Comparative genetic mapping of allotetraploid cotton and its diploid progenitors

C.L. Brubaker, A.H. Paterson, and J.F. Wendel

Abstract: Allotetraploid cotton species (*Gossypium*) belong to a 1–2 million year old lineage that reunited diploid genomes that diverged from each other 5–10 million years ago. To characterize genome evolution in the diploids and allotetraploids, comparative RFLP mapping was used to construct genetic maps for the allotetraploids (AD genome; $n = 26$) and diploids (A and D genomes; $n = 13$). Comparisons among the 13 suites of homoeologous linkage groups permitted comparisons of synteny and gene order. Two reciprocal translocations were confirmed involving four allotetraploid A_t genome chromosomes, as was a translocation between the two extant A genome diploids. Nineteen locus order differences were detected among the two diploid and two allotetraploid genomes. Conservation of colinear linkage groups among the four genomes indicates that allopolyploidy in *Gossypium* was not accompanied by extensive chromosomal rearrangement. Many inversions include duplicated loci, suggesting that the processes that gave rise to inversions are not fully conservative. Allotetraploid A_t and D_t genomes and the A and D diploid genomes are recombinationally equivalent despite a nearly two-fold difference in physical size. Polyploidization in *Gossypium* is associated with enhanced recombination, as genetic lengths for allotetraploid genomes are over 50% greater than those of their diploid counterparts.

Key words: restriction fragment length polymorphism (RFLP), *Gossypium*, evolution, polyploidy.

Résumé : Les espèces allotétraploïdes de cotonnier (*Gossypium*) appartiennent à un lignage vieux de 1 à 2 millions d'années résultant de l'union de génomes diploïdes ayant divergé l'un de l'autre pendant 5 à 10 millions d'années. Afin de caractériser l'évolution génomique chez les diploïdes et les allotétraploïdes, une analyse comparée de cartographie RFLP a été réalisée pour établir des cartes génétiques chez les espèces allotétraploïdes (génome AD; $n = 26$) et diploïdes (génomes A et D; $n = 13$). Une comparaison des treize groupes de liaison homéologues a permis d'étudier la synténie et l'ordre des gènes. L'existence de deux translocations réciproques impliquant quatre chromosomes du génome allotétraploïde A_t a été confirmée, tout comme une translocation entre les deux espèces diploïdes à génome A existantes. Dix-neuf différences quant à l'ordre des gènes ont été décelées parmi les deux génomes diploïdes et allotétraploïdes. La conservation des groupes de liaison parmi les quatre génomes indique que le processus d'allopolyploïdisation n'a pas été accompagné de réarrangements chromosomiques importants chez le genre *Gossypium*. Plusieurs inversions incluaient des loci dupliqués ce qui suggère que le processus menant à des inversion n'est pas parfaitement conservateur. Les génomes allotétraploïdes A_t et D_t de même que les génomes diploïdes A et D sont équivalents sur le plan de la recombinaison en dépit du fait que l'un soit pratiquement deux fois plus grand que l'autre sur le plan de la taille physique. La polyploïdisation chez le genre *Gossypium* s'accompagne d'un accroissement de la recombinaison puisque la somme des distances génétiques chez les génomes allotétraploïdes est plus de 50% supérieure à celle de leurs contreparties diploïdes.

Mots clés : polymorphisme de longueur des fragments de restriction (RFLP), *Gossypium*, évolution, polyploïdie.

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Introduction

Polyploidy is common in plants (Grant 1981; Lewis 1980; Leitch and Bennett 1997; Masterson 1994; Soltis and Soltis

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1993; Stebbins 1950, 1971) and probably has been involved in the evolution of all eukaryotes (Leipoldt and Schmidtke 1982; Sidow 1996; Spring 1997; Wolfe and Shields 1997). In synthetic allopolyploids, illegitimate chromosome pairing often disturbs meioses, and thus it is thought that evolutionary mechanisms which promote exclusively bivalent pairing will be selectively favored in the critical first few generations following natural allopolyploid formation. However, prior to the restoration of diploid-like chromosome behavior, interactions among homoeologous chromosomes may result in the rapid accumulation of structural rearrangements that alter gene order and synteny (Ahn et al. 1993; Feldman et al. 1997; Leipoldt and Schmidtke 1982; Liu et al. 1998; Song et al. 1995).

The cotton genus, *Gossypium* L., is an ideal system for examining genome evolution in polyploids. *Gossypium* com-

prises approximately 45 diploid and five allopolyploid species distributed throughout the arid and semi-arid regions of Africa, Australia, Central and South America, the Indian subcontinent, Arabia, the Galápagos, and Hawaii (Fryxell 1979, 1992). The diploid *Gossypium* species fall into eight cytological groups, or genomes, designated A through G, and K (Beasley 1940; Edwards and Mirza 1979; Endrizzi et al. 1985; Phillips and Strickland 1966; Stewart, 1994). The five allotetraploid species, indigenous to the New World, derive from a single allopolyploidization event that united the Old World A genome with the New World D genome, in an A genome cytoplasm (Wendel 1989; Wendel and Albert 1992).

Judging from differences in meiotic pairing, *Gossypium* allotetraploid genomes appear to be more fully "differentiated" from one another than are the descendents of their diploid progenitors (Endrizzi 1962; Mursal and Endrizzi 1976). Specifically, allotetraploid-derived haploids form an average of less than one bivalent per cell during meiosis, whereas chromosomes of extant A and D diploid F_1's average 5.8 and 7.8 bivalents at metaphase I (Endrizzi and Phillips 1960; Mursal and Endrizzi 1976; Skovsted 1937). These data suggest that stabilization of the newly evolved allotetraploid involved genic or genomic modifications that inhibit homoeologous pairing while promoting exclusively bivalent formation between homologues (Kimber 1961).

To examine whether genome evolution in *Gossypium* allotetraploids was also accompanied by chromosome structural rearrangements, we constructed RFLP maps for the A and D diploid genomes, using a common set of nuclear probes, most of which were previously or simultaneously mapped in the allotetraploids (Reinisch et al. 1994). Comparisons between diploid genomes, between diploid genomes and their corresponding allotetraploid genomes, and between allotetraploid genomes allowed us to address whether allopolyploidization in *Gossypium* was accompanied by rapid chromosomal structural change and compare recombination rates among four homoeologous genomes.

Materials and methods

The A and D genomic linkage maps were based on the multilocus genotypes of 58 A genome (*G. herbaceum* L., $A_2[= A_1]$–97, × *G. arboreum* L., A_2–47) and 62 D genome (*G. trilobum* (Moc. & Sessé ex. DC) Skovsted × *G. raimondii* Ulbr.) F_2 individuals. The AD genomic linkage map was based on a *G. hirsutum* race 'palmeri' × *G. barbadense* K101 F_2 population (see Reinisch et al. 1994 for details). DNA extractions followed Paterson et al. (1993) or Brubaker and Wendel (1994). Restriction digestion, electrophoresis, blotting procedures, RNA probe preparation, hybridization protocols, and autoradiography followed Brubaker and Wendel (1994).

Three hundred and twenty-one mapped DNA probes were selected from six libraries: anonymous *Pst*I nuclear fragments from *G. raimondii* ("G"-series; n = 75), *G. herbaceum* subsp. *africanum* (Mauer) Watt ("A"-series; n = 143), and *G. hirsutum* cultivar TM-1 ["M"-series (n = 1) and "P"-series (n = 16)]; low copy-number nuclear sequences (Zhao et al. 1996) from *G. barbadense* L. cv. Pima S6 ("PXP"-series; n = 8); and anonymous cDNA clones from drought-stressed *G. hirsutum* accession T25 ("pAR"-series; n = 78) (Reinisch et al. 1994). RFLPs between *G. herbaceum* and *G. arboreum* and between *G. raimondii* and *G. trilobum* were revealed using eight (*Eco*RI, *Hin*dIII, *Pst*I, *Dra*I, *Cfo*I, *Bam*HI, *Xba*I, and *Eco*RV) and two restriction enzymes (*Eco*RI and HindIII), respectively, the difference reflecting the relative levels of polymor-

phism between the A genome parents and the D genome parents. We also assayed allelic segregation among the 58 A genome F_2 progeny at seven isozyme loci encoded by six enzyme systems: aconitate hydratase (*ACO1*), arginyl-specific aminopeptidase (*ARG1*), leucyl-specific aminopeptidase (*LEU1*), 6-phosphogluconate dehydrogenase (*PGD1* and *PGD3*), triosephosphate isomerase (*TPI1*). Sample preparation, starch-gel electrophoresis, and nomenclature follow Wendel et al. (1989).

Genetic interpretation of the RFLP phenotypes and nomenclature followed the system of Reinisch et al. (1994). Loci are designated by probe, and arbitrarily assigned lower-case letters to distinguish multiple segregating loci revealed by the same probe. The letters A and D denote the A and D genome linkage groups, respectively, followed by arbitrarily assigned numbers. MapMaker Macintosh 2.0 (Lander et al. 1987) was used to infer the genomic linkage maps (Figs. 1–11). Initial linkage groups were inferred from two-point analyses using a minimum LOD score of 4.0 and a maximum theta value of 0.40. Linkage groups were ordered by selecting a subset of six or seven loci whose most likely order differed from the next most likely order by a LOD of two or more. Remaining loci were added to the initial map using the "try" function. The "ripple" function confirmed the local order around new loci and identified regions of uncertain order. Linkage-1 (Suiter et al. 1983) was used to identify loci whose segregation ratios differed significantly (*P* < 0.05) from Mendelian expectations.

Results

A genome map

The A genome map (Figs. 1–11) comprises 161 loci encoded by 152 nuclear probes and 6 isozyme loci mapping to 18 linkage groups (hereafter LGs). The 18 LGs encompass 856 cM. Individual LGs include 2 to 24 loci (average = 8.94).

There was no discernible bias in allelic or genotypic frequencies. Segregation ratios at only 14 loci (8.7%) from seven LGs deviate significantly from Mendelian expectations (*P* < 0.05). This number is only slightly higher than that expected from chance alone and none of the loci in question occurred in discrete blocks.

