Identification of a genome-specific repetitive element in the *Gossypium* D genome

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Abstract: The Activity of genome-specific repetitive sequence is the main cause of the genome variation between *Gossypium* A and D genomes. Through the comparative analysis of the two genomes, we got a repetitive element (*ICRd* motif), which repeats massively in the diploid *Gossypium raimondii* (D_5) genome while almost absent in the diploid *Gossypium arboreum* (A_2) genome. We further explored the existence of *ICRd* motif in *G. raimondii*, *G. arboreum*, and two tetraploids (AADD) cotton *G. hirsutum* and *G. barbadense* by fluorescence *in situ* hybridization (FISH), and observed the *ICRd* motif exists in D_5 and D-subgenomes but not in A_2 and A-subgenome. The *ICRd* motif was investigated through its two constituents, a length variable tandem repeat region (TR) and a conservative sequence (CS), which highly repeat and evenly distribute in chromosomes of D_5 genome. The *ICRd* motif was revealed as the common conservative region of ancient LTR-TEs. The identifications and investigation of the *ICRd* motif promote the study on the A and D genome differences, facilitate the research on the *Gossypium* genome evolution, and provide assistance to subgenome identification and genome assembling.

Keywords: *Gossypium*; D genome; Repetitive element; Genome-specific; Fluorescence *in situ* hybridization (FISH); Evolution

1. Introduction

Repetitive DNA sequences are common in eukaryotic genomes, account for a huge fraction of the host genome (Ibarra-Laclette et al., 2013) and are highly correlated with the size of the host genome (Feschotte, 2008). Repetitive DNA can be divided into two major groups by their structures: tandem repeats and interspersed repeats (Jurka et al., 2005). The tandem repeats are known as simple repeat sequences (SSR), including micro-satellites, mini-satellites, and satellites (M.Lesk, 2002; Singh, 2015). The Interspersed repeats also were called as transposable elements (TEs) or transposons.

After the first report of the TEs in maize (McCLINTOCK, 1950; Brink & Williams, 1973; Goldschmidt, 2002), TEs were identified in many eukaryotic species (Munoz-Lopez & Garcia-Perez, 2010). There are thousands of different TE families in plants, which display the extreme diversity (Sanmiguel & Bennetzen, 1998; Bennetzen, 2005; Morgante, 2006). Finnegan first proposed a TE classification system, which includes two classes based on their transposition mechanisms: media by RNA (Retrotransposons) or DNA (DNA transposons) (Bowen & Jordan, 2002; Wessler, 2006; Arkhipova, 2018). Wicker unified the TEs nomenclatures and classification system applying mechanistic and enzymatic criteria (Wicker et al., 2007, 2008, 2009; Seberg et al., 2009). TEs play important roles in such as variations in intron size (Deutsch & Long, 1999; Zhang et al., 2011; Koonin, Csuros & Rogozin, 2013), segmental duplication (Del Pozo & Ramirez-Parra, 2015), transfer of organelle DNA to the nucleus (Adams & Palmer, 2003), expansion/contraction of tandem repeats and illegitimate recombination (Finnegan, 1989; Koike, Nakai & Takagi, 2002). Long Terminal Repeat Retrotransposons (LTR-TEs), which are usually scattered throughout

46 genomes, is the most abundant TE type and can cause genome expansion over a short evolutionary period
47 particularly in plant genomes (Piegu et al., 2006). The genome-specific TE is an efficient approach to study species
48 formation and genome evolution in genome comparative research (Dong et al., 2018).

49   *Gossypium* diverged from the common ancestor with *Theobroma cacao* approximately 33.7 MYA (Wang et
50 al., 2012). *Gossypium* comprises eight diploids (2n=2x=26) genomic groups: A, B, C, D, E, F, G, K, and one
51 allotetraploid (2n=4x=52) genomic group: AD (WANG, WENDEL & HUA, 2018). They are good materials for
52 polyploidization, genomic organization and genome-size variation researches due to its dramatic genome diversity:
53 from the smallest New World D genome of an average 885 Mb to the Australian K-genome of an average of 2576
54 Mb (Hendrix & Stewart, 2005). The accumulation of different lineage-specific TEs was thought to be responsible
55 for the variation of genome size in *Gossypium* genomes (Hawkins et al., 2006; Lu et al., 2018). Of the eight genomic
56 groups of *Gossypium*, the A and D groups are the main subjects investigated (Du et al., 2018) in cotton genomics
57 research, because the major cultivated cotton *G. hirsutum* was known as formed from the reuniting of the
58 progenitors of *G. arboreum* (A_2) and *G. raimondii* (D_5) (Paterson et al., 2012). The key trait difference between *G.
59 arboreum* and *G. raimondii* is the former producing spinnable fibers but the latter not, meanwhile in genomics, the
60 former has a genome size (1,746 Mb/1C) that is around two times of the latter (885 Mb/1C) (Hendrix & Stewart,
61 2005). Genome sequencing showed that the numbers of protein-coding genes between A (41,330) and D (37,505)
62 genomes are not obviously different, while the lineage-specific TE content is the main reason for the size gap of A
63 and D genome (Li et al., 2015; Du et al., 2018). Moreover, the transposable elements were suggested to play an
64 important role during cotton genome evolution and fiber cell development (Wang, Huang & Zhu, 2016). Thus the
65 research on the lineage-specific repetitive sequences between A and D genome is a meaningful path to investigating
66 the specification dynamic.

