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Note

Does Chromosome Size Affect Map Distance and Genetic Interference in Budding Yeast?

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ABSTRACT

The hypothesis that chromosome size affects the rate and distribution of meiotic crossovers in budding yeast was tested. Map distance and interference were measured in the same genetic intervals present on either small (340 and 508 kb) or large (917 and 1085 kb) chromosomes, respectively. No differences were observed.

DURING meiosis, crossovers between homologous chromosomes, in combination with sister-chromatid cohesion, create physical connections that promote the accurate segregation of chromosomes to opposite poles at the first meiotic division (Bascom-Slack *et al.* 1997; Petronczki *et al.* 2003). Genetic interference is a phenomenon by which a crossover in one interval decreases the probability that additional crossovers will occur nearby. The distribution of crossovers by interference has been postulated to ensure that every pair of homologs receives at least one. The degree of interference and the number of crossovers per meiosis varies between organisms. For example, in nematodes interference is strong, such that each chromosome pair undergoes only a single crossover (Hillers and Villeneuve 2003). In contrast, in fission yeast there is no interference, but because there is a large number of crossovers and only three chromosomes, the probability that each chromosome will receive a crossover is high (Munz 1994). In budding yeast, there are ~90 crossovers per meiosis (Mortimer *et al.* 1991). There are 16 pairs of homologs and these chromosomes vary in size from 230 to 1530 kb (*Saccharomyces* Genome Database). Mutations that abolish interference increase nondisjunction of small chromosomes preferentially, consistent with the idea that crossovers are distributed to ensure that all chromosomes receive at least one (Sym and Roeder 1994). Studies in the literature using bisected and translocated chromosomes suggest that chromosome size is important in determining the rate of recombination (in centimorgans per kilobase) as well as the degree of interference (Kaback *et al.* 1992, 1999). We sought to test this hypothesis by comparing map distance and interference values obtained from the same genetic intervals present on either small or large chromosomes. Contrary to what has been previously reported, we saw no effect of chromosome size on either of these parameters. We conclude, therefore, that differences in the rate of recombination and interference observed between chromosomes in budding yeast, at least for some intervals, are a function of their DNA sequence as opposed to the size of the chromosome.

To allow direct comparison between the same DNA sequences on different-size chromosomes, ectopic recombination was used to make a reciprocal translocation that exchanges the right arm of chromosome VII for the last ~8 kb of the right arm of chromosome III and vice versa, using the approach described in Borde *et al.* (2000; Figure 1, A and B). Recombination between *ade2* truncation alleles on chromosomes III and VII generated Ade$^+$ cells that were screened by polymerase chain reaction (PCR) and Southern blot analysis to confirm the presence of the translocation (Figure 1B). Three intervals on chromosomes III and VII were then analyzed on either a short chromosome (340 and 508 kb, respectively) or a long chromosome (917 and 1085, respectively). The isogenic diploids, NH598 (which contains the native chromosomes) and NH607 (which contains the translocation chromosomes), were sporulated and dissected. Over 1000 tetrads were analyzed for each diploid. The map distance of each interval was unaffected by the change in chromosome size (Table 1).

