Extensive and Biased Intergenomic Nonreciprocal DNA Exchanges Shaped a Nascent Polyploid Genome, *Gossypium* (Cotton)

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ABSTRACT Genome duplication is thought to be central to the evolution of morphological complexity, and some polyploids enjoy a variety of capabilities that transgress those of their diploid progenitors. Comparison of genomic sequences from several tetraploid (A_tD_t) *Gossypium* species and genotypes with putative diploid A- and D-genome progenitor species revealed that unidirectional DNA exchanges between homeologous chromosomes were the predominant mechanism responsible for allelic differences between the *Gossypium* tetraploids and their diploid progenitors. Homeologous gene conversion events (HeGCEs) gradually subsided, declining to rates similar to random mutation during radiation of the polyploid into multiple clades and species. Despite occurring in a common nucleus, preservation of HeGCE is asymmetric in the two tetraploid subgenomes. A_t-to-D_t conversion is far more abundant than the reciprocal, is enriched in heterochromatin, is highly correlated with GC content and transposon distribution, and may silence abundant A-genome-derived retrotransposons. D_t-to-A_t conversion is abundant in euchromatin and genes, frequently reversing losses of gene function. The long-standing observation that the nonspinnable-fibered D-genome contributes to the superior yield and quality of tetraploid cotton fibers may be explained by accelerated D_t to A_t conversion during cotton domestication and improvement, increasing dosage of alleles from the spinnable-fibered A-genome. HeGCE may provide an alternative to (rare) reciprocal DNA exchanges between chromosomes in heterochromatin, where genes have approximately five times greater abundance of D_t-to-A_t conversion than does adjacent intergenic DNA. Spanning exon-to-gene-sized regions, HeGCE is a natural noninvasive means of gene transfer with the precision of transformation, potentially important in genetic improvement of many crop plants.

GENOME duplication is a potentially rich source of genes with new (Stephens 1951; Ohno 1970) or modified functions (Lynch and Conery 2000), and is thought to be central to the evolution of morphological complexity (Freeling and Thomas 2006). Genome doubling may confer advantages to a polyploid (Comai 2005), via mechanisms such as increased gene dosage, "intergenomic heterosis" conferred by multiple alleles in a polyploid nucleus, or the evolution of novel gene functions (neofunctionalization) (Stephens 1951; Ohno 1970). Over time, duplicated genes may evolve subdivisions of ancestral functions (subfunctionalization) (Lynch and Force 2000) that render them interdependent. Subfunctionalization may sometimes lead to neofunctionalization (He and Zhang 2005).

Polyploids have been suggested to enjoy a variety of capabilities that transgress those of their diploid progenitors. For example, the notion that polyploids may adapt better

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than diploids to environmental extremes has been suggested, based on both their geographic distribution (Muntzing 1936; Love and Love 1949; Stebbins 1950; Grant 1971) and on an inferred abundance of paleopolyploidizations near the Cretaceous–Tertiary extinction (Fawcett *et al.* 2009). A variety of morphological, physiological, and gene expression changes have been associated with polyploidy. Experimental data are available to evaluate causality of only a few such cases in specific adaptations of polyploids, with more data needed but offering some support (as recently reviewed in Madlung 2013).

Angiosperms (flowering plants) are an outstanding model for studying consequences of genome duplication salient to higher eukaryotes. All angiosperms are paleopolyploid (Bowers *et al.* 2003; Jiao *et al.* 2011), and their abundance of multiple independent genome duplications (Paterson *et al.* 2010) provides "natural replicates" for a variety of investigations. Study of the genes from three rounds of ancient whole genome duplications in *Arabidopsis* reveals a short phase of function relaxation followed by diversifying selection (Guo *et al.* 2013). The ability to study multiple independent genome duplications in a lineage also permits inference of the temporal orders and rates at which different duplication-associated events/mechanisms occur. Their larger genome sizes and smaller effective population sizes than microbes that have experienced genome duplication such as yeast (Gu *et al.* 2003; Christoffels *et al.* 2004; Scannell *et al.* 2006) and *Paramecium* (Aury *et al.* 2006), makes angiosperms more appropriate for studying consequences of genome duplication in higher eukaryotes (Lynch *et al.* 2001; Lynch 2006).

The ability to "synthesize" newly polyploid plants by artificial crosses and chromosomal manipulation using colchicine, has revealed striking immediate reactions of genomes to duplication. These reactions include loss and restructuring of low-copy DNA sequences (Song *et al.* 1995; Feldman *et al.* 1997; Ozkan *et al.* 2001; Shaked *et al.* 2001; Kashkush *et al.* 2002; Ozkan *et al.* 2002), activation of genes and retrotransposons (O'Neill *et al.* 2002; Kashkush *et al.* 2003), gene silencing (Chen and Pikaard 1997a, 1997b; Comai *et al.* 2000; Lee and Chen 2001), and subfunctionalization of gene expression patterns (Adams *et al.* 2003, 2004). Gene silencing in the allopolyploid hybrid between *Arabidopsis thaliana* and *Cardaminopsis arenosa* is arguably related to defense response against transposons (Comai *et al.* 2000). Changes of 24-nt siRNA and DNA methylation levels in *Arabidopsis* hybrids are greatest at loci for which two parents differ substantially (Groszmann *et al.* 2011; Greaves *et al.* 2011). Chromosome rearrangement and reactivation of transposable elements are well known when plants are under "genomic stress," which includes formation of polyploids (McClintock 1983). Instability of hybrid genomes has been attributed to bursts of transposition in both animals and plants; cytosine demethylation and deacetylation of lysine residues on histones may be responsible (Fontdevila 2005).

However, to learn whether immediate reactions of genomes to duplication provide raw material for the beginnings of adaptation or are merely symptoms of imminent extinction, it is necessary to investigate naturally formed polyploids that have survived the test of time. The extinction hypothesis seems generally more likely, given that unreduced gametes are produced more or less continuously by organisms but only a tiny fraction result in successful lineages. For example, dramatic early-generation mutations in synthetic *Brassica napus* (Pires *et al.* 2004) are not paralleled in naturally occurring forms (Rana *et al.* 2004).

A particularly intriguing example of possible advantages associated with polyploidy comes from the cotton genus, *Gossypium*, in which two diploids and two tetraploids have each been independently domesticated for production of the same product, seedborne epidermal fibers. A-genome diploids native to Africa, and Mexican D-genome diploids diverged ~5–10 MYA (Senchina *et al.* 2003) . They were reunited ~1–2 MYA by *trans*-oceanic dispersal to the New World of a maternal A-genome propagule resembling *Gossypium herbaceum* (Wendel 1989), hybridization with a native D-genome species resembling *G. raimondii*, and chromosome doubling. The nascent A_tD_t allopolyploid spread throughout the American tropics and subtropics, diverging into at least three subclades and five species, with two of those species (*G. hirsutum* and *G. barbadense*) being independently domesticated.

In India, where scientific improvement programs are active for both ploidies, tetraploid ("A_tD_t" genome) cottons consistently have substantially higher yield and superior fiber qualities than A-genome diploids (Anonymous 1997). Remarkably, the majority of genetic variation among tetraploid cottons has been ascribed to chromosomes from the D-genome diploid progenitor that *does not* produce spinnable fiber, suggesting that postpolyploidy selection for superior fiber yield and quality of tetraploid cottons has preferentially operated upon the D_t genome (Jiang *et al.* 1998; Rong *et al.* 2007).

In the present study, sequencing and careful comparison of several tetraploid *Gossypium* species and genotypes and representatives of their putative progenitor genomes reveals that homeologous gene conversion events (HeGCEs) account for the vast majority of allelic differences between polyploid cottons and their diploid progenitors. High survivorship of alleles that were converted shortly after polyploid formation is suggested to reflect both a rapid rate of conversion at that time and also adaptive significance of many resulting alleles. A second cadre of converted alleles are closely associated with domestication, suggesting a mechanism by which chromosomes from the D-genome diploid progenitor that does not produce spinnable fiber may have come to account for the majority of genetic variation in fiber characteristics among tetraploid cottons. In partial summary, these data suggest that HeGCEs are an early and important mechanism by which genomes adapt to the duplicated state and may also contribute to plant domestication and crop improvement.

Methods

Read mapping and single nucleotide variation detection

Sequences of cotton D-genome v2 (*G. raimondii*), A-genome (*G. herbaceum*), and a tetraploid genome (*G. hirsutum*, Acala 'Maxxa') are from Paterson *et al.* (2012). *G. herbaceum* is sequenced with read depth of 32× and Acala 'Maxxa' with read depth of 82×. Sequences of three additional tetraploid genomes (*G. hirsutum* GA120R1B3 30×, *G. hirsutum* race yucatanese 15×; and *G. mustelinum* 46×) are from Illumina sequencing of paired-end libraries. Reads are aligned to the reference genome using Burrows-Wheeler Aligner (BWA) (Li and Durbin 2009). Single nucleotide variants (SNVs) between D- and A-genomes are called with Samtools/bcftools (Li *et al.* 2009; Li 2011) using reads with mapping quality >30 and base quality >30. Raw SNVs between progenitor genomes are further filtered by keeping those with read depth between 4 and 60. Raw SNVs for the tetraploid species are further filtered by keeping those with read depth between 7 and twice the average effective read coverage. After aligning reads from the tetraploid genome to the reference genome, alleles are assigned to each subgenome by referring to the parental alleles.

Detection of converted alleles

SNVs from the two progenitor genomes are identified by aligning reads from the A-genome to the reference D-genome. For each of the SNV sites, the orthologous alleles in the tetraploid genomes are sorted into subsets corresponding to the respective parental subgenomes (Supporting Information, Figure S6). A locus that differs between the diploid progenitors but for which a tetraploid shows only the allele from one progenitor (*i.e.*, is monomorphic) is regarded as converted, if meeting the following criteria. Assuming that alleles at a locus follow a binomial distribution when sampling tetraploid DNA, read coverage of 7× is necessary to keep the false positive rate under 0.0078. To remove false positives caused by undersampling, we filtered out monomorphic alleles with read coverage under 7. False-positive converted alleles can also be derived from deletion of a progenitor allele in a subgenome. In this event, read coverage of deleted sites would average half that of the sites that show both parental alleles. Average mapped read coverage for the four tetraploid cotton species studied are shown in Figure S7. Reduction of read coverage by half is not observed in either of the conversion categories, compared to sites that show both parental alleles.

Quality control

To rule out the possibility that inferred conversions are due to the A_t subgenome being more divergent than the D_t from the reference genome, the effect of relaxing the value of editing distance (−n flag in BWA) was investigated. If the sequence divergence was a major factor, the magnitude of conversion bias would be reduced after relaxing the editing distance. The default value for −n flag is 0.04. The estimated mean nucleotide divergence between A- and D-genomes is ~0.64%, ranging from 1.13e-5 to 0.012. Theoretically, the default value is sufficiently large to allow the mapping of A_t-genome reads even for most diverged regions. Figure S8A shows the proportion of allele changes resulting from relaxing the value of −n flag to 0.8. The magnitude of conversion bias is not decreased, but increased. A_t-to-D_t converted alleles became the most common one, presumably due to an increase of mapped reads from the D_t subgenome after allowing more mismatches. That is, the relative frequency of A_t alleles declined under the SNP calling threshold because more D_t-subgenome reads aligned to the reference. To test this, we reduced the frequency threshold of calling a heterozygous SNP (Figure S8B). We observed an increase of unchanged alleles and decrease of both converted types. Expectedly, the log ratio of the two conversion types also increased (Figure S9). We also tried reads with mapping quality from 1 to 30 with increments of 5; all of the tests show similar results. We used reads with mapping quality >20 for this study.

We further investigated possible artifacts of read alignment by assessing the sequence divergence between the A- and D-genomes near HeGCE sites. We divided the genome into nonoverlapping 10-kb bins. For each bin, we calculated the number of A_t-to-D_t converted alleles and the nucleotide divergence between the A- and D-genomes. The nucleotide divergence in each bin is normalized by the proportion of sites covered by reads to remove the variation of reads coverage. The Pearson correlation coefficient of nucleotide divergence and A_t-to-D_t conversions is 0.089, which indicates only weak positive correlations (Figure S10A). To compare with other types of allele changes, we also looked into the correlation between D_t-to-A_t converted sites and unchanged sites. The other two types also show similar levels of correlations ($r = 0.0613$ for D_t-to-A_t converted alleles; $r = 0.0979$ for unchanged alleles) (Figure S10, B and C).

Evaluation of gene function impact

Gene function impact of allele changes is measured relative to the cotton reference gene models as described (Paterson *et al.* 2012), by the following four categories: (a) Altered translation initiation site − allele changes in the first codon of a coding sequence that leads to an amino acid other than methionine; (b) altered splicing sites − allele changes disrupting the "GT–AG" conserved sites flanking introns; (c) introduced stop codons − allele changes introducing premature stop codons into the normal protein coding sequence; and (d) altered stop codons − allele changes altering stop codon to encode an amino acid.