67   Fluorescence *in situ* hybridization (FISH) is a versatile tool to visualize the distribution of sequences in
68 chromosomes and plays a vital role in recent cytogenetic research. More and more repetitive sequences in the cotton
69 genome were reported recently with FISH, and the identification and localization of these repetitive sequences
70 would facilitate genome sequencing and understanding the mechanism of genome evolution (Lu et al., 2018). One
71 lineage-specific repetitive element that repeats many times in A genome while absent in D genome was reported and
72 suggested as an important contributor to the size gap between the A and D genome (Lu et al., 2018).

73   The D genomic group represents a diverse group of diploids that diverged from a branch of A, B, C, E, F, G,
74 and K genomic groups about 5-10 million years ago (MYA) (Senchina et al., 2003). Although the D genome is the
75 smallest one in genome size in *Gossypium*, a set of repeat elements with high proliferation in the D genome while
76 absence in A genome was discovered in this work. The discovery and characterization of these novel repetitive
77 elements provided new components for repetitive sequences database and insight into the evolution of *Gossypium*.

78 **2. Materials and Methods**

79 **2.1 Plant Materials**

80   The plant materials were obtained from National Wild Cotton Nursery in Hainan Island, China, sponsored by
81 the Institute of Cotton Research of Chinese Academy of Agricultural Sciences (ICR-CAAS). They were also
82 conserved in the greenhouse at ICR-CAAS' headquarter in Anyang City, Henan Province, China. The DNA and cell
83 came from the plant materials of cotton species listed in Table 1, based on the newest nomenclatures of *Gossypium*
84 species (WANG, WENDEL & HUA, 2018).

85   The genome sequences of *G. raimondii* (Paterson et al., 2012), *G. arboreum* (Li et al., 2014), *G. hirsutum*
86 $(AD)_1$-BGI (Wang et al., 2017), $(AD)_1$-NBI (Zhang et al., 2015; Wang et al., 2019), $(AD)_1$-JGI (Li et al., 2015), *G.*
87 *barbadense* $(AD)_2$-HAU (Yuan et al., 2015) were downloaded from the Cottongen (https://www.cottongen.org/).
88 The other assemblies of *G. barbadense* $(AD)_2$-CAS (Liu et al., 2015) were obtained from the website
89 (http://database.chgc.sh.cn/cotton/index.html).

90   Table 1. The plant materials involved in this work.

Species	Ploidy	Genome	Accession

G. arboreum	2x	A_2	Shixiya-1
G. raimondii	2x	D_5	D5-07
G. hirsutum	4x	$(AD)_1$	CCRI-12
G. barbadense	4x	$(AD)_2$	Xinhai-7

2.2 Characterization of the Repetitive Element and Bioinformatics Analysis

Perl scripts were used in this work to do data management, such as parsing the software results, extracting sequences from genomes or databases, and whole genome insertion analysis. BLASTN was used to identify the element repeats in genomes or other databases, with a threshold of greater than or equal to 80% matching ratio meanwhile 80% similarity, with reference to the 80-80 rules suggested previously (Wicker et al., 2007). The TRs were identified with Tandem Repeats Finder (Benson, 1999). Alignments were performed using MUSCLE (Edgar, 2004). The Unipro UGENE was used to present the alignments and train consensus sequences.(Edgar, 2004) The inner enzyme annotation was realized by online CD-search in NCBI (Marchler-Bauer et al., 2017). RepeatMasker was used to annotate the insertions and estimate the proportion of repetitive sequences in genomes.

The flanking LTRs of LTR-TEs were identified with the LTRharvest (Ellinghaus, Kurtz & Willhoeft, 2008). Subsequently, the Vmatch was used to cluster the LTRs (Kurtz, 2003). The divergence time of the LTR-TEs was estimated using the formula $T = d/2r$, where r represents a substitution rate of 1.3×10^{-8} per site per year (Ma & Bennetzen, 2004), and d means the distances of paired LTRs, which was calculated based on the Kimura two-parameter (Kimura, 1980). The insertions of repetitive sequences in genomes were illustrated by R language (R Core Team, 2014).

2.3 Fluorescence in situ hybridization (FISH)

The probe was designed with the PCR product of *ICRd* motif, which was obtained from the forward primer: TTCTATTTTATCCATCGTTATG, reverse: GGAGATAGGATTTGTTGCT; and followed the amplification procedure: firstly, 95°C for 5 min of pre-degeneration; then 30 cycles at 95°C for 30 s, 52°C for 30 s, and 72°C for 2 min. The final extension was done at 72°C for 6 min. The composition of the reaction mix using the following: gDNA (~5 μg/ml), primers (~0.8 μM), PCR Master Mix (Thermo), and H_2O. The gDNA extracted from leaves of cotton plants (Table 1). The probe was purified and labeled with digoxigenin-dUTP via nick translation, according to the instructions of the manufacturer (Roche Diagnostics, USA). Mitotic chromosome preparation and FISH procedures were conducted using a modified protocol (Wang et al., 2001).

3. Results

3.1. One Specific Repetitive Sequence in *Gossypium* D_5 Genome

One segment in *G. raimondii* (D_5) genome (Chr05: 50639971-50641791) was filtered out as genome-specific in D_5, by comparative genome analysis of *G. raimondii* (Paterson et al., 2012) and *G. arboreum* (A_2) (Li et al., 2014) with BLAST. This sequence is highly repeated and spreading all over 13 chromosomes of the D_5 genome (Supplementary Table 1), while do not exist in A_2 genome. We queried it in Repbase (Chen et al., 2007a) and NCBI, but no related annotation was found. Then we performed LTRharvest (Ellinghaus, Kurtz & Willhoeft, 2008) and CD-search (Marchler-Bauer et al., 2017), which revealed it is neither LTR nor coding sequence.

