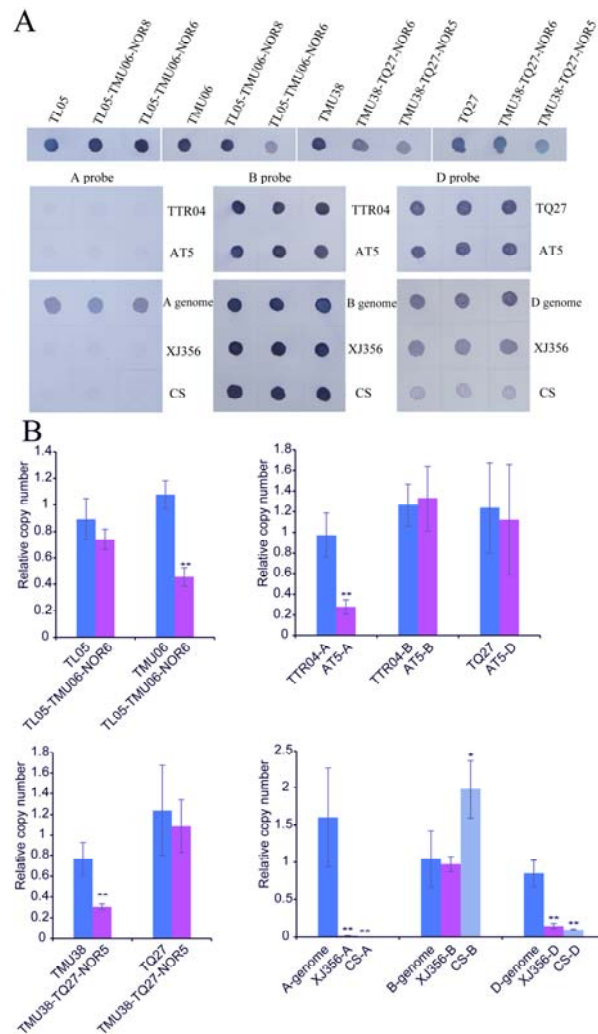


Supplemental Figure 1. FISH Analysis of pHvG38 in the Amphidiploid TL05 × TMU06.

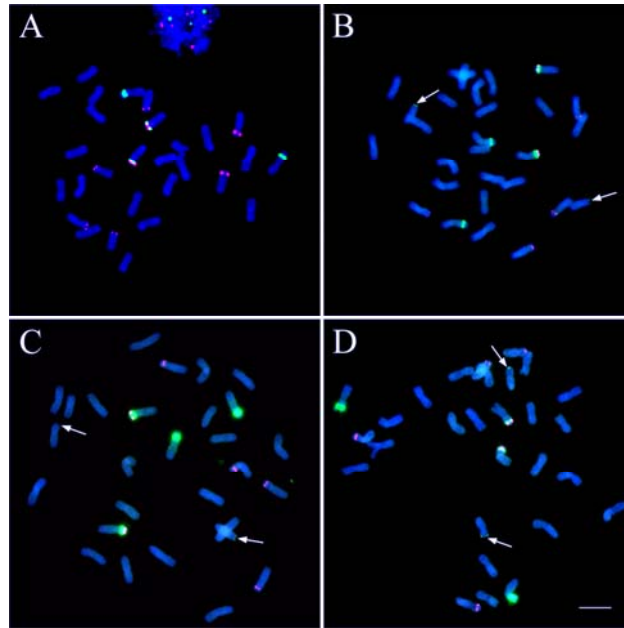
Distribution of pHvG38 signals (green) is studied on the metaphase chromosomes in diploid paternal lines TL05 (A) and TMU06 (B), in synthetic amphidiploid S1 (C), S4 (D), S5 (E) generations, and the natural tetraploid wheat Langdon (F). Bar=10 μ m.



Supplemental Figure 2. Dot-blot and qPCR Analysis of rDNA Copy Number in in Different Natural and Synthetic Amphidiploid Wheat.

