Chromosoma (1996) 105:55–61

CHROMOSOMA
© Springer-Verlag 1996

Distribution of 5S and 18S-28S rDNA loci in a tetraploid cotton (*Gossypium hirsutum* L.) and its putative diploid ancestors

Robert E. Hanson[1], M. Nurul Islam-Faridi[1], Edward A. Percival[2], Charles F. Crane[1], Yuanfu Ji[1], Thomas D. McKnight[3], David M. Stelly[1], H. James Price[1]

[1] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474, USA
(e-mail: monosom@rigel.tamu.edu)
[2] USDA Agricultural Research Service, College Station, TX 77845, USA
[3] Department of Biology, Texas A&M University, College Station, TX 77843, USA

Received: 5 November 1995; in revised form: 16 February 1996 / Accepted: 28 February 1996

Abstract. The most widely cultivated species of cotton, *Gossypium hirsutum,* is a disomic tetraploid (2*n*=4*x*=52). It has been proposed previously that extant A- and D-genome species are most closely related to the diploid progenitors of the tetraploid. We used fluorescent in situ hybridization (FISH) to determine the distribution of 5S and 18S-28S rDNA loci in the A-genome species *G. herbaceum* and *G. arboreum*, the D-genome species *G. raimondii* and *G. thurberi,* and the AD tetraploid *G. hirsutum*. High signal-to-noise, single-label FISH was used to enumerate rDNA loci, and simultaneous, dual-label FISH was used to determine the syntenic relationships of 5S rDNA loci relative to 18S-28S rDNA loci. These techniques provided greater sensitivity than our previous methods and permitted detection of six new G. *hirsutum* 18S-28S rDNA loci, bringing the total number of observed loci to 11. Differences in the intensity of the hybridization signal at these loci allowed us to designate them as major, intermediate, or minor 18S-28S loci. Using genomic painting with labeled A-genome DNA, five 18S-28S loci were localized to the *G. hirsutum* A-subgenome and six to the D-subgenome. Four of the 11 18S-28S rDNA loci in *G. hirsutum* could not be accounted for in its presumed diploid progenitors, as both A-genome species had three loci and both D-genome species had four. *G. hirsutum* has two 5S rDNA loci, both of which are syntenic to major 18S-28S rDNA loci. All four of the diploid genomes we examined contained a single 5S locus. In *G. herbaceum* (A_1) and *G. thurberi* (D_1), the 5S locus is syntenic to a major 18S-28S locus, but in *G. arboreum* (A_2) and *G. raimondii* (D_5), the proposed D-genome progenitor of *G. hirsutum*, the 5S loci are syntenic to minor and intermediate 18S-28S loci, respectively. The multiplicity, variation in size and site number, and lack of additivity between the tetraploid species and its putative diploid ancestors indicate that the behavior of rDNA loci in cotton is nondogmatic, and considerably more complex and dynamic than previously envisioned. The relative variability of 18S-28S rDNA loci versus 5S rDNA loci suggests that the behavior of tandem repeats can differ widely.

Edited by: R. Appels

Correspondence to: R.E. Hanson

Introduction

There are approximately 50 species in the cotton genus *Gossypium*, including 45 diploids and at least 5 tetraploids (Fryxell 1992). Collectively, they grow throughout tropical and subtropical parts of the world and are genomically diversified in relation to their geographic ranges (Skovsted 1934). Seven major diploid genomic groups of *Gossypium* have been identified and are designated by the letters A through G (Beasley 1940, 1942; Edwards and Mirza 1979).

The species *Gossypium hirsutum* is an AD disomic tetraploid (2*n*=4*x*=52) that originated from an interspecific hybridization event(s) between diploid *Gossypium* species thought to be closely related to the A_1-genome species *G. herbaceum* and the D_5-genome species *G. raimondii* (Endrizzi et al. 1985).The *G. hirsutum* chromosomes of A-genome origin are larger, as a group, and are numbered 1–13, whereas those of D-genome origin are numbered 14–26. The A-genome and D-genome diploids are estimated to have diverged from a common ancestor between 6 and 11 million years ago, and the hybridization event(s) leading to *G. hirsutum* is estimated to have occurred 1 to 2 million years ago (Wendel 1989; Wendel and Albert 1992). Previous efforts at interspecific comparisons within *Gossypium* have employed morphological (Fryxell 1992), meiotic (Menzel 1954), karyotypic (Beasley 1940), genetic and molecular criteria (Wendel 1989).

In this study, we have used fluorescent in situ hybridization (FISH) to examine the distribution of 5S and 18S-28S rDNA sites in *G. hirsutum* and closely related diploid species in order to address three objectives. The first objective was to determine the respective homoeologous relationship of chromosomes that bear 5S and

18S-28S rDNA sequences in the diploids. The second objective was to provide new molecular cytogenetic landmarks that will facilitate ongoing construction of a FISH-based karyotype for cotton, and development of an integrated map of recombinational and physical distances among loci defined by molecular markers and cytogenetic landmarks. The third objective was to understand the evolutionary history of rDNA loci of *Gossypium*.

