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The *Uq-ruq* and *En/Spm-I/dSpm* transposable element systems in *Zea mays* L.

by

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For the Major Program

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For the Graduate College

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CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

This dissertation is organized in an alternative format. It is composed of seven chapters including chapters of the general introduction and conclusion. References are placed at the end of each chapter. Chapters include papers: Chapters 4 and 6 are papers published in journals. Chapter 5 is a paper submitted to a journal, and Chapters 2 and 3 are in preparation for submission to journals for formal publication.

Chapter 2 presents a thorough review on the Ubiquitous (*Uq*)-responder to Ubiquitous (*ruq*) transposable element system. Since its discovery (Friedemann and Peterson 1982), several studies have described very interesting phenomena. These include the overlapping function with the *Ac-Ds* system, prevalence in diverse maize genetic and breeding materials, and differential transactivity of individual *Uq*'s in association with *ruq* elements. With no comprehensive review available on the *Uq-ruq* transposable element system, it is the purpose of Chapter 2 to review previous researches, discuss implications in those phenomena, and update them with new information. Each of these characteristics is reviewed and discussed in detail, updating together with current information. This review is further intended to suggest some ideas for future researches on the *Uq-ruq* system.

Chapter 3 characterizes a *Uq*-induced dominant miniature mutant, *Mn::Uq*, which is the first *Uq*-tagged allele. Because both the tagged gene *Mn* and the transposon mutagen *Uq* have not been cloned, it is important to investigate *Mn::Uq* in detail as a first step to clone *Uq*. This chapter presents detailed phenotypes of *Mn::Uq*, discusses the implications in the phenotypes, shows the chromosomal location of *Mn::Uq* through mapping experiments, and describes the molecular experiments that were used to explore the relationship between *Uq* and *Ac* or *Ds1*.

The importance of cloning *Uq* can be found in researches to be made in the future on those interesting phenomena reviewed in Chapter 2 as well as the cloning of *Mn*, which shows pleiotropy on pollen tube growth and kernel development (Pan and Peterson 1989).

Chapters of 4, 5 and 6 on the *En/Spm* transposable element system are additional studies to my Ph. D. program. Whether *En/Spm* transposes after or before host DNA replication has

been questioned. The analysis on the transposition mechanism of *En/Spm* through reciprocal crosses between an autonomous *En* allele and a nonautonomous *En* allele sitting at the *a1* gene led to additional interesting findings, such as excision timing, developmental pattern, and an *En/Spm* modifier that requires two copies for its phenotypic expression. It is concluded that *En/Spm* transposes in most cases before, but can sometimes after, host DNA replication.

Literature Review

Maize transposable element systems

The concept of a transposable element (or transposon) system was developed by McClintock (1948, 1949, 1950, 1951) based on the Mendelian inheritance of instability (or mutability) of unstable (or mutable) loci in maize. Instability typically refers to the phenomenon that a recessive locus is unstable and mutates toward a dominant expression. An unstable locus can mutate autonomously with respect to no other factor required (autonomous instability) or may require a separate factor for instability to be expressed (non-autonomous instability). An example of instability is a maize kernel color variegation when an unstable locus of interest is involved in the anthocyanin pathway. Figure 1 portrays examples of kernel color variegations induced by the *En/Spm-I/dSpm* system.

A transposable element is a DNA unit that can change its chromosomal location via excision from its original site and insertion into another site. An intact functional transposable element codes for a protein(s)/transposase(s) that make the DNA unit self-transposable. Such a self-functioning element is referred to as an autonomous transposable element (Figure 1B). A defective transposable element is typically derived from internal deletions of an autonomous transposable element and does not retain the self-transposing capability. However, a defective element can normally respond to transposases provided *in trans* and transpose. Such a non-functioning but responding element is referred to as a non-autonomous transposable element, receptor or responder element (in that it receives and responds to the *trans*-signal of its autonomous element), or reporter element (in that its

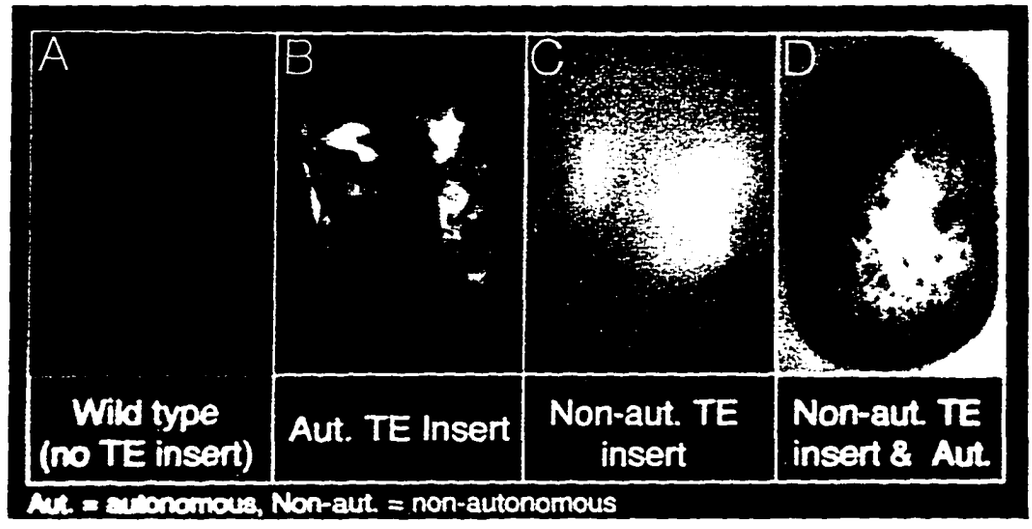

Figure 1. An example of instability: kernel color variegation.

A. A wild-type colored kernel. No transposon insertion occurred in a gene involved in the anthocyanin biosynthetic pathway.

B. A kernel with color variegation: an example of autonomous instability. An autonomous transposon (e.g., *En/Spm*) inserted into the *a1* gene, one of genes involved in the anthocyanin biosynthetic pathway. Autonomous elements can transpose without any *trans*-acting help. Somatic excisions of *En/Spm* from *a1* are detected as color variegation.

C. A colorless kernel. The insertion of a non-autonomous element *I/dSpm* into the *a1* gene blocks the *a1* transcription, so that no color formation occurred.

D. A kernel with color variegation: an example of non-autonomous instability. The non-autonomous element at the *a1* gene in **C** can transpose only in the presence of *trans*-acting factors. Thus, its autonomous element or transposase source is required for color variegation.

expression reports the presence of its autonomous element). The autonomous element is also referred to as the regulatory or controlling element in a sense that it regulates or controls the instability of a non-autonomous mutable locus (Figure 1D).

The interaction between an autonomous element and its derivative non-autonomous element is absolutely specific. In this tenet, a non-autonomous element responds only to transposases of its progenitor autonomous element. Thereby, a transposable element system can be defined as a specific two-component interaction in which a receptor element is mobilized only by the *trans*-acting factors of the same system. By such a specific interaction, a number of transposable element systems have been identified and defined in maize (Table 1). Noticeable is that three pairs of these systems function partially in duplicate. These systems in pairs include *Ac-Ds* and *Uq-Ds1/ruq* (Caldwell and Peterson 1992), *En/Spm-I/dSpm* and *F-En-c1m(r)888104* (Peterson 1997), and *Fcu-rcu* and *Spf-r-R#2* (Gonella and Peterson 1978). The underlying mechanism for these quasi-duplicate relationships has not been resolved.

The typical molecular configuration of a transposable element consists of *cis*-determinant sequences located at both ends that include the terminal inverted repeats (TIRs) and the sub-terminal sequence motifs (SSRs), and the internal coding region that codes for *trans*-acting factors/proteins (Figure 2). Upon insertion, a transposable element generates a target site duplication (TSD). TSD is a short duplication of DNA sequences flanking the insertion site (Saedler and Nevers 1985). The number of sequences to be duplicated is specific and is part of the "signature" for each transposable element system (Table 2). TIRs and TSDs together are enough to make each transposable element system exclusively specific. SSRs have also shown to be specific (Grant *et al.* 1990). During excision, various chromosome rearrangements result, producing diverse mutations and thus creating genetic diversity (Schwarz-Sommer *et al.* 1985).

Among the several maize transposable element systems, the *Uq-ruq* system is described and investigated in detail in this dissertation. A thorough review on the system is presented in Chapter 2. Reviews on other well-known systems such as *Ac-Ds*, *En/Spm-I/dSpm*, and *MuDR-Mu* will not be made here, but can be found in several excellent review

Table 1. Maize transposable element systems.

System*	**Non-autonomous**									
Autonomous	*rDt*	*Ds*	*Ds1*	*I/dSpm*	*c1m(r)*	*r-cu*	*r-R#2*	*Mu1*	*rbg*	*mut*	*mrh*
Dt	+										
Ac(Mp)		+	+								
Uq			+								
En/Spm				+	+						
F-En					+						
Fcu						+	+				
Spf							+				
MuDR								+			
Bg									+		
Mut										+	
Mrh											+

+ indicates a specific system response, in which an autonomous element mobilizes a corresponding non-autonomous element.

**Dt-rDt* (Rhoades 1938), *Ac-Ds* (McClintock 1948), *Uq-Ds1/ruq* (Friedemann and Peterson 1982; Pisabarro *et al.* 1991; Caldwell and Peterson 1992), *En/Spm-I/dSpm*(Peterson 1953), *F-En-c1m(r)888104*(Peterson 1997), *Fcu-r-cu* (Gonella and Peterson 1978), *Spf-r-R#2* (Gonella and Peterson 1978), *MuDR-Mu1* (Robertson 1978), *Bg-rbg* (Salamini 1980), *Mut-mut* (Rhoades and Dempsey 1982), *Mrh-mrh* (Rhoades and Dempsey 1982).

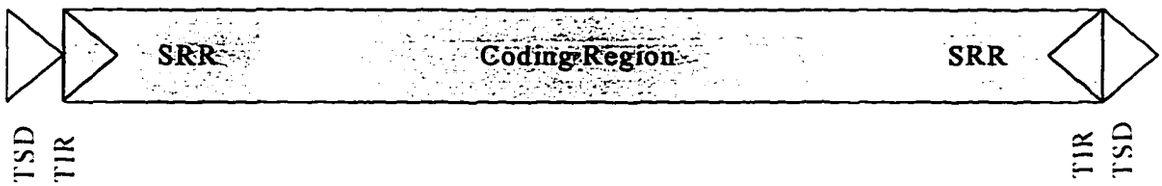

Figure 2. General, schematic configuration of a transposable element.

TSD, target site duplication (created upon insertion); TIR, terminal inverted repeat sequences; SRR, sub-terminal repetitive region (within which transposase-binding motif sequences are arranged). TIR and SRR are cis-requirements for transposition. Coding region codes for a *trans*-acting transposase(s) for transposition.

Table 2. The number of base pairs: terminal inverted repeats (TIRs) and target site duplication (TSD) that are specific for a distinct transposable element system.

	No. of base pairs		
Systems	TIR	TSD	References
Ac-Ds	11	8	Pohlman *et al.* 1984
Dt-rDt	14-19	9	Brown *et al.* 1989
En/Spm-I/dSpm	13	3	Pereira *et al.* 1985
Mrh-mrh	80	9	Shepherd *et al.* 1989
MuDR-Mu	>220	9	Bennetzen *et al.* 1984
Bz-rbg	5	8	Hartings *et al.* 1991

papers (Peterson 1987; Fedoroff 1989; Walbot 1991; Bennetzen *et al.* 1993; Gierl 1996; Kunze 1996; Bennetzen 1996).

Reviewed below are mechanisms of transposable elements that contribute to maize genome evolution, their impact to control or modify gene expression, and how they have been applied in plant genome research. After a half century of studies on transposable element systems, these are the main aspects of general interest, rather than uncovering new systems.

There are a number of references that were not included while reviewing. This is not because they are not appropriate but because there are too many to be included. The reviews below are not to review all related studies in such detail. but to look over the roles that maize transposable elements play in the genome.

Mechanisms by which transposons contribute to maize genome sequence diversity

Studies on the activities or mutageneses of transposable elements in the past one and half decades have provided substantial documentations that transposable elements cause changes in genes and are a major part of the maize genome. Mechanisms by which transposable elements can accelerate the evolution of genomes can be generally and arbitrarily categorized into 1) middle repetitive sequence families of dispersed copies. 2) transposition mechanism of insertion and excision. 3) other mechanisms that are generated often in combination of the first and second mechanisms. and 4) involvement in intron formation.

Repetitive sequence families

Transposon sequences are present in a number of copies. making a dispersed family of middle repetitive sequences in the maize genome. Repetitive sequences of homologous DNA copies have been known to serve as substrates for genome rearrangements through recombination. This has been well demonstrated in the case of a contiguous 280 kb region flanking the maize *Adh1-F* gene with 37 classes of repeat sequences or retrotransposons that accounted for >60% of that region (SanMiguel *et al.* 1996). Various types of chromosomal rearrangements can be induced by transposable elements such as insertions. deletions. inversions. duplications. and translocations (Döring *et al.* 1985: Ralston *et al.* 1988: Döring *et al.* 1990: Robertson *et al.* 1994: Walker *et al.* 1996). Mitotic homologous recombination and asymmetrical synapsis between transposon sequences have also been reported (Athma and Peterson 1991: Lowe *et al.* 1992). Further. a transposon can affect intragenic recombination either in a positive or negative way (Greenblatt 1981: Dooner 1986: Dooner and Kermicle 1986: Brown and Sundaresan 1991). and promote intra-chromosomal crossovers and conversions near the insertion site (Doseff *et al.* 1991).

Transposition mechanism of insertion and excision

The transposition mechanism of insertion and excision (Saedler and Nevers 1985) is a unique and inherent way for transposable elements to generate diverse alleles of a gene. Analyses of footprints of all the transposable elements sequenced have provided support for the role of transposable elements in the creation of genetic diversity, by repeated insertion and excision (Saedler and Nevers 1985; Dennis *et al.* 1986; Shen *et al.* 1992).

The insertion of transposable elements into genes leads to the tandem duplication of the target site sequence (TSD), in which the number of sequences to be duplicated is specific for each transposable element system (Table 2). This process adds that number of nucleotides to the genome. The duplicated and adjacent sequences are further altered by an imprecise repair process during or after excision (Saedler and Nevers 1985). Adjacent sequences may influence DNA repair (Scott *et al.* 1996). Sequence modifications at the excision site are quite variable. Small sequence alterations are commonly made at the insertion site after excision (Schwarz-Sommer *et al.* 1985). The duplicated sequences are retained in a modified form following the deletion of one or a few bases and/or by a base substitution(s) in most revertants (Sachs *et al.* 1983; Bonas *et al.* 1984; Fedoroff *et al.* 1984; Weck *et al.* 1984; Schwarz-Sommer *et al.* 1985; Sutton *et al.* 1984). Complementary transversions of the two central base pairs are often observed at the site of *Ac*, *Ds* and other transposable elements in both native host and transgenic plants (Sachs *et al.* 1983; Baker *et al.* 1986; Dennis *et al.* 1986; Rommens *et al.* 1993). Some terminal sequences of a transposon can be left behind at the excision site, probably by gene conversion (Shen *et al.* 1992).

Diverse alleles generated by the excision process have been well demonstrated especially with *a1*, *adh1* and *wx* genes (Dawe *et al.* 1993; Kloeckener-Gruissem and Freeling 1995; Wessler *et al.* 1986; Schwarz-Sommer *et al.* 1985). In maize, only one out of ten excision events may lead to an 'in-frame' excision without leaving additional nucleotides (Schwarz-Sommer *et al.* 1985). Excision of *Mu1* from *bz1::Mu1* is estimated theoretically to generate more than 500.000 unique alleles given the deletion of up to 30 bases and insertion of 0-5 bases observed thus far in sequenced excision alleles (Nordborg and Walbot 1995).

Other complex mechanisms

There have also been examples that cannot be explained by only one mechanism of recombination, but by two or more mechanisms of recombination in association with transposition (Döring *et al.* 1990; Belzile and Yoder 1994; Kloeckner-Gruissem and Freeling 1995). Double *Ds*-induced chromosomal rearrangements at *sh1* include unequal cleavage of sister chromatids both at the double *Ds* structure and at a certain distance from them, unequal crossover, and complete excision of one *Ds* (Döring *et al.* 1990). *Mu3*-induced promoter scrambling at *adh1* was explained by a fragmentation model that involved deletion, inversions, inverted duplication, and incorporation of sequences of unknown origin (Kloeckner-Gruissem and Freeling 1995). Two neighboring *Ac*'s in transgenic tomato produced derivatives via complex chromosomal rearrangements (Belzile and Yoder 1994).

Intron formation

Splicing events that remove transposable element inserts from primary transcripts and restore the wild-types have suggested that transposable elements are one of sources of new introns (Wessler 1989; Logsdon *et al.* 1994). Transposon inserts that participate in the evolutionary diversification of gene structure as introns are discussed by Purugganan (1993). Examples of transposons as introns have been found mostly with *Ac* and *En/Spm* transposable elements, which have splice sites at the ends, at loci such as *a1* (Tacke *et al.* 1986), *a2* (Thartiparthi *et al.* 1995), *bz* (Raboy *et al.* 1989), *sh2* (Giroux *et al.* 1994), *wx* and *adh1* (Wessler 1989).

Splice sites that are already present and normally used can be utilized as well as those created upon element insertion, located within the element, or located within but not utilized by the wild type. For example, a 5' splice site within the element is joined to a 3' splice site created upon element insertion (*wx-B4*, *adh1-Fm335*), or to a 3' splice site adjacent to the element (*wx-m9*); a normal 5' splice site is joined to a 3' splice site within the element (*bz-m13*); both 5' and 3' splice sites are located within the element (*a2-m1*, whose wild-type has no intron); a normal 5' splice site is joined to a cryptic 3' splice site located within exon 4, which is not used in a wild-type (*adh1-2F11*) (Raboy *et al.* 1989; Tacke *et al.* 1986;

Thatiparthi *et al.* 1995; Wessler 1989). In case of *sh2-m1*, *Ds* and one copy of the target site duplication were precisely removed by alternate splicing (Giroux *et al.* 1994).

The different states of alleles that are derived from one progenitor allele may suggest how a transposon evolves into an intron. The *I/dSpm* allele *a1-m1 6078* (2242 bp) produces highly variegated kernels in the presence of *En/Spm* and near-colorless kernels in its absence. The further *a1-m1 6078* deletion derivatives, *a1-m1 1112* (945 bp) and *a1-m1 5719A-1* (789 bp), produce very low-variegated kernels in the presence of *En/Spm* and almost full-colored kernels in its absence (McClintock 1965; Reddy and Peterson 1984). The *I/dSpm* allele *a2-m1* (2242 bp: original state) and its deletion derivative *a2-m1* (1341 bp: class II state) produce, in the absence of *En/Spm*, very light pale-colored kernels and almost full-colored kernels, respectively (McClintock 1958). This *a2-m1* allele (1341 bp) did not yield germinal transposition out of more than 200.000 cases (Thatiparthi *et al.* 1995). The *I/dSpm* allele *bz-m13* (2242 bp) and its deletion derivative *bz-m13CS9* (903 bp) transposed 307 out of 496 and 3 out of 515, respectively (Raboy *et al.* 1989). These 'changes in state' indicate possible channels of a transposable element into more stable, effective introns. The terms 'stable' and 'effective' refer to less excision and a splicing event that recovers a higher level of activity of a gene product, respectively. This follows from the following sequence of events: an autonomous element changes into a defective, non-autonomous element (via internal deletion); then, a series of further deletions will lead to a lower frequency of transposition and utilization of more efficient splice sites. Optimum states would be selected through an evolutionary process.

Impact of transposons on gene expression

Maize transposable elements, when inserted into or near genes, can alter expression through mechanisms described previously at levels either of transcription or post-transcription. A transposon insertion into a gene is expected to block the expression of the gene. However, expression of a transposon-controlled gene can be quite variable as described below, although mostly on a reduced level. Further, a DNA repair process during or after excision of a transposon can generate numerous alleles of a gene as previously described. Such alleles have

been observed to show a varying range of enzymatic activities, ranging from null or partial activity in most cases to occasional overdominant activity (Wessler *et al.* 1986; Schwarz-Sommer *et al.* 1985; Dawe *et al.* 1993; Giroux *et al.* 1996).

Creation of dominant alleles

While most of the transposon-mediated mutations have been recessive, some dominant mutations have been reported. *C1-I* is a transposon-mediated dominant-negative mutant of the regulatory *c1* locus, and this codes for a repressor function in contrast to the activator function of the *C1* gene product (Paz-Ares *et al.* 1990). Dominance of *Mu* insertion-induced *Kn1* mutations (Greene *et al.* 1994, and references therein) comes from their ectopic expression. What makes the dominance of the *Mu* insertion-induced *Les28* mutation is not known (Martienssen and Baron 1994).

Changes in organ/tissue specificity

Changes in promoter activity or sequences can lead to changes in the levels of organ- or tissue-specific expression of the gene affected. *Adh1-3F1124* (*Mu3* insertion in the TATA box) showed the organ-specific alteration of *Adh1* expression (Chen *et al.* 1987). This *Mu3*-induced *adh1* promoter scrambling led to an unusual expression pattern showing decreased, increased, or similar levels of mRNA accumulation and enzyme activity in different organs, compared to its progenitor allele (Kloeckner-Gruissem *et al.* 1992; Kloeckner-Gruissem and Freeling 1995). A recessive *y1* allele, induced by the insertion of *Mu3* in the 5' end, is expressed by the promoter of *Mu3*. A wild-type *y1* allele expresses at highest levels in seedling, embryo, and endosperm. However, this *Mu3*-controlled *y1* allele was found to accumulate normal levels of *y1* mRNA in seedling and embryo but no detectable level in endosperm (Buckner *et al.* 1996).

Alternative splicing

Read-through transcription produces primary chimeric transcripts that include a transposon insert. As reviewed previously in the involvement of transposons in intron formation, the insert can be removed out through splicing. Splicing events have been observed to be commonly aberrant, resulting in reduced expression. Also known as intron skipping, alternative splicing commonly produces several forms of mRNA. The null *wx-m1* allele (409 bp

Ds1 insertion in exon 9) codes for several *Wx* transcripts due to alternative splicing of *Ds1* that utilizes three 5' splice sites and 3' splice sites, all but one of which are in *Ds1* sequences near the *Ds1* termini (Wessler 1991a). The *wx-m9* allele (4.3 kb *Ds* insertion in an exon) produced two alternatively spliced in-frame transcripts and slightly altered *Wx* proteins (Wessler 1991b). *Mu1* contains RNA processing signals in its central region, however it is related to alternate splicing, that is largely responsible for the decreased levels of steady-state transcripts (Luehrsen and Walbot 1990).

Read-out transcription

Mu1 and *Ds* elements have unusual promoters that promotes transcription outward from them, and these allow the controlled genes to express without excision. In the absence of *Mu* activity, *Mu1* in *hcf106* (a *Mu1* insertion in the 5'-untranslated region) allows the *Hcf* expression or normal phenotype by activating a promoter near the downstream end of *Mu1* that directs transcription outward, into the adjacent *hcf106* gene: transcription initiated at several sites throughout a 70 bp region within and immediately downstream of the *Mu1* insertion (Barkan and Martienssen 1991). In transgenic tomato with the construct of *Ds* and HPT II, the marker gene was observed to express even without the excision of *Ds*. The transcription initiation was mapped to multiple positions over about 300 bp in the sub-terminal part of *Ds* (Rudenko *et al.* 1994).

Gene silencing

Flavell (1994) suggested that gene silencing evolved to help silence the many copies of transposable elements. Co-suppression with transposable elements have been well observed with the *MuDR-Mu* system (for review, Bennetzen *et al.* 1993; Bennetzen 1996). Recently co-suppression between *Mu1*-insertions of *hcf106* and *Les28* was reported (Martienssen and Baron 1994). In *Antirrhinum majus*, paramutation has been reported with *Tam* transposons. The *Tam2*-insertion mutation (*niv-44*, paramutagenic) directed the phenotype of the *Tam1*-insertion mutation (*niv-53*, paramutable) (Krebbers *et al.* 1987). The *Tam3*-excision mediated semi-dominant allele, *niv-525*, had an inverted duplication of 207 bp in its promoter region and this allele was reported to act in trans to reduce a steady-state level of wild-type transcripts (Coen and Carpenter 1988).

Application and contribution of transposons to genome research

Gene tagging and identification

Transposable elements disrupt genes by insertion and subsequent deletions, DNA rearrangements, and point mutations, such that they have been used as valuable mutagens and molecular tags for yet uncloned genes. A number of such cases can easily be found from databases, not only in host species (Fedoroff *et al.* 1984; O'Reilly *et al.* 1985; Cone *et al.* 1986; Theres *et al.* 1987; Hake *et al.* 1989; Martienssen *et al.* 1989; Aukerman and Schmidt 1993; for review, Gierl and Saedler 1992) but also in heterologous species (Aarts *et al.* 1993, 1995; Bancroft *et al.* 1993; Biezen *et al.* 1996; Bishop *et al.* 1996). Several methods of utilizing transposable elements and facilitating their use in gene identification have been developed, especially in heterologous species. Maize transposons as tools and methodologies in gene tagging in heterologous organisms were recently reviewed by Osborne and Baker (1995). Transposon tagging methodologies in different species are carried out mostly with a binary system consisting of a transposase source and a non-autonomous element, either intact or engineered. Heterologous species offer a basic advantage that the introduced transposable element is the only copy in the heterologous genome, while a number of homologous copies are present in host genome.

