A peer-reviewed version of this preprint was published in PeerJ on 3 January 2020.

View the peer-reviewed version (peerj.com/articles/8344), which is the preferred citable publication unless you specifically need to cite this preprint.

Lu H, Cui X, Zhao Y, Magwanga RO, Li P, Cai X, Zhou Z, Wang X, Liu Y, Xu Y, Hou Y, Peng R, Wang K, Liu F. 2020. Identification of a genome-specific repetitive element in the *Gossypium* D genome. PeerJ 8:e8344 https://doi.org/10.7717/peerj.8344

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

Hejun Lu [Equal first author, 1, 2], **Xinglei Cui** [Equal first author, 1], **Yanyan Zhao** [1], **Richard Odongo Magwang** [1, 3], **Pengcheng Li** [1], **Xiaoyan Cai** [1], **Zhongli Zhou** [1], **Xingxing Wang** [1], **Yuling Liu** [4], **Yanchao Xu** [1], **Yuqing Hou** [1], **Renhai Peng** [4], **Kunbo Wang** [Corresp., 1, 5], **Fang Liu** [Corresp. 1]

[1] Research Base of Tarium University, State Key Laboratory of Cotton Biology, Institute of Cotton Research of Chinese Academy of Agricultural Science, Anyang, Henan, China

[2] Gembloux Agro-bio Tech, University of Liège, Gembloux, Namur, Belgium

[3] School of biological and physical sciences,, Jaramogi Oginga Odinga University of Science and Technology (JOOUST), P.O Box, Bondo-Kenya

[4] Anyang Istitute of Technology, Anyang, Henan, China

[5] Tarium University, Alar, Xinjiang, China

Corresponding Authors: Kunbo Wang, Fang Liu
Email address: wkbcri@163.com, liufcri@163.com

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.

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

Hejun Lu[1,2*], Xinglei Cui[1*], Yanyan Zhao[1], Richard Odongo Magwanga[1,3], Pengcheng Li[1], Xiaoyan Cai[1], Zhongli Zhou[1], Xingxing Wang[1], Yuling Liu[4], Yanchao Xu[1], Yuqing Hou[1], Renhai Peng[4], Kunbo Wang[1,5+], Fang Liu[1+]

[1] Research Base of Tarium University, State Key Laboratory of Cotton Biology, Institute of Cotton Research of Chinese Academy of Agricultural Science, Anyang, Henan, 455000, China
[2] Gembloux Agro-Bio Tech, University of Liège, Gembloux, 5030, Belgium
[3] School of biological and physical sciences (SBPS), Jaramogi Oginga Odinga University of Science and Technology (JOOUST), P.O Box 210-40601, Bondo-Kenya
[4] Anyang Institute of Technology, Anyang, Henan, 455000, China
[5] Tarium University, Alar, Xinjiang. 843300, China
[*]These authors contributed equally to this work.
[+]Correspondence: Kunbo Wang, E-mail: wkbcri@163.com; Fang Liu, liufcri@163.com

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

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

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

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

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

2. Materials and Methods

2.1 Plant Materials

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

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

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

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

3.2 LTR-TEs Inserted with *ICRd* Motif

We compared the insertion loci of 72 *ICRd* motifs with the whole genome repeats annotation (gff file) of the D_5 genome (Paterson et al., 2012) and found that each of the motifs is one to one harbored in 72 LTR-TEs (Supplementary Table 3), which meant the former is the inner part of the latter.

We extracted the 72 LTR-TEs sequences from D_5 genome and parsed their structure, which showed all of 72 LTR-TEs are incomplete, lacking either enzyme or flanking LTRs, the required elements for an intact LTR-TE (Wicker et al., 2007). We align all these LTR-TEs together and got their consensus accumulation histogram (Supplementary Figure 1), which showed these TEs have a vast sequence variety among each other, however, an only conservative region was observed, which is just the insertion region of the *ICRd* motif (Figure 4), revealing that the *ICRd* motif is more stable than other elements along with the degradation and the evolution of the TEs.

Of the 72 LTR-TEs, 25 were identified having flanking LTRs, which were used to represent the classification and evolution of these TEs. The LTR cluster results showed except two TEs have similarity in LTR region the other 23 TEs are absolutely different from each other, which further revealed they do not belong to the same family based on the LTR similarity rules (Wicker et al., 2007). The estimated active date curve of these TEs almost all prior to 10 MYA and peak in ~30 MYA (Figure 5), in the close period with that *G. raimondii* and *T. cacao* diverged approximately 33.7 MYA (Wang et al., 2012), but far earlier than the putative divergence time of *Gossypium* A and D genomes (Wendel & Cronn, 2001). These revealed these LTR-TEs are ancient TEs and potentially contribute to the speciation formation of *Gossypium*.

3.3 Abundant Constituents of *ICRd* Motif in D_5 genome

Toward the further analysis of the genomic feature of the *ICRd* motif, we separately investigated its two constituents that TR and CS, on their content and distribution feature in the D_5 genome (Figure 6a). In total 350 *TR* insertions were detected (Supplementary Table 2), which are different in length due to different times of the unit repeating comprising among 2–21, and mainly 2–10 times of the basic unit (Figure 2b). The longest TR insertion in D_5 ($D_5$03: 25689303–25697234) comprising 61 units up to 8 kb, which was extraordinary and unknown on how it formed. On the other hand, in total 463 CSs were found (Supplementary Table 2). Combining analysis of the insertion loci of the two constituents, we found 72 TRs are closely followed by 72 CSs, which just constitute the *ICRd* motifs (Figure 1).

Further analysis proved the TR and CS are evenly distributed on the chromosomes based on $\chi 2$ test (the number of insertions is proportional to the size of the chromosome), where for the TR insertions, $\chi 2 = 5.56$ ($df = 12$, $P > 0.9$), and for the CSs, $\chi 2 = 9.08$ ($df = 12$, $P > 0.5$). The even distributions meant the CS and *TR* are possible ancient repetitive sequences that have evolved along with the chromosomes. The *G. raimondii* genome sequencing work had reported that most TEs in *G. raimondii* are deletion derivatives lacking the domains that are typically necessary for transposition that the only 3% of LTR base pairs derived from full-length LTR-TEs (Paterson et al., 2012). Here the hundreds of constituents of *ICRd* motif in D_5 are potentially the fragments produced from the ancient LTR-TEs.

3.4 The disappearance of ICRd Motif from *Gossypium*

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

syntenic block of the two segments showed the *ICRd* motif together with its host LTR-TE were totally abandoned on the $A_2$01 segment, while their up- and downstream conservative regions remained (Figure 7). In the upstream, we observed two insertions rich in repeat sequences special on $A_2$01 segment (Supplementary Table 4), which was potentially due to the recent TE expanding event happened in A genome (Lu et al., 2018). Thus, we observed that the *ICRd* motifs and host LTR-TEs were directly abandoned from the genome with the recent formation of A genome (Wendel & Cronn, 2001; Wendel, Flagel & Adams, 2012), but remained in the D genome despite mass damage accumulation.

