

# Variation in crossing-over rates across chromosome 4 of *Arabidopsis thaliana* reveals the presence of meiotic recombination “hot spots”

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Crossover (CO) is a key process for the accurate segregation of homologous chromosomes during the first meiotic division. In most eukaryotes, meiotic recombination is not homogeneous along the chromosomes, suggesting a tight control of the location of recombination events. We genotyped 71 single nucleotide polymorphisms (SNPs) covering the entire chromosome 4 of *Arabidopsis thaliana* on 702 F2 plants, representing 1404 meioses and allowing the detection of 1171 COs, to study CO localization in a higher plant. The genetic recombination rates varied along the chromosome from 0 cM/Mb near the centromere to 20 cM/Mb on the short arm next to the NOR region, with a chromosome average of 4.6 cM/Mb. Principal component analysis showed that CO rates negatively correlate with the G+C content ( $P = 3 \times 10^{-4}$ ), in contrast to that reported in other eukaryotes. COs also significantly correlate with the density of single repeats and the CpG ratio, but not with genes, pseudogenes, transposable elements, or dispersed repeats. Chromosome 4 has, on average, 1.6 COs per meiosis, and these COs are subjected to interference. A detailed analysis of several regions having high CO rates revealed “hot spots” of meiotic recombination contained in small fragments of a few kilobases. Both the intensity and the density of these hot spots explain the variation of CO rates along the chromosome.

[Supplemental material is available online at [www.genome.org](http://www.genome.org).]

Meiotic crossovers (COs) and sister chromatid cohesion provide physical links between homologous chromosomes ensuring proper chromosome segregation during the first meiotic division. In most eukaryotes, there is always at least one CO per pair of homologs (obligatory crossover) (Jones 1984, 1987). Cytological, genetic, and molecular studies in many organisms have demonstrated that COs are not evenly distributed along the chromosomes (Jones 1987; Carpenter 1988; Lynn et al. 2002). The tight control of the number and/or localization of COs is crucial. Mutations that reduce CO formation increase chromosome nondisjunction in organisms as diverse as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, *Drosophila melanogaster* (female), *Arabidopsis thaliana*, and the mouse (for review, see Lynn et al. 2004).

In yeast, the distribution of meiotic recombination events (COs and noncrossover gene conversions; NCOs) along chromosomes has been studied in detail by locating DNA double-strand breaks (DSBs), which initiate meiotic recombination (Baudat and Nicolas 1997; Gerton et al. 2000). These studies showed that

DSBs tend to be clustered in chromosomal domains away from telomeres and centromeres (Gerton et al. 2000; Borde et al. 2004). In mammals, COs are also nonrandomly distributed along the chromosomes, with alternate domains having higher or lower levels of recombination (Kong et al. 2002; Nachman 2002). The CO rates tend to be low near the centromeres and increase toward the telomeres. In plants, the CO rates also vary along chromosomes (for review, see Anderson and Stack 2002). In general, centromeric regions have low CO rates compared to telomeric regions. However, in plants, there have been very few high-resolution studies in a single chromosome.

Many sequence parameters have been linked to the variation of CO rates in eukaryotes. In yeast and mammals, several studies have found a correlation between a high G+C content and a high rate of recombination in large domains (Gerton et al. 2000; Fullerton et al. 2001; Yu et al. 2001; Kong et al. 2002; Petes and Merker 2002; Jensen-Seaman et al. 2004). However, within 2–3 kb of the recombination initiation site no correlation between the G+C content and the distribution of COs in both yeast and humans was found (for review, see de Massy 2003) and, second, in human, rat, and mouse, when CpG ratio is included in a multiple regression analysis, correlation with the G+C content becomes negative (Kong et al. 2002; Jensen-Seaman et al. 2004). In wheat, barley, and maize, gene-rich regions are more recombinationally active than gene-poor regions (for review, see

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Schnable et al. 1998). In humans, female CO rates are not correlated with gene density on chromosome 21 (Lynn et al. 2000) whereas male CO rates are correlated, suggesting a different type of control. There are also conflicting results when correlating the density of transposable elements (TEs) and recombination rates (see Wright et al. 2003). Nevertheless, differences in meiotic CO rates between the sexes have been demonstrated in many higher eukaryotes (Lenormand and Dutheil 2005). Therefore, the primary DNA sequence itself cannot explain all of the variation of meiotic recombination.

In *S. cerevisiae* and *S. pombe*, hot spots have been defined as small DNA fragments of 1–2 kb, centered around meiotic DSBs that are repaired, using the homologous chromosome, to produce COs or NCOs (Keeney 2001). In mice, humans, and plants, several such regions have been studied in detail and have been found to share common features with hot spots described in yeast. These include high level of COs and NCOs clustered in small segments (1–2 kb) and a lack of clear consensus sequences (de Massy 2003; Kauppi et al. 2004; Rafalski and Morgante 2004). The distribution of meiotic hot spots along chromosomes is uneven, which suggests a local control of DSB formation. A lot of effort has been made recently to characterize this fine-scale variation of recombination rates mainly in humans but also in other eukaryotes for various reasons among which is to gain insight into the underlying mechanisms, to assist association studies, or to improve inferences from polymorphism data about selection and population history. However, except for *S. cerevisiae*, only a few regions have been characterized at the molecular level in other eukaryotes and more genome-wide studies are needed to unravel the determinants of hot spot activity.