Random mutagenesis. Insertional mutagenesis conventionally mediated via excision and insertion can be classified as 'random mutagenesis', compared to site-selected mutagenesis described below. Random mutagenesis has been used in tagging molecularly unknown genes. Once a mutant induced by a transposon is identified, the mutated gene can be cloned by use of known sequences of the element. A mutant phenotype of a previously unknown gene can be isolated randomly from a transposon-segregating population (non-targeted tagging). A mutant phenotype of a previously known gene can be selectively isolated (targeted tagging). Targeted tagging is facilitated by locating a transposon close to a target gene (Dash and Peterson 1989; Burbridge *et al.* 1995), taking advantage of the tendency of transpositions over short distances. The frequency with which a particular gene in maize can be tagged varies from 10^{-4} to 10^{-6} (Döring 1989). The frequencies can be increased by linking the transposable element to a

desired gene taking advantage of the preference of transposition to linked sites or the location of the transposon to the target site (Peterson 1993).

For heterologous species, engineered binary systems have been developed that consists of an independent transposase source and a non-autonomous element carrying a selectable marker. The marker facilitates the monitoring of excision, reinsertion and segregation of the elements. Transposase sources can either be non-mobile but transposase-producing elements such as stabilized *Ac*'s (Hehl and Baker 1989; Rommens *et al.* 1992; Healy *et al.* 1993; Bancroft *et al.* 1993) or a new *trans*-activating construct such as a T-DNA carrying transposase-coding genes and the selectable marker gene NPT-II for transformation (Cardon *et al.* 1993).

Site-selected mutagenesis. An already cloned gene can also be targeted to analyze phenotypes and to determine the function of the gene by screening a number of individuals for insertion events. In such site-selected reverse genetics, insertion can be identified by PCR amplification using primers from transposons and target genes (Das and Martienssen 1995; Cooley *et al.* 1996). This can be said to be a molecular parallel to fine structure genetic mapping via cyclic insertional mutagenesis, which is described later.

Entrapment strategies. Gene isolation by random mutagenesis has several limitations. It may be difficult or impossible to reveal a mutation that occurred in one of a set of genes conferring a quantitative trait. Knockout of a gene would not be observed if the gene is functionally redundant because a second gene can substitute for the same function. Insertion may not reveal the full role of genes which participate in early as well as late stages of development. Gene or enhancer trap transposon strategies have been developed to overcome these difficulties using the *Ac-Ds* system. (Fedoroff and Smith 1993; Klimyuk *et al.* 1995; Nussaume *et al.* 1995; Sundaresan *et al.* 1995). These strategies use an immobilized *Ac* element as the source of transposase and *Ds* elements constructed to act as enhancer or as gene traps. These systems identify genes by expression patterns during development, which are specific to organs, tissues, cell types, or developmental stages (Sundaresan *et al.* 1995). Expression patterns of trapped enhancers or genes are visualized by the expression of a reporter gene.

An enhancer trap transposon *Ds* carries a reporter gene with a weak or minimal promoter that is activated by a trapped chromosomal enhancer, and selectable marker genes that will facilitate selection of transpositions (Klimyuk *et al.* 1995; Sundaresan *et al.* 1995). When transposition occurs such that the promoter is under control of a trapped chromosomal enhancer, the reporter gene is expressed. A promoter identified in the 3' end of *Ac* is expressed only by neighboring *cis*-acting enhancer elements, suggesting its probable use in enhancer trap strategy (Cocherel *et al.* 1996).

A gene trap transposon *Ds* carries a reporter gene but without a promoter, an intron with splice acceptor sites before the coding region of the reporter gene, and selectable marker genes. Without a promoter, the expression of the reporter gene relies on transcription of a trapped chromosomal gene. Splice acceptor sites are introduced to facilitate the production of fusion proteins when insertion occurs into an intron. Splice donor sites available at the end of the element can be utilized when the element inserts into an exon (Nussaume *et al.* 1995; Sundaresan *et al.* 1995). The reporter gene is expected to be expressed when insertion occurs in the correct orientation.

Transposition-deletion system. This strategy for gene identification and cloning recently suggested by Haaren and Ow (1993) is based on the combination of DNA transposition and site-specific recombination such as the Cre-*lox* system. The T-DNA to be utilized in this system carries a *lox* site, a non-autonomous transposon with a second *lox* site, selectable (positive and negative) markers, and a promoter outside the transposon. The working hypothesis in this system follows. The non-autonomous transposon will transpose in the presence of transposase provided *in trans*, together with the second *lox* site in the middle of it. Subsequent Cre-induced recombination between the two *lox* sites will lead to deletion, inversion, or translocation of DNA segments, depending on the relative orientation and chromosome location of the *lox* sites. Most of the events are expected to result in deletion or inversion when considering the tendency of transpositions predominantly occurring over short distances. Positive excision and negative deletion selectable markers are arranged such that the excision marker is expressed after excision by the promoter and the deletion marker is expressed by the same promoter after the excision marker is removed through

recombination. Mutants obtained are stable even in the presence of transposase because the remaining half *Ds* after deletion cannot further transpose and recombination cannot occur because only one *lox* site remains in the genome. Deletions of different length can occur and likely delimit the location of the gene(s) responsible for the phenotype between the T-DNA and the transposon insertion site. Cre-mediated recombinations between two independent *Dslox* insertions (placed 5.6 cM and 16.5 cM from their T-DNA*lox*) and T-DNA*lox* were reported to be inversions in both cases: the smaller inversion was transmitted as a simple trait but the larger one was not (Osborne *et al.* 1995).

Fine structure genetic mapping and protein characterization

The molecular basis that underlies different phenotypes of transposon-induced mutations of a gene can enable us to allocate specific functions to specific regions within the gene sequence or its protein, making possible fine structure gene mapping (Wessler and Varagona 1985). The strong tendency of transposable elements for the short distance transpositions has been utilized to characterize genes via cyclic insertional mutagenesis in *p* (Athma *et al.* 1992; Moreno *et al.* 1992), *r* (Alleman and Kermicle 1993; Kermicle *et al.* 1989) and *wx* (Weil *et al.* 1992), as can now be done by site-selected mutagenesis described previously.

The variegation patterns conditioned by transposon-induced alleles can reflect structural features of a target gene, such as exon and intron locations (Moreno *et al.* 1992; Alleman and Kermicle 1993), and a critical segment for the protein function (Franken *et al.* 1994; Liu *et al.* 1996). All the exon insertions in the maize *p* gene were shown to produce striping of colorless or very light orange background pigmentation, while most intron insertions were heavier striping (Moreno *et al.* 1992). In like manner, insertions into exons of the maize *r* gene produced sparse variegations and those into introns or flanking regions lead to dense variegations (Alleman and Kermicle 1993). Autonomous *En* insertions of *a1-m(papu)* and *a1-m(Au)* into the second exon of *a1*, produce heavy spotting (Schwarz-Sommer *et al.* 1987). Minimal limits of a structural gene can be examined by investigating the protein products of unstable alleles of the gene (Echt and Schwartz 1981). All of the analyzed *Ac* insertions into *P-rr* have similarly inhibiting effects on pericarp and cob pigmentation, suggesting that *P-rr*

contains a single gene for cob and pericarp expression (Athma *et al.* 1992). Sequence comparisons of three *bz* alleles (*Bz-McC*, *Bz-W22* and *bz-R*) of maize lead to the allocation of specific functional sites within the gene (Ralston *et al.* 1988). The utility of natural mutations for defining functional domains in proteins have been demonstrated (Franken *et al.* 1994; Liu *et al.* 1996). The fine structural analyses of transposon-induced *C1* mutants indicated that the formation of an alpha helix is more important than a negative charge for the activation domain (Franken *et al.* 1994). The *Ds* insertion into the *r* gene altered the nuclear localization of the transcriptional activator R protein, indicating that the carboxyl terminus is far more important than previously considered (Liu *et al.* 1996).

Study of cell lineage and development patterns

Activity of transposons can be observed germinally as well as somatically. Germinal transpositions and those in somatic cells that lead to reproductive organs will be inherited. Somatic events can make genetic mosaics of two different genotypes. The genotypes can differ by the presence and absence of a transposon. The mosaic of an early somatic event will naturally reveal its cell lineage through mitosis. An early somatic event in plant development could reveal a developmental or cell differentiation pattern of an organ as demonstrated with maize ears (Seo and Peterson 1997). This can also be observed with any marker gene (Finnegan *et al.* 1989).

Determination of cell autonomy of a gene product

When the product function of a gene is restricted to the cells when the gene is expressed and does not diffuse into, or influence neighboring, unexpressed cells, the gene product is said to behave in a cell autonomous manner. Taking advantage that transposons can produce genetic mosaics of two different genotypes, one with a transposon and the other without it, the cell autonomy of a gene product can be determined. In such mosaics, sectors of a clear boundary indicate the behavior of a gene product in a cell autonomous manner. Some examples of such observations include *vp5* in maize with a chromosome-breaking *Ds* element (Wurtzel 1992), *rol*C of *Agrobacterium* rhizogenes in transgenic tobacco with *Ac* (Spena *et al.* 1989), *lg3* in maize with *Mu* (Fowler *et al.* 1996), and a selectable marker in transgenic tobacco or tomato (Scofield *et al.* 1994).

Identification of transcription activation domains

TNPA protein, one of *En/Spm*-encoded proteins, binds to sub-terminal motif sequences and acts as a repressor of the unmethylated *En/Spm* (Grant *et al.* 1990). In a yeast GAL4-based hybrid system, more than ten-fold transcriptional activation, compared with the GAL4 DNA-binding domain alone, was repressed by TNPA. On the other hand, 33- to 45-fold activation of the *En/Spm* promoter was observed with VP16 activation domain, compared with the TNPA DNA-binding domain alone (Schläppi *et al.* 1996). Through this study, the authors suggested that the TnpA gene and the TNPA DNA-binding sites in the promoter provide a highly sensitive single-hybrid system for identifying and studying transcription activation domains in plants.

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CHAPTER 2. THE UBIQUITOUS (*Uq*)-RESPONDER TO *Uq* (*ruq*) TRANSPOSABLE ELEMENT SYSTEM IN MAIZE (*Zea mays* L.): A REVIEW PERSPECTIVE

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Abstract

This review covers several features of the *Uq-ruq* transposable element system that have been described in the past fifteen years since the first report and discusses the implications of this system. The *Uq-ruq* system shares some functional activity with the *Ac-Ds* system, which may be explained by a degree of sequence homology between *Uq* and *Ac*. The activation of a silent *Uq* is deliberated as a probabilistic event, depending upon individual genotypes and cellular interactions during the probabilistic stabilization process of a stressed and perturbed genome. Search for *Uq* in assorted maize genetic and breeding lines has led to the isolation of a number of *Uq* elements. The diversity of the functional aspects among *Uq*'s has been revealed by the differential spotting patterns (DSPs) elicited from their interactions with a common reporter allele, *a1-* or *c1-ruq*. Probable factors involved in the DSPs of diverse *Uq*'s are discussed, which include differential methylation, different positions, and sequence differences among *ruq* elements.

Origin and definition of the *Uq-ruq* system

A single variegated kernel, which originated from a line showing the aberrant ratio (AR) following a treatment with the Wheat Streak Mosaic Virus (WSMV) (Sprague and McKinney 1966, 1971), was the origin of the two-element *Uq-a1-ruq* system (Friedemann and Peterson 1980). In characterizing the system and because of the universal prevalence of the regulatory element of this two-element system in numerous tester stocks (*a1 sh2* and *a1 Sh2*), there originated the nomenclature Ubiquitous (*Uq*) for the regulatory/autonomous element and the associated responder (*ruq*) for the non-autonomous element (*Ub* = *Uq*; Friedemann and Peterson 1980, 1982). These autonomous *Uq* and non-autonomous *a1-ruq*

elements have since been used as standard elements (*Uq-St* and *a1-ruq*, respectively) in uncovering and investigating new members of the *Uq-ruq* system.

Prevalence of *Uq*'s in assorted genetic and breeding materials and their role in creating diversity

Uq's that have been discovered and investigated since the original isolation of the *Uq-ruq* system are summarized in Table 1. These *Uq*'s have been uncovered from diverse maize sources by crossing the reporters (*a1-ruq* or *c1-ruq*) to these lines (Pereira and Peterson 1985; Peterson 1985; Peterson and Salamini 1986). Others have been uncovered via spontaneous activation of latent *Uq* elements (Pan and Peterson 1991a, b). The sources include assorted maize genetic testers and breeding lines and populations, in which no conscious selection for the element has been made (Cormack *et al.* 1988; Caldwell and Peterson 1989; Lamkey *et al.* 1991).

Several corn breeding populations were found to carry active *Uq* elements; however no inbred lines, except for one of the original 16 progenitors of BSSS (I159) (Sprague 1946), contained active elements (Cormack *et al.* 1988). These diverse *Uq*'s are distributed over the maize genome; some are clustered on chromosomal segments (Seo and Peterson 1995; see also Figure 2A) and segregate independently of *c1* (Table 1). Under the facts that *ruq* is equal to *Ds1* (Pisabarro *et al.* 1991) and the relationship of *Uq* to *Ds1* is unknown, the origin and distribution of *Uq* remains to be seen, considering that a number of *Ds1*-related elements have been present in *Teosinte*, the immediate wild precursor of maize, as well as in *Tripsacum dactyloides*, a more distantly related species (for review, Peacock *et al.* 1987).

Several studies have associated the abundance of active *Uq* elements in maize lines with relationship to genetic diversity of the maize genome (Peterson 1986, 1988, 1993; Peterson and Salamini 1986; Lamkey *et al.* 1991; Seo and Peterson 1995). Genomic events that occur either during or after transposition of excision and insertion have been well known to generate genetic diversity at or near the site of insertion of a transposable element (Saedler and Nevers 1985; Schwarz-Sommer *et al.* 1985). These have been well documented throughout the past two decades of studies of transposable elements. Recently, direct

Table 1. Diverse *Uq*'s uncovered from assorted maize genetic and breeding materials.

Uq	Source: original cross	Reference[d]	*Uq*[e]
Uq-St	Aberrant Ratio (AR) line[a]	1	*Uq-BSSSC5**
Uq-13	same as above	1	*Uq-BS13(S)C4**
*Uq-16**	same as above	1	*Uq-I159**
Uq-(C sh bz wx Ac)	*C sh bz wx Ac* x *a1-ruq*	1	*Uq-BSLE(ML)C10**
Uq-(a1 sh2)	*a1 sh2* x *a1-ruq*	1	*Uq-BSLE(MS)C15**
Uq-(a1 et)	*a1 et* x *a1-ruq*	1	*Uq-RHP1968**
Uq-(a1 et)(low)	*a1 et* x *a1-ruq*	1	*Uq-SHO1968**
Uq-(a1-dt sh2)	*a1-dt sh2* x *a1-ruq*	1	*Uq-HGC25*
Uq-(c2)	*c2* (from AR line) x *a1-ruq*	2	*Uq-Hiloss*
Uq-(c1)	*c1* (from AR line) x *a1-ruq*	2	*Uq-Jarvis**
*Uq-31**	*A1 C Sh Wx* x[b] *a1-ruq Uq-St*	3	*Uq-LC**
Uq-65, -66, 67**	same as above	3	*Uq-LS**
*Uq-2**	inbreds[c] x *a1-ruq*	4	*Uq-RYD**
Uq-3	same as above	4	*Uq-PDent**
Uq-4	same as above	4	*Uq-EC**
Uq-5	same as above	4	*Uq-SK**
Uq-6	same as above	4	

**Uq*'s tested to be independent of the *c1* locus. [a]Sprague's AR line from the cross *a1 su pr wx* x *A1 Su Pr Wx* (WSMV-treated). [b]This cross was the source of the insertions of *ruq*'s into *c1*, isolated as *c1-ruq31* and *c1-ruq65* (= *c1-ruq66, c1-ruq67*). [c]Inbreds included B70, C103, C123 and 187-2. These inbreds were tested and shown not to have active *Uq*'s. That which cross was the originating sources of these *Uq*'s was not known. [d]1 = Pereira and Peterson (1985), 2 = Peterson (1985), 3 = Caldwell and Peterson (1989), 4 = Pan and Peterson (1991a, b). [e]All uncovered from corn breeding materials by crossing with *c1-ruq* (Cormack *et al.* 1988; Seo and Peterson 1995).

evidence of the contribution of transposons to plant performances useful in plant breeding programs has been reported (Alrefai *et al.* 1994; Mazoti and Broccoli 1995; Giroux *et al.* 1996).

The *Uq-ruq* system *versus* the *Ac-Ds* system

The relationship to *Ac-Ds*

One of the tenets of transposable elements (TEs) is the distinction among the TE systems. This was arrived at genetically (McClintock 1950; Peterson 1965). There is a clear specificity of autonomous elements that recognize non-autonomous elements in an exclusive manner. The relationship of *Uq* and *Ac* represents an exception similar to those of *Fcu* to *Spf* (Gonella and Peterson 1978) and *F-En* to *En* (Peterson 1997). The *Uq-ruq* system is related in function with the *Ac-Ds* system, whereby both *Uq* and *Ac* recognize one of the *Ds* elements (*Ds1*). The *Ac-Ds* system consists of a number of non-autonomous *Ds* elements including standard *Ds*'s and *Ds1*. The molecular analysis shows that all *Ds*'s with the exception of *Ds1*, are *Ac*-deletion derivatives. These include double *Ds* and *Ds2*. The homology of *Ds1* with *Ac* however only lies in the terminal inverted repeats (Sutton *et al.* 1984; Pohlman *et al.* 1984). From genetic tests revealing that *Uq* only recognizes *Ds1*, not the other *Ds*'s, Caldwell and Peterson (1992) indicated that this represents a quasi-relationship. The molecular analysis of a large number of *ruq* elements showed that *ruq* is ~95% homologous to *Ds1*, indicating that *ruq* is *Ds1* (Pisabarro *et al.* 1991, see also Figure 3). Hereafter, *ruq* and *Ds1* are referred to *Ds1/ruq* together when used in the general context.

Dosage effect

Increasing the copy number of *Ac* from one to three was initially observed to result in a delay in the timing but an increase in the frequency of transposition of *Ds* (McClintock 1948), called the 'negative dosage effect'. However, the dosage effects of *Ac*'s at different loci were observed to be inconsistent (Heinlein and Starlinger 1991), suggesting more elaborate investigations to be made. Cycling *Ac*'s at the *p* locus, which are unstable intermediates between active and inactive *Ac*'s that occur somatically via the change in methylation, confer a variegation pattern independent of the copy number of *Ac* and have no

distinct effects on timing and frequency of transposition in association with the copy number (Brutnell and Dellaporta 1994). On the other hand, increasing the *Uq* copy number has been observed to result in an increase in both the timing and frequency of transposition of *ruq* (Friedemann and Peterson 1982; Pereira and Peterson 1985; Pan and Peterson 1991b; Caldwell and Peterson 1989), and this type of dosage response was called the 'positive dosage effect'.

It was hypothesized that the negative dosage effect of *Ac* is not associated with the copy number of *Ac* but with the amount of TPase (*Ac* transposase). This reasoning was based on previous observations that the increased *Ac* copy number results in the increase in TPase and inactive *Ac*'s do not contribute to the dosage effect (Scofield *et al.* 1993 and references therein). However, the inconsistent dosage effects observed by Heinlein and Starlinger (1991) imply an unknown factor(s) involved, such as position, developmental timing, or proteins. The differential variegation patterns by *Ac*'s of *wx-m7* and *wx-m9*, respectively, were suggested to result from allele-specific autonomous regulation (Heinlein 1995). *Ac-st2*, a novel derivative of *Ac*, is identical in sequence to wild-type active *Ac* elements but shows a positive dosage effect (Brutnell *et al.* 1997). The difference between *Ac-st2* and *Ac* lied in different levels of methylation in the ORFa promoter region, leading the authors to suggest a threshold model that *Ac* transposition is dependent on a threshold of transposase accumulation in the cell. If *Uq* is a modified version of *Ac*, the positive dosage effect observed with *Uq* may need a more elaborate testing with a number of diverse *Uq*'s.

How *Uq* is related to *Ac*?

The genetic information (Caldwell and Peterson 1992) that *Uq* transactivates only *Ds1/ruq* while *Ac* transactivates all *Ds*'s and the molecular information that *ruq* is *Ds1* (Pisabarro *et al.* 1991) can only provide some general clues to the structural homology between *Uq* and *Ac*. This includes 11 bp terminal inverted repeat sequences (TIRs) and a possibility that a degree of homology between *Uq* and *Ac* transposases exist. The DNA binding domain of *Ac* TPase has been demonstrated to recognize both the TIRs and sub-terminal motif sequences (A/TCG, A/TCGG) at both ends (Becker and Kunze 1997).

Whether *Uq* is a modified version of *Ac* or vice versa is a wide-open question at present, as is whether *Uq* is the master element of *Ds1*, whose origin has not been known. There have been cases observed that some *Uq*'s change to *Ac* or in a *Uq* search, a silent *Ac* became active (P. A. Peterson, unpublished). If *Uq* is a derivative of *Ac*, it is possible that DNA rearrangements in the internal region of *Ac* had occurred and resulted in a weak *Ac* version identified as *Uq*, perhaps through mechanisms similar to those involved in the generation of *Mu* element subfamilies (Bennetzen and Springer 1994). If *Uq* is not directly related to *Ac*, it is expected that they share a degree of homology, especially in DNA-binding protein domain, enough to recognize the TIRs of *Ds1/ruq*. These questions are at present limited to speculation.

Rationale to the pursuit of *Mn::Uq*

Mn::Uq (a dominant miniature phenotype that strictly co-segregates with *Uq*) was isolated upon treating with 5-aza-2'-deoxycytidine roots of a plant grown from an *a1-ruq/a1-ruq, sectored* kernel (a kernel with a sector of colored spots on a colorless background) (Pan and Peterson 1989). The *a1-ruq/a1-ruq, sectored* kernel was a BC2 progeny kernel derived from backcrosses of inbred lines (B70, C103, C123, and 187-2) with *a1-ruq* as recurrent parent (Pan and Peterson 1988). Since the isolation, the *Mn::Uq* lines have been advanced by crossing with *a1-ruq* more than six generations including backcrosses, so that the *Mn::Uq* line can be considered to be equal (near isogenic) to the *a1-ruq* tester but for *Mn::Uq*. The *a1-ruq* tester has been a long time inbred that has been propagated by selfing or sibbing since its isolation (Friedemann and Peterson 1982).

Under the hypothesis that *Uq* is a version related either to *Ac* or *Ds1*, the attempt to clone *Mn::Uq* has been made. Using enzymes that do not cut *Ac*, the use of a *Ds1* probe (*Ds1* of *r-m1*: a gift from Y. Liu, S. Wessler's laboratory) in Southern hybridization on the genomic DNA of the normal and *Mn::Uq* seedlings from *Mn::Uq* lines showed a number of bands (~40 bands) without identifiable co-segregating bands. The use of the internal 1.6 kb *Hind*III *Ac* fragment (Fedoroff *et al.* 1984) as a probe displayed 8-10 bands, but a unique, co-segregating band with the *Mn::Uq* line could not be detected (data not shown). If the current

hypothesis is correct that *Uq* is derived from *Ac*, more extensive and elaborate investigations are necessary.

Activation of latent inactive *Uq* elements

The finding that *Uq* has been pervasive in assorted maize genetic testers led to a search for *Uq* among various maize sources by crossing with *a1-ruq* reporter (Peterson and Friedemann 1983). The sources included inbred lines [Mo17 (derived from 187-2 x C103), B75 (derived from Iowa stiff stalk synthetic, BSSS), and color-converted W22], a flint variety [Longfellow Flint], and several genetic stocks [*a2 bt*, *a2-m(r)*, and *c sh wx*]. The search found no active *Uq*, but each set of testcrosses gave rise infrequently to kernels of one small colored spot on an otherwise colorless background. This one-spot mutability was primarily explained by the instability of *ruq* at *a1* that releases the control of the locus, but the authors also questioned an alternative that a latent *Uq* can become active later during kernel development and transactivate *a1-ruq*.

Activation of latent *Uq*'s has also been pursued. Pan and Peterson (1988) observed the spontaneous activation of latent *Uq*'s during endosperm development among backcross progeny kernels of four maize inbred lines from the crosses of B70, C103, C123 and 187-2 x *a1-ruq* (recurrent parent). The activation was revealed by a colored sector or a sector of few-spots [earlier mutability than the one-spot mutability observed by Peterson and Friedemann (1983)]. However, none of these sectored kernels had a germinally activated heritable *Uq*. Further advancement of these backcross progeny allowed the authors to isolate 39 fully spotted kernels. Only five of them were further tested and all contained active *Uq*'s (*Uq2*, *Uq3*, *Uq4*, *Uq5* and *Uq6*, respectively; Table 1) (Pan and Peterson 1991a; see also the legend of Figure 1B). Whether these latent *Uq*'s were present in the tested inbreds or in the *a1-ruq* tester was not tested; however, note that upon selfing or sibbing, plants of the *a1-ruq* tester have been observed to produce kernels of one or a few spots at very low frequencies (Pan and Peterson 1988).

