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BREEDING AND GENETICS

Upland Cotton (*Gossypium hirsutum* L.) x Hawaiian Cotton (*G. tomentosum* Nutt. ex Seem.) F_1 Hybrid Hypoaneuploid Chromosome Substitution Series

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ABSTRACT

Interspecific germplasm introgression enables unique opportunities for genetic analysis and improvement of domesticated plants, but is commonly impeded by barriers to transmission and recombination, insufficient genetic resolution, and the difficulty of deriving economically suitable products. Such impediments are mitigated to varying extents by the breeding of chromosome substitution lines; however, the difficulty of developing such lines varies widely among crop species and chromosomes. In this manuscript, the development of 45 interspecific F_1 monosomic (2n=51) and monotelodisomic (2n=52) substitution hybrids of *G. hirsutum* L. inbred TM-1 (2n=52, $[AD]_1$ genome) with *G. tomentosum* Nutt. ex Seem., a wild cotton (2n=52, $[AD]_3$ genome) species endemic to dry and rocky coastal areas of the Hawaiian Islands, is reported. Hypoaneuploid plants that lack specific chromosomes or chromosome arms were identified based on phenotypic syndromes, and conventional meiotic metaphase I configuration analysis of acetocarmine-stained microsporocytes ("pollen mother cells"). Characteristics of the hybrids were largely intermediate compared with those of the parental species, including hairy silvery-green to gray-green palmately veined leaves, a muted but distinctly sulfur-yellow corolla and yellow pollen, no central petal spot, a strongly exerted stigma, the absence of nectaries, and short brown seed fiber. Each hypoaneuploid F_1 interspecific hybrid in the series is heterozygous for all parental nuclear polymorphisms except where *G. tomentosum* loci are rendered hemizygous because of the absence of a specific *G. hirsutum* arm segment or chromosome. These hypoaneuploid chromosome substitution stocks are an additional resource for localization of genomic markers and for development of backcross substitution lines; therefore, these stocks provide a stepping stone toward high-resolution chromosome-specific genetic dissection of complex traits, germplasm introgression, and cotton improvement.

Upland cottons, which descend primarily from the New World species *Gossypium hirsutum L.*, are cultivated widely for textile fibers, food, and ruminant feed, and are prized for their high yields (Barbosa, 1995). Conversely, Sea Island, Egyptian, and Pima cottons, which descend primarily from *G. barbadense L.*, are prized for their fiber length and quality. Both *G. barbadense* and *G. hirsutum* arose as interspecific disomic (2n = 52) tetraploids, perhaps monophyletically, along with three other extant species with 52 chromosomes (Cronn and Wendel, 2004). Although the AD genomes of *G. barbadense* (AD_2) and *G. hirsutum* (AD_1) are now extensively diploidized and their plant morphologies quite distinct, the genomes exhibit high meiotic affinity in hybrids and undergo high rates of recombination (Reinisch et al., 1994). Isozyme polymorphisms indicate *G. hirsutum* is more diverse than *G. barbadense*, but only moderately diverse relative to other crop species (Wendel et al., 1992). Genetic uniformity of the crop has long been recognized and renders the crop vulnerable to biotic and abiotic stresses (National Research Council, 1972). While these species harbor moderate amounts of intraspecific genetic or DNA sequence diversity (Bojinov and Lacape, 2003; Kumpatla et al., 2004), genetic uniformity is very high among the agriculturally elite types (Meredith, 1990; 1991; Wendel et al., 1992; Zhang et al., 2005). Although efforts to incorporate wild germplasm have been undertaken in various cotton breeding programs, pedigrees of few cultivars reveal perceptible alien heritage (Van Esbroeck and Bowman, 1998). The relative paucity of alien germplasm in elite cotton cultivars indicates that specialized and improved methods of breeding are needed to achieve successful introgression in developing germplasm with good breeding values.

