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ORIGINAL PAPER

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Genetic mapping of a cross between *Gossypium hirsutum* (cotton) and the Hawaiian endemic, *Gossypium tomentosum*

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Abstract The existence of five tetraploid species that derive from a common polyploidization event about 1 million years ago makes *Gossypium* (cotton) an attractive genus in which to study polyploid evolution and offers opportunities for crop improvement through introgression. To date, only crosses (HB) between the cultivated tetraploid cottons *Gossypium hirsutum* and *G. barbadense* have been genetically mapped. Genetic analysis of a cross (HT) between *G. hirsutum* and the Hawaiian endemic *G. tomentosum* is reported here. Overall, chromosomal lengths are closely correlated between the HB and HT maps, although there is generally more recombination in HT, consistent with a closer relationship between the two species. Interspecific differences in local recombination rates are observed, perhaps involving a number of possible factors. Our data corroborate cytogenetic evidence that chromosome arm translocations have *not* played a role in the divergence of polyploid cottons. However, one terminal inversion on chromosome (chr.) 3 does appear to differentiate *G. tomentosum* from *G. barbadense*; a few other apparent differences in marker order fall near gaps in the HT map and/or lack the suppression of recombination expected of inversions, and thus remain uncertain. Genetic analysis of a discrete trait that is characteristic of *G. tomentosum*, nectarilessness, mapped not to the classically reported location on chr. 12 but to the homoeologous location on chr. 26. We propose some hypotheses for further study to explore this incongruity. Preliminary quantitative trait locus (QTL) analysis of this small population, albeit with a high probability of false negatives, suggests a different genetic control of leaf morphology in HT than in HB, which also warrants further investigation.

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Introduction

The cotton genus, *Gossypium* L., is an excellent system for examining many fundamental questions relating to genome evolution, plant development, and crop productivity. *Gossypium* comprises approximately 45 diploid and five allopolyploid species that occur naturally 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).

Allotetraploid cottons are all indigenous to the New World and unite the Old World A genome with the New World D genome, in an A-genome cytoplasm (Galau and Wilkins 1989; Wendel 1989; Wendel and Albert 1992). The A- and D-genome progenitors are thought to have diverged from a common ancestor about 6–11 million years ago (mya) and been reunited in a common tetraploid nucleus about 1.1–1.9 mya (Wendel 1989; Wendel and Albert 1992; Senchina et al. 2003; Wendel and Cronn 2003). Polyploidization was followed by radiation and divergence, with the evolution of distinct tetraploid species that are now indigenous to Central America (*Gossypium hirsutum* L.), western South America (*G. barbadense* L.), northeastern Brazil (*G. mustelinum* Miers ex Watt), the Hawaiian Islands (*G. tomentosum* Nuttall ex Seemann), and the Galapagos Islands (*G. darwinii* Watt) (Fryxell 1979). All tetraploid species have 26 gametic chromosomes, exhibit disomic

pairing (Kimber 1961), and have similar genome sizes (Wendel et al. 2002) that have been variously estimated at 2.2–2.9 Gb.

The transmission genetics of crosses between the two cultivated tetraploids, *G. hirsutum* and *G. barbadense*, have been investigated in detail, in part based on a genetic map (hereafter, referred to as the HB map) comprising 2,584 loci at 1.72-cM (approx. 600 kb) intervals (Rong et al. 2004). To date, this cotton map has been linked to a total of 295 quantitative trait loci (QTLs) that influence 26 traits related to plant growth, development, and morphology (Wright et al. 1999; Jiang et al. 2000b), reproductive biology (Lan et al. 1999); fiber yield and quality (Jiang et al. 1998; Paterson et al. 2003; P. Chee and A.H. Paterson, personal communication), disease resistance (Wright et al. 1998), and the preservation of productivity and quality under drought stress (Saranga et al. 2001, 2004). Further, the genetics of reproductive isolation between *G. hirsutum* and *G. barbadense* has been dissected in detail (Jiang et al. 2000a).

Much less is known about transmission genetics and genome organization in the other three naturally occurring allopolyploid cottons. The need for more extensive exploration of the cotton genus for naturally occurring variation has recently been highlighted. Based on information in public data bases (USDA NASS 1998; Rayburn et al. 1999), the rate of gain in cotton yields has steadily diminished since 1985 (Helms 2000). Since 1992, cotton yields have declined in absolute terms, reaching a disturbing rate of 16.8 kg ha^{-1} $year^{-1}$(3.3%) in 1998. Further, year-to-year variations in yield increased almost fourfold in the period from 1980 to 1998 relative to the period from 1960 to 1979, translating into higher risk for the grower. The yield plateau is closely associated with increasing genetic vulnerability (Helms 2000), resulting from the overexploitation of a few genetic backgrounds during the past 15 years. This has been exacerbated by the widespread (approx. 60% of 1999 US hectares) planting of transgenic cultivars that are the result of backcross breeding with an even smaller subset of closely related genotypes (USDA AMS 1999).

Non-domesticated tetraploid species are quite naturally a high priority for the exploration for genes of potential value for the improvement of cultivated genotypes, following on examples from other taxa of discoveries of "cryptic desirable alleles" from otherwise undesirable genotypes (Tanksley et al. 1996; Xiao et al. 1996). Cytogenetic analysis of F_1 hybrids among the various tetraploid cotton species suggests "... only a small (0.1–0.2%), though statistically significant, lowering of chiasma frequency..." (Hasenkampf and Menzel 1980), suggesting the possibility of ample genetic exchange in interspecific populations. We and our collaborators initiated explorations of both basic transmission genetics, and the possibility of extracting agriculturally valuable alleles, in crosses between cultivated and wild tetraploid cotton species. This manuscript describes the first steps in exploring the genome of *G. tomentosum*, the only member of the cotton genus endemic to the Hawaiian archipelago (Fryxell 1979). *G. tomentosum* is particularly noted for its small, rounded leaves with dense pubescence, absence of foliar and bracteole nectaries (which are characteristic of all members of the cotton tribe except *G. gossypioides*), and diminutive floral nectaries (Stephens 1963, 1964, 1967; Fryxell 1979).

Herein, we report a primary genetic map of a cross between *G. hirsutum* and *G. tomentosum* (hereafter HT) and the subsequent comparison of this map with the HB map. This sets the stage for future comparisons of the genetics of reproductive isolation between HB (Jiang et al. 2000a) and HT and the characterization of advanced-backcross introgression stocks for agriculturally desirable variation. In addition, we report the tentative locations of a small number of genes and QTLs for selected traits based on greenhouse studies.

Materials and methods

Plant materials

Genetic mapping used a total of 82 F_2 plants from a cross between single individuals of *Gossypium hirsutum*acc. TMS-22 and *G. tomentosum*acc. WT936. The former is a laboratory strain of the common cytogenetic and genetic stock TM1, whereas the latter is a first-generation wild plant from a population on Niihau. Wild populations of *G. tomentosum* (GT) harbor very little genetic diversity, and individuals exhibit high levels of homozygosity, consistent with a high level of inbreeding (Dejoode and Wendel 1992; Hawkins et al. 2004). Plants were grown in a greenhouse in Athens, Georgia, in 1-gallon pots containing field soil, at a minimum temperature of 28°C and under a 13/11-h (light/dark)photoperiod.