Regardless of their sources, these newly isolated *Uq*'s must have originated from the activation of silent *Uq*'s. The activation of transposable elements is hypothesized to be one of

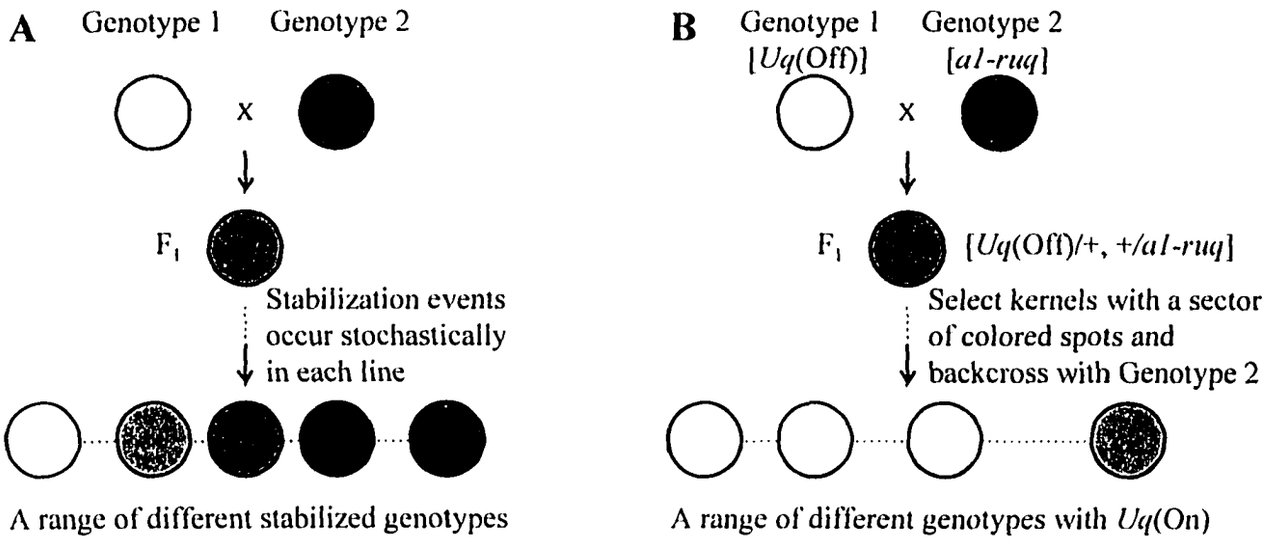
the phenomena that take place in the process of genomic reorganization through 'self-constrained systemic responses to endogenic/exogenic, micro/macro perturbations' caused by genomic stressors (von Sternberg 1996). These genomic stressors can mediate the genome into a period of destabilization followed by stabilization. In case of a new genome reconstructed by bringing together two different genotypes, a major genomic stressor would be heterozygosity, a genome-disturbing factor. The stabilization of a newly constructed genome (assumed to be heterozygous and disturbed) would be mediated by mechanisms such as legitimate and illegitimate recombinations, DNA mutator systems (transposition, gene conversion, unequal crossover, etc.), replication/DNA repair and others such as methylation and demethylation. Meiotic segregation of the rearranged chromosomes would produce a series of different stabilized genotypes with a level of tolerable heterozygosity over the generations (Figure 1A).

One or more silent, latent *Uq*'s are present, at least in some AR lines (Peterson 1985; Sprague 1986) and also in other stocks (Peterson and Friedemann 1983; Pan and Peterson 1988, 1991a), as evidenced by somatic activations or germinal isolations of *Uq*'s. These inactive *Uq*'s, however, are activated occasionally at very low frequencies only in some individual genomes/genotypes. This suggests that the probability of each of the mechanisms to occur in different genotypes, even in the same genotypes of the same clonal origin due to diverse levels of effects from external factors, can not be assumed to be the same. This is probably because the occurrence of a destabilizing mechanism is controlled by several factors such as genotypes (different levels/types of heterozygosity, introduced or inherited), genomic plasticity or responses for the adaptation to environment, and others. So the extent of each mechanism working in the stabilization process seems to be stochastic or probabilistic, as much as in the stochastic model hypothesized to explain cell differentiation (Kupiec 1997), but dependent on individual genotypes and cellular interactions or conditions.

Among those mechanisms, demethylation has been hypothesized to be closely related to the activation of silent *Uq*'s, (and in fact it should occur first to a level for transposase to be provided and transposition to occur). This has been well documented in considering that the activity of transposable elements is correlated to the degree of methylation (Chandler and

Figure 1. **A.** Hypothesized illustration of stochastic or probabilistic stabilization of a newly constructed genome. The stabilization process depends upon individual genotypes and cellular interactions. Without selection, a series of final genotypes will result and be heritable with tolerable heterozygosity.

B. Stochastic activation of a silent *Uq* [*Uq*(Off)] in a newly constructed genome. A combined genome of two, one with *Uq*(Off) and the other without *Uq*. The activation of the *Uq*(Off) is considered to occur stochastically during the stabilization processing events that optimize the genomic conditions. Note that Pan and Peterson (1988, 1991a) were able to select kernels containing a sector of colored spots (an indication of a *Uq* activated during kernel development), and by further selection, continuously observed kernels with larger sectors of colored spots (an indication of a *Uq* activated earlier than in the previous generation), and finally isolated germinally activated *Uq*'s that are heritable in subsequent generations. Fedoroff (1989) also selected kernels showing somatic reactivation of *cryptic* elements, which provided an efficient selection of plants with gradually reactivated *Spm* elements. This series of isolation of kernels showing the progressive activation of an element can be interpreted in the following manner: events occurring during endosperm development reflect a higher probability of those events occurring in the nuclear genome in the stabilization process.


A
Genotype 1
Genotype 2
x
F1
Stabilization events occur stochastically in each line
A range of different stabilized genotypes
B
Genotype 1
[Uq(Off)]
Genotype 2
[a1-ruq]
x
F1
[Uq(Off)/+, +/a1-ruq]
Select kernels with a sector of colored spots and backcross with Genotype 2
A range of different genotypes with Uq(On)

Walbot 1986; Schwartz and Dennis 1986; Chomet *et al.* 1987; Kunze and Starlinger 1989; Dennis and Brettell 1990). It seems that the stabilization progressively occurs over several generations to become germinally heritable, as can be deduced from the progressive activation timing observed in the spontaneously activated *Uq*'s (Pan and Peterson 1988, 1991a) and also from the metastable *programmable Spm* (Fedoroff 1989).

As implied previously, the activation or inactivation or the change in a level of methylation of an element, somatic or germinal, is stochastic. In other words, the chance for a silent element to be activated is a controlled function depending upon genotypes and cellular interactions during the stochastic stabilization process. It is therefore that the activation process of transposable elements can be said to be stochastic (Figure 1B). Once an element becomes active, it would remain active until a type of stress mediate it to become inactive.

On the other hand, it is interesting to discuss that somatic activations of a silent *Uq* late during kernel development can be accounted for by demethylation in association with aging and resultant breakdown of cellular controls, especially in short-lived things. Aging can be an endogenic genomic stressor. The age-related gene expression or epigenetic defects are reviewed and discussed elsewhere by Holliday (1993).

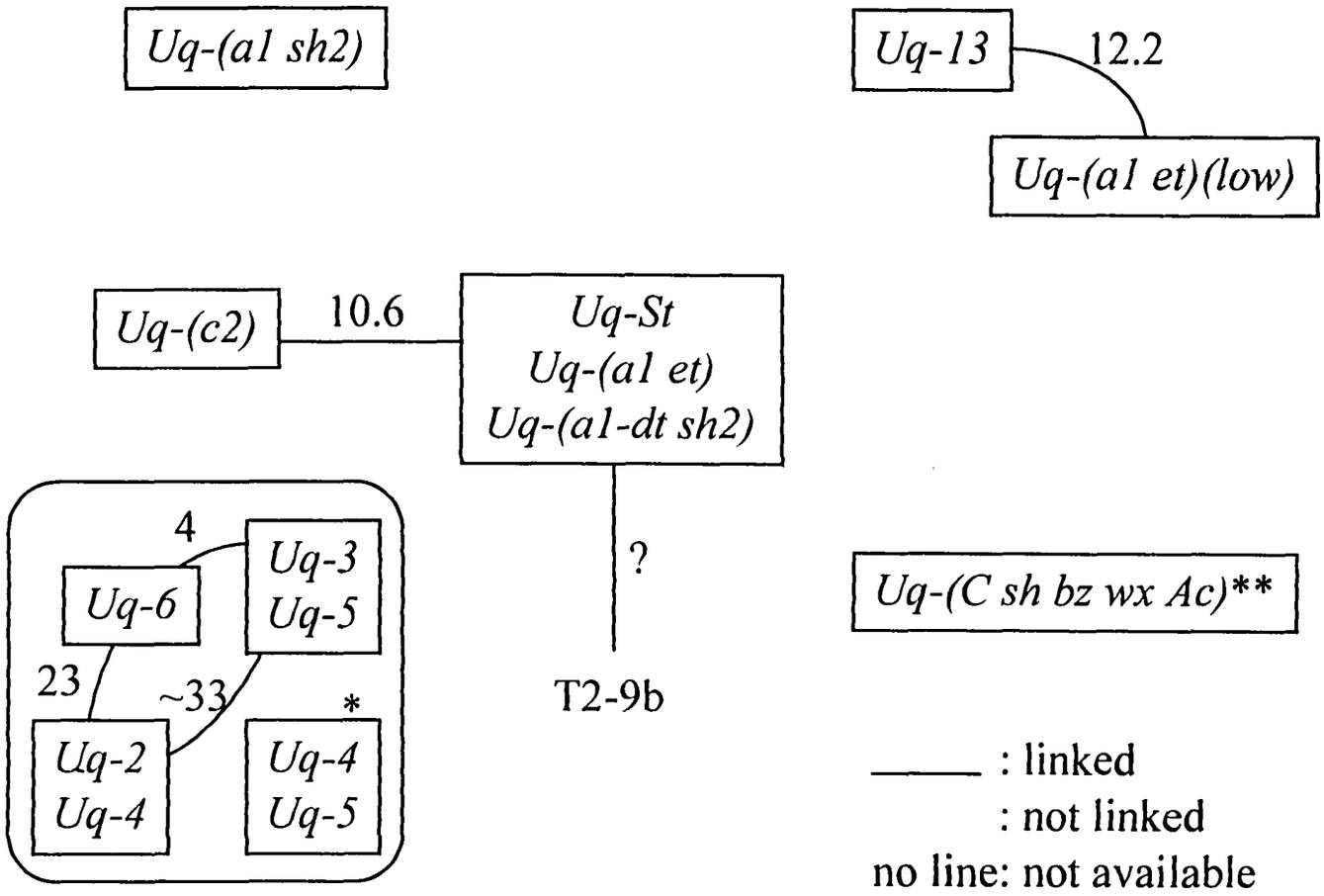
Differential spotting patterns among the members of the *Uq-ruq* system

The search of the *Uq-ruq* system among diverse maize genetic stocks and breeding materials has been described previously (Table 1). A resultant interesting genetic characteristic is that many newly uncovered autonomous *Uq*'s elicit differential spotting patterns (DSPs) in association with *ruq*'s. Figure 2B respectively describes the spotting patterns of *Uq*'s of Figure 2A. In addition, *Mn::Uq* transactivates *a1-ruq* but does not transactivate *c1-ruq67*, a normally *Uq*-responsive allele (Pan and Peterson 1989). *Uq-I159*, a *Uq* element from the inbred I159 (Cormack *et al.* 1988), more often transactivates *a1-ruq* than *c1-ruq67* (P. A. Peterson, unpublished). DSPs observed either from crosses between one *ruq* and different *Uq*'s or from crosses between one *Uq* and different *ruq*'s indicate DNA sequence or position differences among *Uq*'s and *ruq*'s, respectively. Without molecular analyses of these diverse *Uq*'s, genetic studies have described possible causes for this DSP

Figure 2. **A.** Documentation of linkage relationships among diverse *Uq*'s from previous studies. *Uq*'s with an allelic or very close linkage relationship are located in the same rectangle. Previously known map units are indicated between *Uq*'s. **Uq-4* was located at several sites: it was observed to be not only allelic or very close both to *Uq-2* and *Uq-5* but also linked to other *Uq*'s. **This *Uq* from the *Ac* line might be the same *Ac* since *Ac* also transactivates *ruq*, which is *Ds1* (Pisabarro *et al.* 1991; Caldwell and Peterson 1992).

B. Comparison of spotting patterns of respective *Uq*'s listed in **A**: differential interactions with *a1-ruq*. Spotting patterns are as described in the original linkage studies of *Uq*'s: *Uq-St* and *Uq-13* originated and were isolated from the lines that have shown the Aberrant Ratio (AR) phenomenon after the Wheat Stripe Mosaic Virus (WSMV) infection (Friedemann and Peterson 1982; Pereira and Peterson 1985). *Uq-(a et), Uq-(a-dt sh2), Uq-(a sh2), Uq-(a et)(low)* and *Uq-(C sh bz wx Ac)* were uncovered from various testers (indicated in brackets) by crossing with *a1-ruq* (Pereira and Peterson 1985), as was *Uq-(c2)* (Peterson 1985). *Uq-2, Uq-3, Uq-4, Uq-5* and *Uq-6* were germinal isolates that originated through spontaneous activation from backcrossed populations of four inbred lines (C103, C123, B70 and 187-2) by *a1-ruq* (Pan and Peterson 1988, 1991a, 1991b).

A


Uq-(a1 sh2)
Uq-13
12.2
Uq-(a1 et)(low)
Uq-(c2)
10.6
Uq-St
Uq-(a1 et)
Uq-(a1-dt sh2)
4
Uq-3
Uq-5
Uq-6
23
~33
*
Uq-2
Uq-4
Uq-4
Uq-5
?
T2-9b
Uq-(C sh bz wx Ac)**
_____ : linked
: not linked
no line: not available

B

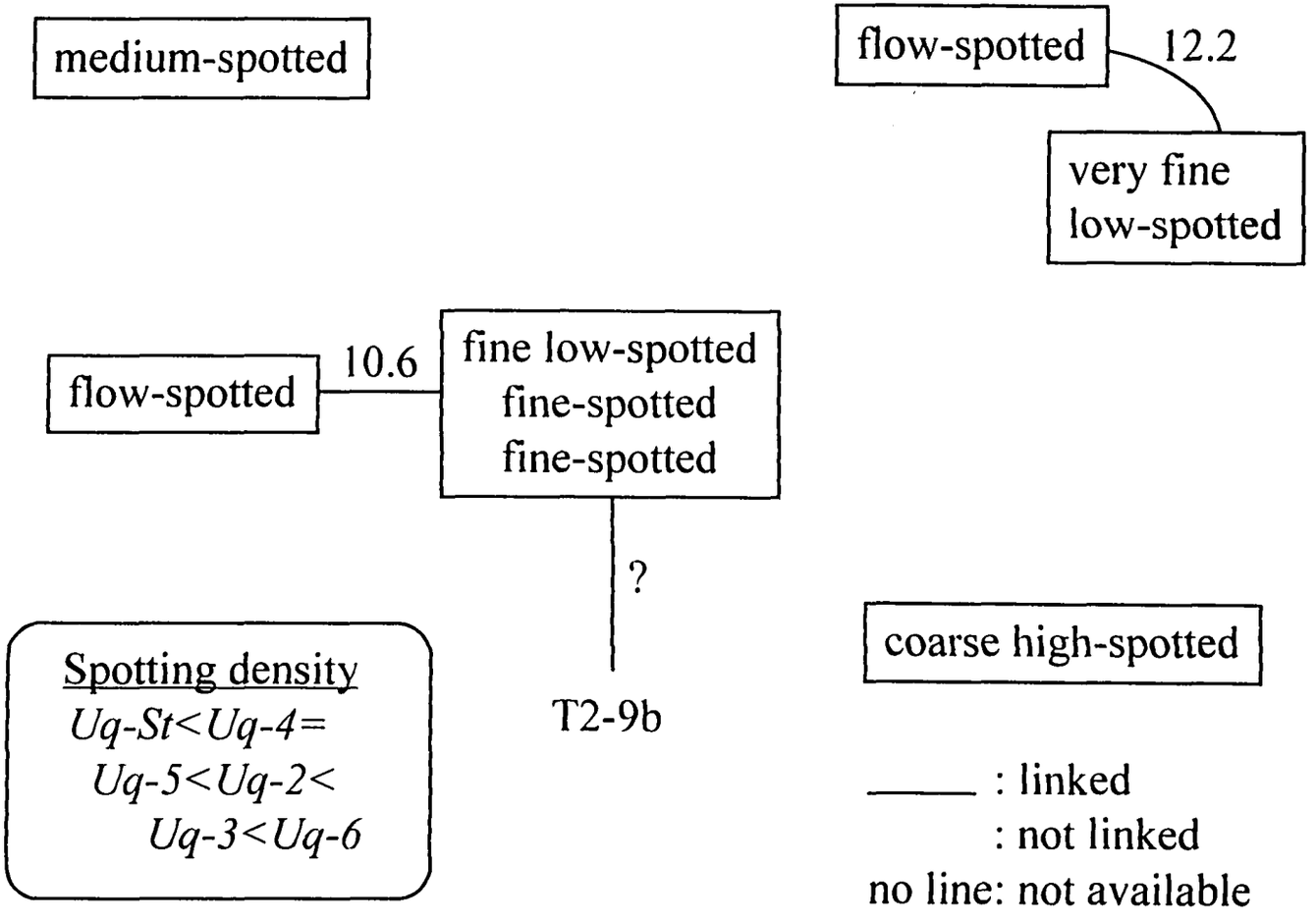


Figure 2 (continued)

phenomenon (Pereira and Peterson 1985; Caldwell and Peterson 1989; Pan and Peterson 1989, 1991a, b).

Differential methylation

As indicated in Table 1, all the crosses that led to the finding of new *Uq*'s included *a1-ruq* as a reporter of an active *Uq* present. The *a1-ruq* tester was derived from the Sprague's AR stock, into which *Uq-St* was introduced from one of the parents (one without WSMV treatment) in the original cross (Peterson 1985). The F_1 progeny of the original cross received half of the genome from the WSMV-treated line and the other half from a different line without WSMV treatment, such that the virus infection and newly introduced heterozygosity would be major genome-stressing factors. Although no support is available, it is possible that *Uq-St* could become inactivated during the stabilization of the reconstructed genome in some lines. It can therefore be challenged whether some of these *Uq*'s (Table 1) were originally the same *Uq-St* that underwent inactivation followed by reactivation, or transposition and inactivation followed by reactivation. If reactivation occurred, it is possible that these reactivated *Uq*'s may show gradient methylation patterns among them. It was suggested that latent *Uq*'s might have graded degrees of potency or varied efficiencies in activating the *a1-ruq* allele (Peterson and Friedemann 1983). It has been well documented that the phase variation between activity and inactivity of maize transposable elements is mediated by DNA modification via methylation (Chandler and Walbot 1986; Schwartz and Dennis 1986; Chomet *et al.* 1987; Banks and Fedoroff 1989; Kunze and Starlinger 1989; Dennis and Brettell 1990). The demethylation process from the inactive to the active has been shown to be progressive (Chandler and Walbot 1986; Schwartz and Dennis 1986; Dennis and Brettell 1990), such that metastable intermediates express different spotting patterns during plant development (Fedoroff 1989). The differential methylation has also been associated with the position effect discussed in the following.

Position effect

The linkages among diverse *Uq*'s previously tested are summarized in Figure 2A and their respective spotting patterns are described in Figure 2B; DSPs and relative chromosomal locations can be compared. In addition, *Uq3*, *Uq4*, *Uq5* and *Uq6* produced spotted kernels at

a frequency less than the expected, suggesting a lesser potency in transactivating the *c1-ruq* alleles (Pan and Peterson 1991a). Interestingly, the three alleles (*a1-ruq*, *c1-ruq31* and *c1-ruq65*) that react differentially to *Uq-13* respond in a similar manner to *Uq-St* (Table 2). What has been clear from allelism tests is that *Uq*'s showing DSPs are located at different sites. The sequence differences among *Uq*'s may exist. Whether different positions of *ruq*'s influence the interactions with a *Uq* is not known. As long as position effect is concerned with one *Uq* and one *ruq*, position-dependent methylation may influence most the activity of a *Uq* and thus the spotting pattern induced by the *Uq*. Independently introduced, immobilized *Ac* lines were shown to differ greatly in their capacity to transactivate one *Ds* in *Arabidopsis* (Altmann *et al.* 1992). It is possible that chromatin organization pattern during development determines the accessibility of the *Uq* transposase to *ruq*'s located at different places. *Mn::Uq* was observed to induce a response not from *c1-ruq67* but from *a1-ruq* (Pan and Peterson 1989). This could be due either to the non-accessibility of *Uq* transposase to *c1-ruq67* or the structural difference between *a1-ruq* and *c1-ruq67* (see below) coupled with a weaker function of *Mn::Uq*. The latter is plausible, considering that *Mn::Uq* is the insertion of a newly activated element from a long-time silent state, in which the element might have undergone modifications.

Table 2. Differential interactions (spotting patterns[a]) among autonomous *Uq*'s and non-autonomous *ruq*'s [adapted from Caldwell and Peterson (1989)].

	Uq-St	*Uq-13*[b]	*Uq-31*	*Uq-66*
a1-ruq	4-6c-e	6-8a-b	4-6c-e	4-6c-e
c1-ruq31	4-6c-e	5-6a	5b-c	na[c]
c1-ruq66	4-6c-e	1-3a	na	4c

[a]The spotting pattern refers to the size (a to e, fine to coarse) and frequency (1 to 6, low to high) of colored spots on an individual kernel expressing mutability for an aleurone color gene. [b]*Uq-13* elicits a flow pattern, in which colored spots are confined to the base of the otherwise colorless kernels (Peterson 1966). [c]na: not available.

Diversity among *ruq* elements

There have been several *ruq* elements inserted into the *a1* and *c1* loci: *a1-ruq*, *a1-ruq13*, *c1-ruq31*, *c1-ruq65*, *c1-ruq66* and *c1-ruq67* (Friedemann and Peterson 1982; Pereira and Peterson 1985; Caldwell and Peterson 1989). Genetic and molecular tests revealed that three of them (*a1-ruq*, *c1-ruq31* and *c1-ruq66*) can be differentiated functionally and structurally: these three alleles respond differentially to the *trans*-signal of *Uq-13* (and also likely to *Uq-31* and *Uq-66*) (Table 2, Caldwell and Peterson 1989) and also show sequence differences among them (Figure 3, Pisabarro *et al.* 1991). *Uq-I159* is observed to transactivate *a1-ruq* more often than *c1-ruq67* (= *c1-ruq66*) (P.A. Peterson, unpublished). Therefore, it seems clear that the diversity among *ruq* elements is in part responsible for the DSPs.

The sequences of *ruq* elements (*a1-ruq*, *c1-ruq31* and *c1-ruq66*) and *Ds1* (*Adh1-fm*) (Sutton *et al.* 1984) are compared (Figure 3; Pisabarro *et al.* 1991). The DNA sequences required *in cis* for transposition in the *Ac-Ds* system are the 11 bp TIRs at both ends and the *Ac* TPase binding motif, AAACGG, in the sub-terminal regions (Kunze and Starlinger 1989). In Figure 3, the 11 bp TIRs are underlined. The TPase binding motif sequences, A/TCG or A/TCGG (Becker and Kunze 1997), are italicized and underlined. The *Ac* TPase not only binds cooperatively to repetitive ACG and TCG sequences showing the highest affinity with an additional G on the 3′ side (i.e., A/TCGG), but also binds to the TIRs although with much lower affinity (Becker and Kunze 1997). The nuclear protein binding sites, GDTAAA (D = G, T, A) (Becker and Kunze 1996; Levy *et al.* 1996), are indicated in boxes. No regulatory role of these sites has been reported in the excision of non-autonomous elements.

Major changes to be considered can probably be noticed on the 5′ side. *a1-ruq* has the G to A base change at position 4 in TIR (terminal inverted repeat). *c1-ruq31* has the G to A base change at position that creates the perfect 16 bp TIRs and a new TPase binding motif, ACGG. *c1-ruq66* has the C to G base change that results in the change in the orientation of the TPase binding site. Whether any of these changes is involved in the DSPs remains to be tested. The AAACGG motif on the 3′ side was shown to be absolutely essential for excision of *Ds1* and some changes by insertions on the 5′ side resulted in reduced or increased

Figure 3. DNA sequences of *ruq* and *Ds1* elements (from Pisabarro *et al*. 1991). Bases different from the consensus sequences are in bold. The terminal inverted repeat sequences (TIRs) at both ends are underlined. The TPase binding motif sequences, A/TCG or A/TCGG (Becker and Kunze 1997), are in italics and underlined. The TPase binding motif, ACGG, within the TIRs of *ruq-31* is in italic and double-underlined. The nuclear protein binding sites, GDTAAA (D = G, T, A) (Becker and Kunze 1996; Levy *et al*. 1996), or homologous (putative) sites are in boxes.