The availability of the *Arabidopsis* genome sequence (The *Arabidopsis* Genome Initiative 2000) and the recent development of powerful high-throughput genotyping techniques (Gut 2001; Kwok 2001), allow us to determine precisely the location and rates of COs on one chromosome. Here, we show that CO rates are highly variable on chromosome 4 of *Arabidopsis*, with some regions having five times more COs than the chromosome average. The CO rates significantly negatively correlate with the G+C content and also significantly correlate with the density of single repeats and with CpG ratio. However, they do not correlate significantly either with genes, pseudogenes, transposable elements, or dispersed repeats. Our data also confirm that COs are subjected to interference on chromosome 4. Finally, we provide evidence of meiotic recombination hot spots and show that both their activity and density contribute to the variation of the CO rates.

## Results

Chromosome 4 of *A. thaliana* is the smallest of its five chromosomes and presents several remarkable features (Fig. 1). It has an acrocentric architecture with a long arm 14.6 Mb long and

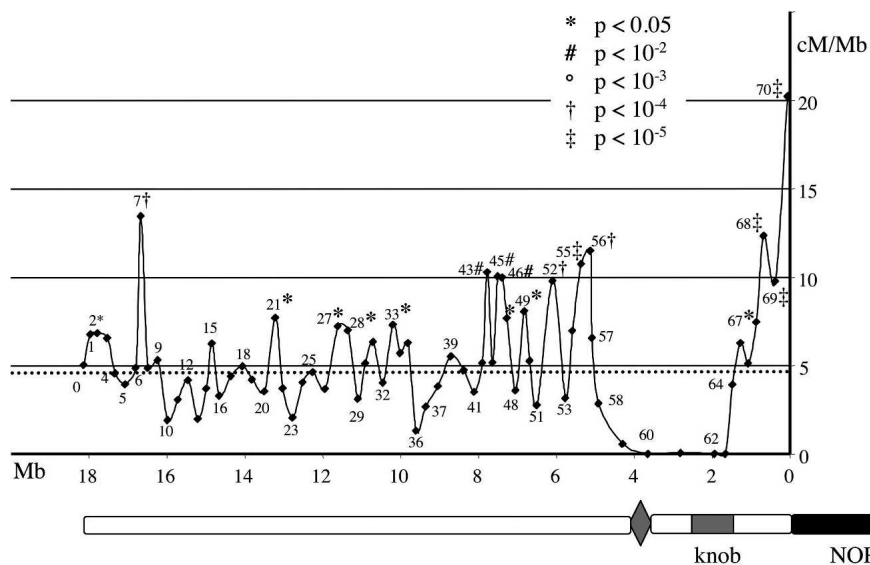
short arm about 8 Mb long tipped by the nucleolar organizer region (NOR). This region is about 3.6–4 Mb long and is constituted of almost homogeneous ribosomal DNA repeats (Haberer et al. 1996). The available short arm sequence starts in the last proximal copy of the rDNA repeat (Mayer et al. 1999; The *Arabidopsis* Information Resource, <http://www.arabidopsis.org/>). In some accessions, including Columbia (Col) but not Landsberg (Ler), the short arm has a heterochromatic region, called the “knob,” identified cytologically (Fransz et al. 2000), primarily comprising transposable elements, in which a few genes are insulated (Mayer et al. 1999; Lippman et al. 2004). Moreover, an approximately 1.5-Mb-long region of the short arm, including the knob, is inverted between the two accessions, Col and Ler (Fransz et al. 2000).

We genotyped a population of 736 F2 plants resulting from a cross between Col and Ler (see Methods) with 71 SNPs (Supplemental Table 1) chosen from the Monsanto database (Jander et al. 2002) to be evenly spaced on the *Arabidopsis* chromosome 4. The average interval between two SNPs was 204 kb on the long arm (60 SNPs) and 239 kb on the short arm (11 SNPs).

### Variation of CO rates across chromosome 4

After SNP genotyping, we analyzed the variation in CO rates in 702 plants (34 plants had missing data for more than 24 markers and were thus discarded). On average, we genotyped 666 plants (thus representing 1332 meioses because in an F2 plant each chromosome comes from an independent meiosis) per interval. We verified that there was no bias in the segregation of each marker. The cumulated genetic distance of the chromosome was estimated to be 83.9 cM, of which 69 cM corresponded to the long arm (Supplemental Table 2).

As the intervals were small, the genetic length of each interval can be simply calculated by dividing the number of recombinant chromosomes by the number of meioses analyzed. Genetic recombination varied greatly along the chromosome, from



**Figure 1.** Variation of the CO rates on chromosome 4 of *A. thaliana*. The numbers refer to the intervals given in Supplemental Table 2. The dotted line represents the average CO rate on chromosome 4 (4.6 cM/Mb). A schematic representation of chromosome 4 of *A. thaliana* is aligned with the diagram. (Black box) NOR (nucleolar organizer region), (gray box) heterochromatic knob, (diamond-shaped box) centromere.

0 cM/Mb next to the centromere, to 20.2 cM/Mb next to the NOR (Supplemental Table 2; Fig. 1). The frequencies of COs in different intervals could not be directly compared because of both the variation in interval length and the number of analyzed chromosomes. Therefore, we developed a statistical approach to unambiguously identify intervals that were significantly either “colder” or “hotter” than the chromosome average. The approach is based on a simply binomial model of the number of COs in each interval, so that the “temperature” of an interval is determined by the probability that the number of COs in it exceeds the expected one, under the assumption that the recombination rate is constant along the chromosome. We implemented a statistical program (TETRA) to compute both the average number of COs per nucleotide, and the significance of the observed values from the binomial model (see Methods).