Manual inspection revealed the structure of the genome-specific sequence having two constituents, a tandem repeats array (referred as TR hereafter) composed of 133 bp basic units, and an unknown conservative sequence (referred as CS hereafter) (Figure 1). Based on this feature, we totally identified 72 sequences from D_5 genome (Supplementary Table 2), for abbreviation, they were termed as the *ICRd* motif naming following our previous work (Lu et al., 2018). Among the 72 *ICRd* motifs, the TRs are length-variable having a variety of the basic unit content that 2-20 basic units (Figure 2a), while the CSs are stable and have an average size ~ 860 bp.

To verify the genome specificity and chromosome distribution of the *ICRd* motif, we used the PCR product of *ICRd* motif from *G. raimondii* to designed the probe for fluorescence *in situ* hybridization (FISH) on the mitotic

131 chromosomes of diploid A_2 and D_5, and tetraploid *G. hirsutum* $((AD)_1)$, *G. barbadense* $((AD)_2)$. The probe generated
132 bright signals covering all the chromosomes of D_5 and D-subgenome, but none signal on the A_2 and A-subgenome
133 (Figure 3). These cytogenetic inspections were in accordance with the genomic comparative analysis and further
134 revealed that the *ICRd* motif is a genome-specific and highly repetitive element in the D_5 genome, as well as in the
135 D-subgenome of tetraploid cotton.

3.2 LTR-TEs Inserted with *ICRd* Motif

137   We compared the insertion loci of 72 *ICRd* motifs with the whole genome repeats annotation (gff file) of the
138 D_5 genome (Paterson et al., 2012) and found that each of the motifs is one to one harbored in 72 LTR-TEs
139 (Supplementary Table 3), which meant the former is the inner part of the latter.
140   We extracted the 72 LTR-TEs sequences from D_5 genome and parsed their structure, which showed all of 72
141 LTR-TEs are incomplete, lacking either enzyme or flanking LTRs, the required elements for an intact LTR-TE
142 (Wicker et al., 2007). We align all these LTR-TEs together and got their consensus accumulation histogram
143 (Supplementary Figure 1), which showed these TEs have a vast sequence variety among each other, however, an
144 only conservative region was observed, which is just the insertion region of the *ICRd* motif (Figure 4), revealing that
145 the *ICRd* motif is more stable than other elements along with the degradation and the evolution of the TEs.
146   Of the 72 LTR-TEs, 25 were identified having flanking LTRs, which were used to represent the classification
147 and evolution of these TEs. The LTR cluster results showed except two TEs have similarity in LTR region the other
148 23 TEs are absolutely different from each other, which further revealed they do not belong to the same family based
149 on the LTR similarity rules (Wicker et al., 2007). The estimated active date curve of these TEs almost all prior to 10
150 MYA and peak in ~30 MYA (Figure 5), in the close period with that *G. raimondii* and *T. cacao* diverged
151 approximately 33.7 MYA (Wang et al., 2012), but far earlier than the putative divergence time of *Gossypium* A and
152 D genomes (Wendel & Cronn, 2001). These revealed these LTR-TEs are ancient TEs and potentially contribute to
153 the speciation formation of *Gossypium*.

3.3 Abundant Constituents of *ICRd* Motif in D_5 genome

155   Toward the further analysis of the genomic feature of the *ICRd* motif, we separately investigated its two
156 constituents that TR and CS, on their content and distribution feature in the D_5 genome (Figure 6a). In total 350 *TR*
157 insertions were detected (Supplementary Table 2), which are different in length due to different times of the unit
158 repeating comprising among 2–21, and mainly 2–10 times of the basic unit (Figure 2b). The longest TR insertion in
159 D_5 ($D_5$03: 25689303–25697234) comprising 61 units up to 8 kb, which was extraordinary and unknown on how it
160 formed. On the other hand, in total 463 CSs were found (Supplementary Table 2). Combining analysis of the
161 insertion loci of the two constituents, we found 72 TRs are closely followed by 72 CSs, which just constitute the
162 *ICRd* motifs (Figure 1).
163   Further analysis proved the TR and CS are evenly distributed on the chromosomes based on $\chi2$ test (the
164 number of insertions is proportional to the size of the chromosome), where for the TR insertions, $\chi2 = 5.56$ (df = 12,
165 P > 0.9), and for the CSs, $\chi2 = 9.08$ (df = 12, P > 0.5). The even distributions meant the CS and *TR* are possible
166 ancient repetitive sequences that have evolved along with the chromosomes. The *G. raimondii* genome sequencing
167 work had reported that most TEs in *G. raimondii* are deletion derivatives lacking the domains that are typically
168 necessary for transposition that the only 3% of LTR base pairs derived from full-length LTR-TEs (Paterson et al.,
169 2012). Here the hundreds of constituents of *ICRd* motif in D_5 are potentially the fragments produced from the
170 ancient LTR-TEs.

3.4 The disappearance of ICRd Motif from *Gossypium*

172   With the aim to observe the disappearance of the *ICRd* motif in the newly formed *Gossypium* A genome, we
173 selected one pair segments from the highly collinear Chromosome 1 in the two cotton species to observe (Li et al.,
174 2014). The segment from Chromosome 1 of *G. raimondii* ($D_5$01) harbor one *ICRd* motif and its homologous
175 segment from $A_2$01 was got based on homologous SSR markers (Supplementary Table 4). The illustration of the

176   syntenic block of the two segments showed the *ICRd* motif together with its host LTR-TE were totally abandoned
177   on the A_201 segment, while their up- and downstream conservative regions remained (Figure 7). In the upstream, we
178   observed two insertions rich in repeat sequences special on A_201 segment (Supplementary Table 4), which was
179   potentially due to the recent TE expanding event happened in A genome (Lu et al., 2018). Thus, we observed that
180   the *ICRd* motifs and host LTR-TEs were directly abandoned from the genome with the recent formation of A
181   genome (Wendel & Cronn, 2001; Wendel, Flagel & Adams, 2012), but remained in the D genome despite mass
182   damage accumulation.