1 70
Ds1 TAGGGATGAAAGTGGTAATCCGAACTGTTAGGATAAATTT AATATTTTAA AATAGATATA TATAAAATTT
ruq-st TAGAGATGAAAGTGGTAATCCGAACTGTTAGAACAAATTT AATATTTTAA AATAGATATG TATAAAATTT
ruq31 TAGGGATGAAAACGGTAATCCGAACTGTTAGAACAAATTT AATATTTTAA AATAGATATA TATAAAATTT
ruq66 TAGGGATGAAAGTGGTAATCGGAACTGTTAGAACAAATTT AATATTTTAA AATAGATATG TATAAAATTT
71 140
Ds1 GATATTGATC TTTTCTTATG TTATCAAGCACATTAGTAGA AATATGAAT- AAATATTACATACATTGTTT
ruq-st GATGTTGATCTTTTCTTATG TTATCAAGCACATTAGTACA AATATGAATAAAATATTATATAAGTTGTTT
ruq31 AATTTTGATC TTTTCTTATG TTATCAAGCACATTAGTACA CATATGAATAAAATATTACACAAATTATTT
ruq66 GATGTTGATCTTTTCTTATG TTATCAAGCACATTAGTACA AATATGAATAAAATATTATATAAGTTGTTT
141 210
Ds1 TATGTATTATTTGCTCCCTA CAACATAAAAGGTTGAAAAA A-TTACCGAA TTTATTTCCGAATCCATACC
ruq-st TATGTATTATTTGCTCCCTA CAACACAAAAAGTTGAAAAA A-TTACCGAA TTTATTTCCGAATCCATACC
ruq31 TATGTATTATTTGCTCCCTA CAACACGAAACGTTG - AAAA ATTTACCGAA TTTATTTCCGAATCCATACC
ruq66 TATGTATTATTTGCTCCATA CAACACAAAAAGTTGAAAAAA-TTACCGAA TTTATTTCCGAATCCATACC
211 280
Ds1 GAAGTTTATATCTATTTATT TGAGAAAATATAGGATGAAT TTGAGGTTTA -CTTTTATGA ATCTTAACAA
ruq-st GAAGTTTATATCTA- TTATTTAAGAAAATGTAGGATGAAT TTGAGGTTTA CCTTTTATGA TTCTTAACAA
ruq31 GAAGTTTATATCTA- TTATTTGAGAAAATATAGAATGAAT TTGAGGTTTA CCTTTCATGA ATCTTTACAA
ruq66 GAAGTTTATATCTA- TTATTTGAGAAAATGTAGGATGGATTTGAGGTTTA CCTTTTATGA ATCTTAACAA
281 350
Ds1 -GCTGGATGTTAAAAACAAGAATACAAATTTGTATTGTATATTCTATATC CTATTTATTCGCAATCAAAG
ruq-st -GCTGGATGTTAAAAACAAGAATACAAATTTGTATTGTATATTCTATATC CTATTTATTCACAATCAAAG
ruq31 -GCTGGACGTTAAAAAAAAGAATACAAATTTGTATTGTAGATTATATATC TTATTTATTCGCAATCAAAG
ruq66 GGCTGGATATTAAAAACAAGAATACAAATTTGTATTGTATATTCTATATC CTATTTATTCGCAATCAAAG
351 409
Ds1 AAAAACGACTAAAAAACTGA TTACCGAAAAAATACCGTTT CCGACCGTTTTCATCCCTA
ruq-st ---------- AAAAAACTGA TTACCGAATA AATACCGTTT CCGACCGTTTTCATCCCTA
ruq31 AAAGACGACTAAAAAACTGA TTACCGAAT - AATACCGTTT CCGACCGTTTTCATCCCTA
ruq66 ---------- AAAAAACTGA TTACCGAATA AATACCGTTT CCGACCGTTTTCATCCCTA

excision frequency (Bravo-Angel *et al.* 1995). The sequence differences and genetic differential responses of *ruq* elements suggest that some of the differences in sequence are responsible for the differential responses to a *Uq*.

In conclusion, the major need in the elaboration of the *Uq* story is the molecular cloning of *Uq*. The cloning of *Uq* will uncover the relationship between *Ac* and *Uq*, and may help establish or find a case or a way(s) that transposable elements evolve and diversify themselves and interact among the members within a large family of the *Ac-Ds* system. *Ac2* (Rhoades and Dempsey 1983) is also still a mystery as to how it originated. Note how *Irma* (Muszynski *et al.* 1993) and *PIF* elements (Walker *et al.* 1997) are related to each other and to *En/Spm*. Also note that there have been parallels to the duplicate relationship of *Ac* and *Uq*. These include the relationship of *Spf* and *Fcu* (Gonella and Peterson 1978) and that of *En* and *FEn* (Peterson 1997).

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CHAPTER 3. CHARACTERIZATION OF THE MUTATION *Mn5::Uq* OF MAIZE (*Zea mays* L.)

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Abstract

Mn::Uq, a dominant mutation that co-segregates with the *Uq* autonomous maize transposon (autonomous - functionally active), was first reported by Pan and Peterson (1989). This report further characterizes *Mn::Uq* by describing and discussing the phenotypes and their genetic implications. Due to non male-transmission that was observed through reciprocal crosses and in vitro pollen germination tests, the progeny of a mutant heterozygote (*Mn::Uq*/+) is theoretically expected and does segregate in a near 1:1 ratio of heterozygous (*Mn::Uq*/+) and normal (+/+) phenotypes; however, due to the variable penetrance, the segregation often falls short of the expected ratio. The mutant kernels have diverse sizes and weights, which result from changeable levels of endosperm development, indicating differential expressivity. The variable penetrance and expressivity are attributable to genetic background, based on genetic observations. The variable penetrance is further explained by vacillating variable levels of expressivity and female transmission. Allelism tests with other known *mn* genes, *wx* translocation mapping, and linkage tests with markers on 2S indicate that this *Mn* is a new gene tagged with *Uq*. Thereby, the locus is designated *mn5*. Given the molecular co-segregation tests of *Mn5::Uq* with *Ac*, it is concluded that *Uq* is not related to *Ac*.

Introduction

The Ubiquitous (*Uq*)-responder to *Uq* (*ruq*) maize transposable element system (Friedemann and Peterson 1982) was identified initially from a single variegated kernel in progeny from lines identified as Aberrant Ratio (AR) stocks that were treated with the wheat stripe mosaic virus (Sprague and McKinney 1966). Since its establishment, *Uq* has been found to be universally prevalent among numerous maize strains and populations but not

necessarily homozygous, and diverse *Uq* elements have been uncovered in assorted maize genetic and breeding materials (Peterson and Friedemann 1983; Pereira and Peterson 1985; Peterson and Salamini 1986; Cormack *et al.* 1988; Pan and Peterson 1991a,b; Lamkey *et al.* 1991).

Non-autonomous *ruq* elements (non-autonomous - respond to autonomous elements) at the *a1* and *c1* loci are designated as *a1-ruq* and *c1-ruq*, respectively (Friedemann and Peterson 1982; Pereira and Peterson 1985; Caldwell and Peterson 1989). These non-autonomous elements report the presence of an active element by excisions (e.g., spots on kernels) that are mediated *in trans* by the protein-coding, active transposable element. For example, the *a1-ruq* tester produces colorless kernels in the absence of an active *Uq* because the *ruq* insert at the *a1* gene interferes with the transcription of the *a1* gene, one of genes involved in the anthocyanin biosynthetic pathway. However, an active *Uq* present in the genome can mediate *in trans* the excision of *ruq*, producing colored spots on an otherwise colorless background.

The *Uq-ruq* system shares the functions of the *Activator-Dissociation* (*Ac-Ds*) system. Genetic analysis indicated that *Ac* transactivates not only all *Ds* elements such as standard *Ds*'s (*Ac*-deletion derivatives), *Ds1*, *Ds2*, and double *Ds*'s but also *ruq*, while *Uq* transactivates only *Ds1* and *ruq* (Caldwell and Peterson 1992). Following molecular analysis, it was revealed that *ruq* is ~95% homologous to *Ds1*, an indication that *ruq* is equal to *Ds1* (Pisabarro *et al.* 1991). Both elements are hereafter referred to as *Ds1/ruq* when used in general context. This duplicate function observed between the two systems has led to the hypothesis that the *Uq-ruq* system is likely a sub-system of the *Ac-Ds* system: is *Uq* a derivative of *Ac*?

To examine spontaneous activation of quiescent mobile elements, Pan and Peterson (1988) backcrossed four maize inbred lines (B70, C103, C123, and 187-2) with the *Uq* tester, *a1-ruq* (recurrent parent) and isolated *sectored* kernels (kernels with a sector of colored spots on a colorless background) among backcross progenies. This is an indication that silent *Uq*'s became spontaneously activated during endosperm development. In further investigation of *sectored* BC2 progeny kernels via the treatment of 5-aza-2'-deoxycytidine, a demethylating

chemical. Pan and Peterson (1989) isolated a miniature mutant, *Mn::Uq* (*Mn::Uq-866248U*), in which an activated *Uq* co-segregates with a miniature kernel phenotype. The assertion that the transposon *Uq* inserted at this *mn* locus was based on two factors. One, there is the absolute co-segregation of spotting (the interaction of *Uq* and *a1-ruq*) with miniature kernels. Spotted kernels, irrespective of size, always give progeny with miniature kernels segregating. Secondly, the miniature phenotype arose simultaneously with the spotted phenotype. The probability of the simultaneous induction of the two mutant phenotypes is very remote.

The characteristics of the *Mn::Uq* phenotype described by Pan and Peterson (1987, 1989) are in brief as follows: 1) Typical *Mn::Uq* kernels have reduced-size embryos and endosperms, but with variable sizes due to inconsistent expressivity, and has colored spots as a result of the transactivation of *a1-ruq* by *Uq*. The variable expressivity of putative *Mn::Uq* kernels can readily be confirmed in a subsequent generation that will continue to segregate the *Mn::Uq* phenotype; 2) The *Mn::Uq* seedling grows slower at the early stage; 3) The segregation into a 1:1 ratio of normal to mutant kernels, supported by no pollen tube growth in *in vitro* germination tests, indicates that mutant gametes are not transmissible to the progeny through microspores but only through megaspores. The genotype of a *Mn::Uq* line is therefore expected to be heterozygous, *Mn::Uq*/+; 4) Dominance is deduced from these sporophytic mutant phenotypes of the heterozygote; and 5) *Mn::Uq* transactivates *a1-ruq*, but does not transactivate a normally *Uq*-responsive *c1-ruq67* allele.

This report further characterizes *Mn::Uq* and to discuss implications in genetic features of *Mn::Uq*. The further genetic descriptions of *Mn::Uq* include the frequent deviation of segregating miniature and normal kernels from the expected 1:1 ratio, the variable penetrance and expressivity of *Mn::Uq* that are attributable to genetic background effects, genetic evidence of no complete transmission of *Mn::Uq* gametes through female, and genetic location of *Mn::Uq* on chromosome 2. Mapping indicates that this *Mn* is a new, fifth *miniature* gene (*mn5*) tagged with *Uq*. The co-segregation tests of *Mn5::Uq* through Southern analysis with an *Ac* probe indicate that *Uq* is not similar to *Ac*.

Materials and Methods

Genetic materials

Mn::Uq is a *Uq*-induced dominant *miniature* allele that was isolated upon treatment of kernels with 5-aza-2'-deoxycytidine. From that treatment, a single kernel with spots (somatic activation of *Uq*) was isolated (Pan and Peterson 1989). The kernel was one of the BC2 progeny kernels from crosses between inbreds (B70, C103, C123, and 187-2) and *a1-ruq* (recurrent parent). The original *Mn::Uq* isolation has been advanced and propagated by recurrently crossing with *a1-ruq* more than six generations including backcrosses, so that these two lines (*Mn::Uq* and *a1-ruq*) can be said to be homogeneous except for the *Uq* insertion, or near-isogenic. Known *mn* genes used for allelism tests are *mn1*, *mn2*, *mn3*, and *mn4*. A set of *wx* translocations and a genetic stock of *ws3 lg1 gl2 b1* were provided by Maize Genetics Cooperation Stock Center.

Identification and selection of mutant kernels

The mutant kernel phenotype includes miniature, etched-like, and spotted in the presence of *a1-ruq* (see Results). In the segregation analysis of mutant kernels from crosses with the *a1-ruq* tester, kernels with any of these three phenotypes were counted as mutant kernels. Such selections have been proven to be correct during the current study. Selections used for DNA extraction, however, had all those three phenotypes for the sake of safety.

Seedling growth tests

From each ear tested, twenty normal kernels were randomly chosen, individually weighed, and the total weights were averaged to produce the standard normal weight. Every mutant kernel was weighed and divided into ten classes, compared to the standard weight of normal kernels, from the first class (0.00-10.00%; almost empty kernels) to the tenth (90.01-100%; nearly normal kernels). Kernels from six classes (first five mutant and normal classes) were grown in a flat in the greenhouse. Seedling heights were measured seven days after planting.

Location of *Mn::Uq* on chromosome

Allelism tests with other *mn* genes and *wx* translocation mapping were first made to genetically locate *Mn::Uq*. Based on the result from *wx* translocation mapping indicating linkage on chromosome 2S. linkage tests with *ws3* and *lg1* were made. Crossing schemes for these tests are shown in Figure 1. respectively. Because of no male transmission. plants of *Mn::Uq/+* were always crossed as female.

Molecular tests

Rationale for probe selection for Southern hybridization

Uq and *Ac* have an overlapping function in that *Uq* transactivates *ruq* and *Ds1* but not other *Ds* elements such as *Ac*-deletion derived *Ds*'s. *Ds2* and double *Ds*'s while *Ac* transactivates all *Ds* elements and *ruq* (Caldwell and Peterson 1992). This indicates a relationship that suggests a quasi-similar protein that recognizes the TIR (terminal inverted repeat) binding site. Subsequently. *ruq* was sequenced and found to be approximately 95% homologous to *Ds1* (Pisabarro *et al.* 1991). Hereafter. *Ds1* and *ruq* are referred to as *Ds1/ruq* when used in general context. These genetic and molecular analyses led us to the working hypotheses that 1) *Uq* is a related version of *Ac*. and 2) *Uq* is the master element of *Ds1/ruq*. whose origin has not been known. In the *Ac* derivative hypothesis. *Ac* experienced sequence modifications or rearrangements and resulted into *Uq* that codes for transposase that can transactivate only *Ds1/ruq*. but not other *Ds* elements. In the *Ds1* master element hypothesis. *Uq* changed into the non-autonomous element *Ds1/ruq*. probably through internal deletion as is typical with other transposable element systems. In this case. the *Uq* protein is expected to share enough homology with the *Ac* protein to recognize *Ds1/ruq*. Based on these two hypotheses. the internal 1.6 kb *Hin*dIII fragment of *Ac* (Fedoroff *et al.* 1984) and the 280 bp *Dde*I-*Hin*dIII fragment of *Ds1* (*Ds1* of *r-m1*: a gift from Y. Liu. S. Wessler's laboratory) were used as probes.

Cross 1	*Mn::Uq*/+, +/+ x +/+, *mn/mn*
	↓
Cross 2	*Mn::Uq*/+, +/*mn* x +/+, *mn/mn*
	↓
If allelic:	all progeny are miniature
If independent:	3 miniature : 1 normal
If linked:	cM = [% normal kernels] x 2

A. Allelism test with other known *mn* genes

Cross 1	*Mn::Uq*/+, +/+ x +/+, *wx-T/wx-T*
	↓
Cross 2	*Mn::Uq*/+, +/*wx-T* x *a1-ruq*, *wx*
	↓
If independent:	*Wx* : *wx* = 1 : 1
If linked: cM = the % of the *Wx* phenotype among the normal kernels	

B. *wx* translocation mapping

Cross 1	*Mn::Uq*/+, + +/+ + x +/+, *ws3 lg1/ws3 lg1*
	↓
Cross 2	*Mn::Uq*/+, + +/*ws3 lg1* x +/+, *ws3 lg1/ws3 lg1*
	↓
Cross 3	all progeny are recorded for the segregation of *ws3* and *lg1*, and selfed for the presence of *Mn::Uq*

C. Linkage test with markers on 2S

Figure 1. Crossing schemes to locate *Mn::Uq* on chromosome

Southern hybridization

Standard procedures were used (Ausubel *et al.* 1989; Sambrook *et al.* 1989). Maize genomic DNA was isolated from seedling tissue using the method of Dellaporta *et al.* (1983). Approximately 10 ug of DNA was digested with 30 units of restriction enzyme, electrophoresed in 0.8% agarose gel and transferred to a nylon membrane (Magnacharge; Micron Separations Inc. Westbro, MA). Membranes were hybridized to DNA probe at 65°C in 6X SSC, 1% sarkosyl, 50 ug/mL denatured salmon sperm DNA (Sigma, St. Louis, MO). The probes were ^{32}P-labeled by the random primer method. The membranes were washed twice in 2X SSC, 0.1% SDS, for 30 min. at 65°C, then once in 0.2X SSC, 0.1% SDS for 30 min. at 65°C, and were exposed to X-ray film for 2-3 days.

Results

Phenotypes of a heterozygous (*Mn::Uq*/+) mutation

Kernel phenotype

A typical kernel phenotype of *Mn::Uq* is miniature, and etched-like. It is miniature because of the insufficient development of the endosperm, resulting in the invagination of the aleurone and/or pericarp making the phenotype etched-like. It is also spotted in the presence of *a1-ruq* because the *Uq* transactivates the *ruq*. Miniature kernels show reduced seedling vigor in the early growth stage. No pollen tube growth and resultant one-way or maternal transmission make the *Mn::Uq* line permanently heterozygous (*Mn::Uq*/+) so that its progeny is expected to segregate in a ratio of 1:1 of miniature to normal kernels. According to Pan and Peterson (1989), *Mn::Uq* is dominant in that the heterozygosity (*Mn* effect) leads to such sporophytic phenotypes of miniature kernels and weak seedling growth.

Miniature kernels of *Mn::Uq* have smaller endosperms and embryos, which are phenotypic expressions of the endosperm genotype (*Mn::Uq*/*Mn::Uq*/+) and embryo genotype (*Mn::Uq*/+), respectively. They are fully viable as long as they are well protected by healthy pericarp. Whether embryo size is also controlled by *Mn::Uq* is not known, as it is likely affected by endosperm development (for review, Birchler 1993), such that smaller endosperms tend to have smaller embryos as observed with miniature kernels (Pan and

Peterson 1989). In this study, the influence of *Mn::Uq* on embryo development has not been further investigated.

Seedling phenotype

The retarded seedling growth could result from insufficient nutrition to support the earlier growth, rather than from the direct role of *Mn::Uq*. Seedling growth can be correlated with seed weight or endosperm development. To test the seedling effect of *Mn*, mutant kernels were weighed and divided into ten weight classes (Materials and Methods). The frequency of kernels in each class is quite dissimilar in some families. However, the overall frequency shows a somewhat even distribution among classes: the frequency of each class is in the range of less than 20% (data not shown).

The comparison of seedling growth rates on the average among seedlings from mutant and normal kernels revealed a consistent positive correlation with seed weight (Table 1). Root growth was also examined and likewise showed the same consistent correlation (data not shown). This consistent positive correlation between seed weight and growth rate in the early stage can be interpreted to demonstrate that the impeded seedling growth might be a consequence of the inadequate endosperm development and subsequent lack of nutrition, rather than a direct effect of *Mn::Uq*.

Table 1. Comparison of germination and seedling growth of normal and among classes of miniature kernels.

Class	First	Second	Third	Fourth	Fifth	Normal[a]
Range of Weight (%)	< 10	10-20	20-30	30-40	40-50	100
Germination (%)	5.29	23.68	22.58	42.50	62.16	100
Seedling Height (cm)	9.51	14.15	15.32	17.18	19.17	19.27

[a]The standard weight of normal is the average weight of twenty plump kernels randomly chosen from each ear tested. Mutant kernels are divided into ten classes by relative weight (%), compared to the standard weight.

Variable penetrance and expressivity

The proportions of mutant kernels from crosses of plants of *Mn::Uq/+* with the *a1-ruq* tester have been observed in the range of approximately 20% to 50% with the average of approximately 39% (expected 50%) (data not shown). This indicates that the penetrance of *Mn::Uq* is variable. Since the spotting and miniature phenotypes were inseparable (Pan and Peterson 1989) and the current analysis was made as such, the lower penetrance is genetic evidence that *Mn::Uq* is not completely transmitted through the female. This is perhaps due to a weak competition of *Mn::Uq* gametes over wild types.

Pan and Peterson (1987) measured the sizes (from top to bottom) of miniature kernels and indicated that the sizes approach the normal distribution with severely expressed, small ones at one end and very weakly expressed, plump ones at the other end. Likewise, the weights of miniature kernels are also variable ranging from very small to almost normal weights, but with a somewhat even distribution for each of ten classes as described previously (Table 1). These observations indicate the variable expressivity of *Mn::Uq*.

Location of *Mn::Uq* on chromosome 2

Mn::Uq was not linked either to *a1* or *c1* (Pan and Peterson 1989). Thus far, three of four *mn* genes have been located; *mn1* on 2S, *mn2* on 7S, *mn3* on 6S. The crossing scheme for allelism tests is shown in Figure 1A. Progeny from crosses of *Mn::Uq* with these *mn* genes showed variable penetrance and expressivity. A very low proportion of *Mn::Uq* kernel phenotypes were observed with *mn3* and *mn4* (data not shown), which is not unusual as observed throughout the analyses of a number of crosses of *Mn::Uq*, especially with the genetic stock of *ws3 lg1 gl2 b1* in linkage tests (see below). However, it is postulated that *Mn::Uq* has no allelic relationship with any of them. First of all, approximately 56% and 62% of miniature kernels were observed in tests with *mn1* and *mn2*, respectively, when 100% (if allelic) and 75% (if independent) are expected. Further, *Mn::Uq* phenotypes are distinguishable from other miniature phenotypes, in that there is no pollen transmission. However, this test does not disallow allelism, especially due to incomplete penetrance of *Mn::Uq*.

A set of *wx* translocations (*wx-Ts*) was used for further mapping of *Mn::Uq*. The crossing scheme is presented in Figure 1B. Progeny of Cross 1 showed variable penetrance and expressivity, and were all colored so that selections were made based on miniature or etched-like phenotype. The selected miniature kernels were backcrossed by each *wx-T* line (Cross 2). *Wx* expression was difficult to detect in severely expressed *Mn::Uq* kernels, so linkage to *wx* was analyzed among normal kernels. The results indicated a loose linkage to T2-9c of ~40 cM and a *Mn::Uq* penetrance of 33% on the average: the linkages (penetrances) of individual crosses were between 36 cM (39%) and 44 cM (33%). Better penetrance will produce less *Wx* phenotypes than observed among normal kernels. Under **35 cM** (arbitrarily chosen based on the observation of 36 cM with 39% penetrance) for correction in consideration of low penetrance, the observed values showed no significance to the expected values (data not shown): this may imply that ~40% penetrance can give a good estimate of linkage with enough samples. T2-9b near to the centromere and T2-9d on 2L showed no linkage. If the *wx-T* mapping holds correct, it is likely that *Mn::Uq* is on the tip of 2S. Is it a coincidence that *Uq-(a et)*, which is allelic to *Uq-St*, was found linked to T2-9b (Pereira and Peterson 1985)? Several *Uq*'s have been located on chromosome 2 (Pereira and Peterson 1985; Seo and Peterson 1995).

Based on the *wx-T* mapping, linkage tests with genes on 2S, *ws3* and *lg1*, were made with a genetic stock of *ws3 lg1 gl2 b1*. The crossing scheme is shown in Figure 1C. A total of 1004 plants grown from seven families of Cross 2 (one ear makes one family) were examined for the segregation of *ws3* and *lg1*. In this population the distance between *lg1* and *ws3* is 10.36 cM (104/1004), which is close to the standard map unit of 11 cM. 960 ears of Cross 3 were harvested and inspected for the presence of *Mn::Uq* kernels. The penetrance was very variable: out of seven, four families showed less than 20% penetrance and only three families with an average of 37.98% were considered for linkage. Combined data from these three families indicated that linkages of *mn*-lg1* and *lg1-ws3* are 15.46 cM and 9.73 cM, respectively (*mn** is a temporarily designated locus for *Mn::Uq*.) (Table 2). One family with the highest penetrance (41.12%) indicated that ***mn*-lg1*** and ***lg1-ws3*** are **14.49 cM** and **10.28 cM**, respectively.

Table 2. Linkage tests of *Mn::Uq* with *ws3* and *lg1* on 2S. Cross: *Mn::Uq/+*, + *+/ws3 lg1* x *+/+*, *ws3 lg1/ws3 lg1* (see Figure 1C).

Genotype[a]	P1	P2	SC1	SC2	SC3	SC4	DC1	DC2	Total
No. Plants	182	231	7	53	5	25	5	16	524

Penetrance of *Mn::Uq* = 37.98% (50% expected), *mn*-lg1* = 15.46, *lg1-ws3* = 9.73

[a]P1 (parent 1) = *Mn Lg1 Ws3*, P2 (parent 2) = + *lg1 ws3*, SC1 (single cross 1) = *Mn lg1 ws3*, SC2 (single cross 2) = + *Lg1 Ws3*, SC3 (single cross 3) = *Mn Lg1 ws3*, SC4 (single cross 4) = + *lg1 Ws3*, DC1 (double cross 1) = *Mn lg1 Ws3*, DC2 (double cross 2) = + *Lg1 ws3*. *mn** is a temporarily designated locus for *Mn::Uq*.

Mapping data of *Mn::Uq* with *wx-Ts* and 2S markers of *lg1* and *ws3* clearly indicate that *Mn::Uq* is a new gene tagged with *Uq*. Hereby this locus is named *mn5*, which is located between *lg1* and *gl2* (Figure 2).

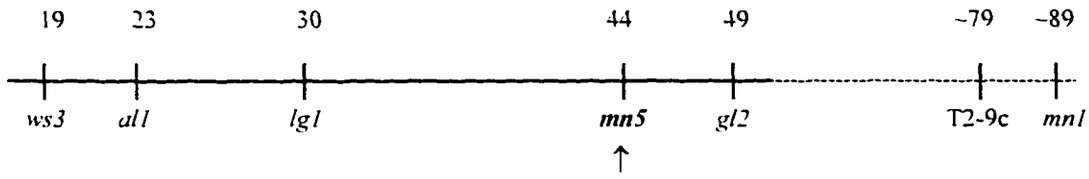

Figure 2. Partial genetic map of 2S with the location of *mn5*.

