# **Supporting Information**

Zhang et al. 10.1073/pnas.1110552108

## Supporting Information Corrected December 29, 2011

#### **SI Materials and Methods**

**BAC Library Screening and Sequencing of the** Q/q Locus. Q/q-containing BACs from the A and B genome of *Triticum turgidum* cv. LDN (1), and from *Aegilops tauschii* accession AL8/78 (2) were isolated by Southern hybridization using probe Tmap2 as previously described (3). One *T. turgidum* BAC of each type was sequenced by Myriad Genetics, and one *Ae. tauschii* BAC was sequenced at the Washington University Genome Sequencing Center. All other BACs were isolated using the PCR method with Q/q-specific primers (Table S4). Q/q-containing BACs from A, B, and D genomes of *Triticum aestivum* cvs. RE and CS were isolated from libraries described previously (4, 5). *Triticum urartu* (URA) and *Aegilops speltoides* (SPE) BACs where isolated from pooled libraries as described previously (6, 7). Shotgun sequencing of RE, CS, URA, and SPE BACs was performed at the Centre National de Sequencage as previously described (8).

Identification of Transposable Elements (TEs). TE annotation and prediction were essentially done as previously described (9). TEs were identified by BLASTn searches against two databases of repetitive elements: TREP (http://wheat.pw.usda.gov/ITMI/Repeats/ index.shtml) (10) and Repbase (http://www.girinst.org/Repbase Update.html) (11). Core domains (nucleic acid coordinates of known elements) were identified through BLASTn alignments against TREPn. Long-terminal repeats (LTRs) and limits were identified using BLASTn and CENSOR (12) alignments against Repbase and TREP databases. Putative polyproteins were identified by BLASTx alignments against TREPprot. No a priori cutoff was imposed for BLASTx and BLASTn. A complete reconstruction of nested insertions of TEs and split elements was conducted. TE prediction and classification followed the 80-80-80 rule recommended by the unified classification system for eukaryotic TEs: a query element belongs to the same family as a subject element referenced in Repbase or TREP if it is longer than 80 bp, and its sequence is at least 80% identical over at least 80% of their coding or internal domain, or in the terminal repeat regions, or both (13).

Novel TE families include elements that did not fit the 80-80-80 rule (13) showing weak or no similarity with the referenced TE families. Novel TEs were confirmed and analyzed by LTR STRUC software (14), and/or BLASTx similarity searches against the NCBI nr database (http://www.ncbi.nlm.nih.gov/), as well as using the DOTTER program (15). When possible (i.e., for complete TEs), target-site duplications were identified to confirm an insertion by transposition. Each BAC sequence was also analyzed using the DOTTER program to identify or confirm direct repeats, LTRs, local duplications, and deletion events as well as MITEs. TEs were named and classified according to the previously suggested nomenclature (i.e., element name, BAC name, discovery appearance rank) and designated as complete, truncated, or degenerated as previously suggested (13) with two exceptions: the Sukkula large retrotransposon derivatives (LARDs) were considered to be Gypsy-like because their LTRs showed sequence BLASTn similarity to Erika (Gypsy-like) TEs. We maintained the Athila designation for those TEs that were initially referenced in TREP (http:// wheat.pw.usda.gov/ITMI/Repeats/) to distinguish them from other Gypsy-like retrotransposons. They were designated Ga (Gypsy retrotransposons, which were initially designated as Athila) in the nomenclature and annotation files.

**Annotation of Other Repetitive DNA.** Short repeats were identified either as inverted repeats (by using EINVERTED with default parameters; http://bioweb.pasteur.fr/seqanal/interfaces/einverted.

html) or tandem repeats (Tandem Repeat Finder, with default parameters; http://tandem.bu.edu/trf/trf.advanced.submit.html. Repeats longer than 100 bp were included in the annotation files.