Gossypium arboreum and *G. herbaceum* differ by a reciprocal translocation (Gerstel 1953) and therefore the F_1 is a translocation heterozygote. As a result, at least one of the A-genome LGs should contain sets of loci for which three point comparisons create unresolvable contradictions (e.g., *locus A maps 5 cM from loci B and C, but locus B and C segregate independently). These contradictory data will be magnified in multipoint comparisons, making map orders ambiguous. Linkage group A5 (Fig. 8) fits this description. Comparisons to homoeologous LGs from the D,* A_t ("A" genome of the allotetraploids), and D_t ("D" genome of the allotetraploids) genomes reinforces this inference.

D genome map

The D genome map (Figs. 1–11) comprises 306 loci encoded by 269 nuclear probes mapping to 17 LGs. The 17 LGs encompass 1486 cM. Individual LGs include 2 to 44 loci (average = 18.0).

In contrast to the A genome, there is evidence for segregation distortion within three genomic regions. Segregation ratios at 37 loci (12.1%) from nine LGs deviate significantly (*P* < 0.05) from Mendelian expectations. Twenty-four of these loci map to three blocks on LGs D1, D5, and D9. On LG D1 (Fig 1), three loci (*pAR168b, A1559,* and *pAR173b*)

Figs. 1–11. RFLP maps of diploid and allotetraploid *Gossypium*. The A and D linkage groups are portrayed with their counterparts from the allotetraploids (A$_t$ and D$_t$; redrawn from Reinisch et al. 1994). Each figure represents homoeologous assemblages (HAs) of A, D, A$_t$, and D$_t$ linkage groups. Loci mapped to more than one linkage group (LG) within an HA are shown in bold. Bracketed numbers beside loci indicate additional HAs (which correspond to Fig. numbers) with potentially homoeologous loci revealed by the same probe. The order of linkage groups within HAs is (from left to right) D$_t$ – D – A – A$_t$, except for in Figs. 7, 8, and 11, which are discussed individually. When a genome is represented by multiple linkage groups, they are arranged in a single column. Lines connecting orthologous and homoeologous loci (see Table 1 and text) indicate statistically supported deviations from colinearity. Loci in boxes are duplicated within that linkage group.

have a lower than expected number of heterozygotes, coupled with a consistent, albeit non-significant, under-representation of *G. raimondii* alleles. On the same chromosome, loci between *G1006* and *pAR168a*, particularly *pAR21a* and *pAR24b*, have fewer *G. raimondii* homozygotes than expected. The loci in the center of LG D5 (Fig. 8) have fewer *G. raimondii* alleles than expected. This is coupled with a slight over-representation of heterozygotes toward the ends of the region. Finally, a 28.8 cM region on the lower end of D9 (Fig. 7) contains five loci with an excess of heterozygotes.

Identification of orthologous and homoeologous LGs

Of the 659 potentially orthologous (related by common ancestry and speciation, *viz.*, A vs. D, A vs. A$_t$, and D vs. D$_t$) and homoeologous loci (related by duplication via allotetraploidy, *viz.*, A$_t$ vs. D$_t$), 424 (64%) fall into 12 suites of loci that unite at least one A, D, A$_t$, and D$_t$ LG. A 13th suite of loci unites one D and one A LG with two different LGs previously assigned to the D$_t$ genome, Chr. 22D and LG D05 (Fig. 2) (Reinisch et al. 1994). Linkage group LG D05 was tentatively assigned to the D$_t$ genome on the basis of a single locus, *P11–23* (Reinisch et al. 1994). The relationships inferred here, however, suggest that LG D05 is of A$_t$ genome origin, an inference supported by the addition of new markers to the allotetraploid map (Paterson unpublished). These 13 suites of loci are used to infer 13 groups of putatively orthologous and homoeologous LGs, the number expected in an allotetraploid derived from two diploids with haploid complements of 13 chromosomes.

For discussion, the putatively orthologous and homoeologous suites of LGs are termed homoeologous assemblages, or HAs, and are denoted by numbers that correspond to their illustrations in Figs. 1–10. The inclusion of multiple LGs for a single genome within one HA implies that they represent segments of the same chromosome (e.g., LG D10 and LG D01 in HA 1; Fig. 1). Figs. 7 and 8 include two and three HAs, respectively, united by A diploid genome translocations and are designated 7A, 7B, 8A, 8B, and 8C (discussed below). Two A genome LGs (A21, A23) and two D genome LGs (D15, D17) could not be included in any of the 13 HAs. Four LGs from the allotetraploid (LG U07, Chr. 17D suppl., LG U02, and LG A04) had loci with putative orthologues in at least one diploid LG but could not be assigned to one of the HAs because of insufficient or contradictory data. These unassigned LGs are shown in Fig. 11, with the map locations of putative orthologs indicated. No loci mapping to allotetraploid LGs U09, A08, D11, or U05 had apparent orthologous loci in either diploid map and are not considered or illustrated. Linkage groups U02 and A04 are unique because all but one of their loci have homoeologues on other

LGs, but almost none of these homoeologous loci map to LGs within the same HA. Either these LGs are artifactual or they represent AD genomic regions that are extensively rearranged. This "mosaicism" also occurs to a lesser extent on the upper end of LG U01 (Fig. 5). The lower end of this LG is clearly homoeologous to the A, D, and A$_t$ LGs in this HA, but potential homoeologues to the loci on the upper end map to a variety of LGs in other HAs, which again suggests extensive rearrangement.

Deviations from colinearity

Comparison of locus orders among LGs within HAs revealed 19 locus order differences for which the assumption of colinearity requires accepting an alternate locus order on one or more LGs that is more than 100 times (LOD > 2) less likely than the preferred order (Table 1). Nine of these involve only two loci, and thus only weakly suggest the possibility of a rearrangement. The remaining 10 involve three or more loci and imply one or more inversions. Because these are low-density maps, we clearly have underestimated the actual number of rearrangements.

Confirmation of A$_t$ genome translocations

Homoeologous assemblages 7A & 7B (Fig. 7) comprise two sets of D$_t$, A, and D LGs (Chr. 20D/D9/A6 and A14/D12/LG D07) with no evident relationship, except that both contain loci with homoeologs mapping to A$_t$ LGs Chr. 5A and 4A. The homoeologous relationships shown (Fig. 7) provide evidence that Chr. 5A and 4A have undergone a reciprocal translocation relative to their D$_t$, A, and D counterparts (i.e., after polyploidization). Both Chr. 4A and 5A have two non-overlapping sets of loci, with each of the two sets showing homology to one of the two sub-assemblages (HA7A and HA7B) in Fig. 7. This inference is strengthened by classical studies that suggest the A$_t$ genome will differ from the D$_t$, D, and A genomes by two reciprocal translocations, one of which involves A$_t$ chromosomes 4 and 5 (Brown and Menzel 1950; Gerstel 1953; Gerstel and Sarvella 1956; Menzel and Brown 1954; Brown 1980; Menzel et al. 1982). Furthermore, the two A$_t$ LGs included in Fig. 7 were assigned by Reinisch et al. (1994) to A$_t$ chromosomes 4 and 5.

Inspection of this compound HA revealed several inconsistencies. The A6 and A14 homoeologs to *pAR219b*, *G1033a*, and *A1172* on Chr. 4A are interspersed with loci whose homoeologs map to Chr. 5A. The intercalation of *G1033* and *A1172* with *A1159* and *pAR206* on LG A14 may reflect a post-translocation inversion (the order of these loci on A14 is inverted relative to D12). Loci *pAR219* and *A1543* on Chr. 4A are also intercalated with loci with homoeologs mapping to Chr. 5A. In this case, it is unlikely that an inver-

Fig. 1. HA1. Probes A1311 and A1437 reveal loci on LG D01 that are inverted relative to their orthologs on D1. The position of *A1311* on D1 is ambiguous and it can be located between *A1437* and *pAR293* (LOD = –0.38). This would place it beside *A1437*, as it is on LG D01 and make D1 and A4 colinear. As a result, however, the position of *A1437*, *A1311*, *pAR168*, and *pAR173* on D1 and A4 is altered relative to LG D01. This suggests an inversion centered around *pAR173*, which is duplicated in LG D01 and D1, and *pAR168,* which is duplicated in D1.
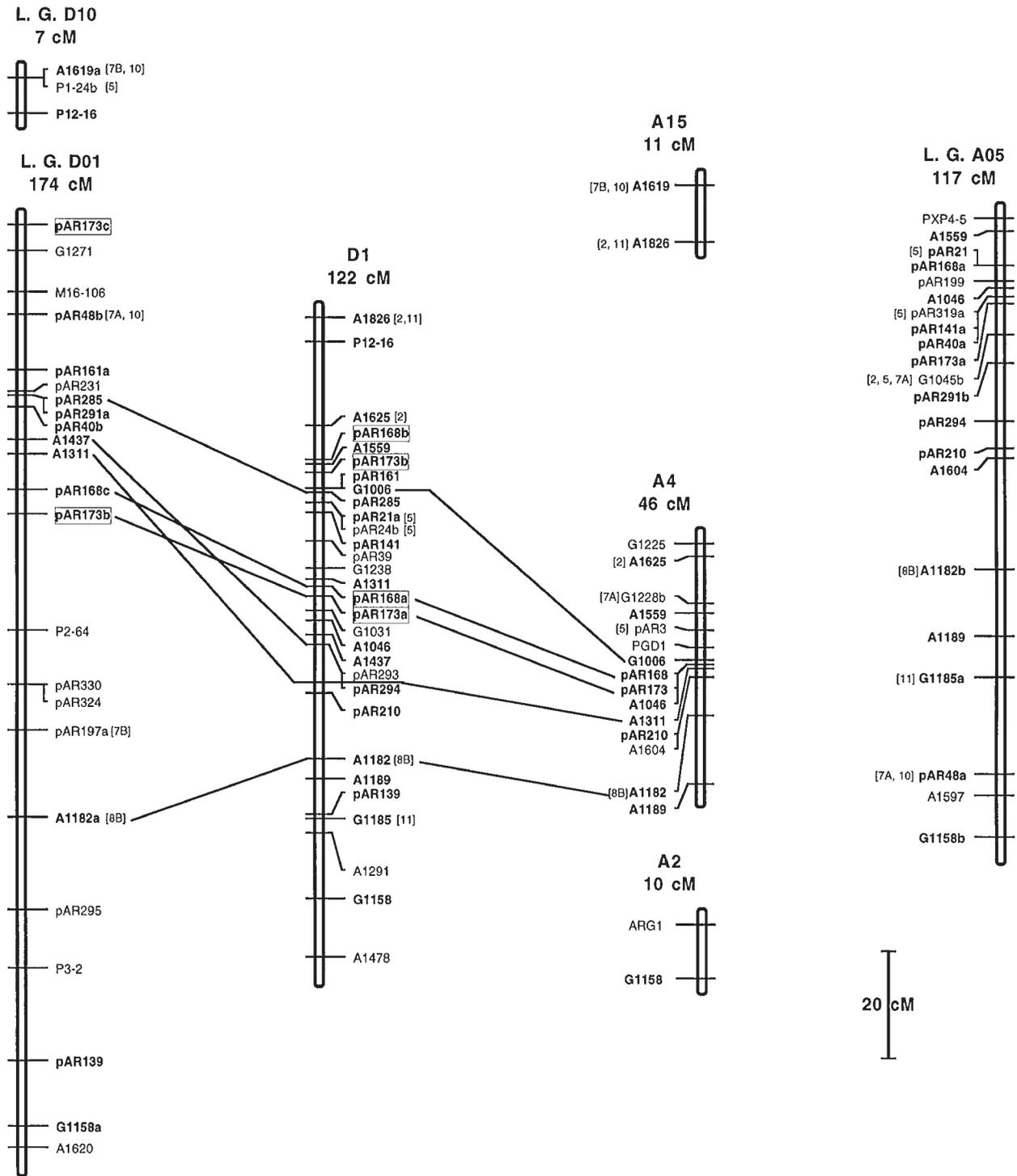
sion is involved. It may be that the inferred homoeology between *pAR219* on Chr. A6 and *pAR219b* on Chr. 4A is incorrect. Finally the relative placements of *pAR138a* on Chr. 4A and *pAR138b* on LG D07 requires the assumption of a post-translocation inversion.