Co-segregation tests of *Mn5::Uq* with *Ac* and *Ds1*

The working hypothesis for these tests is that *Uq* is either a derivative of *Ac* or the master element of *Ds1* as described in Materials and Methods. Genomic DNA from mutant (*Mn5::Uq/+*) and normal (+/+) seedlings, respectively, was digested with *Eco*RI, *Eco*RV, *Kpn*I, *Sst*I, respectively. *Eco*RI has one site in the middle of *Ac* (Fedoroff *et al.* 1984), others have no site. The internal 1.6 kb *Hin*dIII fragment was used as an *Ac* probe. The use of the *Ac* probe in DNA blots displayed seven to nine bands depending on digestions. *Sst*I and *Eco*RI

digestions showed one segregating band. The Southern blot of *Sst*I-digested genomic DNA is shown in Figure 3, in which the ~ 7 kb band is absent in the fifth (lane 5) of lanes of *Mn5::Uq*/+ and present in the third (lane 8) of lanes of wild-type (+/+). *Eco*RI digestions of the same genomic DNA showed one segregating band of ~2 kb, in the same pattern of its presence and absence (data not shown): the ~2 kb band was absent in the fifth lane (lane 5) and present in the third lane (lane 8). Further test of this ~2 kb band for co-segregation, using a *Mn5::Uq* line that segregated mutant and normal kernels in close to the 1:1 ratio (an indication of good penetrance), indicated random segregation (data not shown). The use of the 280 bp fragment as a *Ds1* probe (*Ds1* of *r-m1*: a gift from Y. Liu, S. Wessler's laboratory) displayed approximately 40 bands without a distinctly segregating band (data not shown), and no more tests with *Ds1* were made.

The given molecular tests conclude that *Uq* is not related to *Ac*. If the current hypothesis of the relationship of *Uq* to *Ac* or *Ds1* is correct, more extensive and elaborate investigations are necessary. These may include use of more restriction enzymes, hybridization in lower levels of stringency, and backcrossing with a line having possibly no copy of *Ac* or *Ds1* to help detect the band co-segregating with *Mn5::Uq*.

Discussion

Implications of *Mn5::Uq* in the development of embryo and endosperm

The miniature kernels of *Mn5::Uq* have reduced-size endosperms and embryos. Heterozygous endosperms (*Mn5::Uq/Mn5::Uq*/+) fail to develop to completion. Embryogenesis can be influenced by the extent of endosperm development. It has been commonly observed that smaller miniature kernels have smaller embryos. The extent of embryogenesis is not investigated, for example, by counting the number of leaves. However, no distinct differences have been noticed between matured mutant and normal plants. During germination in greenhouse, however, it has been observed that strongly expressed miniature kernels (in the first class, Table 1) occasionally either fail to germinate or stop growing after a growth of approximately a couple of inches (data not shown).

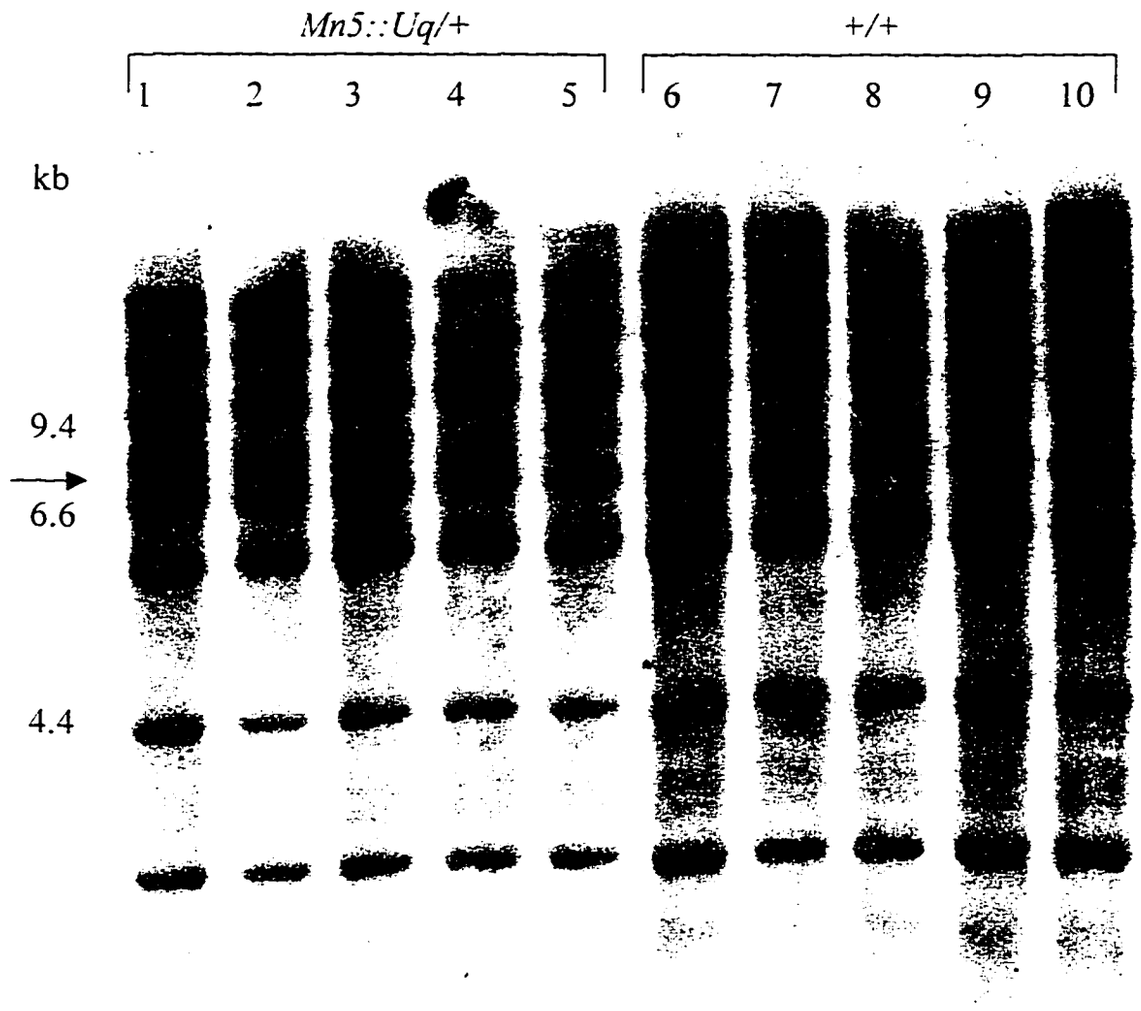

Figure 3. Southern blot analysis of seedlings of *Mn5::Uq*/+ and +/+, respectively. Genomic DNA was digested with *Sst*I and hybridized with the 1.6 kb *Hin*dIII fragment of *Ac*. Lanes 1-5 are DNA from mutant seedlings (*Mn5::Uq*/+) and lanes 6-10 are DNA from wild-type seedlings (+/+). The absence (lane 5) and presence (lane 8) of a ~7 kb band (arrow) that otherwise co-segregated with the mutant phenotype were also observed when the same genomic DNAs were digested with *Eco*RI, in which one co-segregating band of ~2 kb appeared (data not shown).

The dosage theory for the triploid endosperm development has been of interest (for review, Birchler 1993). The insufficient development of the heterozygous endosperm may support the idea that more than one copy of *Mn5* is necessary for the complete development of the endosperm. The insufficient embryogenesis and occasional arrestments of seedling growth may support the argument that the success of the embryo development requires a certain level of endosperm development.

How *Mn5::Uq* influences both endosperm and embryo developments remains to be investigated. In the case of the *mn1* gene of maize, the only *mn* gene molecularly investigated (Miller and Chourey 1992; Cheng *et al.* 1996), the wild type *Mn1* allele encodes a cell wall invertase, CWI-2. CWI-2 is required for normal endosperm development and maternal cells in the pedicel. CWI-2 plays a role spatially and temporally in the appropriate partitioning of sucrose in a developing maize endosperm by metabolizing the incoming sucrose from the plant. It was observed that the invertase-dependent metabolic status of endosperm affected the stability of cell in the pedicel.

Another example can be found in a study of petunia MADS box genes *FBP7* and *FBP11* that are maternally expressed and required indirectly for endosperm development (Colombo *et al.* 1997). Simultaneous down-regulation of both *FBP7* and *FBP11* by co-suppression resulted in their reduced expression more than ten-fold, producing the shrunken seed phenotype. The degree of endosperm development is variable, and so is that of embryo development. Endosperm degeneration occurred at variable (from earlier to later) stages of development. In such aberrant seed development, the embryo developed more slowly, and embryo arrest was observed. Analyses of early and intermediate stages by light microscopy showed that at 18 DAP the endothelium is completely degenerated, so that endosperm development will directly be affected, probably because of a block in transport of nutrition through the seed coat to the developing endosperm and embryo.

Models for the variable penetrance and expressivity of *Mn5::Uq*

In general, the variable penetrance and expressivity of *Mn5::Uq* depend on genetic background. This interpretation seems to be contrary to the high degree of homogeneity

among *Mn5::Uq* lines, which have been advanced by crossing more than six generations with the *Uq* tester *a1-ruq*. The *a1-ruq* tester has been a long time inbred that has been propagated by selfing or sibbing since its isolation (Friedemann and Peterson 1982). However, several observations have been available to support in general the effect of genetic background, whatever the specific mechanism(s) are involved.

The observations are as follows. Penetrance on the average in most outcrosses has been lower than in selfings or crosses with *a1-ruq*. Tests on the heritability of sizes of progeny kernels showed that most plants produced quite variable sizes but some produced somewhat constant sizes of kernels. Crosses of *Mn5::Uq* with an inbred B73 lost mutant kernels completely in BC1 while those with another inbred Tx303 continuously gave rise to them although with a low frequency. Crosses with *mn1* and *mn2* lines gave better expressed kernels than with *mn3* and *mn4* lines. Crosses with a set of *wx-T* lines gave various levels of variability within as well as among crosses. Crosses with a genetic tester of *ws3 lg1 gl2 b1* produced mutant kernels at a low frequency, whose expressions range from well-expressed to very weakly-expressed depending on crosses. Some ears from Cross 2 (Figure 1C) gave no clearly identifiable mutant kernels: many selfed ears (Cross 3, Figure 1C) of such progeny kernels produced only a couple of well-expressed mutant kernels. Occasionally, plants were observed to have more weakly expressed *Mn5::Uq* kernels on second ears than first ears, or vice versa.

Causes for the variable low penetrance of *Mn5::Uq* can be either the incomplete transmission of *Mn5::Uq* through female and thus no kernel development, or no expression of *Mn5::Uq* even if it is transmitted. It has not been tested whether *Mn5::Uq* is completely transmitted through female gametes. However, two observations may support no transmission of some of female gametes of *Mn5::Uq*. One, most of ears have shown good seed set, although some ears have been observed to have either no good seed set or a number of traces of no kernel development. Not all such events could have been counted during analysis, resulting in the less-than-expected penetrance. Secondly, the two phenotypes of miniature and spotting occur simultaneously (Pan and Peterson 1989), so that lower penetrance is equal to lower portion of spotted kernels. This may imply that female gametes

of *Mn5::Uq* may be weak in competition with normal gametes, such that the transmission rate of *Mn5::Uq* female gametes are lower than wild types.

The penetrance of *Mn5::Uq* might also be equal to the expected 50%, but its expressivity might be so weak or none in many cases that normal kernels result more than expected, resulting in the less-than-expected penetrance. Pan and Peterson (1989) made a blind test with normal-looking, spotted kernels and confirmed that miniature kernels segregated in a subsequent generation. In the current study of linkage tests with markers on 2S, a similar test was made although without such purpose to test the segregation of *Mn5::Uq* kernels. Most of ears from Cross 2 (Figure 1C) were observed to have very low proportions of *Mn5::Uq* kernels (no data available): the ear that gave more than 40% penetrance (see Results) segregated a detectable portion of mutant kernels but other ears had almost no mutant kernels as much as one would disregard them. Thus, all the kernels from Cross 2 had to be entered, recorded, and selfed in Cross 3. Each progeny from each ear segregated mutant kernels a lot more than in Cross 2.

It seems clear that non-expressivity and lower transmission rate of *Mn5::Uq* through the female are responsible for lower penetrance than expected. The lower penetrance of *Mu* activity-dependent mutations is also accounted for by no expression of the mutant phenotypes in the absence of *Mu* activity (Barkan and Martienssen 1991; Greene *et al.* 1994 and references therein). In these mutations, segregating *Mu* activity in (segregating) different genetic backgrounds explains the variable penetrance of mutant phenotypes.

The variable expressivity, from none to very severe, suggests that *Mn5::Uq* is transcribed, the mutant transcripts are processed, and the protein is at least in part functional. The total zero-function of *Mn5::Uq* would not lead to variable expressivity. Thus, it is hypothesized that *Uq* regulates the expression of *Mn5* either at the level of transcription or post-transcription, or at levels of both.

Given every known aspect of transposons, the active phase of *Uq* will interfere with the transcription of *Mn5*, and the inactive phase of *Uq* will allow the transcription of *Mn5*. This phenomenon has been observed with non-autonomous elements-induced alleles (Barkan and Martienssen 1991; Martienssen and Baron 1994; Greene *et al.* 1994 and references

therein). This can happen with the autonomous element *Uq* at *Mn5*. Otherwise, what would be the possibility that *Mn5* was tagged with a non-autonomous element of *Uq* and the *Uq* of *Mn5::Uq* is in fact so closely linked that it appears as co-segregating with the mutant phenotype? The activity of the autonomous *Uq* element at the *mn5* locus is, rather than self-controlled, likely regulated or mediated by genetic factors or modifiers that control either *Uq* or *mn5*. Modifier genes regulate penetrance and expressivity of transgene inserts through epigenetic modification (Allen *et al.* 1990). *cis*- and *trans*-acting modifiers may control gene expression by influencing transcription. So far methylation has been the only well-known epigenetic mechanism that regulates the activity of maize transposons (Chandler and Walbot 1986; Schwartz and Dennis 1986; Banks and Fedoroff 1989; Kunze and Starlinger 1989; Dennis and Brettell 1990).

The functionality of proteins derived from the chimeric *Mn5::Uq* transcripts may depend on the insertion orientation and sites of *Uq*. The insertion orientation opposite to the transcriptional direction of the *p* gene were shown to affect background pigmentation (Peterson 1990; Moreno *et al.* 1992). The insertion in the same orientation would result in the premature termination of transcription at *Uq*. An insertion between the TATA box and the coding region, as in *hcf106* (*Mu1*-insertion in the 5′-untranslated region) (Barkan and Martienssen 1991), may produce protein with normal function. What would be the possibility that the read-out transcription occurs with *Uq* as with the construct of *Ds* and HPT II in transgenic tomato (Rudenko *et al.* 1994) and with *Mu1* of *hcf106* in maize (Barkan and Martienssen 1991). Insertions into exons likely result in mutant proteins, since splicing events of transposon inserts have been inaccurate. The mutant proteins may be partially functioning if they are in frame. Insertions in introns may produce functional proteins if splicing sites are conserved. Alternate, aberrant splicing events due to the insert can produce differently processed products with partial function retained (Varagona *et al.* 1992).

With such several possibilities for insertion sites and orientation, the variable expressivity likely comes either from different levels of transcripts or different levels of steady-state mRNA, or from both. If explained at both levels, first, different levels of transcripts depend on different phases of *Uq*, which would be regulated by epigenetic

modification during development. Then, different levels of mRNA stability may depend on different backgrounds, as in the differently isolated, but same mutations of *adh1-S3034* and *adh1-S3034b* (*Mu1*-insertion in the first intron) (Strommer and Ortiz 1989; Ortiz *et al.* 1990). Different levels of enzyme activity between these two same alleles were accounted for at the post-transcriptional level by differences in two genetic backgrounds (BCF and FkF) and in turn differences in levels of steady-state RNA, not by DNA methylation. The same two alleles showed no differences of enzyme activity although with a high variability of up to ~50% in the heterozygote background, but either of two same alleles in two different backgrounds showed distinctly different levels of enzyme activity with a narrowed variability down to 25% in each background. The variability could be due to segregating genetic backgrounds. The frequency of genetic modifiers in different genetic backgrounds is expected to be different, having noticed as background effects.

Action of *Mn5::Uq*: dominance vs. recessiveness

The miniature kernel phenotype and retarded seedling growth of a heterozygote (*Mn5::Uq*/+) suggested to Pan and Peterson (1989) that *Mn5::Uq* is dominant negative. No pollen tube growth from a mutant pollen does not give a clue in deciding between dominance vs. recessiveness. However, under both dosage theory in endosperm development (for review, Birchler 1993) and the observed positive correlation between seedling growth and kernel weight (Table 1), one might have a split opinion that *Mn5::Uq* is either a dominant-negative mutation or a recessive mutation.

For a mutant to be dominant, the mutant should produce a protein that functions dominantly either in a positive or negative way and expresses its phenotype over the normal type. Such transposon-induced dominant mutations have been reported. *C1-I* is a transposon-mediated dominant-negative mutant of the regulatory *c1* locus, and encodes a repressor function in contrast to the activator function of the *C1* gene product (Paz-Ares *et al.* 1990). Dominance of *Mu* insertion-induced *Kn1* mutations (Greene *et al.* 1994) comes from their ectopic expression. What makes dominance of *Mu* insertion-induced *Les28* mutation is not known (Martienssen and Baron 1994).

If *Mn5::Uq* is dominant negative, the repressor case of *C1-I*, or any type of repressors, is likely applicable. One major difference is that *C1-I* originated from footprints created by a transposon through insertion and excision, while *Mn5::Uq* is the insertion of the *Uq*. Then, the *Uq* transcript should be spliced out from the chimeric *Mn5::Uq* transcript, and the resultant protein should function in a negative way. A case that a mutant protein produced via such RNA processing expresses such dominance as *C1-I* has not been reported. Also, under dosage effect of *Mn5* on endosperm development, negative functionality of the *Mn5::Uq* protein would not allow endosperm development because of the presence of mutant and normal proteins in a 2:1 ratio. Otherwise, the amount of the protein from one copy of *Mn5* transmitted via male should be more abundant than the mutant protein from two copies of *Mn5::Uq* transmitted via female. Further, the retarded seedling growth could result from the insufficient nutrition to support the earlier growth, rather than from the direct role of *Mn5::Uq*.

Recessiveness of a mutation comes from no product or non-functionality of its modified protein. Dosage theory supports either no protein from the mutant allele due to the *Uq* interference or non-functionality of the mutant protein. The insufficient endosperm development can slow earlier mutant seedling growth. Such interpretations support the recessiveness of *Mn5::Uq*, thus the dominance of *Mn5::Uq* is not clear. The 1:1 ratio of mutant to normal phenotypes of the *Mn5::Uq*/+ line does not fit into the classical 3:1 ratio of dominant to recessive phenotypes, due to no male transmission. No information has been available on the phenotype of the homozygote (*Mn5::Uq/Mn5::Uq*), which could result in lethal because either no endosperm development or no pollen transmission, likely none of them, is expected under current characterization.

Co-segregation tests of *Mn5::Uq* with *Ac*

A basic tenet of transposon biology is that the specificity of the transposase of functional transposons is the key feature that distinguishes the different families of transposons. Thereby, *En/Spm* does not recognize genetically members of the *Ac* family of transposons. The molecular findings have confirmed this specificity by describing the terminal inverted repeats

(TIR), which have a different configuration (sequence and size) and are recognized only by the specific transposase of the specific family.

The original hypothesis of the functional relationship between *Ac* and *Uq* derived from molecular and genetic findings that (1) *ruq* is ~95% homologous to *Ds1* (Pisabarro *et al.* 1991) and (2) *Ac* transactivates all types of *Ds* elements including *Ds1/ruq* while *Uq* transactivates *Ds1/ruq* only (Caldwell and Peterson 1992). Because *Ac* and *Uq* recognize the same TIR of *Ds1/ruq*, an implication underlying the working hypothesis followed, based on the specificity among the families of transposons, that these two autonomous elements should have somewhat similar proteins and therefore near identical DNA sequences.

This hypothesis was tested by using an *Ac* probe in the Southern hybridization experiments. Genomic DNA from seedlings of *Mn5::Uq/+* and +/+, respectively, was hybridized with the 1.6 kb *Hin*dIII *Ac* fragment to find a co-segregating band unique to *Mn5::Uq/+* lines. No co-segregating, unique band was found. Therefore, given the current stringency tests on the structural relationship, it can be concluded that *Uq* is not related to *Ac*.

The uniqueness of *Uq*, despite of the functional relationship with *Ac*, is an exception to the above-mentioned principle of transposon biology. Then, a minute possibility may exist that *Uq* and *Ac* proteins have no such significant homology, but they still can recognize the TIR of *Ds1/ruq*.

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CHAPTER 4. THE INVETERATE WANDERER: STUDY OF *Enhancer* WANDERING ON CHROMOSOME 3 IN MAIZE

A paper published in Theoretical and Applied Genetics[1,2]

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Abstract

The transposition of the maize transposable element *Enhancer* (*En*) had been focused on one chromosome 3 for several generations. From the *a1-m(Au)* allele with an autonomous *En*, a new *En* reporter allele *a1-m(r)3927-1*, was isolated that undergoes very infrequent and late excision events, producing one or two small spots in the aleurone. This allele is seriously impaired in its capacity to excise. Coincident with the origin of this allele, an *En* was located at a site close to the *a1* locus. From this initial insertion site, the movement of this *En* was followed for three to four generations in 974 families with a higher transposition rate of this *En* (50% of the testcross progeny) than that found in a previous study of *En* transposition. This is the first case reported where a particular *En* was followed for more than 3 generations. The higher rate of wanderings of this *En* along the same chromosome led to the term 'vagabond' *En* (En^{vag}). Genetic evidence that *En* may transpose from a replicated donor site to an unreplicated site is provided. Speculative mechanisms on the origin of *a1-m(r)3927-1* and En^{vag} are discussed.

Key words Maize transposable element, *Enhancer* (*En*), En^{vag} transposition, *En* reporter *a1-m(r)3927-1*

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Introduction

It has been well-established that maize transposable elements move from one chromosome position to another, or even on different chromosomes. These studies were conducted with two autonomous maize transposons, namely, *Activator* (*Ac*) (McClintock 1949; van Schaik and Brink 1959; Greenblatt and Brink 1962; Greenblatt 1984; Dooner and Belachew 1989; Schwartz 1989; Chen *et al.* 1992; Athma *et al.* 1992), and *Enhancer/Suppressor-mutator* (*En/Spm*) (Peterson 1960, 1965; Nowick and Peterson 1981; Pereira *et al.* 1985). Transposition patterns have also been examined in *Drosophila* in genetic and molecular studies of the *P* element (Raymond and Simmons 1981; Levis *et al.* 1985; Daniels and Chovnick 1993; Tower *et al.* 1993; Zhang and Spradling 1993; Golic 1994).

Transposition profiles of *Ac* have been extensively investigated mostly with the mutable pericarp allele (*P-vv*) [*Ac* (= *Mp*) at the *p* locus] [Brink and Nilan (1952) and subsequently by van Schaik and Brink (1959), Greenblatt and Brink (1962), Greenblatt (1984), and Chen *et al.* (1987, 1992)], utilizing the unique feature of the negative dosage effect of *Ac* (McClintock 1950, 1951) combined with the *P-vv* allele that enabled them to isolate germinal events and examine transposition patterns associated with chromosome replication. Several studies have shown that *Ac* and its receptor element *Ds* transpose most often without distinct polarity to target sites over short distances in maize and other species (van Schaik and Brink 1959; Dooner and Belachew 1989; Dooner *et al.* 1991; Moreno *et al.* 1992; Athma *et al.* 1992; Bancroft and Dean 1993).

Transposition of the *Drosophila P* element has been examined in several loci such as *white*, *rosy*, *singed* and others (Levis *et al.* 1985; Tower *et al.* 1993; Zhang and Spradling 1993; Daniels and Chovnick 1993; Golic 1994 and references therein). While the tendency for transposition of *P* to nearby sites has been frequently reported in these studies, some *P* elements in the *rosy* locus transposed more often to independent sites (Levis *et al.* 1985; Golic 1994). These contradictory observations can explain an important role of genomic positions in determining excision and transposition profiles for these elements.

In a study with *En* at the *a1* locus on chromosome 3L, about 20% of the progeny contained a transposed *En* that showed a preference to sites proximal to *a1* in regions up to

30 map units distant (Nowick and Peterson 1981). This report showed that like *Ac*, *En* also moves with regional preferences in both directions. In a study at the *wx-m8* allele (Schwarz-Sommer *et al.* 1985a), the frequency of excision events was estimated to range from 10% to 20%. In *Arabidopsis* an average frequency of germinal excision of *En-1* was 7.5% (Cardon *et al.* 1993). Using somatic observations with appropriate reporter alleles, Dash (1991), and Dash and Peterson (1994) reported that *En* undergoes replicative transposition.

The experiments reported herein demonstrate that an original *En* transposed from the autonomous mutable *a1*-m*(Au)* (Peterson 1978; Nowick and Peterson 1981) to a nearby position. This particular *En* has been followed since 1986 after its initial location was confirmed (Figure 1). This new autonomous *En* 'migrates' on chromosome 3 moving back and forth relative to the *a1* locus approximately 50% of the time; the rest of the time it transposes to a site on chromosome 3 far removed from the *a1* locus or to an independent site. The expectation is that this *En* will continue to move up and down this chromosome indefinitely. Our study is the first case in which one *En* was pursued over an extended period of time, and the results suggest that transposons once on a chromosome will continue to move on that chromosome the majority of the time, likely leaving footprints.

We also report the discovery of a unique reporter allele and an *En* that transposes quite frequently compared with the report of Nowick and Peterson (1981); it is thus termed 'vagabond' *En* (En^{vag}). Coincident with the origin of En^{vag} is the discovery of a new nonautonomous allele, *a1-m(r)3927-1*, which originated upon excision of *En* from the *a1-m(Au)* allele. This allele is unique in that its response to *En/Spm* is expressed with one or two excisions (spots) (Fig. 2D) even in the presence of a very strong *En/Spm*. Provided further in this report is genetic evidence that *En* may also move from a replicated donor site to an unreplicated target site in a manner, similar to that of *Ac* at the *p* locus.