Estimation of the size of biased-conversion tracks

Due to the large variance of measurement of the length of conversion tracks in base pairs (bp), we use number of continuous A_t-to-D_t conversion alleles as a measure. We searched for continuous tracks of conversion alleles both in the genome and in Highly-biased conversion region (HC). If we assume random mutation follows a Poisson distribution,

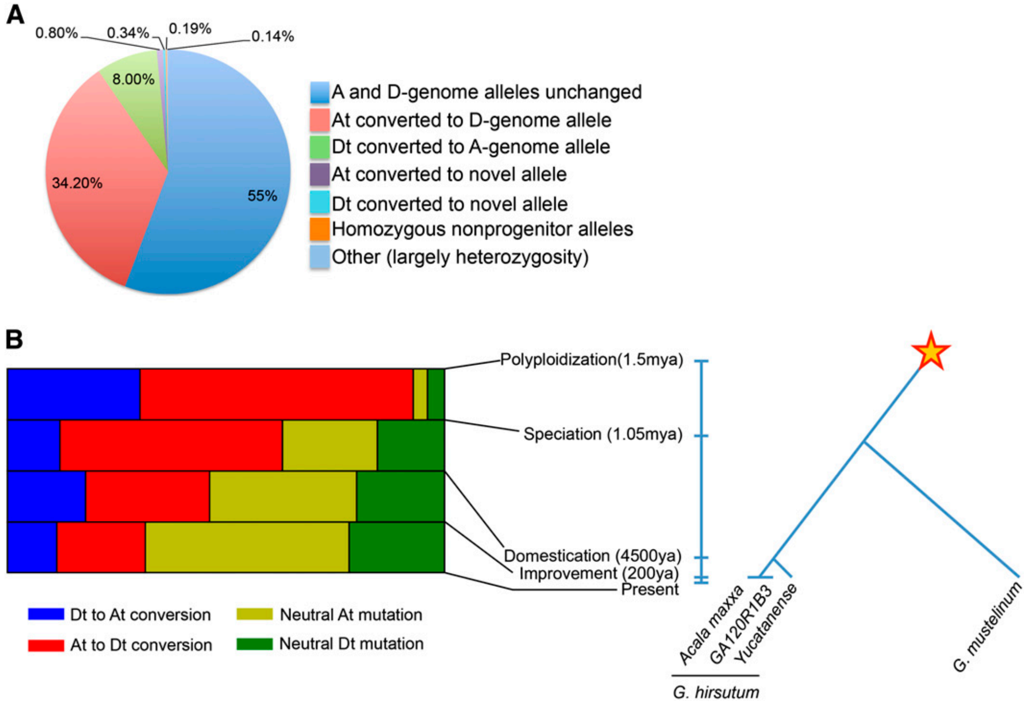

Figure 1 Allelic changes in polyploid cotton. (A) Allelic changes causing striking inferred protein changes between A_t and D_t subgenomes of tetraploid (*G. hirsutum*) Acala 'Maxxa.' (B) Evolutionary history of intergenomic conversion in polyploid cotton. The proportion of A_t and D_t neutral (intergenic) mutations and conversions in both HC and LC are shown in each window in time. The star marks AD allopolyploidization occurring ~1–2 MYA. See Figure S1 for raw data.

the length of continuous mutations should follow an exponential distribution. Both genome-wide and HC conversion tracks show longer continuous tracks than expected (Figure S2). Conversion tracks in the HC show an excess of long ones (four to six alleles) and a deficiency of shorter ones (fewer than three alleles) than the genome-wide set. From the distribution, the average length of conversion tracks across the genome is 3.75 continuous alleles. Genome-wide, the average distance between three continuous conversion alleles is 320.3 bp and 412.6 bp for four. Estimation of the average length of conversion tracks across the genome is (320.3/3 + 412.6/4) × 3.75/2 = 394 bp. In HC, the average length of conversion tracks is 4.32 continuous alleles, with the average length of four continuous A_t-to-D_t conversion alleles being 600.8 bp and 647.3 bp for five. Estimation of the average length of conversion tracks in HC is (600.8/4 + 647.3/5) × 4.32/2 = 604 bp.

Results

Unidirectional DNA exchanges between homeologous loci were the predominant mutational mechanism in the nascent Gossypium polyploid

DNA recombination, typically by reciprocal exchanges between homologous chromosomes ("crossing over"), is a central element of eukaryotic transmission genetics and is also implicated in repair of highly deleterious DNA double-strand breaks (DSBs). Reciprocal exchanges are often accompanied by tracts of unidirectional, local DNA exchanges known as "gene conversion." Most models proposed to account for homologous DSB repair (synthesis-dependent strand annealing, classic double-strand break repair, and break-induced replication, although not single strand annealing) (Helleday *et al.* 2007), also predict the occurrence of tracts of unidirectional gene conversion, even when reciprocal crossing over occurs.

Building on rich evidence of concerted evolution in tandemly repeated sequences such as ribosomal RNA genes (Eickbush and Eickbush 2007) and multigene families such as primate olfactory receptor genes (Sharon *et al.* 1999), we recently showed gene conversion to have occurred in the past ~400,000 years between duplicated rice genes that diverged from a common ancestor 70 million years ago (MYA) (Wang *et al.* 2009). While this is an extreme case, nonrandom similarity between duplicated genes widely distributed across other genomes (Chapman *et al.* 2006; Wang *et al.* 2007) suggests the phenomenon to be widespread.

During the 1–2 MY since its formation, unidirectional DNA exchanges between homeologous chromosomes have greatly outnumbered random mutations in A_tD_t tetraploid cotton. Mapping of 38× Illumina coverage from the A-genome species *G. herbaceum* to the reference D-genome v2 (*G. raimondii*) (Paterson *et al.* 2012) revealed 2,145,177 SNVs between the two, with 60% remaining unchanged in an A_tD_t tetraploid, *G. hirsutum* cultivar Acala 'Maxxa.' Among the 40% of changed sites, 25% now have only D-genome alleles and 10.6% have only A-genome alleles, with only ~4.4% having new mutations. SNVs inferred (by methods previously described) (Paterson *et al.* 2012) to confer striking changes of gene function are even more biased, with 45% of sites changed, 34.2% to D-genome alleles and 8% to A-genome alleles (Figure 1A).

To infer the levels and patterns of occurrence of gene conversions following polyploid cotton formation (Figure 1B), we used a parsimony-based method. For comparison with Acala 'Maxxa,' we resequenced another *G. hirsutum* cultivar

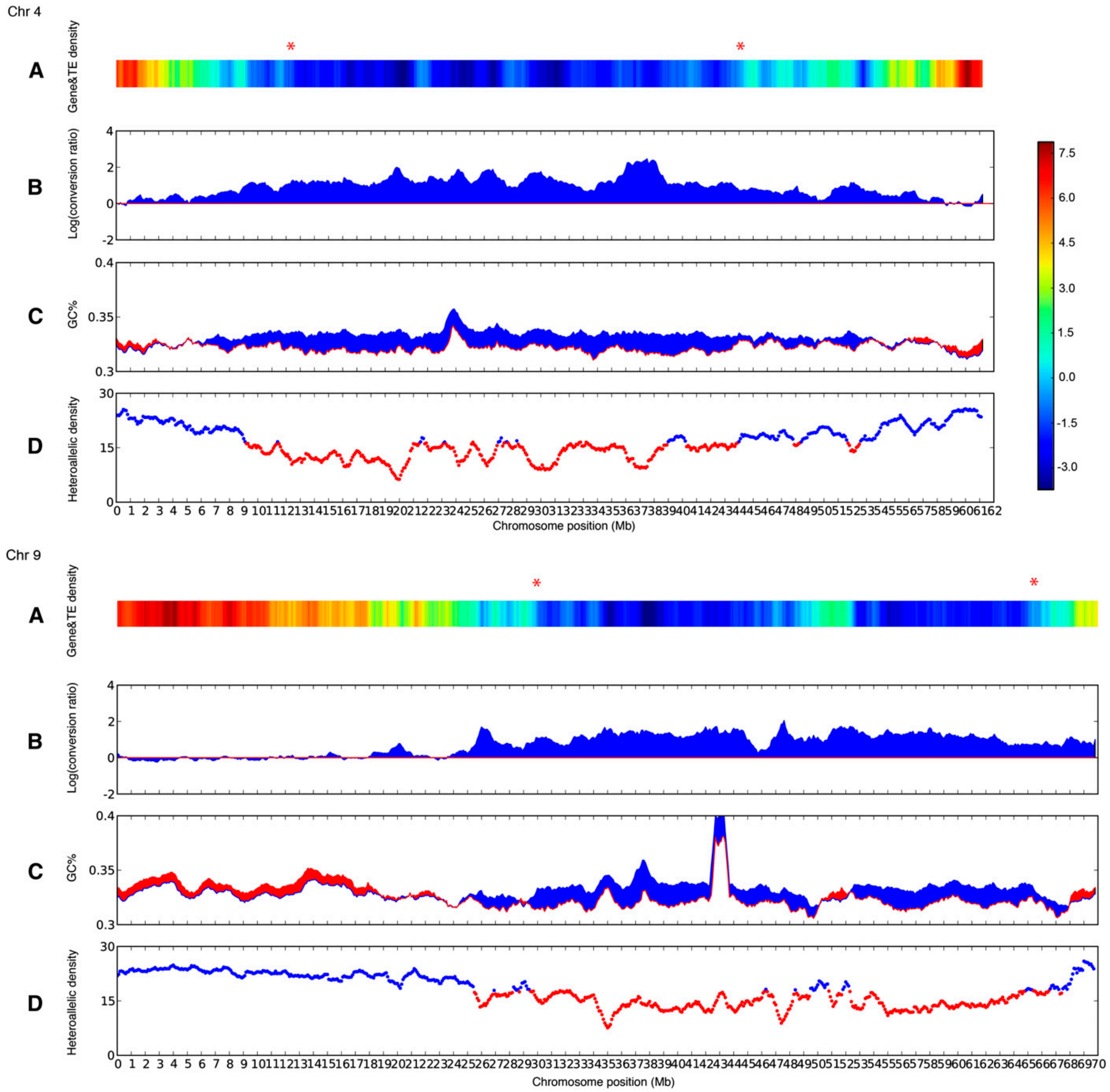

Figure 2 Distributions of biased conversion, heterozygosity, and GC content across chromosomes 4 and 9. Chromosomes are divided into 1-Mb bins using sliding windows with 100-kb step size. (A) Heatmap of gene and transposon density across chromosomes. Log ratio of *Z*-scores of gene *vs.* transposon density in each bin is shown, with blue marking transposon-rich and red marking gene-rich regions. Red asterisks mark inferred heterochromatin boundaries based on densities of genes and transposons. (B) Genomic distribution of log-likelihood ratio of A_t-to-D_t converted alleles to D_t-to-A_t converted alleles in tetraploid cotton, calculated for each bin. Red line denotes log-likelihood ratio of zero. (C) Comparison of GC% between cotton A- and D-genomes. Blue bins indicate GC% of D > A genome; other bins are red. (D) Genomic distribution of heteroallelic density (*i.e.*, with at least one allele differing from the other three) in tetraploid cotton (Acala 'Maxxa'). Heteroallelic density is calculated for each bin, corrected by read coverage, with below-average density indicated by red lines and average or higher density by blue lines. See Figure S3 for other chromosomes.

(GA120R1B3) from a different US production region (*i.e.*, separated by <200 years), a wild *G. hirsutum* (race yucatanese) separated by ∼4500 years, and *G. mustelinum*, the tetraploid cotton species most divergent from *G. hirsutum* separated by ∼1 million yr. If a converted allele is shared by two lineages, we assume that the event occurred in the common ancestor rather than independently in each lineage. Indeed, most conversions were present in all four genomes (85.03–87.54%; Figure 1B), outnumbering omnipresent random mutations by approximately eightfold (χ^2 = 23,599, 87,473 for D_t, A_t, $P<0.001$. Parsimony implies that these HeGCEs occurred prior to speciation in the nascent *Gossypium* polyploid.

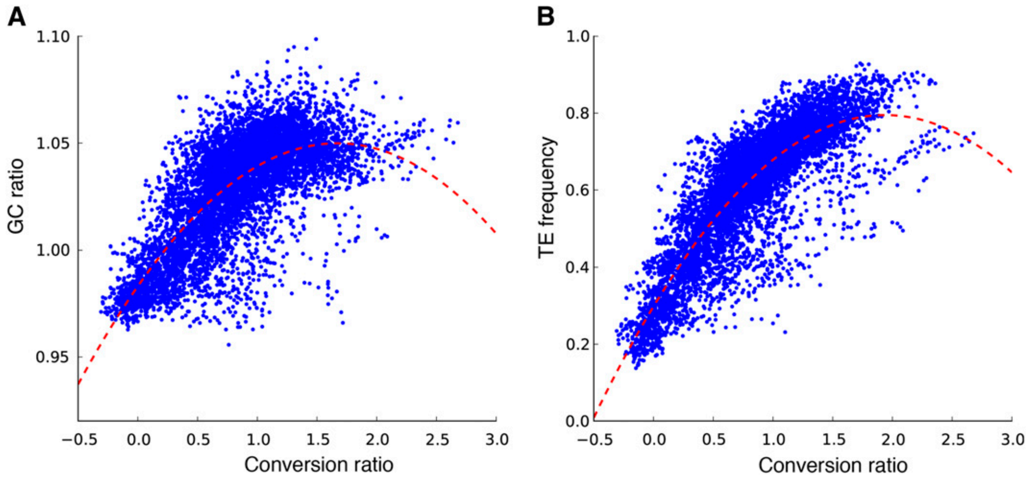

Figure 3 Genome-wide correlations between conversion, GC% ratio of D- to A-genome, and transposon frequency (per base pair), for 1-Mbp bins using 100-kbp sliding windows. Conversion ratio is measured by log-likelihood ratio following Figure 2B. GC% ratio and transposon density are calculated for each bin. (A) Correlation between conversion and GC% ratios (R^2 = 0.506). Data are best fitted with the quadratic function $y = -0.02392x^2 + 0.07992x + 0.9831$. (B) Correlation between conversion ratios and transposon frequency (R^2 = 0.684). Data are best fitted with the quadratic function $y = -0.1322x^2 + 0.5126x + 0.2981$.