3.5 Distributions of *ICRd* Motifs in Tetraploid Cotton

Tetraploid cotton *G. hirsutum* and *G. barbadense* are the major cultivated fiber-producing cotton species. Research on the genome of these two species is an important approach to improve the cotton yield and quality. However, due to the huge amount of homologous segments between A and D-subgenomes, the tetraploid cotton genome assemblage has been a great challenge to molecular geneticists (Bowers et al., 2003; Chen et al., 2007b). Three versions of *G. hirsutum* genome assembly($(AD)_1$-BGI (Li et al., 2015), $(AD)_1$-NBI (Zhang et al., 2015), $(AD)_1$-JGI (Wang et al., 2017)), and two *G. barbadense* versions ($(AD)_2$-HAU (Yuan et al., 2015) and $(AD)_2$-CAS (Liu et al., 2015)) have been reported recently, however, the quality of the sequenced genomes require improvement in order to benefit cotton molecular breeders. Application of the lineage-specific repetitive element (LSR), the *ICRd* motifs are important tools in evaluating the quality of the genome assembly of the tetraploid cotton.

To observe the assembling quality of the *ICRd* motif in tetraploid genomes, we queried it with BLAST in the five tetraploid genome assemblies, including 3 versions of *G. hirsutum* ($(AD)_1$) and two versions of *G. barbadense* ($(AD)_2$) (Table 2). For the $(AD)_1$ assemblies, the blast result from the NBI version was in agreement to the FISH inspection that the *ICRd* motifs only generated the signals on the D-subgenome chromosomes (Figure 3). However, the BGI and JGI versions were inconsistent with the FISH inspection results, that *ICRd* motif was misassembled in the A-subgenome. For the $(AD)_2$ assemblies, the CAS showed a better assembling than HAU, because the *ICRd* motifs were located in all 13 D-sub chromosomes of CAS, but mainly matched with the unassembled scaffolds of HAU (Supplementary Table 5). This means the $(AD)_2$-CAS showed better scaffolds assembling than the $(AD)_2$-HAU. Thus, the *G. hirsutum* genome assembly $(AD)_1$-NBI, and the *G. barbadense* genome assembly $(AD)_2$-CAS are a conclusive version based on the comparison of the BLAST query and our cytogenetic experiment.

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

4. Discussion

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

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

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

4.2 *ICRd* Motif Support Cytogenetic Markers for Tetraploid Cotton

The identification of *ICRd* motif provides new subgenome marker for the accurate assembling of tetraploid cotton (Chen et al., 2007a). Chromosome identification is the foundation of plant genetics, evolution and genomics researches (Saranga, 2007; Xie et al., 2012). Although many species have been sequenced, the rapid identification of subgenome is still useful in applied researches. FISH has been used as a reliable cytological technique for chromosome identification in many species (Wang, Guo & Zhang, 2007). The identification of cotton chromosomes evolved recently with the FISH technique (Gan et al., 2012). In this study, the identified *ICRd* motifs can be used as a new cytological marker in *Gossypium*, especially in tetraploid. And the repetitive sequence probes are easier and more successful to be detected than other probes. Several similar makers have been reported (Liu et al., 2016). The addition of these new cytological markers will enrich the marker database for chromosome identification and facilitate cotton genomic studies.

Eukaryotic genomes have a high proportion of TEs and these TEs make the eukaryotic genomes assembly much more difficult than simple genomes (Treangen & Salzberg, 2012). Many reported genome sequences have gaps because of the high proportion of TEs (Adams et al., 2000). Allopolyploid genomes are especially difficult to assemble homologous fragments from sub-genomes (Chen et al., 2007a). The incorrect assembling of the genomes leads to ambiguity in research, which, in turn, produce biases and errors when interpreting results (Adams et al., 2000). Though the three versions of genome assembly of *G. hirsutum*, two versions of *G. barbadense* have been released, their accuracies are inconsistent as revealed by BLASTN of the *ICRd* motif. Here, our FISH results were in agreement to two tetraploid cotton genome assemblies, $(AD)_1$-NBI of *G. hirsutum*, and $(AD)_2$-CAS of *G. barbadense*. Moreover, the *ICRd* motifs also assist to assure the source of the unpackaged scaffolds in the genome assemblies, for instance, in the scaffolds the presence of the *ICRd* motifs can aid in the assigning of the scaffolds to the D-subgenome chromosomes

5. Conclusions

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

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

Author Contributions: Conceptualization, Kunbo Wang and Fang Liu; Data curation, Hejun Lu and Xinglei Cui; Formal analysis, Hejun Lu and Richard Odongo Magwanga; Funding acquisition, Fang Liu; Investigation, Hejun Lu, Yanyan Zhao, Pengcheng Li and Yuling Liu; Methodology, Xinglei Cui, Yanyan Zhao and Yuqing Hou; Resources, Xiaoyan Cai, Zhongli

Zhou, Yanchao Xu and Renhai Peng; Software, Hejun Lu; Supervision, Kunbo Wang and Fang Liu; Validation, Hejun Lu and Xingxing Wang; Writing original draft, Hejun Lu; Writing-review & editing, Xinglei Cui, Richard Odongo Magwanga, Yanyan Zhao.

Funding: Please add: This research was supported by The National Key Research and Development Plan of China (grants 2016YFD0100306 and 2016YFD0100203) and The Natural Science Foundation of China (grants 31530053 and 31671745).

Acknowledgments: We are indebted to Dr. Syed Shan-e-Ali Zaidi of the University of Liege, Belgium, for his guidance in analysis and interpretation of the data

Conflicts of Interest: The authors declare no conflict of interest.

References

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, George RA, Lewis SE, Richards S, Ashburner M, Henderson SN, Sutton GG, Wortman JR, Yandell MD, Zhang Q, Chen LX, Brandon RC, Rogers YHC, Blazej RG, Champe M, Pfeiffer BD, Wan KH, Doyle C, Baxter EG, Helt G, Nelson CR, Gabor Miklos GL, Abril JF, Agbayani A, An HJ, Andrews-Pfannkoch C, Baldwin D, Ballew RM, Basu A, Baxendale J, Bayraktaroglu L, Beasley EM, Beeson KY, Benos P V., Berman BP, Bhandari D, Bolshakov S, Borkova D, Botchan MR, Bouck J, Brokstein P, Brottier P, Burtis KC, Busam DA, Butler H, Cadieu E, Center A, Chandra I, Michael Cherry J, Cawley S, Dahlke C, Davenport LB, Davies P, de Pablos B, Delcher A, Deng Z, Deslattes Mays A, Dew I, Dietz SM, Dodson K, Doup LE, Downes M, Dugan-Rocha S, Dunkov BC, Dunn P, Durbin KJ, Evangelista CC, Ferraz C, Ferriera S, Fleischmann W, Fosler C, Gabrielian AE, Garg NS, Gelbart WM, Glasser K, Glodek A, Gong F, Harley Gorrell J, Gu Z, Guan P, Harris M, Harris NL, Harvey D, Heiman TJ, Hernandez JR, Houck J, Hostin D, Houston KA, Howland TJ, Wei MH, Ibegwam C, Jalali M, Kalush F, Karpen GH, Ke Z, Kennison JA, Ketchum KA, Kimmel BE, Kodira CD, Kraft C, Kravitz S, Kulp D, Lai Z, Lasko P, Lei Y, Levitsky AA, Li J, Li Z, Liang Y, Lin X, Liu X, Mattei B, McIntosh TC, McLeod MP, McPherson D, Merkulov G, Milshina N V., Mobarry C, Morris J, Moshrefi A, Mount SM, Moy M, Murphy B, Murphy L, Muzny DM, Nelson DL, Nelson DR, Nelson KA, Nixon K, Nusskern DR, Pacleb JM, Palazzolo M, Pittman GS, Pan S, Pollard J, Puri V, Reese MG, Reinert K, Remington K, Saunders RDC, Scheeler F, Shen H, Christopher Shue B, Siden-Kiamos I, Simpson M, Skupski MP, Smith T, Spier E, Spradling AC, Stapleton M, Strong R, Sun E, Svirskas R, Tector C, Turner R, Venter E, Wang AH, Wang X, Wang ZY, Wassarman DA, Weinstock GM, Weissenbach J, Williams SM, Woodage T, Worley KC, Wu D, Yang S, Alison Yao Q, Ye J, Yeh RF, Zaveri JS, Zhan M, Zhang G, Zhao Q, Zheng L, Zheng XH, Zhong FN, Zhong W, Zhou X, Zhu S, Zhu X, Smith HO, Gibbs RA, Myers EW, Rubin GM, Craig Venter J. 2000. The genome sequence of Drosophila melanogaster. *Science* 287:2185–2195. DOI: 10.1126/science.287.5461.2185.