183   **3.5 Distributions of *ICRd* Motifs in Tetraploid Cotton**

184   Tetraploid cotton *G. hirsutum* and *G. barbadense* are the major cultivated fiber-producing cotton species.
185   Research on the genome of these two species is an important approach to improve the cotton yield and quality.
186   However, due to the huge amount of homologous segments between A and D-subgenomes, the tetraploid cotton
187   genome assemblage has been a great challenge to molecular geneticists (Bowers et al., 2003; Chen et al., 2007b).
188   Three versions of *G. hirsutum* genome assembly($(AD)_1$-BGI (Li et al., 2015), $(AD)_1$-NBI (Zhang et al., 2015),
189   $(AD)_1$-JGI (Wang et al., 2017)), and two *G. barbadense* versions ($(AD)_2$-HAU (Yuan et al., 2015) and $(AD)_2$-CAS
190   (Liu et al., 2015)) have been reported recently, however, the quality of the sequenced genomes require improvement
191   in order to benefit cotton molecular breeders. Application of the lineage-specific repetitive element (LSR), the *ICRd*
192   motifs are important tools in evaluating the quality of the genome assembly of the tetraploid cotton.
193   To observe the assembling quality of the *ICRd* motif in tetraploid genomes, we queried it with BLAST in the
194   five tetraploid genome assemblies, including 3 versions of *G. hirsutum* ($(AD)_1$) and two versions of *G. barbadense*
195   ($(AD)_2$) (Table 2). For the $(AD)_1$ assemblies, the blast result from the NBI version was in agreement to the FISH
196   inspection that the *ICRd* motifs only generated the signals on the D-subgenome chromosomes (Figure 3). However,
197   the BGI and JGI versions were inconsistent with the FISH inspection results, that *ICRd* motif was misassembled in
198   the A-subgenome. For the $(AD)_2$ assemblies, the CAS showed a better assembling than HAU, because the *ICRd*
199   motifs were located in all 13 D-sub chromosomes of CAS, but mainly matched with the unassembled scaffolds of
200   HAU (Supplementary Table 5). This means the $(AD)_2$-CAS showed better scaffolds assembling than the $(AD)_2$-
201   HAU. Thus, the *G. hirsutum* genome assembly $(AD)_1$-NBI, and the *G. barbadense* genome assembly $(AD)_2$-CAS
202   are a conclusive version based on the comparison of the BLAST query and our cytogenetic experiment.

203   Table 2. The distribution of *ICRd* motif on different genome assemblies of tetraploid cotton.

Assemblies	Reference	*ICRd* motif
$(AD)_1$-BGI	(Li et al., 2015)	D_h01-D_h13; A_h02, A_h05, A_h07, A_h08
$(AD)_1$-NBI	(Zhang et al., 2015)	D_h01-D_h13; None in A-sub
$(AD)_1$-JGI	(Wang et al., 2017)	D_h01-D_h13; A_h11
$(AD)_2$-CAS	(Liu et al., 2015)	D_b01-D_b13; None in A-sub
$(AD)_2$-HAU	(Yuan et al., 2015)	D_b01, D_b02, D_b06-D_b09, D_b12; None in A-sub

204   **4. Discussion**

205   **4.1 The Identification of *ICRd* Motif and *Gossypium* Evolution**

206   TEs have played an important function in *Gossypium* speciation and the accumulation of different genomic-
207   specific TEs were thought to be responsible for the variation of genome size in *Gossypium* genomes (Hawkins et al.,
208   2006). Through FISH inspection, some A genome-specific repetitive elements have been well identified and
209   characterized (Liu et al., 2016), but the similar work in the D genome has not yet been reported, this may be because
210   the genome-specific repetitive sequences in A genome are much more than that in the D genome (Liu et al., 2018).
211   However, in this work, starting with comparative genomic data, we screened out one kind of specific sequence in the
212   D genome, and subsequently, we identified and characterized TEs.

213   The TEs harboring the *ICRd* motif showed an ancient active date approximately 10 MYA, while the time of
214   divergence of the A and D genomes from the common ancestor is estimated to have occurred 5-10 MYA (Grover et
215   al., 2004), thus the *ICRd* motifs existed in the ancestor and disappeared along with the formation of A genome.
216   Though the previous researches have stated the accumulation of lineage-specific TEs, which is thought to be
217   responsible for the variation of *Gossypium* genomes (Hawkins et al., 2006), and the LTR-TE activities after 5 MYA
218   mainly contributed to the two-fold size difference of the A and D genomes (Zhang et al., 2015). Based on our
219   analysis, we presumed that as same as the activity of new repetitive sequences, the extinction of ancient repetitive
220   sequences, such as the disappearance of *ICRd* motif in the A genome, also contributed significantly to the genome
221   evolution. Through FISH, we observed that the *ICRd* motifs were only distributed in D-subgenome chromosomes,
222   and the results were in agreement to the previous studies which reported that the TE have proliferated in the
223   progenitor genomes but were retained after allopolyploid formation in the D subgenome (Zhang et al., 2015).