Phenotypic analysis

Plants were scored for each of the following traits, as described.

Nectaries Nectaries (*Ne1*) on the abaxial midrib of leaves were scored as present or absent.

Leaf morphology F_2 individuals were measured for 12 leaf size and shape characteristics (Fig. 1): leaf lobe number (lbno); main lobe length (L1) and width (W1); second lobe length (L2) and width (W2); third lobe length (L3) and width (W3); angle (A2) and depth (D1) between the main and second lobes; angle (A3) and depth (D2) between the second and third lobes; leaf width (LW). Average values from two representative leaves per plant were used in QTL analysis, largely following the methodology described by Jiang et al. (2000b).

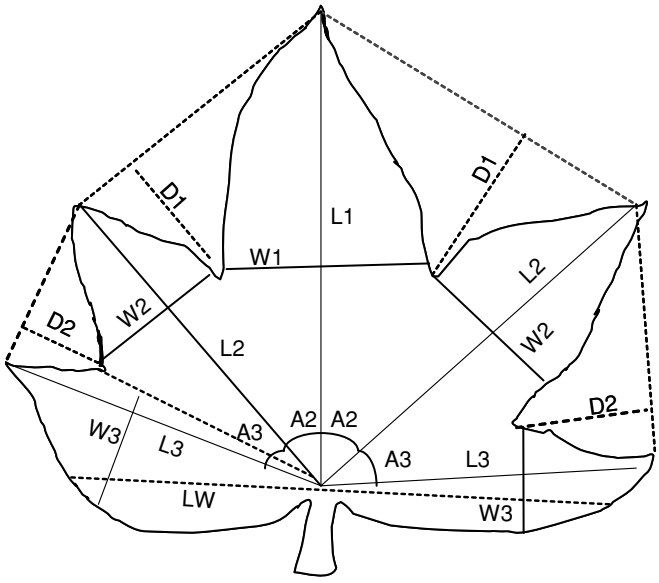


Fig. 1 Leaf morphological traits measured

Genotyping and data analysis

A total of 431 cDNA and genomic DNA probes, largely sampled from a published HB map (Rong et al. 2004) and which were also known (from prior screening) to detect DNA polymorphism between *G. hirsutum* and GT, were analyzed in the F_2 progeny. DNA extractions, electrophoresis, Southern blotting, DNA probe labeling, and autoradiography were done as described by Reinisch et al. (1994). Linkage groups were built using MAPMAKER/EXP 3.0 (Lander et al. 1987) on a PC largely as described (Reinisch et al. 1994). First, for each linkage group, a subset of co-dominant markers (typically five to ten) with a minimum of missing data was used to construct a framework. Linkage groups were initially assembled using the GROUP command with a LOD score of 4. This is a somewhat more stringent threshold than that used for smaller genomes but is appropriate for the approximately 4,500-cM genome of cotton. Initial frameworks were nucleated using a small group of markers and the COMPARE function. Additional markers, including dominant ones, were added into the framework with the TRY command. Final orders were verified using the RIPPLE command. The error detection function provided guidance regarding individual genotypes or autorads that were re-inspected, and scoring or typographical errors were corrected. Recombination fractions were converted to centiMorgans as described (Kosambi 1944). Linkages at distances of greater than 35 Kosambi cM were considered to be non-significant. QTL analyses were performed using MAPMAKER-QTL (Lander and Botstein 1989) and a LOD≥3 threshold ($\alpha = 0.001$ on a nominal basis, or 0.05 after accounting for multiple comparisons; Lander and Botstein 1989) to declare QTLs. Modes of gene action for individual QTLs were calculated and expressed (Table 2) as described (Paterson et al. 1991).

Results

Genetic map

The HT map comprises 589 loci in 52 linkage groups (Fig. 2, Table 1). An additional 26 loci segregated but could not be linked to the map. Most of the mapped loci were based on sequence-tagged sites that had previously been mapped in HB (Rong et al. 2004); however, 86 loci were detected by 63 probes that failed to detect polymorphism in HB. A total of 326 loci are codominant, whereas 141 are dominant for the *G. hirsutum* allele, and 122 are dominant for the GT allele. The relatively high frequency of apparently dominant loci is consistent with the polyploid nature of cotton (Reinisch et al. 1994), where co-migration of one allele with restriction fragments (or PCR amplification products) from a second, duplicated locus necessitates scoring of only two instead of three phenotypes at each segregating codominant locus. The total recombinational length of the present linkage groups is 4,259.4 cM; however, this clearly underestimates the true recombinational length of the HT map due to the presence of gaps and unlinked markers.

The chromosomal and subgenomic affinities of individual HT linkage groups were determined by inference. The HT map was aligned with the current HB map (Rong et al. 2004) by virtue of 165 "anchor loci" (Table 1) at which *G. hirsutum* restriction fragments of indistinguishable size segregated in each of the two populations (based on digestion with the same restriction enzyme). In polyploids such as these, a probe typically hybridizes to two or more restriction fragments; these cases in which the *G. hirsutum* alleles are indistinguishable in the two populations provide the best possible loci for alignment. Only eight (4.6%) loci for which *G. hirsutum* restriction fragments were of indistinguishable size in the two populations mapped to incongruous locations; these included four that mapped to homoeologs, two to non-homoeologous locations, and two comprising a short linkage group (U10) of uncertain location in the HB map (in that it contained one locus from HB chr. 17 and another from HB chr. 26). Further data were provided by 119 "supporting loci" (Table 1) at which *G. hirsutum* restriction fragments were different in the two populations (based on different restriction enzymes or on restriction fragments of different size), but still mapped to locations that were consistent with those of closely linked anchor loci. Together, anchor loci and supporting loci total 284, or 48% of the map. Two small linkage groups could not be identified, and these were designated U10 and U11 so as not to conflict with prior designations (Reinisch et al. 1994). By virtue of this alignment, we can identify the locations of 25 gaps in the HT map, including 14 and 10 on the A-and D-subgenome linkage groups, respectively (and one on an unknown linkage group). Adding 30 cM

for each known gap, the estimated length of the map increases to 4,979.4 cM.

Overall, the average recombination distance between consecutive loci is 8.45 cM, but the density of markers varies between chromosomes, ranging from 5.80 cM (chr. 4) to 14.31 cM (chr. 22). Although the extreme chromosomes in terms of marker density lie in different subgenomes, the overall average in the two subgenomes is similar (8.34 vs. 8.57 cM, a non-significant difference). After allowing for gaps, approximately a threefold variation in chromosome length was observed, ranging from 101.3 cM (chr. 2) to 340.8 cM (LG D08), a similar range to that found in the HB map.

Chromosome structural changes

In most cases, the arrangements of genetic loci along the chromosomes of HT and HB are the same. Most locus order differences are due to reversals of neighboring markers explicable by occasional missing data or scoring errors in either this population (of 82 individuals) or the even smaller HB population (57 individuals). A few additional cases of single, apparently anomalous, marker loci (pAR065y, LG D08; pAR003, LG D02) are likely to be due to the identification of polymorphism at different, paralogous duplications on the same chromosomes in the two crosses.

