Transposon-induced inversions activate gene expression in the maize pericarp

Sharu Paul Sharma,1 Tao Zuo,1 and Thomas Peterson 1,2,*

1Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA, and
2Department of Agronomy, Iowa State University, Ames, IA 50011, USA

*Corresponding author: Email: thomasp@iastate.edu

Abstract

Chromosomal inversions can have considerable biological and agronomic impacts including disrupted gene function, change in gene expression, and inhibited recombination. Here, we describe the molecular structure and functional impact of six inversions caused by Alternative Transpositions between p1 and p2 genes responsible for floral pigmentation in maize. In maize line p1-wwB54, the p1 gene is null and the p2 gene is expressed in anther and silk but not in pericarp, making the kernels white. By screening for kernels with red pericarp, we identified inversions in this region caused by transposition of Ac and fractured Ac (fAc) transposable elements. We hypothesize that these inversions place the p2 gene promoter near a p1 gene enhancer, thereby activating p2 expression in kernel pericarp. To our knowledge, this is the first report of multiple recurrent inversions that change the position of a gene promoter relative to an enhancer to induce ectopic expression in a eukaryote.

Keywords: maize; inversion; alternative transposition; Ac/fAc; pericarp; enhancer; RET

Introduction

Transposable elements are segments of DNA that can move within a genome. The maize Activator (Ac) and Dissociation (Ds) transposable elements are members of the hAT transposon superfamily, which is widespread in eukaryotes (Rubin et al. 2001). Barbara McClintock discovered these transposons while studying the phenomenon of chromosome breakage. She identified Ds as a locus on the short arm of chromosome 9 in some maize stocks where chromosome breaks occurred frequently. She also showed that Ds is dependent on another element Ac which is autonomous and can itself transpose. The Ac/Ds system was also reported to induce a variety of chromosomal rearrangements, such as translocations, deletions, duplications, and inversions (McClintock 1950, 1951). The autonomous Ac element is 4565 bp in length and carries a complete transpose gene. Ds elements vary in size and internal sequence and lack a functional transpose gene, making them non-autonomous (Lazarow et al. 2013). The Ac transpose is known to bind to subterminal motif sequences of Ac/Ds elements and then cut at the transposon 5′ and 3′ TIRs (Terminal Inverted Repeats; 11 bp imperfect repeats) (Becker and Kunze 1997). Ac transpose can recognize and act on the termini of a single element (Standard Transposition), or the termini of two different elements (Alternative Transposition); for example, the 5′ end of Ac and the 3′ end of a second nearby element such as Ds or fractured Ac (fAc) (Ralston et al. 1989; Su et al. 2018). Standard Transposition events change only the position of a single element, while Alternative Transposition events can produce a variety of genome rearrangements, depending on the relative orientations of the TE termini and the location of the target site. When two transposons are in direct orientation, the internal-facing termini are present in a reversed orientation compared to the termini of a single transposon. In this configuration, the two facing termini can undergo Reversed-Ends Transposition (RET) (Zhang and Peterson 2004; Huang and Dooner 2008; Zhang et al. 2009) to induce deletions (Zhang and Peterson 2005a; Zhang et al. 2006), duplications (Zhang et al. 2013), Composite Insertions (Zhang et al. 2014; Su et al. 2018, 2020), inversions (Zhang and Peterson 2004; Yu et al. 2011), and reciprocal translocations (Pulletikuri et al. 2009; Zhang et al. 2009). For example, Zhang et al. (2009) described 17 reciprocal translocations and two large peri-centric inversions derived by RET from a progenitor allele containing Ac and fAc insertions in the maize p1 gene. The frequent occurrence of these structural changes and the fact that Ac inserts preferentially in or near genic regions (Kolkman et al. 2005) suggest that Alternative Transposition events may have a significant impact on the genome and transcriptome. Additionally, inversions provide an opportunity to analyze the function of cis-regulatory elements, such as enhancers, in a native (non-transgenic) context.