When the polyploid *Gossypium* lineage diverged into multiple species, HeGCE appears to have been abating (Figure 1B). This is inferred based on the observation that HeGCEs account for only ~62% (4921) of polymorphisms among the divergent species *G. hirsutum* and *G. mustelinum*, ~47% between wild and cultivated forms of *G. hirsutum*, and 33% between *G. hirsutum* cultivars from different production regions (Figure 1B and Figure S1A). Consideration of the genomic distribution of HeGCEs in these various taxa suggests that it abates sooner in heterochromatin than in euchromatin (Figure S1B).

Asymmetric evolution of the polyploid cotton subgenomes

Despite inhabiting a common nucleus, the evolution of the cotton A_t and D_t subgenomes differs in several striking ways. First, A_t-to-D_t conversion is enriched in heterochromatin (Figure 2). Euchromatin, localized in the terminal regions of cotton chromosomes, shows largely similar rates of the two conversion types (log-likelihood ratio ~0). Occasional 1-Mb bins with D_t-to-A_t biased conversion (log-likelihood ratio <0) unanimously reside in euchromatin.

As in many other eukaryotes (Duret and Galtier 2009) cotton gene conversion is GC biased, and this bias is closely related to the divergent evolution of the A_t and D_t subgenomes. The D-genome has higher GC content than the A-genome in the heterochromatin where A_t-to-D_t conversion is enriched, and the A-genome has higher GC content than the D-genome in the euchromatin where D_t-to-A_t conversion is enriched (Figure 2). The high correlation of tetraploid cotton gene conversion with the GC ratio between the two progenitor genomes ($R^2 = 0.506$) (Figure 3A), may explain the 30% more A-to-G and T-to-C mutations in the D-genome than the A-genome since their divergence (Rong *et al.* 2012).

To investigate genomic features related to HeGCEs, we compared regions with highly (HC)- or little (LC)-biased conversion. Across the genome, the log-ratio of A_t-to-D_t *vs.* D_t-to-A_t conversions per 1-Mb bin averages 0.80 (SD = 0.46). Log ratios above 1.72 (mean + 2 SD) indicate largely A_t-to-D_t conversion (HC), and below −0.12 (mean − 2 SD) indicate D_t to A_t (LC). HC overwhelmingly locates in heterochromatin and LC in euchromatin. Heterochromatic A_t-to-D_t conversion is highly correlated with transposon distribution ($R^2 = 0.684$) (Figure 3B) and enriched for potential DNA methylation sites suitable for transposon silencing (Slotkin and Martienssen 2007). Long terminal repeat (LTR), particularly gypsy-type, retrotransposons are overwhelmingly enriched in HC [>70% of all types of transposable elements, and 10.02% of total length (in base pairs) of HC *vs.* 2.97% of LC] (Figure 4A). A,T-to-G,C conversion, providing potential DNA methylation sites, is more frequent in transposable element (TE) than non-TE regions across the genome (Figure 4B). Further, in HC, A,T-to-G,C significantly outnumber G,C-to-A,T conversions for A_t to D_t (50.04 *vs.* 34.69%), but not D_t to A_t (39.95 *vs.* 41.26%). In LC, A,T-to-G,C conversions are similar to G,C to A,T (43.97 *vs.* 38.13%) for A_t to D_t but differ for D_t to A_t (47.35 *vs.* 32.52%) (Table 1).

In heterochromatic regions where reciprocal DNA exchanges are rare, some conversions might simply be neutral or slightly deleterious relics not yet purged due to inefficient selection, as is true of retroelement insertions (Paterson *et al.* 2009) and other rearrangements (Bowers *et al.* 2005). Longer persistence of harmful mutations in HC than LC may explain an increasing ratio (in HC) of nonsynonymous to synonymous mutations across the phylogenetic tree of the four *Gossypium* species studied (Figure 4C). Indeed, the overall ratio of nonsynonymous to synonymous mutations is larger in heterochromatin than euchromatin (Fisher's exact test, P = 1.334e-6), and heterochromatin conversion tracks are longer than the genome-wide average (Figure S2). All detected conversion tracks in the genome and HC region are listed in Table S1.

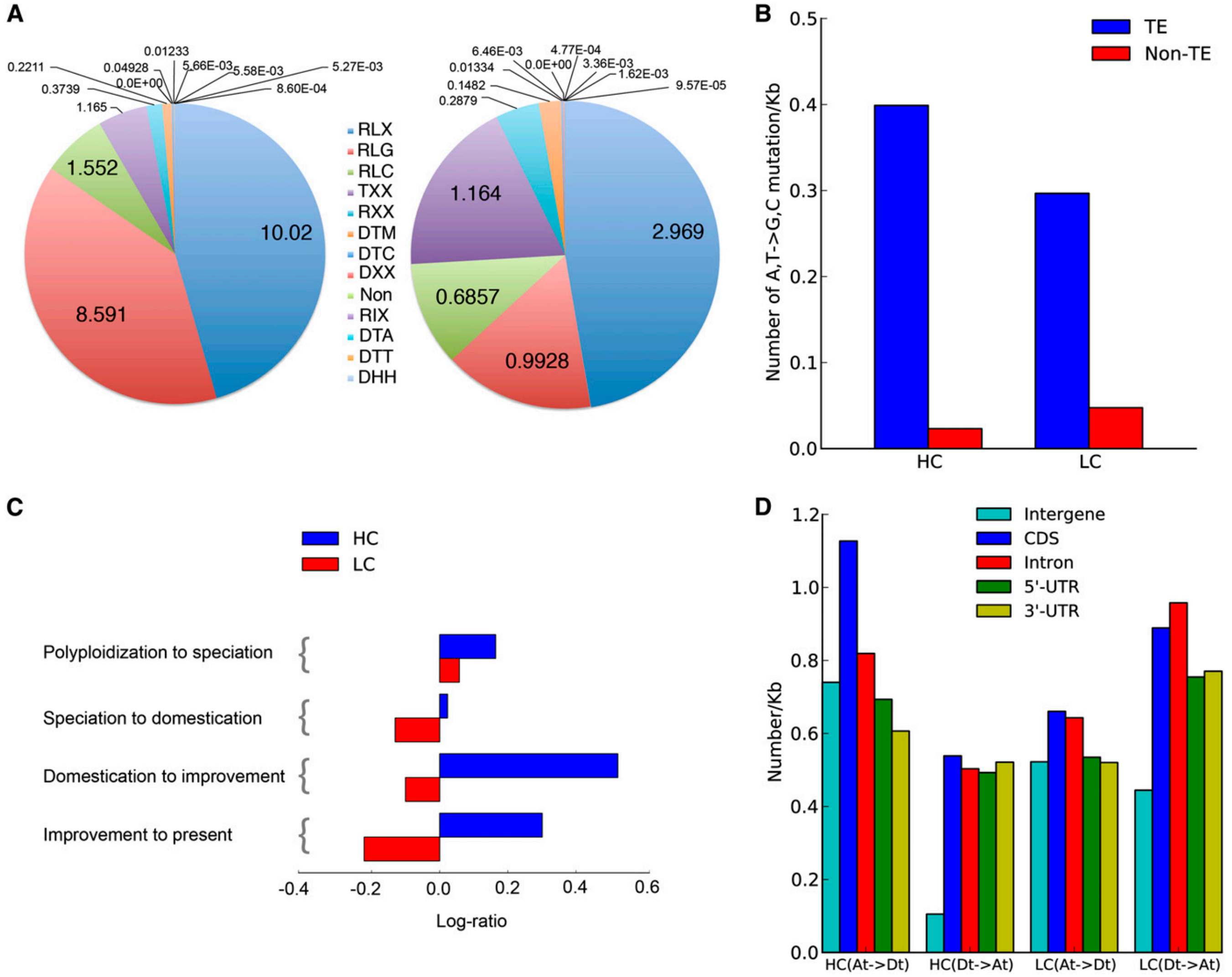

Figure 4 Intergenomic conversion of genes and transposons. (A) Proportion (% by total base pair length) of each transposon type in HC and LC. Transposon types are: RLX, LTR retrotransposon; RLG, Gypsy; TXX, transposon (either transposon or retrotransposon); RLC, Copia; RXX, retrotransposons; DTM, mutator; DTC, CACTA; DXX, DNA transposons; RIX, LINE; Non, others; DTA, hAT; DTT, Tc1-Mariner; DHH, Helitron. See Figure S4 for counts. (B) Proportion of A,T-to-G,C conversion in transposons in HC *vs.* LC. (C) Phylogenetic distribution of the log ratio of nonsynonymous to synonymous conversions in HC and LC. Conversions are more gene-enriched in HC than LC. See Figure S5 for raw data. (D) Density of converted alleles in each functional region.

Gene-altering conversions are of widespread importance

Strong evidence suggests gene-altering conversions to be of widespread functional importance, perhaps serving as an alternative to reciprocal DNA exchanges to form new allele combinations in heterochromatic regions. Heterochromatic D_t-to-A_t conversions are approximately five times more frequent in genes than adjacent intergenic DNA (Figure 4D; Fisher's exact test, $P = 0.0001$) and A_t-to-D_t conversions are also gene enriched ($P = 0.0157$). Conversions more frequently restored gene functions in cotton heterochromatin than euchromatin (Fisher's exact test, $P = 0.0008$). Among 59 HC and 206 LC alleles that experienced striking mutations in the A-genome since its divergence from the F-genome [using the D-genome as outgroup (16), including premature mutations (32 HC and 80 LC), splice site alterations (22 and 89), translation initiation alterations (3 and 19), and stop codon losses (2 and 18)], A_t-to-D_t conversion found in Acala 'Maxxa' restored function to 45.8% (27) of HC *vs.* only 22.3% (46) of LC SNVs. Recent evidence of widespread gene conversion in the centromere cores of maize (Shi *et al.* 2010) and *Arabidopsis* (Yang *et al.* 2012) further supports the importance of this mechanism.

Discussion

Whole genome comparison of four naturally occurring tetraploid cottons and representatives of their progenitor genomes reveals extensive DNA exchanges accumulated during the past 1–2 MY since polyploid formation. Remarkably, the two constituent "subgenomes" of tetraploid cotton

Table 1 Number and proportion (%) of conversion types in HC and LC

	HC ($A_t \rightarrow D_t$)	HC ($D_t \rightarrow A_t$)	LC ($A_t \rightarrow D_t$)	LC ($D_t \rightarrow A_t$)
A → G	6932 (19.63)	1047 (16.62)	2147 (15.90)	2908 (18.88)
T → C	6828 (19.33)	993 (15.76)	2249 (16.65)	3020 (19.61)
G → A	5067 (14.35)	989 (15.70)	2034 (15.06)	1843 (11.97)
C → T	5029 (14.24)	935 (14.84)	2091 (15.48)	1821 (11.82)
T → G	2011 (5.69)	244 (3.87)	772 (5.72)	675 (4.38)
A → C	1902 (5.39)	233 (3.70)	770 (5.70)	690 (4.48)
G → C	1771 (5.01)	407 (6.46)	822 (6.09)	927 (6.02)
C → G	1755 (4.97)	373 (5.92)	780 (5.78)	935 (6.07)
C → A	1091 (3.09)	352 (5.59)	483 (3.58)	656 (4.26)
G → T	1064 (3.01)	323 (5.13)	541 (4.01)	688 (4.47)
A → T	936 (2.65)	200 (3.18)	415 (3.07)	603 (3.92)
T → A	929 (2.63)	203 (3.22)	402 (2.98)	636 (4.13)

have experienced very different evolution while residing within a common nucleus, with more than twice as many conversions of A_t-to-D_t alleles than the reciprocal. The bias is unlikely to be caused by incomplete lineage sorting. Genetic diversity within both A- and D-genomes are estimated to be ~10 times less than the diversity between the two genomes (Van Deynze *et al.* 2009). Comparison of A_t and D_t subgenomes of tetraploid cotton to their corresponding diploid progenitors shows small and comparable sequence divergence (Cronn *et al.* 2002). *G. raimondii* is quite narrow genetically, so there would not be much lineage sorting on the D-side (Cronn *et al.* 2002). The A-genome is a little more heterogeneous when considering the two A-genome species (*G. arboreum* and *G. herbaceum*) (Cronn *et al.* 2002). Given these considerations and keeping in mind the 5–7 MY of divergence between the A- and D-genome diploids (Senchina *et al.* 2003), it seems improbable that there would be segregation for many common alleles.

A key advantage of the cotton system is that polyploids have survived in the wild for 1 MY or more, effectively ruling out the hypothesis that HeGCEs are a symptom of a dysfunctional genome destined for extinction. Extensive gene conversion in the centromere cores of maize and *Arabidopsis* has been described using mapping populations, in which only the first several generations are observed (Shi *et al.* 2010; Yang *et al.* 2012). On the contrary, some studies show that noncrossover gene conversion is relatively rare compared to crossover-associated gene conversion in *A. thaliana* hybrids (Lu *et al.* 2012; Wijnker *et al.* 2013). Cytogenetic studies of recently and naturally formed polyploid species, *Tragopogon miscellus* and resynthesized *B. napus* reveal a prolonged phase of genomic instability coupled with chromosome rearrangement and translocation (Xiong *et al.* 2011; Chester *et al.* 2012). However, in each of these systems and other synthetic or recent polyploids, it is hard to assert whether the sorts of rapid responses to polyploidy that are observed (chromosome rearrangement and translocation) are the beginnings of adaptation or symptoms of pending extinction.