Cytogenetic studies predict that the allotetraploid A_t genome will differ from the genomes of *G. herbaceum* and *G. arboreum* by a second A_t-specific reciprocal translocation confounded by a third translocation specific to the A diploid genome involving the allotetraploid A_t genome chromosomes

Fig. 2. HA2. The order of *G1037* and *pAR101* on Chr. 22D is inverted relative to D8.
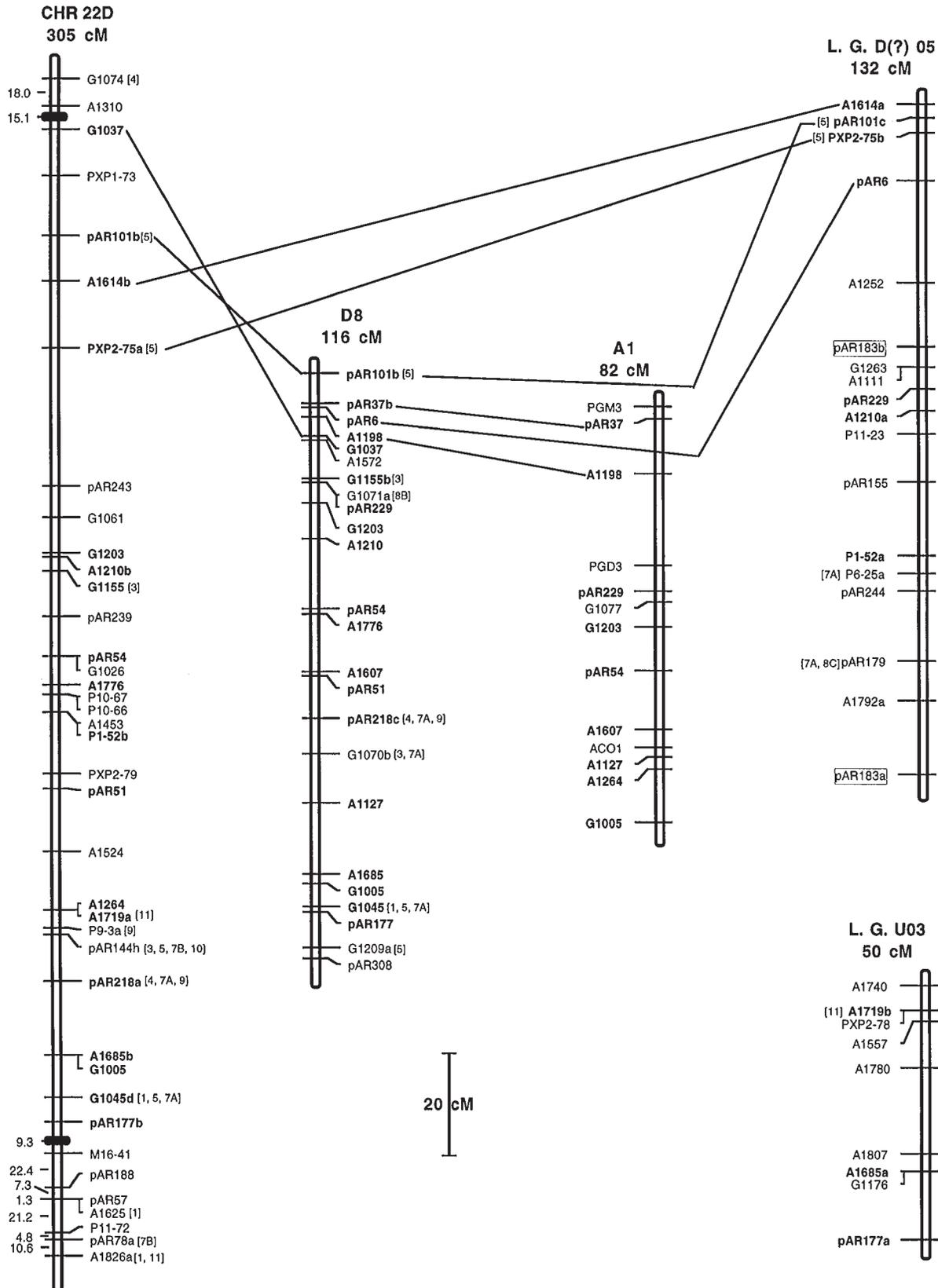
1, 2, and 3 (Menzel and Brown 1954; Brown 1980). Thus, it was not surprising that the compound A genome LG A5 has homologies to three A_t LGs, two D genome LGs, and two D_t LGs (Fig. 8). Reinisch et al. (1994) assigned A_t LGs Chr. 1A and Chr. 2A to A_t chromosomes 1 and 2 (Brown 1980), which is fully consistent with this interpretation (see Menzel

Table 1. Summary of putative rearrangements among the A and D diploid genomes and the A and D allotetraploid genomes, organized by homoeologous assemblages (HAs). Loci in boldface are duplicated in one or more of the LGs.

HA	Linkage groups involved			Loci
HA1	LG D01	vs.	D1 & A4	*A1437*
				A1311
				pAR168
				pAR173
HA2	Chr. 22D	vs.	D8	*G1037*
				pAR101
HA3	D6	vs.	A12	*pAR288*
				A1737
				A1124
				pAR127
	Chr. 23D	vs.	D6	*A1606*
				pAR8
HA4	A16	vs.	D4 & LG D03	*A1168*
				pAR309
				A1590
				pAR118
HA5	D7	vs.	A13	*pAR21*
				pAR319
HA6	D13	vs.	A10	*A1591*
				G1016
				G1125
				A1720
				G1130
				G1174
				pAR49
				pAR8
HA7A	Chr. 20D	vs.	D9	*pAR169*
				pAR65
	D9	vs.	A6	*A1808*
				A1650
				pAR278

Table 1 (*concluded*).

HA	Linkage groups involved			Loci
HA7B	D12	vs.	A14	*G1033*
				A1172
				A1159
				pAR206
HA8A	LG A06	vs.	D5	*G1164*
				A1418
				PXP2–60
HA8B	LG A06	vs.	D3 & Chr. 17D	*pAR185*
				pAR149
				pAR172
HA8C	D2	vs.	Chr. 15D	*A1553*
				A1225
				pAR11
				P1–3
				pAR88
				A1720
	Chr. 1A	vs.	D2	*A1097*
				A1794
				pAR77
HA10	LG D04	vs.	Chr. 10A	*pVNC163*
				A1695
	D11	vs.	A9	*G1257*
				A1286
	D11	vs.	Chr. 10A	*pAR55*
				A1695
	A9	vs.	Chr. 10A	**A1183**
				G1088
	D11	vs.	Chr. 10A	*A1158*
				A1751

and Brown's (1954) Fig. 1). If one accepts this interpretation of the split affinities of A5, a second A_t-specific translocation becomes apparent, this one involving regions of chromosomes marked by LGs Chr. 2A and LG A06. As was the case with Chr. 4A and Chr. 5A in HA7, both of these LGs show split, non-overlapping homologies to two sets of otherwise unrelated assemblages of D, D_t, and A LGs. If these inferences are correct, A_t LG A06 corresponds to the allotetraploid A_t genome chromosome 3 (Menzel and Brown 1954; Brown 1980).

The only contradictory evidence for the interpretation just given involves loci revealed by probes pAR250 and A1536. The former locus maps to LGs in HA8A (A8 and D5) and HA8B (Chr. 17D), whereas *A1536* maps to LGs in HA8B (Chr. 17D) and HA8C (Chr. 1A). Probe A1536 also reveals a locus that maps to A5, but that is not by itself contradictory. In light of the unambiguous homology relationships for a large number of loci, however, this single discrepancy does not weaken the current interpretation.

Homoeologous associations not accounted by HAs

Of the 659 loci that map to at least one other genome, 235 (36%) were not accounted for by the inferred HAs. In Figs. 1–11, locus associations with linkage groups outside of

the HA depicted are indicated in brackets next to the pertinent loci. Many of these inter-HA associations occur in discrete blocks of loci, providing clues to additional rearrangements and past chromosome or chromosome-segment duplication events. Reinisch et al. (1994) described five tentative "nested" associations between segments of putative pairs of A_t and D_t homoeologues. Two of those are supported by the present data: A1759 and P6–57 (Chr. 10A/LG D04 & LG A03/LG D02) and A1751 and P5–61 (Chr. 10A/LG D04 & Chr. 5A). The other three are associated with the translocations and thus "mimic" nested associations. Map comparisons among the four genomes studied here revealed ten "nested" duplications, as follows.

