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ORIGINAL PAPER

The *sh2-R* allele of the maize *shrunken-2* locus was caused by a complex chromosomal rearrangement

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Abstract
Key message **The mutant that originally defined the *shrunken-2* locus of maize is shown here to be the product of a complex chromosomal rearrangement.**
Abstract The maize *shrunken-2* gene (*sh2*) encodes the large subunit of the heterotetrameric enzyme, adenosine diphosphate glucose pyrophosphorylases and a rate-limiting enzyme in starch biosynthesis. The *sh2* gene was defined approximately 72 years ago by the isolation of a loss-of-function allele conditioning a shrunken, but viable seed. In subsequent years, the realization that this allele, termed z*sh2-R* or *sh2-Reference*, causes an extremely high level of sucrose to accumulate in the developing seed led to a revolution in the sweet corn industry. Now, the vast majority of sweet corns grown throughout the world contain this mutant allele. Through initial Southern analysis followed by genomic sequencing, the work reported here shows that this allele arose through a complex set of events involving at least three breaks of chromosome 3 as well as an intra-chromosomal inversion. These findings provide an explanation for some previously reported, unexpected observations concerning rates of recombination within and between genes in this region.

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Introduction

The maize *shrunken-2* gene (*sh2*) on chromosome 3 encodes the large subunit of the starch biosynthetic gene, adenosine diphosphate glucose pyrophosphorylase (AGPase) (Hannah and Nelson 1976; Bhave et al. 1990). This enzyme plays a rate-limiting role in starch biosynthesis as evidenced by a series of mutational and transgenic approaches in which enhanced AGPases were placed into the genomes of cereals and other plants resulting in increased starch synthesis (Stark et al. 1992; Giroux et al. 1996; Smidansky et al. 2002, 2003; Sakulsingharoj et al. 2004; Obana et al. 2006; Wang et al. 2007; Lee et al. 2009; Hannah et al. 2012). Genes encoding the seed AGPase, such as *sh2*, also have significant effects on yield since cereal seed is approximately, 70 % starch.

The *sh2* gene was first identified by the isolation of a loss-of-function allele (Mains 1949) subsequently termed *sh2-R* or *sh2-Reference*. Complete loss of *sh2* function gives rise to a shriveled, shrunken but viable kernel at maturity, while partial loss of gene function as conditioned by the *sh2-i* allele (Lal et al. 1999) gives rise to a less shrunken phenotype.

Laughnan (1953) reported that the *sh2-R* mutant conditioned extremely high levels of sucrose in the seed, reaching levels as high as 50 % of the total dry weight of the developing seed. Following this discovery, the sweet corn industry was revolutionized by incorporation of *sh2-R* into the vast majority of all present-day supersweet sweet corns (Tracy 2000).

Characterization of mutant *sh2* alleles has provided insight into a number of important biological phenomena. For example, the first case of a transposable element functioning as a perfect intron was elucidated in studies of the Dissociation (Ds)-containing allele *sh2-m1* (Giroux et al. 1994). The ability to recognize a mutant dinucleotide AA, derived from the wild type sequence AG, as an intron acceptor site was first noted in plants by characterization of the mutant maize allele *sh2-i* (Lal et al. 1999). In addition, the initial discovery of a family of maize transposable elements termed *Helitrons* was first gleaned from characterization of the mutant *sh2-7527* (Lal et al. 2003).

In this paper, we describe the molecular events that gave rise to the mutant that originally described the *sh2* locus, *sh2-R*. As detailed below, this allele arose from a complex set of events, involving at least three breaks in chromosome 3 and a large inversion. Several genes closely linked to *sh2* in wild type maize, including the anthocyanin gene *a1,* may be located between the two termini of *sh2* in this mutant. These results are relevant to previous fine mapping experiments between *a1* and *sh2* as reviewed below.