Materials and Methods

Genetic testers, phenotypes and terminology

Testers available in our laboratory were used to isolate and study the transposition profiles of a newly isolated, autonomous element, En^{vag}, and to confirm the infrequent

excision state of a new *En*-reporter allele, *a1-m(r)3927-1*. Gene symbols, related phenotypes and terminology are well described in Reddy and Peterson (1984) and also provided in this text whenever necessary. Phenotype abbreviations are used to accommodate the phenotypic expression of genotypes. For example, dominant phenotypes [e.g., pale colored (Cl) and plump (pl)] result from both dominant homozygotes and heterozygotes. Examples are sp pl (= spotted-plump), Cl sh (= pale colored-shrunken), cl pl (= colorless-plump), sp sh (= spotted-shrunken).

Origin of En^{vag} and *a1-m(r)3927-1*

From a testcross of the autonomous *En*-containing mutable *a1-m(Au)* allele (79 0222-21) (cross 1 in Fig. 1) a large number of mutant colorless derivatives arose. Most of these derivatives were proven to be *En*-containing and non-responsive *a1-m(nr)*, which indicates that they arose from excision events at the *a1-m(Au)* allele, leaving the allele non-functional (Peterson 1970; Menssen 1988). One derivative, 80 3927-1 (cross 2 in Fig. 1), though originally isolated as a colorless kernel, expressed a rare spot on some of the kernels among the progeny of a self (Fig. 2D). Further tests with other reporter alleles (Fig. 1) showed that a strongly acting *En* was present (high frequency of spots as in Fig. 2B, C). This observation indicates that the infrequent excision events were due to a drastically impaired response of a new reporter allele to this strong *En*, both named as *a1-m(r)3927-1* and En^{vag}, respectively, as explained in the Results.

Crossing strategy and crossover calculations

This strategy was aided by testers carrying *a1* alleles (*a1*, *a1-m(r)* and *a1-m1*) and closely linked markers (*sh2* and *et1*: 0.25 cM and 12 cM from *a1*, respectively). Genetic crosses used to determine linkage positions of En^{vag} relative to *a1* were as follows: cross 1 *a1-m(r) Sh2 En/a1-m1 sh2* + x *a1 sh2/a1 sh2*; cross 2 *a1-m(r) Sh2 En/a1-m1 sh2* + x *a1 et1/a1 et1*; cross 3 *a1-m(r) Sh2 En/a1 sh2* + x *a1-m1 sh2/a1-m1 sh2*; cross 4 *a1-m1 sh2 En/a1 Sh2* + x *a1-m1 sh2/a1-m1 sh2*.

Figure 1. Origin of *a1-m(r)3927-1* and En^{vag}. Series of crosses that were carried out to isolate *a1-m(r)3927-1* and En^{vag}, and to generate the materials used in this experiment. *a1-m(r)3927-1* is marked with an asterisk (*) to distinguish it from *a1-m(r)*. Parental phenotype or mutability pattern planted in the next generation is indicated in *parenthesis*, with the mutability pattern indicated by scale 1-10 (*1* infrequent excision events, *10* most frequent events) and a-e (*a* very late excision events, colored spot covering one to six aleurone cells, *e* very early events, colored spot covering one-half to one-quarter of the kernel). Final progenies entered into this study were divided into branches for convenience because each assigned branch was planted at the same time in the 1986 summer nursery and linkage was calculated the first time with its progeny. For more details see text.

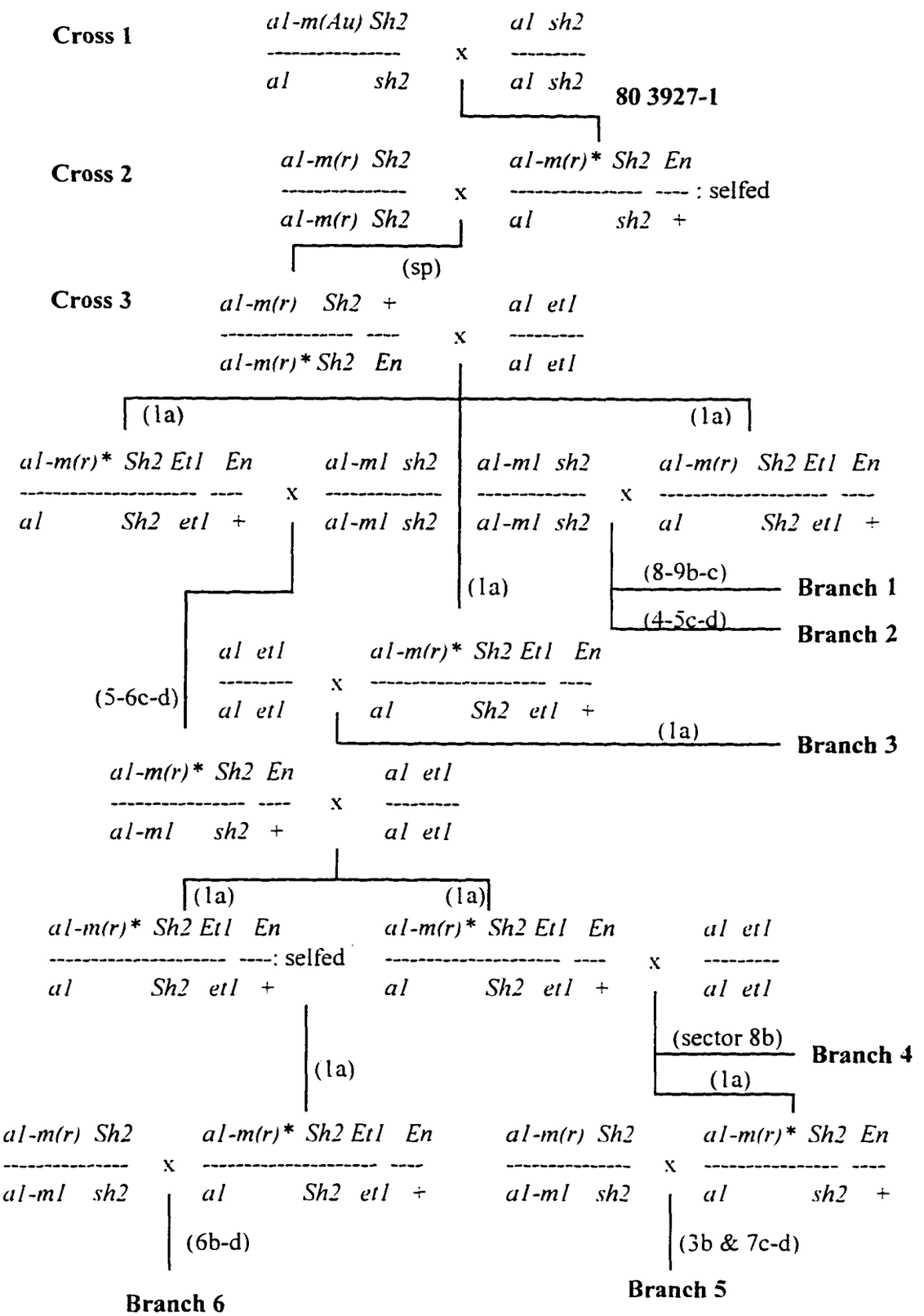
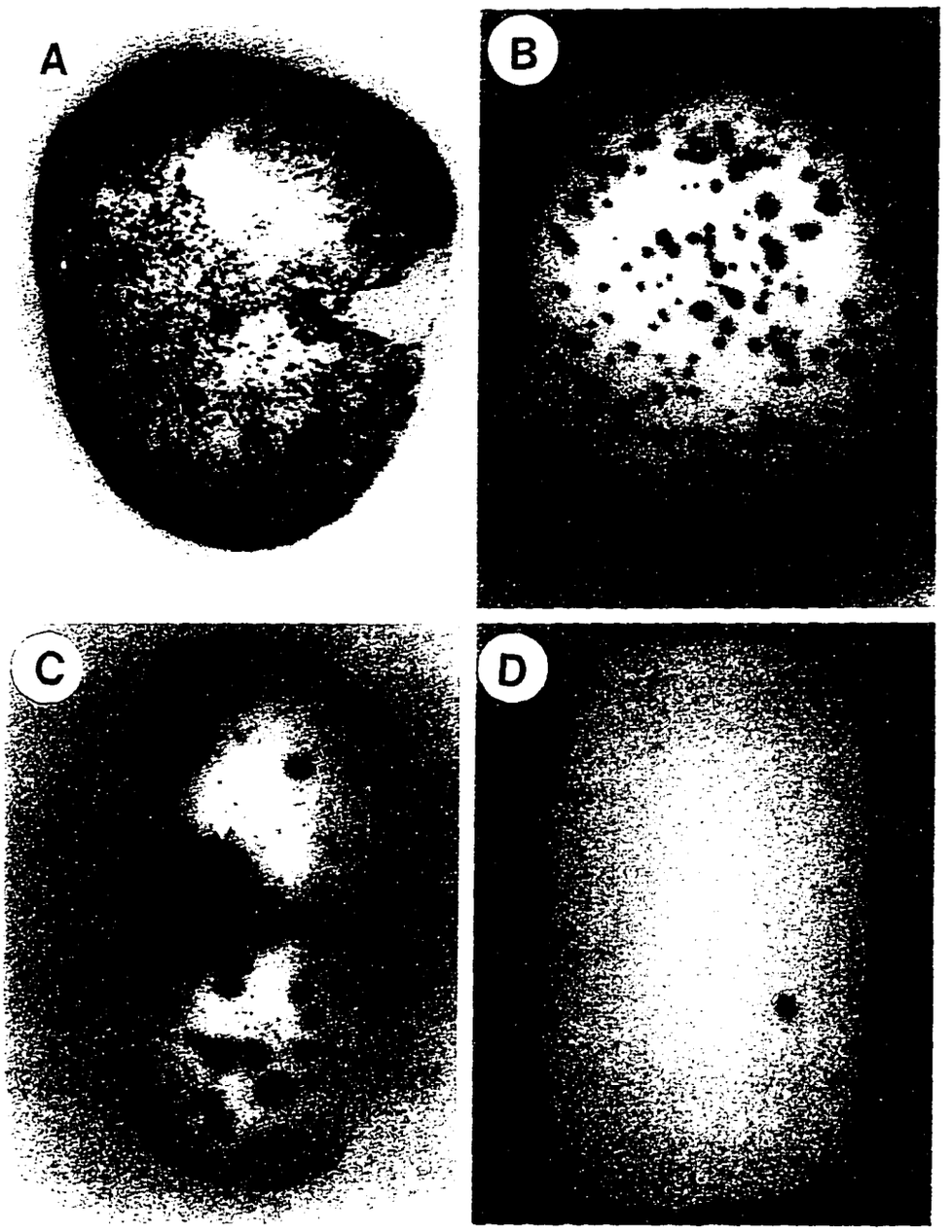

Cross 1
a1-m(Au) Sh2 / a1 sh2 x a1 sh2 / a1 sh2
80 3927-1
Cross 2
a1-m(r) Sh2 / a1-m(r) Sh2 x a1-m(r)* Sh2 En / a1 sh2 + : selfed
(sp)
Cross 3
a1-m(r) Sh2 + / a1-m(r)* Sh2 En x a1 et1 / a1 et1
(1a)
(1a)
a1-m(r)* Sh2 Et1 En / a1 Sh2 et1 + x a1-m1 sh2 / a1-m1 sh2
a1-m1 sh2 / a1-m1 sh2 x a1-m(r) Sh2 Et1 En / a1 Sh2 et1 +
(1a)
(8-9b-c)
Branch 1
(4-5c-d)
Branch 2
(5-6c-d)
a1 et1 / a1 et1 x a1-m(r)* Sh2 Et1 En / a1 Sh2 et1 +
(1a)
Branch 3
a1-m(r)* Sh2 En / a1-m1 sh2 + x a1 et1 / a1 et1
(1a)
(1a)
a1-m(r)* Sh2 Et1 En / a1 Sh2 et1 + : selfed
a1-m(r)* Sh2 Et1 En / a1 Sh2 et1 + x a1 et1 / a1 et1
(sector 8b)
(1a)
Branch 4
(1a)
a1-m(r) Sh2 / a1-m1 sh2 x a1-m(r)* Sh2 Et1 En / a1 Sh2 et1 +
(6b-d)
Branch 6
a1-m(r) Sh2 / a1-m1 sh2 x a1-m(r)* Sh2 En / a1 sh2 +
(3b & 7c-d)
Branch 5

Figure 2. Heritable phenotypes of *a1* alleles in the presence of *En*. **A.** *a1-m(Au)*, an autonomous allele, produces kernels that are almost completely colored with colorless sectors from the cross *a1-m(Au) Sh2/a1 sh2* x *a1 sh2/a1 sh2*. **B.** *a1-m(r) + En*: heritable variegation pattern of 7-9b-d from the cross *a1-m(r) Sh2 En/a1 sh2 +* x *a1 sh2/a1 sh2*. **C.** *a1-m1 + En*: heritable pattern of 4-6b-d on a colorless background from the cross *a1-m1 sh2 En/a1 sh2 +* x *a1-o wx/a1-o wx*. **D.** *a1-m(r)3927-1 + En* from the cross *a1-m(r)3927-1 Sh2 En/a1-m1 sh2 +* x *a1 sh2/a1 sh2*: this new allele produces one or two spots (1a-b), or no spot.


A
B
C
D

Determination of transposition by chi-square (X^2) test

Unlike the situation in other transposition studies where the element of interest was at an autonomously mutable locus. En^{vag} followed in our study was located at a site linked to *a1*. Following a testcross verifying the linkage position of En^{vag} to *a1*. the progenies were evaluated by a chi-square test to confirm the heritability of the En^{vag} position. Because the linkage values between *a1* and transposed *En*'s seemed to be quite variable among sib families from the same parent. chi-square contingency tests for uniformity were performed to confirm the transposed *En*'s (Nowick and Peterson 1981: Snedecor and Cochran 1989). Ears with less than 100 kernels were discarded. When the chi-square test on a set of the sib data from the same parent was significant. some of the sib data were assumed to be the result of transposition events. The test was repeated by excluding one by one the most deviant lines (they can have the largest or smallest c/o values depending upon component values in a set of data) until the test was not significant (Nowick and Peterson 1981). (This test method is named 'exclusion chi-square test' to distinguish from the 'inclusion chi-square test' that was developed for this study). However. in this study. there were sets of data in which at a first glance most of the data in a set were significantly different from their parental linkage (c/o value) to *a1* (data not shown). In these data the exclusion chi-square test could lead to the wrong conclusion that some of the closest values to the parent were transposition events. To avoid this error. we made some modifications to the exclusion chi-square test: (1) inclusion of the parent value. and (2) when significant. inclusion of one by one the closest values to the parent value until just before the test is significant. The test procedure used in this study (= inclusion chi-square test) is illustrated and compared with the exclusion chi-square test in Fig. 3. The exclusion chi-square test can sometimes take an otherwise homogeneous c/o for a heterogeneous c/o or vice versa (e.g. 19.89 and 42.74. respectively. in Fig. 3) and result in a different transposition frequency compared to the inclusion chi-square test (not demonstrated in Fig. 3). While in a data set indicating a low transposition frequency. the exclusion chi-square test may not affect a result. the inclusion chi-square test is appropriate to apply in a data set with a high transposition frequency. One should realize that the chi-square test

Parent c/o	Progeny c/o's								Avg c/o	χ^2-value
29.13	13.74	15.23	19.89	28.79	33.75	40.94	42.22	42.74	29.60	34.83**
			↑	↑	↑	↑	↑		32.45	10.83^{ns}
A			3	1	2	4	5	↑	33.92	13.03*
								6		
B	13.74	15.23	19.89	28.79	33.75	40.94	42.22	42.74	29.66	34.75**
	↑	↑	↑						37.69	4.02^{ns}
	1	2	3							

Figure 3. Comparison of chi-square (χ^2) test procedures: the inclusion chi-square test (**A**) developed for this study and the exclusion chi-square test (**B**) used in the previous study by Nowick and Peterson (1981). $\chi^2 = \Sigma[(O-E)^2/E]$ (O = observed c/o's for individual ears, E = expected value, equals the average c/o of all observed c/o's). *Significant. **Highly significant. ^{ns}Not significant. Part of the data set from 89 1831 and 89 1832 derived from the same parent was used for convenience. *Arrows* with numbers *underneath* indicate the order of sequential inclusion (**A**) and exclusion (**B**) of observed numbers in the chi-square test.

A. The parent c/o is included in the test and when the test is significant, the closest c/o's to the parent are included one by one until just before the test is significant. The test with six c/o's (parent c/o and five progeny c/o's) shows non-significance. The test with seven c/o's including the sixth, 42.74, shows significance. This test indicates that three out of eight (13.74, 15.23, 42.74) are transposition events.

B. The parent c/o is not included in the test and when the test is significant, the most deviant c/o's from the average c/o are excluded one by one until the test is not significant. This test indicates that 13.74, 15.23 and 19.89 are transpositions. In this particular set of data, both tests show the same transposition rate (3/8), although the rate is different depending upon the component c/o's in a set of data. The inclusion chi-square test is developed to avoid errors that are made when most of the values in a set of data are different from their parent value.

There are three situations of *En*-location that can be respectively divided into three percentage classes of spotted kernels: (1) less than 50% (*En* linked to *a1*), (2) 50% (*En* independent of *a1*), and (3) more than 50% (two or more *En*'s present). In the case of recombinant values close to 50%, each was assigned to one of these three classes using the chi-square test for independence. Table 1 shows how this assignment was made with respect to these ambiguous cases. The decision depends on the population size, because the greater the number of kernels on an ear, the greater the accuracy. In the independent situation (class 2), the number of parent and recombinant types are expected to be theoretically equal in the chi-square test. The expected value of each type therefore will be the average of the sum of the observed numbers for parent and recombinant types, respectively. On the basis of this criterion, for example, the location of *En* showing recombination of 45.96% (90 1650-23) was determined to be independent of *a1*.

Table 1. Examples of determination of recombination percentage classes: linked, independent and two or more *En*'s present.

	Segregation			χ^2-test: $\alpha = 0.05$, d.f.=1, value=3.84		
Plant No.	Parent	Recombination	Total	% Recombination	χ^2-value	Class[a]
90 1650-21	294	230	524	43.89	7.82	1
-22	184	271	455	59.56	8.32	3
-23	214	182	396	45.96	2.59	2

[a]Class refers to locations of *En* relative to *a1*; [b]Linked, [c]Independent, [d]Extra *En*'s

Results

Description of the origin of En^{vag} and *a1-m(r)3927-1*

Approximately, one-fifth of the kernels observed on the ear from the cross of *a1-m(Au) Sh2/a1 sh2* x *a1 sh2/a1 sh2* (cross 1 in Fig. 1) were colorless plump, which was not expected. Were these the result of non-responsive alleles (Peterson 1970)? To verify the non-responsiveness of these colorless derivatives, we outcrossed colorless-plump kernels from each of the nine progeny ears to confirm the presence of a transposed *En* and selfed them as

well as to preserve the original derivative (cross 2 in Fig. 1). Outcrossing to a combination reporter genotype [*a1-m(r)/a1-m1*] confirmed that these colorless derivatives contained a strong transposed *En* [*a1-m(r)*, high-spotted kernels (7-9b-d, Fig. 2B) and *a1-m1*, medium-spotted kernels (4-6b-c, Fig. 2C)]. The numbers and letters describing mutability patterns are well explained by Reddy and Peterson (1984). Briefly, scale 1-10 indicates very infrequent (1) to very frequent (10) and a-e, late (a) to early (e) excision events.

Among the outcrosses, the ear from plant 80 3927-1 was an exception. Like the other crosses, this cross to the combination reporter showed the presence of a strongly acting *En*. But in the confirmation of the F_1's by crossing to *a1 et1* (cross 3 in Fig. 1), the kernels of a noticeable number of the progeny were colorless, plump and very lightly spotted. (Fig. 2D). When lightly spotted plump kernels were again tested by *a1-m1* (*a1-m1 sh2/a1-m1 sh2*), densely spotted plump kernels reappeared. When these densely spotted plump kernels were crossed by a null *a1* tester (*a1 et1/a1 et1*), the lightly spotted plump types reappeared among the progeny (Fig. 1). Progeny with a confirmed linkage of *En* to *a1* entered into this study in 1986.

In reviewing these crosses, we were convinced that the original selection (80 3927-1) had a strong *En*, and we later determined it not to be a non-responsive derivative but to be a very low-responding allele to this very strong *En*. To confirm the phenotype of this allele, we reexamined the progeny from the original self (cross 2 in Fig. 1). The reader will appreciate that if spot frequency is variable and if some kernels would include only one spot, one would expect that some kernels would lack spots. The presence of the lightly spotted kernels in the selfed progeny confirmed that this allele originated as a lightly spotting *a1-m(r)*, despite containing a very strongly acting *En*. This new allele was named *a1-m(r)3927-1*, and the autonomous *En* was termed 'vagabond' *En*(En^{vag}) because of the continuous nature of its high transposition frequency over several generations.

Transposition profiles of En^{vag}

Transposition of En^{vag} was evaluated in 974 families over more than three generations (Table 2) and included all three classes of *En* locations. Transposition of this *En* occurred at an average frequency of approximately 45%, based on the chi-square contingency test. Transposition to linked and independent target sites relative to the *a1* locus took place at a similar rate. However, transposition frequencies of sister-emanating lines from an original stem parent varied from 0 to 100% (data not shown).

Table 2. Overall transposition rate of En^{vag} over more than three generations of testcrosses.

	Number of ears considered				% of ears		Ratio (%)[d]
Year	Total	Trans[a]	Indep[b]	Linked[c]	Trans	Indep	Indep : Linked
1988	157	87	45	42	55.41	28.66	51.72 : 48.28
1989	315	149	66	83	47.30	20.95	44.29 : 55.71
1990	502	236	92	144	47.01	18.32	38.98 : 61.02
Average	974	472	203	269	**48.46**	**20.84**	**43.01 : 56.99**
1992	185	46	19	27	**24.86**	**10.27**	**41.30 : 58.70**

[a]Transposition events include both [b]independent and [c]linked transpositions. [d]For example, out of the total 87 transpositions in 1988, 45 were independent and 42 were linked. Therefore, the ratio of indep:linked is 45/87:42/87=51.72:48.28.

Because of the frequent transposition of this *En* in the previous three years of experiments (Table 2), we non-randomly selected a set of lines in 1992 that were genetically homogeneous to the parental lines and that agreed with their parental values within 20 map units from *a1*. The transposition rate of these lines is about 25%, about half the overall rate found in the previous three years (1988-1990). This is not very different from the value reported by Nowick and Peterson (1981). However, in the previous three generations of experiments, no stable genetic position was found. Nowick and Peterson (1981) also described *En* sites giving rise to both transpositions and lacking further transposition within every interval of 2 map units. One could expect that selection of the latter would not result in

as much frequent transpositions as selection of the former. Whether DNA modification such as methylation or rearrangement occurred in these *En*'s is a possibility, as has been reported (Schwartz and Dennis 1986; Bennetzen 1987; Chomet *et al.* 1987; Keller *et al.* 1993).

Two-point mapping between *En* and *a1* does not allow the determination of proximal or distal positions, and the *sh2* marker is too close for use. In the Nowick and Peterson study (1981), transposed *En*'s were found on either side of *a1*. In our study, the distribution of the insertion sites linked to *a1* was also examined, but regardless of direction. Approximately 68% of the transpositions of En^{vag} occurred within 16 map units (data not shown). The rest of the linked transposition events were evenly distributed between 17 and 44 map units from *a1*. Such distributions were likely because parental *En* sites close to *a1* were preferentially selected to test in the next generation.

Exceptional segregation pattern is genetic evidence of transposition from a replicated to an unreplicated chromosome during chromosomal replication

The presence of some individual progeny with unexpectedly large crossover values (>> 50%) indicated that these types might result from the presence of extra *En*'s. According to the replication model of *Ac* (Greenblatt and Brink 1962; Greenblatt 1984; Chen *et al.* 1992), extra *Ac*'s are created by replication of a donor site, followed by replication at a target site after *Ac* has transposed. This model has been supported in studies with *En* (Dash and Peterson 1994). Data signifying the presence of extra *En*'s were chosen and examined to determine the segregation pattern.

This investigation was possible with crosses producing two groups of plump and shrunken kernels, each with parent and recombinant classes. In Cross 1 of Table 3, the plump group includes one parental class (sp pl = spotted-plump) and recombinant class (Cl pl = pale colored-plump). In this same cross the shrunken group includes the other parental (Cl sh = pale colored-shrunken) and recombinant (sp sh = spotted-shrunken) classes. In Cross 2 of Table 3, the shrunken group includes one parent (Cl sh) and recombinant (sp sh), and the plump group includes the other parent (sp pl) and recombinant (Cl pl).

Table 3. Lists of families showing aberrant segregation from testcrosses 1 and 2, and estimation of *En* insertion sites.