**Gene Structure Analysis.** Gene prediction analysis was conducted for the 18.5% non-TE and nonrepeated DNA, using the FGE-NESH gene prediction program (with the Monocot matrix) as well as BLASTn, BLASTx, and tBLASTx alignments against dbEST (http://www.ncbi.nlm.nih.gov/), SwissProt (http://expasy. org/sprot/), and the rice genome databases.

Estimation of Retrotransposon Insertion Dates. Retrotransposons with both 5' and 3' LTRs and target-site duplications (TSDs) were considered to correspond to original insertions. The number of transitions and transversions in pairs of LTRs was calculated using MEGA3 software (16). Retrotransposon insertion dates were calculated using the substitution rate of  $13 \times 10^{-8}$  substitutions/site/year (17). SEs were estimated using the formula T 1/4 K2P/2r (18). All retrotransposon insertion date estimates are presented in Table S1.

Primer Design and PCR-Based Analysis of Helitron-Insertion Haplotype Diversity. The Primer3 program (19) was used to design PCR primers (Table S4) on the basis of the sequence of gene–TE or TE-unassigned DNA junctions as previously described (20). Several pairs of primers were often used. PCR primers targeting internal TE sites were used in control amplifications. PCR amplification was carried out in a final volume of 10  $\mu$ L with 200 nM of each dNTP, 500 nM each of forward and reverse primers, and 0.2 units of Taq polymerase (Perkin-Elmer) using the following "touchdown" procedure: 14 cycles of 30 s at 95 °C, 30 s at 72 °C minus 1 °C for each cycle, 30 s at 72 °C followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C, and 1 cycle of 10 min at 72 °C.

cDNA Amplification and Sequencing. Total RNA was isolated from immature spikes of CS, LDN, and RE (with one-tenth of the length of the full-grown spike) using on-column digestion (RNAeasy Plant mini kit, Qiagen) following the manufacture's instructions. cDNA was prepared using TaqMan reverse transcription reagents with Oligo d(T)16 primer and MultiScribe Reverse Transcriptase (Applied Biosystems) as recommended by the manufacturer. 5Bq and 5Dq cDNAs were PCR amplified using primers based on the predicted LDN 5Bq and Ae. tauschii 5Dq cDNA sequences (Table S4). Gene-specific primers were designed from 5Bq and 5Dq cDNA sequences for rapid amplification of cDNA ends (RACE) using the BD SMART RACE cDNA amplification kit (BD Biosciences). The 5' RACE and 3' RACE ready cDNAs were prepared according to the manufacture's instructions and PCR amplified using primers shown in Table S4. Sequence alignments and amino acid sequence prediction were performed using CLUSTALW and SIXFRAME provided by San Diego Supercomputer Center (SDSC) biology workbench (http://workbench.sdsc.edu/).

**Transcript Analysis.** Steady-state mRNA level in immature spikes collected from CS, CS del143 (*5AQ* deleted), CS 5BL-14 (*5Bq* deleted), CS 5DL-5 (*5Dq* deleted), and the *5AQ*/5Dq double deletion line (CS-5A/5Ddd; both *5AQ* and *5Dq* deleted) was measured by relative quantitative (RQ)-PCR with primers for *5AQ*, *5Bq* (*5Bq.1* and *5Bq.2*), and *5Dq* (*5Dq.1* and *5Dq.2*) using a 7500 Real-Time PCR system (Applied Biosystems). CS-del143 was generated by fast-neutron mutagenesis and contains an interstitial

deletion of  $\sim 2$  Mb encompassing the 5AQ locus (3). CS 5BL-14 and CS 5DL-5 are terminal chromosome deletions that lack the distal 25 and 24% of the chromosome 5B and 5D long arms, respectively (21). CS-5A/5Ddd was generated by crossing CS-del143 and CS 5DL-5. A CS-Triticum dicoccoides 5A disomic chromosome substitution line CS-DIC 5A (22) was also included in the analysis. In CS-DIC 5A, the chromosome substitution replaces 5AQ with 5Aq. An illustration of the genetic structure of these stocks is presented in Fig. S5. The wheat actin gene (Table S4) was used as an internal control for all RQ-PCR experiments. All reactions were done in quadruplicate and each experiment was repeated three times. Specificity of primers designed for 5AQ, 5Bq, and 5Dq (Table S4) was verified using mRNA from the wheat deletion lines. A dissociation analysis was performed after each RQ-PCR assay to confirm that the amplification was specific. The amplification efficiency was tested for each gene using a previously described method (23).

