DOI: 10.1534/genetics.109.101527

Note

Excision of *Helitron* Transposons in Maize

Yubin Li* and Hugo K. Dooner*,†,[1]

**Waksman Institute, Rutgers University, Piscataway, New Jersey 08855 and †Department of Plant Biology, Rutgers University, New Brunswick, New Jersey 08901*

Manuscript received February 5, 2009
Accepted for publication February 27, 2009

ABSTRACT

Helitrons are novel transposons discovered by bioinformatic analysis of eukaryotic genome sequences. They are believed to move by rolling circle (RC) replication because their predicted transposases are homologous to those of bacterial RC transposons. We report here evidence of somatic *Helitron* excision in maize, an unexpected finding suggesting that *Helitrons* can exhibit an excisive mode of transposition.

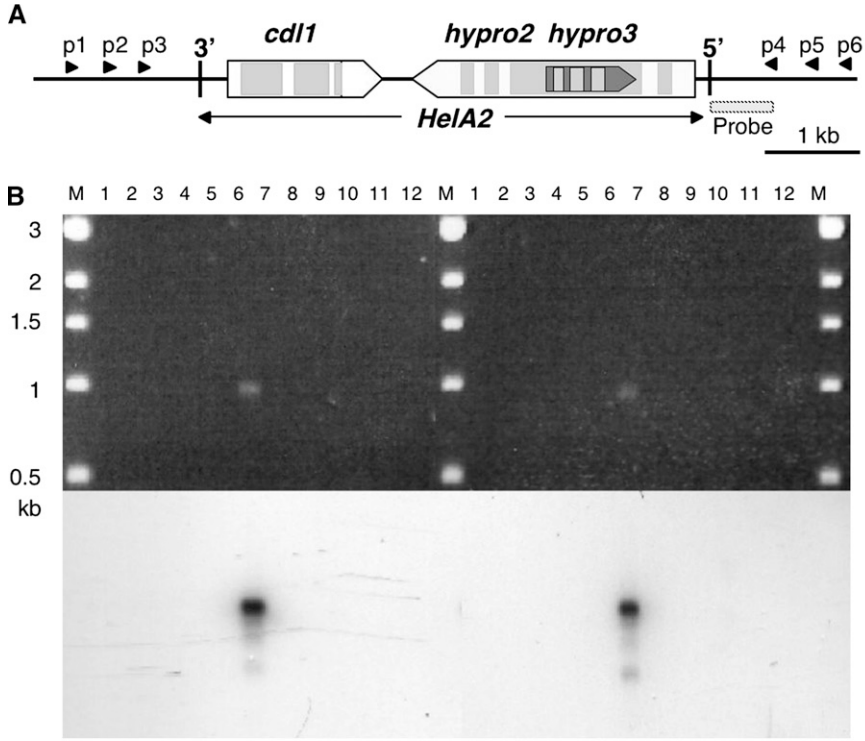
HELITRONS constitute a major superfamily of transposons discovered recently by a computational analysis of genomic sequences from Arabidopsis, rice, and *Caenorhabditis elegans* (Kapitonov and Jurka 2001). These presumed class II elements, unlike most other elements in that class, are postulated to transpose by a copy-and-paste mechanism. The absence of target-site duplications and the homology of the putative transposase encoded by the "consensus" autonomous element to the transposase of bacterial rolling circle (RC) transposons (Mendiola and de la Cruz 1992) led Kapitonov and Jurka (2001) to postulate that *Helitrons* transposed by an RC replication, rather than by an excision-repair, mechanism. *Helitrons* appear to be ubiquitous components of eukaryotic genomes, as they have been found in organisms ranging from fungi to vertebrates, including mammals (Poulter *et al.* 2003; Pritham and Feschotte 2007). However, there is at present no evidence for an autonomous *Helitron* element or a transposition mechanism in any organism.

