DOI: 10.1534/genetics.107.071738

Integrative Mapping of *Gossypium hirsutum* L. by Meiotic Fluorescent *in Situ* Hybridization of a Tandemly Repetitive Sequence (B77)

Yuanfu Ji,[1] Xinping Zhao,[2] Andrew H. Paterson,[3] H. James Price and David M. Stelly[4]

Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77843

Manuscript received February 5, 2007
Accepted for publication February 27, 2007

ABSTRACT

We determined the relative positions of the tandem-repeat molecular cytogenetic marker B77, translocation breakpoints, and telosome arms in *Gossypium hirsutum* cytogenetic stocks by fluorescence *in situ* hybridization (FISH) analysis of meiotic quadrivalents in 16 single and 2 double translocation heterozygotes and five monotelodisomics. Results delimited the B77 FISH locus to the right arm of the *D*-subgenome chromosome 14 (*14R*) and the short arm (*14sh*), respectively. By equating *14R* with *14sh* and *14L* (left) with *14Lo* (long), the findings established a unified nomenclature for the arms of chromosome 14. Previously reported chromosome 14 arm locations were confirmed for four of the five translocations involving chromosome 14, namely *NT1L-14L* (2780), *NT2R-14R* (2B-1), *NT14L-23R* (2777), and *NT14R-24R* (2781), whereas the location of breakpoint *T6L-14L* was not confirmed and was reassigned to arm *14R*. When used as a probe on Southern blots, the B77 signal was associated with a terminus of the *D*-subgenome RFLP linkage group (LG) D04 by linkage analysis of an interspecific F_2 population, now known to be chromosome 20. However, additional codominant DNA marker information in the affected region excluded the B77 polymorphism detected by Southern blot hybridization from chromosome 20 and, indeed, from the remainder of the genome.

FLUORESCENCE *in situ* hybridization (FISH) is a powerful technique for physical localization of DNA sequences to individual chromosomes and subchromosomal regions. Low-copy and unique DNA sequences have been successfully mapped to chromosomes in humans, animals, and plants (Ashley *et al.* 1994; Lemieux *et al.* 1994; Dong and Quick 1995). Repetitive sequences, such as ribosomal DNA sequences, have been detected and mapped in a large range of plant species, such as cotton, wheat, tomato, and others (Jiang and Gill 1994; Ji *et al.* 1999a, 2004). When combined with meiotic analysis of cytogenetic stocks such as translocations, *in situ* hybridization provides a more powerful tool for integrative mapping (Price *et al.* 1990; Crane *et al.* 1993; Stelly *et al.* 1996; Wang *et al.* 2006).

FISH to pachytene bivalents offers numerous advantages over FISH to diakinesis and metaphase chromosomes and has been utilized broadly in plants for gene localization and other studies (Zhong *et al.* 1999; Kim *et al.* 2005). Studies on cotton pachytene bivalents have been limited (Mursal and Endrizzi 1976) and, to our knowledge, there have been no reports of systematic research based on FISH to pachytene bivalents.

Cotton (*Gossypium hirsutum* L.) has long been regarded as an allotetraploid ($2n = 4x = 52$) with A_h and D_h subgenomes (Skovsted 1934; Beasley 1940, 1942). Subgenomic affiliations of the 26 chromosomes of *G. hirsutum* were determined by meiotic analysis of interspecific hybrids between *G. hirsutum* translocation homozygotes and diploid species. Individual chromosomes were identified and numbered according to meiotic analysis of crosses among translocation lines (Menzel and Brown 1978; Brown 1980; Brown *et al.* 1981). Chromosomes of *A*- and *D*-subgenomes were designated as chromosomes 1–13 and 14–26, respectively. On the whole, *A*-subgenome chromosomes are discernibly larger than *D*-subgenome chromosomes (Skovsted 1934), but chromosomes of the two subgenomes overlap in size, precluding reliable subgenomic assignment on the basis of size alone (Kimber 1961).

A high-resolution genetic recombination map of sequence-tagged sites for Gossypium genomes was coalesced into 26 linkage groups (LGs), 20 of which were assigned to individual chromosomes (Rong *et al.* 2004). The remaining six groups were eventually assigned to individual chromosomes by meiotic *in situ* hybridization analysis of related translocation stocks with linkage-group-specific bacterial artificial chromosome clones (Wang *et al.* 2006), thus leading to complete

The authors acknowledge and express their appreciation of the late H. James Price for his unending enthusiasm for science, collaboration, and the positive things in life.

[1]*Present address:* Gulf Coast Research and Education Center, University of Florida, Wimauma, FL 33598.

[2]*Present address:* Neurology Department, University of Michigan Medical Center, Ann Arbor, MI 48109.

[3]*Present address:* Center for Applied Genetic Technologies, University of Georgia, Athens, GA 30602.

[4]*Corresponding author:* Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843. E-mail: stelly@tamu.edu

identification of the 26 cotton chromosomes. As a result, the 13 homeologous chromosome pairs have also been completely established, which were supported by numerous prior studies, including conventional cytogenetics (Endrizzi *et al.* 1985), molecular cytogenetics (Crane *et al.* 1993), and linkage mapping of various molecular markers (Reinisch *et al.* 1994; Lacape *et al.* 2003; Nguyen *et al.* 2004; Rong *et al.* 2004; Han *et al.* 2006).

A total of 62 translocations have been maintained in the Cotton Cytogenetics Collection (Stelly 1993). The breakpoints affect 25 of the 26 chromosomes, and most have been localized to an arm and mapped relative to each other and their respective centromeres (Menzel *et al.* 1985). Menzel *et al.* (1985) arbitrarily designated the two arms of each chromosome as "right" (*R*) and "left" (*L*). Telosomes were designated as "short" (*sh*) and "long" (*Lo*) according to their relative size (Endrizzi and Ramsay 1979, 1980; Endrizzi *et al.* 1985). The correspondence of "*L*" and "*R*" to "*Lo*" and "*sh*" designations was reported for a number of the chromosomes (Menzel *et al.* 1985), but a limited investigation subsequently revealed that some assignments were incorrect (Stelly *et al.* 1996). Thus, two partially independent systems of nomenclature exist for the chromosome arms of *G. hirsutum*, one based on translocations and the other on telosomes. Moreover, the modest map of genes governing conventional traits, the extensive molecular marker map (Reinisch *et al.* 1994; Rong *et al.* 2004), and the breakpoint map (Menzel *et al.* 1985) are currently independent and thus need to be integrated.