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Interspecific germplasm introgression can greatly expand opportunities for crop improvement (Tanksley and McCouch, 1997). Obvious candidates include readily observed traits, such as resistance to pathogens, and new morphological or physiological traits that might occur in wild accessions or related species but not in the domesticated species or forms. Less obvious, but perhaps more important, are beneficial alleles for multigenic traits, e.g. yield. These can often be introgressed from wild species and accessions, even if their phenotypes seem to indicate that they are useless for the respective trait(s) (Tanksley and McCouch, 1997; Gur and Zamir, 2004). But wide-cross introgression typically requires considerable time and effort to circumvent or overcome biological, genetic, and cytogenetic hurdles, and the choice of breeding strategies to introgress germplasm can greatly influence success. The two simplest approaches are to hybridize and then inbreed repeatedly, or to hybridize, backcross repeatedly, and then inbreed repeatedly. Alien gene retention and genetic combinations are often very limited nonrandom products from these methods. A complementary approach is introgression through chromosome substitution.

In wheat (*Triticum aestivum* L.), chromosome substitution has facilitated extensive introgression, marker localization, genetic trait dissection, gene localization, and germplasm improvement (Berke et al., 1992; Friebe et al., 1991; Campbell et al., 2003; Erayman et al., 2004). The infusion of alien germplasm into wheat has been crucial to avoiding and curbing pathological epidemics and broadening its germplasm base (Knott, 1987; Friebe et al., 1991). The development of the bread wheat chromosome substitution lines was made possible by prior development of the respective hypoaneuploids of *Triticum aestivum*. In species lacking such hypoaneuploids, it has been possible to bring about chromosome substitutions through the intensive use of molecular markers for selection at each generation (Nadeau et al., 2000; Singer et al., 2004). Another approach for genome-wide introgression employs markers to track segments of the donor at the levels of both individuals and populations in each generation of a backcross-inbred program, while exercising selection to recover specific segments of the genome in each backcross line, and more or less genome-wide coverage among the overall set of lines. Of course, these methods also have limitations, e.g. incomplete detection of recombination, conversion and double cross-overs between marker loci, and the number of markers required is proportional to linkage map length.

The difficulty of developing hypoaneuploids varies widely among crops, but is feasible in Upland cotton ($[AD]_1$ genome, n = 26), where existing hypoaneuploid stocks already provide about 75% coverage of the genome and continues to increase (Stelly, 1993; Raska et al., 2005). Interspecific chromosome substitution F_1 stocks and backcross-derived chromosome substitution lines of Upland cotton have been created only with alien chromosomes from *G. barbadense* ($[AD]_2$ genome, n = 26) line 3-79 (Kohel et al., 1977; Saha et al., 2004; Stelly et al., 2005). Another potential source is the Hawaiian species *G. tomentosum* Nutt. ex Seem. ($[AD]_3$ genome, n = 26), which is closely allied with *G. hirsutum*, but nevertheless quite different from both *G. hirsutum* and *G. barbadense* in terms of phenotype, isozymes, and markers (Fryxell, 1979; Saha and Zipf, 1997). *Gossypium tomentosum* is endemic to dry and rocky coastal areas of the Hawaiian Islands. Its habitat has been reduced by rapid coastal development, rendering this species at risk of becoming endangered (Center for Plant Conservation, 2006). It is one of the most heat-resistant species of the genus (Percival et al., 1999, Akhtar et al., 1996). "The sparse, coarse lint of *G. tomentosum*" may have "attracted the attention of early domesticators" because "the lint is convoluted and could be used for many of the non-textile uses" (from Brubaker et al., 1999: p. 17). Stephens (1964) reported that *G. tomentosum* originated in Hawaii on old volcanic outcrops as opposed to friable soil. The local name of this species is ma'o (yellow green) or hulu hulu (hairy hairy) (DeJoode and Wendel, 1992). The species is a medium-sized shrub with coarse texture and is considered likely to become endangered in the near future. The species is already extinct in the wild on Kaua'i of Hawaii. This species is very distinct from the other tetraploid species in morphology. It has hairy, silvery-green to gray-green, palmately veined leaves, sulfur-yellow corollas without petal spots, and forms strongly exerted stigmas, but it is devoid of leaf, bracteole, and extra floral nectarines (a source of the nectariless insect-resistance trait). Capsules are 3-celled, strongly attached, and each contains 6 to12 seeds covered with reddish brown short fibers lacking differentiation in two layers (Meyer and Meyer, 1961; Meyer and Meredith, 1978; DeJoode and Wendel, 1992; Akhtar et al., 1996; Percival et al., 1999). The fruits are small bolls that produce a tan or brown fiber that is fine and strong (DeJoode and Wendel, 1992).