Helitrons have few constant structural features. They have conserved 5′-TC and CTRR-3′ termini, carry a 16- to 20-bp palindrome of variable sequence ~10–12 bp upstream of the 3′ terminus, and insert invariably between host nucleotides A and T. The reconstructed putative autonomous elements in Arabidopsis and rice are large (5.5–15 kb) and encode proteins with homology to a DNA helicase and an RPA-like, single-stranded DNA-binding protein (Kapitonov and Jurka 2001). A recently described 11.5-kb *Helitron* in Ipomoea carries both of these genes, but with prematurely terminating mutations in each (Choi *et al.* 2007). The vast majority of *Helitrons* in Arabidopsis and *C. elegans* are shorter nonautonomous elements that do not encode proteins. Like other transposons, *Helitrons* can be mutagenic and have been reported in spontaneous mutations in maize (Lal *et al.* 2003; Gallavotti *et al.* 2004; Chuck *et al.* 2007) and in morning glory (Choi *et al.* 2007). The mutagenic maize *Helitrons* are large and have captured fragments of several unrelated genes. The *Helitrons* that contribute to the apparent breakdown in genetic colinearity among *bz* haplotypes (Fu and Dooner 2002) are also large and carry sequences from yet other genes (Lai *et al.* 2005; Morgante *et al.* 2005). In fact, the diversity of element complement is greater in maize than in other plant species (Hollister and Gaut 2007; Sweredoski *et al.* 2008). Smaller *Helitrons* lacking gene fragments and resembling the agenic *Helitrons* of Arabidopsis and *C. elegans* were recently identified in maize from a vertical comparison of 8 *bz* haplotypes (Wang and Dooner 2006). In spite of the extensive *Helitron* insertion polymorphisms found in maize and other species, an actual *Helitron* transposition event has yet to be reported.

Somatic excision of the 6.0-kb *HelA2 Helitron* in *5S*: Several *Helitrons* are polymorphic among *9S bz* haplotypes (Wang and Dooner 2006). The simplest explanation for the +/− polymorphism is that the unoccupied site was never visited. Nevertheless, because the actual mechanism of transposition of *Helitrons* is not known, the site lacking a *Helitron* has been referred to as "vacant" to distinguish it from the footprint-bearing "empty sites" produced by the excision of most class II DNA transposons (Lai *et al.* 2005). *Helitron HelA1* is 5.8 kb long, contains sequences for three genes, and

Supporting information is available online at http://www.genetics.org/cgi/content/full/genetics.109.101527/DC1.

[1]*Corresponding author:* Waksman Institute, Rutgers University, Piscataway, NJ 08855. E-mail: dooner@waksman.rutgers.edu