A restriction fragment length polymorphism (RFLP) detected using a tandemly repeated sequence, B77 (572 bp), as a probe, was previously loosely associated (19 cM, no flaking markers) with a single locus in *D*-subgenome LG D04 (Zhao *et al.* 1998), which was later assigned to chromosome 20 (Rong *et al.* 2004). Interesting features of B77 include its subgenomic specificity, tandem nature, and genetic variability (Zhao *et al.* 1998). The large size of the B77 locus (~0.5 Mb) presented a facile opportunity to jointly investigate B77, LG D04, and the use of FISH for integrative mapping. At the outset of the study reported here, we endeavored (1) to identify which chromosome bears B77 and thus to identify LG D04; (2) to further localize B77 with respect to subchromosomal regions (arm and segment) defined by translocation and/or telosome breakpoints; and (3) to test previous arm assignments of cytogenetic landmarks for their respective chromosomes.

MATERIALS AND METHODS

Plant material: Single and double reciprocal translocation heterozygotes used in molecular-meiotic analyses were developed from translocation lines maintained in the Cotton Cytogenetics Collection at Texas A&M University (Stelly 1993). Single translocation heterozygotes (*NT*s) were produced by crossing the translocation homozygotes to the genetic standard line *TM-1*. Double translocation heterozygotes (*dNT*s) were produced by intercrossing the translocation homozygotes. Monotelodisomic translocation heterozygotes (*TeNT*s) were produced by intermating monotelodisomics and chromosomally related translocation homozygotes, the latter serving as pollen parent. Progeny were screened phenotypically and meiotically to identify the *TeNT* aneuploids. A segmental duplication-deficiency (*dp-df*), which was produced by outcrossing *NT14R-24R* to *TM-1* and was shown by FISH to be deficient for *14*R (Ji *et al.* 1999b), was also used in this study. The *NT*s, *dNT*s, and *TeNT*s used in this study are listed in Table 1.

Chromosome preparation: Meiotic chromosome spreads were prepared according to the procedures of Crane *et al.* (1993) with some modifications. Briefly, upon removal of calyx and corolla, meiotic buds are fixed in two or more changes of 2:1 (v/v) acetone:acetic acid with 1% polyvinylpyrrolidone (Sigma, St. Louis; M_r 40,000) at room temperature for 24 hr, washed in distilled water, and stored in distilled water for several hours to a couple of weeks or in 70% ethanol for several months at 4°. Buds were individually macerated in 1% acetocarmine and screened for metaphase I (MI) under a microscope; selected macerates were transferred to a clean slide and squashed under a silicolized coverslip at 75–80° on a temperature-controlled hot plate. Slides were frozen in liquid nitrogen and then stored in a freezer at −135°.

Probe labeling and *in situ* hybridization: A biotin-labeled probe was prepared by nick translation (BRL BioNick kit) of a plasmid containing B77 element, a 572-bp clone from a tandemly repeated (~900 times) sequence of *G. barbadense* ($2n = 4x = 52$; Zhao *et al.* 1998). The probe mixture contained probe DNA (final concentration 1.2 ng/μl), *Escherichia coli* DNA (final concentration 240 ng/μl), 50% deionized formamide, 20% dextran sulfate, and 2× SSC. The procedures of *in situ* hybridization and signal detection followed Ji *et al.* (1997).

TABLE 1

Single, double, and monotelodisomic translocation heterozygotes used in this study

*NT*s			
Line no.[a]	Name	*dNT*s	*TeNT*s
2780	*NT1L-14L*	*dNT[7L-18R, 20L-22R]*	*Te14LoNT1L-14L*
2B-1	*NT2R-14R*	*dNT[14L-23R, 19R-24R]*	*Te14LoNT2R-14R*
AZ-7	*NT6L-14R*[b]		*Te14LoNT6L-14L*
4659	*NT7L-18R*		*Te14LoNT14R-24R*
2767	*NT15R-16R*		*Te14LoNT14L-23R*
6340	*NT9L-17Rb*		
2772	*NT9R-20L*		
2870	*NT9L-25*		
4675	*NT10L-21L*		
2925	*NT13R-19R*		
2777	*NT14L-23R*		
2781	*NT14R-24R*		
SL15	*NT15R-20R*		
7-3F	*NT19R-21R*		
2786	*NT19R-24R*		
DP4	*NT20L-22R*		

[a] From Brown *et al.* (1981).

[b] Previous designation as *NT6L-14L* was found to be incorrect (see text).

Fluorescence microscopy: Slides were screened and photographed with an Olympus AX-70 microscope equipped with UV and blue and green excitation filter sets. Photographs were taken on Fujicolor 400 professional film. Prints were digitally scanned, processed, and reproduced.

Chromosomal and subchromosomal localization: The physical association of B77 FISH signal with translocation-bearing multivalents was used to discern the chromosomal location of B77. Positions of B77 FISH signals on metaphase I multivalents were used to subchromosomally localize B77 relative to the translocation breakpoints. The numbers and positions of FISH signals on *TeNT IV*s were used to define relationships among B77, translocation breakpoints, and telosome-defined arms. The requisite interpretations were based on principles detailed previously (Price *et al.* 1990; Crane *et al.* 1993; Stelly *et al.* 1996).

Detection of major nucleolar organizing regions: Differentially bright propidium iodide (PI; red) fluorescence of nucleolar organizing regions (*NOR*s) in somatic and meiotic metaphase chromatin [when doubly stained with DAPI (4′, 6-diamidino-2-2-phenylindole) and PI] often allows for facile detection of major *NOR*s (Hanson *et al.* 1996; Ji *et al.* 1997). At metaphase (when most chromosome regions are well contracted), the *NOR*s are brighter red than other chromatin. Therefore, the three major *NOR*s of *G. hirsutum* (Bergey *et al.* 1989) can be detected by their differential PI fluorescence. In this study, we used this technique for dual detection of the major *NOR*s and B77 in meiocytes probed only with B77 (fluorescein isothiocyanate detection) and stained with DAPI and PI.

