabidopsis tetrads (vertical axis). Intervals are not drawn to scale but are represented as equal sized boxes (horizontal axis). Chromosome arms are defined by the centromere positions (grey circle) described in COPENHAVER et al. (1999).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chrm 2</th>
<th>Chrm 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrads scored</td>
<td>143</td>
<td>143</td>
</tr>
<tr>
<td>Total CO observed</td>
<td>239</td>
<td>212</td>
</tr>
<tr>
<td>Average COs per meiosis</td>
<td>1.67</td>
<td>1.48</td>
</tr>
<tr>
<td>Total COs on short arms</td>
<td>42</td>
<td>30</td>
</tr>
<tr>
<td>Average COs on short arms</td>
<td>0.29</td>
<td>0.21</td>
</tr>
<tr>
<td>Total COs on long arms</td>
<td>197</td>
<td>182</td>
</tr>
<tr>
<td>Average COs on long arms</td>
<td>1.38</td>
<td>1.27</td>
</tr>
<tr>
<td>Short arms w/o COs</td>
<td>100</td>
<td>113</td>
</tr>
<tr>
<td>Long arms w/o COs</td>
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<td>1</td>
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### TABLE 2

Estimates of interference parameters

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<tr>
<th>Chromosome</th>
<th>Extended model</th>
<th>Null model</th>
<th>Likelihood-ratio</th>
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<tr>
<td></td>
<td>$m$ estimate$^a$</td>
<td>$m$ estimate</td>
<td>$p$ estimate</td>
</tr>
<tr>
<td>2</td>
<td>5.43</td>
<td>8.7</td>
<td>0.029</td>
</tr>
<tr>
<td>4</td>
<td>4.74</td>
<td>9.3</td>
<td>0.054</td>
</tr>
</tbody>
</table>

$^a$ Best value of $m$ if $p$ is set at zero.

$^b$ Probability (obtained via simulations) that the difference between the observed $p$ estimate and zero could be due to sampling error alone.
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