Cross 1. *a1-m(r) Sh2 En/a1 sh2 +* x *a1-m1 sh2/a1-m1 sh2*

Plant No.	Parent C/O[b]	Segregation[a] sp pl (P)	Cl pl (R)	Cl sh (P)	sp sh (R)	Total	Donor C/O[c]	Trans C/O[d]
88 1623-26	11.46	264	22	100	130	516	7.69	56.52
88 1624-23	6.79	174	15	82	94	365	7.94	53.41
88 4659-25	2.94	120	4	28	72	224	3.23	72.00
89 1803-24	2.48	206	19	106	115	446	8.44	52.04
90 1615-21	3.90	97	11	52	42	202	10.19	44.68
90 1617-21*	11.02	192	64	92	112	460	25.00	54.90
90 1617-27	11.02	49	3	33	36	121	5.77	52.17
90 1621-22	1.60	245	6	171	40	462	2.39	18.96
90 1647-27*	33.45	205	12	147	72	436	5.53	32.88
90 1648-31	10.40	193	25	112	103	433	11.47	47.91
90 1652-29*	8.70	132	81	54	114	381	38.03	67.86
90 1653-29	8.70	235	11	48	143	437	4.47	74.87
90 1654-28	7.33	208	6	69	77	360	2.80	52.74
92 5012-12*	13.13	135	1	40	105	281	0.74	72.41
92 5013-2	6.54	146	10	98	42	296	6.41	30.00
92 5013-10	6.54	101	4	53	35	193	3.81	39.77
92 5014-16	6.54	125	13	49	59	246	9.42	54.63

Cross 2. *a1-m1 sh2 +/a1 Sh2 En* x *a1-m1 sh2/a1-m1 sh2*

Plant No.	Parent C/O	Segregation sp pl (P)	Cl pl (R)	Cl sh (P)	sp sh (R)	Total	Donor C/O	Trans C/O
90 1645-25	16.13	142	81	178	23	424	11.44	36.32
90 1645-29	16.13	109	93	167	20	389	10.70	46.04
90 1646-21	16.13	134	89	185	23	431	11.06	39.91
90 1646-26*	16.13	17	17	30	1	65	3.23	50.00
90 1646-27	16.13	101	71	144	29	345	16.76	41.28
90 1646-28	16.13	114	86	163	22	385	11.89	43.00

[a]For abbreviations, sp pl = spotted-plump, Cl sh = pale colored-shrunken, Cl pl = pale colored-plump, sp sh = spotted-shrunken, (P)arent and (R)ecombinant types. [b]Parent C/O is the previously estimated, original *En* site. [c]Donor C/O, the site occupied by *En* that will

transpose to trans c/o. e.g., 88 1623-26: donor c/o = 7.69 [=22/(264 + 22)]. [d]Trans C/O. the target site of the transposed *En*. e.g., 88 1623-26: trans c/o = 56.52 [=130/(100 + 130)]. [e]The calculated trans c/o is close to parent c/o. Thus this family, unlike other families, has donor c/o of 32.88 and trans c/o of 5.53, indicating a transposition event toward *a1* regardless of the donor site. *These families are possible indications of two times of transposition when inferred from the difference between parent c/o and donor c/o (see text for details).

Among the progeny segregating all four distinct classes, 23 entries showed an unexpected segregation that indicated extra *En*'s present (Table 3). In the classical segregation with four different classes, the number of progeny one parent class is expected to be equal to that in the other class and this is also expected for recombinant classes. All the families listed in Table 3, however, showed an unexpected segregation; the size of the opposite parental or recombinant segregates are quite different (cross 1: sp pl *vs*. Cl sh, Cl pl *vs*. sp sh, cross 2: sp pl *vs*. Cl sh, Cl pl *vs*. sp sh), and this segregation pattern was consistent among all of them. Recombination can be calculated within one group of one parental class and one recombinant class. If segregation occurred as expected with *En* at a site, two crossover values from plump and shrunken groups, respectively, should be similar or homogeneous. But linkage calculation from these two groups showed two values significantly different from each other. Linkage values from the plump group of Cross 1 (shrunken group of cross 2) were similar to parent values and those from the shrunken group of Cross 1 (plump group of cross 2) were in most instances larger than 50. In the case of 88 1623-26 (cross 1, Table 3), the crossover value of the plump group was 7.69 [=22/(264+22)] and that of the shrunken group 56.52 [=130/(100+130)]. In these instances, we believe that two positions were occupied by transposable elements: one by the donor *En*, the other by the transposed *En* (*trEn*). The original or parental site was designated as "parent c/o" and the site inserted by the donor as "donor c/o". This differentiation was made because parent c/o and donor c/o are not always the same (see plant no. with asterisks in crosses 1 and 2, Table 3). The site occupied by the *trEn* was abbreviated as "trans c/o".

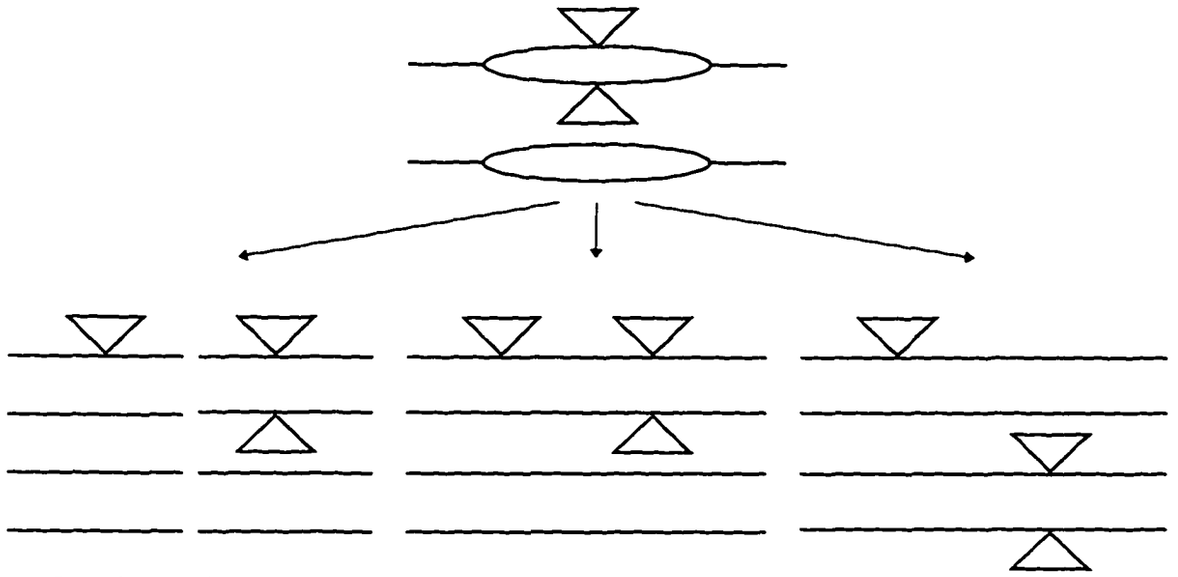
It seems that in most situations the donor c/o was very close to the parent c/o, whereas the trans c/o was significantly different from both the parent c/o and the donor c/o (Table 3). This difference could be explained if one of the *En*'s transposed after replication (Fig. 4). This aberrant segregation with extra *En*'s follows the replicative transposition model of *Ac* (Greenblatt and Brink 1962; Greenblatt 1984; Chen *et al.* 1992). Under this assumption the expected segregation pattern was tested and confirmed by chi-square test (Tables 4 and 5). The segregation ratios can be divided into three cases according to the locations of *trEn*'s (Table 4). Table 4 describes the chi-square test procedure to verify the expected segregation, depending upon each of the cases, as will be indicated in the next paragraphs (cases 1, 2 and 3). The results from the chi-square test confirmed and classified each entry in Table 3 into a specific case, which indicates whether the donor *En* transposed to an independent site (relative to donor c/o) (case 1), a linked site (case 2) or a site on a sister chromatid (case 3). These results are summarized in Table 5.

Case 1

Transposition of *En* to independent target sites relative to donor c/o (e.g. 88 1624-23, cross 1, Table 3). Case 1 results from model A (Fig. 4), in which one of the *En*'s after replication transposes to an unreplicated site, independent of donor c/o. The transposition site can be either on the same chromosome or on any other chromosome (intra/inter-chromosome transposition). The parental classes are (1) sp pl [*a1-m(r) Sh2 En trEn/a1-m1 sh2* + +] and (2) Cl sh [*a1 sh2/a1-m1 sh2*], but here *trEn* will act independently of *a1*. Therefore, half will be segregating with *trEn* (thus sp sh). The donor *En* could affect the number of this type but this would not be significant because the distance between *a1* and *En* is small. The recombinant classes are (3) Cl pl [*a1-m(r) Sh2/a1-m1 sh2*] and (4) sp sh [*a1 sh2 En/a1-m1 sh2* +], plus sp sh [*a1 sh2 trEn/a1-m1 sh2* +] from the second parent, Cl sh.

Case 2

Transposition of *En* to sites linked to donor c/o (e.g. 90 1621-22, cross 1, Table 3) is illustrated with model B (Fig. 4). Transposition occurs to an unreplicated site, linked to donor c/o, on the same chromosome (intra-chromosome transposition). Thus, the proportion of the second parental phenotype (Cl sh) will be calculated by the subtraction of half the number of



A. Inter/Intra-chromosome transposition

B. Intra-chromosome transposition

C. Inter-homologous chromosome transposition

Figure 4. Replicative transposition models of *En* to explain the aberrant segregation due to extra *En*'s. For each case, refer to Table 4.

A. Transposition occurs after replication of *En* to an unreplicated site independent of the donor site, leading to a second replication. Whether the insertion site is on the same chromosome or on another chromosome is not known. This model accounts for case 1 in Table 4.

B. Transposition occurs to a site linked to the donor site on the same chromosome. This model explains case 2 in Table 4.

C. *En* transposes after its replication to an as yet unreplicated target site, likely closely linked to the donor site in repulsion, on the homologous chromosome, resulting in three out of four chromatids possesses with one element. This pattern supports case 3 in Table 4.

Table 4. Methods of genetic and chi-square tests to verify aberrant segregation (d.f. = 3, α = 0.05, value = 7.81). All cases were selected from Cross 1 in Table 3.

Case 1. Transposition of *En* to sites independent of its donor site.

	Plant No. 88 1624-23			
Phenotype[a]	Obs.	Exp.[b]	Exp.[c]	χ^2-value
sp pl (P)	174	168.01	168.01	0.21
Cl sh (P)	82	168.01	84.00[d]	0.05
Cl pl (R)	15	14.49	14.49	0.02
sp sh (R)	94	14.49	98.50[e]	0.20
Total	365	365.00	365.00	0.48

Case 2. Transposition of *En* to sites linked to the donor site.

	Plant No. 90 1621-22			
Phenotype	Obs.	Exp.	Exp.	χ^2-value
sp pl (P)	245	225.48	225.48	1.69
Cl sh (P)	171	225.48	187.21[f]	1.40
Cl pl (R)	6	5.52	5.52	0.04
sp sh (R)	40	5.52	43.79[g]	0.33
Total	462	462.00	462.00	3.46

Case 3. Transposition of *En* to sites on a different sister chromatid.

	Plant No. 88 4659-25			
Phenotype	Obs.	Exp.	Exp.	χ^2-value
sp pl (P)	120	108.39	108.39	1.24
Cl sh (P)	28	108.39	27.10[e]	0.03
Cl pl (R)	4	3.62	3.61	0.04
sp sh (R)	72	3.62	84.90[f]	1.96
Total	224	224.00	224.00	3.28

[a]For abbreviations, refer to Table 3. [b]Expected number under the condition that transposition does not occur, i.e., with *En* only at the donor site. [c]Expected number under the assumption that one of the duplicated *En*'s transposed and replicated again, i.e., with *En* at the donor and *trEn*'s at the target site (Figure 4). **Case 1.** In 88 1624-23, donor c/o is 7.94, trans c/o is 53.41 and distance between donor *En* and *trEn* is 45.47 (= 53.41-7.94) (Tables 3 and 5). Provided that transposition occurred to an independent site, the independent segregation of the tr*En*

turns half the parent Cl sh into sp sh ([d]84 = 168/2), and the half add to the number of the recombinant sp sh ([e]98.49 = 14.49 + 84). **Case 2.** In 90 1621-22, donor c/o is 2.39, trans c/o is 18.96 and distance is 16.57 (Tables 3 and 5). Given that one *En* transposed to a linked site on the same chromosome, the expected number of Cl sh is after the subtraction of half the crossovers between donor c/o and trans c/o [[f]187.21 = 225.48 - 38.27 (= (462 x 0.1657/2)], and the half (sp sh) add to the recombinant sp sh ([g]43.79 = 5.52 + 38.27). **Case 3.** Given that transposition occurred to the homologous chromosome, three-quarters of gametes have carry *En*, allowing a quarter of the parent Cl sh to have no *En*, remaining Cl sh ([h]27.10 = 108.38/4) and three-quarters to have *En*, becoming sp sh, which is the same as the recombinant sp sh ['84.90 = 3.62 + (108.38 - 27.10)].

single crossovers between donor c/o and trans c/o (total number of kernels x distance/2) from the number that is expected under no transposition. Distance between donor c/o and trans c/o is shown in Table 5. The subtracted portion, i.e. sp sh, adds to the second recombinant phenotype, sp sh.

Case 3

Trans c/o values around 70 (e.g. 88 4659-25, cross 1, Table 3). To accommodate these values, transposition to an unreplicated target site must occur on the homologous chromosome, the site linked to donor c/o in repulsion (inter-homologous chromosome transposition). This event can be explained by model C (Figure 4). Under this condition, three of the four chromatids will have a donor *En* or *trEn*. The chance of the second parental phenotype (Cl sh) to get a donor *En* or *trEn* therefore increases up to approximately 75%, which are recovered as sp sh. In case 3, trans c/o cannot be detected. Trans c/o's (Table 3) and distance (Table 5) are regarded as not real.

This assumed replicative transposition model (Fig. 4) seems to be adequate to explain this segregation based on the chi-square test. There was only one deviation to this rule. Line 90 1654-28 has a chi-square value of 12.84, which was highly significant (12.92>11.34, α = 0.01) (Table 5). There is no plausible explanation for this. Also, several families were not

Table 5. Chi-square test of families from Table 3 to classify each into a specific case demonstrated in Table 4 (d.f. = 3, $\alpha = 0.05$, value = 7.81).

Cross 1. *a1-m(r) Sh2 En/a1 sh2 +* x *a1-m1 sh2/a1-m1 sh2*

Plant No.	Parent C/O	Segregation[a]				Total	Case[b]	Distance[c]
		sp pl (P)	Cl pl (R)	Cl sh (P)	sp sh (R)			
88 1623-26	11.46	2.81	0.23	3.06	0.57	6.67	1	48.83
88 1624-23	6.79	0.21	0.02	0.05	0.20	0.48	1	45.47
88 4659-25	2.94	1.24	0.04	0.03	1.96	3.28	3	68.77
89 1803-24	2.48	0.02	0.00	0.01	0.01	0.04	2	43.59
90 1615-21	3.90	0.44	0.05	0.27	0.22	0.97	2	34.50
90 1617-21	11.02	2.20	0.73	1.33	1.61	5.88	2	29.90
90 1617-27	11.02	1.13	0.07	0.71	0.50	2.40	1	46.40
90 1621-22	1.60	1.69	0.04	1.40	0.33	3.46	2	16.57
90 1647-27	33.45	0.00	0.01	0.00	0.00	0.01	2	27.35
90 1648-31	10.40	0.01	0.00	0.01	0.0	0.02	2	36.44
90 1652-29	8.70	1.65	1.01	0.85	1.81	5.31	2	29.83
90 1653-29	8.70	3.31	0.15	0.34	3.27	7.07	3	70.40
90 1654-28	7.33	6.24	0.18	3.04	3.39	12.84**	2	49.94
92 5012-12	13.13	0.14	0.00	0.76	0.00	0.90	3	71.68
92 5013-2	6.54	0.40	0.03	0.30	0.13	0.86	2	23.59
92 5013-10	6.54	0.72	0.03	0.45	0.30	1.50	2	35.96
92 5014-16	6.54	1.66	0.17	0.83	1.00	3.66	2	45.21

Cross 2. *a1-m1 sh2 +/a1 Sh2 En* x *a1-m1 sh2/a1-m1 sh2*

Plant No	Parent C/O	Segregation				Total	Case	Distance
		sp pl (P)	Cl pl (R)	Cl sh (P)	sp sh (R)			
90 1645-25	16.13	0.51	0.07	0.36	0.21	1.14	2	24.88
90 1645-29	16.13	0.26	0.03	0.16	0.13	0.58	2	35.34
90 1646-21	16.13	0.23	0.03	0.16	0.10	0.52	2	28.85
90 1646-26	16.13	0.07	0.00	0.10	0.00	0.18	1	46.77
90 1646-27	16.13	0.00	0.00	0.00	0.00	0.00	2	24.52
90 1646-28	16.13	0.26	0.03	0.17	0.13	0.58	2	31.11

[a]For abbreviations, refer to Table 3. [b]See Table 1. [c]Distance = donor c/o - trans c/o. **Highly significant

significant at the 5% level, but had rather high chi-square values. This might be due, in part, to the crossover between *a1* and donor *En*, which was not considered in the calculation because of the small distance.

In four families (90 1617-21, 90 1652-29, 92 5012-12 and 90 1646-26: see plant no. with asterisks in crosses 1 and 2, Table 3) it is possible that these *En*'s transposed two times as inferred from the difference between parent and donor c/o's. Whereas parent and donor sites of *En*'s in these four families seem to be heterogeneous to each other (11.02 *vs.* 25.00, 8.70 *vs.* 38.03, 13.13 *vs.* 0.74 and 16.13 *vs.* 3.23, respectively), genetic donor sites in other families were quite close to the parental sites. These data certainly indicate that *En* sometimes moves a second time in one generation, likely conservative transposition in the first, i.e. before replication and replicative transposition in the second.

Discussion

It has been shown that a transposed *En* from *a1-m(Au)*, named En^{vag}, has been followed for several generations and found to transpose at a high rate on chromosome 3. Coincident with the origin of En^{vag}, a new reporter allele *a1-m(r)3927-1* arose (Figure 1), with a low-spotting phenotype (1a-b) (Figure 2D). En^{vag} expresses strong activity with other reporter alleles, *a1-m1* and *a1-m(r)* (Figures 2B and C), showing full mutator function of *En/Spm*.

Isolation of the state of *a1-m(r)3927-1*

With the isolation of the phenotype/state of *a1-m(r)3927-1* allele, differentiation among the three *En*-reporter alleles is definable by somatic excision rates: *a1-m(r)* excises at a high frequency, *a1-m1* at a medium frequency, and *a1-m(r)3927-1* at an extremely low rate (Fig. 2).

The influence of transposable elements inserted into the *a1* gene has been well-documented by molecular studies (Schwarz-Sommer *et al.* 1987). The insertion site of the defective element *I* in *a1-m(r)* and *En* in *a1-m(Au)* is in the same orientation 20 bp from the

5' side of exon 2 (Menssen *et al.*, in preparation). Two states of the *a1-m1* alleles, *I6078* and its deletion derivative *I5719A-1*, are at the 3' end of exon 2. Part of one side of the terminal inverted repeats is deleted in *I5719A-1* (Schwarz-Sommer *et al.* 1987; Tacke *et al.* 1986). Such differences are also possible for the different patterns of *a1-m(r)* and *a1-m(r)3927-1*. Only by isolating and sequencing the *a1-m(r)3927-1* allele will this pattern difference be resolved.

Transposition profiles of En^{vag}

Overall, this *En* transposed among approximately 45% of the progeny through three generations, with half of the transpositions being to target sites independent of *a1* (Table 2). Insertion sites linked to *a1* were distributed around 68% of the time within a 16 map unit region flanking *a1*, indicating a preference for short-distance transposition. Even if recombination values of sister lines were homogeneous based on the chi-square test, they look quite variable from one another, probably because the test can not detect very short movements along the chromosome. This suggests that the actual transposition frequency could be higher than that detected by the chi-square test.

The three-point mapping transposition study of *En* by Nowick and Peterson (1981) illustrated that proximal transposition events were predominant and that distal transposition events were all within 24 map units from *a1*, which may imply the maximum distal map unit location of a target site of *En* on 3L. The direction on chromosome 3 with respect to *a1* could not be determined in this two-point mapping study. However, close examination of linked replicative transposition events (Tables 3 and 5) may reveal that transpositions occurred in both directions relative to *a1*, taking advantage of three markers of *a1*, donor c/o and trans c/o plus a probable maximum distal unit of 24 from *a1*. Larger trans c/o's than donor c/o's indicate transposition events away from *a1*, and smaller trans c/o's are transposition events toward *a1*. Except for one family (90 1647-27), all trans c/o's were larger than donor c/o (Table 3), likely indicating that the 90 1647-27 family is the latter case and the remaining, the former. Further, distances between donor c/o and trans c/o larger than 24 map units in these latter families imply that these were likely proximal transposition events. It can be inferred

that replicative transposition may preferentially occur in one direction depending upon the insertion site relative to a replicon initiation site.

Replicative nature of *En*

Somatic genetic studies by Dash and Peterson (1994) showed that *En* transposes after replication, as in the case of *Ac* in the *P-vv* allele (Greenblatt and Brink 1962; Greenblatt 1984; Chen *et al.* 1992). Families of exceptional segregation that were selected in this study support these observations. Extra *En*'s in the progeny relate to about 5% of the experimental lines with four distinct types (data not shown). A characteristic of these families is that the number of kernels of one parental type is always, and in most cases significantly, larger than that of the other (e.g., cross 1 in Table 3: sp pl *vs.* Cl sh). This is because half the latter contained *trEn* and thus became part of one recombinant class (sp sh). The recombination value calculated from one group (shrunken) is therefore always much larger than that from the other group (plump). Provided that one of two *En*'s moves from an already replicated site to an as yet unreplicated site, either independent of or linked to the donor site on the same chromosome, leading to a second replication, both chromatids should have one or two *En*'s. Transpositions to independent sites on the same chromosome are not distinguished form those on other chromosomes. The genetic data reported here support both intra/inter-chromosome transposition (Fig. 4A) and intra-chromosome transposition (Fig. 4B).

There are three lines of case 3 (88 4659-25, 90 1653-29 and 92 5012-12) that show one difference from other lines of cases 1 and 2 (Tables 3 and 5): the number of pale colored-shrunken (Cl sh) in case 3 is approximately one quarter of that expected. This can be explained by the three-quarters of the Cl sh being recovered as spotted-shrunken (sp sh). This phenomenon can result if inter-homologous chromosome transposition occurs (inter-homologous chromosome transposition, Fig. 4C), giving rise to three of the four chromatids possessing an *En*. The transposition site in case 3 is expected to be linked close to the donor c/o in repulsion, otherwise the transposition is to an independent site and not identifiable from case 1.

Transposition along the chromosome

Genetic and molecular proofs have focused on transposition mechanisms. These include (1) 'cut-and-paste' by Saedler and Nevers (1985) and (2) synapsis of homologous sequences (Robbins *et al.* 1989; Dooner *et al.* 1994). In the Dooner *et al.* (1994) studies unlinked receptor sites of transposed *Ac* from the *bz-m2(Ac)* were mapped, and the results suggested to the authors that a physical chromosome association between donor and receptor sites during transposition accounts for non-random distribution of target sites. These sites were associated with a spatial ordering of the chromosomes in the interphase nucleus, which was also consistent with the aberrant chromosome rearrangement studied for *Tam3* from *Antirrhinum majus* (Robbins *et al.* 1989). En^{vag} was followed for several generations and was found to move frequently. In some of the progeny, En^{vag} remains on chromosome 3 and is assumed to transpose indefinitely on this chromosome. Instead of a free complex, a physical link of donor and target sites during transposition supports our study.

The molecular studies are also supportive of the physical link model. Because the TNPAs (transposase A - one of *En/Spm* proteins) bound to each of the subterminal motif sequences are in contact with each other through a dimerization domain (Frey *et al.* 1990; Trentmann *et al.* 1993), it is possible that a physical association between donor and target sites during transposition can be mediated by this TNPA dimerization domain (Masson *et al.* 1991). The predominance of the short-genetic-distance transposition may support the assumption that the interaction between two TNPAs, one bound to a TNPA binding site (Grant *et al.* 1993) and the other to a sequence having some homology to the TNPA binding site, occurs in most situations before the cutting by TNPD (transposase D - another *En/Spm* protein). Quasi-homologous TNPA binding sequences (Masson *et al.* 1991) may be distributed over the genome, but TNPA is assumed to be mostly localized in the adjacent area of the original *En* insertion site. It could also happen, although not often (Masson *et al.* 1991), that if TNPD cutting takes place before the interaction of the TNPAs between the donor and recipient sites, a transposition intermediate can be free to move and can reinsert.

Speculation on the origin of *a1-m(r)3927-1* and En^{vag}

The simultaneous isolation of both a reporter allele and an autonomous allele in a single event is not common. McClintock (1955) reported, without providing further explanation or data, that *Ac* excised from the *bz1* locus creates and thus leaves a *Ds* at *bz1*, even though an *Ac* was still present. This newly generated *Ds* led to a marked increase in the frequency of occurrence of somatic mutation at the *bz1* locus, which is in contrast to the low somatic mutability of *a1-m(r)3927-1*. This similar phenomenon of increased transposition frequency of nonautonomous elements was observed with a *bronze*-mutable allele, *bz-m13* (*dSpm* at *bz1*), where the germinal transposition frequency of *dSpm* was high (50-83%) (Nelson and Klein 1984; Raboy *et al.* 1989). This increase could be attributed to the altered structure of the nonautonomous elements relative to respective autonomous elements, with smaller elements typically moving faster than larger elements. However, this phenomenon does not seem to be common, and all the independent nonautonomous derivatives of the *bz-m13* allele resulted in a drastically reduced transposition frequency both somatically (Schiefelbein *et al.* 1985) and germinally (Raboy *et al.* 1989). To our knowledge, such a high transposition rate of an autonomous *En/Spm* has not been reported.