Materials and methods

Probe DNA isolation and labeling. DNA of pAm033 (a 470 bp BamHI fragment of the 5S rDNA repeat of *Acacia melanoxylon* in pUC118, kindly provided by Rudi Appels) and pGMR3 (a 4.5 kb EcoRI fragment of the 18S-28S ribosomal repeat of *Glycine max* in pBR325, kindly provided by Elizabeth Zimmer) was isolated by alkaline lysis in plasmid maxipreps as described by Silhavy et al. (1984). Total genomic DNA was isolated from immature leaves of *G. raimondii* ($2n=2x=26=D_5D_5$) and *G. arboreum* ($2n=2x=26=A_2A_2$) (accessions given below) using the technique described by Paterson et al. (1993). Whole plasmid and total genomic DNAs were labeled with biotin-14-dATP (Gibco BRL) using the Gibco BRL BioNick Labeling System or with digoxigenin-11-dUTP (Boehringer-Mannheim) using the Boehringer-Mannheim nick-translation kit.

Plant material, pretreatment and metaphase preparation. Root tips of *G. hirsutum* ($2n=4x=52=[(AD_1)]^2$) (Deltapine 50, Delta and Pine Land Co.), *G. arboreum* (Accession no. A2-92) and *G. herbaceum* ($2n=2x=26=A_1A_1$) (Accession no. unknown) were clipped into 1–2 cm lengths and pretreated for 4 h with 2.5 mM 8-hydroxyquinoline (aq) at room temperature. Root tips of *G. raimondii* (Accession no. D5-1 A22) and *G. thurberi* ($2n=2x=26=D_1D_1$) (Accession no. D1-1) were clipped into 2–3 cm lengths and pretreated with 2.5 mM 8-hydroxyquinoline (aq) at room temperature for 5 h. Following pretreatment, root tips were fixed overnight in 4:1 ethanol:acetic acid at room temperature. All accessions were obtained from the National Collection of *Gossypium* Germplasm, College Station, Texas. Metaphase spreads were prepared as described by Jewell and Islam-Faridi (1994).

In situ hybridization. The procedure used was a modification of that of Islam-Faridi and Mujeeb-Kazi (1995). Slides were immersed in 30 µg/ml RNase in 2×SSC for 45 min at 37°C, denatured at 70°C in 70% formamide, 2×SSC for 2.5 min and dehydrated in 70%, 85%, 95% and 100% ethanol for 2 min each at –20°C.(1×SSC is 0.15 M NaCl, 0.015 M sodium citrate). The probe mix was denatured at 80°C for 5 min, applied to the air-dried slide in a 25 µl volume, covered with a 20 mm×40 mm coverslip and sealed with rubber cement. The slides were then put in a humidity chamber in an 80°C oven for 7 min and allowed to incubate overnight at 37°C. Each 25 µl of hybridization mixture contained 50% deionized formamide, 10% dextran sulfate, 9 µg *Escherichia coli* DNA, and 90 ng of biotin-labeled probe DNA in 2×SSC. For dual labeling, 90 ng of biotin-labeled 18S-28S probe and 90 ng of digoxigenin-labeled 5S rDNA probe were used per slide. For genomic "painting" of the A-subgenome 50 ng of biotin-labeled total genomic DNA of *G. arboreum*, 1 µg unlabelled genomic DNA of *G. raimondii* and 8 µg *E. coli* DNA were used per slide.

Following overnight incubation at 37°C, coverslips were removed and slides were rinsed at 40°C in: 2×SSC, 5 min; 2×SSC, 5 min; 2×SSC, 50% formamide, 10 min; 2×SSC, 5 min; 2×SSC, 5 min; and 4×SSC, 5 min. Signal from biotin-labeled probes was then amplified with sequential 30 min applications of 5 µg/ml fluorescein isothiocyanate (FITC)-avidin DCS (Vector) in 4×SSC, 0.2% Tween 20, 10 µg/ml biotinylated anti-avidin D (Vector) in 4×SSC, 0.2% Tween 20 and 5 µg/ml FITC-avidin in 4×SSC, 0.2% Tween 20. For two-probe detection, a final incubation with 20 µg/ml rhodamine anti-digoxigenin (Boehringer Mannheim) was added. After each step three washes were performed in 4×SSC, 0.2% Tween 20 for 5 min each at 37°C. Slides were stained in 2 µg/ml 4',6-diamidino-2-phenylindole (DAPI) in McIlvaines buffer (9 mM citric acid, 80 mM $Na_2HPO_4{\cdot}H_20$, 2.5 mM $MgCl_2$) for 20 min at room temperature (RT), then with propidium iodide (PI; 20 µg/ml in 2×SSC) for 30 min at RT, and finally, antifade was applied under a 22 mm×40 mm coverslip. For two-probe detection, no PI was used. After FISH detection of 18S-28S rDNA loci of *G. hirsutum* and photography of selected metaphase spreads was complete, coverslips were soaked off in 2×SSC, allowed to destain in 2×SSC for 30 min, dehydrated in a 70%, 85% and 95% ethanol series at RT, and air-dried. Genomic painting was then carried out on the slides as described above.