HA1 versus HA2

In HA1, *A1826* and *A1625* map to the upper end of D1; *A1826* maps to A15, which is associated with the upper end of A4 to which the other locus, *A1625*, maps. *A1826* and *A1625* also, however, map to the lower end of Chr. 22D in HA2.

HA1 versus HA5

A number of loci mapped in HA1 have duplicate loci that map to HA5. Specifically, *pAR21* and *pAR24* map to D1;

Fig. 3. HA3. The order of *pAR288, A1737, A1124*, and *pAR127* on D6 is inverted relative to A12, as is *A1606* and *pAR8* on Chr. 23D versus D6.
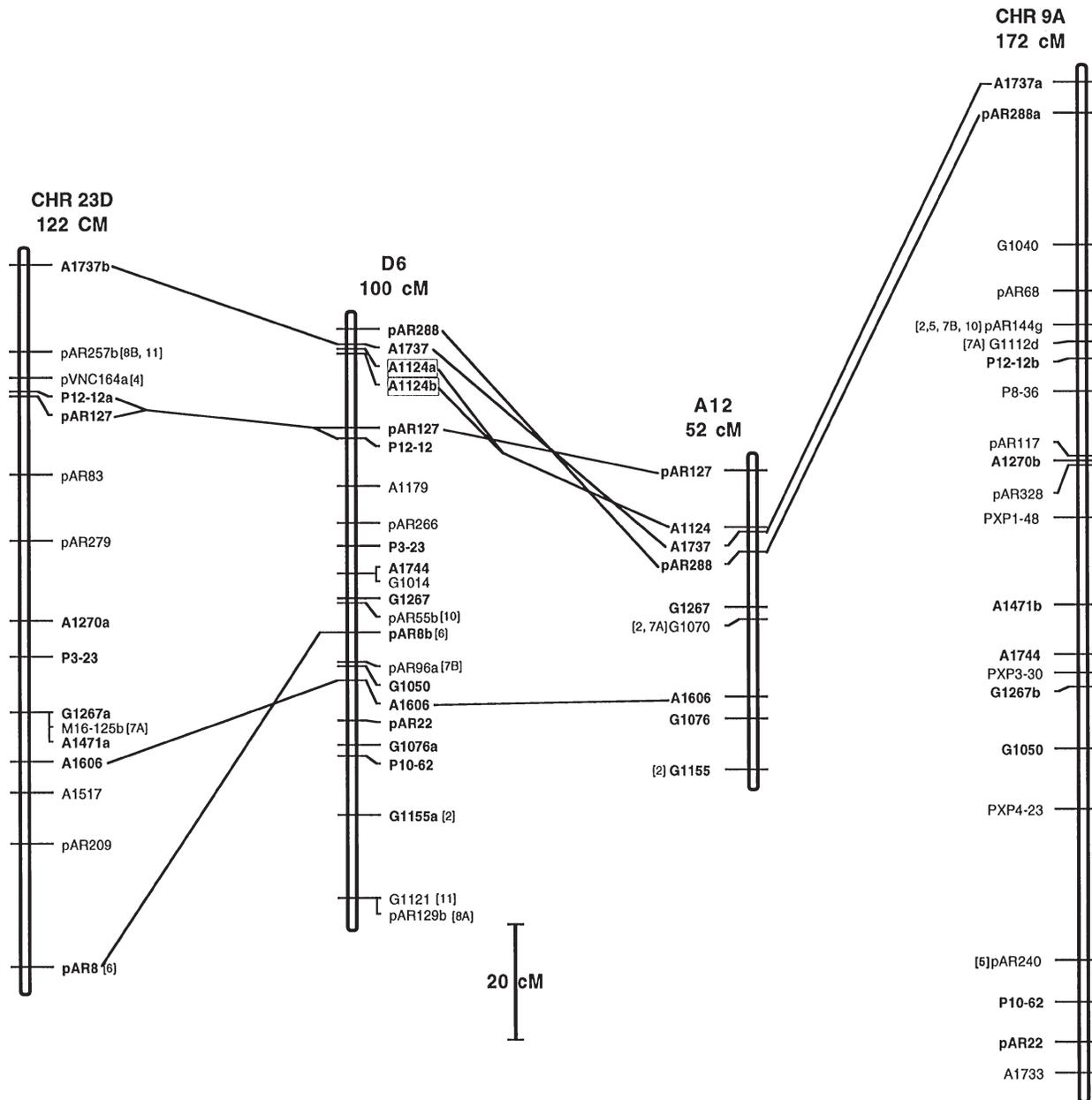
pAR3 maps to a corresponding region of A4; and *G1045b*, *pAR319a*, and *pAR21* map to the corresponding region of LG A05. In HA5, these loci variously map to median regions of LG D02, D7, and A13, and the upper region of LG A03.

HA2 versus HA3

In HA2, Probes G1155 and G1070 reveal loci mapping to D8 and G1155 reveals a locus on Chr. 22D; in HA3 these probes reveal loci that map to D6 and A12.

HA2 versus HA5

In HA2, *pAR101* and *PXP2–75* map to the upper regions of Chr. 22D and LG D05 and the former locus maps to D8.

In HA5, one or both of these loci map to the upper region of D7 and the median region of LG U01, which itself represents the most distal region of homology with the other linkage groups in HA5.

HA2 versus HA7A

pAR218, G1045, G1070, P6–25, and *pAR179* map to submedian regions of Chr. 22D, D8, and LG D05. In HA7A, homologous loci map to D9 and Chr. 20D, albeit not as a discrete block.

HA4 versus HA5

P10–56 and *A1214* map to the lower ends of LG D03 and LG A02, respectively, in HA4. In HA5, duplicates of both

Fig. 4. HA4. The order of *A1168*, *pAR309*, *A1590*, and *pAR118* on A16 is inverted relative to D4 and LG D03.
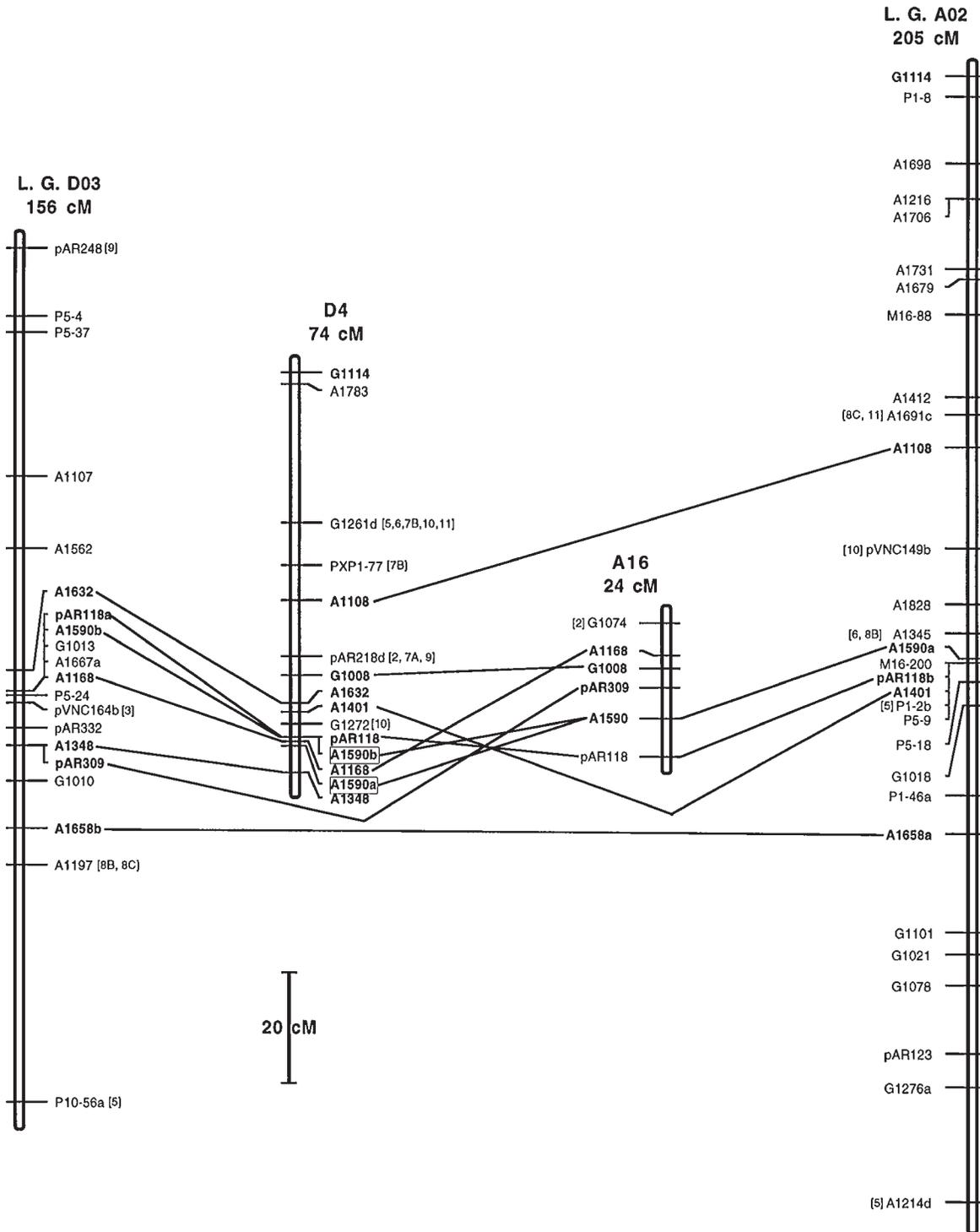
loci map to the lower end of LG D02 whereas only the latter locus is duplicated on LG A03. Both of these chromosome segments show evidence of homology with an additional chromosomal region marked by HA10.

HA5 versus HA10

The loci *PXP4–75, G1261, A1759, pAR144, P6–57* are variously distributed on LG U01 + LG D02, D7, and LG

A03 in HA5. Duplicates of these loci map to HA10, where one or more of the constituent loci map to LG D04, D11, and Chr. 10A.