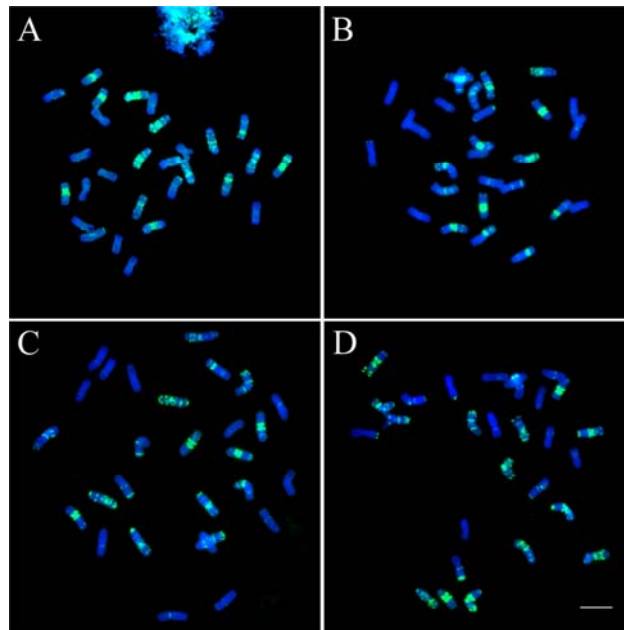
(A) Dot-blot analysis of rDNA copy number changes in amphidiploid TL05 × TMU06, TMU38 × TQ27, synthetic (AT5), semi-wild (XJ356) and natural hexaploid wheat (CS). NOR8 and NOR6 present the amphidiploid containing eight and six NOR loci, respectively. Specific PCR products of NOR promoter from A, B, D genome were used as A, B and D probes.

(B) qPCR analysis of rDNA copy number changes. -A, -B and -D present copy number of A, B and D genomes. The columns and error bars represent the mean relative level and SD, respectively. Each line had three biological replications. Differences between lines were compared by Student's t test. P values: *P < 0.05 and **P < 0.01, respectively.

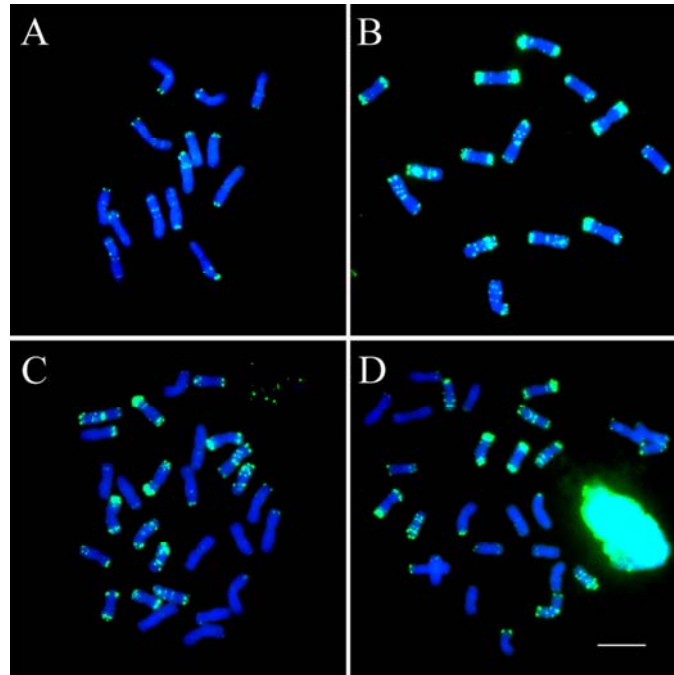


Supplemental Figure 3. FISH Analysis of rDNA Distribution on Somatic Metaphase Chromosomes in Natural Tetraploid Wheat.

The 45S (green) and 5S (red) rDNA loci are detected in *T. dicoccoides* (TTD14), *T. durum* (13-1), *T. turgidum* (AS2255) and *T. polonicum* (PI286547), respectively, in (A) to (D). The NORs from the A genome are indicated by white arrows. Bar=10 μ m.