Adams KL, Palmer JD. 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Molecular phylogenetics and evolution* 29:380–95.

Arkhipova IR. 2018. Neutral theory, transposable elements, and eukaryotic genome evolution.

Molecular Biology and Evolution 35:1332–1337. DOI: 10.1093/molbev/msy083.

Bennetzen JL. 2005. Transposable elements, gene creation and genome rearrangement in flowering plants. *Current Opinion in Genetics and Development* 15:621–627. DOI: 10.1016/j.gde.2005.09.010.

Benson G. 1999. Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Research* 27:573–580. DOI: 10.1093/nar/27.2.573.

Bowen NJ, Jordan IK. 2002. Transposable elements and the evolution of eukaryotic complexity. *Current Issues in Molecular Biology* 4:65–76.

Bowers JE, Chapman BA, Rong J, Paterson AH. 2003. Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* 422:433–438. DOI: 10.1038/nature01521.

Brink RA, Williams E. 1973. Mutable R-navajo alleles of cyclic origin in maize. *Genetics* 73:273–296.

Chen ZJ, Scheffler BE, Dennis E, Triplett BA, Zhang T, Guo W, Chen X, Stelly DM, Rabinowicz PD, Town CD, Arioli T, Brubaker C, Cantrell RG, Lacape J-M, Ulloa M, Chee P, Gingle AR, Haigler CH, Percy R, Saha S, Wilkins T, Wright RJ, Van Deynze A, Zhu Y, Yu S, Abdurakhmonov I, Katageri I, Kumar PA, Mehboob-ur-Rahman, Zafar Y, Yu JZ, Kohel RJ, Wendel JF, Paterson AH. 2007a. Toward Sequencing Cotton (Gossypium) Genomes. *Plant Physiology* 145:1303–1310. DOI: 10.1104/pp.107.107672.

Chen ZJ, Scheffler BE, Dennis E, Triplett BA, Zhang T, Guo W, Chen X, Stelly DM, Rabinowicz PD, Town CD, Arioli T, Brubaker C, Cantrell RG, Lacape J-M, Ulloa M, Chee P, Gingle AR, Haigler CH, Percy R, Saha S, Wilkins T, Wright RJ, Van Deynze A, Zhu Y, Yu S, Abdurakhmonov I, Katageri I, Kumar PA, Mehboob-ur-Rahman, Zafar Y, Yu JZ, Kohel RJ, Wendel JF, Paterson AH. 2007b. Toward Sequencing Cotton (Gossypium) Genomes. *PLANT PHYSIOLOGY* 145:1303–1310. DOI: 10.1104/pp.107.107672.

Deutsch M, Long M. 1999. Intron-exon structures of eukaryotic model organisms. *Nucleic Acids Research* 27:3219–3228. DOI: 10.1093/nar/27.15.3219.

Dong G, Shen J, Zhang Q, Wang J, Yu Q, Ming R, Wang K, Zhang J. 2018. Development and Applications of Chromosome-Specific Cytogenetic BAC-FISH Probes in S. spontaneum. *Frontiers in Plant Science* 9. DOI: 10.3389/fpls.2018.00218.

Du X, Huang G, He S, Yang Z, Sun G, Ma X, Li N, Zhang X, Sun J, Liu M, Jia Y, Pan Z, Gong W, Liu Z, Zhu H, Ma L, Liu F, Yang D, Wang F, Fan W, Gong Q, Peng Z, Wang L, Wang

X, Xu S, Shang H, Lu C, Zheng H, Huang S, Lin T, Zhu Y, Li F. 2018. Resequencing of 243 diploid cotton accessions based on an updated A genome identifies the genetic basis of key agronomic traits. *Nature Genetics* 50:796–802. DOI: 10.1038/s41588-018-0116-x.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* 32:1792–7. DOI: 10.1093/nar/gkh340.

Ellinghaus D, Kurtz S, Willhoeft U. 2008. LTRharvest, an efficient and flexible software for de novo detection of LTR retrotransposons. *BMC Bioinformatics* 9. DOI: 10.1186/1471-2105-9-18.

Feschotte C. 2008. Transposable elements and the evolution of regulatory networks. *Nature reviews. Genetics* 9:397–405. DOI: 10.1038/nrg2337.

Finnegan DJ. 1989. Eukaryotic transposable elements and genome evolution. *Trends in Genetics* 5:103–107. DOI: 10.1016/0168-9525(89)90039-5.

Gan Y, Liu F, Peng R, Wang C, Li S, Zhang X, Wang Y, Wang K. 2012. Individual chromosome identification, chromosomal collinearity and genetic-physical integrated map in Gossypium darwinii and four D genome cotton species revealed by BAC-FISH. *Genes & genetic systems* 87:233–41.

Goldschmidt RB. 2002. Marginalia to McClintock's Work on Mutable Loci in Maize. *The American Naturalist* 84:437–455. DOI: 10.1086/281640.

Grover CE, Kim HR, Wing RA, Paterson AH, Wendel JF. 2004. Incongruent patterns of local and global genome size evolution in cotton. *Genome Research* 14:1474–1482. DOI: 10.1101/gr.2673204.

Hawkins JS, Kim HR, Nason JD, Wing RA, Wendel JF. 2006. Differential lineage-specific amplification of transposable elements is responsible for genome size variation in Gossypium. *Genome Research* 16:1252–1261. DOI: 10.1101/gr.5282906.

Hendrix B, Stewart JM. 2005. Estimation of the nuclear DNA content of Gossypium species. *Annals of Botany* 95:789–797. DOI: 10.1093/aob/mci078.

Ibarra-Laclette E, Lyons E, Hernández-Guzmán G, Pérez-Torres CA, Carretero-Paulet L, Chang TH, Lan T, Welch AJ, Juárez MJA, Simpson J, Fernández-Cortés A, Arteaga-Vázquez M, Góngora-Castillo E, Acevedo-Hernández G, Schuster SC, Himmelbauer H, Minoche AE, Xu S, Lynch M, Oropeza-Aburto A, Cervantes-Pérez SA, De Jesús Ortega-Estrada M, Cervantes-Luevano JI, Michael TP, Mockler T, Bryant D, Herrera-Estrella A, Albert VA, Herrera-Estrella L. 2013. Architecture and evolution of a minute plant genome. *Nature*

498:94–98. DOI: 10.1038/nature12132.