C

HelA2 Site	3'-end Junction	*HelA2*	5'-end Junction
B73	GTATATATATATATA	*CTAGGTGAGTGCCCGTGCGT*	..... *GCCTTATAGAGTAGTAGAGA*	TAATGTCTATATACACG
B73 (e)	GTATGTATATATATA	--TATATATATATATATATATATATATATATATATATATATATATA	--TAATGTCTATATACACG
B73 (e)	GTATGTATATATATA	------TATATATATATATATATATATATATATATATATATATA	------TAATGTCTATATACACG
A188	GTATATATATATATA	*CTAGGTGAGTGCCCGTGCGT*	..... *GCCTTATAGAGTAGTAGAGA*	TAATGTCTATATACACG
A188 (e)	GTATGTATATATATA	----TATATATATATATATATATATATATATATATATATATATA	----TAATGTCTATATACACG
A188 (e)	GTATGTATATATATA	-TATATATATATATATATATATATATATATATATATATATATATA	-TAATGTCTATATACACG
BSSS53	GTATATATATATATA	*CTAGGTGAGTGCCCGTGCGT*	..... *GCCTTATAGAGTAGTAGAGA*	TAATGTCTATATACACG
BSSS53 (e)	GTATGTATATATATA	-------TATATATATATATATATATATATATATATATATATA	-------TAATGTCTATATACACG
Mo17	GTATATATATATATA	*CTAGGTGAGTGCCCGTGCGT*	..... *GCCTTATAGAGTAGTAGAGA*	TAATGTCTATATACACG
Mo17 (e)	GTATGTATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATAATGTCTATATACACG		
Mo17 (e)	GTATGTATATATATA	-------TATATATATATATATATATATATATATATATATATATA	-------TAATGTCTATATACACG
M14	GTATATATATATATA	*CTAGGTGAGTGCCCGTGCGT*	..... *GCCTTATAGAGTAGTAGAGA*	TAATGTCTATATACACG
M14 (e)	GTATGTATATATATA	--------TATATATATATATATATATATATATATATATATATA	--------TAATGTCTATATACACG
M14 (e)	GTATGTATATATATA	--TATATATATATATATATATATATATATATATATATATATATATA	--TAATGTCTATATACACG
H99	GTATATATATATATA	*CTAGGTGAGTGCCCGTGCGT*	..... *GCCTTATAGAGTAGTAGAGA*	TAATGTCTATATACACG
H99 (e)	GTATGTATATATATA	--TATATATATATATATATATATATATATATATATATATATATA	--TAATGTCTATATACACG
H99 (e)	GTATGTATATATATA	---TATATATATATATATATATATATATATATATATATATATATA	---TAATGTCTATATACACG
H99 (e)	GTATGTATATATATA	----TATATATATATATATATATATATATATATATATATATATA	----TAATGTCTATATACACG
H99 (e)	GTATGTATATATATA	-----TATATATATATATATATATATATATATATATATATATA	-----TAATGTCTATATACACG

FIGURE 1.—Evidence of *HelA2* excision in *5S*. (A) *HelA2* structure in B73, showing the location of primers p1–p6 used for PCR. The exons and introns of the *cdl*, *hypro2*, and *hypro3* gene fragments are open and shaded, respectively, pointing in their direction of transcription. (B, top) Agarose gel stained with ethidium bromide. *HelA2* excision was tested by nested PCR on leaf DNA with the primer combinations p2/p6 and p3/p4 (left) or p1/p5 and p3/p4 (right). Lane 1, A188; lane 2, A636; lane 3, B73; lane 4, BSSS53; lane 5, H99; lane 6, M14; lane 7, Mo17; lane 8, 4Co63; lane 9, W23; lane 10, W22; lane 11, McC; lane 12, BAC b0511l12; lane M, DNA markers. The band in inbred M14 has the expected size of a *Helitron* excision product. (B, bottom) Hybridization of gel to a probe adjacent to *HelA2*. (C) Sequence of *HelA2* full and empty (e) sites. Only part of the *HelA2* 3′ and 5′ junctions is shown, with the body of *HelA2* indicated by dots. A sample of excision footprints from different inbreds is shown beneath the respective full sites. All excision footprints consist of a variable number of TA repeats (16–24). The AT host dinucleotide at the *HelA2* insertion site is in boldface type and the *HelA2* termini are in italic type (dashes introduced for alignment). No variability in TA repeat number was seen when multiple clones of a wild-type vacant site were sequenced (supporting information, Figure S1), ruling out *in vitro* DNA replication artifacts.

is found only in the McC and W22 *bz* haplotypes (DOONER and HE 2008). A nearly identical copy of *HelA1*, designated *HelA2*, is found in *5S* in the inbred lines B73, McC, W22, A188, A636, B73, H99, M14, Mo17, and BSSS53, but not in W23. Thus, most of the Corn Belt lines examined lack the *HelA* copy in *9S* and have only one in *5S* (LAI *et al.* 2005; WANG and DOONER 2006).