Microscopy. Images were photographed directly on Fuji ASA 400 film with Olympus AX-70 (Figs. 1A, B, D, E, 3B, C, E, 4B–D), Olympus Vanox (Figs. 1C, 3A, D), Zeiss Axioscope (Fig. 2A) and Zeiss Axiophot (Fig. 4A) epifluorescence microscopes using standard filter sets for DAPI, FITC and FITC/rhodamine/DAPI excitation.

Results

To detect 18S-28S rDNA loci with the greatest possible sensitivity, single-label FISH was used rather than dual-label FISH. The description and number of 18S-28S rDNA loci we detected using this method are given in Table 1; photomicrographs of the results are shown in Fig. 1A–E. For simplicity, 18S-28S rDNA FISH sites

Table 1. The distribution of 18S-28S rDNA loci in *Gossypium hirsutum* and closely related diploid species

Haploid genome or subgenome	Major 18S-28S rDNA loci	Intermediate 18S-28S rDNA loci	Minor 18S-28S rDNA loci	Total
G. hirsutum ([AD]1)	3	1	7	11
(A)	1	1	3	5
(D)	2	0	4	6
G. herbaceum (A1)	3	0	0	3
G. arboreum (A2)	2	0	1	3
G. raimondii (D5)	2	1	1	4
G. thurberi (D1)	4	0	0	4

Headings A and D represent the A- and D-subgenomes of *G. hirsutum*, as determined by genomic painting with labeled total A-genome (*G. arboreum*) DNA

were described as follows. Major sites were defined as those giving very large signals observable in all interphase and metaphase cells. Much smaller FISH signals, which were nevertheless detected with high frequency in interphase and metaphase cells, were described as intermediate sites. The smallest FISH signals, which were generally detectable in at least 40% of metaphase cells, were described as minor sites. Based on results from FISH of bacterial artificial chromosome clones of *G. hirsutum* (Hanson et al. 1995), we estimate that the smallest rDNA signals reported herein represent loci in the 25–35 kb size range.

The four largest 18S-28S rDNA loci of *G. hirsutum* were previously mapped by molecular-meiotic configuration analysis to chromosomes 7, 9, 16 and 23 (Price et al. 1990; Crane et al. 1993). A smaller fifth site was observed but not mapped. Using somatic metaphase preparations and improved protocols for in situ hybridization (ISH) and detection, we were able consistently to detect an additional six minor sites, bringing the total reported 18S-28S rDNA loci to 11 (Fig. 1E). Similar results have also been obtained in our laboratory using meiotic chromosomes. To avoid confusion of signals with background noise, which was extremely low, only loci at which a pair of signals was visible, one per chromatid, were scored. Eleven loci were observable in greater than 30% of the approximately 70 *G. hirsutum* metaphase spreads we examined. A minimum of 30 metaphase spreads was examined for enumeration of diploid 18S-28S rDNA loci.

To determine the subgenomic locations, A versus D, of 18S-28S loci in the *G. hirsutum* genome, metaphase spreads that had been used for FISH with the 18S-28S rDNA probe were destained, dehydrated in an ethanol series, air-dried and then reprobed with labeled A2-genome DNA (Fig. 2). It was found that addition of a 20-fold excess of unlabeled total genomic DNA of *G. raimondii* to the probe mix was sufficient to suppress cross-hybridization of sequences that are shared between the two genomes, with the exception of major loci of the highly conserved 18S-28S rDNA repeat. Results show that five 18S-28S loci can be localized to the *G. hirsutum* A-subgenome and six to the D-subgenome (Table 1). The number of 18S-28S loci in the A-subgenome is two more than the additive total observed in each A-diploid, and the number of 18S-28S loci in the D-subgenome is two more than was observed in each of the D-diploids (see Table 1). We observed no chromosomes with painted and unpainted segments, other than the 18S-28S rDNA loci bearing D-subgenome chromosomes, which suggests that no large intergenomic translocations have occurred following polyploidization. This conclusion was supported by genomic painting results using D-diploid DNA as a probe on other metaphase spreads of *G. hirsutum* chromosomes (not shown).