Sh2-R materials and methods

Southern blot

Genomic DNA was prepared from maize leaves using the method of Dellaporta et al. (1983), digested with *EcoR*V (Bethesda Research Labs, BRL) and electrophoresed through a 1 % agarose gel (UltraPure agarose, BRL). The Southern transfer was carried out according to Maniatis et al. (1982). The probe was prepared by random primer labeling (BRL) of the 600 bp *EcoR*V fragment of *sh2* DNA that had been isolated from an agarose gel. The Southern blot was probed as described in Maniatis et al. (1982).

Genomic DNA library construction

Leaf genomic DNA from the 12-day-old seedlings of the supersweet sweet corn W1065A was isolated using the CTAB method from the CIMMYT Applied Molecular Genetics Laboratory as based on method of Saghai-Maroof et al. (1984). A total of 5 µg of DNA was digested with *EcoR1* (New England BioLabs, NEB) for 6 h at 37 °C as described by the supplier. The digested DNA was fractionated on a 1 % SeaPlaque agarose (Lonza) Tris-Acetate-EDTA (TBE) gel run 16 h at 40 volts. The gel was stained with ethidium bromide and DNA in the 3–8 kb range was extracted using standard techniques and resuspended at 50 ng/µl in TE buffer. *EcoR1* digested DNA libraries in Lambda ZapExpress (3–7 kb insert size) and Lambda DASHII (9–20 kb insert size) were synthesized using protocols provided by the manufacturer. The CopyControl Fosmid library production kit (Epicentre) was used to generate fosmid libraries for screening. The libraries were constructed as described by the supplier (Epicentre).

DNA hybridization probes

PCR was used to generate probes from 5′ and 3′ side of the *sh2-R* insertion in W1065A DNA. The 3′insertion primers were (5′-GATAACACTGAACATCCAACGT-3′) and (5′- GATCCATCAGCAAAGTTGATCCCGCC -3′). This generates a 442 bp amplicon.

The 5′ primers were (5′-GGGAGTTCTATACTTCTGTTGGACTGG -3′) and (5′- CGTAGCTCTTGTGCTTGTCAGA -3′), 506 bp amplicon. The *x1* probe 5′ and 3′ primers were (5′- CAGGTGGTGGGAAAAAAAGC-3′) and (5′- CACTACTACTTACAGTAGACA -3′); *yx1* 5′ and 3′ primers were (5′- CATGTACATCTTCCTCACTG -3′) and (5′- CAGGTTTGCAAATGTGAGG -3′), 690 bp amplicon; *a1* 5′ and 3′ primers were (5′- TTTTTGCATACATCCACTCAG-3′) and (5′- TAATTACTAACAAAACACTCGG -3′), 600 bp amplicon; CL11820 5′ and 3′ primers were (5′- TTGGAATAAGTACAATTCT -3′) and (5′- ACAAATTCTCCGTGAGCATAT - 3′), 410 bp amplicon. PCR was completed with the following conditions (94 °C, 4 min), 35 cycles (94 °C, 30 s, X °C, 30 s, 72 °C, 1 min) with the annealing temperate (*X*) varying with primer pair Tm. The reaction contained 1X Expand buffer, 1U Expand DNA polymerase (Roche), 200 uM dNTP, 50 ng DNA, 50 ng primers, and nuclease-free water to 50 µl. PCR products were fractionated in 1 % SeaPlaque-TAE agarose gel and the isolated gel slice was treated with Gelase as described above. Probes were labeled with EasyTide (ά-32P) dCTP 3,000 Ci/mmol (Perkin Elmer) using Rediprime II random primer labeling system (Amersham Biosciences). Unincorporated nucleotides were removed using BioRad Micro Bio-Spin 30 columns. Probes were heated at 95 °C for 5 min before addition to hybridization solution.