The objective of this paper is to report on the development of the aneuploid F_1 substitution stocks in *G. hirsutum* for the chromosomes and chromosome arms of *G. tomentosum*.

MATERIALS AND METHODS

The original hypoaneuploids of *G. hirsutum* arose in a number of genetic backgrounds. Quasi-isogenic monosomic and monotelodisomic plants of *G. hirsutum* were derived by repeated backcrossing of each type of hypoaneuploid as female with the inbred line TM–1 of *G. hirsutum*, with phenotypic selection of hypoaneuploid progeny and cytological identification (meiotic metaphase I analysis) of each generation, which result in BC_nF_1 ($n > 4$) plants. The inbred TM-1 was derived from the commercial cultivar Deltapine 14 (Delta and Pine Land Co.; Scott, MS) and inbred over 40 generations by self pollination, and now serves as the primary genetic standard for cotton genetics, cytogenetics, and molecular genetics for *G. hirsutum* (Kohel et al., 2001). The hypoaneuploids of *G. hirsutum* were hybridized as the seed parent with *G. tomentosum*, and the respective hypoaneuploid F_1 progenies were identified according to methods described below. The hypoaneuploids included monosomes (25 II + I) for chromosomes 1, 2, 3, 4, 6, 7, 9, 10, 12, 16, 17, 18, 20, and 25, and monotelodisomes (25 II + Ii or 25II + slightly unequal II or 25II + 2I) for the chromosomes arms Te01Lo, Te01sh, Te02Lo, Te02sh, Te03Lo, Te03sh, Te04Lo, Te04sh, Te05Lo, Te06Lo, Te06sh, Te07Lo, Te07sh, Te08Lo, Te09Lo, Te10Lo, Te10sh, Te11Lo, Te12Lo, Te14Lo, Te15Lo, Te16Lo, Te17sh, Te18sh, Te18Lo, Te20Lo, Te20sh, Te22Lo, Te22sh, Te25Lo, and Te26sh. Each substitution monosomic F_1 plant contains a full haploid complement from the donor *G. tomentosum*, but is missing one chromosome from the TM-1-like haploid complement. Similarly, the substitution monotelodisomic F_1 plant for long-arm telosome of chromosome-1 (Te01Lo) contains a normal haploid complement from the *G. tomentosum* donor, plus 25 normal chromosomes and the telosome (long arm) from the parent that was like TM-1. The Te01Lo F_1 is thus disomic and extensively heterozygous for the long arm but hemizygous for all loci of *G. tomentosum* opposite the missing short-arm segment**.** Major features of monosomic and monotelodisomic plants of *G. hirsutum*, including their cytogenetic behavior, transmission, and inheritance have been described elsewhere (Endrizzi et al., 1985). Each hypoaneuploid condition elicits a characteristic phenotypic syndrome, and most are transmitted with good fidelity from the maternal parent, occurring in 10 to 50% of progeny.

The desired hypoaneuploids were each recovered as a segregate in an interspecific progeny family from the respective hypoaneuploid maternal parent of *G. hirsutum*. In concert with the pedigree, the desired segregates were tentatively identified by plant phenotype; however, some related monotelodisomics and monosomics cause similar phenotypic syndromes. Moreover, mis-division of the univalent occasionally results in formation of monotelodisomic progeny by cotton monosomic seed parents, as in wheat (Sears, 1952), and meiotic numerical nondisjunction in monotelodisomics can lead to formation of monosomic progeny by monotelodisomic seed parents, e.g. H01 from Te01sh. The cytogenetic constitution of the prospective segregates was confirmed by meiotic metaphase I configuration analysis of acetocarmine-stained microsporocytes (pollen mother cells) using conventional cytogenetic methods (Endrizzi and Ramsay, 1980).