RESULTS

Chromosomal localization of B77: The RFLP locus B77 was previously mapped by linkage analysis to a terminus of linkage group D04, ~19 cM away from the nearest marker G1016 of this linkage group (Zhao *et al.* 1998). Although the LOD score was statistically significant, the lack of flanking markers renders the linkage of terminal markers tenuous. This uncertainty is made even greater in that the marker was "dominant"; *i.e.*, segregation was for presence *vs.* absence of the B77 allele with no ability to detect heterozygotes. Association of B77 with a *D*-subgenome chromosome was further confirmed by FISH (Zhao *et al.* 1998). LG D04 was recently assigned to chromosome 20 (Lacape *et al.* 2003; Rong *et al.* 2004). Accordingly, we first subjected B77 to molecular-meiotic tests by hybridization to *NT*s and *dNT*s involving chromosome 20. However, in metaphase I spreads of *NT9R-20L*, *NT15R-20R*, *NT20L-22R*, and *dNT[7L-18R, 20L-22R]*, the pair of B77 signals invariably occurred on one bivalent per cell, and the respective *IV* was devoid of FISH signal (data not shown), indicating that B77 is neither in chromosome 20 nor in other *D*-subgenome chromosomes 15, 18, and 22. To expedite subsequent identification efforts, we hybridized B77 to metaphase I spreads of *dNT[14L-23R, 19R-24R]*, which involved four of the remaining *D*-subgenome chromosomes, *i.e.*, 14, 19, 23, and 24. FISH signals were observed, both of which were associated with one *IV*/cell (Figure 1A), indicating that one of the four chromosomes involved in the translocations carries the B77 sequence. The presence of a *NOR* on chromosome 23 was then used to discriminate between the two *IV*s. Following PI staining, the three differentially PI-fluorescing major *NOR*s were observed on two bivalents and one of the two *IV*s (Figure 1, B and C). The B77 FITC signals were invariably associated with the *NOR*-bearing *IV*, *i.e.*, with *NT14L-23R IV*, suggesting that B77 lies in chromosome 14 or 23, not 19 or 24.

To deduce whether B77 is located in chromosome 14 or 23, we first determined the relative physical positions of B77 and *NOR* sites on *NT14L-23R IV*s. We observed that they were located on opposite sides of *NT14L-23R IV*s (Figure 1D), indicating that B77 is in chromosome 14, not 23. To further test this inference, we determined whether or not B77 was associated with *IV*s of the other four translocations involving chromosome 14 that are maintained in the Cotton Cytogenetics Collection. Association was detected in all cases, *i.e.*, for *NT1L-14L* (Figure 1E), *NT2R-14R* (Figure 1F), *NT6L-14L* (Figure 1G), and *NT14R-24R* (Figure 1H). Supporting evidences also came from nonassociation of B77 with the remaining *D*-subgenome chromosomes 16, 17, 21, and 25 (data not shown) except 26, for which no translocation stock is available in the current collection. Given that translocations constitute the basis for the existing nomenclature for chromosomes of *G. hirsutum* (Menzel and Brown 1978; Brown 1980; Brown *et al.* 1981), these data demonstrated that the B77 FISH site is in chromosome 14.

Subchromosomal localization of B77 by analysis of *NT*s: Metaphase I spreads of translocation heterozygotes were analyzed for each of the five translocations that affect chromosome 14. Two of the five *NT*s rarely form interstitial chiasmata, whereas the other three *NT*s have at least one breakpoint recombinationally distal from their respective centromere and therefore tend to form interstitial chiasmata, which lead to "barbell"-*IV*s and other types of *IV*s (Menzel *et al.* 1985). Interstitial chiasmata constrain the shape of *IV*s and provide "reference points" useful to subchromosomal localization by molecular-meiotic methods (Stelly *et al.* 1996).

"Barbell"-*IV*s were observed in *NT1L-14L* and *NT14L-23R* microsporocytes. The B77 signals on "barbell"-*IV*s of *NT14L-23R* (Figure 1D) and *NT1L-14L* (Figure 1E) were located on the "exterior" of the *IV*s, indicating that B77 is located in the arms opposite the *T1L-14L* and *T14L-23R* breakpoints in chromosome 14. According to the map of breakpoints (Menzel *et al.* 1985), both the *T1L-14L* and the *T14L-23R* chromosome 14 breakpoints are in the left arm (*14L*). Therefore, the B77 cluster must be located in the right arm of chromosome 14 (*14R*).

"Frying pan"-*IV*s were observed in *NT2R-14R* microsporocytes. Such *IV*s result when crossing over occurs in just one of the two interstitial regions. In all the observed "frying pan"-*IV*s, the B77 signals were always