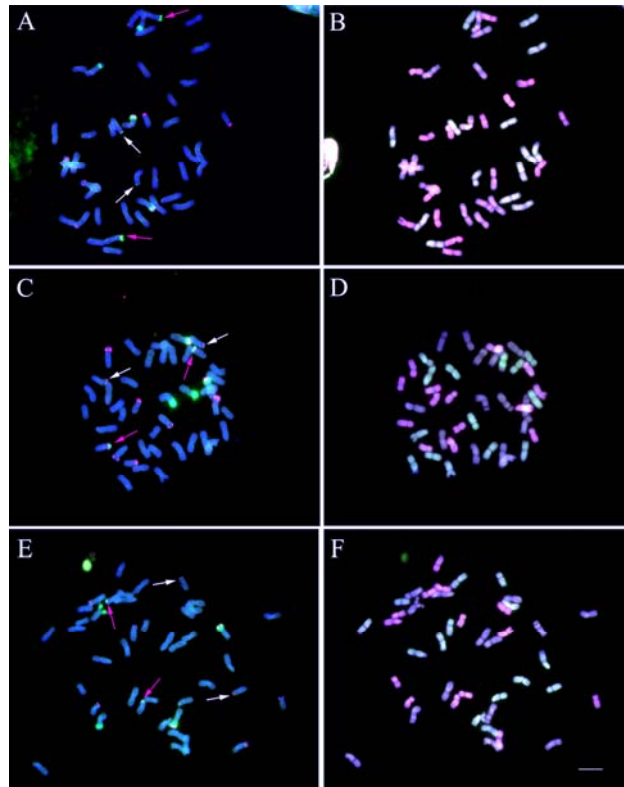


Supplemental Figure 4. FISH Analysis of pHvG38 in Natural Tetraploid Wheat. pHvG38 signals (green) are detected on the metaphase chromosomes in *T. dicoccoides* (A), *T. durum* (B), *T. turgidum* (C) and *T. polonicum* (D). Bar=10 μ m.



Supplemental Figure 5. FISH Analysis of pAs1 in the Amphidiploid TMU38 × TQ27.

pAs1 signals (green) are detected in TMU38 (A), TQ27 (B), S3 (C) and S4 (D) generation of TMU38 × TQ27. Bar=10 μm.



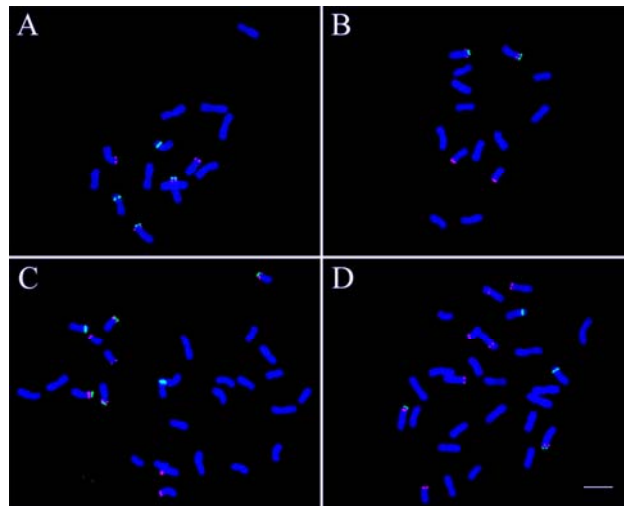
Supplemental Figure 6. FISH and Multicolor FISH Analysis of Somatic Metaphase Chromosomes in Hexaploid Wheat.

In (A), (C) and (E), 45S rDNA sequences are labeled in green, and 5S rDNA sequences are labeled in red. DAPI is blue. The red and white arrows indicate the 45S signals from the D genome and the A genome, respectively. The other 45S signals are from the B genome. In (B), (D) and (F), the A genome DNA is labeled in green, the D genome DNA is labeled in red and the B genome DNA is used as a block. Bar=10 μm .

(A) and (B) FISH and multicolor FISH of synthetic hexaploid wheat 960. This line shows strong 45S signals from the B and D genomes.

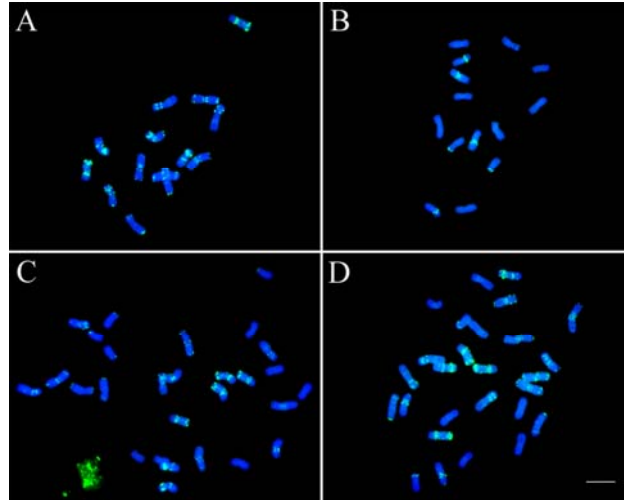
(C) and (D) FISH and multicolor FISH of semi-wild hexaploid wheat AS329. This line has major 45S loci in the B genome and minor 45S loci in the D and A genomes.