Jurka J, Kapitonov V V., Pavlicek A, Klonowski P, Kohany O, Walichiewicz J. 2005. Repbase Update, a database of eukaryotic repetitive elements. *Cytogenetic and Genome Research* 110:462–467. DOI: 10.1159/000084979.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120. DOI: 10.1007/BF01731581.

Koike A, Nakai K, Takagi T. 2002. The Origin and Evolution of Eukaryotic Protein Kinases. *Genome Letters* 1:83–104. DOI: 10.1166/gl.2002.010.

Koonin E V., Csuros M, Rogozin IB. 2013. Whence genes in pieces: Reconstruction of the exon-intron gene structures of the last eukaryotic common ancestor and other ancestral eukaryotes. *Wiley Interdisciplinary Reviews: RNA* 4:93–105. DOI: 10.1002/wrna.1143.

Kurtz S. 2003. The Vmatch large scale sequence analysis software. *Ref Type: Computer Program*.

Li F, Fan G, Lu C, Xiao G, Zou C, Kohel RJ, Ma Z, Shang H, Ma X, Wu J, Liang X, Huang G, Percy RG, Liu K, Yang W, Chen W, Du X, Shi C, Yuan Y, Ye W, Liu X, Zhang X, Liu W, Wei H, Wei S, Huang G, Zhang X, Zhu S, Zhang H, Sun F, Wang X, Liang J, Wang J, He Q, Huang L, Wang J, Cui J, Song G, Wang K, Xu X, Yu JZ, Zhu Y, Yu S. 2015. Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. *Nature Biotechnology* 33:524–530. DOI: 10.1038/nbt.3208.

Li F, Fan G, Wang K, Sun F, Yuan Y, Song G, Li Q, Ma Z, Lu C, Zou C, Chen W, Liang X, Shang H, Liu W, Shi C, Xiao G, Gou C, Ye W, Xu X, Zhang X, Wei H, Li Z, Zhang G, Wang J, Liu K, Kohel RJ, Percy RG, Yu JZ, Zhu YX, Wang J, Yu S. 2014. Genome sequence of the cultivated cotton gossypium arboreum. *Nature Genetics* 46:567–572. DOI: 10.1038/ng.2987.

Liu Z, Liu Y, Liu F, Zhang S, Wang X, Lu Q, Wang K, Zhang B, Peng R. 2018. Genome-wide survey and comparative analysis of long terminal repeat (LTR) retrotransposon families in four gossypium species. *Scientific Reports* 8. DOI: 10.1038/s41598-018-27589-6.

Liu Y, Peng R, Liu F, Wang X, Cui X, Zhou Z, Wang C, Cai X, Wang Y, Lin Z, Wang K. 2016. A Gossypium BAC clone contains key repeat components distinguishing sub-genome of allotetraploidy cottons. *Molecular Cytogenetics* 9. DOI: 10.1186/s13039-016-0235-y.

Liu X, Zhao B, Zheng HJ, Hu Y, Lu G, Yang CQ, Chen JD, Chen JJ, Chen DY, Zhang L, Zhou

Y, Wang LJ, Guo WZ, Bai YL, Ruan JX, Shangguan XX, Mao YB, Shan CM, Jiang JP, Zhu YQ, Jin L, Kang H, Chen ST, He XL, Wang R, Wang YZ, Chen J, Wang LJ, Yu ST, Wang BY, Wei J, Song SC, Lu XY, Gao ZC, Gu WY, Deng X, Ma D, Wang S, Liang WH, Fang L, Cai CP, Zhu XF, Zhou BL, Chen ZJ, Xu SH, Zhang YG, Wang SY, Zhang TZ, Zhao GP, Chen XY. 2015. Gossypium barbadense genome sequence provides insight into the evolution of extra-long staple fiber and specialized metabolites. *Scientific Reports* 5. DOI: 10.1038/srep14139.

Lu H, Cui X, Liu Z, Liu Y, Wang X, Zhou Z, Cai X, Zhang Z, Guo X, Hua J, Ma Z, Wang X, Zhang J, Zhang H, Liu F, Wang K. 2018. Discovery and annotation of a novel transposable element family in Gossypium. *BMC Plant Biology* 18. DOI: 10.1186/s12870-018-1519-7.

M.Lesk A. 2002. Introduction to Bioinformatics Introduction to Bioinformatics VSN-S. *Introduction to Bioinformatics* 2002:1–16.

Ma J, Bennetzen JL. 2004. Rapid recent growth and divergence of rice nuclear genomes. *Proceedings of the National Academy of Sciences* 101:12404–12410. DOI: 10.1073/pnas.0403715101.

Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY, Bryant SH. 2017. CDD/SPARCLE: Functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research* 45:D200–D203. DOI: 10.1093/nar/gkw1129.

McCLINTOCK B. 1950. The origin and behavior of mutable loci in maize. *Proceedings of the National Academy of Sciences of the United States of America* 36:344–55.

Morgante M. 2006. Plant genome organisation and diversity: the year of the junk! *Current Opinion in Biotechnology* 17:168–173. DOI: 10.1016/j.copbio.2006.03.001.

Munoz-Lopez M, Garcia-Perez J. 2010. DNA Transposons: Nature and Applications in Genomics. *Current Genomics* 11:115–128. DOI: 10.2174/138920210790886871.

Paterson AH, Wendel JF, Gundlach H, Guo H, Jenkins J, Jin D, Llewellyn D, Showmaker KC, Shu S, Udall J, Yoo MJ, Byers R, Chen W, Doron-Faigenboim A, Duke M V., Gong L, Grimwood J, Grover C, Grupp K, Hu G, Lee TH, Li J, Lin L, Liu T, Marler BS, Page JT, Roberts AW, Romanel E, Sanders WS, Szadkowski E, Tan X, Tang H, Xu C, Wang J, Wang Z, Zhang D, Zhang L, Ashrafi H, Bedon F, Bowers JE, Brubaker CL, Chee PW, Das S, Gingle AR, Haigler CH, Harker D, Hoffmann L V., Hovav R, Jones DC, Lemke C, Mansoor S, Rahman MU, Rainville LN, Rambani A, Reddy UK, Rong JK, Saranga Y,

Scheffler BE, Scheffler JA, Stelly DM, Triplett BA, Van Deynze A, Vaslin MFS, Waghmare VN, Walford SA, Wright RJ, Zaki EA, Zhang T, Dennis ES, Mayer KFX, Peterson DG, Rokhsar DS, Wang X, Schmutz J. 2012. Repeated polyploidization of Gossypium genomes and the evolution of spinnable cotton fibres. *Nature* 492:423–427. DOI: 10.1038/nature11798.

Piegu B, Guyot R, Picault N, Roulin A, Saniyal A, Kim H, Collura K, Brar DS, Jackson S, Wing RA, Panaud O. 2006. Doubling genome size without polyploidization: Dynamics of retrotransposition-driven genomic expansions in Oryza australiensis, a wild relative of rice. *Genome Research* 16:1262–1269. DOI: 10.1101/gr.5290206.

Del Pozo JC, Ramirez-Parra E. 2015. Whole genome duplications in plants: An overview from Arabidopsis. *Journal of Experimental Botany* 66:6991–7003. DOI: 10.1093/jxb/erv432.