HA6 versus HA8B

In HA6, *A1267* and *A1345* map to A17; in HA8B duplicates of these loci map to D3. We note that *A1345* also maps to LG A02 on HA4, raising the possibility that A17 is im-

Fig. 5. HA5. The order of *pAR21* and *pAR319* on D7 is inverted relative to A13.
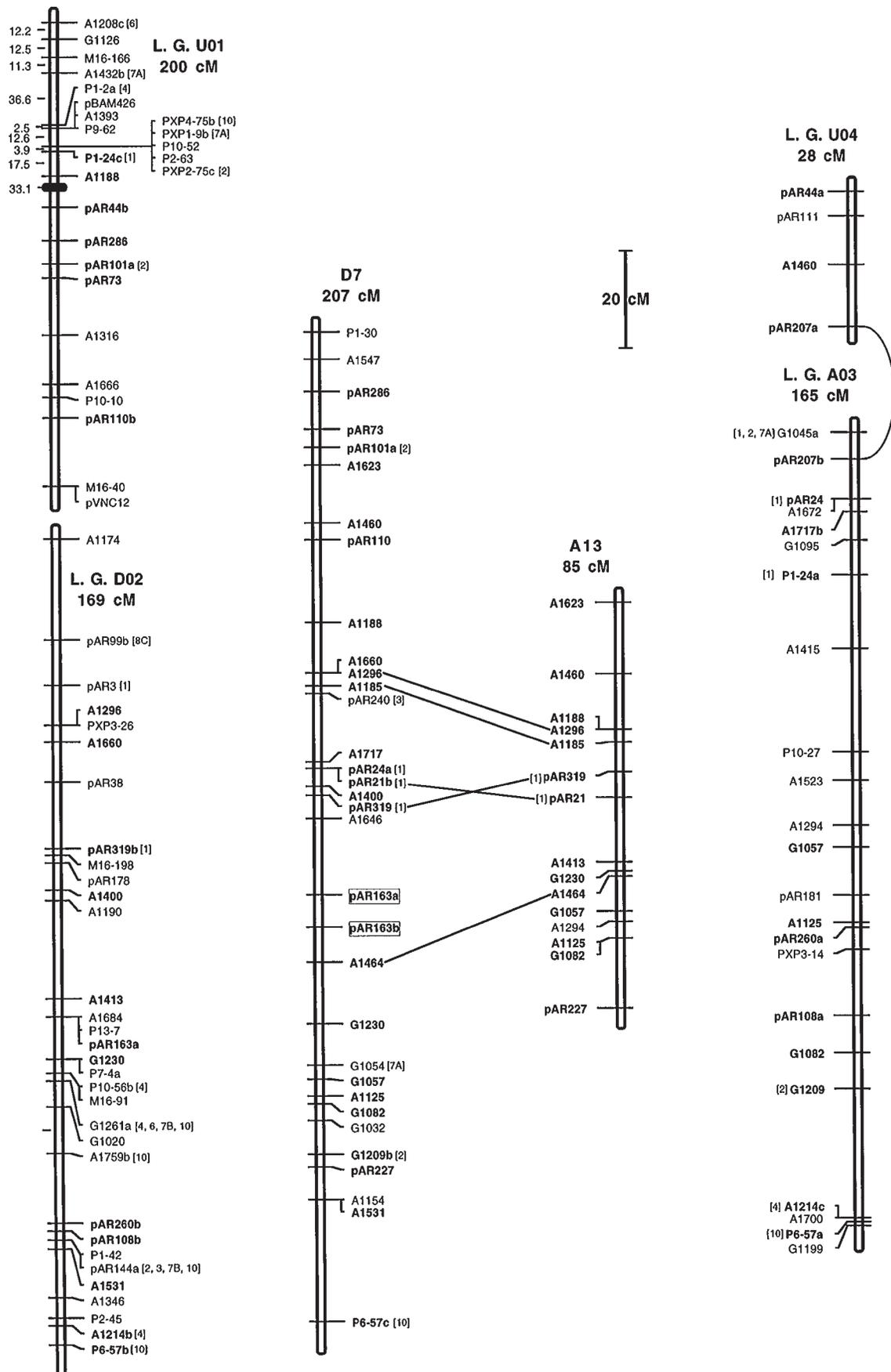
Table 2. Genetic length differences among the diploid (A, D) and allotetraploid (A_t, D_t) *Gossypium* mapping populations.

Shorter genome	L_1 (cM)[a]	Longer genome	L_2 (cM)[a]	Percentage difference[b]
A	532.73	D	563.77	5.8%
D_t	532.40	A_t	557.89	4.8%
A	134.00	A_t	203.00	51.5%
D	769.20	D_t	1219.38	58.5%

[a]L_1 and L_2 were calculated by summing the genetic distance between each adjacent pair of loci.
[b]Calculated as L_1–L_2/L_1, where L_1 = shorter genome length, and L_2 = longer genome length.

properly associated with HA6. A more highly saturated A genome map should resolve this issue.

HA7A versus HA9

In HA7A, *pAR26* and *P12–20* map to orthologous regions of D9 and Chr. 5A, respectively. Locus *pAR218* also maps to D9. Each of these three loci maps to one or more corresponding regions of HA9 on linkage groups Chr. 25D, D10, and Chr. 10A.

HA7A versus HA10

P5–61, A1751, and *pAR48* map to corresponding regions of Chr. 5A, A6, D9, and Chr. 20D in HA7A; in HA10 these loci map to the subterminal regions of LG D04, D11, and Chr. 10A.

Variation in recombination rates among genomes

To evaluate potential differences in recombination rates among the mapped genomes, recombinational distances (cM) between pairs of loci in colinear regions were summed across the A, D, A_t, and D_t LGs (data available from CLB). To remove any bias due to map density, each summation was calculated directly from recombination fractions (Haldane 1919) for every pair of loci. When summed across all loci, the two diploid F_2 populations differ in recombinational length by 5.8% (Table 2). Similarly, the two genomes of the allotetraploid F_2 population are recombinationally equivalent (4.8% difference overall). The two diploid F_2 populations, however, were genetically shorter than their A_t and D_t counterparts, differing by 52% and 59%, respectively.

Discussion

Genome evolution in *Gossypium*

Comparisons among the four maps demonstrate that gene order and synteny are sufficiently conserved among the A, D, D_t, and A_t genomes that the expected 13 assemblages of homoeologous linkage groups were easily identified. The resulting maps confirm and clarify some of the inferred homoeologies between linkage groups described previously for allotetraploid cotton (Reinisch et al. 1994).

These comparisons among the A, D, D_t, and A_t linkage groups provide genetic evidence for structural rearrangements that have altered gene order and synteny. Map comparisons revealed two reciprocal translocations that differentiate the A diploid genome from the A_t genome (Figs. 7 and 8). Colinearity in these same genomic regions among the A, D, and D_t linkage groups suggests that these translocations occurred in the A_t genome subsequent to allotetraploid formation. In addition, RFLP evidence identifies the genomic location of a translocation that differentiates *G. arboreum* and *G. herbaceum* (Fig. 8).

In addition to these translocations, comparisons of gene order among linkage groups within HAs revealed 19 putative inversions. These inversions differentiated two or more of the four genomes in 12 of the 13 HAs (Table 1). In most cases the loci involved in the inversions were not polymorphic in all four genomes and hence could not be mapped in one or more of the four genomes. Because of this, for 13 of the rearrangements it is unclear whether the event occurred at the diploid or allotetraploid level, or in which particular genome. The remaining six inversions probably arose subsequent to polyploidization. Five of these differentiate D from D_t while one differentiates A from A_t.

These inferences are remarkably congruent with those based on chromosome pairing behavior in diploid and allotetraploid hybrids. Classical cytogenetic approaches to comparative genome analysis have shown that the two A diploid genome species, *G. arboreum* and *G. herbaceum*, differ by a single translocation, and that genomes of these two species differ from the A_t genome of allotetraploid cotton by two and three translocations, respectively (Brown 1980; Brown and Menzel 1950; Gerstel 1953; Gerstel and Sarvella 1956; Menzel and Brown 1954; Menzel et al. 1982). Beasley (1942) inferred the presence of one or more inversions between the A and A_t genomes, claimed that the D and D_t chromosomes differ by a minimum of four structural differences (by implication inversions), and suggested that structural differences exist between all of the A and D chromosomes.

If we accept the congruence between Beasley's observations and ours as evidence that a minimum of 13 inversions and one translocation occurred during the divergence of the A and D diploid genomes, and set aside the caveats regarding the timing and genomic location of these events, the estimated rate at which inversions and translocations arose is 1.4 to 2.8 events per million years (13 inversions and one translocation divided by 5 to 10 million years). Similarly, the rate of fixation for translocations and inversions in the allotetraploids is estimated as 2.6 to 5.2 events per million years (6 inversions and 2 translocations divided by 1 to 2 million years, minus the rate for diploids). This suggests that the rate of fixation of inversions and translocations in *Gossypium* allotetraploids may be similar to or only moderately higher than that which occurred in the A and D diploid genomes. These calculations clearly are approximations and are subject to several sources of error. Nonetheless, the

Fig. 7. This figure includes two HAs (HA7A and HA7B) joined by a reciprocal translocation between A_t LGs Chr. 5A and Chr. 4A. A heavily dotted line separates HA7A from HA7B. A lightly dotted line connects two loci whose putative homoeology may contradict the inferred linkage group homologies depicted (see text). (A) HA7A. The orders of *pAR169* and *pAR65* on Chr. 20D relative to D9 and *A1808*, *A1650*, and *pAR278* on D9 relative to A6 are inverted. The locations of *G1112* and *G1066* on Chr. 20D differ from their locations on Chr. 5A and A6, respectively. (B) HA7B. The order of *G1033* and *A1172* on D12 relative to A14 is inverted.