(E) and (F) FISH and multicolor FISH of Natural hexaploid wheat Jing411. This line shows major 45S loci in the B genome and minor 45S loci in the D and A genomes.



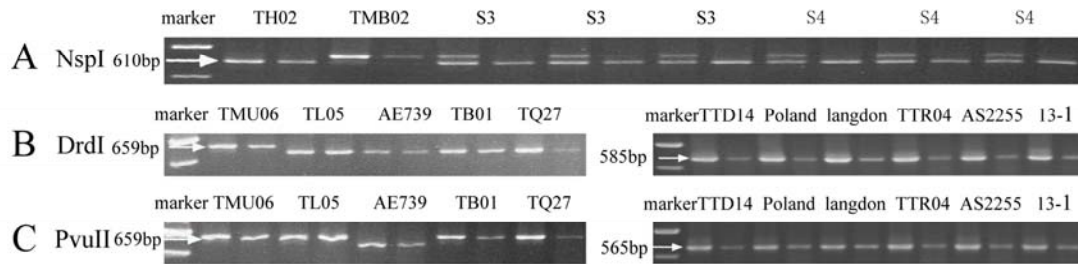
Supplemental Figure 7. FISH Analysis of rDNA Distribution on Somatic Metaphase Chromosomes in Amphidiploid TB01 × TQ27.

The 45S (green) and 5S (red) rDNA loci are shown in diploid maternal lines TB01 (A) and TQ27 (B), and in the amphidiploid TB01 × TQ27 in S3 (C) and S4 generations (D). Bar=10 μm.



Supplemental Figure 8. FISH Analysis of pHvG38 in the Amphidiploid TB01 × TQ27.

The pHvG38 signals (green) is detected on metaphase chromosomes in TB01 (A), TQ27 (B), and the S3 (C) and S4 (D) generations of the synthetic amphidiploid. Bar=10 μ m.



Supplemental Figure 9. Analysis of the Expression Pattern of rRNA Genes in Natural Tetraploid Wheat.

In each set (two lanes per set), samples were derived from products amplified from DNA template (the first lane) and RNA template (cDNA, the second lane), and subsequently were examined by digestion with restriction enzymes. The restriction enzymes used for analysis are indicated on the left in different lines.

(A) The digested product by *NspI* from parental lines TH02, TMB02 and the S4 generation of the amphidiploid.

(B) The digested product by *DrdI* from parental lines TMU06, TL05, AE739, TB01, TQ27 and natural tetraploid wheat TTD14, poland, langdon, TTR04, AS2255 and 13-1.

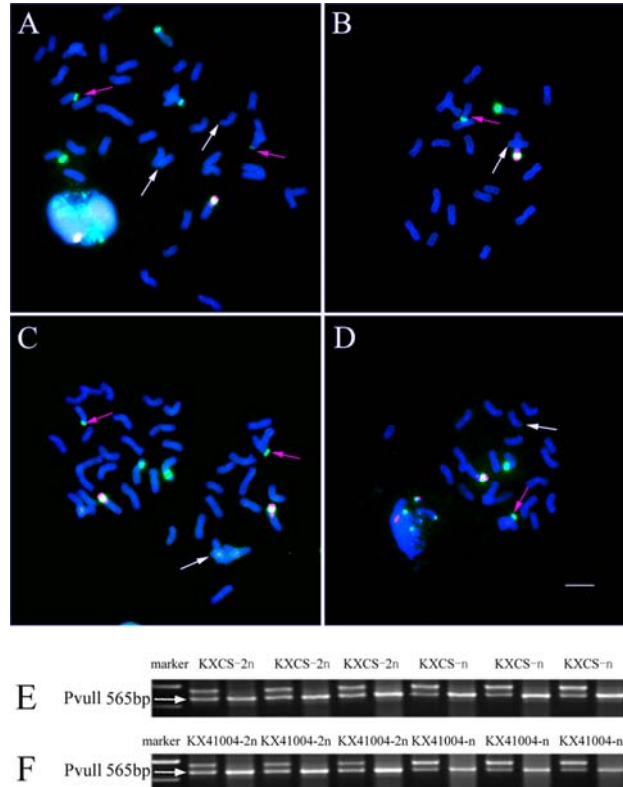
(C) The digested product by *PvuII* from parental lines TMU06, TL05, AE739, TB01, TQ27 and natural tetraploid wheat TTD14, poland, langdon, TTR04, AS2255 and 13-1.