Characterization of the hypoaneuploid F_1 plant phenotypes was based on within-family comparative progeny observations in the field or, more rarely, in greenhouse environments, and, later, by comparisons of identified hypoaneuploids (across families) to each other and to euploid F_1 controls from euploid x euploid crosses. Fields and greenhouses were located in both College Station, Texas, and Mississippi State, Mississippi. The hypoaneuploids were examined for unusual characteristics, especially those known to be often associated with the respective aneuploid conditions. To maintain or increase plants across seasons and locations, plants were ratooned, or vegetatively increased by "air-layer" propagation. This study did not include replicated testing to assess agronomically relevant traits.

RESULTS AND DISCUSSION

F_1 plants were identified as specific hypoaneuploids based on maternal cytogenetic type, F_1 plant phenotypes, and meiotic metaphase I analyses (Table 1, Fig. 1A-C, 2A-B). The aneuploid F_1 plants were morphologically intermediate between *G. tomentosum and G. hirsutum* in most regards (Fig. 1A). Like their euploid siblings, most of the hypoaneuploid hybrids were fertile and autonomously fecund, whereas *G. tomentosum* only occasionally sets self-seed under field and greenhouse conditions. The

Table 1. Monosomic and monotelodisomic *G. hirsutum* x *G. tomentosum* interspecific F_1 hybrid chromosome substitution stocks deficient for specific *G. hirsutum* chromosomes and chromosome arms

Original plant identity	Cytological configuration[z]	Designation	Deficiency (*G. hirsutum*)	Phenotypic syndrome
200108072.06	25 II + large I	CS-T-H01-F1	1	Smaller plant, small narrow leaf, narrow or twisted bracts, small oblong boll
200108072.08	25 II + large I	CS-T-H02-F1	2	Smaller plant, smaller leaf with cupped margins, shorter sympodia, smaller round bolls with mid-locule furrow
200108073.05	25 II + large I	CS-T-H04-F1	4	Bushy plant, wavy margins near leaf base, long peduncle and boll
200108073.07	25 II + very large I	CS-T-H06-F1	6	Slower plant growth, reduced branching and clustered sympodia and small bolls
200108073.09	25 II + large I	CS-T-H07-F1	7	Light green plant with large crinkled leaf with secondary lobing; contorted bracteole and calyx; toothy bracteole, shorter sympodia.
200108074.03	25 II + large I	CS-T-H09-F1	9	Slower growing and rigid plant. Pitted boll.
200108074.10	25 II + very large I	CS-T-H10-F1	10	Slower plant with fewer branches. Large, round, flat leaf. Clustered sympodia. Large abnormal stigma. Larger seed.
200200121.01	25 II + large I	CS-T-H12-F1	12	Smaller, narrower and crinkled leaf. Larger flower with petals more open. Semi-sterile (poor pollen shed).
200108075.04	25 II + small I	CS-T-H16-F1	16	Lighter green plant. Crinkled leaf. Shorter sympodia. Smaller boll.
200108075.09	25 II + small I	CS-T-H17-F1	17	Smaller plant. Smaller, narrower and more pointed leaf. Longer style and stigma. Seed usually larger.
200108076.02	25 II + small I	CS-T-H18-F1	18	Smaller plant. Smaller, narrower and more pointed leaf. Longer peduncle.
200100283.01	25 II + small I	CS-T-H20-F1	20	Darker green plant with less branching. Very large leaf. Clustered sympodia. Large abnormal style and stigma. Larger seed.
200108076.08	25 II + medium I	CS-T-H25-F1	25	Slower plant. Smaller leaf. Clustered sympodia.
200100323.12	25 II + large Ii	CS-T-Te01Lo-F1	Short arm of 1	Smaller and narrower leaf. Narrow or twisted bracteole.
200108076.10	25 II + large Ii	CS-T-Te01sh-F1	Long arm of 1	Smaller and narrower leaf. Narrow or twisted bracteole. Small oblong boll.
200100324.01	25 II + large Ii	CS-T-Te02Lo-F1	Short arm of 2	Slower plant. Slightly smaller and narrow leaf. Round boll.
200108077.02	25 II + large Ii	CS-T-Te02sh-F1	Long arm of 2	Smaller plant, smaller narrow leaf. Shorter sympodia. Most bolls with mid-locule furrow.
200108077.04	25 II + large slightly unequal II	CS-T-Te03Lo-F1	Short arm of 3	Leaf more rounded with slight crinkling
200108077.07	25 II + large Ii	CS-T-Te03sh-F1	Long arm of 3	Rounded leaf, usually glossy and less pubescent.
200108078.01	25 II + large slightly unequal II	CS-T-Te04Lo-F1	Short arm of 4	Indistinguishable from euploid.
200100325.02	25 II + large Ii	CS-T-Te04sh-F1	Long arm of 4	Bushy plant. Longer peduncle and boll.
200108078.05	25 II + large Ii	CS-T-Te05Lo-F1	Short arm of 5	Narrow leaf with shallow base. Narrow bracteole.
200108078.07	25 II + v. large Ii	CS-T-Te06Lo-F1	Short arm of 6	Reduced branching. Clustered sympodia. Small boll.
200108078.09	25 II + v. large Ii	CS-T-Te06sh-F1	Long arm of 6	Shorter sympodia. Smaller, rounder boll.