224   **4.2 *ICRd* Motif Support Cytogenetic Markers for Tetraploid Cotton**

225   The identification of *ICRd* motif provides new subgenome marker for the accurate assembling of tetraploid
226   cotton (Chen et al., 2007a). Chromosome identification is the foundation of plant genetics, evolution and genomics
227   researches (Saranga, 2007; Xie et al., 2012). Although many species have been sequenced, the rapid identification of
228   subgenome is still useful in applied researches. FISH has been used as a reliable cytological technique for
229   chromosome identification in many species (Wang, Guo & Zhang, 2007). The identification of cotton chromosomes
230   evolved recently with the FISH technique (Gan et al., 2012). In this study, the identified *ICRd* motifs can be used as
231   a new cytological marker in *Gossypium*, especially in tetraploid. And the repetitive sequence probes are easier and
232   more successful to be detected than other probes. Several similar makers have been reported (Liu et al., 2016). The
233   addition of these new cytological markers will enrich the marker database for chromosome identification and
234   facilitate cotton genomic studies.
235   Eukaryotic genomes have a high proportion of TEs and these TEs make the eukaryotic genomes assembly
236   much more difficult than simple genomes (Treangen & Salzberg, 2012). Many reported genome sequences have
237   gaps because of the high proportion of TEs (Adams et al., 2000). Allopolyploid genomes are especially difficult to
238   assemble homologous fragments from sub-genomes (Chen et al., 2007a). The incorrect assembling of the genomes
239   leads to ambiguity in research, which, in turn, produce biases and errors when interpreting results (Adams et al.,
240   2000). Though the three versions of genome assembly of *G. hirsutum*, two versions of *G. barbadense* have been
241   released, their accuracies are inconsistent as revealed by BLASTN of the *ICRd* motif. Here, our FISH results were in
242   agreement to two tetraploid cotton genome assemblies, (AD)$_1$-NBI of *G. hirsutum*, and (AD)$_2$-CAS of *G.*
243   *barbadense*. Moreover, the *ICRd* motifs also assist to assure the source of the unpackaged scaffolds in the genome
244   assemblies, for instance, in the scaffolds the presence of the *ICRd* motifs can aid in the assigning of the scaffolds to
245   the D-subgenome chromosomes

246   **5. Conclusions**

247   We identified a kind of repetitive sequence in *Gossypium* D genome but absent in A genome, the *ICRd* motifs,
248   were found to be retained in D-subgenome and not in A-subgenome. We analyzed their structure, genomic
249   distribution, affiliation, and evolution, which revealed a conserved region which harbored the ancient LTR-TEs, in
250   the D genome. The identification and characterization of *ICRd* motif provided new insight into understanding the TE
251   evolution along with the formation of the cotton genomes as well as providing a convenient and applicative tool to
252   distinguish the A and D genome subsets of the tetraploid cotton genome assembly.

253   **Supplementary Materials:**, Figure S1: Supplementary Figure 1. The whole alignment of the 72 LTR-TEs, Table S1: Blast of the
254   1.8 kb sequences in *G. raimondii* genome, Table S2: The ICRd motifs and their constituents, Table S3: The structures of the
255   LTR-TEs harboring the ICRd motif, Table S4: The information of the two homologous segments, Table S5: Blast results of the
256   ICRd motif with tetraploid cotton.

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523

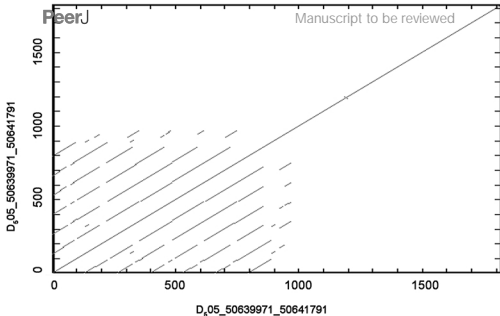
524

The structure of *ICRd* motif

a: The self-blast of the *ICRd* motif showed the inner repeats; b: The structure of *ICRd* motif; c: The basic TR unit; d: The examples of the structure illustration of the LTR-TEs inserted with *ICRd* motif.

a

(plot: y-axis labeled D₆05_50639971_50641791 ranging 0 to ~1700; x-axis labeled D₆05_50639971_50641791 ranging 0 to ~1700)

b

GTAGAATTACTGGCTTCAATGTACTCCACTGCAACTTCATGGAGGTAAAATCCGCCATCTTCGATCTGCTCCACTACTGCTTAGGGAGACAAGACCTGAAATCTTCAACCTGCTCCACTGTCTCGAGGGA

c

ICRd motif

| Variable unit repeat | unit | unit | |

TR ←——————————→ CS ←——————————→

d

D₆01_7929175_7938465

D₆02_14597849_14606769

D₆07_19182817_19192364

D₆05_57995613_58007404

Legend: TR, TR-c, LTR

1 Kb

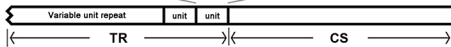
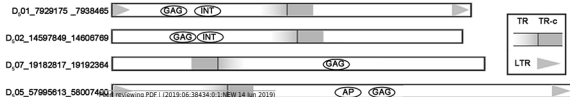



Figure 2

The content of the basic unit in the TRs

a: The basic unit content in the TRs involved in the *ICRd* motifs, displayed from small to large; b: The number of *ICRd* TRs that harboring different unit content, the x-axis adopt the intervals of unit content for convenient exhibition.