RC transposons are not supposed to excise (DEL PILAR GARCILLAN-BARCIA *et al.* 2002), so lines carrying *HelA2* in *5S* are not expected to produce PCR products equivalent to transposon "empty" sites. We tested this prediction in DNA from different inbreds by performing nested PCR with various combinations of primers flanking *HelA2* (Figure 1A). Unexpectedly, we obtained PCR products of a size similar to excision empty sites in some of the inbreds that carry *HelA2* in *5S*. One set of results is shown in Figure 1B, where M14 displays an ~1-kb empty-site-sized PCR band that also hybridizes to a probe from a *HelA2*-adjacent sequence present in single copy in the maize genome (data not shown). Other inbreds, *e.g.*, A188 and B73, gave empty-site-sized bands with other primer combinations. This difference among inbreds could reflect variability in *HelA2* excision activity and/or polymorphisms for the primer sites, which were based on the sequence of B73 (LAI *et al.* 2005).

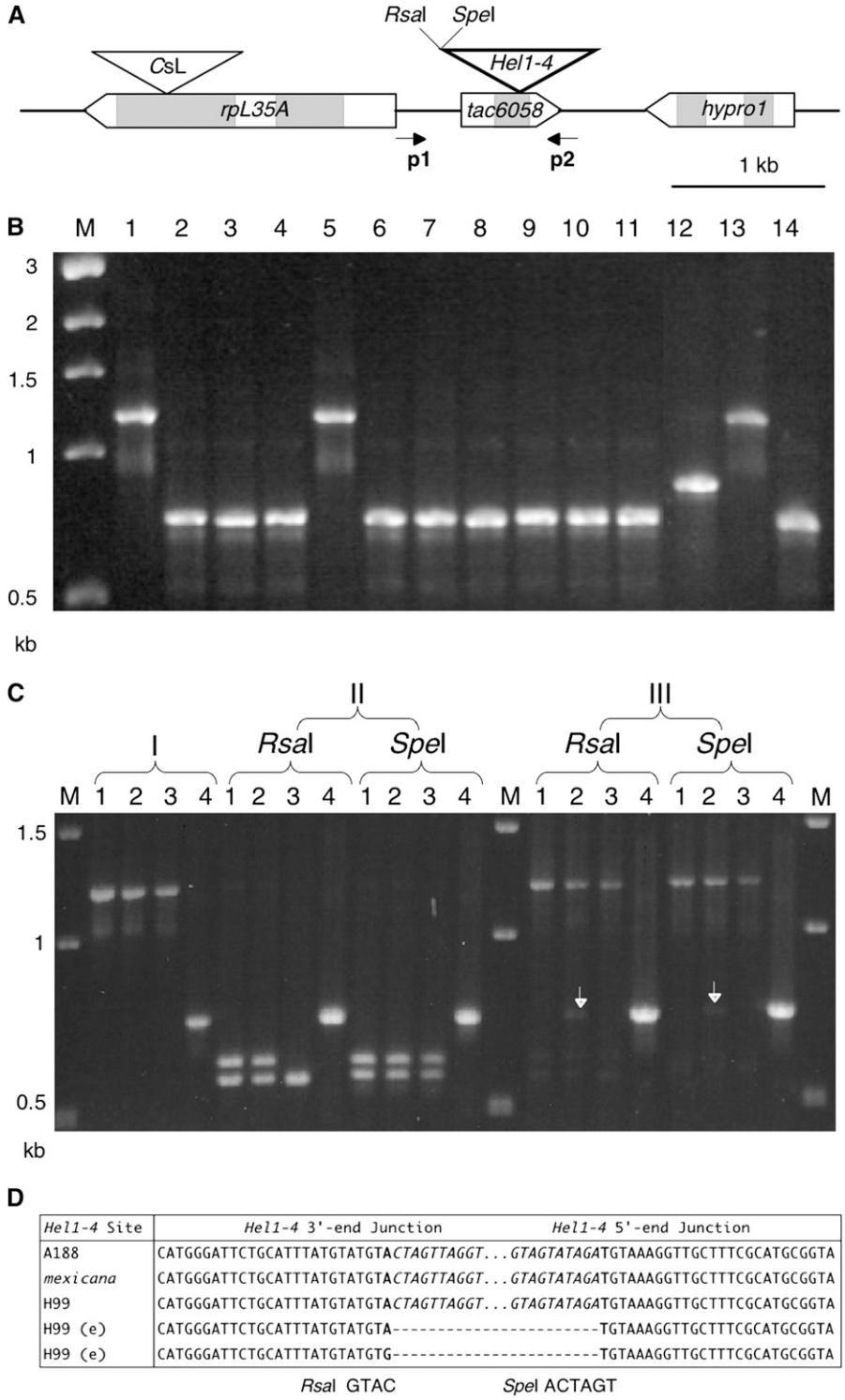


FIGURE 2.—Evidence of *Hel1-4* excision in *9S*. (A) *Hel1-4* structure in the A188 *bz* haplotype, showing the location of primers p1 and p2 used for PCR. (B) Agarose gel stained with ethidium bromide. The presence or absence of *Hel1-4* in a series of inbreds was tested on leaf DNA by PCR with primers 1 and 2. Lane 1, A188; lane 2, A636; lane 3, B73; lane 4, BSSS53; lane 5, H99; lane 6, M14; lane 7, Mo17; lane 8, 4Co63; lane 9, W23; lane 10, W22; lane 11, McC; lane 12, I137TN, lane 13, *Zea mays* ssp. *mexicana*; lane 14, *Z. mays* ssp. *parviglumis*. (C) Agarose gel stained with ethidium bromide. Lane 1, A188; 2, H99; 3, *Zea mays* ssp. *mexicana*; lane 4, McC (negative control). I, PCR amplification with p1 and p2 primers. II, PCR products from I were digested with *Rsa*I and *Spe*I, which cut at the *Hel1-4* 3′ junction (A and D). *Rsa*I also cuts within the *mexicana Hel1-4* element, producing the polymorphic *Rsa*I