Table 1. Continued

Original plant identity	Cytological configuration	Designation	Deficiency (*G. hirsutum*)	Phenotypic syndrome
200100326.07	25 II + slightly unequal II	CS-T-Te07Lo-F1	Short arm of 7	Normal to larger leaf. Narrower bracteole.
200108079.02	25 II + large Ii	CS-T-Te07sh-F1	Long arm of 7	Bright green plant. Large crinkled leaf. Contorted bracteole and calyx. Toothy bracteole. Shorter sympodia.
200100433.02	25 II + v. large slightly unequal II	CS-T-Te08Lo-F1	Short arm of 8	Small dark green plant. Smaller leaf and flower. Small boll with pointed tip.
200108079.03	25 II + slightly unequal II	CS-T-Te09Lo-F1	Short arm of 9	Shorter sympodia.
200100326.12	25 II +v. large Ii	CS-T-Te10Lo-F1	Short arm of 10	Large leaf. Shorter sympodia. Large abnormal stigma.
200108079.10	25 II +v. large Ii	CS-T-Te10sh-F1	Long arm of 10	Narrow leaf. Short style.
200108080.03	25 II + v. large slightly unequal II	CS-T-Te11Lo-F1	Short arm of 11	Slower, bushy plant. Darker and glossy leaf. Smaller flower. Short bracteole. Smaller boll with mid-locule furrow.
200100861.18	25 II + v. large Ii	CS-T-Te11sh-F1	Long arm of 11	Compact bushy plant. Dark and glossy leaf. Small flower and boll. Deep mid-locule furrow.
200108080.05	25 II + large Ii	CS-T-Te12Lo-F1	Short arm of 12	Larger leaf. Narrow bracteole. Longer style and stigma.
200108080.09	25 II + medium-small slightly unequal II	CS-T-Te14Lo-F1	Short arm of 14	Most plants slower. Slightly smaller leaf. Semi-short sympodia. Mid-locule furrow.
200108081.01	25 II + small slightly unequal II	CS-T-Te15Lo-F1	Short arm of 15	Narrow, twisted bracteole.
200108081.04	25 II + small slightly unequal II	CS-T-Te16Lo-F1	Short arm of 16	Indistinguishable.
200108081.06	25 II + small Ii	CS-T-Te17sh-F1	Long arm of 17	Smaller plant. Smaller and narrower leaf.
200108081.08	25 II + small slightly unequal II	CS-T-Te18Lo-F1	Short arm of 18	Slightly smaller, semi-crinkled leaf.
200108081.10	25 II + small Ii	CS-T-Te18sh-F1	Long arm of 18	Smaller leaf. Longer peduncle and style.
200108082.01	25 II + small slightly unequal II	CS-T-Te20Lo-F1	Short arm of 20	Reduced branching. Larger and usually darker leaf. Semi-short sympodia. Larger but shorter stigma.
200100334.07	25 II + small Ii	CS-T-Te20sh-F1	Long arm of 20	Reduced branching. Larger darker leaf. Clustered sympodia.
200108082.06	25 II + medium slightly unequal II (often 25 II + 2 I)	CS-T-Te22Lo-F1	Short arm of 22	Lighter green , concave leaf. Long style and stigma. Long bracteole teeth and boll.
200108082.08	25 II + medium Ii	CS-T-Te22sh-F1	Long arm of 22	Bushy plant. Narrow bracteole. Longer peduncle.
200108082.10	25 II + medium-small slightly unequal II	CS-T-Te25Lo-F1	Short arm of 25	Slightly smaller leaf. Shorter sympodia.
200108083.03	25 II + small Ii	CS-T-Te26sh-F1	Long arm of 26	Smaller, narrower leaf. Short sympodia. Prolific flowering. Petals more open.