R Core Team. 2014. R Language Definition V. 3.1.1. *https://www.r-project.org/*:Accessed Nov 2015.

Sanmiguel P, Bennetzen JL. 1998. Evidence that a recent increase in maize genome size was caused by the massive amplification of intergene retrotransposons. *Annals of Botany* 82:37–44. DOI: 10.1006/anbo.1998.0746.

Saranga Y. 2007. Special Issue: A century of wheat research - from wild emmer discovery to genome analysis. *Israel Journal of Plant Sciences* 55:207–313.

Seberg O, Petersen G, Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH. 2009. Reply: A unified classification system for eukaryotic transposable elements should reflect their phylogeny. *Nature Reviews Genetics* 10:276. DOI: 10.1038/nrg2165-c4.

Senchina DS, Alvarez I, Cronn RC, Liu B, Rong J, Noyes RD, Paterson AH, Wing RA, Wilkins TA, Wendel JF. 2003. Rate variation among nuclear genes and the age of polyploidy in Gossypium. *Molecular Biology and Evolution* 20:633–643. DOI: 10.1093/molbev/msg065.

Singh GB. 2015. Introduction to bioinformatics. In: *Modeling and Optimization in Science and Technologies*. 3–10. DOI: 10.1007/978-3-319-11403-3-1.

Treangen TJ, Salzberg SL. 2012. Repetitive DNA and next-generation sequencing: Computational challenges and solutions. *Nature Reviews Genetics* 13:36–46. DOI: 10.1038/nrg3117.

Wang K, Guo W, Zhang T. 2007. Detection and mapping of homologous and homoeologous segments in homoeologous groups of allotetraploid cotton by BAC-FISH. *BMC Genomics*

8. DOI: 10.1186/1471-2164-8-178.

Wang K, Huang G, Zhu Y. 2016. Transposable elements play an important role during cotton genome evolution and fiber cell development. *Science China Life Sciences* 59:112–121. DOI: 10.1007/s11427-015-4928-y.

Wang M, Tu L, Yuan D, Zhu D, Shen C, Li J, Liu F, Pei L, Wang P, Zhao G, Ye Z, Huang H, Yan F, Ma Y, Zhang L, Liu M, You J, Yang Y, Liu Z, Huang F, Li B, Qiu P, Zhang Q, Zhu L, Jin S, Yang X, Min L, Li G, Chen LL, Zheng H, Lindsey K, Lin Z, Udall JA, Zhang X. 2019. Reference genome sequences of two cultivated allotetraploid cottons, Gossypium hirsutum and Gossypium barbadense. *Nature Genetics* 51:224–229. DOI: 10.1038/s41588-018-0282-x.

Wang K, Wang Z, Li F, Ye W, Wang J, Song G, Yue Z, Cong L, Shang H, Zhu S, Zou C, Li Q, Yuan Y, Lu C, Wei H, Gou C, Zheng Z, Yin Y, Zhang X, Liu K, Wang B, Song C, Shi N, Kohel RJ, Percy RG, Yu JZ, Zhu YX, Cang J, Yu S. 2012. The draft genome of a diploid cotton Gossypium raimondii. *Nature Genetics* 44:1098–1103. DOI: 10.1038/ng.2371.

Wang KB, Wang WK, Wang CY, Song GL, Cui RX, Li SH, Zhang XD. 2001. [Studies of FISH and karyotype of Gossypium barbadense]. *Yi chuan xue bao = Acta genetica Sinica* 28:69–75.

Wang Q, Wang S, Zhu X, Fang L, Hu Y, Chen X, Huang X, Du X, Chen S, Wan Q, Guo W, Chen J, Liu C, Han B, Chen H, Li X, Pan M, Chen ZJ, Mei G, Chang L, Wu H, Huang T, Xiang D, Wang Y, Cai C, Liu B, Zhou B, Zhang T, Gong H, Fang DD. 2017. Genomic insights into divergence and dual domestication of cultivated allotetraploid cottons. *Genome Biology* 18. DOI: 10.1186/s13059-017-1167-5.

WANG K, WENDEL JF, HUA J. 2018. Designations for individual genomes and chromosomes in Gossypium. *Journal of Cotton Research* 1. DOI: 10.1186/s42397-018-0002-1.

Wendel JF, Cronn RC. 2001. Polyploidy and the evolutionary history of cotton. *Advances in Agronomy* 78:139–186. DOI: 10.1016/S0065-2113(02)78004-8.

Wendel JF, Flagel LE, Adams KL. 2012. Jeans, genes, and genomes: Cotton as a model for studying polyploidy. In: *Polyploidy and Genome Evolution*. 181–207. DOI: 10.1007/978-3-642-31442-1_10.

Wessler SR. 2006. Transposable elements and the evolution of eukaryotic genomes. *Proceedings of the National Academy of Sciences* 103:17600–17601. DOI: 10.1073/pnas.0607612103.

Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P,

Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH. 2007. A unified classification system for eukaryotic transposable elements. *Nature Reviews Genetics* 8:973–982. DOI: 10.1038/nrg2165.

Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH. 2008. A universal classification of eukaryotic transposable elements implemented in Repbase. *Nature Reviews Genetics* 9:414–414. DOI: 10.1038/nrg2165-c2.

Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH. 2009. Reply: A unified classification system for eukaryotic transposable elements should reflect their phylogeny. *Nature Reviews Genetics* 10:276–276. DOI: 10.1038/nrg2165-c4.

Xie Y, Dong F, Hong D, Wan L, Liu P, Yang G. 2012. Exploiting comparative mapping among Brassica species to accelerate the physical delimitation of a genic male-sterile locus (BnRf) in Brassica napus. *Theoretical and Applied Genetics* 125:211–222. DOI: 10.1007/s00122-012-1826-6.

Yuan D, Tang Z, Wang M, Gao W, Tu L, Jin X, Chen L, He Y, Zhang L, Zhu L, Li Y, Liang Q, Lin Z, Yang X, Liu N, Jin S, Lei Y, Ding Y, Li G, Ruan X, Ruan Y, Zhang X. 2015. The genome sequence of Sea-Island cotton (Gossypium barbadense) provides insights into the allopolyploidization and development of superior spinnable fibres. *Scientific Reports* 5. DOI: 10.1038/srep17662.

Zhang T, Hu Y, Jiang W, Fang L, Guan X, Chen J, Zhang J, Saski CA, Scheffler BE, Stelly DM, Hulse-Kemp AM, Wan Q, Liu B, Liu C, Wang S, Pan M, Wang Y, Wang D, Ye W, Chang L, Zhang W, Song Q, Kirkbride RC, Chen X, Dennis E, Llewellyn DJ, Peterson DG, Thaxton P, Jones DC, Wang Q, Xu X, Zhang H, Wu H, Zhou L, Mei G, Chen S, Tian Y, Xiang D, Li X, Ding J, Zuo Q, Tao L, Liu Y, Li J, Lin Y, Hui Y, Cao Z, Cai C, Zhu X, Jiang Z, Zhou B, Guo W, Li R, Chen ZJ. 2015. Sequencing of allotetraploid cotton (Gossypium hirsutum L. acc. TM-1) provides a resource for fiber improvement. *Nature Biotechnology* 33:531–537. DOI: 10.1038/nbt.3207.