Fig. 6. HA6. The order *of A1591, G1016, G1125, A1720, G1130, G1174, pAR49*, and *pAR8* on D13 is inverted relative to A10.
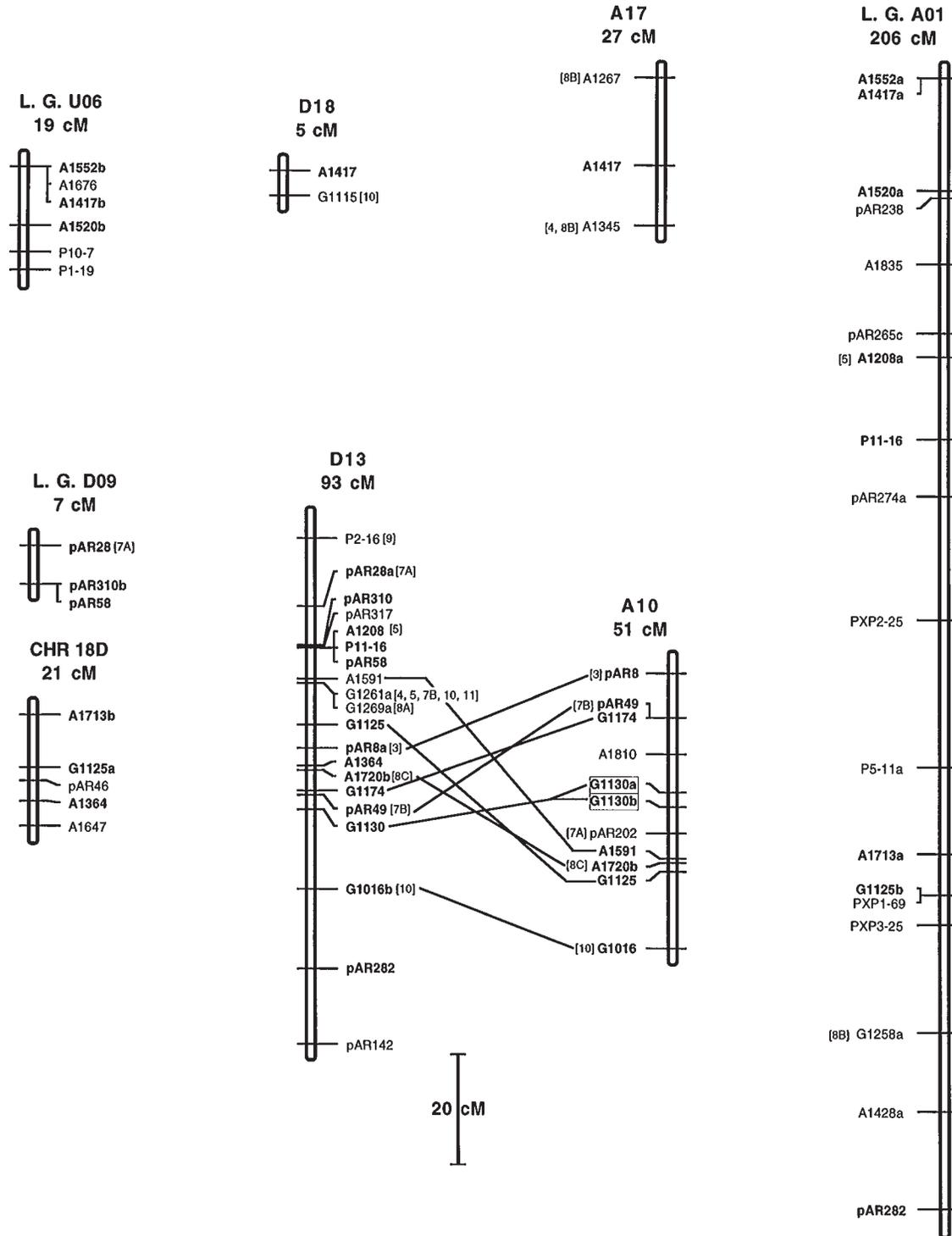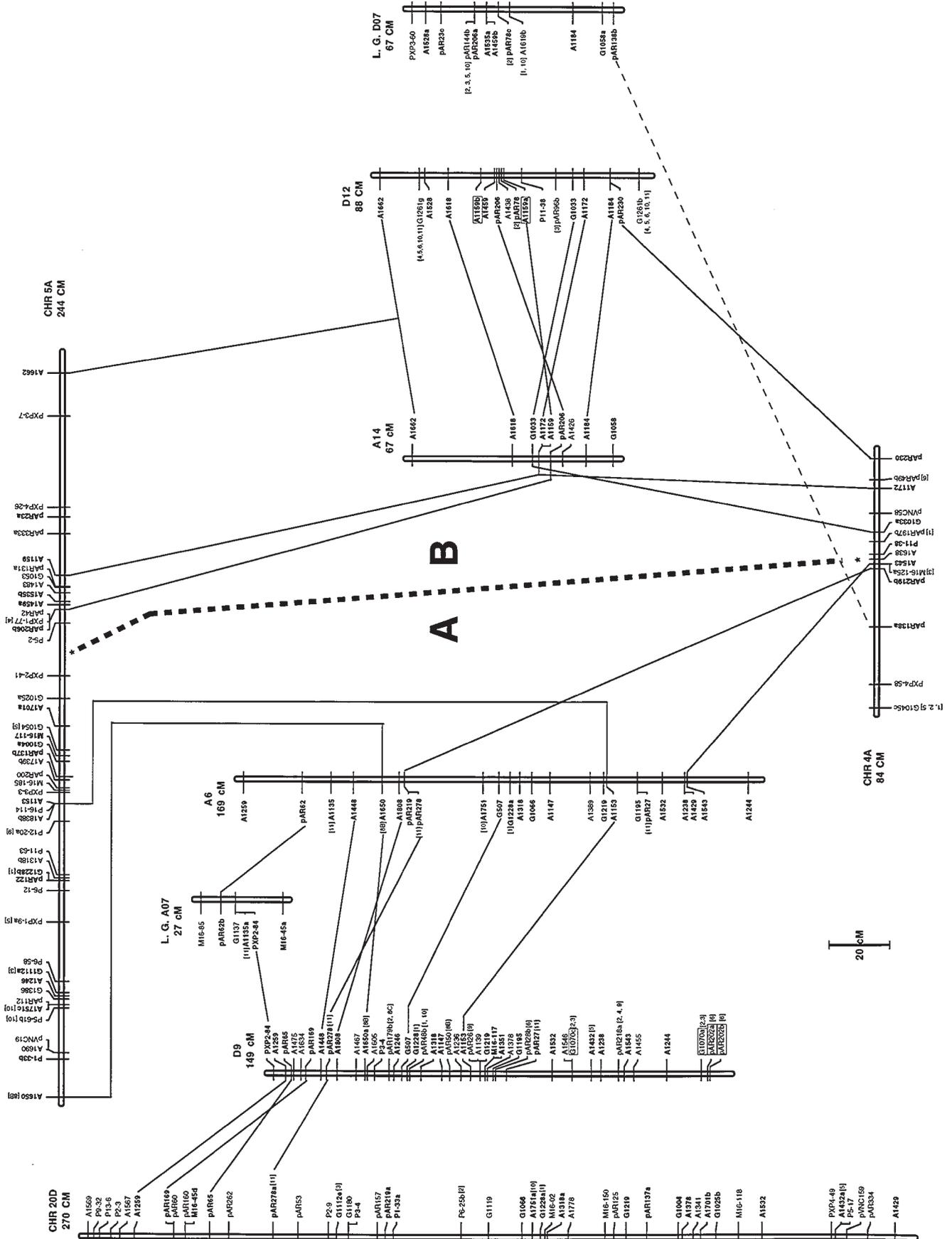
Fig. 8. This figure includes three HAs (HA8A, HA8B, and HA8C) united by a reciprocal translocation involving A_t linkage groups Chr. 2A, and LG A06 and a confounded A linkage group (A5) that arises because the A genome diploid F_1 parent of the F_2 progeny is heterozygous for a reciprocal translocation. A heavily dotted line separates HA8A from HA8B from HA8C. Lightly dotted lines connect loci whose putative homoeology may contradict the inferred linkage group homoeologies depicted (see text). (A) HA8A. The order of *G1164*, *A1418*, and *PXP2–60* on LG A06 is inverted relative to D5. (B) HA8B. Some of the HA8B D_t, D, and A_t loci have putative counterparts mapping to the confounded A genome linkage group, A5. Interspersed among these loci on A5 are loci with homologues mapping to linkage groups in HA8C which otherwise comprise one A_t (Chr. 1A), one D (D2), and one D_t (Chr. 15D) linkage groups. Within HA8B, the order of *pAR185*, *pAR149*, and *pAR172* on LG A06 is inverted relative to D3 and Chr. 17D. (C) Within HA8C, the order of *A1553*, *A1225*, *pAR11*, *P1–3*, *pAR88*, and *A1720* on D2 is inverted relative to Chr. 15D, and the locations of *A1097* and *A1794* on Chr. 1A differ from their positions on D2.

Table 3. Intra-linkage group duplications in the A, D, and AD genetic linkage maps. Probes revealing loci located within or near putative rearrangements are denoted in boldface.

Probes revealing two loci within a linkage group	Map location	Map location of homoeologs	
A genome			
G1130	A10	D13	(Fig. 6)
D genome			
A1124	D6	A12	(Fig. 3)
A1159	D12	A14, Chr. 5A	(Fig. 7B)
A1590	D4	A16, LG D03, LG A02	(Fig. 4)
G1070	D9		(Fig. 7A)
pAR145	D11		(Fig. 10)
pAR163	D7	LG D02	(Fig. 5)
pAR168	D1	A4, LG A05, LG D01	(Fig. 1)
pAR173	D1	A4, LG A05, LG D01	(Fig. 1)
pAR202	D9		(Fig. 7A)
AD genome			
pAR173	LG D01	D1, A4, LG A05	(Fig. 1)
pAR183	LG D(?)05		(Fig. 2)
A1183	Chr. 10A		(Fig. 10)

RFLP evidence is unambiguous with respect to the magnitude of structural changes: allotetraploidy in *Gossypium* was not accompanied by extensive restructuring.