[z] Configuration symbols: II = bivalent, I = univalent, Ii = unequal bivalent

F_1 aneuploid plants were silvery gray-green, and flowered abundantly with bright yellow corollas, yellow pollen, and elongated stigmas. Flowers of most aneuploid F_1 plants remained open at night, similar to the *G. tomentosum* parent. In contrast, flowers of most *Gossypium* species, which open in the morning and close in the evening. While the seed of *G. tomentosum* are covered with reddish brown short fibers that were distinctly different in length and color from Upland cotton (Fig. 1B), lint of F_1 hybrids was of intermediate length and color. Phenotypes of some aneuploid stocks were quite distinctive, in a few cases unexpectedly (Table 1). For example, plants missing chromosome 10 (CS-T-H10-F1) and the short arm of chromosome 10 (CS-T-Te10Lo-F1) had abnormal styles (Fig. 1C).

The value of such cytogenetic stocks is well exemplified by recent progress in genome mapping of wheat, including physical mapping, assigning markers to chromosomes or arms, and, in the case of wheat deletion lines, creating bins within chromosomes or arms (Erayman et al., 2004). As tools for physical mapping, these cotton substitution F_1 aneuploids will provide several capabilities that are complementary to linkage mapping, especially at early phases of linkage map development, and enable validation, which is essential at all stages (Stelly and Saha, 2004). Individually and collectively, all cotton genome maps exhibit one or more of the limitations as follows: the number of linkage groups exceeds the haploid chromosome number, many loci remain unlinked, chromosome identification is incomplete, and multiple nomenclatures exist for linkage groups (Reinisch et al., 1994; Lacape et al. 2003; Han et al., 2004; Nguyen et al., 2004). The aneuploid stocks reported here can aid in map development, for example, by enabling portable markers to be grossly binned and localized by chromosome or segment. Aneuploid stocks can also reveal previously unrecognized synteny between loosely linked linkage groups and/or loci (Gao et al., 2004). Moreover, by enabling integration of laboratory and population-specific maps, they can establish a biologically based, common nomenclature for chromosomes and linkage groups. Several complementary physical mapping methods are available for cotton genomics (Stelly and Saha, 2004), including one for deficiency analysis of hypoaneuploid interspecific F_1 hybrids (Ulloa et al., 2004; Karaca et al., 2002; Liu et al., 2000).