Supplemental Data. Guo et al. (2014). Plant Cell 10.1105/tpc.114.129841

A_IGS.SEQ	CCCCACACCTCTCCTTCATCCACTTAGGTTGAGATGCTA	44
B_IGS.SEQ	CCCCACAACTCTCCTTCACCAACTAAGGTTGAGATATGG	44
D_IGS.SEQ	CCCCACAACTCTCCTTCACCAACTAAGGTTCCAAAATGG	44
Consensus	ccccaca ctctccttca c a t aggttc a	
A_IGS.SEQ	CAAGAAAATTCTACATGCCTAAGTCATGGTAAAAGAAAT	84
B_IGS.SEQ	TTCCGAAATTCTCCACCCTAAGTCATGGTAAAAGAAAT	84
D_IGS.SEQ	TAGCGAAATTCTCACTCTAAGTCATGGTAAAAGAAAT	84
Consensus	c aaattct ca ctaagtcatggt aaaagaat	
A_IGS.SEQ	GGCAAAGAC.....	93
B_IGS.SEQ	GGCAAAGCCCTTGTAAGACTTAAGCAATCACCCGATAAG	124
D_IGS.SEQ	GGCAAAGCCCTTGTAAGACATAAGCAAGCACCCGATAAG	124
Consensus	ggcaaaag c	
A_IGS.SEQ	93
B_IGS.SEQ	GCCAGCGGAAACAACACTCAAACTATATGTGCCAAATGA	164
D_IGS.SEQ	GCCAGCGGAAACAACACTCAAACTATATGTGCCAAATGA	164
Consensus		
A_IGS.SEQGATTCATGTCATGGCATCGTCACA	118
B_IGS.SEQ	CCAAGATACTTGGCCGATTCATGCGCATGCCGTCGTACA	204
D_IGS.SEQ	CCAAGATACTTGGCCGATTCATGCGCATGCCGTCGTACA	204
Consensus	gattcatgt c atggcatcg tc caca	
A_IGS.SEQ	GGCTACACG.....	127
B_IGS.SEQ	GGCTACACGCCCTAAGTCATGGTCAAGCAAATGGTAAAGT	244
D_IGS.SEQ	GGCTACACGCCCTAAGTCATGGTCAAGCAAATGGTAAAGT	244
Consensus	ggctacacg	
A_IGS.SEQGACCGTAA	136
B_IGS.SEQ	CCCTTATATGACATATGCAATCACTCCATAAGCCAGTGG	284
D_IGS.SEQ	CCCTTATATGACATATGCAATCACTCCATAAGCCAGTGG	284
Consensus	g cc gt	
A_IGS.SEQ	CGGCTG.....GTATGAGCAAGGGAAGAAAABA	165
B_IGS.SEQ	CGAGCACACTCAAACTATATCTGCAAGTGACCAAGAAA	324
D_IGS.SEQ	CGAGCACACTCAAACTATATCTGCAAGTGACCAAGAAA	324
Consensus	cg g tatg g caag ga aa a a	
A_IGS.SEQ	TTC.....TCGACGCC.....	177
B_IGS.SEQ	CTTGACCGATTTCATGCGGATGCCCTTCGTCCAGGCTACAC	364
D_IGS.SEQ	CTTGACCGATTTCATGCGGATGCCCTTCGTCCAGGCTACAC	364
Consensus	t c ga gcc	
A_IGS.SEQGTCGTGGACGGAGC.....	191
B_IGS.SEQ	GGCTAAGTCTTGGTCAAGCAAATGGTAAAGTCCCTTATA	404
D_IGS.SEQ	GGCTAAGTCTTGGTCAAGCAAATGGTAAAGTCCCTTATA	404
Consensus	gtc tgg c c	
A_IGS.SEQ	191
B_IGS.SEQ	TGACATACACAATCACTCGATAAGGCCAGTCGCGAGCACA	444
D_IGS.SEQ	TGACATACGCAATCACTCGATAAGGCCAGTCGCGAGCACA	444
Consensus		
A_IGS.SEQTGGAC.	196
B_IGS.SEQ	CTCAAACTATTTGTGCCAAGTGACCAAGATACTTGGCC	484
D_IGS.SEQ	CTCAAACTATTTGTGC. AAGTGACCAAGACACTTGGCTG	483
Consensus	tgg	
A_IGS.SEQACCCCAATGA	208
B_IGS.SEQ	ATTCATACATGGGATGTATCACAACGAAGCTTTATCGG	524
D_IGS.SEQ	ATTCATACATGTGATGTATCACAAGAACTTTAAAG	523
Consensus	a g g a g	
A_IGS.SEQ	AAACAGGGCAAAG.....C	223
B_IGS.SEQ	AGACAGGGCCAAAGATCATGGACGGAAGTGGACGCGCAGC	564
D_IGS.SEQ	AGACAGGGCCAAAGATCATGGACGGAAGTGGACGCGCAGC	563
Consensus	a aca gg aa c	
A_IGS.SEQ	ATATACGATTCACGA...TCGGTACAGGACCCAG	256
B_IGS.SEQ	ATGGACAACCTAGGCAAAACCAAGTACACGAACTCTT	600
D_IGS.SEQ	ATGGAAAACCTAGGCAAAACCAAGTACACGAACTCTT	599
Consensus	at a at c a cgtaca ac c	