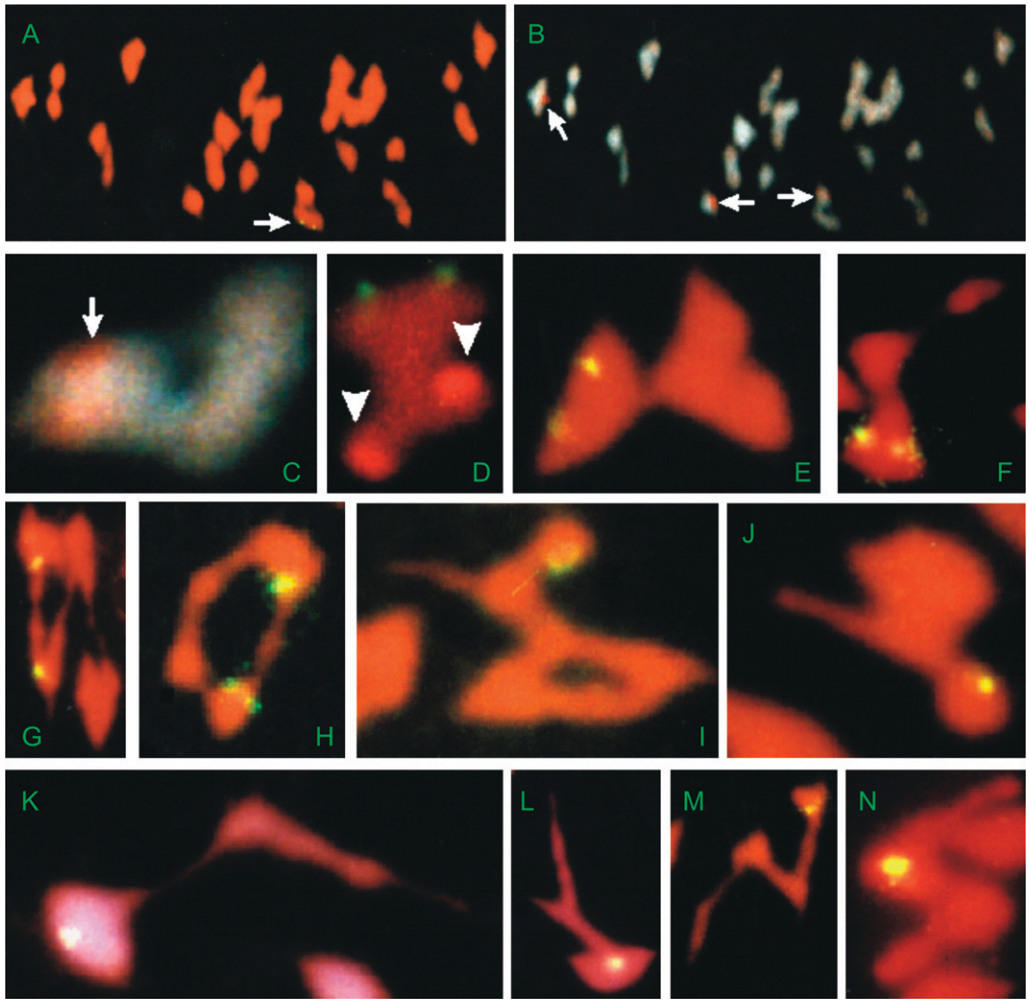

FIGURE 1.—Meiotic MI chromosome spreads of single reciprocal translocation heterozygotes (*NT*s), double reciprocal translocation heterozygotes (*dNT*s), and monotelodisomic translocation heterozygotes (*TeNT*s) from *G. hirsutum* after fluorescence *in situ* hybridization of clone B77. (A–D) *dNT[14L-23R, 19R-24R]*, where A–C are from the same chromosome spread. (A) A pair of hybridization signals were associated with just one (arrow) of the two *IV*s. (B and C) DAPI staining of the same spread at low and high magnifications, respectively. (B) The *IV* associated with FISH signals is shown here to be associated with one of the three major *NOR* sites (arrows) on chromosomes 9, 16, and 23, each demarcated by a red spot from differentially brighter PI staining (HANSON *et al.* 1996). (C) High magnification of *IV* only (rotated image). (D) *NT14L-23R* "barbell"-*IV* from a different meiotic spread, showing the pair of green B77 FISH signals to be on the "exterior" and opposite the major *NOR* in chromosome 23 (brighter red; arrowheads). (E) *NT1L-14L* "barbell"-*IV*, showing the pair of B77 FISH signals (yellow) on the *IV* "exterior." (F) *NT2R-14R* "frying pan"-*IV* showing the pair of B77 FISH signals in the "proximal" *IV* region. (G) A pair of FISH signals on the same side of an *NT6L-14L* alternate "ring"-*IV*. (H) A pair of FISH signals on the same side of an *NT14R-24R* adjacent "ring"-*IV*. (I) A single unpaired FISH signal on the "knob" (unpaired arm at the nontelosomic end of the chain) of a *Te14LoNT1L-14L* "wheel barrow"-*IV*. (J) A single unpaired FISH signal on the "knob" of a *Te14LoNT14L-23R* "wheel barrow"-*IV*. (K and L) A single unpaired FISH signal was in the "exterior" of the *Te14LoNT6L-14R* "frying pan"-*IV*s. (M) A single unpaired FISH signal on the "knob" of a *Te14LoNT14R-24R* "N-shaped chain"-*IV*. (N) A single unpaired FISH signal on a *Te14LoNT2R-14R* "U-shaped chain"-*IV*.

located on the inner side of the "pan" (Figure 1F), indicating that the crossing over occurs in the interstitial region in *14R* and that B77 is "proximal" to the respective *T2R-14R* breakpoint. These results indicated that B77 is located in the interstitial region between the centromere of chromosome 14 and the *T2R-14R* breakpoint in chromosome 14. According to the map of breakpoints (MENZEL *et al.* 1985), the *T2R-14R* breakpoint is in the right arm of chromosome 14 (*14R*). Therefore, B77 must be in the right arm of chromosome 14 (*14R*). This conclusion was consistent with the results from *NT1L-14L* and *NT14L-23R*, indicating that the relative arm assignments by MENZEL *et al.* (1985) were internally consistent for chromosome 14 breakpoints of *T1L-14L*, *T2R-14R*, and *T14L-23R*.

"Ring"-*IV*s were observed in both *NT6L-14L* (alternate; Figure 1G) and *NT14R-24R* (adjacent; Figure 1H) metaphase I cells. In each type of *NT*, the B77 signals were located on just one side of the "ring"-*IV*s, indicating that the B77 site must be "opposite" or "distal" to the respective translocation breakpoint in chromosome 14. These data indicated that if (and only if) the *T6L-14L* and *T14R-24R* breakpoints are indeed in separate arms, as indicated by MENZEL *et al.* (1985), then B77 must be "distal" to one breakpoint and "opposite" the other. Our findings, which indicate that B77 is proximal to neither breakpoint, are concordant with the previous report that both breakpoints are near the chromosome 14 centromere (MENZEL *et al.* 1985). However, our *NT-IV* data do not indicate the arm in which B77 is located relative to these two translocation breakpoints. For this purpose, we tried a newer procedure, based on molecular-meiotic analysis of monotelodisomic translocation heterozygotes (*TeNT*s).

Subchromosomal localization of B77 by analysis of *TeNT*s: The relationship between a breakpoint and a

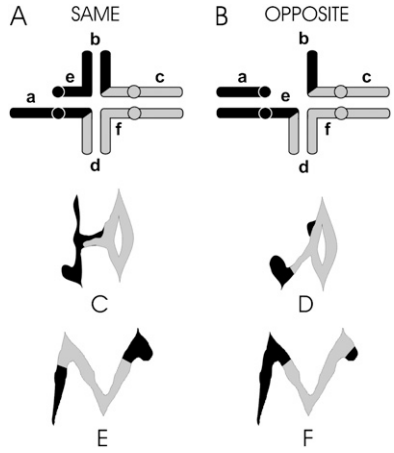

Figure 2.—Diagrams of monotelodisomic translocation heterozygote quadrivalents (*TeNT IV*s) and their corresponding MI configurations, following the nomenclature of Menzel *et al.* (1985) for chromosome segments. (A) A pachytene representation of a *TeNT IV* with the telosome and the breakpoint affecting the same arm. (B) A pachytene representation of a *TeNT IV* with the telosome and the breakpoint affecting different arms. (C) An MI "wheel barrow"-*IV* produced from A with segments b, c, d, e, and f being chiasmate. (D) An MI "frying pan"-*IV* produced from B with segments a, c, d, and f being chiasmate. (E) An MI "chain"-*IV* produced from A with segments b, c, and d being chiasmate. (F) An MI "chain"-*IV* produced from B with segments a, c, and d being chiasmate.

related telosome can be deduced from several types of "critical configurations" in which one or both interstitial regions are chiasmate (Menzel *et al.* 1985; Stelly *et al.* 1996). "Wheel barrow"-shaped *TeNT IV*s were observed in *Te14LoNT1L-14L* (Figure 1I) and *Te14LoNT14L-23R* metaphase I cells (Figure 1J). This type of *TeNT IV* configuration is critical in that it can arise only when the telosome is homologous to the breakpoint-bearing arm; it is formed if both "distal" segments, the single disomic opposite arm, and one or both of the "interstitial" regions are chiasmate (Figure 2, A and C). Therefore, the *T1L-14L* and *T14L-23R* breakpoints in chromosome 14 must be located in the arm homologous to the telosome, *i.e.*, the long arm of chromosome 14 (*14Lo*). The FISH signals were observed on the highly contracted hemizygous end of the chain, which looks like a terminal "knob" from Figure 1, I and J and corresponds to region "a" in Figure 2C. The *TeNT* results concomitantly indicated that B77 is in arm *14sh*, that *14sh* = *14R*, that the arm designations of the chromosome 14 breakpoints in *T1L-14L* and *T14L-23R* are internally consistent, and that B77 lies in the arm "opposite" the respective breakpoints, *i.e.*, *14Lo*.