In all the diploids examined, only a single major pair of 5S rDNA FISH signals was observed per haploid genome, located near the centromere on the short arm of the respective chromosome (Fig. 3A–D). Two pairs of signals of unequal size were detected in *G. hirsutum*. The larger 5S rDNA locus was in the long arm near the

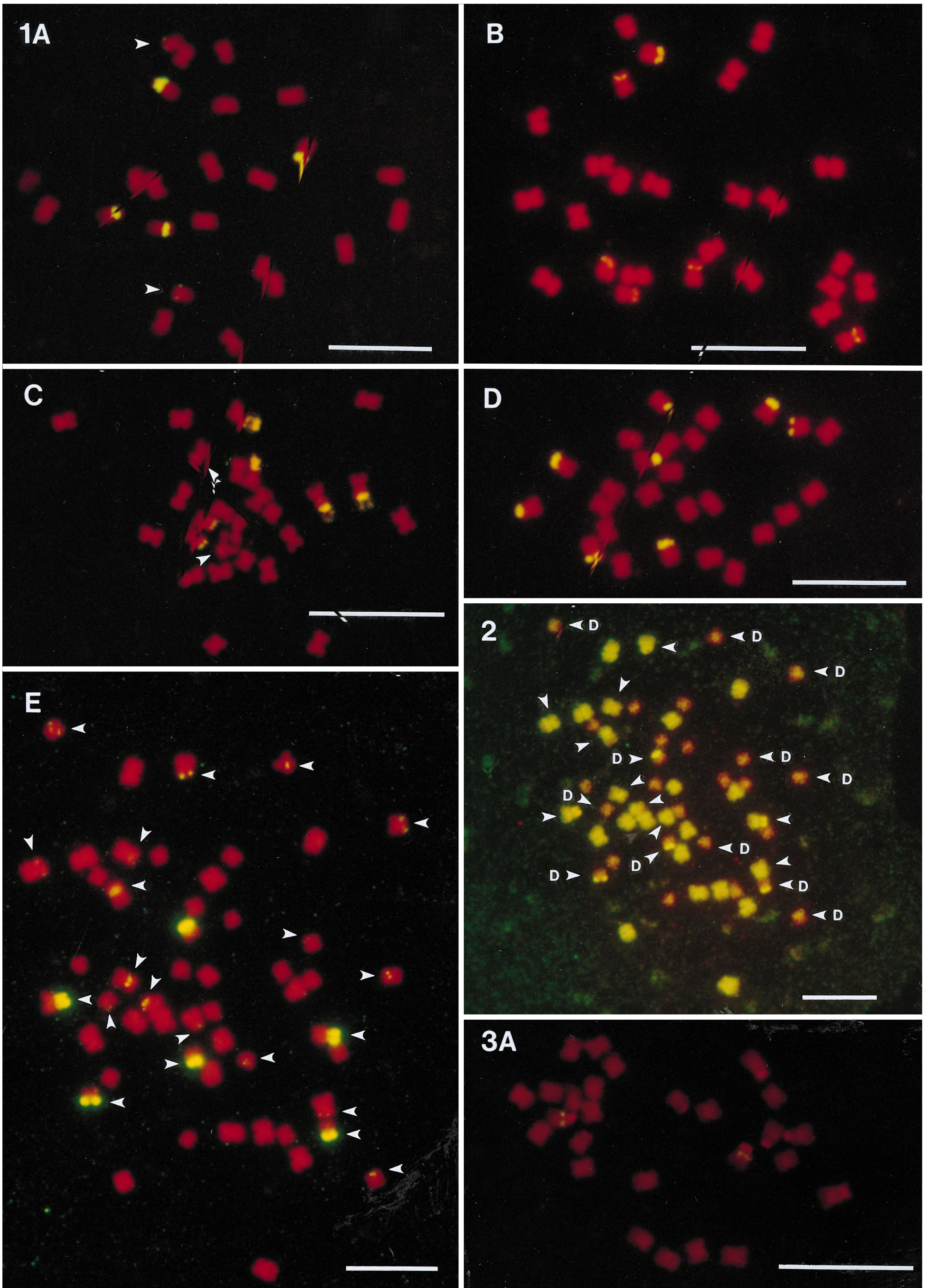
→

Fig. 1A–E. Fluorescence photomicrographs of cotton metaphase chromosomes hybridized with a biotin-labeled 18S-28S rDNA probe. Signals were detected with fluorescein isothiocyanate (FITC)-conjugated avidin and chromosomes were counterstained with propidium iodide (PI). **A** Partial metaphase from *G. arboreum* (A_1), showing all three 18S-28S loci. Two major loci and one minor site (*arrowheads*) are visible. **B** *G. herbaceum* (A_2); three major loci are visible. **C** *G. raimondii* (D_5) shows two major sites, an intermediate site, and one minor site (*arrowheads*). The minor site is extremely small and is located near the centromere on the short arm of the chromosome. **D** *G. thurberi* (D_1); four major loci are visible. **E** *G. hirsutum* ($[AD]_1$); *arrowheads* indicate the 11 observable loci. Three loci are major sites, one is an intermediate site and seven are minor. Bars represent 10 µm