One clear structural rearrangement appears to differentiate *G. tomentosum* from *G. barbadense*. Based on anchor loci detected by probes G1164, pXP3-89a, and pAR764a, HT chr. 3 differs from HB chr. 3 by a terminal inversion. The affected region appears to be collinear between HT chr. 3 and homoeologous chr. 14 (both HT and HB). The corresponding regions span about 47 cM in HT compared to only 19 cM in HB; indeed, the three central markers of the group span 28 cM in HT but only 3 cM in HB.

Two additional regions also show marker incongruities for which alternative marker orders are found unlikely by several LOD units. Based on one anchor locus (A1183a) and three supporting loci (pAR572, pAR709, pXP1-30), an inversion appears to distinguish HT chr. 10 from HB. Two of the affected markers are about 30 cM apart in HT, but they cosegregate in HB, perhaps consistent with a possible inversion. However, a gap in the HT linkage map that we were not able to close (despite a concerted search for informative polymorphisms), leaves the comparison of this region open to question. Based on anchor loci detected by probes A1686 and A1553, HT chr. 15 appears to differ in marker arrangement both from the corresponding HB chromosome (15) and from the homoeologous HT and HB chromosomes (1). However, we find no evidence of recombinational suppression, and two gaps in the HT map further complicate interpretation of this region.

Two of the rearranged chromosomes show evidence suggesting the possibility of additional rearrangements. Three anchor loci (Gate4DB12b, A1126a, and pAR784) are inverted between HT and HB chr. 15 near the upper end. While alternative arrangements are unlikely in both cases by several LOD units, we are cautious about the interpretation of this in view of the close proximity of the loci in HB (< 5 cM) and also their proximity to a 25-cM gap in HT (the gap is shared by HB and appears to be a region that is rich in recombination and/or poor in genes). Further, because the diagnostic HB markers co-segregate on the homoeolog, more data are needed before any inference can be made about the ancestral chromosome configuration. Similarly, two anchor markers on chr. 10 (pGH588, G1271) show an alternative order in HB and HT, in a region that is collinear with HB homoeologous chr. 20. Before even proposing that this is an inversion, we would prefer to see data from at least one additional locus.

Fig. 2 *Gossypium hirsutum* × *G. tomentosum* (HT) genetic map and its alignment to the *G. hirsutum* × *G. barbadense* (HB) map. The HT genetic map is drawn in Kosambi centiMorgans, and its alignment to the HB map (same scale) is illustrated. "Anchor loci" at which *G. hirsutum* restriction fragments of indistinguishable size based on digestion with the same restriction enzyme segregated in both the HT and HB populations are connected by *solid lines*. Loci at which *G. hirsutum* restriction fragments segregated on the basis of different restriction enzymes (hence orthology is not certain) are connected by *dotted lines*. Each HT locus indicates the restriction enzyme used to map it (*E3 Eco*RI, *E4 Eco*RV, *E5 Hin*dIII), and the dosage of the segregating polymorphism (*C*odominant, *DG. hirsutum* allele dominant, *R G. tomentosum*allele dominant). In cases where a particular DNA probe detected additional loci that are not shown by *solid* or *dotted* lines, these were listed *in parenthesis* to the *right* of the subject locus (for both HB and HT, and indicating the chromosomal or linkage group location in each)

Recombination patterns

The total recombinational lengths of corresponding chromosomes were compared between HT and HB and found to be correlated at $r = 0.58$ ($P < 0.01$). The two subgenomes are similar in this regard, with the length of the respective A-subgenome chromosomes correlated at $r = 0.55$ ($P = 0.05$) and the D-subgenome chromosomes correlated at $r = 0.71$ ($P < 0.01$).

While the relative lengths of the HT and HB chromosomes are similar, the absolute lengths of HT chromosomes were generally higher. Genomic regions that were free of gaps and could be compared based on orthologous DNA marker loci are summarized in Table 1 of the electronic supplementary material—HT shows 15.8% (185.1 cM) more recombination in corresponding regions of the A subgenome and 35.9% (439.2 cM) more recombination in the D subgenome. These differences are not uniformly distributed across the chromosomes; instead, each subgenome includes some chromosomes that are substantially shorter, and others that are substantially longer, in HT than in HB.

Curiously, although the lengths of individual chromosomes in HB are predictive of those in HT (or