Supplemental Figure 10. Sequence Alignments of IGS Regions from Different Parental Genomes.

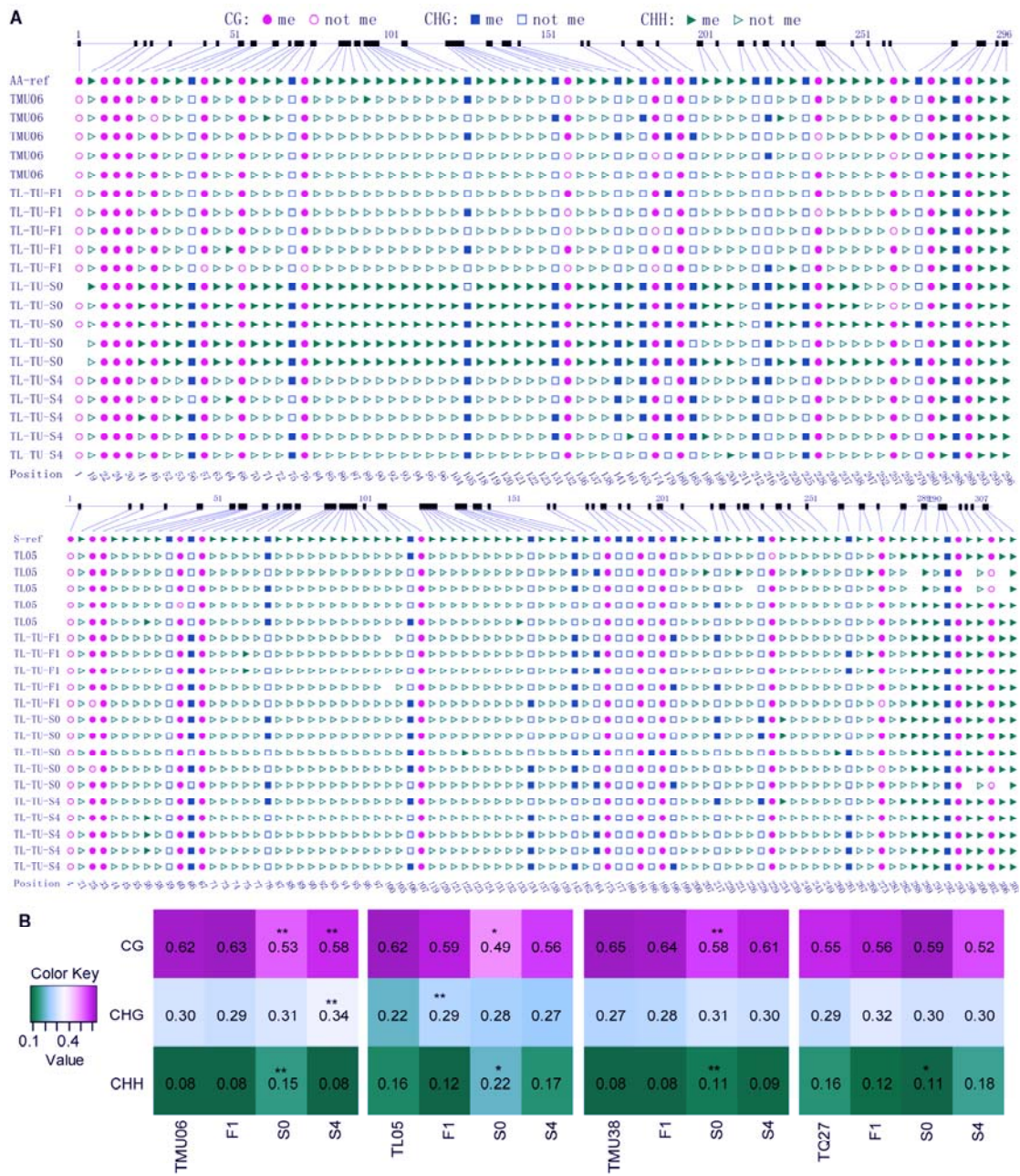
A 425-bp repetitive sequence is indicated by red arrows and is only found in the B and D genomes, not the A genome.