The hypoaneuploid F_1 hybrids reported here will facilitate the localization of co-dominant or dominant chromosome–specific markers of *G. hirsutum*, including simple sequence repeats (SSRs). These stocks and markers also will help validate and integrate map information from different labs into a consensus map that will benefit the entire cotton community. Until now, the only interspecific aneuploid series that has been available for cotton genomics was derived from hybrids of *G. hirsutum* x *G. barbadense* (Liu et al., 2000; Han et al., 2004), a DNA panel that has been

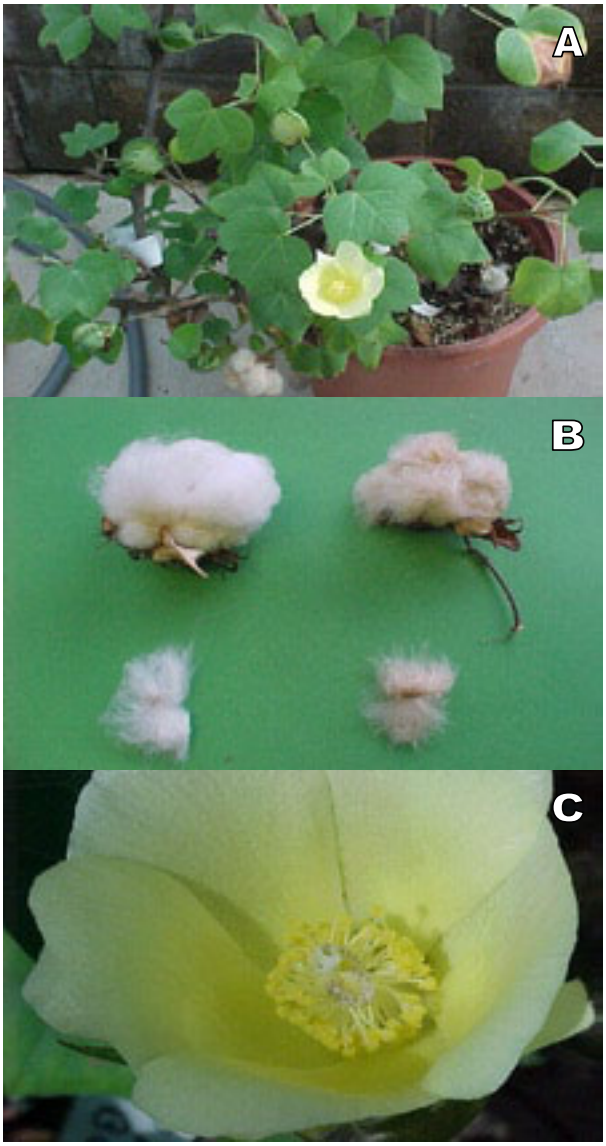

Figure 1. Images of monosomic substitution interspecific F_1 hybrid plants. A. monosomic substitution interspecific F_1 hybrid plant (CS-T-10-F1) hemizygous for the chromosome of *G. tomentosum*. B. Comparative phenotype of a boll and seed with fiber between TM-1 (*G. hirsutum*) (left) and *G. tomentosum* (right). C. Flower of a monosomic substitution interspecific F_1 hybrid (CS-T-H10-F1) hemizygous for chromosome 10 of *G. tomentosum*. Note the abnormal style and stigma.

shared with several labs developing public markers. While most of the deficiencies in *G. hirsutum* in the new plant series of *G. tomentosum* are analogous to those in the existing DNA panel of *G. barbadense*, the panel of *G. tomentosum* differentially lacks (-) or contains (+) deficiencies for the chromosomes and chromosome arms (sh or Lo, for short or long arm, respectively) as follows: +2sh, +2Lo, -3, +5sh, +6sh, +11Lo, +16sh, -16Lo, +22Lo, and -23. The deficiencies common to both aneuploid series provide opportunities for mutual validation across panels, and the additional deficiencies among the *G. tomentosum* series affords extra genomic coverage, and thereby improves the overall ability to orientate linkage groups with respect to the two telomeres of several chromosomes. The new series of substitution aneuploids of *G. tomentosum* also offers a somewhat complementary array of polymorphisms to *G. hirsutum* than *G. barbadense*, so it will enable chromosomal localization of additional molecular marker loci.