Lambda phage screen

The phage library was plated at a density of 50,000 pfu per 150 × 25 mm L-agar plate as described by supplier (Stratagene), a total of 10 plates, 500,000 pfu were plated per library screened with the 5′ and 3′ *sh2* probes. Plates were incubated overnight at 37 °C. Plates were placed at 4 °C for 1 h prior to filter lifts. BioRad C/P or Hybond NX (GE Amersham) 137 mm filter circles and filters were treated as described by the supplier. Lifts were completed as follows: filters were placed on plates for 1 min, using forceps, then lifted and place colony side up on Whatman paper soaked with 0.5 M NaOH for 5 min. Membranes were then placed on Whatman paper soaked in 2X SSC for 5 min. The DNA was linked to the filters using a UV Stratalinker 2400 set at

2,000 × 100 µJ (Stratgene) and then air-dried on Whatman paper. The filters were pre-hybridized for 4 h in 250 mM NaPO4, pH 7.0, 7 % SDS, 1 % BSA at 65 °C and then fresh solution was added and allowed to equilibrate to 65 °C before the addition of probe. The filters were washed in 2X SSC, 0.5 % SDS for 30 min at 65 °C, followed by 0.2X SSC, 0.2 % SDS for 30 min at 65 °C. Filters were exposed to Kodak BIOMAX XAR film overnight with intensifying screens at −80 °C.

Colony hybridization screen

The fosmid library was plated at a density of 5,000 cfu per 150 × 25 mm L-agar plus 15 µg/ml chloramphenicol plate. A 100,000 cfu or 20 plates were screened for the *x1, yx1, a1* and *CL1180* probes. Hybond NX (GE Amersham) 137 mm filter circles were used in lifts. Lifts were completed as follows: filters were placed on plates for 5 min, using forceps, membranes were lifted from agar surface and placed colony side up on Whatman paper soaked with 0.5 M NaOH for 5 min. Membranes were then placed on Whatman paper soaked in 2X SSC for 5 min. The DNA was linked to the filters using a UV Stratalinker 2400 set at 2,000 × 100 µJ (Stratgene) and then air-dried on Whatman paper. The filters were pre-hybridized for 4 h in 250 mM NaPO4, pH 7.0, 7 % SDS, 1 % BSA at 65 °C and then fresh solution was added and allowed to equilibrate to 65 °C before the addition of probe. The filters were washed in 2X SSC, 0.5 % SDS for 30 min at 65 °C, followed by 0.2X SSC, 0.2 % SDS for 30 min at 65 °C. Filters were exposed to Kodak BIOMAX XAR film overnight with intensifying screens at −80 °C.

Isolation of phage DNA and fosmid DNA for sequencing

Lambda DASHII phage DNA was isolated using the Qiagen Lambda Mini Kit. The isolated lambda DNA was amplified using the GE GenomPhi Whole Genome Amplification kit for sequencing. Lambda Zap Express phage were converted to plasmid using the In Vivo Excision Protocol described in the Lambda Zap Express manual (Stratagene). Fosmid DNA was isolated using the Sigma PhasePrep BAC DNA kit.

Sequencing

DNA preparations for 2–4 independent clones were processed following the manufacturer's instructions (Qiagen). DNA was subjected to sequencing analysis using the BIGDYE™Terminator Kit according to manufacturer's instructions (ABI). Sequencing made use of primers designed to both strands of the predicted nucleotide sequence of interest. DNA sequencing was performed using standard dye-terminator sequencing procedures and automated sequencers (models 373 and 377; Applied Biosystems, Foster City, Calif.). All data were analyzed and assembled using the Phred/Phrap/Consed software package (University of Washington) to an error ratio equal to or less than 10^{-4} at the consensus sequence level.

Fosmid DNA was treated with the EZ-Tn5 insertion kit (Epicentre) to generate random insertions for rapid sequencing of the 35–40 kb fosmid inserts. Lambda DNA was sequenced by primer walking. Fosmid clones were digested with restriction enzymes, fragments isolated and ligated into BlueScript (Stratagene) and sequenced. This allowed the assembly of repetitive regions that the transposon mutagenesis (Epicentre EZ-Tn kit) or primer walking failed to complete alone. Assembly of sequences was completed with the program Sequencher (GeneCodes). All sequences have been deposited in Genbank under the accession numbers KJ811995 for the sequences attached to the 5′ *Sh2* region and KJ811996 for the 3′ *Sh2* segment.