Zhang X, Tolzmann CA, Melcher M, Haas BJ, Gardner MJ, Smith JD, Feagin JE. 2011. Branch point identification and sequence requirements for intron splicing in plasmodium falciparum. *Eukaryotic Cell* 10:1422–1428. DOI: 10.1128/EC.05193-11.

Figure 1(on next page)

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.

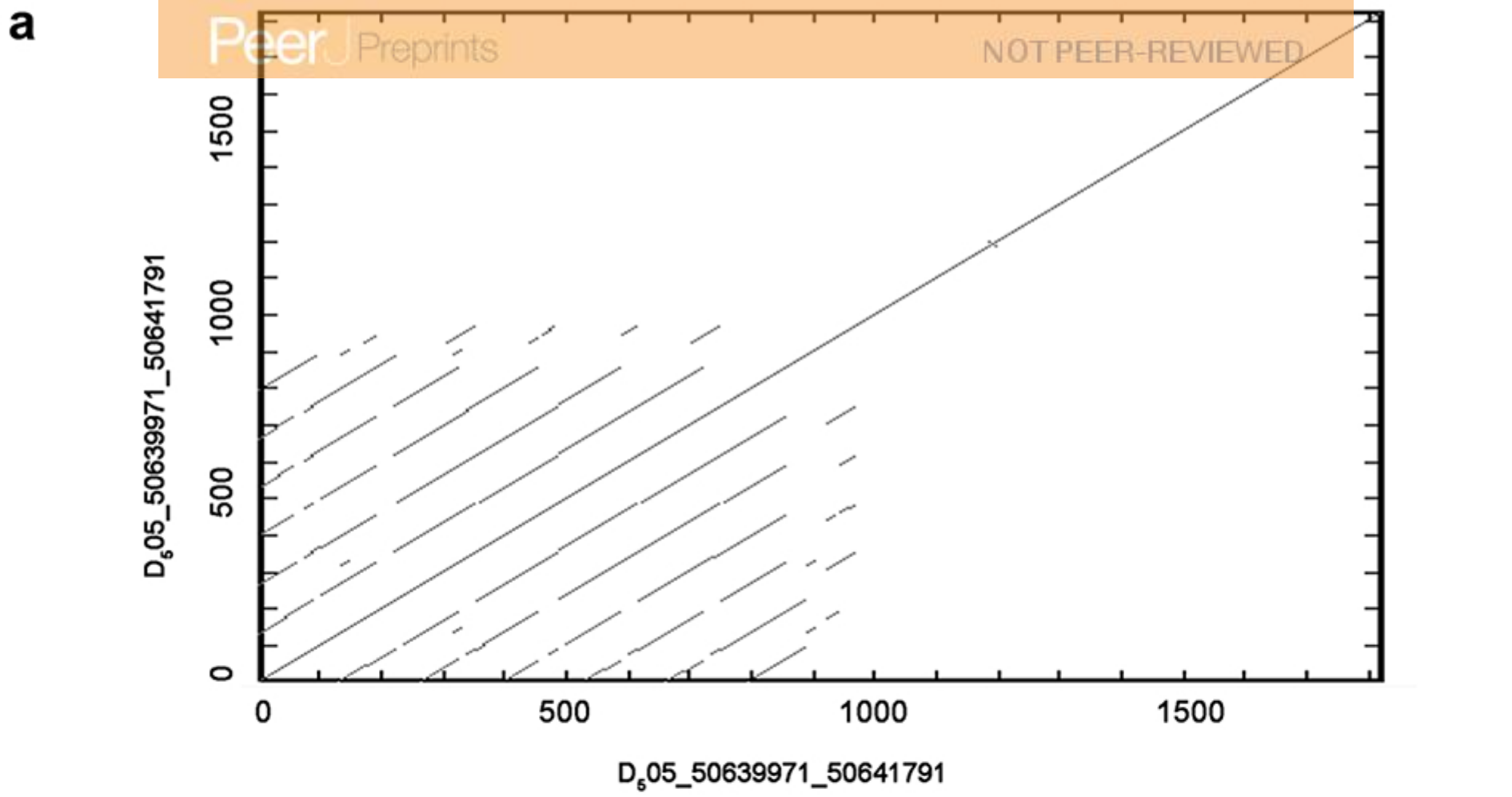


Figure 2

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.