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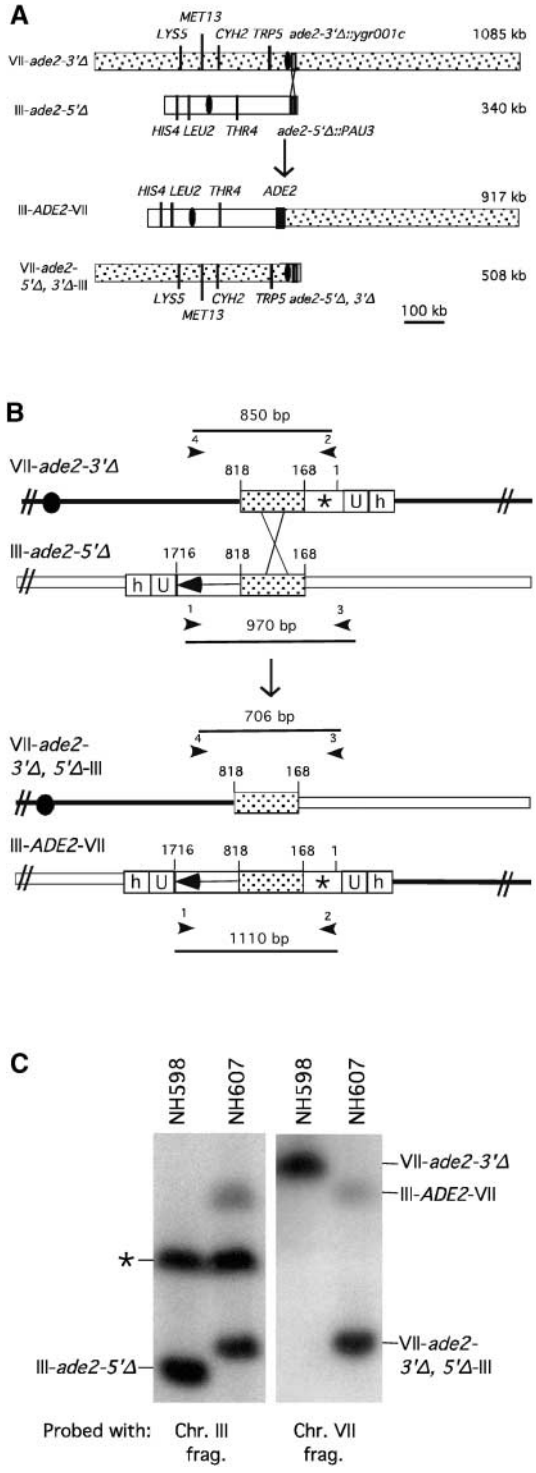

FIGURE 1.—Construction of a reciprocal translocation between chromosome III and VII by ectopic recombination. (A) Overview of the translocation strategy drawn to scale. A cassette containing a truncation of the 3′ end of the *ADE2* gene, *URA3*, and *hisG* was integrated 680 bp from the centromere on chromosome VII. A cassette containing *hisG*, *URA3*, and a 5′ truncation of *ADE2* was integrated 7.6 kb from the telomere of the right arm of chromosome III. Recombination between the two truncated *ade2* alleles produces Ade$^+$ colonies containing two reciprocal translocation chromosomes (BORDE *et al.* 2000). Solid ovals represent centromeres; rectangles represent *ade2* cassettes; the stippled bar indicates chromosome VII sequences while the open bar represents chromosome III. (B) Expanded view of the *ade2* truncation cassettes and their recombination products (not drawn to scale). h, *hisG*; U, *URA3*; dotted bar, the region of *ADE2* shared between the two truncation cassettes; the arrow and the asterisk indicate the 3′ and 5′ ends of the *ADE2* gene, respectively. To create the strains, a 576-bp fragment from chromosome III (coordinates 308386–308962 from the *Saccharomyces* Genome Database, located between *PAU3* and *ADH7*) and a 583-bp fragment from chromosome VII (coordinates 497716–498299, one end of which is within *YGR001c*) were amplified and cloned into pVZ1. Site-directed mutagenesis was used to introduce an *Sph*I and a *Xho*I site into the III and VII sequences, respectively. A 4.9-kb *Sph*I fragment from pMJ436 containing the *hisG-URA3-ade2-5′*Δ cassette was cloned into the *Sph*I site to generate pTS93. A 5.3-kb *Xho*I fragment from pMJ437 containing the *ade2-3′*Δ*-URA3-hisG* cassette was inserted into the *Xho*I site to make pTS87. *Bam*HI/*Sac*I-digested pTS93 and *Sph*I/*Sac*I-digested pTS87 were used to transform *MAT***a** and *MAT*α derivatives of two SK1 strains of complementary genotypes (G1 and G2) using *URA3* as the selectable marker. To generate G1 and G2 strains that contained both truncation cassettes, the G1 VII::*ade2-3′*Δ haploid was crossed to the G1 III::*ade2-5′*Δ haploid. The diploid was sporulated and dissected and Ura$^+$ segregants were screened by Southern blot analysis for the presence of both cassettes. The G2 strains were treated similarly. G1 and G2 haploids carrying both *ade2* truncations were crossed to create the nontranslocation diploid, NH598. This diploid was converted to Ade$^+$ by transformation of an *ADE2* fragment. To generate the translocations, G1 and G2 haploids carrying both cassettes were plated on SD-ade medium. Ade$^+$ colonies arose at a frequency of 2–4 × 10^{-7}. The Ade$^+$ colonies were screened for the VII-III translocation using yeast colony PCR (see http://www.fhcrc.org/labs/hahn/methods/mol_bio_meth/per_yeast_colony.html). The presence of this translocation was indicated by the formation of a 706-bp fragment when the TL3 and TL4 primers were used for amplification and the concomitant loss of the 850- and 970-bp fragments obtained using the TL4/TL2 and TL1/TL3 primer combinations, respectively. Primers are indicated by arrowheads. Primer sequences were TL1, 5′-TTCCGCCATAC TGGAGGC-3′; TL2, 5′-ATGGATTCTAGAACAGTTGG-3′; TL3: 5′-ATACA CACATAAGTAGGCAC-3′; TL4, 5′-CGGTTTCATTAAGATGTAAG-3′). A G1 translocation haploid was mated to a G2 translocation haploid to make the diploid NH607. Strain genotypes are as follows:

NH598: $\dfrac{his4\ \ leu2\ \ MAT\alpha\ \ THR4\ \ III::hisG\text{-}URA3\text{-}ade2\text{-}5'\Delta}{HIS4\ LEU2\ MAT\mathbf{a}\ \ thr4\ \ III::hisG\text{-}URA3\text{-}ade2\text{-}5'\Delta}\ \dfrac{ADE2}{ade2}\ \dfrac{ura3}{ura3}$

$\dfrac{lys5\ \ MET13\ \ cyh2\ \ TRP5\ \ VII::ade2\text{-}3'\Delta\text{-}URA3\text{-}hisG}{LYS5\ met13\ \ CYH2\ trp5\ \ VII::ade2\text{-}3'\Delta\text{-}URA3\text{-}hisG}$

NH607: $\dfrac{his4\ \ leu2\ \ MAT\alpha\ \ THR4\ \ III::hisG\text{-}URA3\text{-}ADE2\text{-}URA3\text{-}hisG::VII_{right\ arm}}{HIS4\ LEU2\ MAT\mathbf{a}\ \ thr4\ \ III::hisG\text{-}URA3\text{-}ADE2\text{-}URA3\text{-}hisG::VII_{right\ arm}}$

$\dfrac{lys5\ \ MET13\ \ cyh2\ \ TRP5\ \ VII::ade2\text{-}3'\Delta,\ 5'\Delta::III_{telomere}}{LYS5\ met13\ \ CYH2\ trp5\ \ VII::ade2\text{-}3'\Delta,\ 5'\Delta::III_{telomere}}\ \dfrac{ade2}{ade2}\ \dfrac{ura3}{ura3}$

(C) Southern blot confirming the formation of the translocation chromosomes. NH598 and NH607 were grown to stationary phase in YEPD and cell plugs were made as described in BORDE *et al.* (1999). The chromosomes were fractionated on a contour-clamped homogeneous electric field gel, transferred to a nylon membrane and probed with either the chromosome III or the chromosome VII amplified fragments. The chromosome III fragment was observed to cross-hybridize with a second chromosome indicated by an asterisk.