Supplemental Figure 11. Analysis of the Distribution and Expression of rRNA Genes in K-salmon × CS, K-salmon × 41004 and their Haploid Lines.

The distribution of NOR loci are shown in the hybrid of K-salmon × CS (A), a haploid induced by K-salmon × CS (B), K-salmon × 41004 (C) and a haploid induced by K-salmon × 41004 (D). 45S rDNA sequences are labeled in green, rye genomic DNA is labeled in red, and DAPI is blue. The red and white arrows indicate the 45S signals from the D and A genomes, respectively. Bar=10 μm.

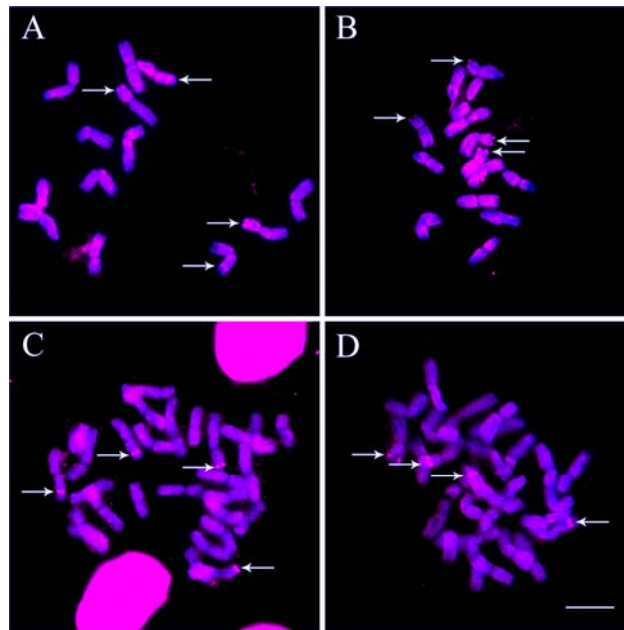
Analysis of expression pattern of rRNA genes in K-salmon × CS, K-salmon × 41004 and their haploid lines (E-F). The products amplified from K-salmon × CS and its haploid lines, and were examined by digestion with *PvuII* (E). The products amplified from K-salmon × 41004 and its haploid lines, and were examined by digestion with *PvuII* (F).



Supplemental Figure 12. Analysis of DNA Methylation and relative DNA methylation ratio in Amphidiploids TL05 × TMU06 and TMU38 × TQ27.

(A) Bisulfite sequencing analysis of DNA methylation changes in amphidiploids TL05 × TMU06 at a sequence level. Five clones were randomly selected from 90 clones for each sample.