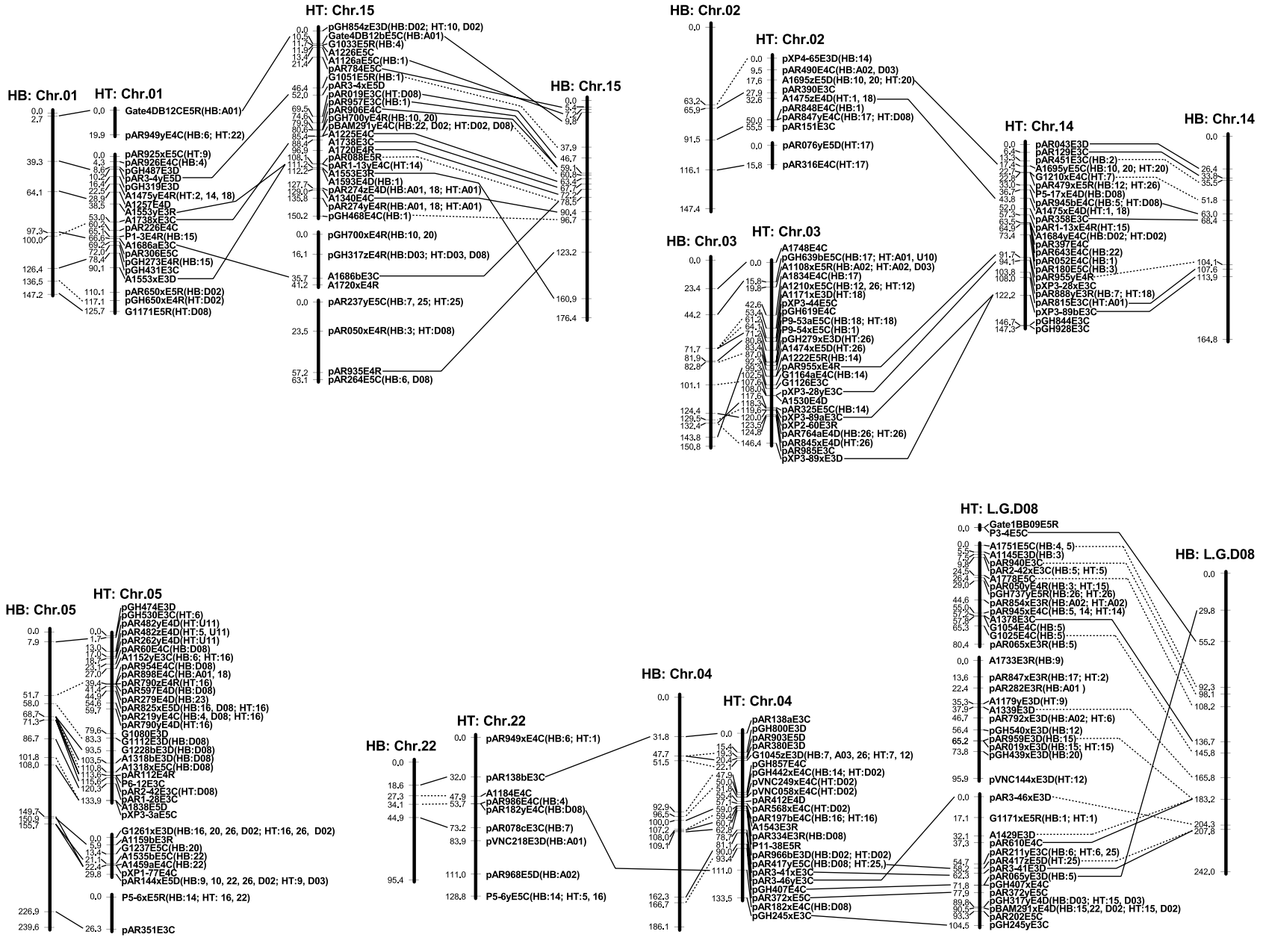
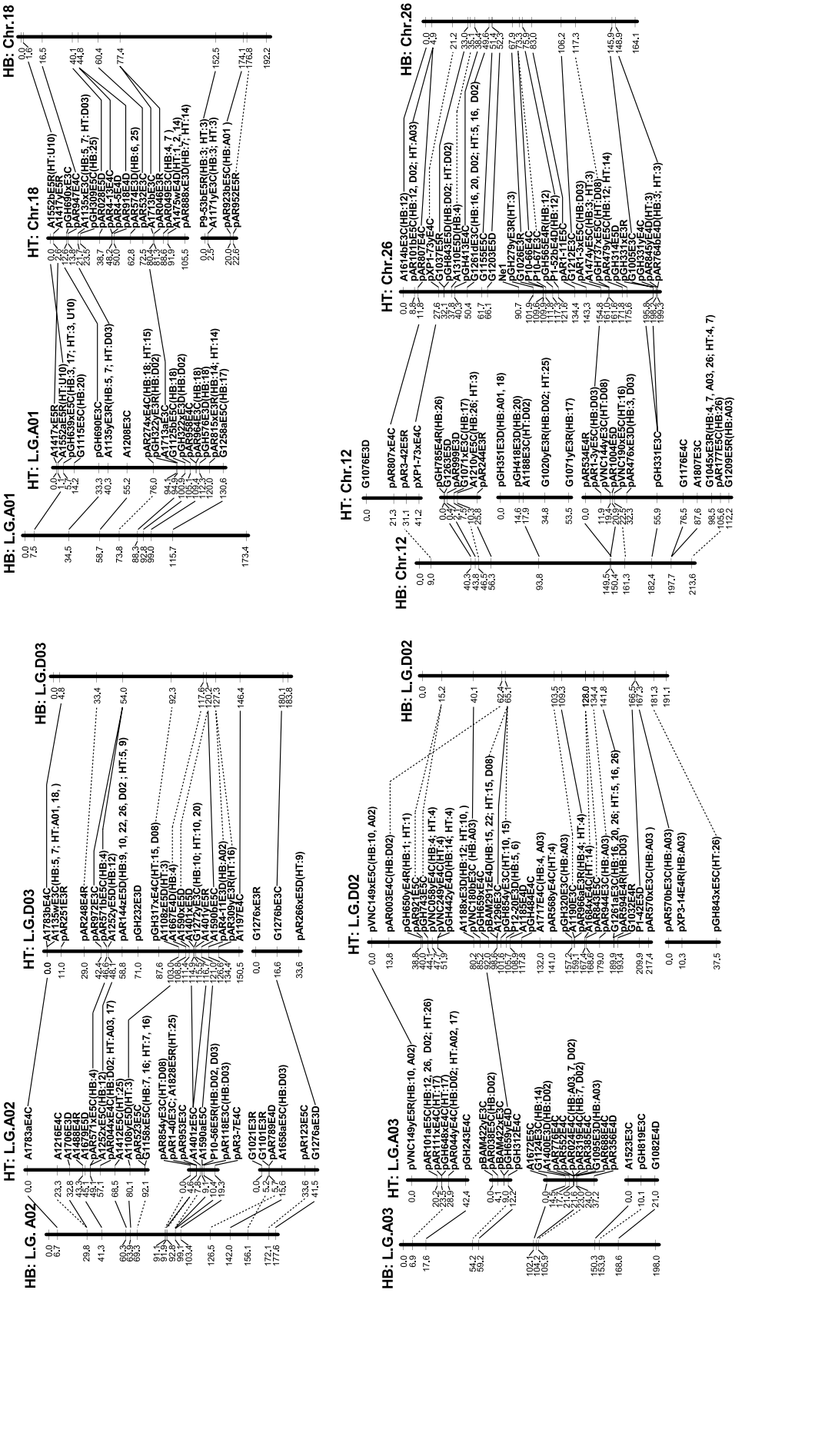