"Frying pan"-shaped *TeNT IV*s were observed in *Te14LoNT6L-14L* (previously designated) metaphase I cells (Figure 1, K and L). This type of *TeNT IV* configuration is critical in that it can arise only when the telosome is opposite the breakpoint-bearing arm; it is formed if both opposite arms, the single "distal" segment, and the single "interstitial" region are chiasmate (Figure 2, B and D). Therefore, the *T6L-14L* breakpoint in chromosome 14 must be located in the arm opposite the telosome, *i.e.*, the short arm of chromosome 14 (*14sh*). A single FISH signal was asymmetrically associated with the "pan" of each "frying pan" *IV*, indicating that the B77 cluster is in the hemizygous distal segment corresponding to segment "B" in Figure 2D, *i.e.*, *14L*, according to the previous arm assignments by Menzel *et al.* (1985). According to assignments by Menzel *et al.* (1985), the FISH result would indicate that B77 was in *14L* and that *14sh* = *14L*, which is contrary to our above inference based on *NT1L-14L* and *NT14L-23R*. As shown below, arm assignments of *NT2R-14R* and *NT14R-24R* are correct for the respective chromosome 14 breakpoints. Therefore, the result indicated that the previous assignment of the *T6L-14L* breakpoint to arm *14L* by Menzel *et al.* (1985) is internally inconsistent with the other four chromosome 14 translocations. On the basis of these observations, we correct the previous assignment of *T6L-14L* (Menzel *et al.* 1985) by redesignating it as *T6L-14R*.

"Chain"-*IV*s were observed in *Te14LoNT14R-24R*, and the FISH signal occurred at a single site on the "knob" (Figure 1M). "Chain" configurations lack interstitial chiasmata and, in themselves, do not define whether a telosome is homologous to a breakpoint-bearing arm or not (Figure 2, E and F). "Chain" configurations thus constitute "noncritical configurations." The hemizygosity and position of the FISH signal concordantly indicated, however, that B77 lies in the arm opposite the telosome (*14Lo*), *i.e.*, short arm of chromosome 14 (*14sh*), a finding consistent with the results from the previously mentioned *TeNT* critical configurations. The *TeNT*-based analyses indicated that B77 lies distal to or opposite the chromosome 14 breakpoint, but could not distinguish between these possibilities without additional information, *e.g.*, interstitial chiasmata.

"Chain" *IV*s of *Te14LoNT2R-14R* were also observed to bear the B77 signal at just one location per *IV*, indicating hemizygosity (Figure 1N). The position of the signal, which corresponds to region "E" of Figure 2, B and F, indicated that the B77 locus is proximal to the breakpoint and that the breakpoint is in the hemizygous arm (*14sh*) and, thus, that *14R* = *14sh*. The findings for *Te14LoNT2R-14R* confirm the previous assignment by Menzel *et al.* (1985) of the *T2R-14R* breakpoint to arm *14R*.

Confirmation of *T14R-24R* chromosome 14 breakpoint on arm *14R*: In lieu of critical configurations in *Te14LoNT14R-24R* meiocytes, we FISHed B77 to segmental *dp-df* stocks to test the arm assignment of the chromosome 14 breakpoint in *T14R-24R*. A *dp-df* plant from *NT14-24* was shown by molecular cytogenetics to be hemizygous for B77 (Ji *et al.* 1999b). Such a plant would be adjacent-2 *dp24L-df14R*, if the *T14-24* chromosome 14 breakpoint were in *14L* (Figure 3A), or adjacent-1 *dp24R-df14R*, if the *T14-24* chromosome