The question naturally arises as to whether this structural conservation is true for other allopolyploids. At present, this is a difficult question to address, as few comparative mapping data exist for diploids and their allotetraploid descendants. Perhaps the best example is *Brassica*. The three diploid genomes in *B. rapa* (A), *B. nigra* (B), and *B. oleracea* (C) are extensively rearranged relative to each other (Lagercrantz and Lydiate 1996; Quiros et al. 1994), but the A and C genomes of the allotetraploid *B. napus* are highly conserved relative to *B. rapa* and *B. oleracea* (Bohuon et al. 1996; Lydiate et al. 1993; Parkin et al. 1995). Cheung et al. (1997) inferred that major genome rearrangements differentiate *B. napus* and *B. oleracea*, but they did not distinguish between the A and C genomes in *B. napus*. Because the A and C diploid genomes are structurally different, many of the rearrangements they observed probably reflect differences between the C genome of *B. oleracea* and the A genome of *B. napus*. Nonreciprocal translocations may arise from homoeologous recombination in doubled haploids of *Brassica napus* (Sharpe et al. 1995), but the relevance of this observation to rearrangement in natural allopolyploids is not clear. Similarly, it may be that the unexpected gains

and losses of restriction fragments observed in synthetic allopolyploids (Song et al. 1995) were partly due to genome rearrangement on the scale detectable by comparative RFLP mapping, but other mechanisms are perhaps more likely (Feldman et al. 1997; Liu et al. 1998; Song et al. 1995). Thus in *Brassica*, as in *Gossypium*, there is little direct evidence for structural rearrangement directly associated with polyploidy itself (Parkin et al. 1995).

Genome duplication and paleopolyploidy in *Gossypium*

One of the more notable features of genetic maps of diploid plants is that many genomic regions appear to be duplicated. For example, approximately 50% of loci in the *Brassica* A and C genomes are duplicated (Quiros et al. 1994), and in *Sorghum* 41% of probes detected more than one restriction fragment (Pereira et al. 1994). While there are a number of mechanisms by which loci may become duplicated, the occurrence of conserved linkage blocks shared by two chromosomes within a diploid genome or two chromosomes within a polyploid genome has been taken as evidence of paleopolyploidy (e.g., Chittenden et al. 1994; Helentjaris et al. 1988; Kianian and Quiros 1992; Kowalski et al. 1994; Whitkus et al. 1992). Given an evolutionary history of repeated cycles of polyploidization in angiosperms, ancient duplicated linkage blocks should be detectable in

Fig. 9. HA9. There are no statistically supported deviations from colinearity among these four linkage groups.
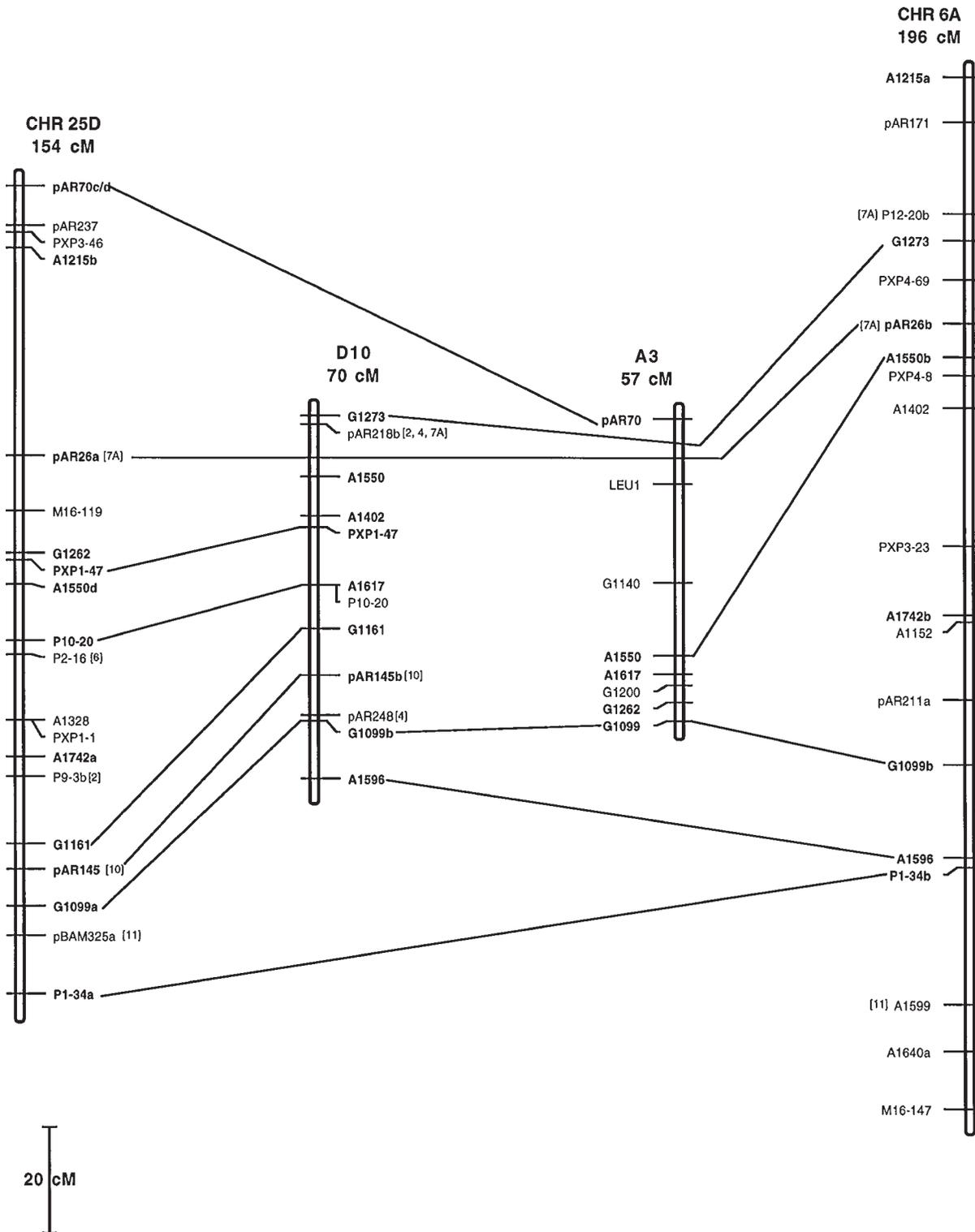
map comparisons, and may even be detectable across kingdoms (Paterson et al. 1996). Within the grasses and *Brassica,* chromosomes appear to be mosaics of conserved linkage blocks. In *Brassica*, the A, B, and C genomes may be paleohexaploids that combine three sets of eight linkage blocks that have been differentially rearranged in each lineage as they diverged from a com-

mon ancestor (Lagercrantz and Lydiate 1996). Similarly, most grass genomes can be reconstructed using 19 linkage blocks (Moore et al. 1995; Moore et al. 1997). This suggests that diploid genome evolution may be accompanied by extensive shuffling of conserved linkage blocks that are flanked by centromeric and telomeric sites (Moore et al. 1997).

Fig. 10. HA10. Five putative inversions were identified: *pVNC163* and *A1695* (LG D04 vs. Chr. 10A), *G1257* and *A1286* (D11 vs. A9), *pAR55* and *A1695* (D11 vs. Chr. 10A), *A1183* and *G1088* (A9 vs. Chr. 10A), and *A1158* and *A1751* (D11 and Chr. 10A). Two differences in locus placement are also evident: *A1548* on D11 relative to A9 and LG D04, and *G1059* on A9 relative to Chr. 10A and D11.
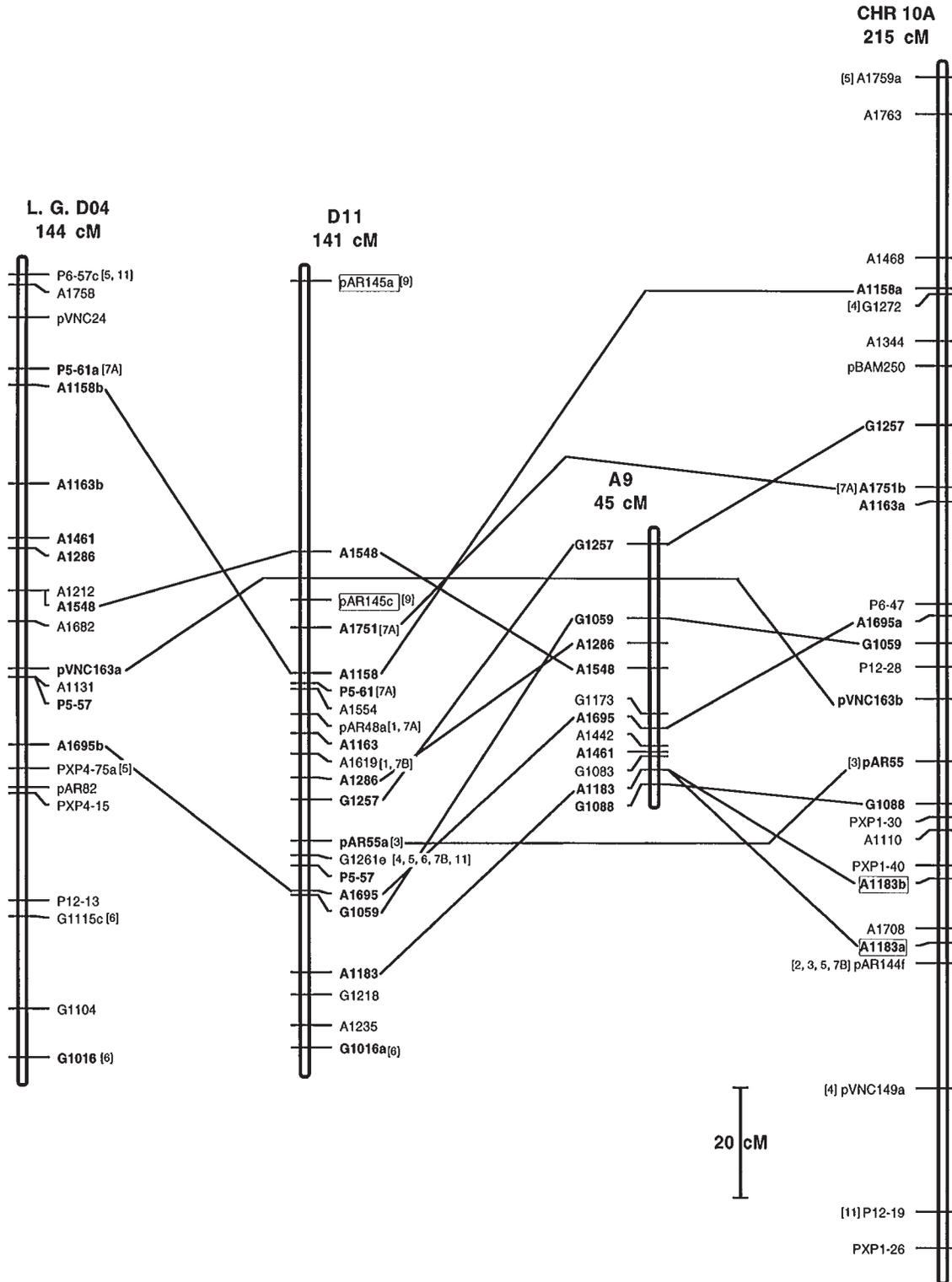
In *Gossypium*, previous cytogenetic, biochemical, and genetic mapping evidence suggested that the diploid species are paleopolyploids (reviewed by Reinisch et al. 1994). Thus, in the present study we expected to see blocks of loci shared by two or more linkage groups in one HA to be reiterated in linkage groups located in another HA. Summed across HAs, there were 235 loci (36% of those mapped) in the A, D, D_t, and A_t genomes that had putative orthologs