(B) Changes of relative DNA methylation (the ratio of CG, CHG and CHH methylation in total DNA methylation) in the parental lines and their two amphidiploids.



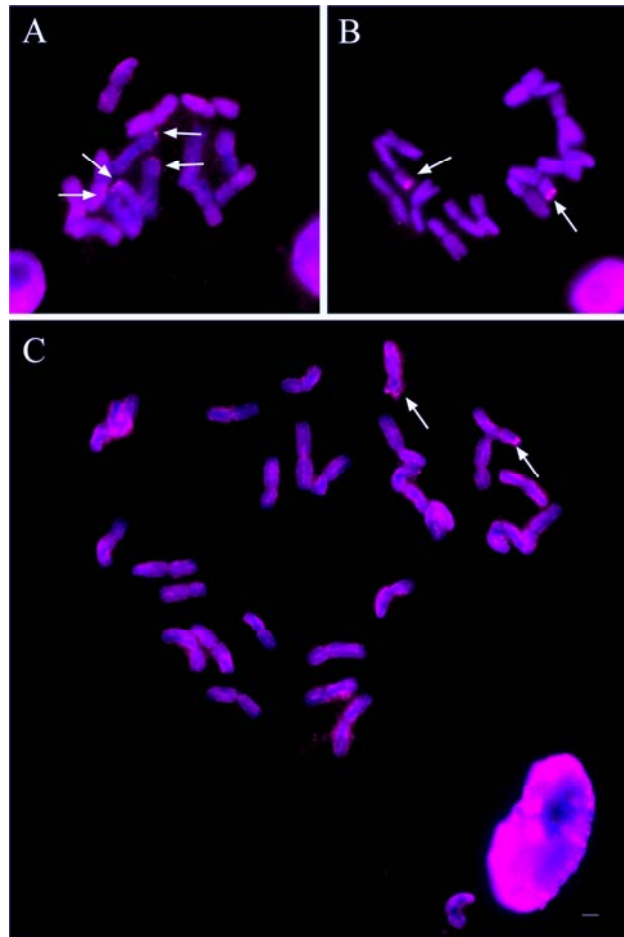
Supplemental Figure 13. Immunolocalization Analysis of H4K12 Acetylation in the Amphidiploid TL05 × TMU06.

(A) Maternal line TL05. The four NORs are accompanied by strong H4K12ac modification, as indicated by the arrows. The H4K12 acetylation signals are red and DAPI is blue in all panels. Bar=10 μ m.

(B) Paternal line TMU06. The four NORs are accompanied by strong H4K12ac modification.

(C) S4 generation of the amphidiploid TL05 × TMU06. The NORs from TL05 are enriched for H4K12ac modification, and those from TMU06 exhibit decreased H4K12ac modification.

(D) Natural tetraploid wheat Langdon. The major NORs show high levels of H4K12ac modification.



Supplemental Figure 14. Immunolocalization Analysis of H4K12 Acetylation on Metaphase Chromosomes of the Amphidiploid TMU38 × TQ27.

H4K12ac modification (red) is detected on metaphase chromosomes in TMU38 (A), TQ27 (B) and the S4 generation of the amphidiploid TMU38-TQ27 (C). Bar=10 μ m.

Supplemental Table 1. Primers Used in This Study

Primer name	Primer sequence
ITS-F	CTGCGGAAGGATCATTGTCG
ITS-R	TGCTTAAACTCAGCGGGTAGTC
IGS-F	GCCGGATTATGACTGAACGCCTCTA
IGS-R	GAGCCATTCGCAGTTTCACAGTTCA
TIS-F-AA	CAAGGTGTTTCGGGAAAAACG
TIS-R-AA	CGTTGGGAAGGAGTTGGGCT
TIS-F-AA-Bisulfite	TAAGGTGTTTGGGAAAAATG
TIS-R-AA-Bisulfite	TGTTGGGAAGGAGTTGGGTT
TIS-F-BB	GTACGAGAGCTCCGGGAGGA
TIS-R-BB	CGTTGGGAAAGGATGGGCAT
IGS-B repeat-F	AATCACCCGATAAGGCCAG
IGS-B repeat-R	TGGCCGTGTCTCCGATAAC