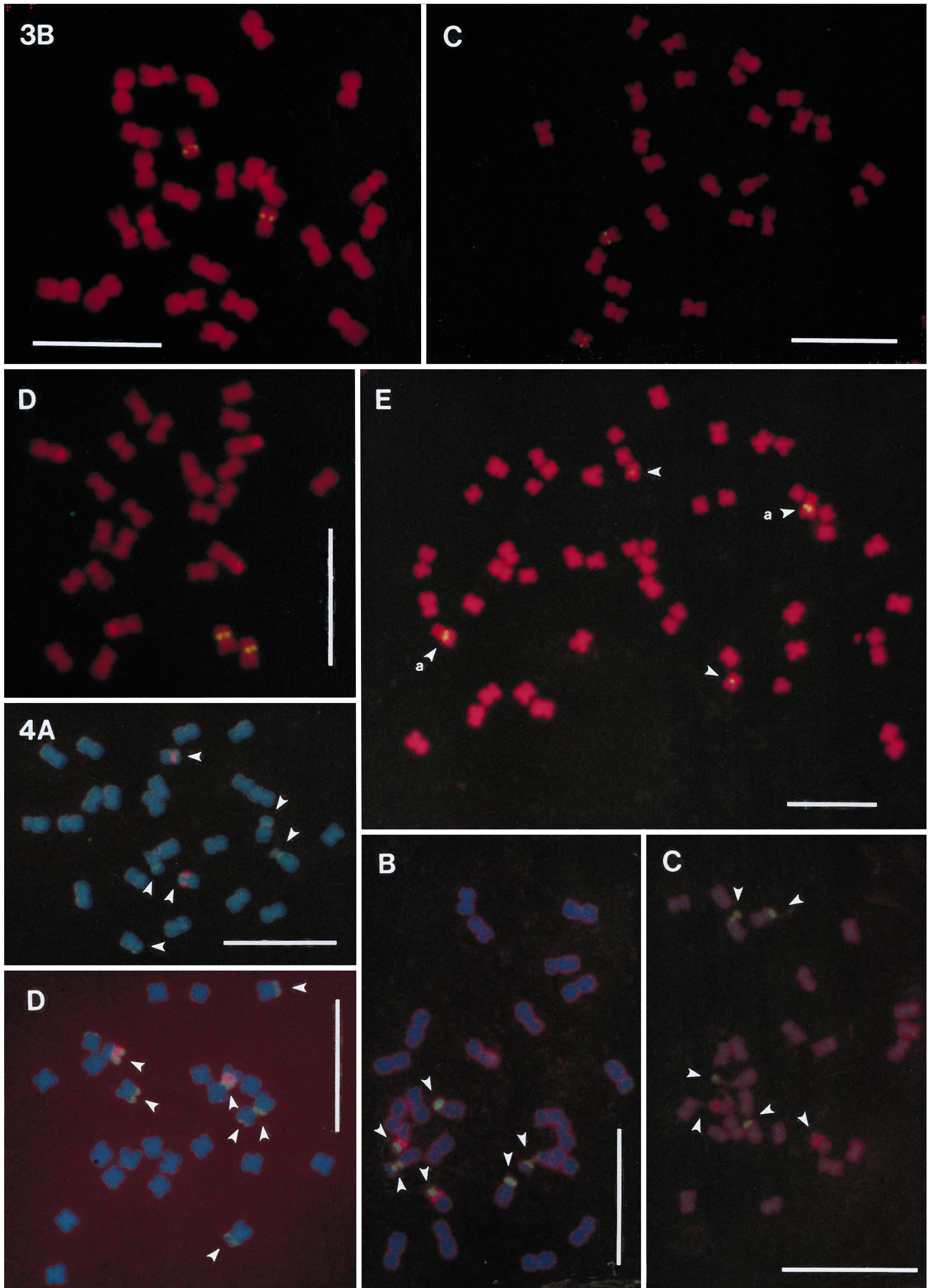
Fig. 2. Fluorescence photomicrograph of the metaphase spread of *G. hirsutum* shown in Fig. 1E rehybridized with biotin-labeled total A-genome (*G. arboreum*) DNA. Signal was detected with FITC-conjugated avidin and chromosomes were counterstained with PI. Unlabeled total D-genome (*G. raimondii*) DNA was used in a 20-fold excess to block cross-genomic hybridization. *Arrowheads* show chromosomes that bear 18S-28S rDNA loci; *arrowheads labeled D* represent chromosomes from the D-subgenome and *unlabeled arrowheads* represent chromosomes from the A-subgenome. The strong signals on two pairs of D-subgenome chromosomes are due to hybridization of the highly conserved 18S-28S rDNA repeat. No other areas of "painted" and "unpainted" segments can be observed on A- or D-subgenome chromosomes. Bar represents 10 µm