banding pattern in lane 3. No *Hel1-4* excision products are seen if the genomic DNA is cut prior to PCR (data not shown). III, Detection of *Hel1-4* excision by loss of restriction sites. The *Rsa*I and *Spe*I digests from II were reamplified with primers p1 and p2. The bands indicated by the arrows in H99 represent *Hel1-4* excision products. (D) Sequence of *Hel1-4* full and empty (e) sites. Only part of the *Hel1-4* 3′ and 5′ junctions is shown, with the body of *Hel1-4* indicated by dots. A sample of sequenced excision footprints from H99 is shown beneath the H99 full site. The AT host dinucleotide at the *Hel1-4* insertion site is in boldface type and the *Hel1-4* termini are in italic type (dashes introduced for alignment).

To investigate the nature of these putative excision products, the PCR bands from various inbreds (B73, A188, BSSS53, Mo17, M14, and H99) were cloned and sequenced (Figure 1C). Interestingly, all *HelA2* excision site footprints consisted of a variable number of TA repeats (16–24) at the previous site of *HelA2* insertion. The sequence adjacent to *HelA2* is TA-rich and consists of a string of (TA/G) repeats at the 3′-end. The sequences in Figure 1C are clearly empty sites derived from previous sites of *HelA2* insertion because they differ from each other by the same SNPs that distinguish the parental *HelA2* insertion sites (located beyond the sequences shown in Figure 1C). Oligo-TA stretches were also found recently adjacent to TAFT elements, novel transposons that do not appear to be related to *Helitrons*, but rather to elements of the *Mutator* superfamily (WANG and DOONER 2006). The meaning of this coincidence is unclear, but may have more to do with a

general mechanism of double-strand break repair by the host than with the specifics of transposition of the elements because excision of *Helitrons* from insertion sites lacking flanking TAs did not generate TA repeats at the excision sites (see below).