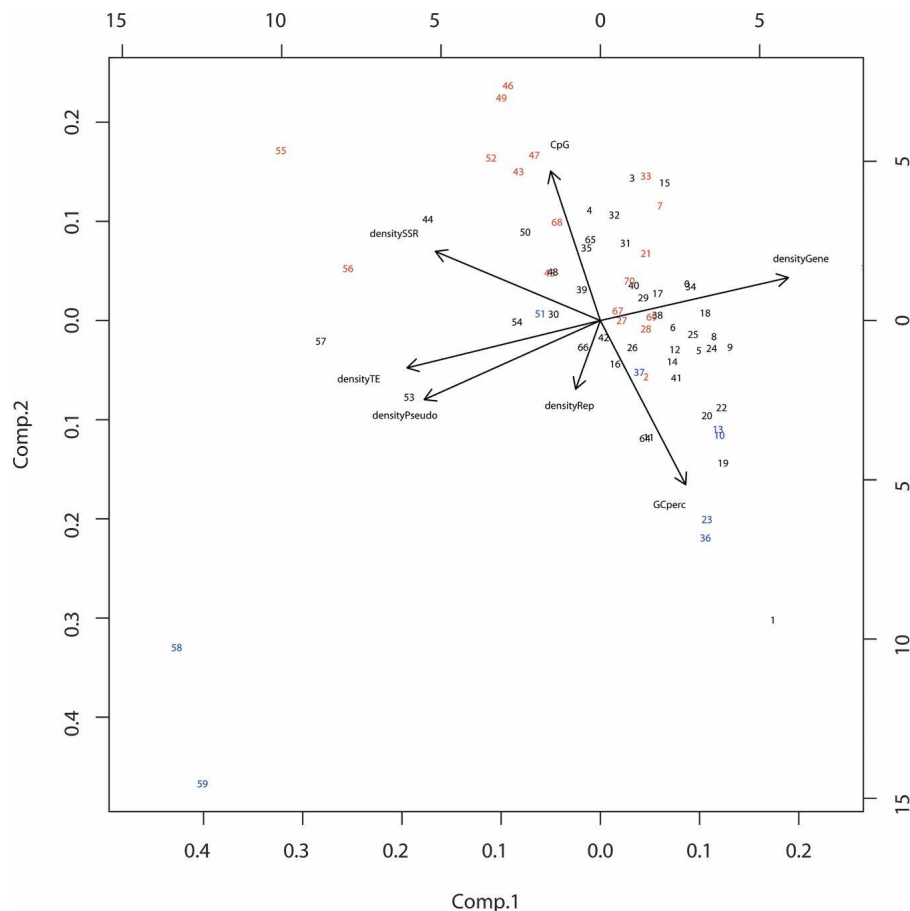
TETRA calculated an average of  $4.6 \times 10^{-8}$  COs/nucleotide, which is, on average, 1 cM for 217 kb for chromosome 4. Among the 70 intervals tested, TETRA identified 30 intervals with a significant deviation from the average rate of COs; 12 intervals had a significantly lower rate (cold) and 18 had a significantly higher rate (hot) ( $P > 0.95$  and  $P < 0.05$  for the cold and hot intervals, respectively; Supplemental Table 2). The hot intervals were not randomly distributed: four (intervals 67–70) were clustered on the short arm next to the NOR and eight (intervals 43–56) were clustered in a 3-Mb region on the long arm next to the centromere (Fig. 1). There was almost no genetic recombination in the centromeric and inverted region (intervals 58–63) and no clustering of the cold intervals was observed outside the centromeric region. In the middle of the long arm, there were alternate hot and cold intervals, although the “temperature” of most of these intervals was not significantly different from the chromosome average. In summary, the COs were unevenly distributed along chromosome 4 with alternating hot and mildly cold regions.

### Correlation of CO rates with primary sequence features








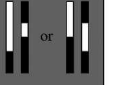



We performed a principal component analysis to determine the most relevant genome features that correlated with the observed CO frequencies. The genes, pseudogenes, G+C content, and CpG log ratio, as well as repeated sequences, such as transposable elements (TEs) and single repeats (SSR), were carefully listed from both publicly available data and in-house computed analysis (see Methods). In each interval, we took the G+C content and the CpG ratio (see Methods) and calculated the density of each of the other features. We analyzed the whole chromosome, excluding the intervals 60–63 contained in the inverted region. On the first principal component axis, accounting for 46.4% of the variation, we found that gene, pseudo-

gene, and TE densities contribute the most to the composition diversity of the intervals (Fig. 2). However, this axis shows that these features do not occur randomly along the chromosome, but follow two opposite gradients: The gene density is low in the pericentromeric and subtelomeric regions and high in the middle of chromosome arms, whereas the opposite is true for pseudogene and TE density. On the second principal component axis, adding 22.6% to the explained variation, the GC content and the CpG ratio appear to be more relevant to CO rates' variation. The intervals showing a significantly higher rate of COs tend to cluster in regions of low G+C content where the CpG ratio is high. Conversely, the intervals with a low CO rate cluster in regions of high G+C content where the CpG ratio is low (Fig. 2).