Fig. 3A–E. Fluorescence photomicrographs of cotton metaphase chromosomes hybridized with a biotin-labeled 5S rDNA probe. Signal was detected with FITC-conjugated avidin and chromosomes were counterstained with PI. In all the diploid species (**A–D**), one locus is visible, located near the centromere on the short arm of a homologous pair of chromosomes. **A** *G. thurberi* (D_1); note the PI staining of major 18S-28S rDNA loci. **B** *G. arboreum* (A_2). **C** *G. herbaceum* (A_1). **D** *G. raimondii* (D_5). **E** *G. hirsutum* ($[AD]_1$); two loci are visible. The larger pair of signals (*arrowheads labeled a*) are located on the long arm of chromosome 9 and the smaller pair of signals (*unlabeled arrowheads*) are located on the short arm of chromosome 23. Propidium iodide staining allows for the visualization of major 18S-28S rDNA loci syntenic to both 5S rDNA loci, on the short arm of the chromosome. Bars represent 10 µm

Fig. 4A–D. Fluorescence photomicrographs of metaphase chromosomes of diploid cotton simultaneously tested with a biotin-labeled 18S-28S probe and a digoxigenin-labeled 5S rDNA probe. The 18S-28S rDNA probe was detected with FITC-conjugated avidin (*yellow/green*) and the 5S rDNA probe was detected with rhodamine-conjugated anti-digoxigenin antibodies (*red*). Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI). *Arrowheads* indicate 18S-28S rDNA loci. In each figure the red-colored 5S rDNA signals are clearly visible. **A** *G. arboreum* (A_2). Three 18S-28S loci are visible; the 5S site is syntenic to the minor 18S-28S site, which can very faintly be seen on the short arm of the chromosome. **B** *G. herbaceum* (A_1). All three major 18S-28S loci are clearly visible; the 5S site is syntenic to one of the two telomerically located major loci. **C** *G. raimondii* (D_5). Three 18S-28S loci can be seen; the 5S site is syntenic to an intermediate 18S-28S site. **D** *G. thurberi*(D_1) shows the four major 18S-28S rDNA loci; signal is not visible in the photograph for one chromosome. The 5S site is syntenic to a major 18S-28S site. Bars represent 10 µm



Figs. 1A–3A (for legends see p. 57)



Figs. 3B–4D (for legends see p. 57)

centromere of a medium-sized pair of A-subgenome chromosomes, whereas the smaller locus was in the short arm near the centromere of a small- to medium-sized pair of D-subgenome chromosomes (Fig. 3E).

Differentially bright PI fluorescence allowed for the simultaneous visualization of major 18S-28S rDNA loci (red) and FITC-avidin-detected 5S rDNA loci (yellow/green) in *G. hirsutum*. Major 18S-28S rDNA loci were observed to be syntenic to each of the two 5S rDNA loci of *G. hirsutum*, corroborating the positions established by Crane et al. (1993) using molecular meiotic ISH (Fig. 3E). In the diploid species, the syntenic relationships of the 5S rDNA site relative to the 18S-28S rDNA site were determined by simultaneous detection of a biotin-labeled 18S-28S rDNA probe and a digoxigenin-labeled 5S rDNA probe (Fig. 4A–D). The results revealed that the 5S rDNA loci of both *G. herbaceum* (A1) and *G. thurberi* (D1) were syntenic to the major 18S-28S rDNA loci (Fig. 4B, D); whereas those of *G. arboreum* (A_2) *and G. raimondii* (D_5) were syntenic to minor and intermediate 18S-28S rDNA loci, respectively, and not to major 18S-28S rDNA loci, as would be expected based on the *G. hirsutum* data (Fig. 4A, C).

Discussion

A number of hypotheses may explain why all 18S-28S rDNA loci of *G. hirsutum* were not accounted for in diploid progenitors. First, the number of copies of the rDNA repeat present at some A- and D-diploid loci may be too few to be detected. Hence, a discrepancy in the number of detectable 18S-28S rDNA loci could be explained either by elimination of copies of the 18S-28S repeat in modern A- and D-diploids relative to the ancestral genome donors, or by post-polyploidization amplification of those 18S-28S rDNA loci of the tetraploid that originally bore few repeats. Given an estimated limit of 25–35 kb for consistent signal detection, and the length of the probe used (4.5 kb), we infer that our current threshold for detection of 18S-28S rDNA repeats is approximately five to eight copies. A second possible explanation is that translocations with breakpoints located in the middle of 18S-28S rDNA loci may have bisected and thus duplicated sites following polyploidization of *G. hirsutum*. Third, increases and/or decreases in site number could arise by the formation of translocations with breakpoints proximal to the rDNA sites and subsequent fixation of duplication-deficiencies. Fourth, new rDNA loci may have been formed in the tetraploid by transposition of sequences containing rDNA repeats. Fifth, deletions may have eliminated loci in modern A- and D-diploids. And finally, the diploids examined may not represent the ancestral donors of the tetraploid A- or D-subgenomes.