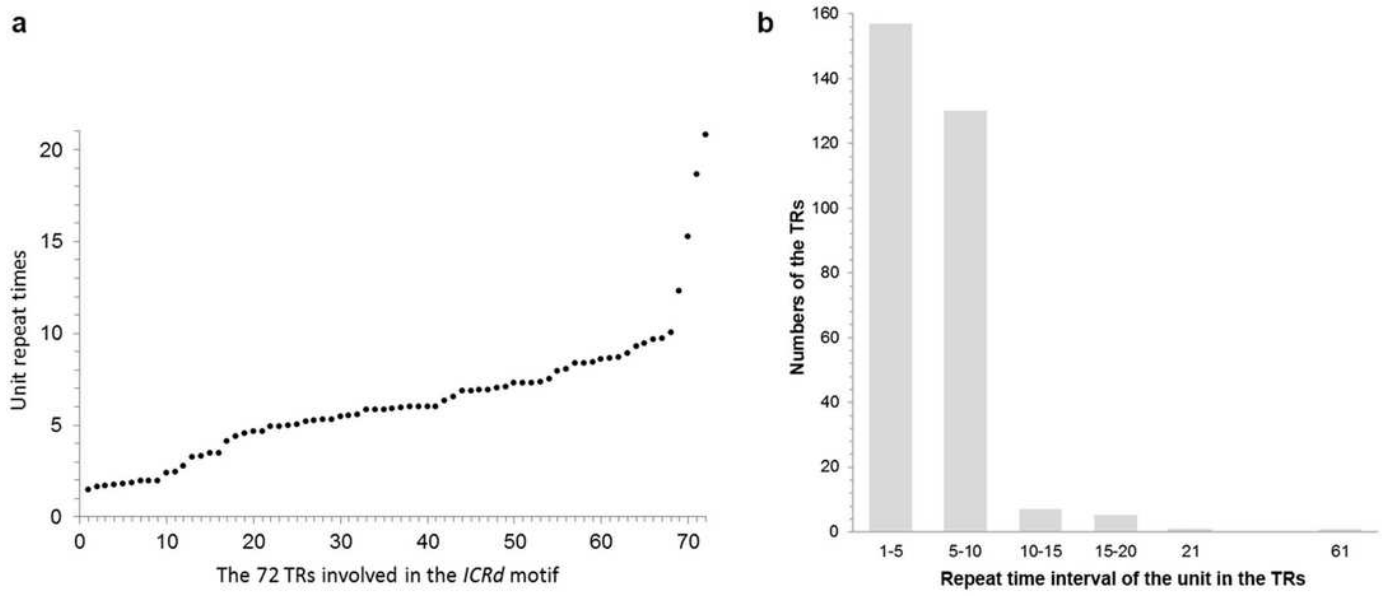
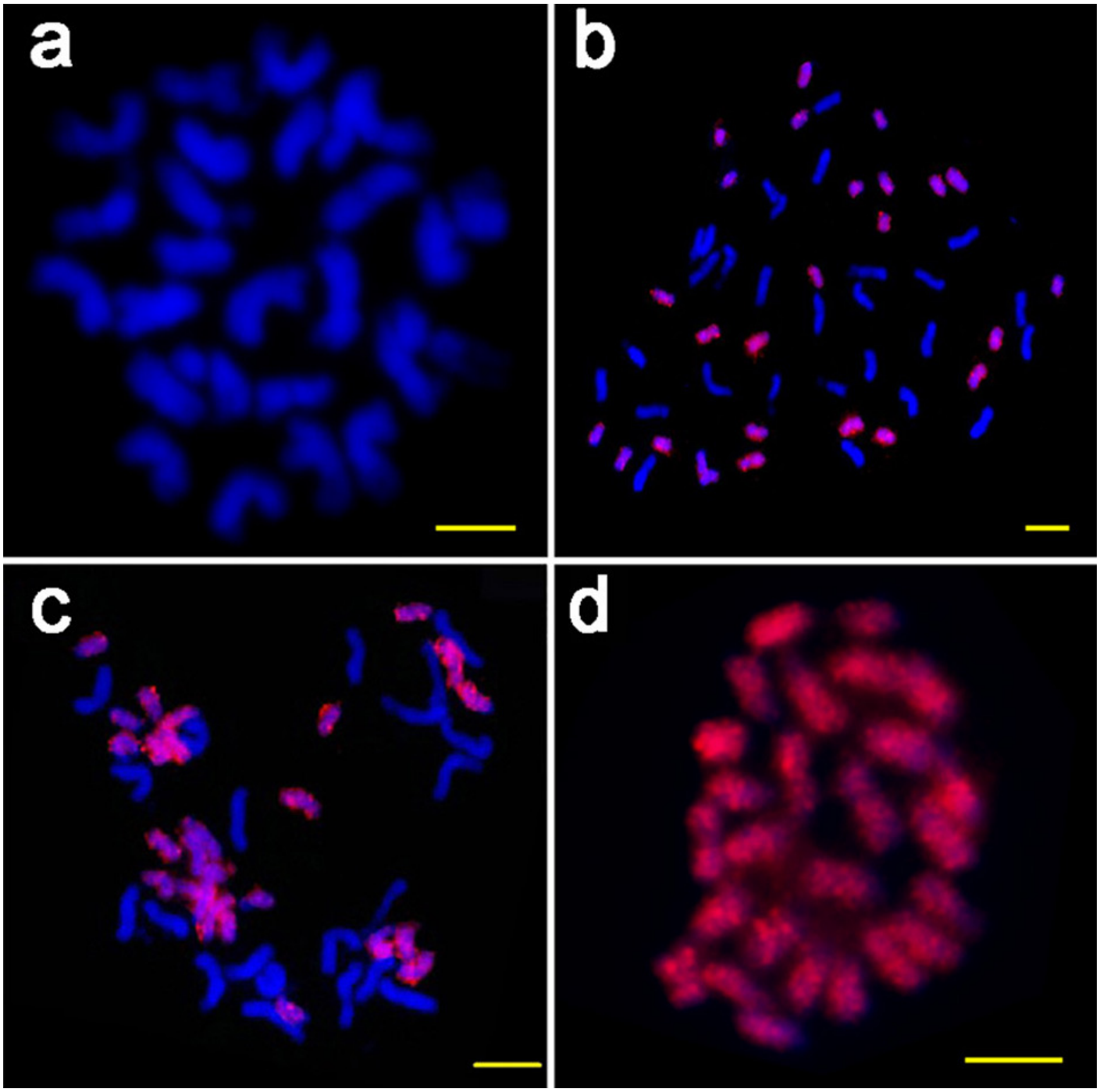


Figure 3

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.



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27806v1 | CC BY 4.0 Open Access | rec: 16 Jun 2019, publ: 16 Jun 2019

Figure 4(on next page)

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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27806v1 | CC BY 4.0 Open Access | rec: 16 Jun 2019, publ: 16 Jun 2019

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

Figure 5

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

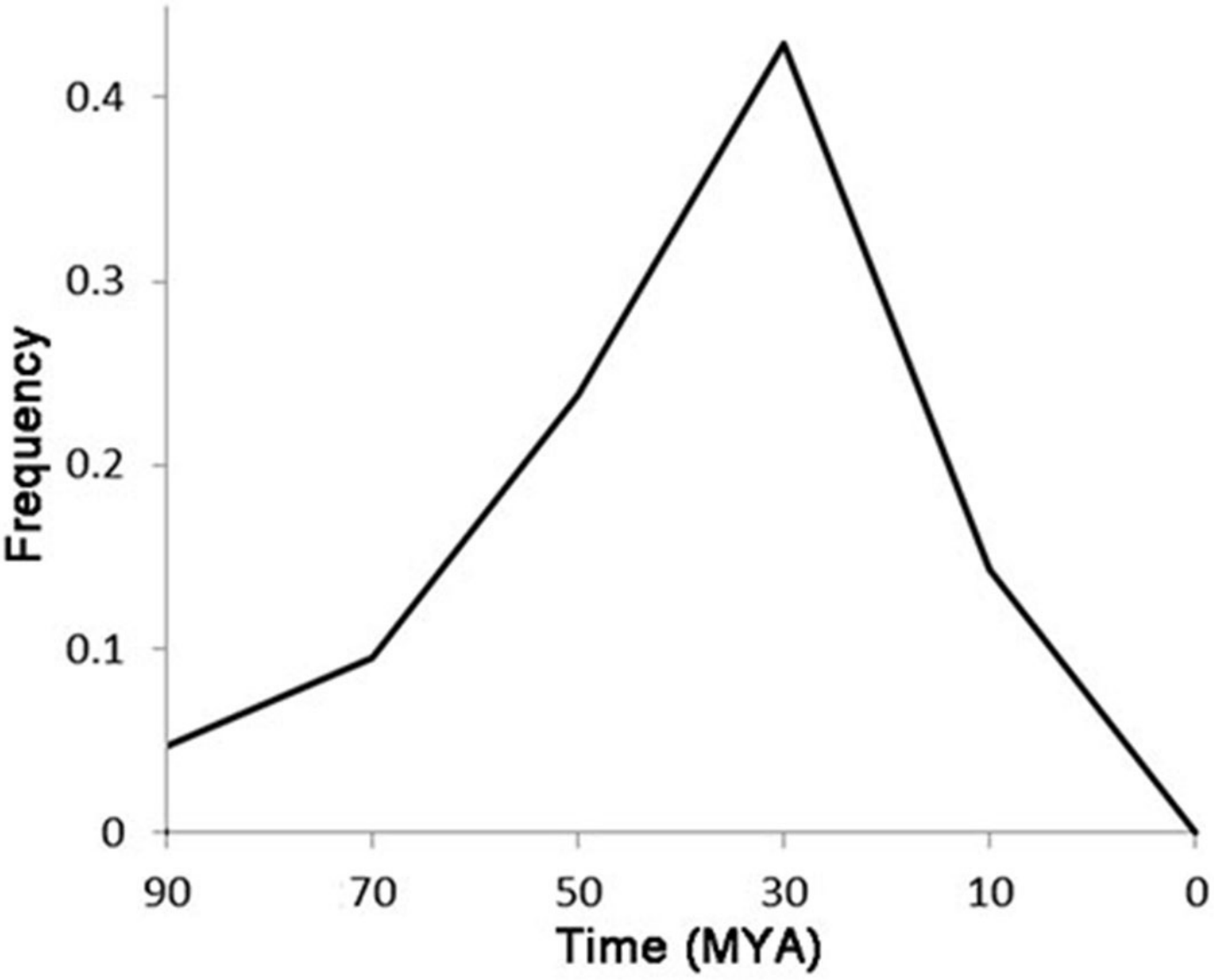


Figure 6(on next page)