TABLE 1

Map distances and NPD ratios from translocation and nontranslocation diploids

	Map distance (cM)[a]																							
	Chromosome III markers[b]												Chromosome VII markers[b]											
	HIS4-LEU2[c]				*LEU2-MAT*				*MAT-THR4*				*LYS5-MET13*				*MET13-CYH2*				*CYH2-TRP5*			
Strain	P	N	T	cM	P	N	T	cM	P	N	T	cM	P	N	T	cM	P	N	T	cM	P	N	T	cM
NH598	804	3	228	11.9	563	18	438	26.8	709	3	312	16.1	623	5	366	19.9	807	1	217	10.9	348	41	655	43.1
NH607	781	2	229	11.9	600	16	373	23.7	672	6	310	17.5	574	7	412	22.9	769	0	231	11.5	311	42	667	45.0
NPD ratio[b]																								
NH598	0.40 ± 0.23				0.51 ± 0.13				0.20 ± 0.11				0.22 ± 0.10				NA				0.36 ± 0.06			
NH607	0.26 ± 0.18				0.65 ± 0.17				0.38 ± 0.15				0.22 ± 0.09				NA				0.30 ± 0.06			

	HIS4-MAT		*LEU2-THR4*		*LYS5-TRP5*	
	Map distance	NPD ratio	Map distance	NPD ratio	Map distance	NPD ratio
NH598	37.0	0.57 ± 0.10	43.6	0.50 ± 0.08	64.9	0.68 ± 0.12
NH607	35.2	0.57 ± 0.11	41.2	0.75 ± 0.12	70.1	0.86 ± 0.13

[a] Spore viability for NH598 was 94.7% (1305 tetrads) and for NH607, 96.4% (1282 tetrads).

[b] Tests for statistical significance were performed using the program available at http://groik.com/stahl/. NPD ratios were calculated by the Papazian equation (Papazian 1952) using the software at this website. NA, not applicable because too few N tetrads.

[c] Map distances were calculated using the Perkins formula (Perkins 1949). P, parental ditype; N, nonparental ditype; T, tetratype.

Gene conversion was also unaffected (data not shown). To monitor interference, the ratio of observed nonparental ditypes (or NPDs, which arise from four-strand double crossovers) to those expected assuming no interference, was calculated. An NPD ratio of 1 indicates no interference, while an NPD ratio of 0 indicates complete interference (Snow 1979). Although differences in NPD ratio were observed, these differences are not likely to be significant as the standard deviations of all the intervals overlap, with the exception of the *LEU2-THR4* interval (where at least 48 NPDs were scored for each diploid; Table 1). In the *LEU2-THR4* interval, the NPD ratio is increased in the translocation diploid, in contradiction to what was expected from the literature. The prediction was that moving this interval from a small chromosome to a large chromosome should increase the amount of interference (*i.e.*, decrease the NPD ratio).

Our experiments unambiguously demonstrate that changes in chromosome size, by either an increase of 2.6-fold or a decrease of 2.1-fold, have no effect on the rate of recombination or the degree of interference for specific intervals on chromosomes III and VII. Why do these results differ from those in the literature? One possible explanation is that intervals on different chromosomes are differentially sensitive to changes in chromosome size. In the work by Kaback *et al.* (1992, 1999), recombination was measured using translocations between chromosomes I and II, while this work used translocations between chromosomes III and VII. Alternatively, there may be genetic differences between the SK1 strains used in this study and the non-SK1 strains used by the Kaback lab that are responsible. Whatever the reason, the main finding of this article is that the phenomenon of chromosome size affecting recombination rates and interference is not a general one.

Different intervals in our experiment clearly exhibited different rates of recombination as measured by centimorgans per kilobase. The highest rates were observed for the chromosome III intervals *MAT-THR4* and *HIS4-LEU2*, irrespective of chromosome size (Table 2). Interestingly, chromosome III also contained the interval that exhibited the lowest rate of recombination. Not surprisingly, the rate of recombination correlates well with the frequency of double-strand breaks (DSBs) formed in or near these intervals (Baudat and Nicolas 1997; Gerton *et al.* 2000; Borde *et al.* 2004). Whereas chromosome III has well-defined hotspots for DSB formation, the breaks on chromosome VII are more evenly distributed (Gerton *et al.* 2000; Borde *et al.* 2004). Therefore, differences in recombination rates between chromosomes are likely due to DNA sequences that lead to different chromosomal contexts that influence the frequency of DSB formation, rather than to chromosome size.

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