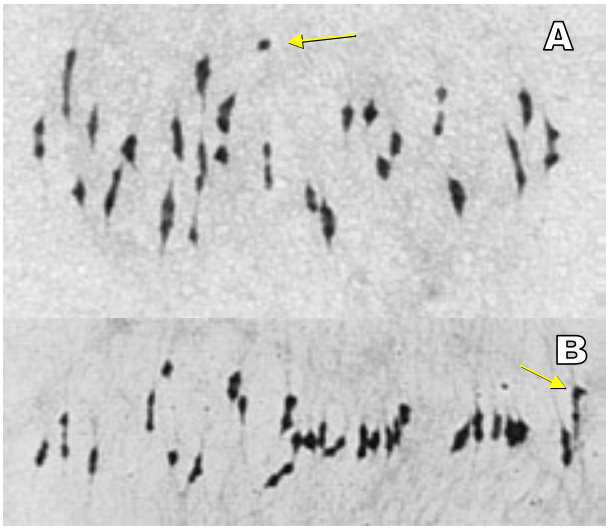

Figure 2. Cytological detection of monosomy and monotelodisomy by metaphase I chromosome configuration analysis of microsporocytes. A. Univalent (arrow) revealing monosomy of cotton plant missing one copy of chromosome 17 (25 II + I). B. Strongly asymmetric bivalent (arrow) revealing monotelodisomy of cotton plant with one normal chromosome 3 and one long-arm telosome of chromosome 3.

The distinctiveness of *G. tomentosum* has been only partially exploited for improving the cultivated tetraploid cotton species. The nectariless trait in *G. tomentosum* is characterized by the lack of nectaries in the leaves, bracts, and extra-floral regions and contributes to the reduction of certain insect populations, since no leaf or extra-floral nectar is present as a food source for adult insects (Lukefahr and Rhyne, 1960). Scientists have introgressed the nectariless trait from *G. tomentosum* into germplasm of *G. hirsutum* to improve insect resistance in Upland cotton (Meyer and Meyer, 1961; Meyer and Meredith, 1978). Meredith et al. (1973) reported that nectariless cottons reduce tarnished plant bug numbers (50%), fleahoppers (50%), boll rot (20%) and bollworm damage (20%). They also observed that nectariless lines produced lint yield and fiber quality equal to their isogenic commercial parents. *G. tomentosum* also has strong fiber (Meyer and Meredith, 1978) and is the most heat-resistant species in *Gossypium* (Percival et al., 1999; Aktar et al., 1996). These aneuploid substitution lines provided a stepping-stone toward backcross chromosome substitution line development, a key to successful wide-cross alien gene transfer for improved agronomic performance, fiber traits and yield, and biotic and abiotic resistance traits. Previous studies have indicated that these traits are genetically complex, and relatively difficult to improve through wide-cross introgression using conventional breeding methods (Van Esbroeck and Bowman, 1998). Their conclusions suggest that successful use of alien germplasm in cotton requires specialized breeding approaches that force the retention of alien chromosome germplasm, extensive recombination, and enable genetic dissection as part of the breeding process. Chromosome substitution is one such method. We expect to use the F_1 hypoaneuploids of *G. tomentosum* as parents for developing backcross substitution lines, which can then be used then to create chromosome-specific interspecific recombinant inbred lines that enable high-resolution quantitative trait loci mapping with the aid of molecular markers, as has been done effectively in wheat (Campbell et al. 2003). Analogous chromosome-specific recombinant inbred lines are now under development using chromosome substitution lines of *G. barbadense* (Stelly et al., 2005; Saha et al., 2004).

In conclusion, these new aneuploid chromosome substitution stocks are useful for cotton genome map development, validation of genome maps, and constitute a critical stepping-stone toward germplasm introgression, genetic dissection of important traits, and their genetic improvement.

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