The maize p1 and p2 genes are closely linked paralogous genes located on the short arm of chromosome 1 that originated by duplication of an ancestral P′′′′ gene, approximately 2.75 mya (Zhang et al. 2000a). These genes are separated by a ~70 kb intergenic region and coincide with a major QTL for levels of silk maysin, a flavone glycoside with antibiotic activity toward corn earworm (Zhang et al. 2003; Meyer et al. 2007). Both p1 and p2 encode highly similar R2R3 Myb transcription factors involved in controlling the structural genes c2, chi, and a1, encoding chalcone synthase, chalcone isomerase, and dihydro-flavonol reductase.
respectively (Dooner et al. 1991; Grotewold et al. 1994). These enzymes of the flavonoid biosynthetic pathway produce red phlobaphene pigments in maize floral organs. The \( p1 \) gene is expressed in maize kernel pericarp, cob, and silk, while \( p2 \) is active in anther and silk (Zhang et al. 2000a; Goettel and Messing 2009). Different \( p1 \) alleles are indicated by a two-letter suffix indicating their expression in kernel pericarp and cob glumes; for example, \( p1\-ww \) specifies white (colorless) pericarp and white cob, while \( P1\-wr \) indicates white pericarp and red cob.

The robust visual phenotypes and abundance of alleles with \( Ac \) insertions (Athma et al. 1992; Moreno et al. 1992) make the \( p1/p2 \) cluster an ideal genetic system to analyze the genetic impact of Alternative Transposition events. The \( p1\-wwB54 \) allele has a deletion of \( p1 \) exons 1 and 2 along with insertions of \( Ac \) and \( fAc \) elements upstream of \( p1 \) exon 3 (Yu et al. 2011). Because exons 1 and 2 encode most of the essential Myb DNA binding domain (Grotewold et al. 1991) their deletion renders the \( p1 \) gene non-functional leading to white kernel pericarp and white cob. The \( 5' \) \( Ac \) and \( 3' \) \( fAc \) termini are in a reversed orientation, separated by a 331 bp inter-transposon segment. These elements exhibit frequent RET, leading to chromosome breakage and rearrangements such as deletions and inversions (Yu et al. 2011).

Materials and methods
Screening for inversions derived from RET

The inversion alleles described here were derived from \( p1\-wwB54 \) (Figure 1). Stock \( J \) (\( p1\-ww[4Co63] r1-m3::Ds \)) (described in Zhang et al. (2003)) was used as common genetic background and to detect the presence of \( Ac \) by excision of \( Ds \) from \( r1-m3 \). The occurrence of red kernel pericarp in \( p1\-wwB54 \) was used as a visual screen for \( p2 \) activation in the pericarp (see Materials and Methods in Su et al. (2020)). \( p1\-wwB54 \) has white kernels, but approximately one in eight ears were found to have a single red kernel, and \( \sim1 \) in 40 ears had a multi-kernel red sector (Figure 1, F86). The occurrence of a sector of red-colored pericarp on single or multiple kernels reflects the stage of ear and kernel development at which an activating mutation (e.g. transposition) occurred. Events that occurred sufficiently early (prior to embryo formation) can be inherited (Emerson 1917). The red kernels were selected and planted, and in cases where the new structure was transmitted through meiosis, the resulting plants would produce whole ears with red kernels (Figure 1, S25). The pericarp is maternal tissue and hence the red color phenotype is independent of the pollination parent.

DNA extraction, gel electrophoresis, and southern blotting

Genomic DNA was extracted from maize seedling leaves by a modified CTAB method (Saghai-Maroof et al. 1984) and digested with different restriction enzymes according to the manufacturer’s instructions. For Southern blotting, genomic DNA digests were done with \( KpnI \), \( HpaI \), and \( EcoRV \). Agarose gels (0.7%) were run under 30–50 volts for 18–24 h for maximum separation of large fragments. The DNA was then transferred to a membrane for 24 h, followed by probing each membrane with fragment-15 (\( f15 \)), a 411 bp sequence two copies of which are located within the enhancer of the \( p1 \) gene (Zhang and Peterson 2005a).