The two 5S rDNA loci of *G. hirsutum* were previously mapped using ISH. A large site of 5S rDNA ISH was mapped to the long arm of chromosome 9 and a somewhat smaller site was localized to the short arm of chromosome 23 (Crane et al. 1993). Based on the differences in chromosomal arm location of 5S rDNA loci, Crane et al. (1993) proposed that following divergence of the A- and D-genomes, either a pericentric inversion or a transposition had occurred in the homolog of chromosome 9 or chromosome 23. Our data, showing 5S rDNA loci located near the centromere in the short arm of all A- and D-diploids observed (Fig. 3A–D), suggest that the rearrangement occurred on chromosome 9 and that this occurred after the polyploidization event leading to *G. hirsutum*. This conclusion is concordant with independent restriction fragment length polymorphism (RFLP) mapping data (Reinisch et al. 1994; J. F. Wendel, personal communication).

The results from simultaneous FISH of 5S and 18S-28S rDNA probes to chromosomes of *G. herbaceum* (A_1) and *G. thurberi* (D_1) were concordant with results from the tetraploid in that the 5S rDNA sites were syntenic to major 18S-28S rDNA loci (Fig. 4B, D). The results for *G. arboreum* (A_2) and *G. raimondii* (D_5) were not as predicted, however, as neither of the 5S sites was syntenic with a major 18S-28S rDNA locus (Fig. 4A, C). The *G. raimondii* data are particularly interesting, as it has been reported that this species is the most likely D-genome ancestor of the extant tetraploids (Endrizzi et al. 1985). If this is indeed the case, then our results indicate that, barring reciprocal rearrangement between minor and major rDNA loci, the 18S-28S rDNA cluster of *G. hirsutum* on the D-subgenome chromosome 23 has been significantly amplified in size following polyploidization, or that this same locus has been significantly deamplified in modern *G. raimondii* lineages.

The size distribution of the 18S-28S rDNA sites observed in the diploids was not as expected. Based on our genomic painting data from *G. hirsutum* and the previously mentioned ISH mapping data (Crane et al. 1993), the expected pattern in the A-genome diploid species included one major site corresponding to chromosome 9, one intermediate site corresponding to chromosome 7, and three minor sites. The expected pattern of the D-genome species included two major 18S-28S rDNA sites corresponding to chromosomes 16 and 23, and four minor sites. In A-genome diploids no fewer than two major 18S-28S rDNA sites were observed, suggesting that at least one major site was significantly deamplified in the A-subgenome of the tetraploid following polyploidization, or that at least one site has been greatly amplified in modern diploid ancestors of the A-subgenome donor. In *G. raimondii* the number of major 18S-28S loci observed was as expected, although neither of the two major 18S-28S rDNA loci we observed was located on the predicted chromosome 23 homolog, which bears a 5S site. It is therefore suggested, that one of these two major 18S-28S sites in *G. raimondii* is homologous to the major site on chromosome 16 of *G. hirsutum*, while the other has either been significantly amplified in *G. raimondii* relative to the ancient D-subgenome donor or greatly deamplified in the tetraploid following polyploidization.

Several mechanisms may account for the changes in sizes of rDNA loci that we observed. One proposed way that tandem repeats such as rDNA loci can be significantly amplified or deleted is through both homologous and nonhomologous unequal crossing over (Arnheim et al. 1980; Seperack et al. 1988). Anderson and Roth

(1981) have shown that in *Salmonella typhimurium* unequal crossing over takes place much more frequently at rDNA loci than at 37 other loci observed. Recent data obtained by Wendel et al. (1995) have demonstrated the occurrence of interlocus concerted evolution among rDNA loci in *Gossypium* tetraploids following polyploidization, which may suggest that unequal crossing over takes place between nonhomologous rDNA loci in *G. hirsutum*. The fact that rDNA-bearing chromosomes appear to be nonrandomly associated with each other at mitotic metaphase (Figs. 1A–D, 3B–C, 4A–C) may support the theory that somatic exchange takes place between nonhomologous loci, in which case homogenization and changes in rDNA copy number could occur especially quickly, particularly in plants and other organisms without germ lines that are differentiated early in development. In rye, it has been shown that the telomeres of some "associated" chromosomes are more alike in their arrays of repeats than those of "un-associated" chromosomes (Bennett 1982). Significant to this potential mechanism is that 12 of 14 18S-28S rDNA loci observed in the diploid species and all 3 major 18S-28S loci of *G. hirsutum* are located at either telomeric or slightly subtelomeric positions, as this would possibly allow significant rearrangements to occur between rDNA loci without deleterious effects to the cell. It therefore seems reasonable to propose that in species with mostly telomerically located 18S-28S rDNA repeats there will be observed on average: a greater degree of interlocus concerted evolution, a higher number of loci, and more variability in locus number and size, both within and between closely related species.