A regression analysis carried out between the CO rate and the G+C content or CpG ratio confirmed these trends with  $R^2$  of 0.18 ( $P = 3 \times 10^{-4}$ ) for G+C content and an  $R^2$  of 0.20 ( $P = 1.3 \times 10^{-4}$ ) for the CpG ratio. The regression was stronger when analyzing only the long arm of the chromosome, with  $R^2 = 0.36$  ( $P = 4 \times 10^{-7}$ ) for G+C content and  $R^2 = 0.22$  ( $P = 1.7 \times 10^{-4}$ ) for the CpG ratio. Of the other regressions tested (gene density, pseudogenes, etc.), only the SSR density had a significant correlation with CO rates ( $R^2 = 0.13$ ;  $P = 3 \times 10^{-3}$ ). Therefore, unlike the results obtained in several other eukaryotes, in which a high CO rate tends to correlate with a high G+C content, we suggest that on chromosome 4 of *A. thaliana* a high



**Figure 2.** Principal component analysis of chromosome 4 of *A. thaliana*. Numbers refer to the intervals given in Figure 1 and Supplemental Table 2. Hot intervals are indicated in red; cold intervals are indicated in blue.

COs / plant	COs / chromatid: non ambiguous (number of plants)	Examples	COs / chromatid: ambiguous (number of plants)	Examples
0	0+0 (100)	 or 	none	
1	0+1 (223)		none	
2	1+1 (97)		2: 1+1 or 0+2 (123)	 or 
2	0+2 (38)			
3	1+2 (57)	 or 	3: 0+3 or 2+1 (41)	 or 
>3	none		> 3 (23)	

**Figure 3.** Number of CO events per chromatid deduced from the genotype of an F2 plant. When an F2 plant displays two COs, either the extremities of both chromosomes are homozygous, and the COs are on one chromatid or both, or the extremities are heterozygous and there is ambiguity between one event on each chromatid or two events on the same chromatid. A similar approach has been used to analyse F2 plants that displays three COs. When an F2 plant displays more than three COs, the recombination history cannot be inferred from the genotype. In the columns “COs/chromatid,” the numbers in parentheses represent the number of plants in each category. (Light gray box) plants used in the interference analysis. (Dark gray box) plants containing a chromatid with two COs but that could not be used in the interference analysis.

CO rate correlates with a low G+C content. The CpG ratio and SSR density also weakly correlate with CO rates.

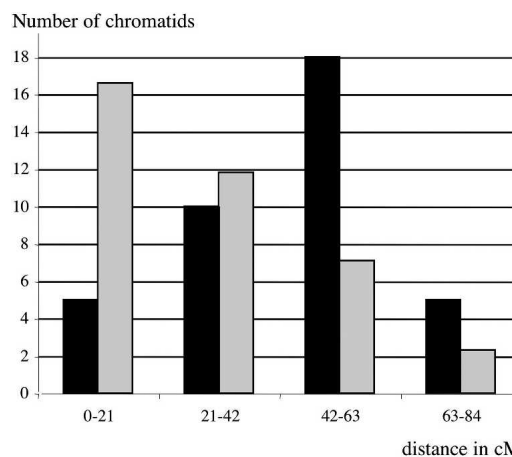
#### Interference on chromosome 4

We obtained 1171 COs for 1404 analyzed meioses. This corresponded to an average of 0.8 events per chromatid and per meiosis, corresponding to 1.6 COs per pair of homologous chromosomes (bivalents) per meiosis. There were, on average, 1.3 events on the long arm and 0.3 events on the short arm. However, if we take into account the 1.5 Mb that are inverted between the two parental lines, and therefore “forbidden” from forming and/or recovering COs, the ratio of COs per megabase on the short arm was double that of the long arm (0.18 vs. 0.09).

For 515 pairs of chromosomes, we were able to determine unequivocally the number of exchanges that each chromatid had undergone (0, 1, or 2 COs) during meiosis (Fig. 3). For 123 pairs of chromosomes harboring two exchanges, we could not unambiguously attribute the recombination events to one or the other chromatid. We reassigned them either to the “1 + 1” or the “2 + 0” class (see Fig. 3) on the basis of the prorate between the sizes of these latter classes in the nonambiguous class with two exchanges. The 41 pairs that exhibit three exchanges that could not be credited to one or the other chromatid were considered to

fall in the “2 + 1” class (that is, we assumed “3 + 0” pairs to be very rare). For the remaining pairs (23), which display four or more CO, we could not attribute CO unambiguously to parental chromosomes, so we discarded them. As expected, one exchange event was the most common occurrence (692 chromatids). Furthermore, we compared the observed distribution of the number of COs to what is expected under a Poisson distribution (Supplemental Table 3), Test of  $\chi^2$  goodness-of-fit shows that the null Poisson hypothesis can be strongly rejected ( $\chi^2 = 121.8$ ,  $P < 5 \times 10^{-4}$ ). Hence, we can conclude that multiple COs do not occur on chromosome 4 independently one from each other.

For each of the 38 plants having two precisely located COs on the same chromatid (Fig. 3, light gray box), we calculated the genetic distance between the two COs. The distance varied from 1.17 to 62.8 cM with a mean distance of 44.1 cM. The mean expected value for randomly distributed double COs was one-third of the chromosome, being 27.9 cM (see Methods). We then classified the 38 plants into four groups: group 1, with events separated by less than 25% of the chromosome (0–21 cM); group 2, with events separated by more than 25% but less than 50% of the chromosome (21–42 cM); group 3, with events separated by more than 50% but less than 75% of the chromosome (42–63 cM); and group 4, with events separated by more than 75% of the chromosome (63–83.9 cM) (Fig. 4). We compared the observed distribution with the expected distribution if COs were located independently of each other. We found a very strong probability ( $\chi^2 = 27.9$ ,  $P < 5 \times 10^{-3}$ ) that double COs were not located independently of each other. The same analysis on only the long arm also showed that the observed distribution and the observed mean distance (36 cM) were very different from the theoretical values (23 cM; data not shown). We then looked at the effect of the centromere on interference. For the 12 chromosomes having one CO on the short arm and the other on the long arm, the mean distance was 59.6 cM, that is, 70% of the genetic length of the chromosome, while the mean distance between two COs occurring on the long arm represents 52% of the genetic length of the long arm. These results confirm that CO location on chromosome 4 is affected by interference and that the centromere is not a barrier to interference.