HB: Chr.01
HT: Chr.01
Gate4DB12CE5R(HB:A01)
pAR949yE4C(HB:6; HT:22)
pAR925xE5C(HT:9)
pAR926E4C(HB:4)
pGH487E3D
pAR3-4yE5D
pGH319E3D
A1475yE4R(HT:2, 14, 18)
A1257E4D
A1553yE3R
A1738xE3C
pAR226E4C
P1-3E4R(HB:15)
A1686aE3C
pAR306E5C
pGH273E4R(HB:15)
pGH431E3C
A1553xE3D
pAR650xE5R(HB:D02)
pGH650xE4R(HT:D02)
G1171E5R(HT:D08)
HT: Chr.15
HB: Chr.15
pGH854zE3D(HB:D02; HT:10, D02)
Gate4DB12bE5C(HB:A01)
G1033E5R(HB:4)
A1226E5C
A1126aE5C(HB:1)
pAR784E5C
G1051E5R(HB:1)
pAR3-4xE5D
pAR019E3C(HT:D08)
pAR957E3C(HB:1)
pAR906E4C
pGH700yE4R(HB:10, 20)
pBAM291yE4C(HB:22, D02; HT:D02, D08)
A1225E4C
A1738E3C
A1720E4R
pAR088E5R
pAR1-13yE4C(HT:14)
A1553E3R
A1593E4D(HB:1)
pAR274zE4D(HB:A01, 18; HT:A01)
A1340E4C
pAR274yE4R(HB:A01, 18; HT:A01)
pGH468E4C(HB:1)
pGH700xE4R(HB:10, 20)
pGH317zE4R(HB:D03; HT:D03, D08)
A1686bE3C
A1720xE4R
pAR237yE5C(HB:7, 25; HT:25)
pAR050xE4R(HB:3; HT:D08)
pAR935E4R
pAR264E5C(HB:6, D08)
HB: Chr.02
HT: Chr.02
pXP4-65E3D(HB:14)
pAR490E4C(HB:A02, D03)
A1695zE5D(HB:10, 20; HT:20)
pAR390E3C
A1475zE4D(HT:1, 18)
pAR848E4C(HB:1)
pAR847yE4C(HB:17; HT:D08)
pAR151E3C
pAR076yE5D(HT:17)
pAR316E4C(HT:17)
HB: Chr.03
HT: Chr.03
A1748E4C
pGH639bE5C(HB:17; HT:A01, U10)
A1108xE5R(HB:A02; HT:A02, D03)
A1834E4C(HB:17)
A1210xE5C(HB:12, 26; HT:12)
A1171xE3D(HT:18)
pXP3-44E5C
pGH619E4C
P9-53aE5C(HB:18; HT:18)
P9-54xE5C(HB:1)
pGH279zE3D(HT:26)
A1474xE5D(HT:26)
A1222E5R(HB:14)
pAR955xE4R
G1164aE4C(HB:14)
G1126E3C
pXP3-28yE3C
A1530E4D
pAR325E5C(HB:14)
pXP3-89aE3C
pXP2-80E3R
pAR764aE4D(HB:26; HT:26)
pAR845xE4D(HT:26)
pAR985E3C
pXP3-89xE3D
HT: Chr.14
HB: Chr.14
pAR043E3D
pAR129E3C
pAR451E3C(HB:2)
A1695yE5C(HB:10, 20; HT:20)
G1210xE4C(HT:7)
pAR479xE5R(HB:12; HT:26)
P5-17xE4D(HB:D08)
pAR945bE4C(HB:5; HT:D08)
A1475xE4D(HT:1, 18)
pAR358E3C
pAR1-13xE4R(HT:15)
A1684yE4C(HB:D02; HT:D02)
pAR397E4C
pAR643E4C(HB:22)
pAR052E4C(HB:1)
pAR180E5C(HB:3)
pAR955yE4R
pXP3-28xE3C
pAR888yE3R(HB:7; HT:18)
pAR815E3C(HT:A01)
pXP3-89bE3C
pGH844E3C
pGH928E3C
HB: Chr.05
HT: Chr.05
pGH474E3D
pGH530E3C(HT:6)
pAR482yE4D(HT:U11)
pAR482zE4D(HT:5, U11)
pAR262yE4D(HT:U11)
pAR60E4C(HB:D08)
A1152yE3C(HB:6; HT:16)
pAR954E4C(HB:D08)
pAR898E4C(HB:A01, 18)
pAR790zE4R(HT:16)
pAR597E4D(HB:D08)
pAR279E4D(HB:23)
pAR825xE5D(HB:16, D08; HT:16)
pAR219yE4C(HB:4, D08; HT:16)
pAR790yE4D(HT:16)
G1080E3D
G1112E3D(HB:D08)
G1228bE3D(HB:D08)
A1318bE3D(HB:D08)
A1318xE5C(HB:D08)
pAR112E4R
P6-12E3C
pAR2-42E3C(HT:D08)
pAR1-28E3C
A1838E5D
pXP3-3aE5C
G1261xE3D(HB:16, 20, 26, D02; HT:16, 26, D02)
A1159bE3R
G1237E5C(HB:20)
A1535bE5C(HB:22)
A1459aE4C(HB:22)
pXP3-77E4C
pAR144xE5D(HB:9, 10, 22, 26, D02; HT:9, D03)
P5-6xE5R(HB:14; HT: 16, 22)
pAR351E3C
HB: Chr.22
HT: Chr.22
pAR949xE4C(HB:6; HT:1)
pAR138bE3C
A1184E4C
pAR986E4C(HB:4)
pAR182yE4C(HB:D08)
pAR078cE3C(HB:7)
pVNC218E3D(HB:A01)
pAR968E5D(HB:A02)
P5-6yE5C(HB:14; HT:5, 16)
HB: Chr.04
HT: Chr.04
pAR138aE3C
pGH800E3D
pAR903E5D
pAR380E3D
G1045zE3D(HB:7, A03, 26; HT:7, 12)
pGH857E4C
pGH442xE4C(HB:14; HT:D02)
pVNC249xE4C(HT:D02)
pVNC058xE4C(HT:D02)
pAR412E4D
pAR568xE4C(HT:D02)
pAR197bE4C(HB:16; HT:16)
A1543E3R
pAR334E3R(HB:D08)
P11-38E5R
pAR966bE3D(HB:D02; HT:D02)
pAR417yE5C(HB:D08; HT:25,)
pAR3-41xE3C
pAR3-46yE3C
pGH407E4C
pAR372xE5C
pAR182xE4C(HB:D08)
pGH245xE3C
HT: L.G.D08
HB: L.G.D08
Gate1BB09E5R
P3-4E5C
A1751E5C(HB:4, 5)
A1145E3D(HB:3)
pAR940E3C
pAR2-42xE3C(HB:5; HT:5)
A1778E5C
pAR050yE4R(HB:3; HT:15)
pGH737yE5R(HB:26; HT:26)
pAR854xE3R(HB:A02; HT:A02)
pAR945xE4C(HB:5, 14; HT:14)
A1378E3C
G1054E4C(HB:5)
G1025E4C(HB:5)
pAR065xE3R(HB:5)
A1733E3R(HB:9)
pAR847xE3R(HB:17; HT:2)
pAR282E3R(HB:A01)
A1179yE3D(HT:9)
A1339E3D
pAR792xE3D(HB:A02; HT:6)
pGH540xE3D(HB:12)
pAR959E3D(HB:15)
pAR019xE3D(HB:15; HT:15)
pGH439xE3D(HB:20)
pVNC144xE3D(HT:12)
pAR3-46xE3D
G1171xE5R(HB:1; HT:1)
A1429E3D
pAR610E4C
pAR211yE5C(HB:6; HT:6, 25)
pAR417zE5D(HT:25)
pAR3-41E3D
pAR065yE3D(HB:5)
pGH407xE4C
pAR372yE5C
pGH317yE4D(HB:D03; HT:15, D03)
pBAM291xE4D(HB:15,22, D02; HT:15, D02)
pAR202E5C
pGH245yE3C

Fig. 2b (Contd.)


HB: L.G. A02
HT: L.G.A02
HT: L.G.D03
HB: L.G.D03
HB: L.G.A03
HT: L.G.A03
HT: L.G.D02
HB: L.G.D02
HB: L.G.A01
HT: L.G.A01
HT: Chr.18
HB: Chr.18
HB: Chr.12
HT: Chr.12
HT: Chr.26
HB: Chr.26