**Phylogenetic Analysis.** DNA sequence alignments were created using ClustalX 2.0.12 (24) and further analyzed and edited, where necessary, using MacClade 4.08 (Sinauer Associates). Synonymous substitution rates and neighbor-joining phylogenetic trees were calculated using the Nei-Gojobori method with Jukes-Cantor correction for multiple substitutions as implemented in MEGA5 (16). All positions with gaps were excluded. CS 5Bq sequence was excluded from the initial calculations because of the large deletion in the gene and was added later assuming one observed synonymous difference from RE 5Bq. Divergence times of the Q/q genes were calculated using 11.6 Mya for divergence between barley and wheat (17). Errors of the substitution rate calculations in

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herent in the analyzed sequence data sets (multiple alignments), and divergence times derived from them, were evaluated by the bootstrap method (500 repetitions) implemented by MEGA5. MEGA5 was used to perform molecular clock (third codon position) and Tajima's relative rate tests. Pairwise nucleotide substitution rates for noncoding sequences were calculated using Microsoft Excel after manual parsing sequence alignments with all gap positions excluded. These calculations used a 1-kb window sliding in 1-bp steps.

Morphology and Trait Analysis. The genetic stocks CS, CS-DIC 5A, CS-del143, CS 5BL-14, CS 5DL-5, and CS-5A/5Ddd were grown in a completely randomized design with eight replications to evaluate various morphological and agronomic traits. Plants were grown in a greenhouse at 22-25 °C with a 16-h photoperiod. Eight plants for each genetic stock were grown with one plant per pot. Spike emergence time was measured from planting date to the date that the first spike completely emerged from the boot. Plant height from the soil surface to the tip of the tallest spike was measured when plants were mature (completely senesced) immediately before harvest. At the same time, spike length and number of spikelets per spike were measured for three spikes of each plant. Number of tillers, number of seeds per plant, 1,000-kernel weight, and grain yield per plant were also measured. Number of seeds per spike was calculated by dividing the number of seeds per plant by the number of tillers for the same plant. Spike morphology (square/speltoid) was assessed by manual inspection as previously described (22). Glumes were also manually inspected for shape, thickness, and rigidity. Spikes were threshed manually to evaluate threshability and glume/rachis disarticulation.

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**Fig. S1.** The amphiploidization events involved in the evolution of common bread wheat illustrated with photographs of spikes of species/cultivars used for BAC sequence analysis of the *Q/q* loci. Designation of accessions used in this research is indicated in blue. Taxonomical names, genome constitutions, and *Q* loci genotypes are shown in parentheses with strikethrough indicating a pseudogene.