Frequent conversion of mostly heterochromatic A_t alleles by GC-rich D_t DNA may have helped to silence abundant A-genome-derived retrotransposons, perhaps stabilizing the early polyploid. It seems intuitive that D-genome alleles, from the progenitor native to the New World habitat of the polyploid, may confer many adaptations that are lacking from the Old World A-genome. However, this explanation of enrichment for A_t-to-D_t conversions is not consistent with the strong heterochromatic bias observed for these conversions. The approximately two times larger physical size of the A- than the D-genomes is largely due to retrotransposons, mostly in heterochromatin (Paterson *et al.* 2012) and some having spread to the D_t genome following polyploidy (Zhao *et al.* 1998). Bursts of retrotransposition following hybridization (McClintock 1983; Fontdevila 2005) can cause many DSBs (Gasior *et al.* 2006) that are fatal to cells if not repaired (Van Gent *et al.* 2001). More quickly than random mutations, GC-biased gene conversion may have provided the A_t genome with (D_t derived) targets for DNA methylation-based transposon silencing. Transcripts from A_t-derived retrotransposons may enter the RNAi pathway for RNA-dependent DNA methylation (Slotkin *et al.* 2009; Groszmann *et al.* 2011). An existing example similar to this process is the *Drosophila* P cytotype female that produces a repressor protein and piRNA to inhibit *P*-element transposition in gametes (Brennecke *et al.* 2008). Correlation between DNA methylation and gene conversion implies that the two are mechanistically related (Colot *et al.* 1996) by an as-yet-unknown process. In primates, biased gene conversion is shown as a major force for stabilizing constitutively methylated CpG islands (Cohen *et al.* 2011).

Meiotic recombination tends to be concentrated in small regions on chromosomes known as "recombination hotspots." Recombination is initiated by introduction of double-strand breaks to the hotspot alleles. When a DSB occurs at a hotspot that is heterozygous with an inactive ("cold") hotspot allele, the hotspot alleles are replaced with cold alleles by gene conversion during DNA repair. The process will cause a rapid loss of the recombination hotspot in the genome. The existence of recombination hotspots is therefore considered a "hotspot-conversion paradox" (Boulton *et al.* 1997). The paradox predicts small numbers of hotspots in the region with a high conversion rate. Consistent with this prediction, the high rate of conversion in the heterochromatin region identified in this study might be partially explained by the paradox.

The ability to "copy" existing alleles via HeCGEs may expedite the ability of polyploids to evolve new or more exaggerated phenotypes, by achieving allele dosages that exceed those of their progenitors. For example, D_t-to-A_t conversion steadily declines following polyploid formation, but accelerates during cotton domestication and improvement (Figure 1B and Figure S1). This may provide a mechanism to explain the long-standing irony that so many QTL for yield and quality of tetraploid cotton map to chromosomes derived from an ancestor (D) that lacks spinnable fibers (Jiang *et al.* 1998). This genetic process may complement paramutations that copy epigenetic information. In *Arabidopsis* hybrids, for example, methylation levels of one parental allele change to match the other (Greaves *et al.* 2011). Our findings show that nonreciprocal exchange of both genetic and epigenetic information may be important to the integrity of hybrid genomes.

HeCGEs may have practical value in crop improvement. The length of cotton conversion tracts averages 455 bp, ranging from 279 to 3650 bp (see *Methods*), somewhat longer than in mammals (Chen *et al.* 2007) and often spanning entire exons or occasional genes. Cotton and many other neopolyploid crops are genetically depauperate and occasional crosses with exotic or synthetic polyploids for crop improvement may be prone to bursts of transposition, DSBs, and conversion. A fascinating area for further study is whether occasional "successes" in extracting valuable alleles from exotic germplasm (*e.g.*, Campbell *et al.* 2011) might have occurred by gene conversion rather than crossing over. Introgression by gene conversion might solve the widespread challenge of extracting desirable alleles from exotic germplasm while leaving behind nearby undesirable ones, *i.e.*, with the precision of transformation but by a natural noninvasive means.

While their early evolution involved extensive intergenomic exchange, modern tetraploid cottons show strict disomy (Kimber 1961). The gradual decline of intergenomic conversion during the course of polyploid cotton evolution (Figure 1B) may have been due in part to fewer DSBs as the nascent polyploid was stabilized, perhaps by silencing of abundant A-genome-derived retrotransposons. The evolutionary success of a newly formed polyploid may require a delicate balance between genomic-stress induced novel variation (McClintock 1983) and stabilization via such quantitative factors as we suggest or qualitative factors such as the *ph1* locus enforcing pairing specificity of wheat (Griffiths *et al.* 2006).

Data Access

The *G. raimondii* genome sequence is in the National Center for Biotechnology Information (NCBI) with BioProject accession PRJNA171262. Other sequences are available at the NCBI short read archive for *G. herbaceum* (accession F1-1, SRA061660), *G. hirsutum* (accession GA120R1B3, SRA068148), *G. hirsutum* (race yucatanese, SRA068479), and *G. mustelinum* (SRA068485).

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GENETICS

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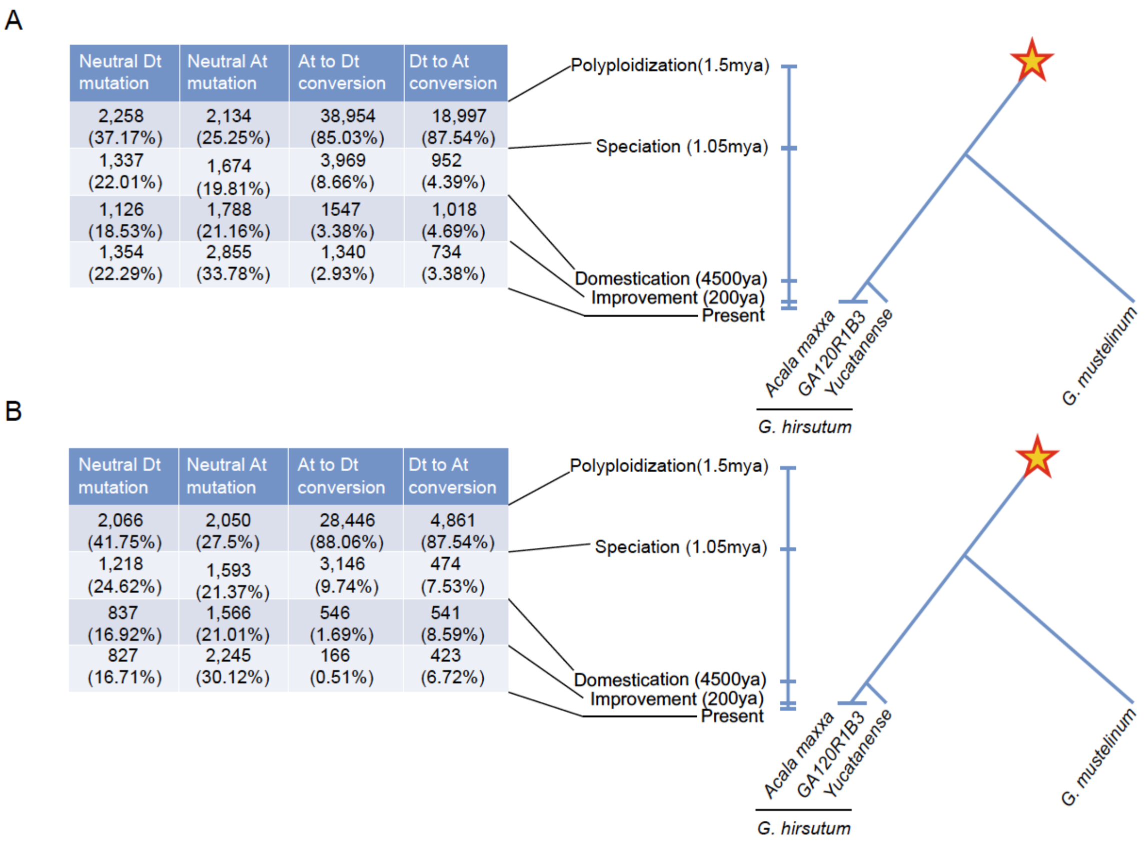
Extensive and Biased Intergenomic Nonreciprocal DNA Exchanges Shaped a Nascent Polyploid Genome, *Gossypium* (Cotton)

Hui Guo, Xiyin Wang, Heidrun Gundlach, Klaus F. X. Mayer, Daniel G. Peterson, Brian E. Scheffler, Peng W. Chee, and Andrew H. Paterson

DOI: 10.1534/genetics.114.166124



A

Neutral Dt mutation	Neutral At mutation	At to Dt conversion	Dt to At conversion
2,258 (37.17%)	2,134 (25.25%)	38,954 (85.03%)	18,997 (87.54%)
1,337 (22.01%)	1,674 (19.81%)	3,969 (8.66%)	952 (4.39%)
1,126 (18.53%)	1,788 (21.16%)	1547 (3.38%)	1,018 (4.69%)
1,354 (22.29%)	2,855 (33.78%)	1,340 (2.93%)	734 (3.38%)

B

Neutral Dt mutation	Neutral At mutation	At to Dt conversion	Dt to At conversion
2,066 (41.75%)	2,050 (27.5%)	28,446 (88.06%)	4,861 (87.54%)
1,218 (24.62%)	1,593 (21.37%)	3,146 (9.74%)	474 (7.53%)
837 (16.92%)	1,566 (21.01%)	546 (1.69%)	541 (8.59%)
827 (16.71%)	2,245 (30.12%)	166 (0.51%)	423 (6.72%)

Figure S1 Timing of inter-genomic conversion. The star marks AD allopolyploidization occurring ~1-2 million years ago. (A) The numbers (proportion) of At, Dt neutral mutations (in intergenic region) and At to Dt conversions in both HC and LC are shown in each evolutionary time scale. (B) The numbers (proportion) of At, Dt neutral mutations (in intergenic region) and At to Dt conversions in HC are shown in each evolutionary time scale.