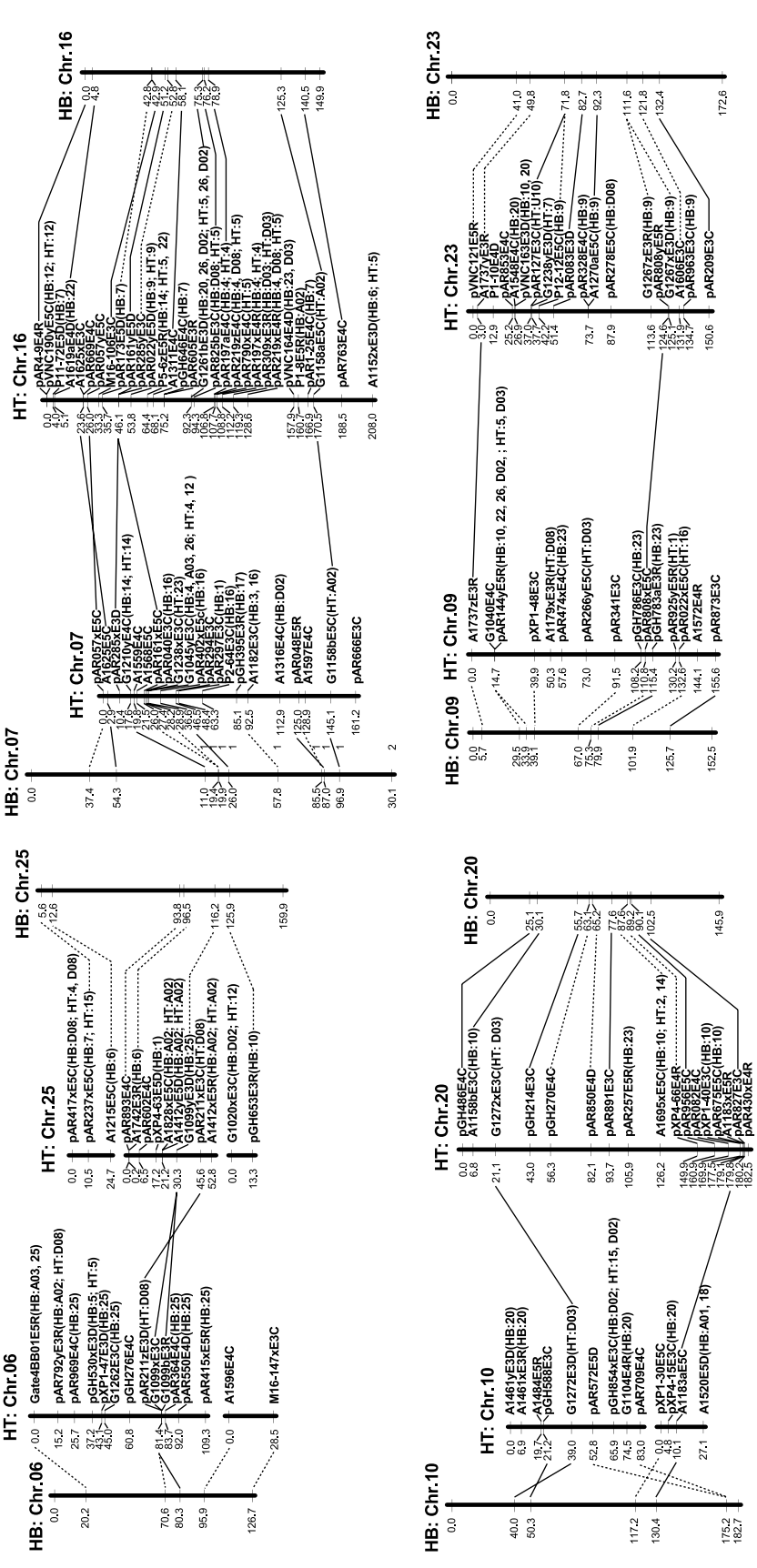

HB: Chr.06
HT: Chr.06
HT: Chr.25
HB: Chr.25
HB: Chr.07
HT: Chr.07
HT: Chr.16
HB: Chr.16
HB: Chr.10
HT: Chr.10
HT: Chr.20
HB: Chr.20
HB: Chr.09
HT: Chr.09
HT: Chr.23
HB: Chr.23
HT: Chr.17
HB: Chr.17
L.G.U10
L.G.U11

Fig. 2c (Contd.)

vice versa), the spacing of polymorphic DNA markers along each chromosome are not related in the two crosses. The average distance between loci for each HT chromosome is reported in Table 1 (column 5), and that for each HB chromosome is reported elsewhere (Rong et al. 2004). Correlation coefficients for the average distance between loci along each chromosome were 0.20, −0.11, and 0.02 for the A subgenome, D subgenome, and overall (respectively, all non-significant).

Patterns of DNA marker locus duplication

Among the 431 different probes mapped, 90 detected two loci, 31 detected three loci, and two detected four loci. The distributions of duplicated loci (Fig. 2) were generally consistent with the homoeologous relationships among chromosomes that are well established in the HB map (Rong et al. 2004), albeit with a few exceptions. Unexpected concentrations of duplicated loci were found between chrs. 3 and 26, chr. 4 and LG D08; chrs. 5 and 16, and chr.17 and LG A03.

Trait mapping

While we recognized that the small population size would permit us to resolve only a few QTLs of large effect (i.e., causing many false negatives), we measured and analyzed one discrete phenotype (nectarilessness) and several quantitative phenotypes (related to leaf morphology, see Fig. 1) in the HT progeny and attempted to map these traits.

Nectarilessness was mapped as a discrete marker (*Ne1*) and was located in the central region of chr. 26. The trait maps 11.2 cM from *pGH279y* and 24.6 cM from *G1203*. In the absence of the *Ne1* locus, the distance between the affected markers is estimated at 23.1 cM, suggesting that there may be a modest amount of mis-scoring associated with the nectariless phenotype.

For the quantitative measures of leaf morphology, 11 locus-trait associations met a LOD threshold of 3.0 (necessary to preclude false-positives in the recombinationally large genome of cotton) and five putative QTLs that fell close to this threshold are also noted (Table 2, Fig. 3). The 16 associations (including putatives) represent 13 different genomic regions. Close correspondence

Table 1 Summary statistics for *Gossypium hirsutum* × *G. tomentosum* genetic map

Chromosome name	Number of loci	Actual map length (cM)	Estimated map length (cM) with gaps	Average distance between loci (cM)	Number of anchor loci	Number of supporting loci	New loci
A subgenome							
Chr. 1(A)	21	145.6	175.6	8.4	6	2	5
Chr. 2(A)	10	71.3	101.3	10.1	3	1	1
Chr. 3(A)	25	146.4	146.4	5.9	7	6	5
Chr. 4(A)	23	133.5	133.5	5.8	6	6	5
Chr. 5(A)	35	190	250.0	7.1	13	4	7
Chr. 6(A)	15	137.8	167.8	11.2	1	4	1
Chr. 7(A)	21	161.2	161.2	7.7	5	5	3
Chr. 9(A)	15	155.6	155.6	10.4	2	7	4
Chr. 10(A)	13	110.1	140.1	10.8	3	3	1
Chr. 12(A)	27	218.1	308.1	11.4	9	5	2
A01	17	130.6	130.6	7.7	7	1	1
A02	27	152.9	212.9	7.9	12	8	2
A03	25	112.8	202.8	8.1	5	6	7
Subtotal	274	1,865.9	2,285.9	8.3	79	58	44
D subgenome							
Chr. 14(D)	23	147.3	147.3	6.4	6	3	5
Chr. 15(D)	32	254.5	314.5	9.8	13	4	3
Chr. 16(D)	30	208	208.0	6.9	10	2	3
Chr. 17(D)	14	94.8	124.8	8.9	4	3	1
Chr. 18(D)	21	128.1	158.1	7.5	9	2	3
Chr. 20(D)	17	182.5	182.5	10.7	6	4	2
Chr. 22(D)	9	128.8	128.8	14.3	2	2	0
Chr. 23(D)	19	150.6	150.6	7.9	4	6	3
Chr. 25(D)	14	90.8	150.8	10.8	0	6	1
Chr. 26(D)	29	199.3	199.3	6.9	15	4	3
D02	33	254.9	284.9	8.6	6	10	6
D03	23	184.1	214.1	9.3	6	6	3
D08	40	280.8	340.8	8.5	5	9	5
Subtotal	304	2,304.5	2,604.5	8.6	86	61	38
Unlinked							
Linkage group (LG) U10	6	57	57	9.5			1
LG U11	5	32	32	6.4			3
LG U	11	89	89	8.1			
Total	589	4,259.4	4,979.4	8.5	165	119	86