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**Fig. 52.** Composition of the *Q/q* loci in wheat. Vertical numbers show an overall % identity of the overlapping sequences marked in gray, violet, and green. Diagonal numbers show TE insertion date estimates in million years ago (Mya). See also Table S1. Nineteen large intragenome insertions and deletions and breaks in genome colinearity marked with numbers are as follows: (1) 5,101–5,105 bp sequence repeated in tandem (blue arrows in the figure: an additional repetition in RE); (2) complete solo-LTR RLC *Angela* (insertion in polyploids); (3) complete *gypsy* RLG Fatima1 (recombined to solo-LTR in URA); (4) unassigned segment with complete DTM Argo (insertion in A genomes of polyploids); (5) newly identified complete CACTA (insertion in A genomes of polyploids); (6) complete *copia* RLC Maximus (insertion in URA); (7) unclassified TE (insertion in URA); (8) partial class II TE (insertion in all B genomes); (9) class II TE (insertion in all B genomes); (10) class II TE (insertion in all B genomes); (11) class I TE (insertion in B genome of RE); (12) break in colinearity, nonalignable (NA) sequences between B and S genomes; (13) Insertion of a complete helitron transposon in the B genome of RE; (14) class II TE (partial deletion in RE); (15) Tandem duplication of four copies of *copia* retrotransposon *Angela* in RE, through a mechanism of unequal homologous recombination; (16) complete *copia* retrotransposon RLC *Angela* (insertion in TAU); and (19) partial *copia* retrotransposon RLC Angela and complete *gypsy* retrotransposon RLG If is (insertion in RE and CS).



**Fig. S3.** Analysis of the 9,535-bp helitron inserted in opposite orientation in the *5Bq* gene of *T. aestivum* cv. RE. (A) Intron/exon structure of the helitron, predicting a putative gene of four exons (shown in green), where the putative RepHel protein is of 1,405 amino acids. A gene encoding a 128-amino-acid fragment of a glyoxalate reductase-like protein interrupted by a frameshift is also present in the helitron (shown in red) inserted in the same orientation as *q*. (*B*) Comparison of the 5'- and 3'-end sequences of the identified helitron shows a typical AT insertion site, the 5' TC, the 3' CTAG, and the sequences leading to the hairpin structure formation at 3' ends, show high conservation with rice and maize helitron consensus sequences. (*C*) Bootstrapped neighbor-joining tree of predicted RepHel proteins encoded by helitrons from various species [found in repbase (http://www.girinst.org/repbase/update/index.html)]. Reference helitron sequences and species are indicated. The protein encoded by the *T. aestivum* cv. RE *SBq* helitron is shown in the red box. It is 76% similar to RepHel encoded by Helitron4 Os of rice (*AC105746.1*) and 87% similar to a putative *Brachypodium distachyon* helicase (*Bradi5g20077*). The insertion site, presence of the RepHel-like gene, and fragments of other genes are characteristic features of helitrons (1, 2).

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**Fig. S4.** Pairwise comparisons of the noncoding parts of the *Q*/*q* genes and the neighboring intergenic regions. Nucleotide substitution rates in a 1-kb window sliding by 1 bp in *5AQ*/*q*, *5Bq*, and *5Dq* for different pairs of wheats are shown in *A*, *B*, and *C*, respectively. Introns, light gray; UTRs, dark gray; and conserved sequences at the 5' and 3' ends of the *Q*/*q* gene, medium gray. Red lines indicate average substitution rates for the marked region (intergenic or intron) and green lines indicate synonymous nucleotide substitution rates for the *Q*/*q* coding sequence.

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**Fig. S5.** Homoeologous group 5 chromosome constitutions for the genetic stocks used for *Q/q* expression and phenotypic analysis. Locations of homoeologous *Q/q* loci on the long arms of group 5 chromosomes are indicated by yellow stars. Absence of a *Q/q* locus is indicated by a red strike through. Black and gray regions along the chromosomes indicate dark and light cytological C bands, respectively.