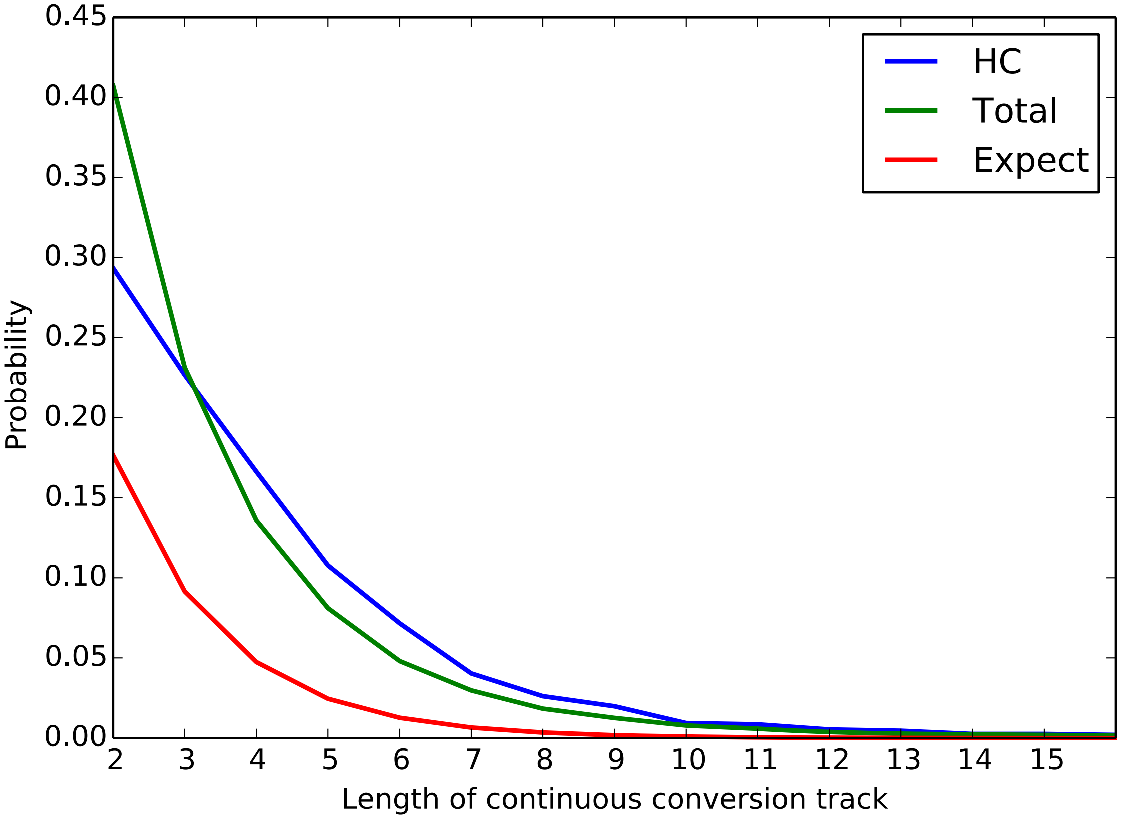
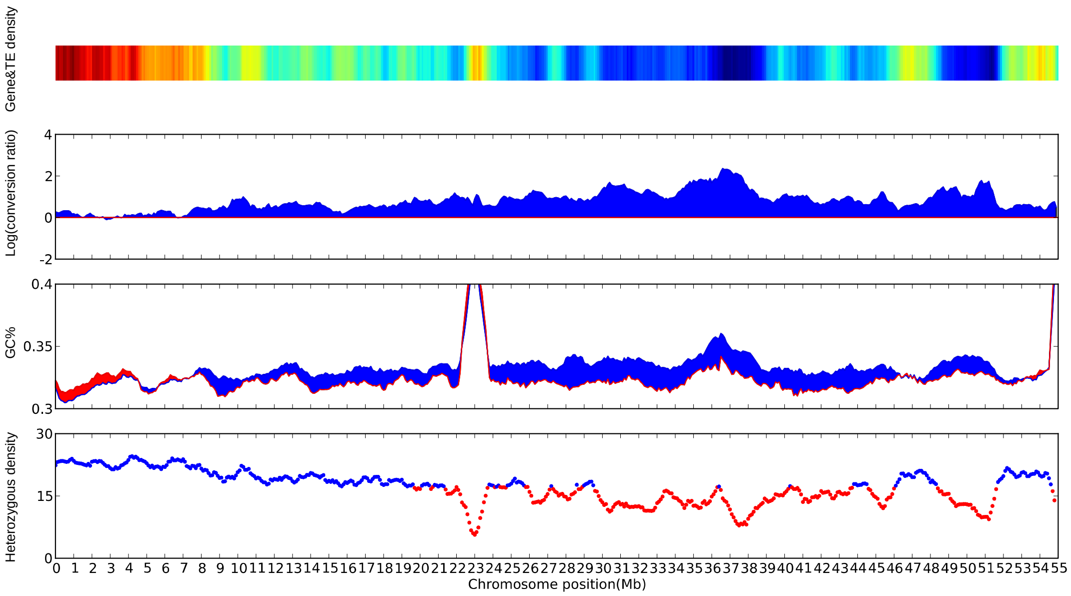
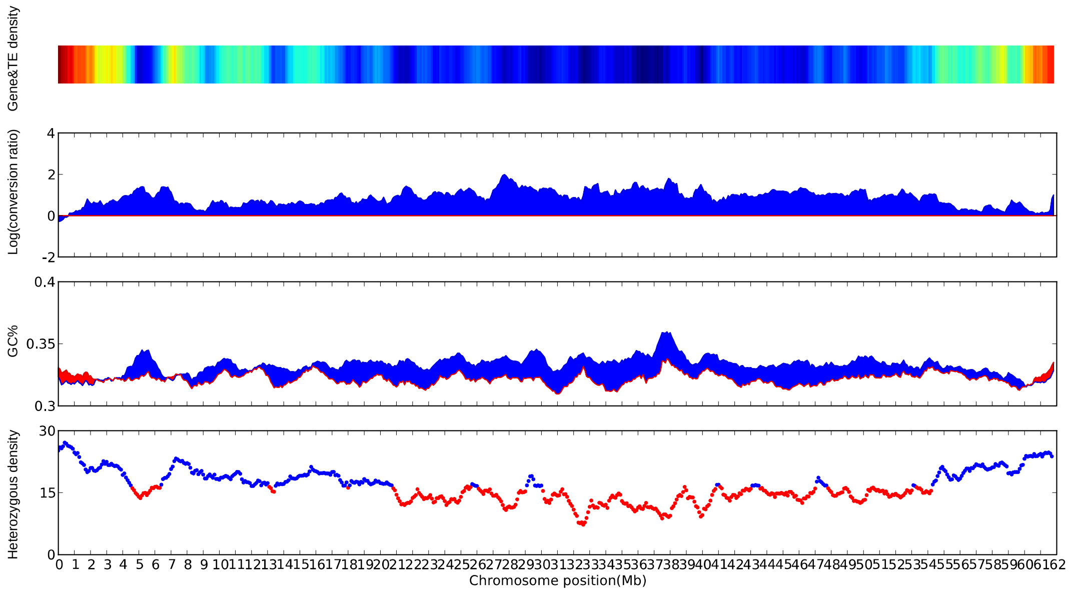


Figure S2 Frequency distribution of the length of conversion tracks measured by number of continuous converted alleles. Red line is expected distribution (exponential) of the length of continuous random mutations. Green line is the distribution of total number of conversions in the genome. Blue line is the distribution of conversions in HC.

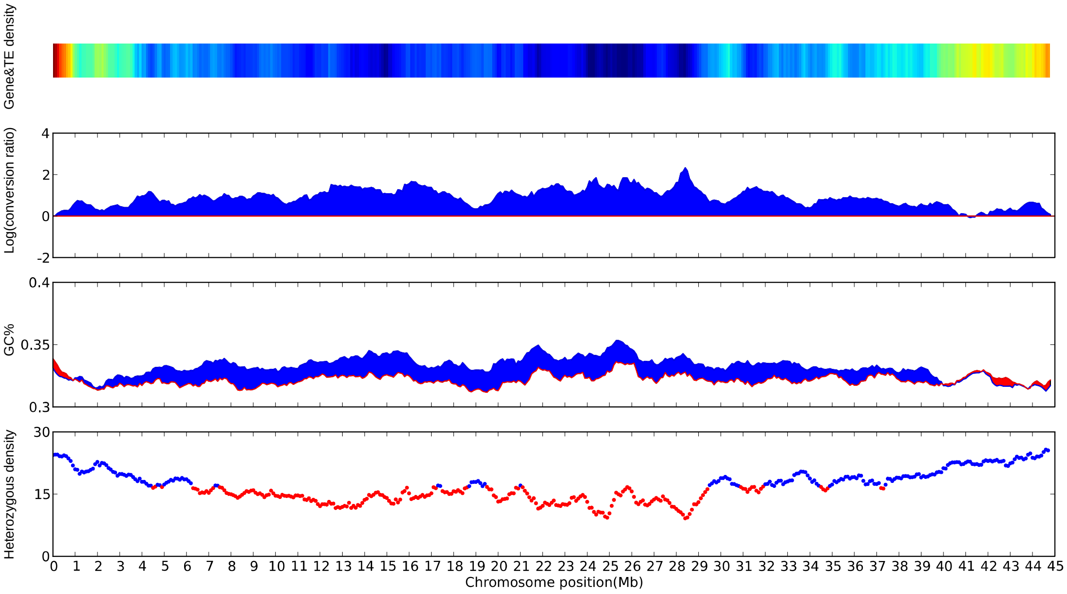
Chr1


Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55
Chromosome position(Mb)

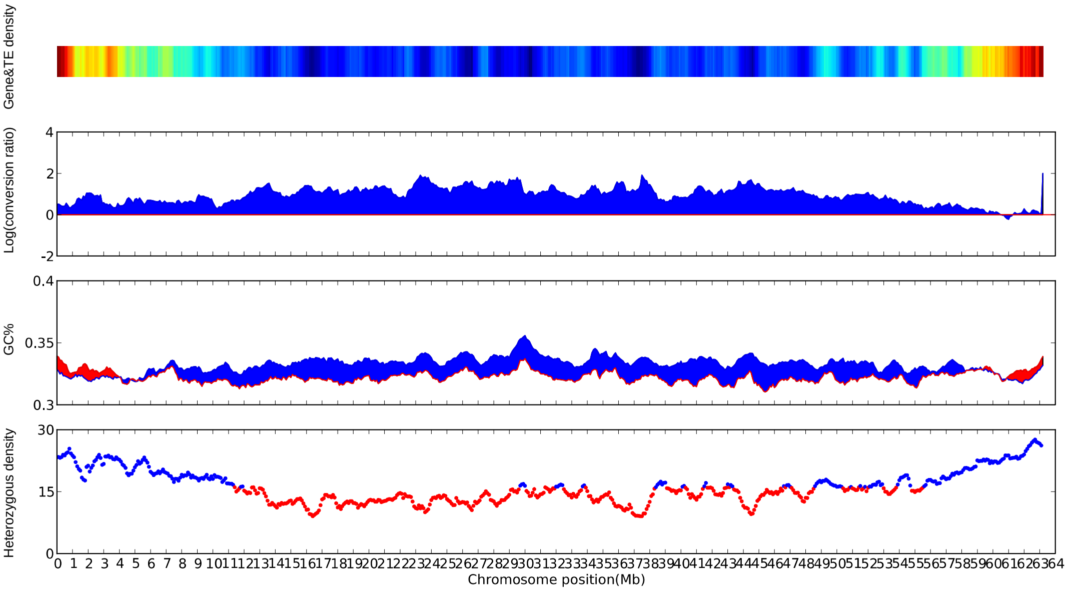
Chr2

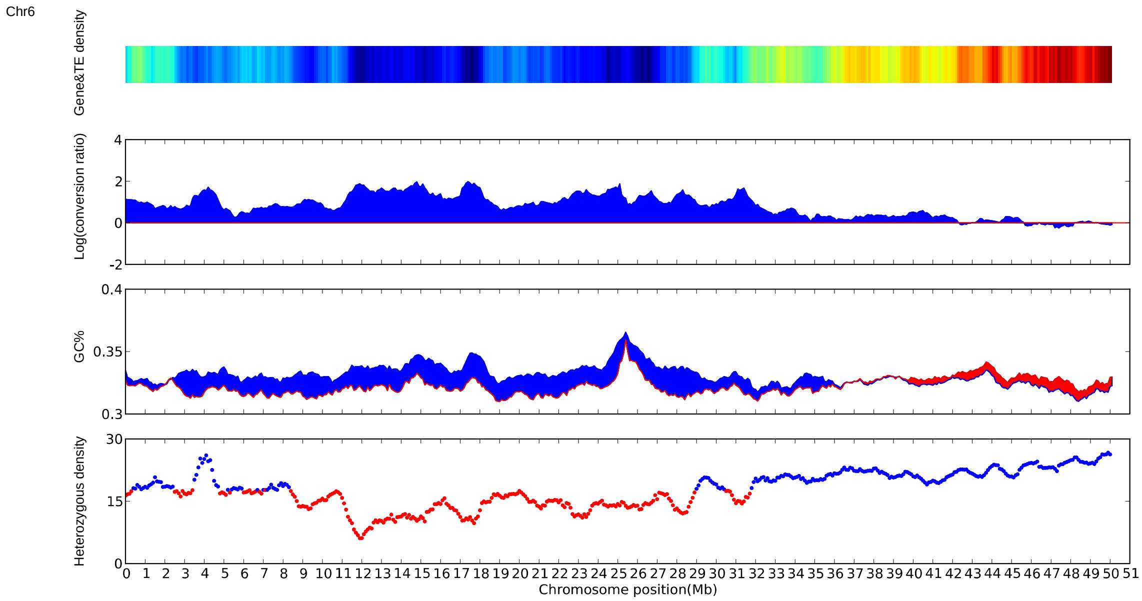

Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62
Chromosome position(Mb)

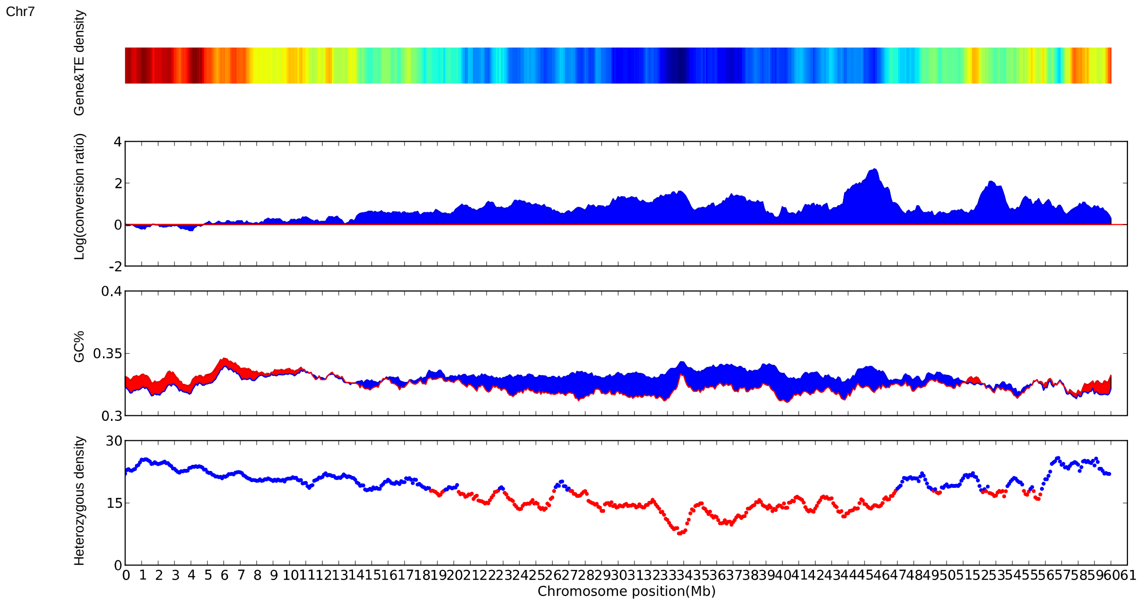
Chr3

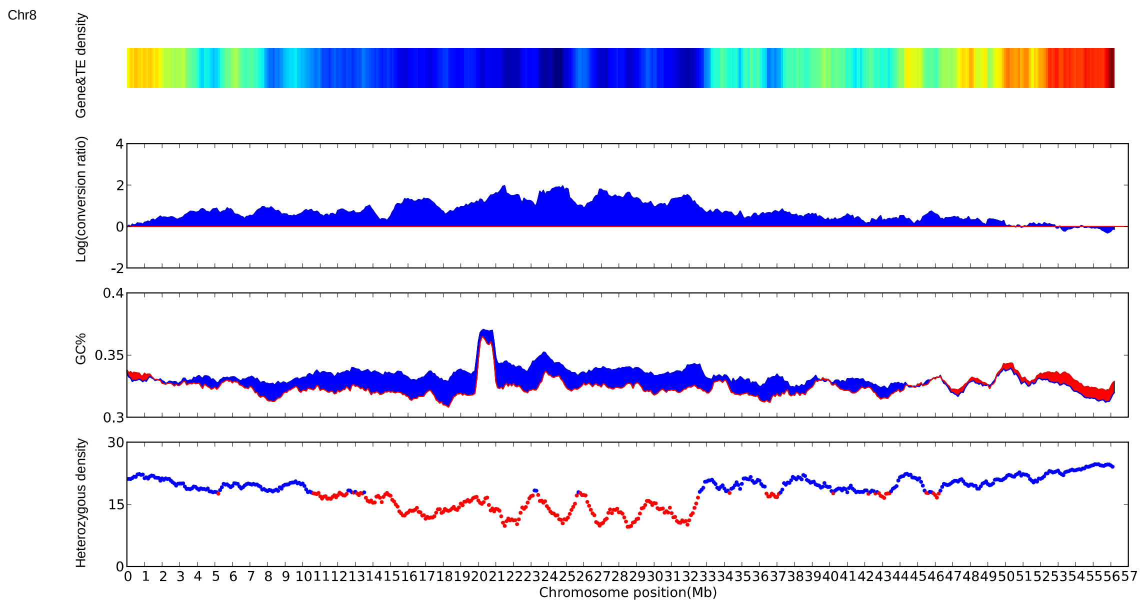

Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Chromosome position(Mb)