Table 2 Biometrical parameters of QTLs affecting leaf morphology in *G. hirsutum* × *G. tomentosum*

Locus	LOD	PVE[a]	a	d	d/a	Mode[b]
Lbno-chr. 10	5.85	79.3	−0.81	0.75	−0.93	D
Lbno-chr. 18	3.14	19.1	0.53	0.21	0.40	AD
Lbno-chr. 22	3.28	30.8	0.63	−0.07	−0.11	AR
LW-chr. 15	2.97	45.0	0.34	−2.26	−6.64	
A2-chr. 15	4.50	29.6	−5.21	6.15	−1.18	R
A3-chr. 9	2.77	33.1	−3.21	−1.56	0.48	DA
D1-chr. 4	2.99	18.3	−0.32	−0.47	1.48	D
D1-chr. 4	4.14	23.8	−0.78	−0.55	−0.72	DA
D1-D02	2.98	21.0	0.36	−0.47	−1.31	R
D2-chr. 4	3.69	37.8	−0.26	−0.42	1.64	D
D2-chr. 12	2.98	33.9	−0.69	−0.75	1.09	D
W1-chr. 9	3.12	18.8	0.49	0.13	0.26	AD
W2-chr. 9	3.16	18.8	0.40	0.09	0.23	AD
W2-chr. 15	3.90	55.1	0.08	−1.01	−13.14	
W3-chr. 2	3.04	66.0	−0.43	−0.37	0.86	D
W3-chr. 6	3.71	50.9	−0.19	0.65	−3.44	

[a]Percentage of phenotypic variance explained
[b]Mode of gene action, as described (Paterson et al. 1991). In cases where no mode is assigned, additive [a], dominant [d] and recessive [r] models could all be deemed statistically unlikely, suggesting underdominance or overdominance (as indicated by sign of d/a ratio)

between QTLs for D1 and D2 on chr. 4, W1 and W2 on chr. 9, and W2 and LW on chr. 15 is consistent with the high phenotypic correlations among these traits (0.77–0.90). No cases of possible homoeology among these QTLs were found, and only two show possible correspondence to previously mapped QTLs for related traits in HB (Jiang et al. 2000b). While this level of association between QTLs may be explained by chance, the affected QTLs are W3 (HT) and W2 (HB) on chr. 6; and lbno (HT) and L3 (HB) on chr. 10.

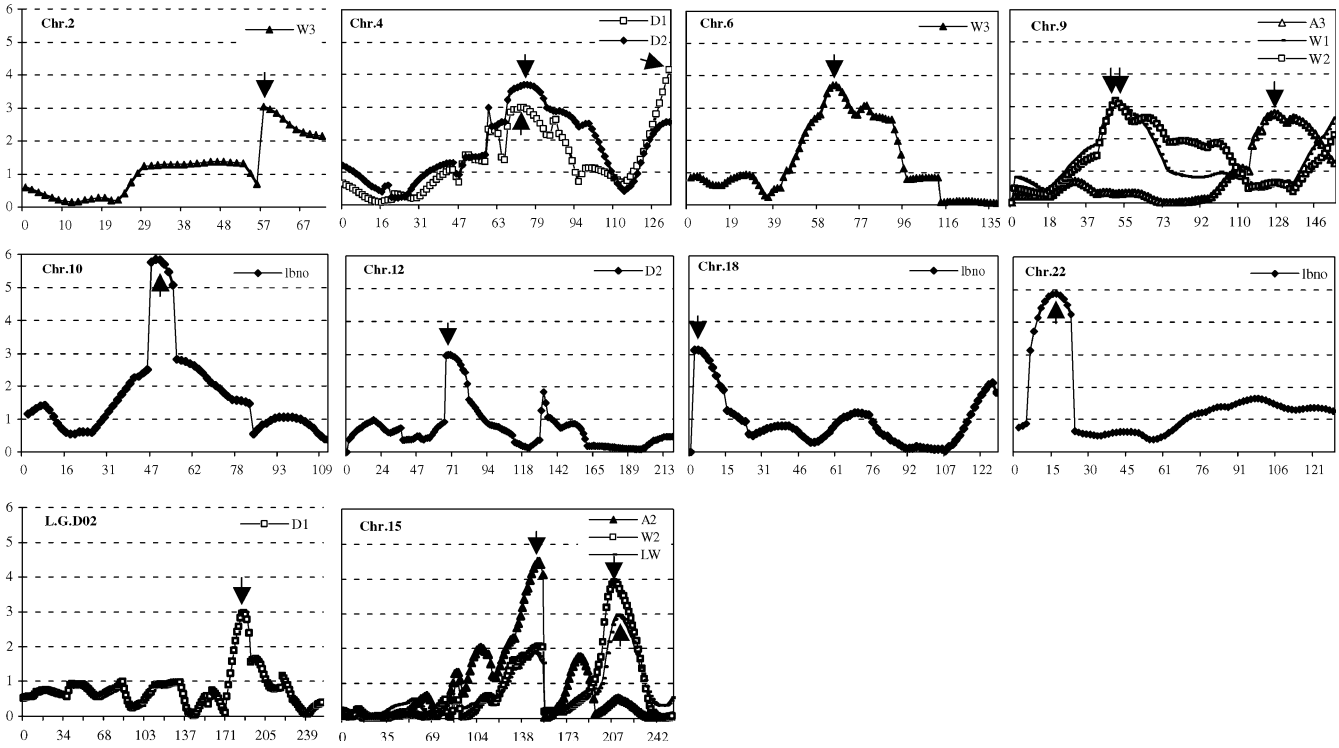


Fig. 3 *G. hirsutum* × *G. tomentosum* (HT) QTL likelihood plots. Chromosome numbers and trait identifiers are indicated in each plot. Maps are plotted in Kosambi centiMorgans. Likelihood peaks for each QTL are indicated by *arrows*

Discussion

Genome divergence following polyploid formation

Herein we provide early molecular evidence on the extent of chromosomal restructuring that has occurred since the divergence of the polyploid cottons from a

common ancestor. While our data support prior evidence that chromosome arm translocations have *not*-played a role in the divergence of *G. hirsutum*, *G. barbadense*, and *G. tomentosum* (Gerstel and Sarvella 1956), at least one intra-chromosomal inversion (and perhaps more) has accompanied the divergence of *G. barbadense* from *G. hirsutum* and *G. tomentosum*.

There was a general increase in the amount of recombination between corresponding loci in the HT cross, which consistent with the somewhat closer relationship of *G. hirsutum* to *G. tomentosum* than to *G. barbadense*. The relatively greater expansion of recombination in the D than the A subgenome of HT leads to a greater overall length of the D-subgenome chromosomes; in contrast, in HB, the two subgenomes have very similar recombinational lengths. Further, the diploid D genome is shorter than both the diploid A genome and each of the tetraploid subgenomes. The larger number of markers found for the D subgenome (304 vs 274 for the A subgenome, Table 1) reconciles this expanded recombination with the non-significant difference in marker density reported above. The basis for differential expansion of recombination in the D subgenome of HT warrants further investigation.