PCR, iPCR, and sequencing

PCR was performed with 20\( \mu \)L reaction volumes under the following temperature conditions: 95\( ^\circ \)C for 2 min, then 35 cycles at...
95° for 30 s, 60° for 30 s, and 72° for 1 min per 1-kb length of the expected PCR product, then final extension at 72° for 5 min. For initial PCR screening of new alleles, a high-efficiency agarose gel electrophoresis method was used to visualize PCR products (Sharma and Peterson 2020). Inversion breakpoint junctions ending with fAc elements were obtained by inverse-PCR (iPCR; Ochman et al. 1988). Inversion breakpoints at Ac elements were isolated by Ac casting (Singh et al. 2003; Wang and Peterson 2013). This method relies on the occurrence of frequent Ac transpositions to closely linked sites during plant development. For each inversion, genomic DNA was isolated from seedling leaf tissue and then the region containing the breakpoint was amplified by two pairs of nested PCR primers (Set 1 and then Nested in Supplementary Table S1). The inversion breakpoint regions from i-PCR and Ac casting were sequenced by the Iowa State University DNA Sequencing Facility. Sequences were analyzed using Snapgene (snapgene.com) and BLAST (Zhang et al. 2000b).

RT-PCR detection of p2 expression
Pericarps were peeled from kernels 15–20 days after pollination (DAP) and flash-frozen in liquid nitrogen. Three biological replicates (pericarps from three sibling ears) were pooled to extract RNA. RNA was isolated using Purelink Plant RNA Reagent, treated with NEB DNaseI, and reverse transcribed to cDNA using Invitrogen™ SuperScript™ II Reverse Transcriptase kit using protocols recommended by the product suppliers. Two technical replicates of reverse transcription were used per sample. cDNAs were amplified by PCR using primers specific to exons 1 and 3 of the p2 gene transcript (Supplementary Table S3). Primers specific to the maize Beta-tubulin gene were used as an internal control.

Data availability
Maize genetic stocks are available by request to T.P. The data underlying this article are available in the article and in its online supplementary material. Supplemental material available at figshare: https://doi.org/10.25386/genetics.14388596.

Results
Due to the deletion of p1 exons 1 and 2, the p1-wwB54 allele was expected to be a stable null. We were surprised to see ears carrying p1-wwB54 produced red kernel pericarp sectors of varying sizes (Figure 1). We hypothesized that the p2 gene, which is normally not expressed in kernel pericarp, could be activated by inversions generated by RET (Zhang and Peterson 2004; Zhang et al. 2006; Huang and Dooner 2008; Zhang et al. 2009, 2013; Yu et al. 2011; Su et al. 2020). A diagram of this model showing an inversion with breakpoints in the p2 promoter region is shown in Figure 2. According to this model, RET would begin with excision of the Ac 5’ end and fAc 3’ end in p1-wwB54, followed by insertion of the excised termini into a new target site unique for each event (Figure 2, a/b). If the 5’ end of Ac (solid red arrowhead, Figure 2) joined with the “a” side of the target sequence, and 3’ end of fAc (white arrowhead, Figure 2) joined with the “b” side of the target site, the segment from 5’ end of Ac up to the target site a/b will be inverted (for animation, see Supplementary Material). The resulting structure (Figure 2, Lower) contains an inversion of the p1-p2 interval, if the p2 gene promoter region is inserted sufficiently near the p1 3’ pericarp enhancer (Sidorenko et al. 2000), p2 may be expressed in the kernel pericarp.

Screening for inversions
To obtain RET-induced inversions, ears from several thousand plants carrying the p1-wwB54 allele were screened for kernels with red pericarp (example in Figure 1, third ear from left). Selected red kernels were grown and propagated to obtain stable lines with various shades of red kernel pericarp. Genomic DNA preparations from these lines were tested for structural rearrangements by PCR using sets of primer pairs (Supplementary Table S1) that can amplify the Ac and fAc junctions in p1-wwB54: primer set 1 detects the p1/3’ Ac junction, and primer set 2 detects the 5’ Ac/p1/3’ fAc segment (Figure 3A). Simple Ac transposition or RET-induced deletion (Yu et al. 2011) would result in negative PCR for both sets 1 and 2, while the formation of Composite Insertions (Su et al. 2020) results in retention of both junctions. Whereas, RET-induced inversion would result in retention of the p1/3’ Ac junction (Set 1 positive), and loss of the 5’ Ac/ p1/3’ fAc segment (Set 2 negative). Using this test, several cases of putative inversions were detected (Figure 3B). These cases were further tested using primers flanking the downstream fAc/p1 junction (Supplementary Table S1) to confirm the retention of fAc at its original position next to p1 exon 3. Following confirmation of potential inversions, the new Ac and fAc inversion breakpoint junction sequences (a/Ac and fAc/b in Figure 3A) were amplified from genomic DNA using direct PCR, Ac Casting, or iPCR (see Materials and methods) along with nested PCR. Once obtained, both inversion breakpoint junctions were sequenced (list of primers in Supplementary Table S2). Junction sequences were examined to confirm expected orientations based on the