Somatic excision of the 0.4-kb *Hel1-4 Helitron* in *9S*: The short, 0.4-kb *Hel1-4* element found in an intron of the single-copy *tac6058* gene in the A188 *bz* haplotype (Wang and Dooner 2006) is present at the same location in inbred H99 and an accession of Chalco teosinte. Figure 2 shows PCR and sequence data documenting that *Hel1-4* can excise from *tac6058* in H99. Figure 2D presents the sequence of two empty sites, one without an excision footprint and one with a simple A-to-G transition at the "universal" AT dinucleotide insertion site. Polymorphisms beyond the *tac6058* empty sites shown in Figure 2D were identical with those of H99. Thus, inbred H99 has the ability to excise both large and small *Helitrons* of the *Hel1* family (Dooner *et al.* 2007).

Somatic excision of a 2.8-kb *Helitron* from *10L*: Several putative maize *Helitrons* were identified recently in the GenBank nr database (Du *et al.* 2008). Primers flanking the putative *Helitrons* were used to analyze a panel of maize inbreds, and the PCR products were sequenced to identify +/− polymorphisms. One such polymorphism was identified for a 2.8-kb putative *Helitron* in *10L*, with inbreds W22 and W23 lacking the insertion. In addition to the occupied site, B73 gave a faint empty-site-sized PCR product that lacked a footprint and was otherwise identical to the sequence flanking the insertion in the bacterial artificial chromosome (BAC) (Du *et al.* 2008). Lack of sequence polymorphisms in the adjacent sequences prevented the authors from excluding the possibility that this PCR product arose from DNA contamination in the B73 DNA preparation. However, considered against the data presented here, it is very likely that this band represents a *Hel* somatic excision product. Whether empty *Helitron* sites have or lack footprints may depend on the nature of the adjacent sequence.

Conclusions: The data presented here clearly establish that *Helitrons* can excise somatically from their chromosomal sites, suggesting that, like *Tn7* (Craig 2002) and *Mutator* (Walbot and Rudenko 2002), they may exhibit both replicative and excisive modes of transposition. *Helitron* excision activity was detected in some lines, but not in others, so it may be a polymorphic trait in maize. When present, the excision activity (excision band intensity) varies among lines. A screen for *Helitron* excision from an aleurone-pigmenting reporter construct would be valuable in tracking this activity. Two lines carrying *Helitrons* at more than one site, B73 and H99, produced empty sites for both, supporting the notion that the *Helitron* empty sites represent a general *Helitron* excision mechanism, rather than an unusual property of the adjacent sequences.

We are grateful to Jun Huang and Qinghua Wang for helpful comments on the manuscript. The work was supported by National Science Foundation grant DBI-03-20683.

LITERATURE CITED

Choi, J. D., A. Hoshino, K. I. Park, I. S. Park and S. Iida, 2007 Spontaneous mutations caused by a *Helitron* transposon, *Hel-It1*, in morning glory, *Ipomoea tricolor*. Plant J. **49:** 924–934.

Chuck, G., R. Meeley, E. Irish, H. Sakai and S. Hake, 2007 The maize *tasselseed4* microRNA controls sex determination and meristem cell fate by targeting *Tasselseed6/indeterminate spikelet1*. Nat. Genet. **39:** 1517–1521.

Craig, N. L., 2002 *Tn7*, pp. 422–456 in *Mobile DNA II*, edited by N. L. Craig, R. Craigie, M. Gellert and A. M. Lambowitz. ASM Press, Washington, DC.

del Pilar Garcillan-Barcia, M., I. Bernales, M. V. Mendiola and F. de la Cruz, 2002 IS91 rolling-circle transposition, pp. 891–904 in *Mobile DNA II*, edited by N. L. Craig, R. Craigie, M. Gellert and A. M. Lambowitz. ASM Press, Washington, DC.