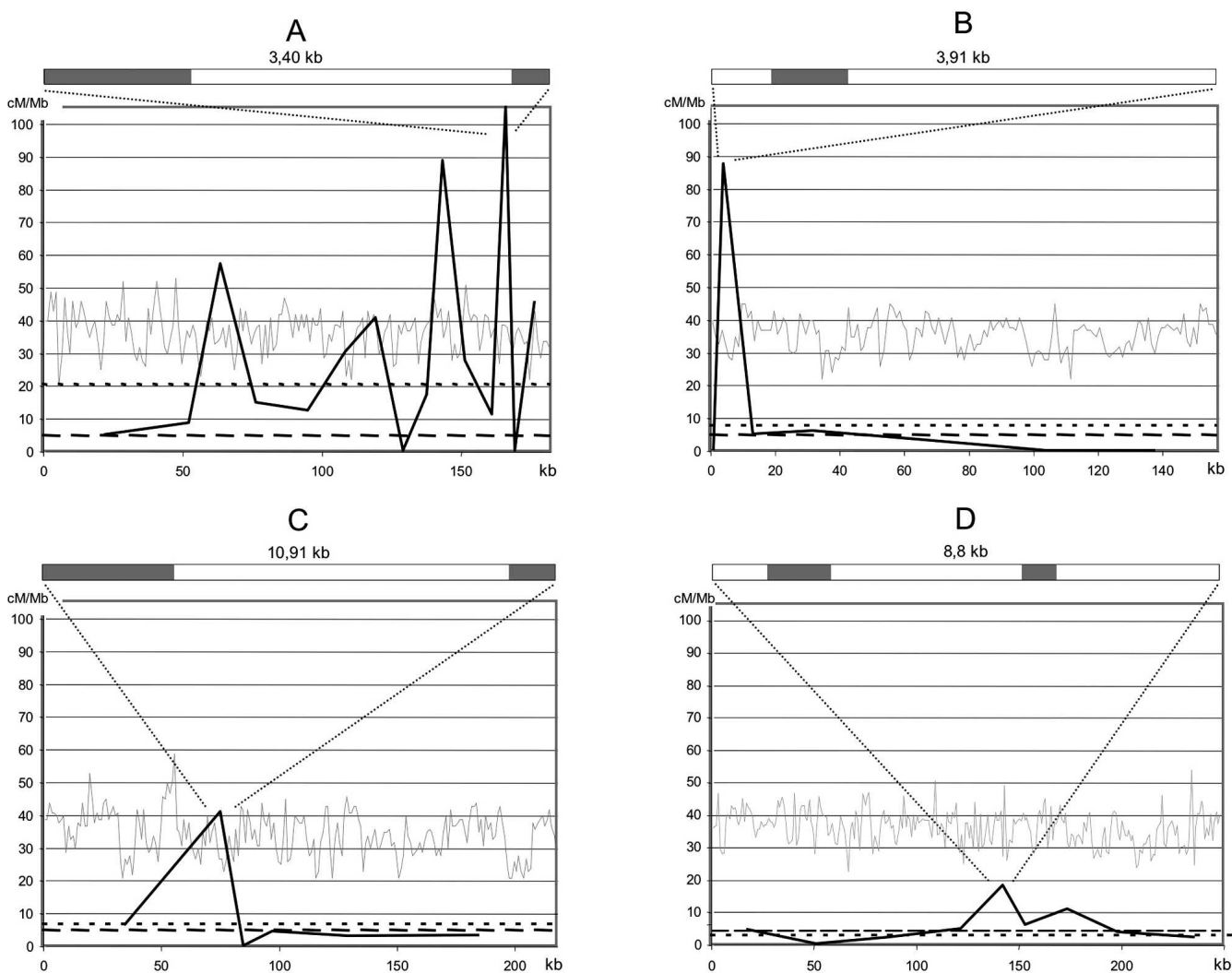
**Figure 4.** Distribution of the distances in centiMorgans between double COs. (Histogram in black) observed distribution of double COs in our F2 (see text); (histogram in gray) theoretical distribution of double COs if the position of one CO is independent of the second (Methods).

## Evidence for the existence of hot spots of recombination

We further investigated several of the 14 intervals having the highest CO rates together with one interval with a slightly above average CO rate and one cold interval. For each interval, we genotyped the corresponding recombinant plants using a set of SNP or indel markers, giving precise locations of the exchange points. We divided the hottest interval (interval 70; Fig. 1) into 15 parts to map the COs at a precision of a few kilobases (Fig. 5A). We found a clearly nonhomogeneous distribution of exchange events. Two very small fragments (3.4 and 3.2 kb) 20 kb apart exhibited a very high rate of COs (>85 cM/Mb), being 15 times higher than the chromosome average (4.6 cM/Mb) and four times higher than the interval average (20.2 cM/Mb). We found that two other fragments in interval 70 had moderately high rates of genetic recombination (40 and 55 cM/Mb, 8 to 10 times

the chromosome average). We also analyzed another hot interval (interval 21, Fig. 5B) in the middle of the long arm (Fig. 1). We found one DNA fragment displaying a large increase of genetic recombination in this interval. The recombination rates in the remainder of this interval were mostly lower than the chromosome average. We also observed the same type of “spotty” CO distribution in the other hot intervals that we investigated (7, 55, 56 and 68, 69; data not shown).