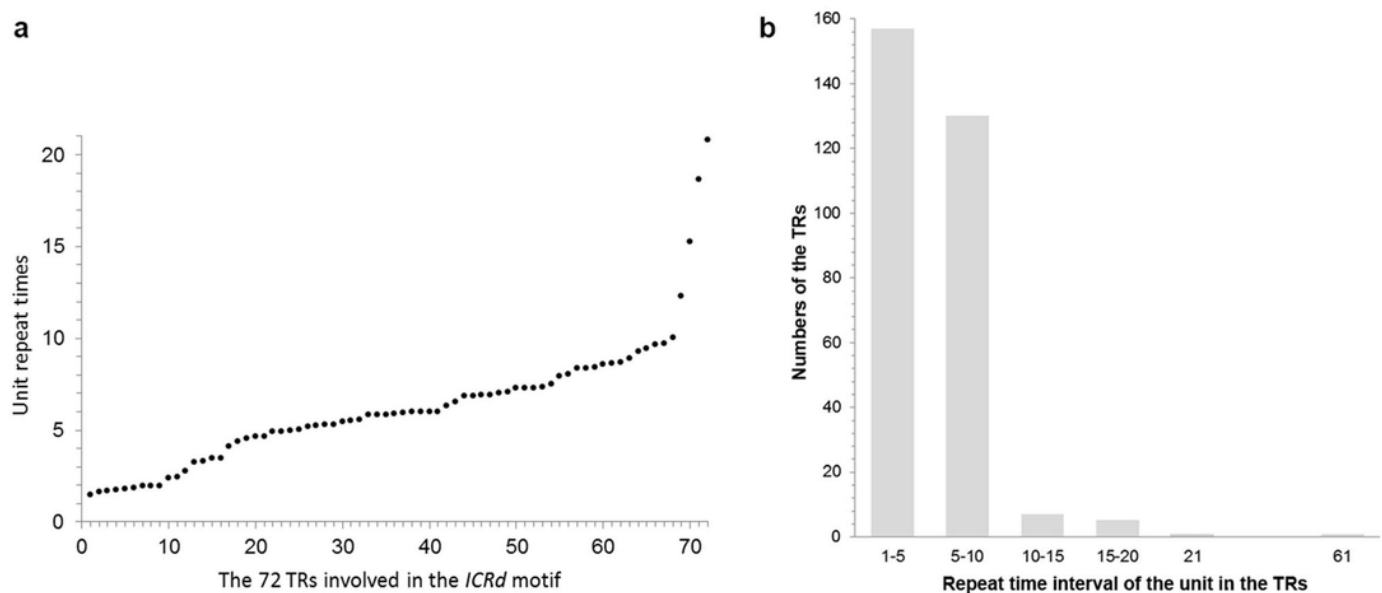
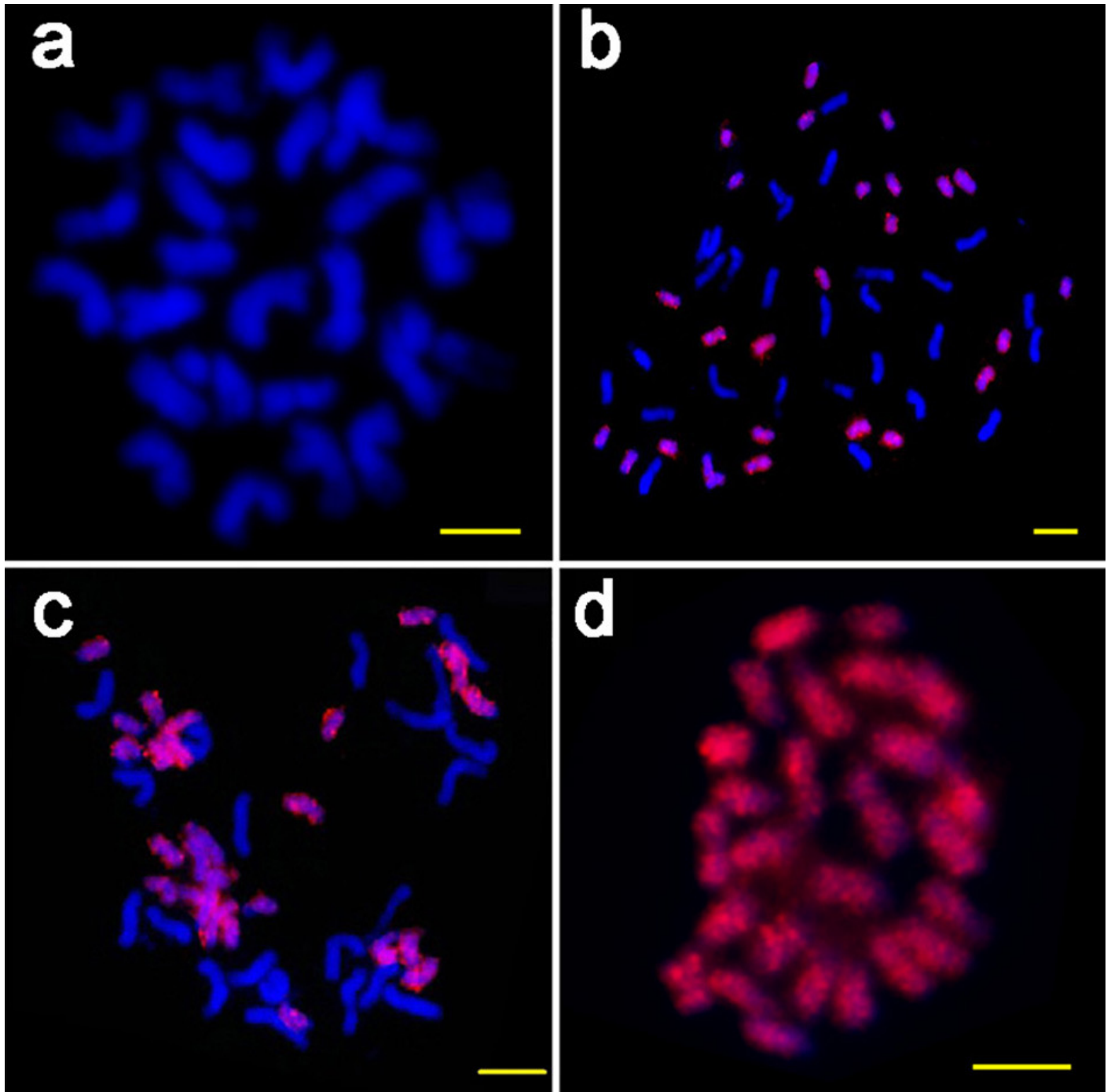



Figure 3

The FISH images of *ICRd* motif (red) hybridized to mitotic chromosomes of four species.

a: *G. arboreum* (AA); b: *G. hirsutum* (AADD); c: *G. barbadense* (AADD); d: *G. raimondii* (DD).
Bar = 5μm.