Figure 2 Model of RET-induced inversion leading to p2 activation. Upper: Diagram of progenitor allele p1-wwB54 and nearby p2 gene. Purple and blue boxes indicate exons of p2 and p1 genes, respectively. Red arrows represent Ac (with two arrowheads) and fAc (with single arrowhead) elements. Red boxes indicate two copies of p1 enhancer fragment f15. Dashed lines indicate Ac/fAc excision by RET and re-insertion at a/b target site upstream of p2. The 331 bp DNA fragment between Ac and fAc (blue line) is lost during the transposition event. The same symbols and coloring scheme are used in other figures in this paper. Lower: Inversion. Inverted segment extends from point a (Ac junction) to point b (fAc junction) and includes Ac, p1-p2 intergenic region, and p2 gene. In the inversion allele, the proximity of the p2 promoter to the p1 3’ enhancer may activate p2 expression in the pericarp.
established p1 and p2 genomic sequence data (Zhang et al. 2006) and the presence of 8 bp TSDs (Target Site Duplication) characteristic of Ac transposition (Figure 3A, yellow box and Supplementary Table S4).

Structure of inversions

The structures of six independent inversions with red kernel pericarp were determined. Ears produced by plants carrying these inversions are shown in Figures 1 and 4. The inversion junctions were PCR-amplified and sequenced as described above, and their sequences compared with established p1 and p2 genomic sequences to identify the breakpoint locations. One breakpoint common to all cases is at the 5’ end of Ac (Figure 5, vertical blue line), as expected for inversions originating by RET of Ac and fAc elements. The second breakpoint unique to each allele is at the transposition target site, located in a ~1kb window from 2.6 to

**Figure 3** PCR test for inversions. (A) Progenitor p1-ww854 and derived inversion allele structures showing locations of primers (black arrows) used in PCR tests. Primer Set 1 detects the p1-Ac junction which is present in both p1-ww854 and Inversion; Primer Set 2 detects the Ac/p1/fAc segment which is present in p1-ww854 and absent in Inversion. Yellow box is the 8 bp target site duplicated in inversion. (B) Agarose Gel image showing an example PCR using Primer Set 1 (upper) and Primer Set 2 (lower). Lane 1, positive control (p1-ww854); Lane 2, negative control (p1-ww Stock J); Lanes 3–7, candidates tested. Only lane 6 (allele 132, not one of the cases described here) is positive for Set 1, negative for Set 2, as expected for inversions.

**Figure 4** Representative ears of five inversion alleles. Ears have varying shades of red kernel pericarp due to p2 activation. The sixth inversion case S25 is shown in Figure 1. Some kernels have purple or purple-sectored aleurone due to Ac-induced excision of Ds from r1-m3 leading to anthocyanin pigmentation.
The expression of the p2 gene in plants homozygous for the inversion alleles was analyzed by RT-PCR. RNA was extracted from pericarp tissue and reverse transcribed to cDNA. PCR with primers from (A) p2 exons 1 and 3, and (B) Beta-tubulin as an internal control. Lane 1, J (p1-ww) is negative control; Lane 2, P1-rr4B2 is positive control for p1 expression; Lane 3, p1-wwB54 is the progenitor and lacks p2 expression; Lane 4, 140; Lane 5, E1; Lane 6, E1; Lane 7, TZ3-4; Lane 8, SP1-18; Lane 9, S25; and Lane 10, TZ2-7. All six inversion alleles are positive for p2 expression.

p2 expression in inversions

The expression of the p2 gene in plants homozygous for the inversion alleles was analyzed by RT-PCR. RNA was extracted from pericarps of homozygous plants collected 15–20 DAP (days after pollination) (Figure 7). P1-rr4B2 was used as a positive control (Figure 7, lane 2) because p1 is expressed in P1-rr4B2 pericarp and the same p2 primers can amplify p1 transcripts due to sequence similarity. The six inversion alleles were derived from the

Figure 5 Map of the six inversion alleles. The vertical blue line is one breakpoint, and the red lines indicate the second breakpoint unique to each inversion. Numbers on red lines correspond to alleles, 1, 140, 2, E1, 3, TZ3-4; 4, SP1-18, 5, S25; 6, TZ2-7. Numbers below the figure are distances in kbs.