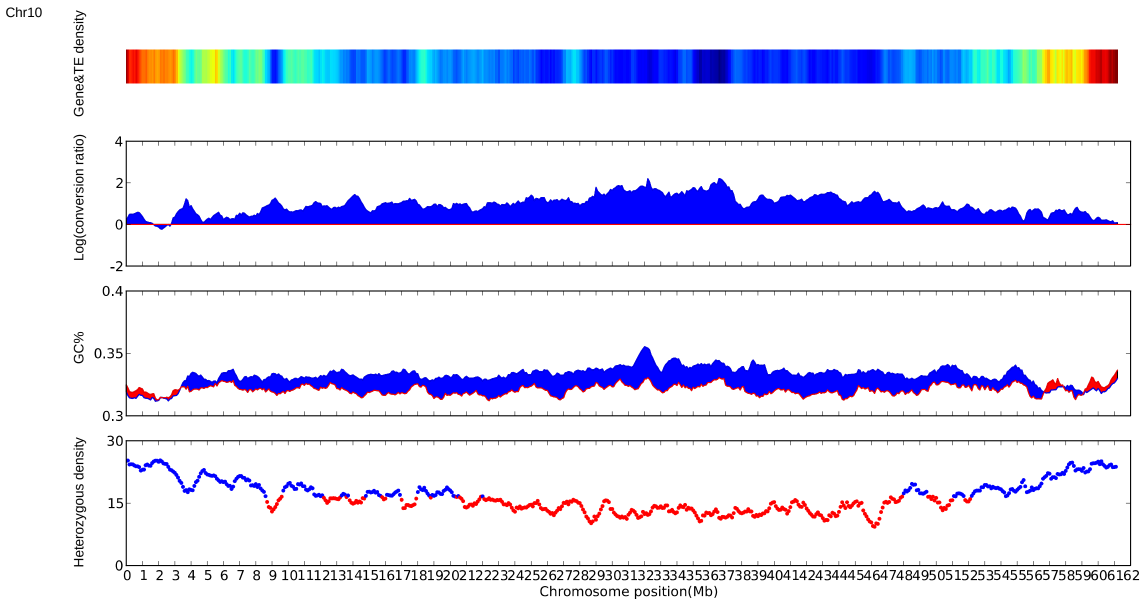
Chr5


Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10111213141516171819202122232425262728293031323334353637383940414243444546474849505152535455565758596061626364
Chromosome position(Mb)

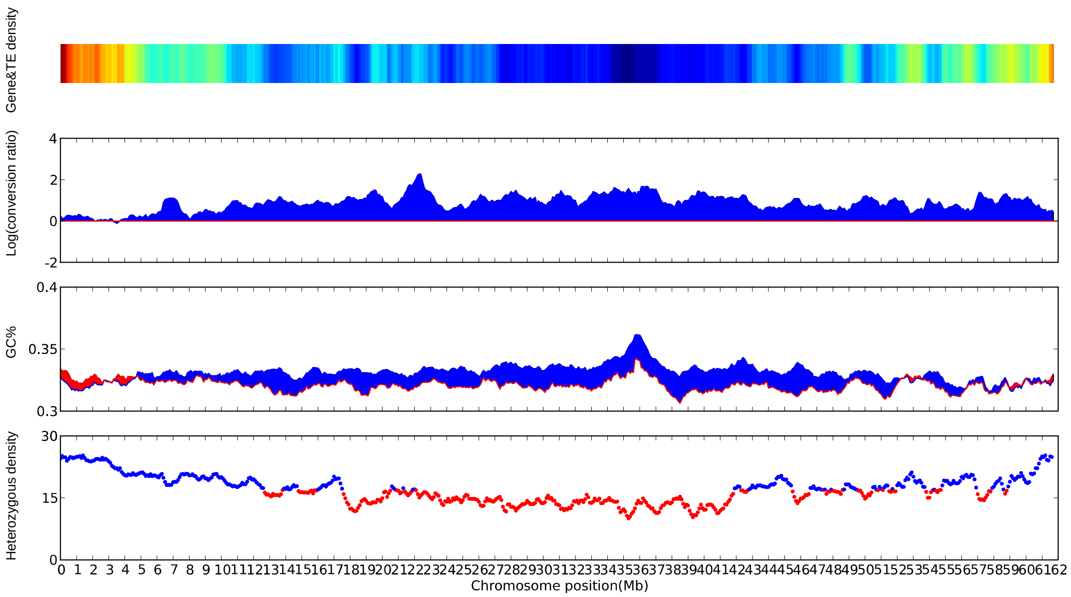

Chr6
Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51
Chromosome position(Mb)


Chr7
Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61
Chromosome position(Mb)

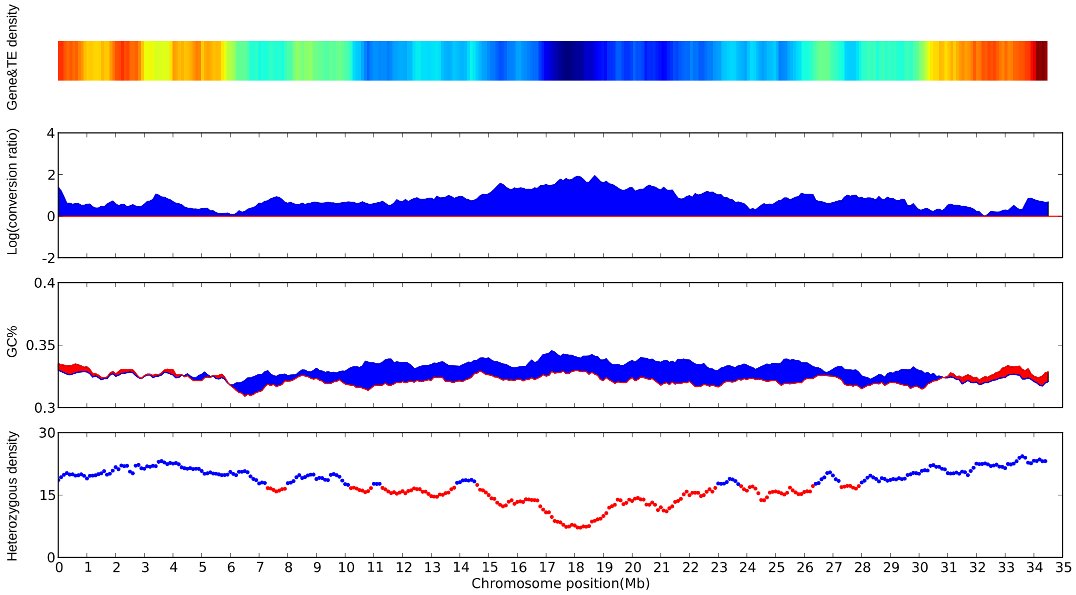

Chr8
Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57
Chromosome position(Mb)


Chr10
Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62
Chromosome position(Mb)

Chr11


Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62
Chromosome position(Mb)

Chr12


Gene&TE density
Log(conversion ratio)
4
2
0
-2
GC%
0.4
0.35
0.3
Heterozygous density
30
15
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Chromosome position(Mb)

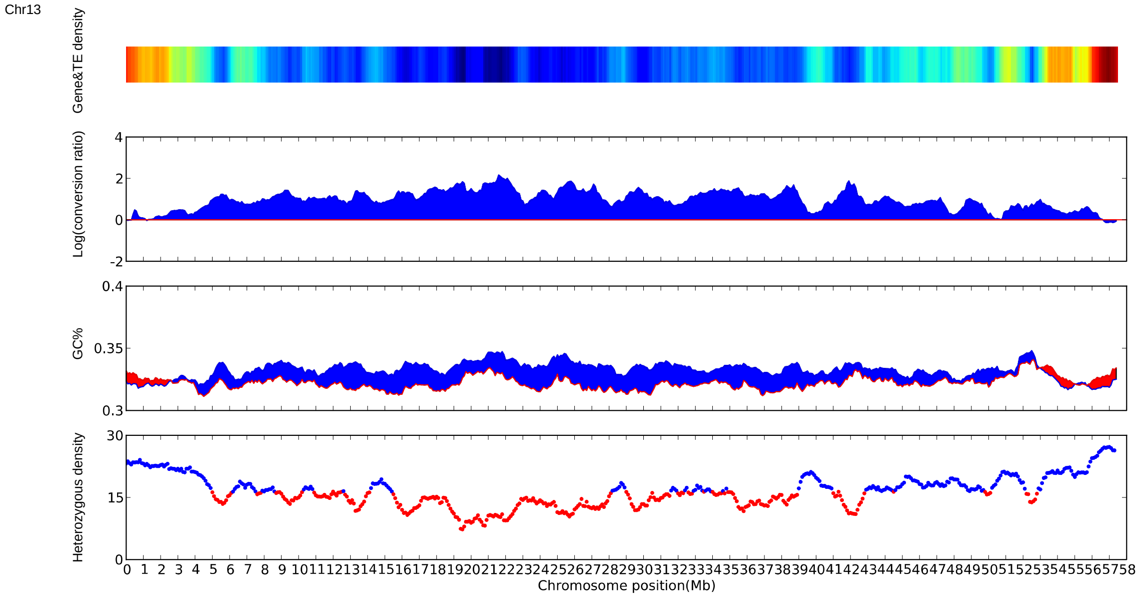


Figure S3 Genome distribution of biased conversion, heterozygosity and GC-content. See Fig. 2 for detailed legend.

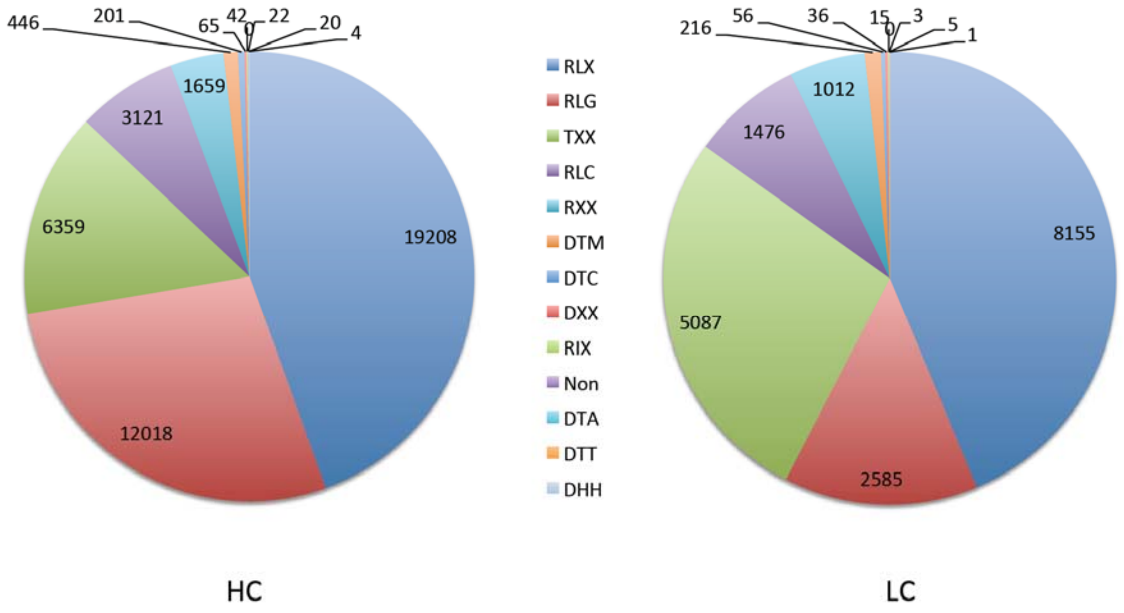


Figure S4 Number of each transposon types in HC and LC. Transposon types are: RLX, LTR-retrotransposon; RLG, Gypsy; TXX, transposon (either transposon or retrotransposon); RLC, Copia; RXX, retrotransposons; DTM, mutator; DTC, CACTA; DXX, DNA transposons; RIX, LINE; Non, others; DTA, hAT; DTT, Tc1-Mariner; DHH, Helitron.