TE name	Insertion date (Mya)	Divergence	Std error
A_ContigQRG1_Ale1.fasta	0.66	0.0172	0.0084
A_ContigQRG1_Fatima1.fasta	1.25	0.0326	0.0083
A_ContigQRG1_Melina1.fasta	1.07	0.0279	0.0048
A_ContigQRG1_Wis1.fasta	1.06	0.0276	0.004
A_ContigQURA_Ale1.fasta	0.66	0.0172	0.0083
A_ContigQURA_Claudia1.fasta	0.28	0.0073	0.0022
A_CS12224M17Q_Ale1.fasta	0.66	0.0172	0.0085
A_CS12224M17Q_Fatima1.fasta	1.25	0.0326	0.0083
A_CS12224M17Q_Melina.fasta	1.01	0.0263	0.0047
A_CS12224M17Q_Sabrina2.fasta	1.12	0.029	0.0046
A_CS12224M17Q_Wis1.fasta	1.06	0.0276	0.0039
A_LDN376H15_Angela1.fasta	0.36	0.0093	0.0025
A_LDN376H15_Angela2.fasta	1.95	0.0507	0.0055
A_LDN376H15_Fatima1.fasta	1.06	0.0275	0.0072
A_LDN376H15_Melina1.fasta	1.23	0.032	0.0053
A_LDN376H15_Sabrina1.fasta	1.49	0.0387	0.005
A_LDN376H15_Sabrina2.fasta	1.30	0.0338	0.005
A_LDN376H15_Sabrina3.fasta	0.93	0.0241	0.0039
A_LDN376H15_Wis1.fasta	1.02	0.0264	0.0039
B_1255P23QRG4_Angela1.fasta	1.11	0.0288	0.0046
B_1255P23QRG4_Derami1.fasta	2.42	0.0629	0.0067
B_1255P23QRG4_Jeli1.fasta	1.20	0.0312	0.0075
B_1255P23QRG4_Wis1.fasta	0.15	0.004	0.0016
B_1255P23QRG4_Wis2.fasta	0.33	0.0086	0.0022
B_CS102N4QF_Angela1.fasta	1.14	0.0296	0.0045
B_LDN1004P5_Angela1.fasta	1.11	0.0289	0.0043
B_LDN1004P5_Barbara1.fasta	1.28	0.0334	0.0043
B_LDN1004P5_Derami1.fasta	2.52	0.0654	0.0073
B_LDN1004P5_Fatima3.fasta	1.31	0.034	0.0079
B_LDN1004P5_Laura1.fasta	0.36	0.0094	0.0015
B_LDN1004P5_Wis1.fasta	0.33	0.0086	0.0022
D_AeT20P19_Angela2.fasta	1.08	0.0282	0.0043
D_AeT20P19_Angela3.fasta	1.02	0.0265	0.0037
D_AeT20P19_Barbara1.fasta	1.02	0.0266	0.0042
D_AeT20P19_Derami1.fasta	1.09	0.0283	0.0042
D_AeT20P19_Maximus1.fasta	1.01	0.0262	0.0041
D_AeT20P19_NewQ04.fasta	0.67	0.0174	0.0068
D_AeT20P19_Valerie1.fasta	0.88	0.023	0.0034
D_AeT20P19_Valerie2.fasta	0.03	0.0009	0.0006
D_ContigQRG5_Ifis1.fasta	1.17	0.0305	0.0077
D_ContigQRG5_Ifis2.fasta	0.55	0.0142	0.005
D_ContigQRG5_Maximus1.fasta	0.87	0.0225	0.0037
D_ContigQRG5_Sabrina1.fasta	0.53	0.0139	0.0031
D_ContigQRG5_Wis1.fasta	0.34	0.0088	0.0022
D_CS201F2QGD_Angela3.fasta	1.04	0.0271	0.0043
D_CS201F2QGD_Ifis1.fasta	0.52	0.0136	0.005
D_CS201F2QGD_Maximus1.fasta	0.84	0.0218	0.0038
D_CS201F2QGD_NewQ04.fasta	0.78	0.0204	0.0076
D_CS201F2QGD_Wis1.fasta	0.32	0.0082	0.0022
S_SH120_23G16_Quinta2.fasta	0.16	0.0041	0.0021
S_SH120_23G16_Quinta3.fasta	0.88	0.0228	0.0047
S_SH120_23G16_Quinta4.fasta	0.83	0.0217	0.0046

Table S1. Divergence and insertion dates estimated for LTR retrotransposons with both left andright LTRs, and target site duplications identified

Insertion dates were calculated using the substitution rate of 13 x 10-9 substitutions/site/year. SEs were estimated using the formula T 1/4 K2P/2r (1).

1. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120.

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### Table S2. Helitron is found only in hexaploid wheat cvs Renan and Mironovskaya 808

Helitron	
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Haplotype	Primers	21F / 21R	22F / 22R	32F / 32R	32F / 32bR
44 - AABBDD- <i>T. aestivum</i> cv. Arminda			1		1
45 - AABBDD-T. aestivum cv. Chinese Spring			1		1
46 - AABBDD- T. aestivum ssp. macha 1793N	1A86112		1		1
47 - AABBDD- T. aestivum ssp. compactum r	ufulum 71VREB68368		1		1
48 - AABBDD- T. aestivum ssp. compactum c	rebicum 72VREB68369		1		1
49 - AABBDD- T. aestivum ssp. compactum e	rinaceum 75VREB68372		1		1
50 - AABBDD- T. aestivum ssp. spaerococcun	n tumidum		1		1
perciv globosum REB77050					
51 - AABBDD-T. aestivum cv. Recital			1		1
52 - AABBDD- <i>T. aestivum</i> cv. Renan		1		1	
52 - AABBDD-T. aestivum cv. Mironovskaya	308	1		1	
53 - AABBDD-T. aestivum cv. Soisson			1		1
54 - AABBDD-T. aestivum ssp. spelta SP8130	0		1		1
55 - AABBDD- T. aestivum ssp. spelta SP8605	54		1		1
56 - AABBDD- <i>T. aestivum</i> cv. Vilmorin			1		1
13 - AABBDD- <i>T. aestivum</i> cv. Chopin			1		1
5 - AABB-T. turgidum ssp. dicoccoides DIC 1	18		1		1
6 - AABB- T. turgidum ssp. dicoccoides DIC 1	19		1		1
7 - AABB- T. turgidum ssp. dicoccoides DIC 1	20		1		1
8 - AABB- T. turgidum ssp. dicoccoides DIC 1	32		1		1
9 - AABB- T. turgidum ssp. dicoccoides DIC 1	96		1		1
10 - AABB- T. turgidum ssp. dicoccoides DIC	44		1		
11 - AABB- T. turgidum ssp. dicoccoides DIC	46		1		
12 - AABB- T. turgidum ssp. dicoccoides DIC	54		1		
13 - AABB- T. turgidum ssp. dicoccoides DIC	55		1		1
14 - AABB- T. turgidum ssp. dicoccoides DIC	87		1		4
15 - AABB-1. turgiaum ssp. aicoccum DIC 29	8		1		1
16 - AABB-1. turgiaum ssp. aicoccum DIM-0	-		1		1
17 - AABB-1. turgidum ssp. dicoccum DIM-1			1		1
10 AABB T turgidum ssp. ducucum HTTH01	5		1		1
20 - AABB-T. turgidum ssp. dicoccum TTC01			1		1
21 - AABB-T, turgidum ssp. dicoccum TTC02			1		1
22 - AABB-T. turgidum ssp. dicoccoides TTD	01		1		1
23 - AABB- T. turgidum ssp. dicoccoides TTD	03		1		1
24 - AABB- T. turgidum ssp. dicoccoides TTD	06		1		1
25 - AABB- T. turgidum ssp. dicoccoides TTD	09		1		1
26 - AABB- T. turgidum ssp. dicoccoides TTD	121		1		1
27 - AABB- T. turgidum ssp. dicoccoides TTD	125		1		1
28 - AABB- T. turgidum ssp. dicoccoides TTD	15		1		1
29 - AABB- T. turgidum ssp. dicoccoides TTD	20		1		1
30 - AABB- T. turgidum ssp. dicoccoides TTD	23		1		1
32 - AABB- T. turgidum ssp. dicoccoides TTD	48		1		1
33 - AABB- T. turgidum ssp. dicoccoides TTD	58		1		
34 - AABB- T. turgidum ssp. dicoccoides TTD	72		1		
35 - AABB- T. turgidum ssp. dicoccoides TTD	86		1		
36 - AABB- T. turgidum ssp. dicoccoides TTD	88		1		
37 - AABB-T T. turgidum ssp. dicoccoides TT	095		1		
38 - AABB-T. turgidum ssp. polinicum TTP03			1		
39 - AABB-T. turgidum ssp. durum TTR04			1		
40 - AABB-T. turgidum ssp. durum TTR05			1		
41 - AABB-T.turgidum ssp durum TTR16			1		1
42 - AABB-T. turgidum ssp durum TTR19			1		1
43 - AABB-T.turgidum ssp.turgidum TTT02			1		1
11 - AABB- T. turgidum ssp. durum cv. Lange	don		1		1
12 - AABB- T. turgidum ssp. durum cv. Cape	lli		1		1
1 - AA- <i>T. monococcum</i> TMB02			1		
2 - AA-T. urartu TMU38			1		
1 - AA - T. beoticum-no_ref			1		
2 - AA- T. beoticum-no_ref			1		
3 - AA- T. beoticum-no_ref			1		