PCR confirmation of 3′ lambda DASH clone 6

Because the 3′ Lambda DASH clone 6 derived from an *EcoR1 sh2-R* DNA digest, contained an internal *EcoR1* site, PCR was used to confirm that clone 6 was not chimeric but rather contiguous within the genome. The clone contains an *EcoRI* site at 16,060 bp. Primers were designed to amplify from clone 6 into the coding sequence of the *sh2* gene. The primers were 2098f (5′-GCACTGTGCTCAT CATCCCTT-3′) and 741r (5′-AGAAAATTTGACTGGAA GTCTC-3′). The following cycle condition were used: (94 °C, 4 min), 35 cycles (94 °C, 30 s, 52 °C, 30 s, 72 °C, 2 min). The reaction contained 1X Expand buffer, 1U Expand DNA polymerase (Roche), 200 µM dNTP, 50 ng of *sh2-R* DNA, 50 ng primers, and nuclease-free water to 50 µl. This produced the expected 2.1 kb amplicon from *sh2-R* DNA.

Results

The molecular lesion causing *sh2-R* was first assessed by Southern blotting (Fig. 1). Genomic DNA from two maize lines containing a wild type *sh2* allele, Black Mexican Sweet and a wild type revertant of *sh2-m1*, *Rev 25* (Giroux et al. 1994), and the *sh2-R* containing sweet corn 'Florida Stay Sweet' was digested with *Eco*RV and probed with a 600 bp *sh2 Eco*RV fragment beginning 284 bp from the 3′ terminus of intron 2 and terminating 33 bp into exon 4. As expected, a 600 bp fragment hybridized to this probe in the two wild type lines. In contrast, two fragments of approximately, 3.6 and 4.8 kb hybridized in DNA from *sh2-R*. PCR

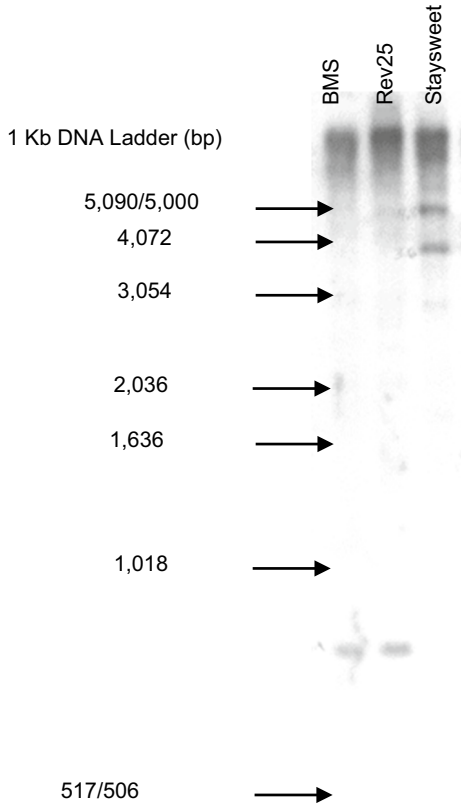

Fig. 1 Southern blot of genomic DNA digested with EcoRV and probed with the 632 bp EcoRV fragment from *Sh2* containing sequences from internal intron 2–33 bp of the 5′ end of exon 4. BMS (Black Mexican sweet) = *su Sh2*, Rev25 (field #34046) = *Su Sh2*, Staysweet = *Su sh2*-R

analysis spanning this region of the mutant resulted in no product and amplification of the remaining portion of the gene detected no differences from wild type. The simplest interpretation of the observations to this point was that *sh2* had suffered the insertion of a DNA fragment of at least 8 kb in length.

Further insight into *sh2-R* arose from sequencing *sh2-R* containing genomic lambda and fosmid clones. Informative clones are listed in Table 1. The 5′ or 3′ designations refer to clones hybridizing to the portion of *sh2* either 5′ or 3′ to the insertion, respectively. The presence or absence of genes known to be closely linked to *sh2* is also shown. In total, 69,686 bp from the 3′ end of the insertion and 32,426 bp from the 5′ end were sequenced. Attempts to isolate clones overlapping the 5′ and 3′ termini were unsuccessful.