	HC		LC	
	At -> Dt	Dt -> At	At -> Dt	Dt -> At
Polyploidization to speciation	735/472	338/263	1473/1116	1779/1733
Speciation to domestication	48/48	10/7	37/47	37/53
Domestication to improvement	12/1	8/5	11/17	12/12
Improvement to present	4/2	0/0	2/2	4/8

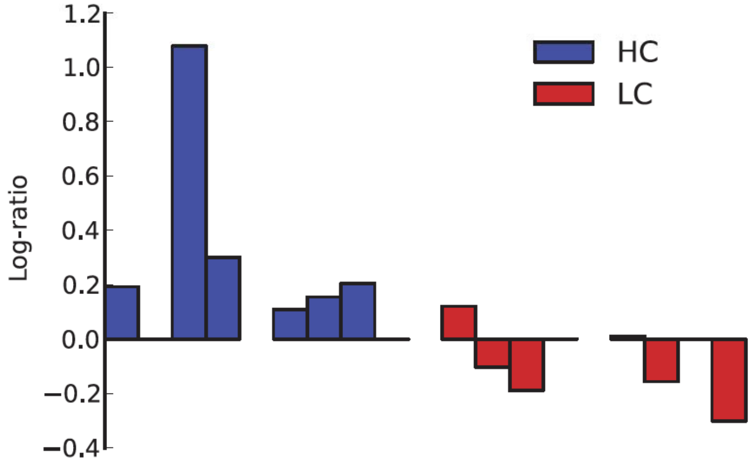


Figure S5 Phylogenetic distribution of the ratio of non-synonymous to synonymous conversions. Barplot shows logarithm of the ratio for each cell in the above table.

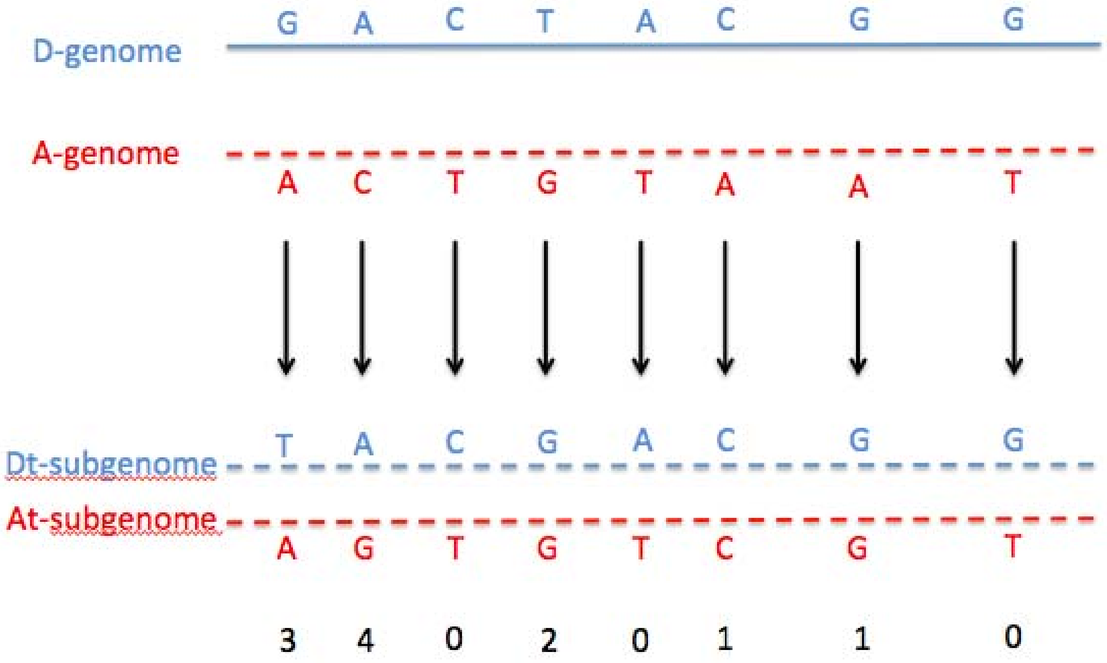
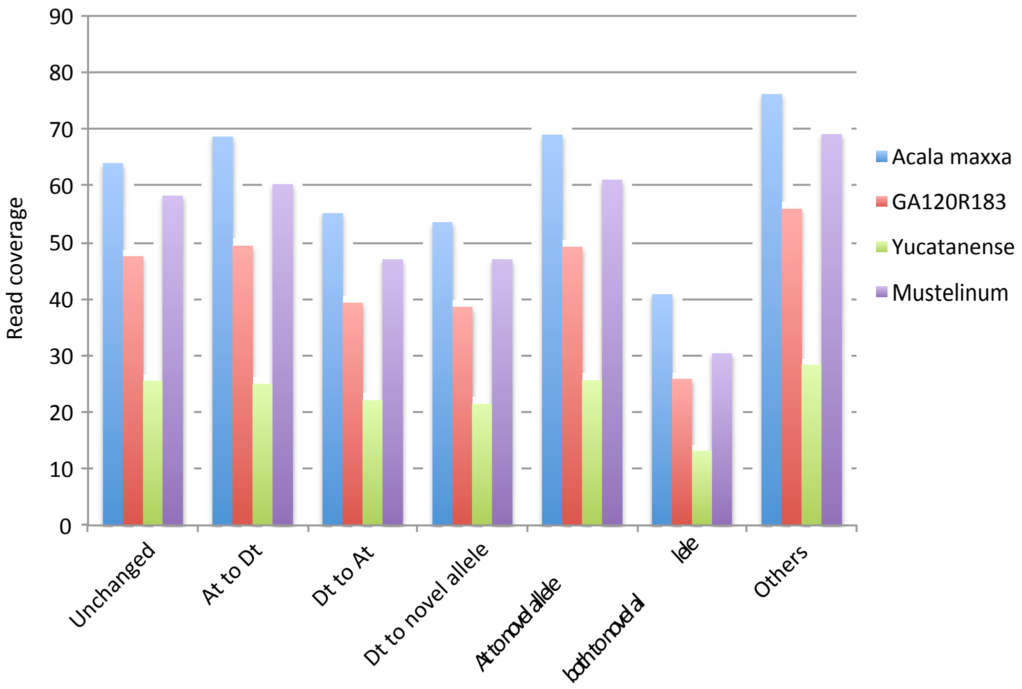
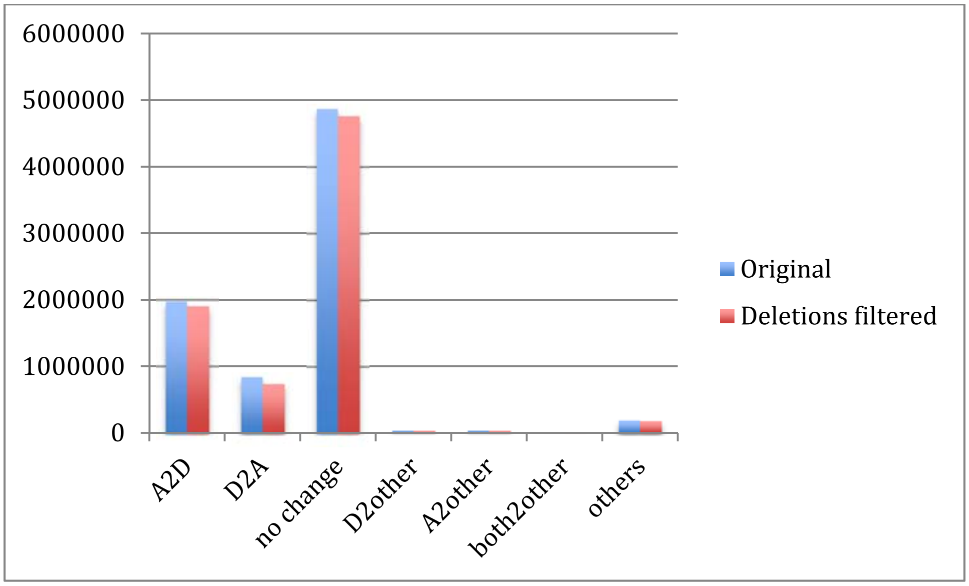


Figure S6 Identification of converted alleles. A-genome and D-genome are diploid progenitor genomes. At- and Dt-subgenome are tetraploid genomes with At-genome derived from A-genome and Dt-genome derived from D-genome. Solid line represents cotton reference genome and broken line indicates re-sequenced genomes. Sites with number “0” represents no allele changes in the tetraploid genomes, likewise, “1”: At to Dt conversion; “2”: Dt to At conversion; “3”: Dt mutation; “4”: At mutation.

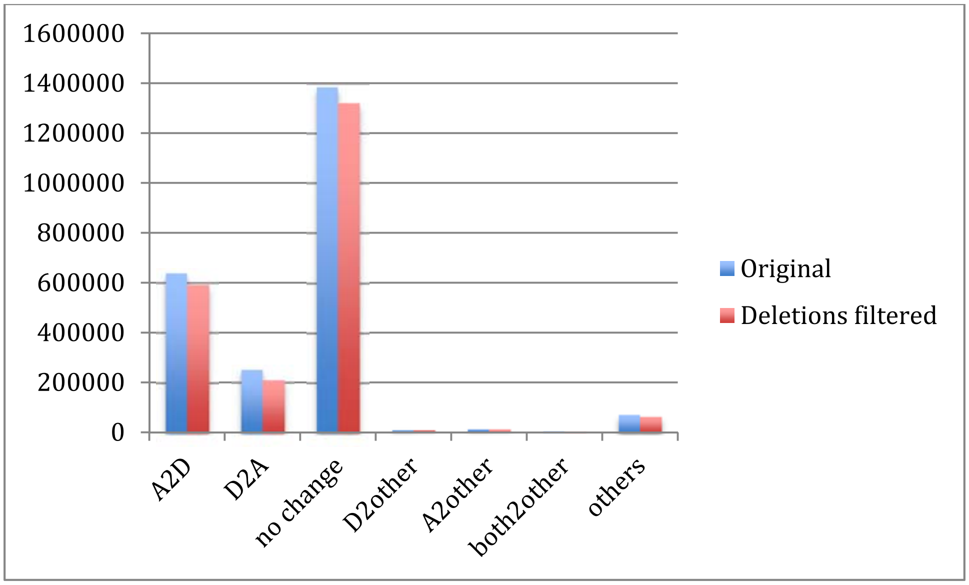
A


90
80
70
60
50
40
30
20
10
0
Read coverage
Unchanged
At to Dt
Dt to At
Dt to novel allele
At to novel allele
both to novel al
lele
Others
Acala maxxa
GA120R183
Yucatanense
Mustelinum

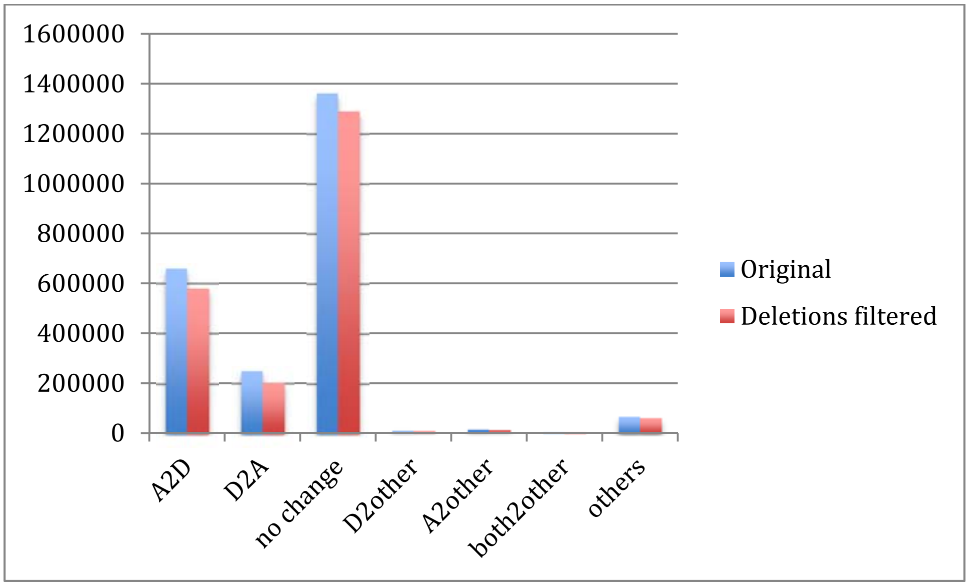
B


6000000
5000000
4000000
3000000
2000000
1000000
0
A2D
D2A
no change
D2other
A2other
both2other
others
Original
Deletions filtered

C


1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
A2D
D2A
no change
D2other
A2other
both2other
others
🟦 Original
🟥 Deletions filtered

D


1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
A2D
D2A
no change
D2other
A2other
both2other
others
🟦 Original
🟥 Deletions filtered