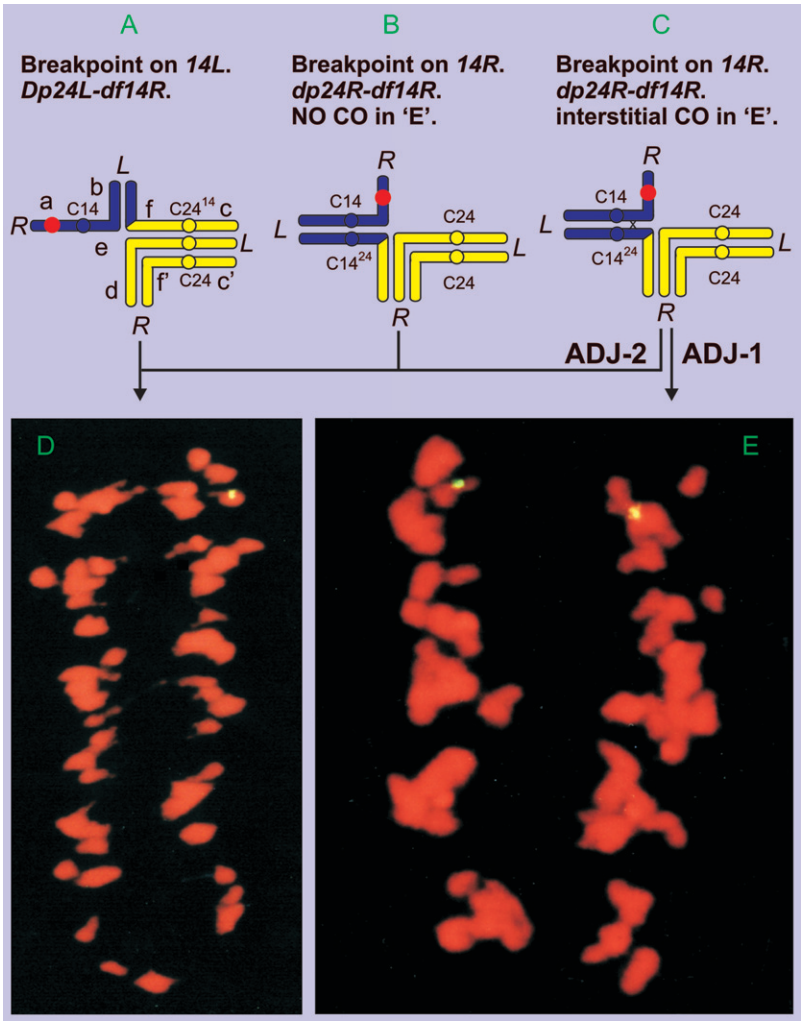

Figure 3.—Relationship between A–C pachytene representations of the FISH signal-adorned *dp-df*'s (hemizygous for FISH site) from *NT14-24* under two scenarios: the chromosome 14 breakpoint is located in *14R* or *14L*, respectively, and D and E photomicrographs of AI produced from the corresponding *dp-df*'s. (A) *NT14-24* breakpoint in chromosome 14 located on *14L* [adjacent (ADJ)-2 *dp24L-df14R*]. (B) *NT14-24* breakpoint in chromosome 14 located on *14R* (ADJ-1 *dp24R-df14R*) with no crossing over (CO) in the interstitial region "e." (C) *NT14-24* breakpoint in chromosome 14 located on *14R* (ADJ-1 *dp24R-df14R*) with an interstitial CO in region "e." (D) AI spread of the *dp-df*'s showing that the FISH signal goes to one pole, which is not diagnostic of the breakpoint's arm location. (E) AI spread of the *dp-df* showing the segregation of FISH signal to two poles, diagnostically indicating the *T14-24* breakpoint in chromosome 14 is on *14R*.

14 breakpoint were in *14R* (Figure 3, B and C). The expected distribution of B77 signals at anaphase I (AI) is quite different for the two types of *dp-df*'s. For the former, only one of the two meiosis I products would bear the B77 FISH signal, unless sister centromeres were to separate precociously (Figure 3, A and D). For the latter, one or both meiotic I products would bear the B77 signal, depending on recombination in the interstitial segment (Figure 3, B and E). The equal distribution at meiosis I of a hemizygous FISH locus (Figure 3E) of the involved *dp-df* can occur only under the following situations: (i) the *T14R-24R* breakpoint in chromosome 14 must be located in the arm carrying the FISH site, *i.e.*, *14R*; (ii) crossing over must occur in the interstitial region proximal to this breakpoint; and (iii) homologous centromeres must undergo adjacent-1 disjunction. The equal distribution of the FISH signal was observed in ~5% of anaphase I–metaphase II spreads of the *dp-df* stock that was deficient for *14R*, strongly indicating that the *T14-24* breakpoint in chromosome 14 is located to arm *14R* and thus confirming the arm assignment by Menzel *et al.* (1985). If the breakpoint were in the opposite arm, precocious sister-centromere separation would be required at anaphase I to produce signal in both meiotic I products. Moreover, nondisjunction would have been expected to arise occasionally from such precociously separated sister centromeres, whereas none was observed on the basis of the B77 signal.

DISCUSSION

In this study, we localized the B77 FISH locus to the right arm of chromosome 14 (*14R*), demonstrated that *14R* is the short arm of chromosome 14 (*14sh*), and confirmed the previous arm designations (*R vs. L*) for all the chromosome 14 translocation breakpoints, except *T6L-14L*, for which the breakpoint was redesignated to *14R*.

Relationship between RFLP and physical maps: In constructing a detailed RFLP map of cotton, Reinisch *et al.* (1994) associated a linkage group (~149 cM) with chromosome 14 by deficiency analysis using an interspecific F_1 monotelodisomic lacking the *G. hirsutum* chromosome arm *14sh*. A recent study concatenated LG *U09* to chromosome 14, bringing its length up to ~165 cM (Rong *et al.* 2004). Chromosome 14 was initially

hypothesized to be homeologous to the *A*-subgenome chromosome 2 on the basis of monosomic plant description (Endrizzi *et al.* 1985). Their homeology was further confirmed by duplicated DNA markers on both chromosomes (Reinisch *et al.* 1994; Lacape *et al.* 2003; Rong *et al.* 2004). The tandem repeat family (B77), isolated from *G. barbadense*, was mapped to the terminal of LG D04, now assigned to chromosome 20 (Zhao *et al.* 1998; Rong *et al.* 2004). However, our findings cytologically associate B77 FISH signals with chromosome 14. Given the strong evidence supporting the identity of chromosome 14, we proposed the following hypothesis for the conflicting data.

We hypothesized that B77 was incorrectly assigned to LG chromosome 20, previously known as LG D04. As noted above, B77 was mapped to a terminus of the linkage group, ~19 cM away from the nearest marker (Zhao *et al.* 1998). Although the linkage was statistically significant, the lack of flanking markers renders the linkage of terminal markers speculative. This uncertainty is made even greater in that the marker was "dominant"; *i.e.*, segregation was for presence *vs.* absence of the B77 allele with no ability to detect heterozygotes. A few additional markers were recently mapped to the terminus of the same linkage group; B77 was slightly closer (~16 cM) to G1016, but did not fit with the other nearby markers or anywhere else in the genome (A. H. Paterson, unpublished data). The best fit for B77 is still chromosome 20, but it is no longer statistically significant. One possible explanation may be that there are small groups of B77 repeats at multiple locations in the genome, in addition to the primary array on chromosome 14. The polymorphism tenuously associated with chromosome 20 may be confounded with B77 alleles resulting from loss of restriction sites at some other locus as well. In any case, we must conclude that if there is a B77 locus on chromosome 20, it is composed of a relatively small number of elements and that the primary locus appears to be on chromosome 14.

Position of B77 relative to translocation breakpoints in chromosome 14: The association of B77 with chromosome 14 was revealed by association of B77 FISH signal with the *IV*s of five different euploid chromosome 14 translocation heterozygotes and the five respective *Te14Lo*-bearing monotelodisomic translocation heterozygotes. B77 was subchromosomally localized by more detailed analysis of the position of B77 signal(s) on the respective multivalents and allowed placement of B77 relative to the centromere and respective breakpoints. For the three translocations with high frequencies of interstitial chiasmata (*T1L-14L*, *T2R-14R*, and *T14L-23R*), the heterozygotes were sufficient for mapping, whereas, for the other two (*T6L-14L*, *T14R-24R*), the monotelodisomic translocation heterozygotes and chromosomal segmental *dp-df*s were used for mapping.