A search for *EcoR*V sites in the sequences revealed that the 5′ *sh2*/insert fragment is 4.6 kb and the 3′ *sh2*/insert fragment is 3.5 kb, in accordance with the Southern blot in Fig. 1. The repetitive nature of the 5′ sequence hindered further sequencing and analysis. Much of the 5′ insert has sequences similar to transposable elements, such as Gypsy and Copia, based on analysis using the POPcorn (PrOject Portal for corn) search algorithm for repeat sequences found at MaizeGDB (http://www.maizegdb.org/).

The 3′ insertion sequence yielded more useful information. In wild type maize (i.e. B73, the inbred used for the genome sequencing project), *sh2* is found on the long arm of chromosome 3 and is closely linked to the gene *a1*. The genes *yz1* and *x1* are found between *a1* and *sh2* and all four of these genes are arrayed along the chromosome with the direction of transcription toward the centromere (Fig. 2). These genes are also found in the sequence of the 3′ insert and occur in the same order. However, the sequence of *sh2* begins with the last 3 bp of exon 3 and continues to the stop codon. In other words, a 5′-truncated *sh2* gene occurs in the opposite direction from wild type vis-à-vis the other genes

Table 1 *sh2-R* clone insertion size and gene content

Clone name	Clone containing 5′ or 3′ *sh2*	Size (kb)	*x1* gene	*yz1* gene	*a1* gene	*CL11820* gene
Lambda Zap clone 8	5′	4.85				
Fosmid clone 11-1	5′	37.450				
Lambda DASH clone 6	3′	18.879	Yes			
Fosmid clone 1-3-1	3′	33.865	Yes	Yes	Yes	
Fosmid clone 15-2-1	3′	35.902	Yes	Yes	Yes	
Fosmid clone 7-2-1	3′	42.099		Yes	Yes	Yes
Fosmid clone 15-1-1	3′	35.145		Yes	Yes	Yes
Fosmid clone 4-1-1	3′	41.020		Yes	Yes	Yes

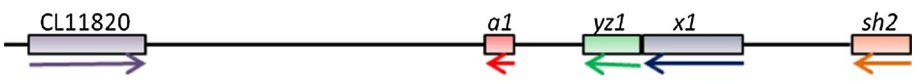

Fig. 2 Diagram of the wild type chromosome 3 gene arrangements of sequences detected in the 3′ junction fragment. *Boxes* represent genes and the *arrows* below indicate the direction of transcription

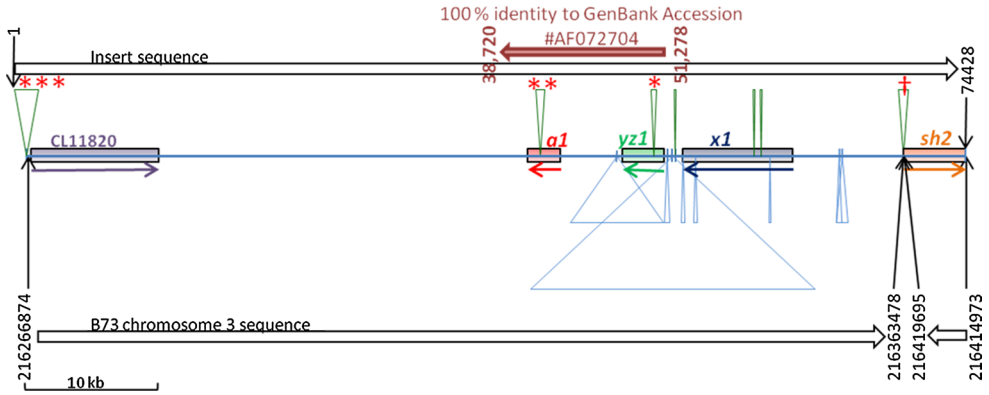

Fig. 3 Diagram of the 3' insert. *Boxes* represent genes with *arrows* below indicating transcription direction. The *blue line* indicates sequence similarity between the insert and B73 chromosome 3 with >97 % identity and ≤2 % gaps. The *green triangles* above the *blue line* indicate sequences that are in the insert and not in B73 and the *light blue triangles* below the *line* represent sequences in B73 that are not in the insert. The *brown arrow* indicates sequence that has 100 % identity to GenBank accession #AF072704 and the *numbers* indicate the position in the insert