E

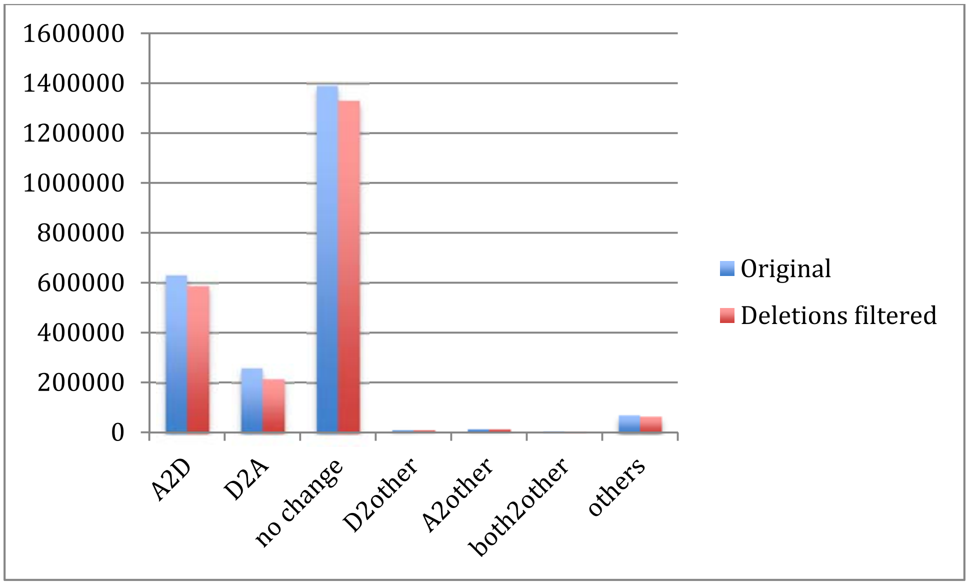


Figure S7 (A) Average mapped read coverage for different mutation categories in four cotton species. (B-E) Effects of deletion. Blue bar shows number of sites in each mutation category. Red bar shows number of sites after removal of the ones with reads coverage less than or equal to half of the average read coverage of each chromosome. (B) *Acala Maxxa*, (C) *GA120R183*, (D) *Yucatanense*, (E) *G. mustelinum*

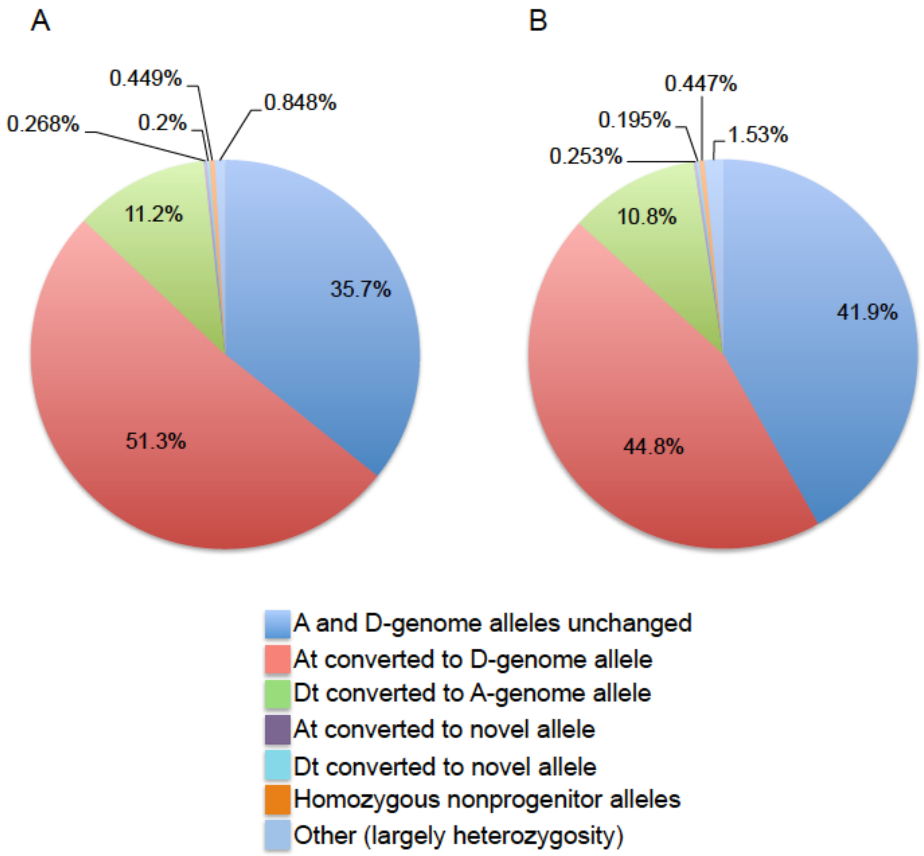


Figure S8 Allelic changes in polyploidy cotton using relaxed read editing distance (0.8). (A) Allelic changes with all other parameters unchanged. (B) Allelic changes with reduced frequency threshold to call heterozygous genotype.

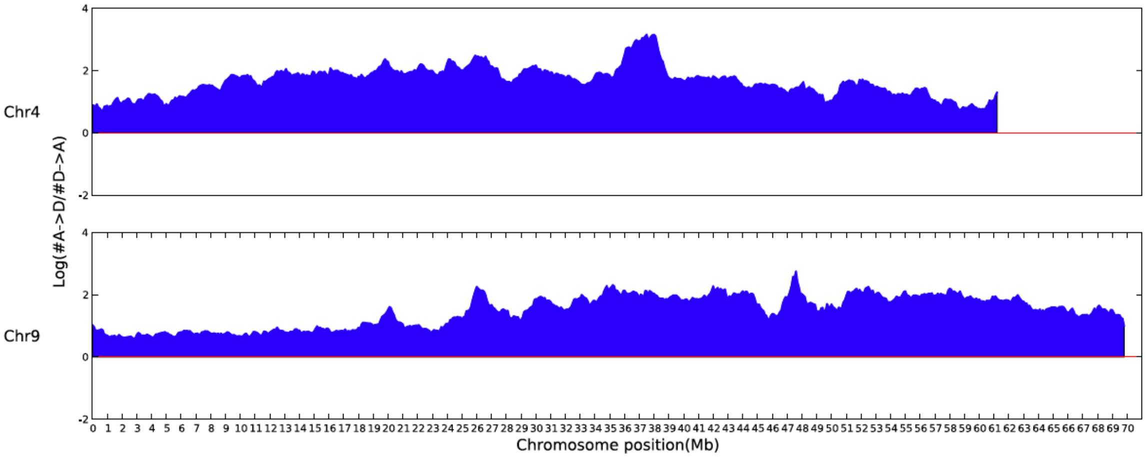
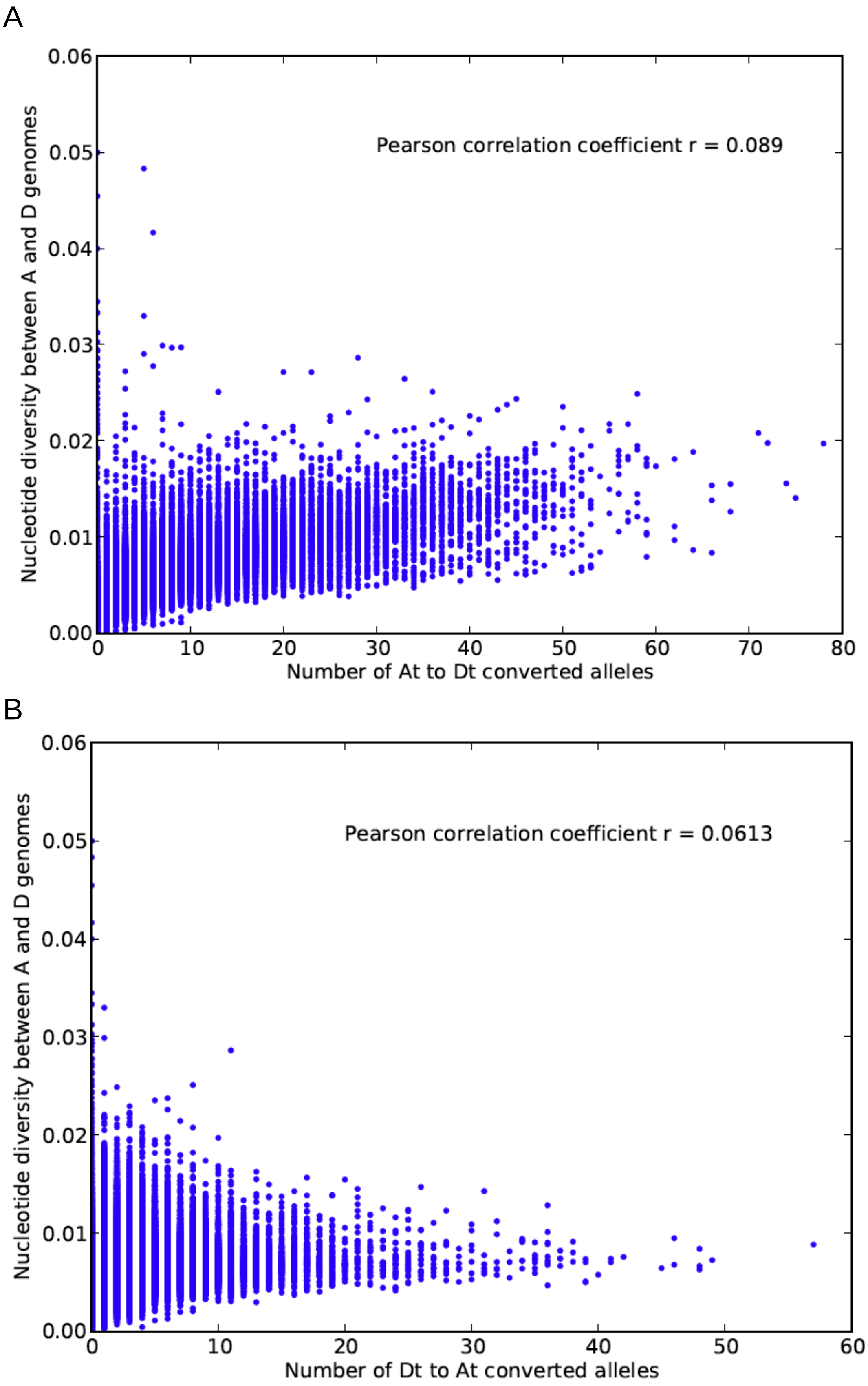


Figure S9 Genome distribution of conversion bias using relaxed read editing distance (0.8).


A
Pearson correlation coefficient r = 0.089
Nucleotide diversity between A and D genomes
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0
10
20
30
40
50
60
70
80
Number of At to Dt converted alleles
B
Pearson correlation coefficient r = 0.0613
Nucleotide diversity between A and D genomes
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0
10
20
30
40
50
60
Number of Dt to At converted alleles

C

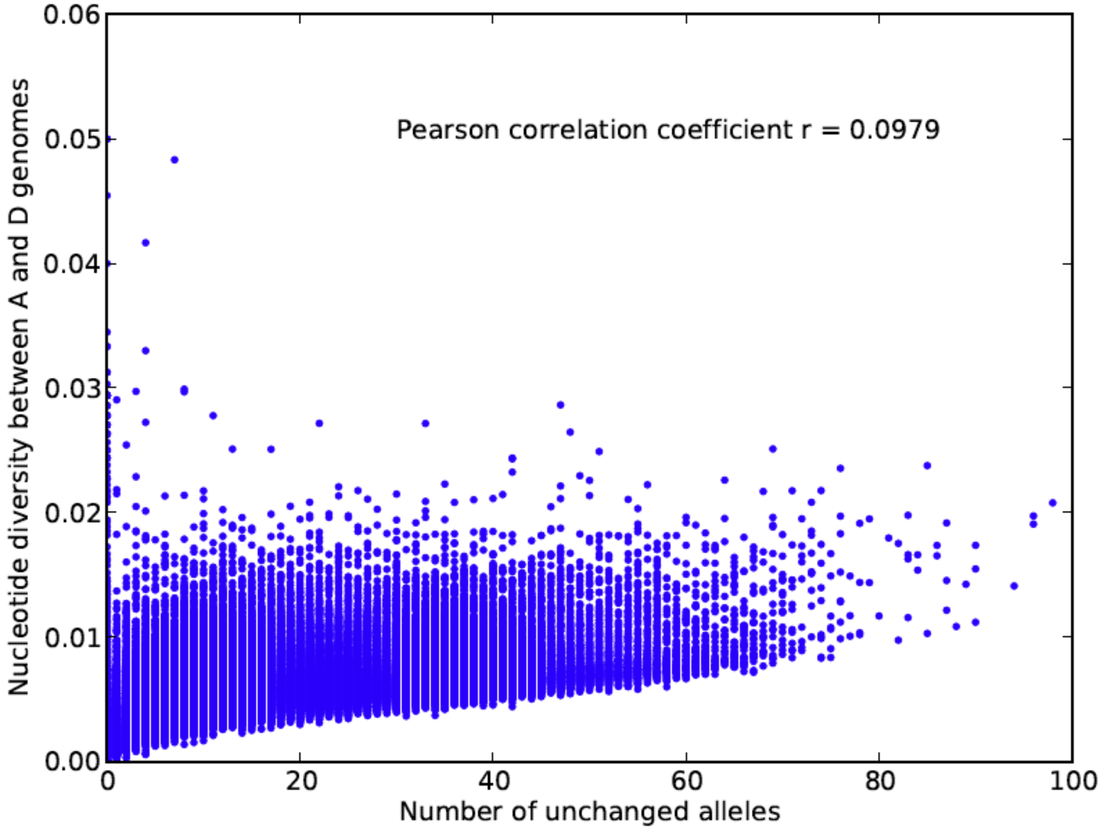


Figure S10 Correlation between number of allele changes and nucleotide divergence between A and D genomes. Genome is divided into non-overlap 10Kb bins. For each bins, the number of At to Dt converted alleles and the nucleotide divergence between the A and D genomes are calculated. (A) The number of At to Dt conversions and nucleotide divergence between A and D genomes. (B) The number of Dt to At conversions and nucleotide divergence between A and D genomes. (C) The number of unchanged alleles and nucleotide divergence between A and D genomes.

Table S1 is available for download as an Excel file at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.166124/-/DC1.