The *NT* and *TeNT* data for these five translocations indicated that the relative arm assignments by Menzel *et al.* (1985) were correct for at least three of the five chromosome 14 breakpoints (*T1L-14L*, *T2R-14R*, and *T14L-23R*), but incorrect for *T6L-14L*, which was redesignated as *T6L-14R*. The incorrect assignment of the *T6L-14L* breakpoint to *14L* was also indicated as a footnote in a previous report, but data were not shown (Menzel and Dougherty 1987). The analysis of the segregation of B77 on the hemizygous segment of a *dp-df* stock (*dp24R-df14R*) supported the original assignment of the *T14R-24R* chromosome 14 breakpoint to arm *14R* (Menzel *et al.* 1985), but did not support its later reassignment to *14L*, which was noted as a footnote in a previous report (Menzel and Dougherty 1987). Our data and those of Menzel *et al.* (1985) concordantly indicate that the *T6L-14R* and *T14R-24R* breakpoints are recombinationally very close to the centromeres. Two of the five translocation breakpoints in chromosome 14, *i.e.*, the *T1L-14L* and *T14L-23R* breakpoints, affect arm *14L*, whereas the other three, *i.e.*, the *T2R-14R*, *T6L-14R*, and *T14R-24R* breakpoints, affect the opposing arm, *14R*. In addition, our data show that *14sh* = *14R* and *14Lo* = *14L*.

Our analysis allows placement of B77 relative to the translocation breakpoints, providing a seminal integrated map. B77 was mapped to *14R*, which was opposite the two translocation breakpoints in *14L*. Analysis of *NT2R-14R* and *Te14LoNT2R-14R* placed B77 in the interstitial region of *NT2R-14R* in chromosome 14, *i.e.*, between the chromosome 14 centromere and the *T2R-14R* breakpoint. Analyses of *NT6L-14R*, *NT14R-24R*, *Te14LoNT6L-14R*, and *Te14LoNT14R-24R* placed B77 distal to *T6L-14R* and *T14R-24R* breakpoints. According to Menzel *et al.* (1985), *T6L-14R* and *T14R-24R* breakpoints in chromosome 14 were ~4.5 and 2.7 cM from the centromere, respectively. High-resolution mapping with a *dp-df* stock indicated that the latter was 2.57 cM (our unpublished data). Our analyses place the breakpoints in the same arm; thus the data from Menzel *et al.* (1985) and the map from the *dp-df* suggest that the *T6L-14R* breakpoint in chromosome 14 may be farther from the centromere relative to the *T14R-24R* breakpoint in chromosome 14. The combined data suggest that B77 is between the *NT2R-14R* breakpoint (2B-1) and the *NT6L-14R* breakpoint in *14R* or *14sh*. A revised map for chromosome 14 breakpoints and B77 is shown in Figure 4.

Perspectives: Our findings have several ramifications. By anchoring B77 to the chromosome 14 map, we have rendered it a useful molecular genetic/cytogenetic marker for that specific chromosome and segment. Most significantly, the results demonstrate the feasibility of integrative mapping, where one or more unknown(s) can be mapped relative to other types of known loci, centromeres, translocation breakpoints, and telomeres (Reyes-Valdés and Stelly 1995; Reyes-Valdés *et al.* 1996). A skeletal map of molecular cytogenetic loci will facilitate subsequent mapping of repetitive sequences,

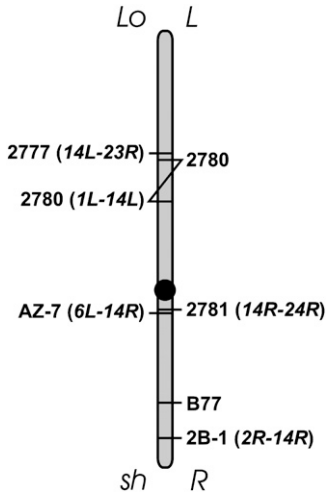

FIGURE 4.—Chromosome map of chromosome 14 breakpoints and B77. *L*, left arm; *R*, right arm; *sh*, short arm; *Lo*, long arm.

and mature integrated maps will improve genome comparisons, interspecific introgression, analysis of transformant gene activity (position effects), and rapid assessment of karyotypic variation in wild germplasm.

The authors gratefully acknowledge the support of this work in part by the U. S. Department of Agriculture (USDA) grant 91-37300-8819 and Texas Advanced Technology Research Program (TATRP) grant 999902148 for cloning, recombination mapping, and characterization of B77; USDA grant NR1 CGP 92-37300-7655 (classical cytogenetics) for development of cytogenetic stocks; TATRP grant 999902090 (molecular cytogenetics) for physical mapping by FISH; and a Tom Slick Senior Graduate Fellowship at Texas A&M University awarded to the senior author.

LITERATURE CITED

ASHLEY, T., T. RIED and D. C. WARD, 1994 Detection of nondisjunction and recombination in meiotic and post meiotic cells from XY-Sxr (XY, Tp(Y)1Ct) mice using multicolor fluorescence *in situ* hybridization. Proc. Natl. Acad. Sci. USA **91:** 524–528.

BEASLEY, J. O., 1940 The origin of American tetraploid *Gossypium* species. Am. Nat. **74:** 285–286.

BEASLEY, J. O., 1942 Meiotic chromosome behavior in species, species hybrids, haploids and induced polyploids of Gossypium. Genetics **27:** 25–54.

BERGEY, D. R., D. M. STELLY, H. J. PRICE and T. D. MCKNIGHT, 1989 *In situ* hybridization of biotinylated DNA probes to cotton meiotic chromosomes. Stain. Technol. **64:** 25–37.

BROWN, M. S., 1980 The identification of chromosomes of *Gossypium hirsutum* L. by means of translocations. J. Hered. **71:** 266–274.

BROWN, M. S., M. Y. MENZEL, C. A. HASENKAMPF and S. NAQI, 1981 Chromosome configurations and orientations in 58 heterozygous translocations in *Gossypium hirsutum*. J. Hered. **72:** 161–168.

CRANE, C. F., H. J. PRICE, D. M. STELLY and D. G. CZESCHIN, JR., 1993 Identification of a homeologous chromosome pair by *in situ* DNA hybridization to ribosomal RNA loci in meiotic chromosomes of cotton (*Gossypium hirsutum*). Genome **36:** 1015–1022.

DONG, H., and J. S. QUICK, 1995 Detection of a 2.6 kb single/low copy DNA sequence on chromosomes of wheat (*Triticum aestivum*) and rye (*Secale cereale*) by fluorescence *in situ* hybridization. Genome **38:** 246–249.

ENDRIZZI, J. E., and G. RAMSAY, 1979 Monosomes and telosomes for 18 of the 26 chromosomes of *Gossypium hirsutum*. Can. J. Genet. Cytol. **21:** 531–536.

ENDRIZZI, J. E., and G. RAMSAY, 1980 Identification of ten chromosome deficiencies of cotton. J. Hered. **71:** 45–48.

ENDRIZZI, J. E., E. L. TURCOTTE and R. J. KOHEL, 1985 Genetics, cytology, and evolution of *Gossypium*. Adv. Genet. **23:** 271–375.