(Fig. 3). The physical orientation of the genes in relation to the centromere of the *sh2-R* chromosome is unknown. As can be seen in Fig. 3, the area between *a1* and *sh2* diverges from that of B73 having additional sequence between the *a1* and *yz1* genes and between the *yz1* and *x1* genes. B73 is likely not the progenitor of *sh2-R* and so these differences most likely are separate from those that created *sh2-R*. A search of GenBank maize sequences detected 100 % identity to accession AF072704, a genomic sequence of *a1* to *yz1* from the stock *a1::rdt sh2* (Xu et al. 1995), suggesting that the *sh2* allele in the latter stock corresponds *to sh2-R*.

Assuming the progenitor wild type, *Sh2* allele and *sh2-R* sequences have had little sequence change other than those causing the disruption of *sh2* and assuming the 5′ and 3′ sequences here are contiguous, the most parsimonious scenarios for the rearrangement are illustrated in Figs. 4 and 5. Diagram 1 of Fig. 4 portrays this section of chromosome 3 from the progenitor. The solid line represents the sequence from the 3′ portion of *sh2* and the dashed line indicates the sequence from the 5′ terminus. A loop is formed by a segment of the long arm of chromosome 3 bringing *sh2* in close proximity to sequences downstream from *sh2* (Diagram 2). The rearrangement is depicted in Diagram 3 where breaks occur close to *sh2* and in the looped segment in the distal portion of chromosome 3. A third break takes place 3 bp internal to the 3′ end of *sh2* exon 3 and the chromosome is repaired by attaching to the breakpoint distal to *sh2* (Diagram 4). The 3′ portion of *sh2* is inverted in the ligation process.

The scenario above creates an inversion with *a1*, its accompanying genes and surrounding sequences inserted between the ends of *sh2*. It is also possible that both *sh2* pieces are on the same side of the other genes (Fig. 5). The loop of chromosome 3 may have come from the telomere side of the genes to separate the gene pieces. However, this scenario must also involve a break within *sh2,* and inversion of the 3′ terminus of *sh2* before reinsertion into the chromosome.

We considered the possibility that the rearrangement is more complex and the two junction sequences are not contiguous. More than the three breaks discussed above are required for the two sequences to be dispersed to different locations in the genome and it may then be possible to separate the two *sh2* termini through recombination. In previous analysis of newly created *sh2* mutations that are phenotypically distinguishable from *sh2-R* (Shaw et al. 1995), we analyzed 140 seeds exhibiting the *sh2-R* phenotype that were derived from self-pollination of plants heterozygous for *sh2-R*. All 140 samples contained both the 5′ and the 3′ junction fragments. Recombination was not observed. We conclude that the two termini of *sh2* are closely linked in *sh2-R*.

Discussion

Here, we show that the *sh2-R* allele, the allele that originally defined the *sh2* locus, was caused by a rearrangement of chromosome 3 that also may have involved the closely linked and previously described genes *a1*, *yz1* and *x1*. While homozygosity for deletions spanning the *a1* to *sh2* region conditions severely shrunken, unviable seed (Yandeau-Nelson et al. 2006a), it is interesting to note that *sh2-R* is viable. Rates of germination and seedling vigor in *sh2-R* mutants are reduced; however, these differences are likely due to inadequate starch reserves in the mature mutant seed. This viability then points to the presence and function of the gene within this region that is required to complete a life cycle.