We then analyzed interval 57 (Fig. 1), which did not appear to have a significantly high rate of genetic recombination when analyzed by TETRA (6.6 cM/Mb;  $P = 0.08$ ). We found one DNA fragment of 12 kb displaying a high rate of genetic recombination (40 cM/Mb) whereas the remainder of the interval displayed CO rates below the chromosome average (Fig. 5C). Interval 37 was found to be significantly cold when analyzed by TETRA (2.7 cM/Mb;  $P > 0.98$ ). We performed the same kind of analysis as for



**Figure 5.** Fine-scale analysis of the distribution of CO breakpoints in four intervals. (A) interval 70: 44.5kb-3; 16.4kb-2; 6.2kb-5; 19.5kb-4; 17.5kb-3; 9.4kb-4; 12.3kb-7; 8.1kb-0; 8.3kb-2; 3.2kb-4; 13.0kb-5; 6.4kb-1; 3.4kb-5; 3.3kb-0; 11.0kb-7. (B) interval 21: 2.5kb-0; 3.9kb-5; 13.8kb-1; 23.6kb-2; 45.7kb-2; 29.2kb-0; 38.6kb-0. (C) interval 57: 69.5kb-6; 12.2kb-7; 7.1kb-0; 17.4kb-1; 46.5kb-2; 65.0kb-3. (D) interval 37: 35.2kb-2; 32.7kb-0; 38.1kb-1; 8.8kb-2; 12.6kb-1; 28.3kb-1; 20.2kb-1; 49.2kb-2; 38.7kb-1. For each interval the size of each DNA fragment is given and the number of recombinant plants. (Light gray line) G+C content calculated in 1-kb windows. (Black line) CO rates in centiMorgans per megabase. (Small dotted line) interval COs rate average. (Large dotted line) chromosome 4 COs rate average. Above each major peak is the gene organization of the fragment. (Gray box) gene.

the other intervals. We found a dispatch of the 12 CO exchanges in 9 of the 10 fragments studied (Fig. 5D) with a maximum of two events in an 8.8-kb fragment. This small fragment seems to exhibit a slightly higher CO rate than the genome average (Fig. 5D). However, more plants would be needed to confirm this difference. For the four regions analyzed, hot spots did not seem to correlate with G+C content or gene organization (Fig. 5A–D).

## Discussion

We obtained a very detailed genetic map of chromosome 4 of *A. thaliana* by genotyping a series of 71 SNP markers on 702 F2 plants issued from an F1 Col/Ler hybrid. The total size of the genetic map was estimated at 83.9 cM, which is consistent with other maps obtained from crosses of the same accessions: the classical map (76 cM; Meinke et al. 1998), the RFLP map (74.4 cM; Schmidt et al. 1995; Liu et al. 1996), tetrad analysis using the *quartet* mutation (85 cM; Copenhaver et al. 1998; Lam et al. 2005), and first versions of the RIL genetic map (76 cM; Lister and Dean 1993).

We found that, on average, a chromosome 4 bivalent undergoes 1.6 crossovers per meiosis. Copenhaver et al. found an average of 1.5 COs on chromosome 4 in male meiosis in a Col/Ler cross (Copenhaver et al. 1998; Lam et al. 2005). Meiotic recombination has also been assessed using cytology by recording the numbers and locations of chiasmata on metaphase I bivalents in pollen mother cells of several accessions, including Col and Ler (Sanchez-Moran et al. 2002). Both the genetic and cytological methods gave consistent results, with the mean chiasma frequency being 1.6 for chromosome 4. Therefore, CO frequency on chromosome 4 during meiosis of a Col/Ler F1 hybrid is not greatly different from that in the parents.

In most eukaryotes, “positive interference” (i.e., the probability of COs occurring next to each other is lower than expected) affects the distribution of multiple COs on a single chromosome (see Zickler and Kleckner 1999). However, not all the COs seem to interfere, and recent data suggest two pathways for crossovers in *S. cerevisiae*, in humans, and in *A. thaliana*: one pathway being sensitive to interference (class I) and the other insensitive (class II) (Copenhaver et al. 2002; Housworth and Stahl 2003; Higgins et al. 2004; Hollingsworth and Brill 2004; Stahl et al. 2004; Lam et al. 2005; Mercier et al. 2005). We show also that COs are subjected to interference on chromosome 4. Double COs on the same chromatid are significantly further than one-third of the chromosome length apart, contrary to what is expected for randomly distributed COs. In addition, our results suggest that interference is insensitive to centromere as previously proposed by Colombo and Jones (1997) and that the centromere may increase the strength of interference on chromosome 4. However, there is not complete interference, as we observed double COs only a few centiMorgans apart. We could assume, as suggested by previous studies (Copenhaver et al. 2002; Lam et al. 2005) that these close double COs are insensitive to interference and the distant double COs are sensitive to interference. In yeast, the level of interference has been shown to depend on the size of the chromosome with the short chromosomes harboring less interference (Kaback et al. 1999). However, the disparity in size of the chromosomes is less pronounced in *Arabidopsis*, with the shortest chromosome being more than two-thirds of the size of the longest chromosome, while in yeast there is a fourfold difference in size. Moreover, Lam et al. (2005) recently provided evidences that in *Arabidopsis* NOR-bearing chro-

somes (i.e., chromosomes 2 and 4) exhibit more interference than the others and suggested that the NOR region itself rather than the size of the chromosome could influence interference. Further analyses are needed to determine whether interference varies along the chromosome, as recently suggested in a study on rice (Esch 2005).