Although there is a good general correlation between HT and HB with respect to overall chromosome length, corresponding intervals delineated by common DNA markers show recombination rates between *G. hirsutum* and *G. barbadense* that are uncorrelated to those between *G. hirsutum* and *G. tomentosum*. Some of these differences may have non-genetic causes—for example, the study populations were small and grown in different environments. However, striking differences such as 180 cM versus 77 cM (chr. 20), 150 cM versus 91 cM (chr. 23), 105 cM versus 64 cM (chr. 12), and 93 cM versus 135 cM for corresponding regions—in populations that assay 114 and 164 gametes, respectively—are not reasonably attributed to chance. Alternative explanations include interspecific differences in local recombination rates, perhaps due to cryptic differences in genome structure beyond the resolution of our present data, differential action of selection to preserve particular assemblies of alleles, or other factors. Once again, further investigation is warranted.

Our comparison of homologous and homoeologous gene arrangements permits us to make inferences about the likely origins of chromosome structural rearrangements among polyploid cottons. In the clearest case, on chr. 3, the affected region is collinear between HT chr. 3, and homoeologous chr. 14 of both HT and HB. Apparent preservation of chromosome structural organization between the homoeologs, derived from ancestors that are thought to have diverged about 6–11 mya (Wendel 1989; Wendel and Albert 1992; Senchina et al. 2003; Wendel and Cronn 2003) suggests that the incongruity of the HB chr. 3 gene order may have occurred in the *G. barbadense* lineage subsequent to its divergence from *G. tomentosum*. If true, this would predict that HB should show recombination suppression in the region while HT should not, a prediction that our data corroborate. A similar approach might be applied to inferring the evolutionary history of other possible chromosomal rearrangements that are suggested herein, if additional data should corroborate them.

Further investigation of additional tetraploid cottons should add greater resolution to our understanding of the course of chromosomal evolution subsequent to polyploid formation. It would be ideal to have maps among the tetraploids crossed in all possible combinations as well as maps of intraspecific crosses for each tetraploid. While the former is now possible, the latter will need to await DNA marker approaches that are inexpensive enough to scan the vast numbers of loci necessary to overcome the relatively low levels of intraspecific DNA polymorphism in *Gossypium*. Especially important is the investigation of *G. mustelinum*, a Brazilian endemic representing the alternate branch of the first split in the polyploid cotton lineage. The comparison of *G. barbadense* and *G. tomentosum* spans the second split. Analysis of *G. hirsutum* × *G. darwinii*, the latter being a sister to *G. barbadense*, should reveal whether the chr. 3 rearrangement predates or postdates the divergence of these sister taxa.

Genetic control of nectaries

A particularly interesting feature of *G. tomentosum* is the absence of leaf nectaries. Most cotton species exhibit pronounced vegetative nectaries on the abaxial midrib, approximately one-third of the way from the petiole junction to the leaf apex (Rudgers et al. 2004). Nectaries are secretory ducts that may attract pollinators or recruit ants to act as mercenaries against herbivores. In cultivated cottons, nectaries tend to attract insect pests, reducing yield (Lukefahr and Rhyne 1960; Lukefahr et al. 1965, 1966; Holder et al. 1968; Meredith et al. 1973; Wilson and Wilson 1977; Thomson et al. 1987; Bhat et al. 1989). Because Hawaii has no native ants and because cotton is largely self-pollinating, there may have been little advantage to nectaries in the ancestor of *G. tomentosum*, and loss-of-function (nectariless) mutations may have become fixed (or nearly so) in this species.

Nectarilessness has been attributed to mutations in each of two homoeologous genes, with the nectariless condition reflecting double-recessive mutations (Meyer and Meyer 1961). *Ne1*has a primary effect on leaf nectaries, with a modifying effect on bract nectaries. *Ne2* has a primary effect on bract nectaries, with a modifying effect on leaf nectaries (Holder et al. 1968). *Ne1* and *Ne2* have been assigned (based on the use of aneuploid genetic stocks) to homoeologous chrs. 12 and 26, respectively (Endrizzi et al. 1984). Genetic mapping of a *G. hirsutum* mutant lacking leaf nectaries tentatively supported this assignment (Jiang et al. 2000b), with the strongest ($P = 0.0053$) of four regions associated with the trait locating to what is now (Rong et al. 2004) known to

be chr. 12, although it was at the time referred to as LG D05.

In the present study, we found the absence of leaf nectaries to be completely explained by the *G. tomentosum* allele in a region of chr. 26 that is approximately homoeologous to the previously mapped location on chr. 12 (Endrizzi et al. 1984). The basis for this incongruence with previous studies, including those of some of the co-authors, warrants further study. Our prior mapping study used a multiple mutant stock as a nectariless source (Jiang et al. 2000b)—the origins of this source are not detailed by its authors (Thomson et al. 1987) but are widely assumed to trace to the same *G. tomentosum* introgressants (Meyer and Meyer 1961) that led to the initial characterization of the trait. If indeed the nectariless sources are all the same, this would suggest that an unusual event such as homoeologoous exchange may have occurred in the historical introgression of this trait from *G. tomentosum* into *G. hirsutum* (Meyer and Meyer 1961). If the sources of the trait are different, then it raises the possibility that duplicate genes at the ancestral *Ne* locus may have taken different evolutionary paths in different lineages, a phenomenon referred to as subfunctionalization (Force et al. 1999; Lynch and Force 2000; Altschmied et al. 2002). At least one other case of possible differential subfunctionalization of cotton QTL alleles has been reported (Saranga et al. 2001). Approaches such as RT-PCR-SSCP (Adams et al. 2003) may provide a generalizable means by which to explore the extent to which differential subfunctionalization affects expression of large numbers of genes.

Genetic control of leaf morphology

The small population size studied here necessitates caution in the interpretation of our QTL data. Nonetheless, we have found at least one group of QTLs that has not been identified previously in HB (Jiang et al. 2000b), suggesting that the small rounded leaves of *G. tomentosum* are under novel genetic control. Several QTLs in the present study fall on the same chr. 15 as a concentration of QTLs previously associated with the okra leaf trait (Jiang et al. 2000b); however, the two groups of QTLs do *not* correspond, falling at opposite ends of the chromosome. The high probability of false negatives in our small population suggests that additional loci remain to be demonstrated at which *G. tomentosum* alleles influence leaf morphology.

The present study sets the stage for future work designed to explore whether barriers to gene transmission between *G. hirsutum* and *G. tomentosum* are similar to those that have previously been identified between *G. hirsutum* and *G. barbadense* (Jiang et al. 2000a). Further insight into chromosomal evolution subsequent to polyploid formation will emerge from crosses with additional tetraploid cottons. Finally, advanced-generation (BC_3F_2) progeny that have been phenotyped for a suite of agriculturally relevant traits will enable us to explore for cryptic desirable alleles that may be useful in reducing the genetic vulnerability of cultivated cottons.

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