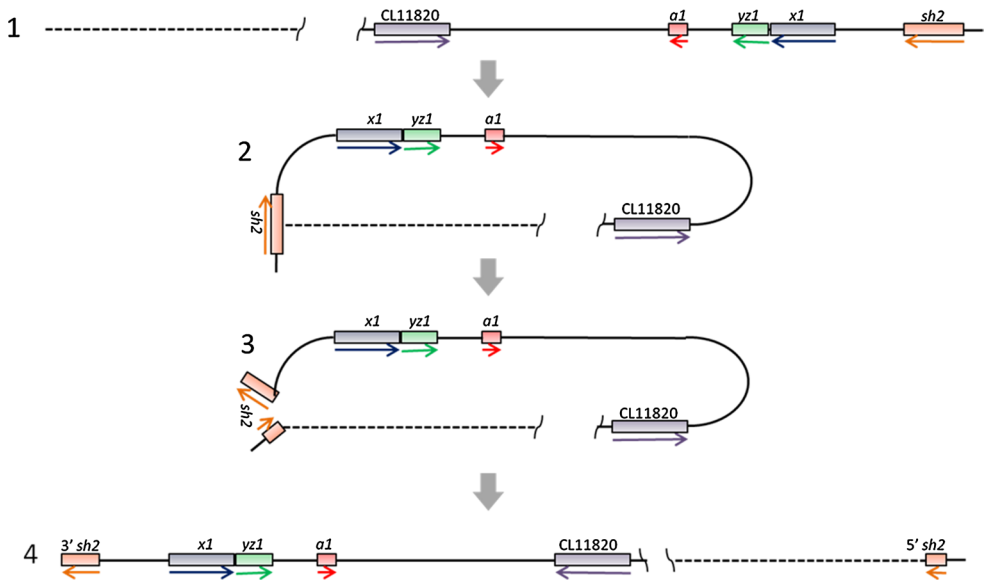

Fig. 4 The simplest scheme for formation of an inversion with *a1* between the termini of *sh2*. *Diagram 1* represents the progenitor form of part of chromosome 3. The *solid line* is the area of chromosome 3 containing the genes CL11820 to *sh2*, *colored boxes* represent the genes and the *arrows* below the *boxes* indicate the direction of transcription. The *dashed line* indicates the sequence proximal to *sh2* that will break and rejoin with sequences within and adjacent to *sh2*. The chromosome is contiguous, but our sequences do not overlap; hence, the gap between the *dashed* and *solid lines*. *Diagram 2* illustrates the loop in the chromosome that brings *sh2* in proximity with upstream DNA. In diagram 3, *sh2* breaks in two with the 5′ terminus attaching in the upstream area and the 3′ piece flipping before reattaching near the original break. The newly formed inversion is represented in *diagram 4*