Numerous studies have attempted to understand the factors responsible for genetic recombination variations and to identify primary sequence features that may correlate with this variability. In many sexual organisms, such as mammals, birds, yeast, *Drosophila*, and nematodes, positive correlations between the CO rates and G+C content have been observed at the scale of several hundred of kilobases (Hurst et al. 1999; Gerton et al. 2000; Fullerton et al. 2001; Marais et al. 2001; Takano-Shimizu 2001; Yu et al. 2001; Birdsell 2002; Kong et al. 2002; Jensen-Seaman et al. 2004). Gerton et al. (2000) suggested that regions of high G+C content stimulate recombination. Alternatively, several recent studies have proposed that high levels of recombination may create regions with high G+C content, probably through a biased gene conversion (BGC) toward G+C. In other words, meiotic recombination modifies the base composition through the average density of recombination hot spots (see below; Galtier et al. 2001; Birdsell 2002; Montoya-Burgos et al. 2003; Meunier and Duret 2004). However, in humans, rats, and mice, when CpG ratio is included in a multiple regression model, the correlation with the G+C content becomes negative (Kong et al. 2002; Jensen-Seaman et al. 2004).

In contrast, we found that regions of low G+C content and high CpG ratio on chromosome 4 of *A. thaliana* tend to have higher rates of genetic recombination. Therefore, the BGC hypothesis suggested to explain the correlation found in other eukaryotes may not apply in *Arabidopsis*. However, homologs of genes believed to participate in G+C-biased mismatch repair in other organisms exist in the genome of *Arabidopsis* (Birdsell 2002). It is also possible that in *Arabidopsis* BGC cannot affect the nucleotide content due to the high level of inbreeding of the plant that does not favor the formation of heteroduplex DNAs. However, this would explain an absence of correlation but not a negative correlation. In contrast to our results, a study recently reported no correlation between the G+C content and CO rates in *Arabidopsis* (Marais et al. 2004). We suggest that our observation is due to the higher precision of our recombination map because we studied 702 plants compared to the 101 RILs used in the study of Marais et al. (2004). Therefore, our study in *Arabidopsis* questions the assumptions made for G+C correlation and so, the problem of causation remains an open query. Data from more species are needed and may reveal a species-specific lineage in the evolution of recombination.

A fine-scale analysis of several intervals showed peaks in crossover activity. For example, in the hottest interval (interval 70; Fig. 1), CO breakpoints are found in 12 of the 14 fragments tested, even though there is clustering in two small regions 20 kb apart (Fig. 5A). In other intervals, including one not having a significantly high CO rate, one small DNA fragment accounts for most of the genetic recombination of the interval. In the genome of *A. thaliana*, this punctuate distribution of CO activity strongly suggests recombination hot spots where recombination events group around an initiation site. In plants, several hot spots of CO activity have been described. The 140-kb *a1-sh2* region in maize has peaks of CO activity (three to six times the genome average) in three small intervals (1.7–3.4 kb) (Yao et al. 2002). Other loci in maize or in rice, such as *bronze* or *waxy*, show some properties

of hot spots (Dooner and Martinez-Ferez 1997; Okagaki and Weil 1997; Inukai et al. 2000). For the *bronze* locus, unlike for yeast and mammals, it has been suggested that recombination is initiated uniformly along the gene and not at a preferential site. Therefore, although existence of hot spots seems to be the rule rather than the exception in plants and other higher eukaryotes, there may be some differences. However, all studies on higher eukaryotes looked at no more than one or two intervals that were often selected for a phenotype associated with the recombination event. Therefore, it is difficult to determine whether the observed hot spot patterns in these intervals can be applied to the whole genome. Here, we show that a punctuate distribution of hot spots is a general feature of the chromosome that is not restricted to significantly recombinogenic regions. Our results also strongly suggest that recombination is initiated at preferential sites all along the chromosome. However, both the intensity and the density of the recombination sites influence the variation of recombination, as hot regions contain one or several very hot spots whereas a mildly warm interval would contain only one mild hot spot. Cold regions may contain few spots with a higher rate of recombination than the genome average, but it remains to be demonstrated. A similar result has recently been obtained in the human genome, where strong hot spots have been detected in narrow regions of strong LD and weak hot spots in regions of strong marker association (Jeffreys et al. 2005). We have identified more than 10 small DNA fragments that may behave as hot spots on chromosome 4 of *A. thaliana*. Further experiments are needed to confirm the strength and the precise location of the initiation site of these hot spots. Their fine characterization and analysis in other genetic backgrounds is needed to determine the factors that govern their activity and distribution.

## Methods

### F2 recombinant population construction, genomic DNA extraction

The two *Arabidopsis* accessions, Columbia and Landsberg *erecta*, were crossed to obtain an F1 hybrid. Self-fertilization from a single F1 was carried out to obtain F2 seeds. Seeds were grown in soil in long-day conditions in the greenhouse. At the rosette stage, the whole material of 736 F2 plants together with plant material from the two parental accessions was collected. DNA was extracted as described (Loudet et al. 2002).

### Selection of SNPs

Most of the SNPs were chosen from the Monsanto database (Jander et al. 2002). When convenient SNPs were not found in the database, DNA fragments were amplified at the desired position on the genomic DNA of the two parental accessions and sequenced to identify a SNP suitable for genotyping. A list of the SNPs used in this study is given in Supplemental Table 1. A couple of primers were designed for each SNP to obtain a PCR fragment containing the predicted SNP. A list of the PCR primers used in this study is given in Supplemental Table 1. The PCRs were carried out on the parental accession DNAs using standard conditions: 94°C 4 min, (94°C 45 sec, 52°C 45 sec, 72°C 1 min) × 35 cycles, 72°C, with Eurobio 1× reaction buffer and Taq polymerase. PCR fragments were sequenced (Genome Express) to check the presence and position of the SNPs.