HAN, Z. G., C. B. WANG, X. L. SONG, W. Z. GUO, J. Y. GOU *et al.*, 2006 Characteristics, development and mapping of *Gossypium hirsutum* derived EST-SSRs in allotetraploid cotton. Theor. Appl. Genet. **112:** 430–439.

HANSON, R. E, M. N. ISLAM-FARIDI, E. A. PERCIVAL, C. F. CRANE, Y. JI *et al.*, 1996 Distribution of 5S and 18S rDNA loci in a tetraploid cotton (*Gossypium hirsutum* L.) and its putative diploid ancestors. Chromosoma **105:** 55–61.

JI, Y., W. A. RASKA, T. D. MCKNIGHT, M. N. ISLAM-FARIDI, C. F. CRANE *et al.*, 1997 Use of meiotic FISH for identification of a new monosome in *Gossypium hirsutum* L. Genome **40:** 34–40.

JI, Y., M. DE DONATO, C. F. CRANE, W. A. RASKA, M. N. ISLAM-FARIDI *et al.*, 1999a New ribosomal RNA gene locations in *Gossypium hirsutum* mapped by meiotic FISH. Chromosoma **108:** 200–207.

JI, Y., W. A. RASKA, M. DE DONATO, M. N. ISLAM-FARIDI, J. H. PRICE *et al.*, 1999b Identification and distinction among segmental duplication-deficiencies by fluorescence in situ hybridization (FISH)-adorned multivalent analysis. Genome **42:** 763–771.

JI, Y., R. PERTUZE and R. T. CHETELAT, 2004 Genome differentiation by GISH in interspecific and intergeneric hybrids of tomato and related nightshades. Chromosome Res. **12:** 107–116.

JIANG, J., and B. S. GILL, 1994 New 18S.26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. Chromosoma **103:** 179–185.

KIM, J. S., M. N. ISLAM-FARIDI, P. E. KLEIN, D. M. STELLY, H. J. PRICE *et al.*, 2005 Comprehensive molecular cytogenetic analysis of sorghum genome architecture: distribution of euchromatin, heterochromatin, genes and recombination in comparison to rice. Genetics **171:** 1963–1976.

KIMBER, G., 1961 Basis of the diploid-like meiotic behavior of polyploid cotton. Nature **191:** 98–100.

LACAPE, J. M., T. B. NGUYEN, S. THIBIVILLIERS, B. BOJINOV, B. COURTOIS *et al.*, 2003 A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. Genome **46:** 612–626.

LEMIEUX, N., B. MALFOY, R. FETNI, M. MULERIS, N. VOGT *et al.*, 1994 *In situ* hybridization approach at infragenic level on metaphase chromosomes. Cytogenet. Cell Genet. **66:** 107–112.

MENZEL, M. Y., and M. S. BROWN, 1978 Reciprocal chromosome translocations in *Gossypium hirsutum*. J. Hered. **69:** 383–390.

MENZEL, M. Y., and B. J. DOUGHERTY, 1987 Transmission of duplication-deficiencies from cotton translocations is unrelated to map lengths of the unbalanced segments. Genetics **116:** 321–330.

MENZEL, M. Y., K. L. RICHMOND and B. J. DOUGHERTY, 1985 A chromosome translocation breakpoint map of the *Gossypium hirsutum* genome. J. Hered. **76:** 406–414.

MURSAL, I. E. J., and J. E. ENDRIZZI, 1976 Re-examination of diploid-like meiotic behavior of polyploid cotton. Theor. Appl. Genet. **47:** 171–178.

NGUYEN, T. B., M. GIBAND, P. BROTTIER, A. M. RISTERUCCI and J. M. LACAPE, 2004 Wide coverage of the tetraploid cotton genome using newly developed microsatellite markers. Theor. Appl. Genet. **109:** 167–175.

PRICE, H. J., D. M. STELLY, T. D. MCKNIGHT, C. F. SCHEURING, D. RASKA *et al.*, 1990 Molecular cytogenetic mapping of a nucleolar organizer region in cotton. J. Hered. **81:** 365–370.

REINISCH, A. J., J. DONG, C. L. BRUBAKER, D. M. STELLY, J. F. WENDEL *et al.*, 1994 A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: chromosome organization and evolution in a disomic polyploid genome. Genetics **138:** 829–847.

REYES-VALDÉS, M. H., and D. M. STELLY, 1995 A maximum likelihood algorithm for genome mapping of cytogenetic loci from meiotic configuration data. Proc. Natl. Acad. Sci. USA **92:** 9824–9828.

REYES-VALDÉS, M. H., Y. JI, C. F. CRANE, J. F. TAYLOR, M. N. ISLAM-FARIDI *et al.*, 1996 ISH-facilitated analysis of meiotic bivalent pairing. Genome **39:** 784–792.

RONG, J. K., C. ABBEY, J. E. BOWERS, C. L. BRUBAKER, C. CHANG *et al.*, 2004 A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (Gossypium). Genetics **166:** 389–417.

Skovsted, A., 1934 Cytological studies of cotton. II. Two interspecific hybrids between Asiatic and New World cotton. J. Genet. **28:** 407–424.

Stelly, D. M., 1993 Interfacing cytogenetics with the cotton genome effort, pp. 1545–1550 in *Proceedings of the Beltwide Cotton Conferences*, edited by D. J. Herber and D. A. Richter. National Cotton Council of America, Memphis.

Stelly, D. M., C. F. Crane, R. E. Hanson, T. D. McKnight, and H. J. Price, 1996 Molecular-meiotic analysis of cotton, pp. 99–111 in *Methods of Genome Analysis in Plants: Their Merits and Pitfalls*, edited by P. P. Jauhar. CRC Press, Boca Raton, FL.

Wang, K., X. L. Song, Z. G. Han, W. Z. Guo, J. Z. Yu *et al.*, 2006 Complete assignment of the chromosomes of *Gossypium hirsutum* L. by translocation and fluorescence *in situ* hybridization mapping. Theor. Appl. Genet. **113:** 73–80.

Zhao, X., Y. Ji, X. Ding, D. M. Stelly and A. H. Paterson, 1998 Macromolecular organization and genetic mapping of a rapidly-evolving chromosome-specific tandem repeat family (B77) in cotton (*Gossypium*). Plant Mol. Biol. **38:** 1031–1042.

Zhong, X. B., J. Bodeau, P. F. Fransz, V. M. Williamson, A. van Kammen *et al.*, 1999 FISH to meiotic pachytene chromosomes of tomato locates the root knot nematode resistance gene *Mi-1* and the acid phosphatase gene *Aps-1* near the junction of euchromatin and pericentromeric heterochromatin of chromosome arms 6S and 6L, respectively. Theor. Appl. Genet. **98:** 365–370.

Communicating editor: J. A. Birchler