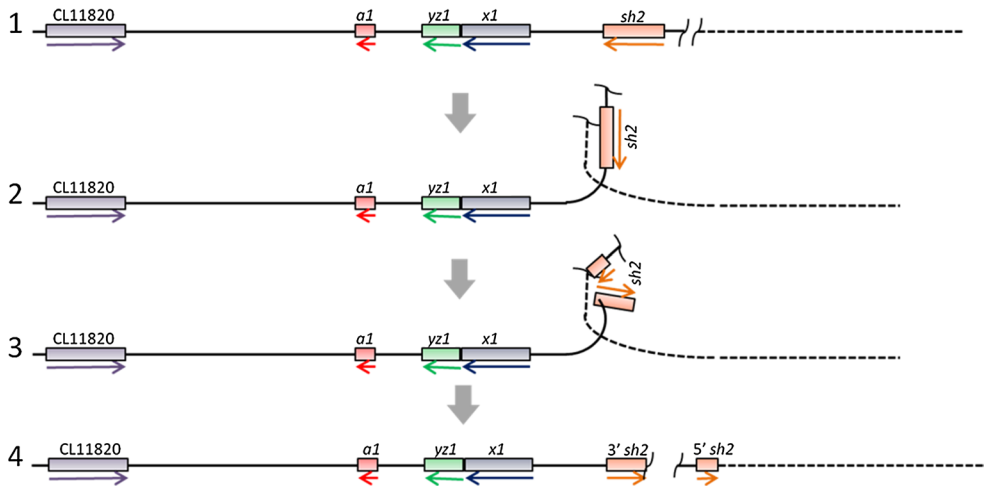

Fig. 5 A scenario for the rearrangement of *sh2* in *sh2-R* involving exchange with sequences distal to the gene. *Diagram 1* represents the progenitor form of part of chromosome 3. The *solid line* is the area of chromosome 3 containing the genes CL11820 to *sh2*, *colored boxes* represent the genes and the *arrows* below the *boxes* indicate the direction of transcription. The *dashed line* indicates the sequence distal to *sh2* that will exchange DNA with *sh2*. The chromosome is contiguous, but our sequences do not overlap; hence, the gap between the *dashed* and *solid lines*. Diagram 2 demonstrates the loop involving *sh2*. In diagram 3, *sh2* breaks and DNA is transferred leading to the illustration in diagram 4 containing inverted 3′ and 5′ pieces of *sh2* encompassing an unknown sequence

A large series of recombinational studies have been performed within the *a1–sh2* region using mutant alleles of *sh2* and *a1* that are easily distinguishable by eye (Yandeau-Nelson et al. 2006a, b and references cited therein). The mutant *sh2* allele used in these studies was derived from the sweet corn 'Sweet Belle' and PCR analysis for the junction sequences unique to *sh2-R* revealed that this sweet corn does contain the *sh2-R* allele (data not shown). The presence of a complex rearrangement in a heterozygote would be expected to reduce the rate of observed recombination.

As expected, while the rate of recombination between *a1* and *sh2* measured in heterozygotes involving the *sh2-R* allele is 0.09 ± 0.01 cM (Civardi et al. 1994), the recombinational distance determined from employment of RFLPs and wild type alleles is approximately, 0.5 cM (Schaeffer et al. 2006).

The structure of *sh2-R* reported here likely bears on the elegant recombinational studies from the Schnable laboratory (Civardi et al. 1994; Yao et al. 2002; Yandeau-Nelson et al. 2006a, b). Recombinants in the *a1-sh2* region were selected from plants containing *sh2-R* and a functional *sh2* allele as well as phenotypically distinguishable alleles of the closely linked *a1* locus. DNA polymorphisms were then used to more precisely place and quantify exchange events within this 0.1 cM region. These investigators noted that while genes are considered hot spots for recombination, as seen in their studies for the *a1* and *yz1* genes, the *x1* gene lying in this region appears to be an exception. The rate of recombination in *x1* in these stocks is comparable to the rate found in non-genic regions. Furthermore, recombination in the non-genic region separating *x1* and *sh2* is virtually non-existent. The data reported here provide an explanation for this abnormality. We note here that *x1* is relatively close to the chromosomal rearrangement underlying *sh2-R*. This large structural polymorphism and the resulting difficulty of chromosomal pairing with wild type *sh2* may then provide an explanation for the reduced recombination in *x1* and in the region separating *x1* and *sh2*.

It is interesting to note that the vast majority of events that changed the linkage relationship of the *a1* and *sh2* alleles are single reciprocal exchange events in the *x1, yz1, a1* region. This then provides evidence that the *x1, yz1, a1* region is in the same orientation in the wildtype and *sh2-R* parents since single meiotic crossover events in inversions produce dicentric and acentric chromosomes. These are genetically unstable, normally lost in subsequent divisions and hence not recovered.

While the *sh2-R* mutation defined the *shrunken-2* locus and is presently the cornerstone of the sweet corn industry, its origin is unknown. The mutation occurred at least 72 years ago (as reported by Mains 1949) and was found in a stock of unknown parental origin. Shrunken seeds were found from self-pollination of plants having a female parent derived from open pollination of an unknown commercial line while the male parent was an inbred termed LA. Because numerous chromosomal breaks occurred in the origin of this allele, it is interesting to note that no mention of mutagenesis with X-rays or other mutagens was made in the original report describing this mutant.

Author contribution statement VK performed the genomic cloning and sequencing, interpreted data and helped to prepare the manuscript, JRS performed Southern analyses, interpreted data and helped to prepare the manuscript, MLS conceived the experiments and aided in data interpretation and preparation of the manuscript. LCH furnished genetic stocks, interpreted molecular, genetic and recombinational data and prepared and finalized manuscript preparation.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The experiments in this study comply with the current laws of the USA.

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