The multiplicity of sites, facility of detection and relative variability of rDNA loci indicate that rDNA loci will have considerable utility in future molecular cytogenetic studies of *G. hirsutum* and its diploid relatives. Further, the data indicate that rDNA loci have much to offer as a tool for analyzing the evolution and behavior of tandemly repeated gene families, how they contribute to genomic elasticity, and how they may promote or constrain changes relevant to polyploidization. Subsequent studies using FISH of both repetitive and single-copy sequences will likely add to the information we have presented and contribute greatly to our understanding of the homologous relationships between the chromosomes of *G. hirsutum* and the chromosomes of A- and D-diploids, and more generally, to genome evolution per se.

Acknowledgements. Various aspects of this work were supported by the Texas A&M University Office of University Research, the Texas Agricultural Experiment Station REP grant, NRICGP Plant genome grant 92-37300-7655, and Texas Advanced Technology & Research Program grant 999902-090. We wish to thank Dr. Wayne Smith for supplying seed of *G. hirsutum*, Dr. Jonathan Wendel for comments on the manuscript, and Wayne Raska, Don Czeschin, Karen Kelly and Lori Jackson for technical assistance.

References

Anderson P, Roth R (1981) Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA cistrons. Proc Natl Acad Sci USA 78: 3113–3117

Arnheim N, Krystal M, Schmickel R, Wilson G, Ryder O, Zimmer E (1980) Molecular evidence for genetic exchanges among ribosomal genes on nonhomologous chromosomes in man and ape. Proc Natl Acad Sci USA 77: 7323–7327

Beasley JO (1940) The origin of American tetraploid *Gossypium* species. Am Nat 74: 285–286

Beasley JO (1942) Meiotic chromosome behavior in species, species hybrids, haploids and induced polyploids of *Gossypium*. Genetics 27: 25–54

Bennett MD (1982) Nucleotypic basis of the spatial ordering of chromosomes in eukaryotes and the implications of the order for genome evolution and phenotypic variation. In: Dover GA, Flavell RB (eds) Genome evolution. Academic Press, London, pp 239–262

Crane CF, Price HJ, Stelly DM, Czeschin DG, McKnight TD (1993) Identification of a homeologous chromosome pair by *situ* hybridization to ribosomal RNA loci in meiotic chromosomes of cotton (*Gossypium hirsutum* L.). Genome 36: 1015–1022

Edwards GA, Mirza MA (1979) The designation of a new G genome for *Gossypium bickii*. Can J Genet Cytol 21: 367–372

Endrizzi JE, Turcotte EL, Kohel RJ (1985) Genetics, cytology, and evolution of *Gossypium.* Adv Genet 23: 271–375

Fryxell PA (1992) A revised taxanomic interpretation of *Gossypium* L. Rheedea 2: 108–165

Hanson RE, Zwick MS, Choi SD, Islam Faridi MN, McKnight TD, Wing RA, Price HJ, Stelly DM (1995) Fluorescent in situ hybridization of a bacterial artificial chromosome. Genome 38: 646–651

Islam-Faridi MN, Mujeeb-Kazi A (1995) *Secale cereale* DNA in wheat germplasm; visualization by fluorescent in situ hybridization and breeding application. Theor Appl Genet 90: 595–600

Jewell DC, Islam-Faridi MN (1994) Details of a technique for somatic chromosome preparation and C-banding of maize. In: Freeling M, Walbot V (eds) The maize handbook. Springer, Berlin Heidelberg New York, pp 484–493

Menzel MY (1954) A cytological method for genome analysis in *Gossypium*. Genetics 40: 214–223

Paterson AH, Brubaker KL, Wendel JF (1993) A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. Plant Mol Biol Rep 11: 122–127

Price HJ, Stelly DM, McKnight TD, Scheuring CF, Raska D, Michaelson MJ, Bergey D (1990) Molecular cytogenetic mapping of a nucleolar organizer region in cotton. J Hered 81: 365–370

Reinisch AJ, Dong J, Brubaker CL, Stelly DM, Wendel JF, Paterson AH (1994) A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: chromosome organization and evolution in a disomic polyploid genome. Genetics 138: 829–847

Seperack P, Slatkin M, Arnheim N (1988) Linkage disequilibrium in human ribosomal genes; implications for multigene family evolution. Genetics 119: 943–949

Silhavy TJ, Berman ML, Enquist LW (1984) Experiments with gene fusion. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 144–146

Skovsted A (1934) Cytological studies in cotton. II. two interspecific hybrids between Asiatic and New World cottons. J Genet 28: 407–424

Wendel JF (1989) New World tetraploid cottons contain Old World cytoplasm. Proc Natl Acad Sci USA 86: 4132–4136

Wendel JF, Albert VA (1992) Phylogenetics of the cotton genus (*Gossypium* L.): character-state weighted parsimony analysis of chloroplast DNA restriction site data and its systematic and biogeographic implications. Syst Bot 17: 115–143

Wendel JF, Schnabel A, Seelanan T (1995) Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc Natl Acad Sci USA 92: 280–284