Figure 6 Genomic southern blot analysis of inversion alleles. (A) Southern blot of genomic DNA samples from inversion homozygotes digested with KpnI and probed with fragment f15 from the p1 enhancer (red boxes in B, C and D). Lane 1, DNA ladder (arrow points to 10 kb band); Lane 2, J (p1-ww); Lane 3, P1-rr4B2; Lane 4, progenitor p1-wwB54 (top band is 13.5 kb); Lane 5, 140; Lane 6, E1; Lane 7, TZ3-4; Lane 8, SP1-18; Lane 9, S25; Lane 10, TZ2-7. The six inversions (lanes 5–10) are arranged in order of decreasing band sizes (from 12 to 10.5 kb). (B–D) Diagrams showing KpnI restriction sites (vertical blue arrows) in (B) P1-rr4B2, (C) progenitor p1-wwB54 and (D) inversions. Southern blot band sizes reflect differences in the sites of fAc insertion in the p2 promoter (breakpoint b).

Figure 7 RT-PCR. Agarose gel images showing RT-PCR results using RNA extracted from pericarp tissue and reverse transcribed to cDNA. PCR with primers from (A) p2 exons 1 and 3, and (B) Beta-tubulin as an internal control. Lane 1, J (p1-ww) is negative control; Lane 2, P1-rr4B2 is positive control for p1 expression; Lane 3, p1-wwB54 is the progenitor and lacks p2 expression; Lane 4, 140; Lane 5, E1; Lane 6, E1; Lane 7, TZ3-4; Lane 8, SP1-18; Lane 9, S25; and Lane 10, TZ2-7. All six inversion alleles are positive for p2 expression.
Discussion

Mechanisms of inversions

A variety of molecular mechanisms are known to induce inversions. The double-strand break (DSB) mechanism involves breakage and then repair by Non-Homologous End Joining (NHEJ) (Moore and Haber 1996). If two DSBS occur on the same chromosome, re-ligation of the DNA molecule via NHEJ can form inversions (Hefferin and Tomkinson 2005), deletions, or inversions flanked by inverted duplications, if the DSBS are staggered cuts (Ranz et al. 2007). Additionally, inversions can result from ectopic recombination (Non-Allelic Homologous Recombination, NAHR) between dispersed repeated sequences including transposons (Delprat et al. 2009), retrotransposons (Kupiec and Petes 1988), interspersed repeat sequences (Montgomery et al. 1991), or interspersed duplications (Cáceres et al. 2007). For example, NAHR between pairs of homologous TEs present in opposite orientations at different positions on a chromosome can lead to inversions of the DNA segment between the two TEs (Delprat et al. 2009). Recently, CRISPR has also been used to induce inversions in mammals (Guo et al. 2015) and maize (Schwartz et al. 2020).

Here we show that DNA transposons, in addition to serving as passive substrates for ectopic recombination, can also directly induce inversions via Alternative Transposition reactions. Our results are consistent with a model of RET-induced inversion, in which the ends of two nearby DNA transposons are involved in a single transposition reaction. In this model, two TE copies present in direct orientation will have their adjacent termini in a reversed orientation (i.e., the 5' end of one TE faces 3' end of a second TE). Recognition of the terminal sequences of the two TEs by the transposase will lead to an RET event in which the TE termini face each other attempt to transpose to a genomic target site. Because each TE remains linked to the donor sequences by one un-transposed end, RET results in inversion of a flanking segment, and loss of the fragment originally between the two TEs (Figure 2). Specifically, the DNA segment from one TE end to the new insertion site is inverted. The resulting inversion has TEs present at each breakpoint, one within the inversion and another just outside the second endpoint (Figure 6D). The TE insertion is accompanied by TSDs flanking the TE termini at the inversion breakpoints. As in standard transposition, TSDs result from the staggered cuts made by transposase followed by gap-filling and DNA ligation (Lazarow et al. 2013).