### SNP genotyping

At each of the SNP sites, DNA extracted from the F2 plants was genotyped either by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, as described by Sauer et al. (2000) or by fluorescence based techniques: the Amplifluor technology (Serological Corporation) or the TaqMan technology (Applied Biosystems). For a number of SNPs, the results obtained with one technique (usually mass spectrometry) were confirmed with one of the two other methods. The techniques used for each SNP are given in Supplemental Table 1.

### Statistical analysis of CO rates: TETRA

We define  $P$  as the probability of having a CO in a specific position of the chromosome, and assume that  $P \ll 1$ . The probability  $P_i$  of observing one CO in the  $i$ th interval on a chromosome is approximated as  $PL_i$ , where  $L_i$  stands for the length of the  $i$ th interval. If the number of informative chromosomes for the interval  $i$  (i.e., the number of chromosomes for which both SNPs delimiting the interval are available) is written as  $V_i$ , then the number,  $N_i$ , of COs in the  $i$ th interval is distributed according to a binomial  $B(V_i, PL_i)$  under the null hypothesis that the COs rate is constant along the chromosome. More explicitly, we have for all

$$k: P(N_i = k) = \binom{V_i}{k} (P \times L_i)^k (1 - P \times L_i)^{V_i - k}.$$

According to the observed values,  $n_i$ , of the number of COs in the different intervals, TETRA computes the average CO rate,  $P$ , along the whole chromosome. It then computes the  $P$ -value,  $T_i$ , of the observed number of COs under the above binomial model, that is:

$$P(N_i \geq n_i) = \sum_{k=n_i}^{V_i} \binom{V_i}{k} (P \times L_i)^k (1 - P \times L_i)^{V_i - k}.$$

This  $P$ -value can be interpreted as the probability that the number of COs in the  $i$ th interval exceeds its observed value under the model of homogeneous CO rate along the chromosome.

### Statistical analysis of COs interference

We derive the probability distribution function of the distribution of distances between the two COs by assuming that the locations of the two COs are independently uniformly distributed random variables.  $L$  is the length of the chromosome, and  $x$  and  $y$  are locations of the two COs.  $x$  and  $y$  are uniformly distributed in  $[0, L]$ . The distance  $r = (x - y)$ . The distribution of  $x$  and  $y$  is symmetric under the exchange of  $x$  and  $y$ ; we can condition on  $x > y$ . The probability distribution function  $P(r)$  is proportional to the length of the segment, in the  $x, y$  plane, between the points of coordinates  $(r, 0)$  and  $(L, L - r)$ , which is itself proportional to  $L - r$ . Imposing the normalization of the probability distribution function, we obtain  $P(r) = 2(1 - r/L)$ . The expectation value of  $r$  is thus  $\int_0^L P(r)r dr = L/3$ . We can deduce the probabilities  $P_1, P_2, P_3, P_4$  of  $r$  being in each of the four bins  $[0, L/4], [L/4, L/2], [L/2, 3L/4], [3L/4, L]$ . For instance  $P_1 = 2 \int_0^{L/4} (1 - x) dx = 7/16$ . Similarly, we find  $P_2 = 5/16, P_3 = 3/16, P_4 = 1/16$ . These probabilities are used in the analysis of Figure 4.

### Correlation studies

The *A. thaliana* genomic sequence and its annotation were downloaded from the TIGR Web site ([http://ftp.tigr.org/pub/data/a\\_thaliana/ath1/](http://ftp.tigr.org/pub/data/a_thaliana/ath1/)). Gene and pseudogene annotations have been

extracted from TIGR-XML files from release 5 of the genome annotation. Transposable elements have been re-annotated using the RMBL procedure from the TE annotation pipeline described by Quesneville et al. (2005). The TE reference set used is derived from the *A. thaliana* RepeatMasker repeat library (March 6, 2004). The same TE family consecutive TE fragments (on both the genome and the reference TE) have been automatically joined if separated by a sequence composed of more than 80% of other TE insertions (in this case we have a nested TE). Otherwise they are joined if a gap of 5000 nucleotides or a region of mismatches 500 nucleotides long separate them.

Single repeats (SSR) were found using the Tandem Repeat Finder program (Benson 1999), and repeats by a BLASTN all-by-all using BLASTER and GROUPER (Quesneville et al. 2005) without using any simple link clustering coverage constraint. G+C content and CpG were counted with in-house python scripts. CpG ratios were computed by taking the  $\log_{10}$  of the C+G dinucleotide frequency divided by the product of G and C frequencies. All data and analysis results were stored in a MySQL database and retrieved by SQL queries.

Statistical analyses were carried out using the R software environment (<http://cran.r-project.org>)

### Detection of hot spots

We halved each interval by choosing a convenient SNP (or indel) either from the Monsanto database or by DNA sequencing (see above). We then sequenced the DNA fragment containing the SNP in the recombinant plants and finally distributed the plants according to their genotype within one or the other half interval. This was iteratively repeated until the location of CO breakpoints was obtained within a few kilobases. A list of the SNPs, indels, and corresponding primers is given in Supplemental Table 1. Genomic DNA from plants was amplified by PCR in standard conditions (see above) with an annealing temperature adapted for each set of primers.

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