The consensus accumulation histogram from the whole alignment of the 72 LTR-TEs .

The region marked with the black line is the *ICRd* motif region.

The consensus accumulation from the whole alignment of the 72 LTR-TEs

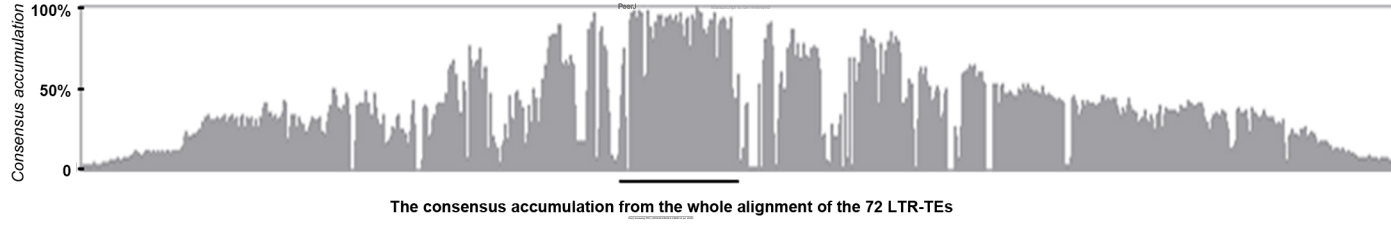
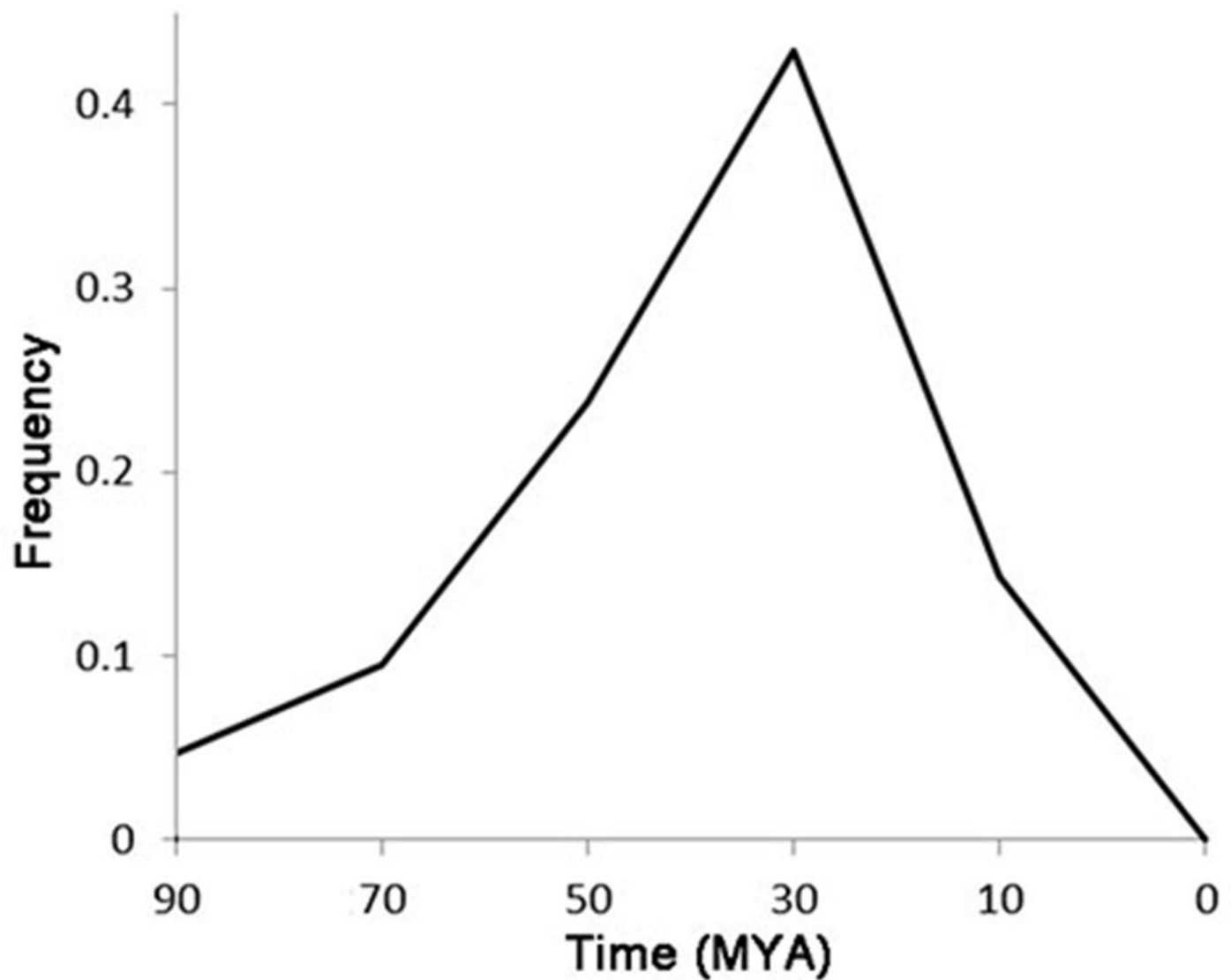
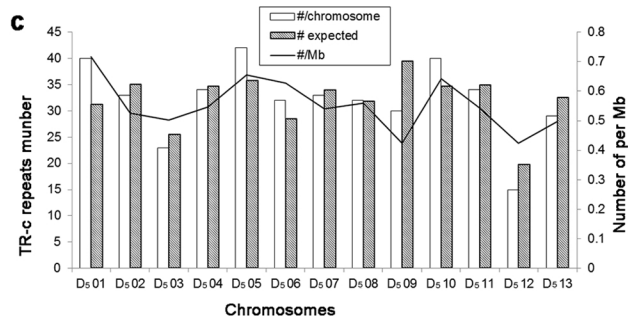
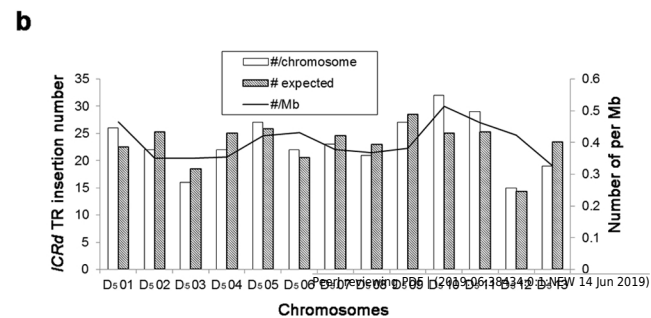
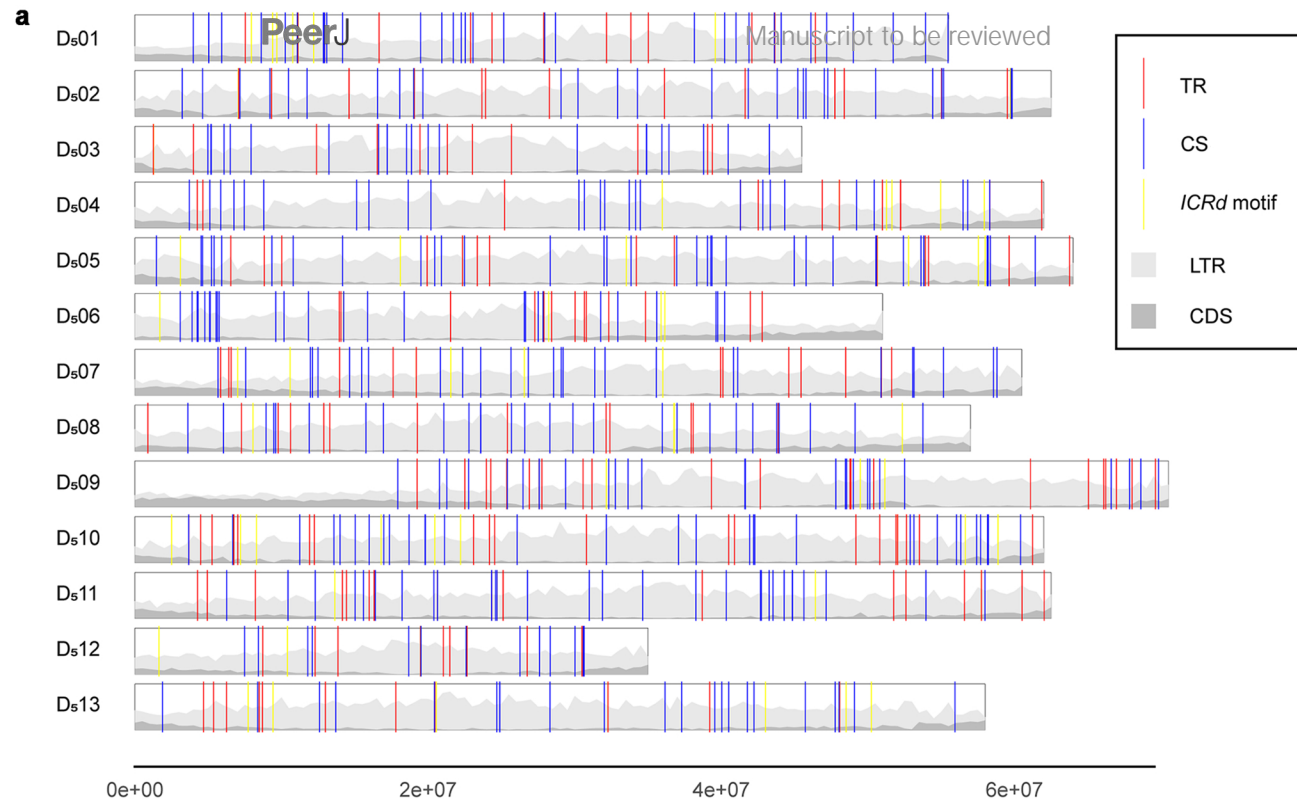



Figure 5

The accumulation of putative active date of the LTR-TEs.

The distribution of the *ICRd* motif and its constituent in the D_5 genome

a: Insertions of the *ICRd* motif and its constituents in the D_5 genomes; b, c: *ICRd* TR and TR-c chromosomal distribution, the expected (grey) and actual (white) distributions across all chromosomes are illustrated; in addition, the density per megabase is shown for each chromosome.

The colinearity of the two homologous segments.

D₈01_9633633_9699033

ICRd motif
LTR-RT
homologous genes

2 kb

A₈01_129978952_130050452