There are several important differences between inversions resulting from ectopic recombination (NAHR) between two inversely oriented TEs and those caused by RET. First, inversions formed by NAHR will not have a newly generated TSD; instead, the TSDs flanking the internal TE termini will also be inverted, resulting in TEs with (usually) non-identical TSDs. Second, NAHR between two inversely oriented TEs can only flip the intervening segment; whereas, RET can induce inversions of varying lengths on either side of each TE. Third, RET will only operate on Class II TEs that transpose via ‘cut-and-paste’ mechanism, and will not occur with Class I elements that utilize a retro-transposition mechanism. Fourth, RET requires the expression of a DNA transposase and transposition-competent TE termini in appropriate orientation, whereas, NAHR proceeds via the action of host recombination machinery on substrate sequences of sufficient homology and orientation.

The maize Ac/Ds system is not the only TE system that can induce inversions and other rearrangements. Like Ac/Ds elements in maize, the F-elements in Drosophila are also known to cause inversions and other chromosomal rearrangements through Alternative Transpositions (Gray et al. 1996; Tanaka et al. 1997). Other examples of such rearrangements via non-standard transposition include impala elements in the fungus Fusarium (Huang et al. 2002) and Sleeping Beauty transposons in transgenes of mice (Geurts et al. 2006).

In addition to RET, Ac/Ds elements can also undergo Sister Chromatid Transposition (SCT) (Zhang and Peterson 2005b; Zhang et al. 2013). While RET targets TEs on the same chromosome, SCT involves TEs on sister chromatids. After DNA replication, a pair of Ac 5' and 3' termini in direct orientation can move to an un-replicated region where they can undergo a second round of replication. This results in inverted duplications and Composite Insertions (Wang et al. 2020). Both SCT and RET can lead to major rearrangements in the genome. Transposition in the Ac/Ds system is non-random (Vollbrecht et al. 2010) as Ac transposes preferentially into hypomethylated DNA (Kolkman et al. 2005) often associated with genic regions (Cowperthwaite et al. 2002). This insertion preference likely increases the potential genetic impact of Ac/Ds-induced Alternative Transposition events.

Class II TIR-containing TEs are quite numerous in maize genomes: in B73, the hAT transposon superfamily alone has over 30,000 copies of intact elements (Su et al. 2019), and likely many more partial or fractured copies (Ralston et al., 1989). One may reasonably surmise that many TE systems may have closely-linked family members with termini in suitable orientations to undergo either SCT or RET. Either of these Alternative Transposition (AT) events can lead to chromosome breakage (Huang and Dooner 2008; Yu et al. 2010). However, specific tests for breakage of chromosomes 1 and 9 have not indicated significant numbers of breaks occurring in the absence of a known TE system under study (Dooner and Belachew 1991; Yu et al. 2010). These results are consistent with the idea that the great majority of TE systems are currently inactive. Most TEs are highly methylated (Regulski et al. 2013; West et al. 2014) and transcriptionally silenced (Anderson et al. 2019). Some TEs, such as the maize Mutator and Ac/Ds systems, can become silenced through the formation of inverted copies of the TE, whose transcription generates dsRNA leading to the production of siRNA and TE methylation (Burgess et al. 2020; Wang et al. 2020). In the Ac/Ds system, cytosine methylation is associated with transcriptional
repression and loss of transposition competence, even in the presence of an active Ac element (Len et al. 1992). Nevertheless, TEs have been active in the past and may have undergone AT events during maize genome evolution. Even with active Ac/Ds elements competent to undergo both Standard and Alternative Transposition, AT events occur less frequently (Zhang and Peterson 1999; Huang and Dooner 2008). Not surprisingly, the frequency of AT is inversely proportional to the distance between the two elements (Dooner and Belachew 1991; Zhang et al. 2011). Finally, transposons capable of AT events leading to chromosome breaks, large deletions, or duplications may be selected against or preferentially silenced by the host.