Dooner, H. K., and L. He, 2008 Maize genome structure variation: interplay between retrotransposon polymorphisms and genic recombination. Plant Cell **20:** 249–258.

Dooner, H. K., S. K. Lal and L. C. Hannah, 2007 Suggested guidelines for naming helitrons in maize. Maize Genet. Coop. News Lett.. **81:** 24–25.

Du, C., J. Caronna, L. He and H. K. Dooner, 2008 Computational prediction and molecular confirmation of *Helitron* transposons in the maize genome. BMC Genomics **9:** 51.

Fu, H., and H. K. Dooner, 2002 Intraspecific violation of genetic colinearity and its implications in maize. Proc. Natl. Acad. Sci. USA **99:** 9573–9578.

Gallavotti, A., Q. Zhao, J. Kyozuka, R. B. Meeley, M. K. Ritter *et al.*, 2004 The role of *barren stalk1* in the architecture of maize. Nature **432:** 630–635.

Hollister, J. D., and B. S. Gaut, 2007 Population and evolutionary dynamics of *Helitron* transposable elements in *Arabidopsis thaliana*. Mol. Biol. Evol. **24:** 2515–2524.

Kapitonov, V. V., and J. Jurka, 2001 Rolling-circle transposons in eukaryotes. Proc. Natl. Acad. Sci. USA **98:** 8714–8719.

Lai, J., Y. Li, J. Messing and H. K. Dooner, 2005 Gene movement by *Helitron* transposons contributes to the haplotype variability of maize. Proc. Natl. Acad. Sci. USA **102:** 9068–9073.

Lal, S. K., M. J. Giroux, V. Brendel, C. E. Vallejos and L. C. Hannah, 2003 The maize genome contains a *Helitron* insertion. Plant Cell **15:** 381–391.

Mendiola, M. V., and F. de la Cruz, 1992 IS91 transposase is related to the rolling-circle-type replication proteins of the pUB110 family of plasmids. Nucleic Acids Res. **20:** 3521.

Morgante, M., S. Brunner, G. Pea, K. Fengler, A. Zuccolo *et al.*, 2005 Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in maize. Nat. Genet. **37:** 997–1002.

Poulter, R. T., T. J. Goodwin and M. I. Butler, 2003 Vertebrate helentrons and other novel *Helitrons*. Gene **313:** 201–212.

Pritham, E. J., and C. Feschotte, 2007 Massive amplification of rolling-circle transposons in the lineage of the bat *Myotis lucifugus*. Proc. Natl. Acad. Sci. USA **104:** 1895–1900.

Sweredoski, M., L. DeRose-Wilson and B. S. Gaut, 2008 A comparative computational analysis of nonautonomous helitron elements between maize and rice. BMC Genomics **9:** 467.

Walbot, V., and G. N. Rudenko, 2002 *MuDR/Mu* transposable elements of maize, pp. 533–564 in *Mobile DNA II*, edited by N. L. Craig, R. Craigie, M. Gellert and A. M. Lambowitz. ASM Press, Washington, DC.

Wang, Q., and H. K. Dooner, 2006 Remarkable variation in maize genome structure inferred from haplotype diversity at the *bz* locus. Proc. Natl. Acad. Sci. USA **103:** 17644–17649.

Communicating editor: J. A. Birchler

GENETICS

Supporting Information
http://www.genetics.org/cgi/content/full/genetics.109.101527/DC1