Fig. 11. Two A (A21, A23), two D (D15, D17), and four allotetraploid (LG U07, Chr. 17D, LG U02, and LG A04) linkage groups containing loci with orthologues or homoeologues in one of the 13 HAs (they were not incorporated into the HAs because of contradictory or insufficient evidence). Locations of the putative orthologues and homoeologues are indicated to the right of each locus.
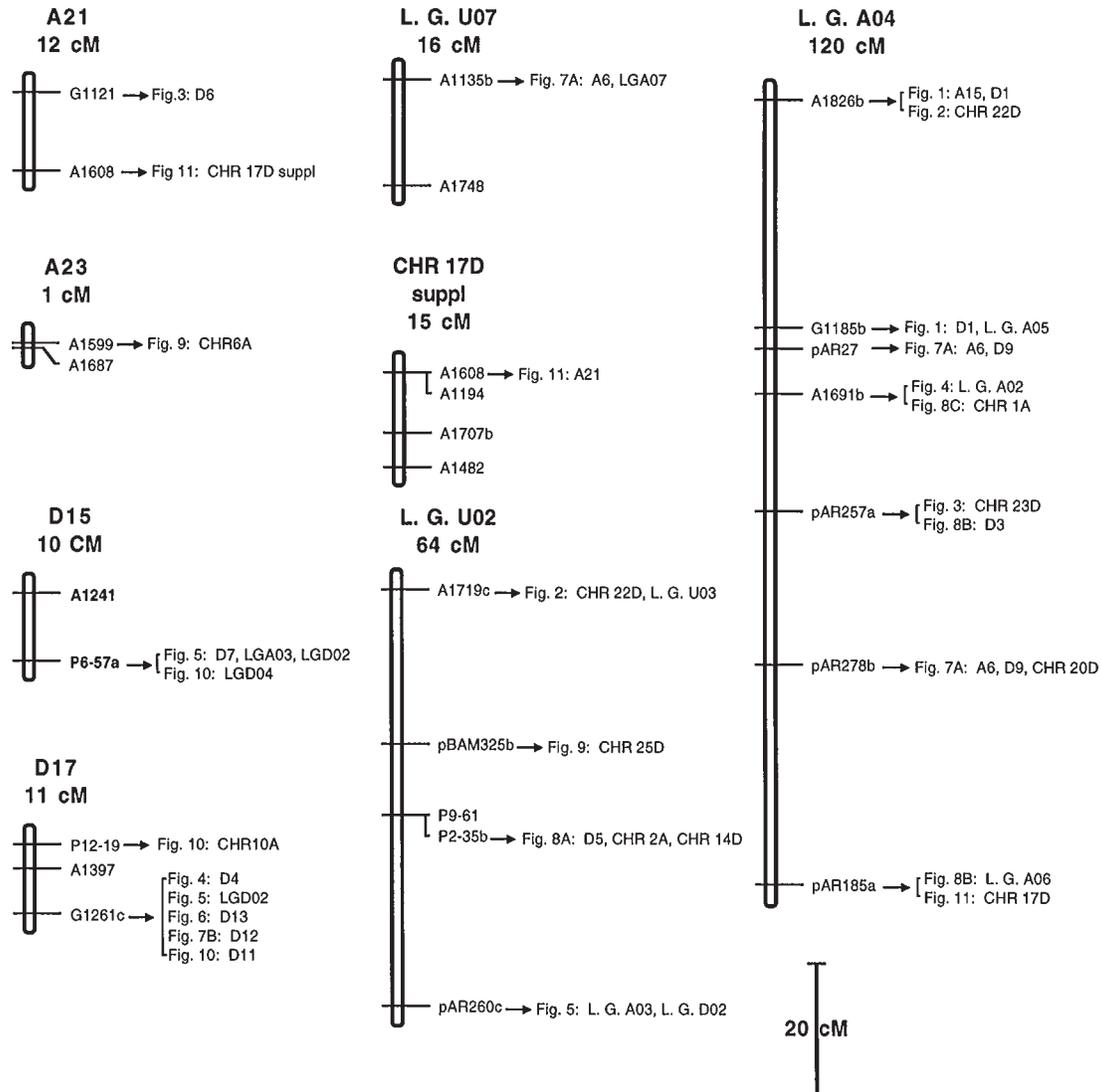
and homoeologs on linkage groups in other HAs. For the most part, however, these duplicated loci did not fit the simple mapping pattern expected for ancient duplicated chromosome segments. The 10 nested duplications that fit the predictions of paleopolyploidy (see "Results") accounted for 76, or about one third, of the loci under consideration. Moreover, there was no simple pattern of duplicated loci among the various HAs. It is probable that some duplications were generated by processes other than paleopolyploidy. Map densities also may be insufficient to identify ancient, duplicated linkage groups. In addition, it may be that the amount of time that has elapsed since paleopolyploidization has been long enough that subsequent mutational change has disrupted or obscured most of the ancient linkage groups. In this respect, we note that the entire tribe to which *Gossypium* belongs (the Gossypieae) is based on a chromosome number of *n* = 13, implying that paleopolyploidization

antedates the origin of the tribe, which may be 20 to 40 million years old (Seelanan et al. 1997, and unpublished data).

Are intrachromosomal duplications in *Gossypium* inversion footprints?

Twelve probes revealed 13 pairs of loci that mapped to a single A, D, or AD linkage group (Table 3). Although intrachromosomal duplication of a locus may not be especially noteworthy, we draw attention to the correlation between these duplications and the rearrangements detected. Ten of the 13 locus pairs are located within or near putative rearrangements. Of the ten putative rearrangements involving three or more loci, six include duplicated loci on at least one linkage group. Only two intra-linkage group duplications are not associated with putative inversions. This suggests that the duplications may be "footprints" of inversions and that the process or processes that give rise to inversions

may not be fully conservative, giving rise to duplications or deficiencies. Although duplications are evident, identifying deletions requires finding probes that differentially hybridize to the genomes being mapped. This possibility was not evaluated because probes were selected to maximize intergenomic comparisons.

Allopolyploidy in *Gossypium* is associated with increased recombination

A growing body of evidence indicates that recombination rates are not correlated with chromosome size. Maize has six times as much DNA per nucleus as rice and three times as much as sorghum, yet all three genomes are recombinationally similar across conserved linkages (Ahn and Tanksley 1993; Binelli et al. 1992; Whitkus et al. 1992). Similarly, *Capsicum* genomes are four times larger as those of tomato, yet the two species are recombinationally similar (Tanksley et al. 1988). Conversely, conserved linkages between potato and tomato differ in recombinational length by a factor of 1.4 to 3.6 but are of similar physical sizes (Bonierbale et al. 1988; Tanksley et al. 1992).

In *Gossypium,* chromosomes in the A genome diploids are nearly twice as large as those in the D genome diploids, and the A_t and D_t genome chromosomes retain the sizes of their diploid antecedents in allotetraploid cells (Endrizzi et al. 1985; Skovsted 1934). These size differences parallel incongruities in nuclear DNA content. The A genome has almost twice as much DNA per nucleus as does the D genome (2C = 4 vs. 2 pg; Bennett et al. 1982; Edwards et al. 1974; Edwards and Endrizzi 1975; Kadir 1976; H.J. Price, personal communication) with near additivity in the allotetraploids (2C = 5.6–5.8 pg; Gomez et al. 1993; Michaelson et al. 1991; H.J. Price, personal communication). Despite this nearly two-fold difference in size, recombination in linkage blocks conserved between the A and D diploid genomes and between the A_t and D_t allotetraploid genomes are essentially equivalent (5.8% and 4.8%, respectively; Table 2). This result verifies previous reports of a lack of correlation between genome size and total recombination in a particularly satisfying way, in that the two allotetraploid genomes are in the same nucleus, thereby controlling for all of the various life-history, population genetic, and ecological covariables that might be suspected of effecting recombination rates.

An unexpected result was that although there is no significant difference in recombination between genomes that vary in size by a factor of two, at either the diploid or allotetraploid level, there was an increase in recombination in the allotetraploid genomes (Table 2). The D_t genome was 58.5% larger, recombinationally, than its diploid counterpart, with a similar increase in recombination in the A_t genome (51.5% greater than A). These differences are remarkably similar to each other, suggesting that the responsible mechanism operates genome-wide in the allotetraploid. Although these results are based on only a single allotetraploid mapping population, Antonio et al. (1996) demonstrated that genetic distance for 300 markers in five different populations of rice did not vary significantly in any of 12 chromosomes. Our results should be considered suggestive and in need of verification with additional crosses, but at present, they suggest that allotetraploidy in *Gossypium* has been accompanied

by an increased frequency of recombination. Whether this is true for polyploids in general, relative to their diploid ancestors, is worthy of investigation, as is the question of why this might be the case. At present, a satisfactory explanation for enhanced recombination in allotetraploids is wanting.

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