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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27806v1 | CC BY 4.0 Open Access | rec: 16 Jun 2019, publ: 16 Jun 2019

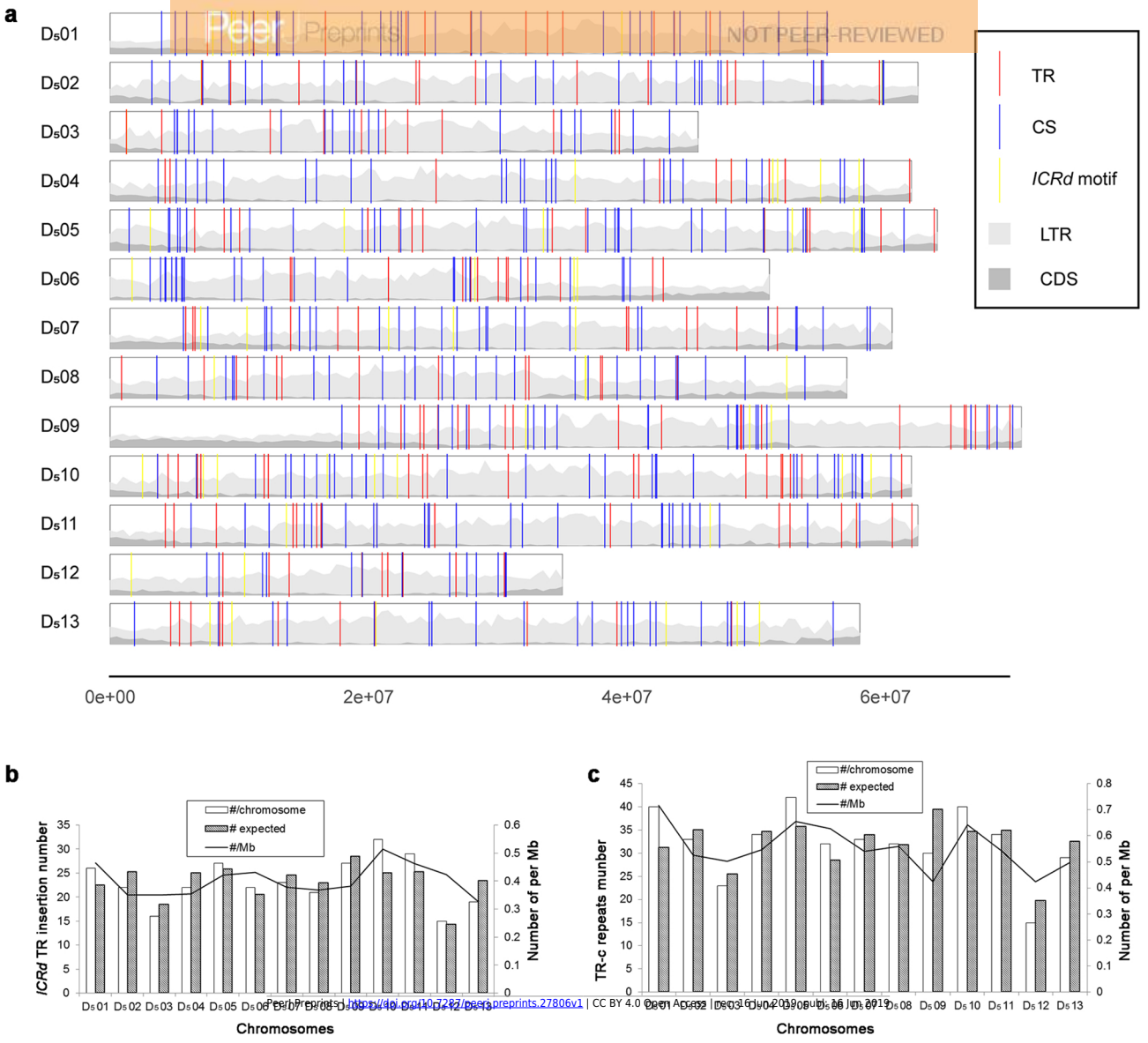
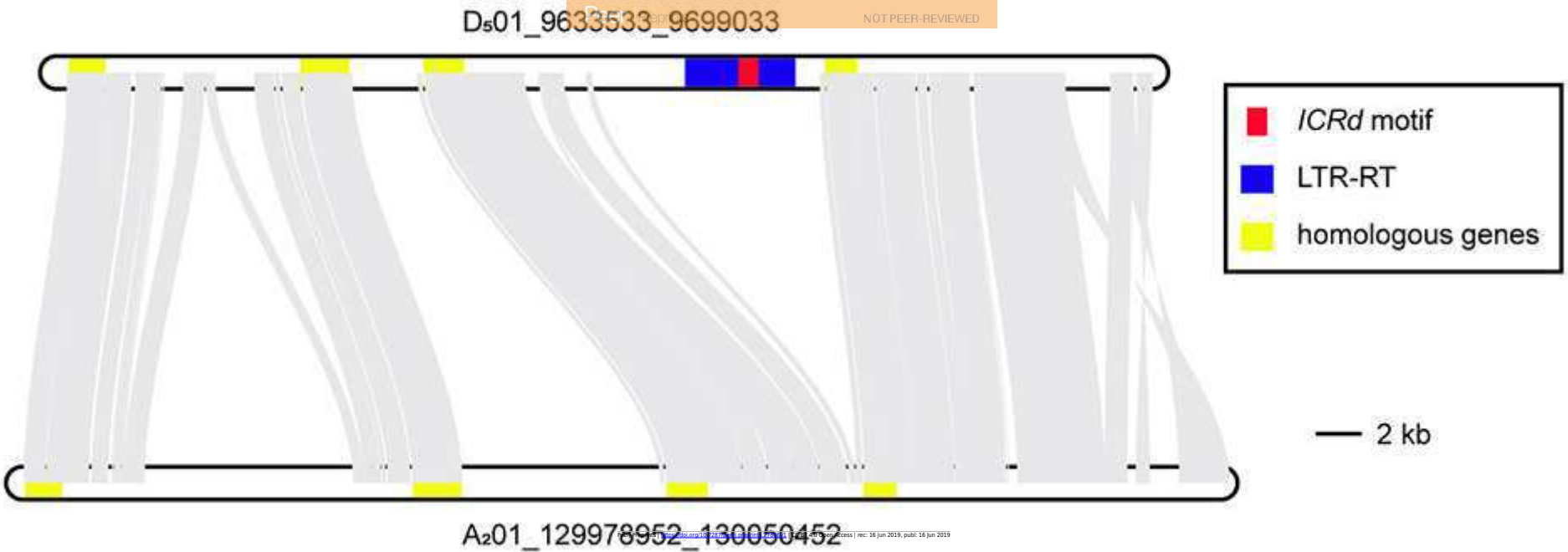


Figure 7 (on next page)

The colinearity of the two homologous segments.



doi: https://doi.org/10.1101/... | rec: 16 Jun 2019, publ: 16 Jun 2019