Frequency of inversions and other rearrangements

In a previous study, Yu et al. (2011) screened alleles with reverse-oriented Ac/fAc insertions in an active p1 gene for RET-induced loss-of-function mutants. Out of 100 mutants obtained, 89 were identified to have undergone major structural changes. Approximately half (47 out of 89) were inversions, and the rest were primarily deletions plus some other rearrangements. This result is consistent with the RET model which predicts that inversions and deletions are equally likely to occur, because the outcome is determined by which transposon end (Ac or fAc) is ligated to which side (a or b) of the transposition target site. Here, we screened ears from roughly 4000 plants of p1-uwb54/p1-uw@ genotype for red kernels indicating putative rearrangements. About 400 unique red kernel events were found and propagated. The red pericarp phenotype was inherited in 97 cases; 83 of these were characterized as rearrangements due to RET. Among these 83, only 14% (12 out of 83) were inversions, 35% (29) were deletions, and 51% (42) were Composite Insertions. The markedly different proportion of inversions recovered here (14%) compared to Yu et al. 2011 (53%) is most likely due to the different screens used to detect RET events. The 2011 study began with a functional p1 gene and selected for loss-of-function events, yielding mostly deletions and inversions; most Composite Insertions would not be detected because they leave the original donor loci intact (Zhang et al. 2014; Su et al. 2018, 2020). Whereas, this study began with a non-functional p1 allele, and required gain-of-function (red pericarp sectors). This selection favored recovery of p2-expressing alleles caused by inversions and Composite Insertions near p2 (Su et al. 2020). Indeed, all six of the cases described here have inversion breakpoints within 3.5 kb upstream of the p2 gene. To our knowledge, this is the first example of multiple independent inversions that bring a promoter near an enhancer, thus activating a gene in a tissue in which it is not normally expressed.

The six inversion cases described here have no other detectable rearrangements. However, we also obtained seven other cases of inversions which contain other more complicated structural rearrangements. These cases of complex inversions are currently being characterized and will be described elsewhere.

Effects of inversions on fitness

Inversions can have a variety of effects, such as causing position effect variegation of white gene in Drosophila (Muller 1930; Levis et al. 1985; Lerach et al. 2006; Bao et al. 2007), suppressing recombination (Jiang et al. 2007), and playing a vital role in the evolution of sex chromosomes (Wright et al. 2016). Inversions are also associated with local adaptation and reproductive isolation (Lowry and Willis 2010), as many closely related species are thought to have diverged via inversion polymorphisms (Oneal et al. 2014; Twyford and Friedman 2015). Inversion of boundary elements may also change higher-order organization in mammalian genomes, due to the directional nature of CTCF binding sites (Guo et al. 2015). By altering topologically associated domains boundaries, inversions can cause misexpression and disease by changing the relative position of enhancers and their target promoters (Lupiáñez et al. 2015; Bompade and Andrey 2019).

Some inversions can result in major adaptive advantages; for example, the paracentric inversion in Arabidopsis thaliana induced by Vandal transposon activity is strongly associated with fecundity under drought conditions (Fransz et al. 2016). Inversions can even affect the spread of disease: a chromosome 2La inversion in Anopheles gambiae is associated with susceptibility of the vector to malaria infection (Riehle et al. 2017). Inversions are also involved in local adaptation in teosinte populations (Pyhäjärvi et al. 2013). A large (13 Mb) inversion called Inv4m found in Mexican highland maize populations affects expression of a large number of genes regulating various developmental and physiological processes contributing to local adaptation to highland environments (Crow et al. 2020).

The phlobaphene pigments controlled by the maize p1 gene are non-essential, and many modern corn varieties lack significant kernel pericarp color. However, a recent study reported that high phlobaphene levels were associated with increased kernel pericarp thickness and reduced mycotoxin contamination when compared to isogenic colorless pericarp lines lacking an active p1 gene (Landoni et al. 2020). Because the p1 and p2-encoded proteins are highly similar and regulate the same flavonoid biosynthetic pathway (Zhang et al. 2000a), similar effects are likely induced by the expression of p2 in the pericarp. Thus, the transposon-induced inversions identified here may provide an adaptive benefit. Small (<1Mb) inversions are difficult to detect by genetic and cytological methods, and so their frequency in plant populations is often unknown. Our results show that even small, cytologically undetectable inversions between linked genes may positively affect fitness. In summary, these findings suggest that Alternative Transposition events may play a critical role in altering gene expression and generating adaptive variation during genome evolution.

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Conflicts of interest

None declared.

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