PNAS PNAS

#### Table S2 Cont.

PNAS PNAS

	Helitron	+	-	+	-
Haplotype	Primers	21F / 21R	22F / 22R	32F / 32R	32F / 32bR
6 - AA- <i>T. urartu-</i> no_ref			1		
7 - AA- <i>T. beoticum-</i> no_ref			1		
8 - AA- <i>T. beoticum-</i> no_ref			1		
9 - AA- <i>T. urartu-</i> no_ref			1		
70 - AAGG-T. timopheevii ssp. armeniacum TIA02	2		1		
71 - AAGG-T. timopheevii ssp. armeniacum TIA06	6		1		
72 - AAGG- T. timopheevii ssp. armeniacum TIA2	.8		1		
73 - AAGG- T. timopheevii ssp. armeniacum TIA5	50		1		
74 - AAGG- T. timopheevii ssp. timopheevii TIM0	)1		1		
75 - AAGG- T. timopheevii ssp.timopheevii viticu	losum TIM05		1		
77 - AAGG- T. timopheevii ssp.timopheevii zhuk	(k38555) TIM07		1		
78 - AAGG- T. timopheevii TIM09			1		
79 - AAGG- T. timopheevii ssp. typicum TR103			1		
80 - AAGG-T. araraticumTR94			1		
81 - AAGG-T. araraticumTR95			1		
82 - AAGG-T. araraticumTR96			1		
83 - AAGG-T. araraticumTR99			1		
14 - AAGG- T T. timopheevii ssp. timopheevii var	r typica-no_ref		1		
15 - AAGG- T. timopheevii ssp. araticum var Schu	ulze-motel-no_ref		1		
16 - AAGG- T. timopheevii ssp. militinae -no_ref			1		
18 - AAGG- T. timopheevii ssp. timopheevii -no_r	ref		1		
84 - DD-Ae. kotschyi var. palaestina AEG 506_1			1		

Empty cells, no PCR amplification; cells showing number 1, PCR amplification confirming helitron insertion.

#### Table S3. Traits of genetic stocks with various combinations of Q/q homoeoalleles and chromosomal deletions including the Q/q locus