Note

Excision of *Helitron* Transposons in Maize

Yubin Li and Hugo K. Dooner

doi:10.1534/genetics.109.101527

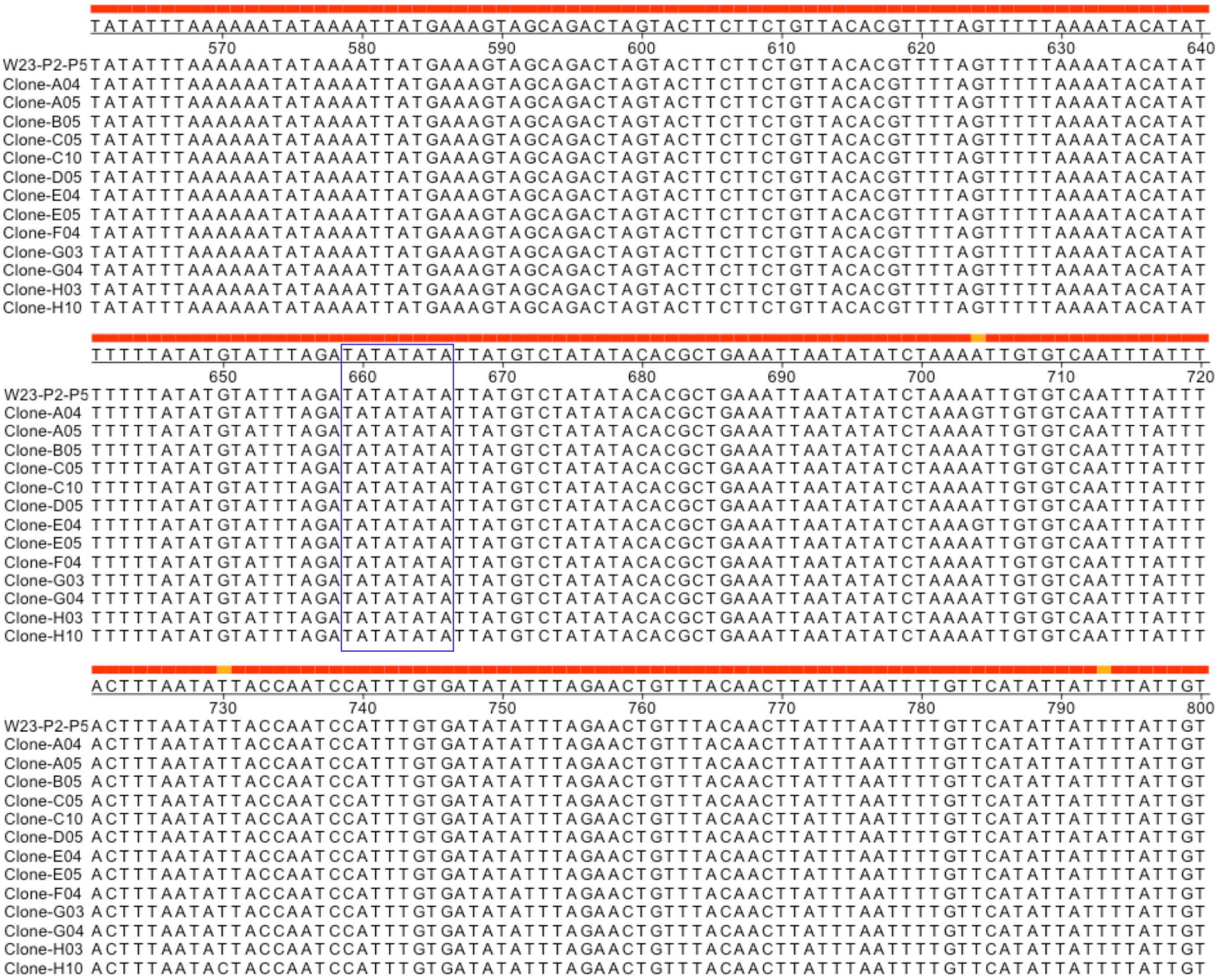


Figure S1.— No TA repeat variation detected at the W23 *HelA2* vacant site. The absence of a W23 vacant site in Figure 1B results from a deletion of the p6 priming site in this inbred. The vacant site was amplified with a combination of p2 and p5 primers (Figure 1A) and 13 clones were sequenced. All had the same number of TA repeats (boxed in blue). The W23 consensus sequence is shown above the line.