		Spike emergence (days)	Plant height (cm)	Yield components					
Wheat line <i>Q/q</i> genotype (AABBDD)	Spike length (mm)			Tillers/ plant	Spikelets/ spike	Seeds/ spike	Seed weight (mg)	Grain yield (g/plant)	
CS 5DL-5 (QQ <del>99</del> –)	67 (63–72)	114 (112–117)	102 (96–108)	11.4 (9.2–13.6)	20 (18.4–21.2)	12 (6–19)	20 (18–23)	3.0 [3.0] (1.2–4.7)	
CS (QQ <del>ባባ</del> qq)	72 (69–75)	80 (78–82)	118 (116–120)	10.3 (9.3–11.2)	25 (24.6–25.5)	36 (30–42)	31 (28–33)	11 [11] (9.6–13.0)	
CS 5BL-14 ( <i>QQ – qq</i> )	62 (57–67)	76 (72–79)	98 (88–107)	8.6 (7.1–10.2)	20 (18.4–21.8)	9 (4–14)	18 (16–19)	1.6 [1.4] (0.5–2.7)	
CS-5A/5Ddd (– <del>99</del> –)	81 (78–83)	113 (110–115)	110 (105–115)	12.1 (9.7–14.6)	17 (16.1–17.9)	6 (5–7)	18 (17–20)	1.4 [1.3] (1.0–1.7)	
CS-del143 (– <del>👎</del> qq)	101 (98–105)	73 (71–75)	123 (119–127)	10.8 (9.7–11.8)	25 (24.6–26.1)	37 (32–42)	29 (28–30)	12 [12] (9.8–13.3)	
CS-DIC 5A (qq 👎 qq)	110 (107–114)	68 (66–69)	129 (122–134)	9.9 (8.2–11.5)	28 (27.2–28.3)	47 (43–50)	29 (27–31)	13 [13] (11.6–14.7)	

9, pseudogene; -, chromosomal deletion including the Q/q locus; 95% confidence intervals are shown in parentheses; grain yield calculated on the basis of the yield components is shown in brackets.

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Primer Helitron haplotype diversity 21F 21R 22F 22R 32F 32R 32bR BAC library screening AP2start (FW) AP2.12R (RW) AP2.13R (RW) 5Bq cDNA amplification 5BqF2 5BqR2 5Dq cDNA amplification 5Dq1bF 5Dq3aR 5Bq RACE 5Bq5'race1 5Bq3'race1 5Dq RACE 5Dq5′race3 5Dq3'race1 5AQ/q RQ-PCR RQAQ/q.F1 RQAQ/qR2 5Bq RQ-PCR RQBSF1 ROBSR1 5Dg RQ-PCR 5D3'race1 Dq3'UTR.R2 Wheat Actin RQ-PCR Actin.F Actin.R 5Bq 2-bp deletion analysis 5Bq2bp.del.F1 5Bq2bp.del.R3

CTGATGATGATGCGTGATTT TACTGTATATGGCATCTGATTAGAG AGGAGCACACGCAAGTAGTACCTCT CTACAAAGGGCATGATCGAAC GCAGGTAATCATCTAAGCTACTATAAGC GGATTGGAGCGAGTGTAAGATTTC CAGCTTGTGAATTTCTTTTCAACGTAATCTTGTA ATGGTGCTGGATCTCAATGTGGAGTCGCCGGCGGA CAAAGTACCTTGCAGCTTCAACTTCGCTGTCAA CTCTTGGGATCGTGCGCGGTGGGTTGCGACATC TGCTTAACTCCGCGGACGCCGGCGGCTT GTGCGCGGTAGGTTGCGACATCCGA GGATGATGACGGGGCAGCTGGCA TGGGGAAGCACGACGGCGGCTCAGGGGGCCTTGG CATATATTATCCGGCGCACTGACTACC GGTAGTCAGTGCGCCGGATAATATATG CGCCACCCGCTGCGCCATCACG CCCCTGAGCCGCCGTCGTGCTTCCCCA GGATCTGCGGATGTCGCAACCC ATGCACAGGCCACTGGGAC CAGTGCGCCGGATAATATATG ATGTGCACGAACTCCTCCTTG CCCCTGAGCCGCCGTCGTGCTTCCCCA CAGCCAGTCACACTCACACATGGCCT ATGGAAGCTGCTGGAATCCAT CCTTGCTCATACGGTCAGCAATAC

CAGCCCTCTCATCTCCCTACTCA AATGGATTCAAACGAGGCCTGA