A critical mechanism may account for the coincident discovery in this study of a residue left at the locus [origin of *a1-m(r)3927-1*] and an autonomous transposon (En^{vag}). For excision to occur, elements should contain both the 13-bp terminal inverted repeats (TIRs) and at least a minimum region of 12-bp subterminal motif sequences, to which TNPD and TNPA bind, respectively (Frey *et al.* 1990). This coincident occurrence of the two elements can be explained by two hypotheses. First, in the "independent origin model" the origin of a new nonautonomous element (*I/dSpm*) from an autonomous element (*En/Spm*) can be accounted for by at least three mechanisms: 1) incomplete gap repair at the donor site following transposition, 2) transposition of the *En* on one chromosome or chromatid at the same time as an internal deletion of the *En* on the same chromosome or chromatid occurs and 3) internal deletion unrelated to transposition. The first mechanism requires the parent to be homozygous. Otherwise, the 3' fragment of the new element will be absent, which is necessary for transposition. The parent of the *a1-m(r)3927-1* is heterozygous (Cross 1 in

Figure 1), and this can be excluded. The occurrence of the second mechanism in the heterozygous parent, in this study *a1-m(Au)/a1* (Cross 1 in Fig. 1), will produce no kernels of the *a1-m(Au)* allele phenotype (Fig. 2A). The observed phenotypes of *a1-m(Au)* in the progeny of Cross 1 therefore eliminate the second mechanism. The third is a possible mechanism under the condition that there was another autonomous element present nearby *a1* but not detected. At present, however, this cannot be confirmed. The insufficient explanation for the simultaneous discovery of the *a1-m(r)3927-1* and En^{vag} led us to adopt a second hypothesis for the heterozygous parent from the gap repair model for the homozygous parent by Engels *et al.* (1990).

The 'double-strand gap repair' model to explain the coincident origin of the two elements was suggested by Engels *et al.* (1990) to account for the frequent occurrence of internal deletion derivatives of the *P* element in *Drosophila melanogaster*. This model requires the homozygosity of two copies for the fill-in process of both strands to occur at the same time. However, Cross 1 in Figure 1 shows that *En* was present in the hemizygous state. Given that one *En* excised after replication and became a new element, En^{vag}, the remaining one copy should be used as a template. But this time, the gap repair synthesis occurred in one strand only. In this instance, if the repair process is interrupted, the non-synthesized segment is lost, leading to the loss of all of the 3' *cis*-determinants. However, *cis*-determinants can bring together and align themselves in association through TNPA binding. If this happened during the time of the gap repair process, which in turn was interrupted possibly by TNPA bound to a 12-bp motif, the fill-in process could jump to the opposite strand and proceed to synthesize the 3' *cis*-determinants. We would name this mechanism the 'single-strand gap repair' model. We assume that this gap repair model in the heterozygous parent is a very rare event. One should realize that this model is limited to the explanation for the simultaneous origin of *a1-m(r)3927-1* and En^{vag} and not extended to models for replicative transposition events listed in Tables 3 and 5. We expect this element to contain sufficient *cis*-determinants to produce the low-mutating pattern of *a1-m(r)3927-1* allele (1a - late and infrequent

spotting). This can be and will be resolved from molecular cloning of this allele. What makes the transposition of En^{vag} more frequent than its progenitor still remains a question.

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CHAPTER 5. TRANSPOSITION OF THE *En/Spm* TRANSPOSABLE ELEMENT SYSTEM IN MAIZE (*Zea mays* L.): RECIPROCAL CROSSES OF *a1-m(Au)* AND *a1-m(r)* ALLELES UNCOVER DEVELOPMENTAL PATTERNS

A paper submitted to Genetics[1]

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Abstract

Transposition studies of *En/Spm* have dealt with some aspects of the timing of excision with regard to DNA replication and during plant development, but without describing details of the process. By analyzing ears from reciprocal crosses between an autonomous *En* allele, *a1-m(Au)*, and a nonautonomous *En* allele, *a1-m(r)*, we hereby describe several features on the *En* of *a1-m(Au)* that take place during ear development and microsporogenesis. First, *En* undergoes epigenetic change in activity during kernel development. Second, the distribution of kernel phenotypes on an ear illustrates that *En* transposes late in most of the events during ear development. Third, the phase change of *En* (presence and absence of activity) is observed during cob development. Fourth, discordant kernel phenotypes of two resulting ears from a reciprocal cross with parental phenotype can result from the transposition of *En* during microsporogenesis and subsequent fertilization. The phase change and discordance are analyzed to result in part from transposition after host DNA replication. It can be concluded that the activity of *En* of *a1-m(Au)* is not limited to a specific stage or timing during plant development.

Introduction

Transposition of a mobile genetic element through the cut-and-paste process, by which an element excises from a donor site and reinserts into another site (Saedler and

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Nevers 1985), appears to be applicable for most plant DNA transposable elements with a probable exception of the *Mutator* (*Mu*) element (Alleman and Freeling 1986; Lisch *et al.* 1995). However, it is not clear whether the timing of transposition is compatible among such elements during plant development and with regard to host DNA replication.

During cob development, the timing and mechanism of transposition of *Activator* (*Ac*) has been well understood with the advantage of two features: (1) the *P-vv* allele controlling the phenotype of the cob and pericarp of maternal tissue and (2) the negative *Ac* dosage effect (Brink and Nilan 1952; van Schaik and Brink 1959; Greenblatt and Brink 1962; Greenblatt 1984; Chen *et al.* 1987, 1992). The pericarp color is a reflection of cob color in the same cell lineage, thereby the somatic transposition event in a cell during cob development is manifested as the phenotype of the pericarp of the same cell origin. Twin sectors (red and light variegated mutations on medium variegated ears) on the ear of the *P-vv* plant are observed and are clearly differentiated when *Ac* undergoes somatic transposition during cob development. That differentiation is clear due to the negative *Ac* dosage effect in which no *Ac*, one *Ac* and two *Ac*s produce red, medium, and light variegated pericarps, respectively. This genetic observation has been explained by the transposition after replication (Greenblatt 1984) and molecularly proven (Chen *et al.* 1987, 1992).

Based on the observation that *Mu* elements do not leave their previous sites, Alleman and Freeling (1986) discussed that *Mu* elements might transpose by a mechanism similar to a replicative transposition model proposed by Shapiro (1979) and proven for some transposable elements in bacteria. Subsequently, Lisch *et al.* (1995) proposed a duplicative transposition model likewise based on no excision from previous sites by using a Minimal *Mutator* Line loaded with relatively few *Mu* elements and applying simple Mendelian principles. Both duplications and deletions of *MuDR* have been explained by a gap-repair model (Lisch *et al.* 1995; Hsia and Schnable 1996), which was originally proposed for the recovery of the *Drosophila P* element at the donor site following excision by a cut-and-paste mechanism (Engels *et al.* 1990). In reference to the replicative transposition of *Mu*, one of the *Mu* transposases, MURA, shares an amino acid sequence motif with the putative transposases of a group of bacterial insertion sequences (Eisen *et al.* 1994).

A demonstrated parallel on the transposition mechanism of the *Enhancer/Suppressor-mutator* (*En/Spm*) element with that of *Ac* includes twinned sectors (Fedoroff 1983) that reveal changes in *Spm* dosage on kernels carrying the *a2-m1* allele and *Spm-c* element (McClintock 1971). Whether *En* transposition is associated with DNA replication could not be determined from transposition studies of *En* and applied statistical methods (Peterson 1970; Nowick and Peterson 1981).

The first genetic case that *En* transposition is also associated with chromosome replication was the observation of somatic post-excision loss of *En* (Dash and Peterson 1994). That study utilized the continuity of cell lineage in the endosperm (*wx* to *Wx* assay) and aleurone (*a1* to *A1* assay) of the kernel and the characteristics of several *En/Spm* alleles. The continuity of the cell lineage between the endosperm and aleurone enabled Dash and Peterson (1994) to observe simultaneous transposition events of *a1-* and *wx-mutable* alleles in both endosperm and aleurone tissues. The autonomous *En* allele *wx-844* and unique features of *En* reporter alleles [*a1-m(r)* expressing response to only the M (mutator) function, *a2-m1*(II) expressing response only to the S (suppressor) function and revealing *En* dosage changes, and *a1-m1 5719* to the M and S functions] allowed these authors to monitor the excision of *En* in the endosperm tissue and post-excision loss in the aleurone tissue. Such features made it possible to observe several phenotypes, which are accountable for by different *En* excision processes: "loss and gain type" twin sectors of colored aleurone (no *En*) and fine aleurone (two *En*s) or "no loss and gain type" twin sectors of *Wx* endosperm with coarse (early excision events) aleurone and *wx*-mutable endosperm with fine (late excision events) aleurone. Dash and Peterson (1994) proposed that the transposition of *En* **after replication** explains all of these observations, and post-excision loss and the twin sectors of loss and gain type are the consequences of the transposition into a site of an already-replicated sister chromatid.

Further genetic support that *En* can also transpose after replication was described by Seo and Peterson (1996). In classical Mendelian segregation, the number of one parent class is expected to be equal or similar to that of the other class as well as for the two recombinant classes, thereby the calculated linkage values from each group are expected to be equal or

similar. However, Seo and Peterson (1996) noticed ears with kernels of four distinct phenotype classes segregating (two groups of plump and shrunken kernels, each group with parent and recombinant classes), and that they showed an unexpected segregation ratio, producing different segregation ratios and thus different linkage values between two classes. This aberrant segregation was statistically explained as the consequence of *En* transposition after replication and subsequent recombination between *En*s.

This study consists of the genetic analyses of the reciprocal crosses between *a1-m(Au)*, the autonomous *En*-containing allele, and *a1-m(r)*, the non-suppressible defective *En* allele. Several features were noticed in the reciprocal crosses. First, epigenetic change in activity of *En* during kernel development. Second, the distribution of kernel phenotypes on an ear illustrates that *En* transposes late in most of the events during ear development. Third, the phase change of *En* (presence and absence of activity) is observed early during cob development. Fourth, several pairs of two reciprocal ears showed a discordant phenotype, in that both ears from a reciprocal cross had same kernel phenotypes but no parental phenotype. Such discordant phenotypes were analyzed to in part come from transposition of *En* during microsporogenesis and subsequent fertilization that can result in the disconcordant genotype between endosperm and embryo. The phase change and discordance are analyzed to result in part from transposition after host DNA replication. These observations suggest that *En* also occasionally transposes after replication.

Materials and Methods

Genetic stocks, gene symbols and phenotypes

The *a1* gene codes for flavonoid:4-reductase catalyzing the carbonyl reduction of dihydroflavonols at the 4-position (Reddy *et al.* 1987) and is involved in the anthocyanin biosynthesis throughout the plant. The *a1-m(Au)* allele carries the full-size *En* insert in the second exon of the *a1* gene (Menssen 1988). This *En* self-transposes and produces almost fully colored kernels with colorless sectors [***a1-m(Au)* type** kernel: Figure 1A] (Nowick and Peterson 1981). The *En/Spm* reporter allele *a1-m(r)* is a derivative of *a1-m(Au)* and was isolated in *pale green mutable* stocks (Peterson 1956). This *En*-deletion derivative *I* element

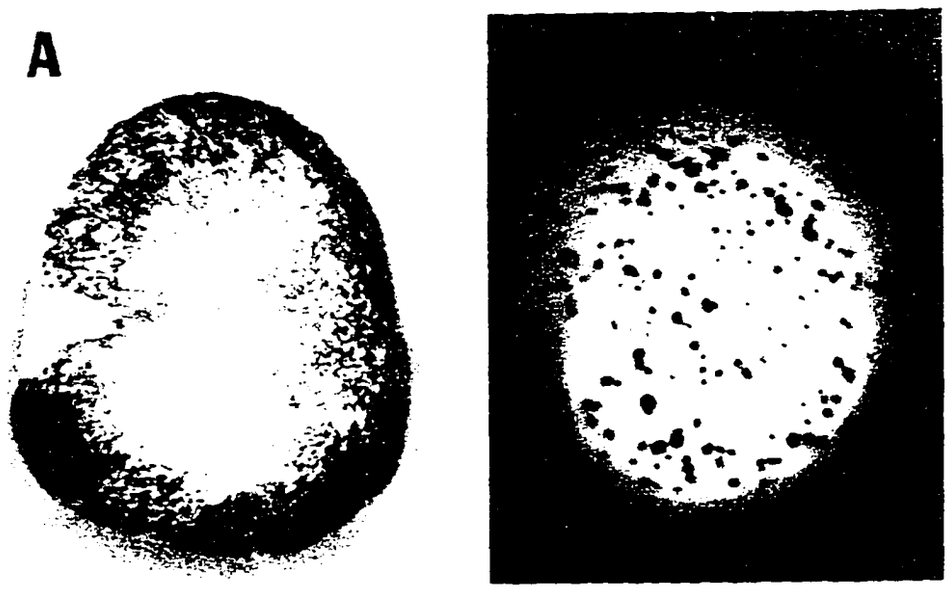


Figure 1. A. An *a1-m(Au)* type kernel (an almost colored kernel with colorless sectors) from the cross *a1-m(Au) Sh2/a1 sh2* x *a1 sh2/a1 sh2*. *a1-m(Au)* is an autonomous *En* allele at *a1*.

B. An *a1-m(r)* type kernel (a highly variegated kernel) from the cross *a1-m(r) Sh2/a1 sh2, En* x *a1 sh2/a1 sh2*. *a1-m(r)* is a deletion derivative of *En* and responds to the trans-signal of *En*.

(Cuypers *et al.* 1988) cannot excise by itself, thereby gives rise to colorless kernels but can be excised in the presence of *En/Spm* transposases provided in *trans*, giving rise to variegated kernels [***a1-m(r)* type** kernel: Figure 1B]. Unexpected **off-type** kernel refers to any type of variegation that belongs neither to *a1-m(Au)* type kernel nor to *a1-m(r)* type kernel.

Genetic crosses

Cross 1 is the testcross in which each *a1-m(Au)* line was crossed as a male on an *a1-m(r)* plant. From this cross, some unexpected, off-type kernels arose. These off-type kernels were selected from the progenies of Cross 1 for further tests in Crosses 2A and B, where they

were crossed reciprocally by and on a randomly chosen *a1-m(r)* plant. In 3A and B reciprocal crosses, the standard *a1-m(Au)* type kernels screened via Cross 1 were crossed reciprocally with *a1-m(r)* to assay the transposition of *En*.

Cross 1: *a1-m(r) Sh2/a1-m(r) Sh2* x *a1-m(Au) Sh2/a1 sh2*

[**Off-type** kernels and standard ***a1-m(Au)* type** kernels are advanced to Crosses 2A and B with *a1-m(r) Sh2* and to Crosses 3A and B with *a1-m(r) Sh2*, respectively.]

Cross 2A: **Off-type** [*a1-m(Au) Sh2/a1-m(r) Sh2*] x *a1-m(r) Sh2/a1-m(r) Sh2*

Cross 2B: *a1-m(r) Sh2/a1-m(r) Sh2* x **Off-type** [*a1-m(Au) Sh2/a1-m(r) Sh2*]

Cross 3A: ***a1-m(Au)* type** [*a1-m(Au) Sh2/a1-m(r) Sh2*] x *a1-m(r) Sh2/a1-m(r) Sh2*

Cross 3B: *a1-m(r) Sh2/a1-m(r) Sh2* x ***a1-m(Au)* type** [*a1-m(Au) Sh2/a1-m(r) Sh2*]

Hereafter, the *sh2* gene symbol is only specified whenever necessary.

Goodness-of-fit χ^2 test

This χ^2 method was utilized for the two sets of data obtained from a reciprocal cross (Crosses 3A and B) to test whether the segregation ratio in one set (of progeny of 3A) fits into the ratio in the other (of progeny of 3B), specifically to compare the transposition rates when *En* is transmitted as female and male. The formula is $\chi^2 = \sum[(O - E)^2/E]$ (O = observed value, E = expected value) (Snedecor and Cochran 1989). The observed values were converted or standardized in percentage so that they are on the same magnitude for comparison. The summed χ^2 value is compared with the tabular value with the same degree of freedom. Table 1 describes the procedures of goodness-of-fit χ^2 test in comparing the transposition rates with two standardized sets of data in a reciprocal cross. Only when both calculated values showed significance were transposition rates of an *En* considered different in a reciprocal cross.

Detection of the transposition mechanism hypothesis

Based on the mutable pericarp studies (Greenblatt 1984) of transposition of *Ac* after replication at the *p* locus and the associated dosage effect of *Ac*, it is hypothesized that a

Table 1. Goodness-of-fit χ^2 test: comparison of transposition rates of *En* transmitted through female (F) and male (M) in reciprocal crosses

	No. (%) of			Goodness-of-fit χ^2 test[a]		
Entry No.	*a1-m(Au)* type	*a1-m(r)* type	Colorless	F = E[b]	M = E[c]	
A-1 (F)	173 (42.30)	34 (8.31)	202 (49.39)	19.46		**
(M)	108 (35.06)	63 (20.45)	137 (44.48)		9.24	
A-4 (F)	281 (56.09)	6 (1.20)	214 (42.71)	23.69		ns[d]
(M)	161 (49.24)	21 (6.42)	145 (44.34)		5.26	
A-7 (F)	197 (52.67)	6 (1.60)	171 (45.72)	10.41		*
(M)	131 (40.43)	15 (4.63)	178 (54.94)		7.23	

[a] $\chi^2 = \Sigma[(O - E)^2/E]$ (O = observed value, E = expected value). Tabular values with 2 d.f. are 5.99 at $\alpha = 0.05$, 9.21 at $\alpha = 0.01$. [b] When standardized values from crosses in which *En* was transmitted through female and male are used as expected and observed values, respectively. [c] When standardized values from crosses in which *En* was transmitted through male and female are used as expected and observed values, respectively. [d] not significant because 5.26 is not significant although 23.69 is. * significant at $\alpha = 0.05$, ** highly significant at $\alpha = 0.01$, [ns] Not significant.

parallel to the *P-vv* twin sector on the female ear can be established from Cross 3A in which *a1-m(Au)* is used as female. The parallel in this study is deduced from the distribution of kernel phenotypes on an ear. The *a1-m(Au)* allele produces almost fully colored kernels (Figure 1A). The *a1-m(r)* allele gives rise to colorless kernels without *En* but variegated kernels with *En* (Figure 1B). The working hypothesis is as follows: 1) with *no transposition*, the ear contains only *a1-m(Au)* type and colorless kernels (no sector), 2) *transposition before replication* at a certain cell during the cob development results in an ear with a sector of *a1-m(r)* type and colorless kernels only within the same cell lineage, and 3) *transposition after replication* would result in an ear with a twin sector, in which one part of the twin has the three phenotypes *a1-m(Au)* type, *a1-m(r)* type, and colorless kernels and the other part has the two phenotypes *a1-m(r)* type and colorless kernels. However, because the boundary of

the twin sector is not as clear as the twin sectors of the *Ac* dosage effect, the identified twin sector should be large enough not to escape one's imagination.

Results

Initially, to examine the transposition of *En* from the *a1-m(Au)* allele, 150 plants from *a1-m(Au)* type kernels with the *a1-m(Au)/a1* genotype as a sample population were crossed on *a1-m(r)* (Cross 1). There were 140 crosses with more than 100 kernels used in the analysis. Many of them segregated kernels of expected and unexpected off-type variegation patterns. The following describes the analyses of those unexpected off-type kernels and *En* transposition profiles drawn from the progenies of the five selected out of 140 crosses.

Off-type kernels come from phase changes of *En* at *a1*

Among the Cross 1 progenies, kernel color variegations that ranged from full-colored to almost colorless with a few, very late mutations were observed. Each of these off-types were selected and tested to see the heritability of their variegation patterns in reciprocal crosses with *a1-m(r)* (Crosses 2A and B). The selected full-colored kernels transmitted mostly the same full-colored ones but also produced the typical *a1-m(Au)* type kernels, indicating that these full-colored types represent the heavy mutability of the typical *a1-m(Au)* expression. Most of the low-variegated phenotypes fully recovered the *En* activity and produced the *a1-m(Au)* type kernels, and only a few transmitted a lower *En* activity, segregating a smaller proportion of the *a1-m(Au)* types and more of off-type and colorless kernels.

Estimation of transposition frequency

The transposition of *En* at *a1-m(Au)* is expected to be detected by observing the *a1-m(r)* type kernels (Figure 1B) from Crosses 3A and B. Surprisingly, out of the 140 crosses rescued from the sample population (Cross 1), 133 crosses had one or more *a1-m(r)* type kernels whose frequency on an ear ranges from 0.32% up to 51.23% (data not shown). With this high frequency (133/140), five out of 140 crosses were selected for further test

(designated as groups A to E): two (C and D) without *a1-m(r)* type kernels (the absence of *a1-m(r)* type kernels was assumed to be evidence of no previous transposition and/or the absence of extra *En*s) and three (A, B and E) with some *a1-m(r)* type kernels (Table 2). Fifty standard *a1-m(Au)* type kernels from each of the five groups were randomly selected, and reciprocal crosses were made with *a1-m(r)* (Crosses 3A and B).

Table 2. Selected groups and their progenies.

Group	Plant No.	*a1-m(Au)* type	*a1-m(r)* type	Colorless	No. Progeny[a]
A	95 1847-3	108	1	72	32, 0, 15
B	95 1847-6	104	4	122	42, 0, 3
C	95 1848-7	103	0	98	43, 1, 1
D	95 1848-12	79	0	70	44, 0, 2
E	95 1852-6	185	7	145	42, 3, 2

[a] Number of progeny ears rescued from a selection of fifty *a1-m(Au)* type kernels in each group. The order is the number of reciprocal crosses in which both ears were harvested, one ear was rescued only when *En* was crossed as female, and one ear was rescued only when *En* crossed as male, respectively.

Following the detection of the transposition mechanism (see Materials and Methods), examination of the female ear morphology of kernel phenotypes revealed that *a1-m(r)* type kernels are scattered all over the ear. No clear sectors as a probable indication of somatic transpositions before or after replication during cob development were not readily detected. Thus, it is hypothesized that the random distribution of *a1-m(r)* type kernels, where *En* was transmitted as a female, suggests that *En* transpositions are, if not all, independent events. Transposition events can, therefore, be interpreted to occur late during ear development, likely in a mitotic cell to make a single megasporocyte through meiosis prior to fertilization. The assumption of independent events agrees with the previous observations, based on which *En* transposition studies have been made (Peterson 1970; Nowick and Peterson 1981; Seo and Peterson, 1996).

With independent transpositions, the transposition frequency should be measured by calculating the proportion of *a1-m(r)* type kernels out of the number of expected *a1-m(Au)* type kernels, which is the sum of observed *a1-m(Au)* type and *a1-m(r)* type kernels because the latter is expected to result from transpositions of *En* of *a1-m(Au)*. Thereby, transposition rates were estimated with the combined data from Crosses 3A and B for each group (Table 3). The estimated frequencies range approximately from 6% to 17% on average. Groups A and E showed higher transposition rates than other groups (B, C and D), but no correlation is observed with the presence of *a1-m(r)* type kernels in the selected groups (A, B and E). All the groups have lower transposition rates than that of the sample, whose rate was estimated with the data from Cross 1, in which *a1-m(Au)* was used as male only. The higher transposition rate of the sample could be due to several factors: a higher transposition of *En* when transmitted as male, an overall rate of different crosses, the presence of extra *En*s in some sample individuals, or very likely a combination of these factors.

Table 3. Overall transposition frequencies of *En*.

	Sample	Group A	Group B	Group C	Group D	Group E
a1-m(Au) type	15.431	10.284	13.102	12.140	10.593	14.250
a1-m(r) type	4.450	2.151	847	1.102	808	1.972
Total	19.881	12.435	13.949	13.242	11.401	16.222
Trans. Freq. (%)[a]	22.38	17.30	6.07	8.32	7.09	12.16

[a] Transposition frequency = (*a1-m(r)* type/Total) x 100

Reciprocal difference in the transposition frequency of *En*

With the observed difference in transposition frequencies between the sample and the selected groups (Table 3), transposition rates of *En* were compared when transmitted as female and male within each group (Table 4). The frequencies range from approximately 4.5% to 16% with *En* as female and from 7.5% to 18% with *En* as male. The comparison of transposition frequencies indicates that the probability of overall transposition events is consistently higher as male than as female. More than 60% of the ears from Cross 3A (*En* as

Table 4. Comparison of transposition frequencies of *En* when transmitted through female (F) versus male (M).

	Group A		Group B		Group C		Group D		Group E	
	F	M	F	M	F	M	F	M	F	M
a1-m(Au) type	4.874	5.410	6.838	6.264	6.314	5.826	5.287	5.306	7.917	6.333
a1-m(r) type	943	1.208	333	514	317	785	261	547	882	1.090
Total	5.817	6.618	7.171	6.778	6.631	6.611	5.548	5.853	8.799	7.423
Trans. Freq. (%)[a]	16.21	18.25	4.64	7.58	4.78	11.87	4.70	9.35	10.02	14.68

[a] Transposition frequency = (*a1-m(r)* type/Total) x 100

female) showed none or less than 5%, while most of ears from Cross 3B (*En* as male) were distributed over up to 25% (Figure 2).

Each reciprocal cross (of two ears from Cross 3A and Cross 3B, respectively) was examined to detect the difference in the transposition frequency by the goodness-of-fit χ^2 test as described in Table 1. Out of the total 203 reciprocal crosses, 24 crosses revealed the highly significant difference at $\alpha = 0.01$ (3 in A, 5 in B, 8 in C, 6 in D, and 2 in E), and, additionally, 22 more crosses showed the significant difference at $\alpha = 0.05$ (5 in A, 3 in B, 5 in C, 2 in D, and 7 in E), resulting in the total 46 crosses. Out of these 46 crosses in this test, only seven families showed higher mutation rates through females than males.

Predetermination of cells destined to ear and tassel

It has been reported that different cell lineages destined to ear (2-4 cells) and tassel (2-4 cells) are formed in the dormant embryo (Regiroli and Gavazzi 1976; Coe and Neuffer 1978; Johri and Coe 1983). The ear illustrated in Figure 3A from the cross *a1-m(Au)/a1-m(r)* x *a1/a1-m(r)* is equally divided into two halves; one half of *a1-m(Au)* type and colorless kernels and the other of only colorless kernels. This whole ear with the twin sectors indicates that there are two initial cells destined in the embryo for the development of the ear. This change in *En* activity in one of the two cells results in the ear illustrated in Figure 3A. With the *a1-m(r)*, an *En* reporter allele involved in the cross, the colorless half is likely due to the

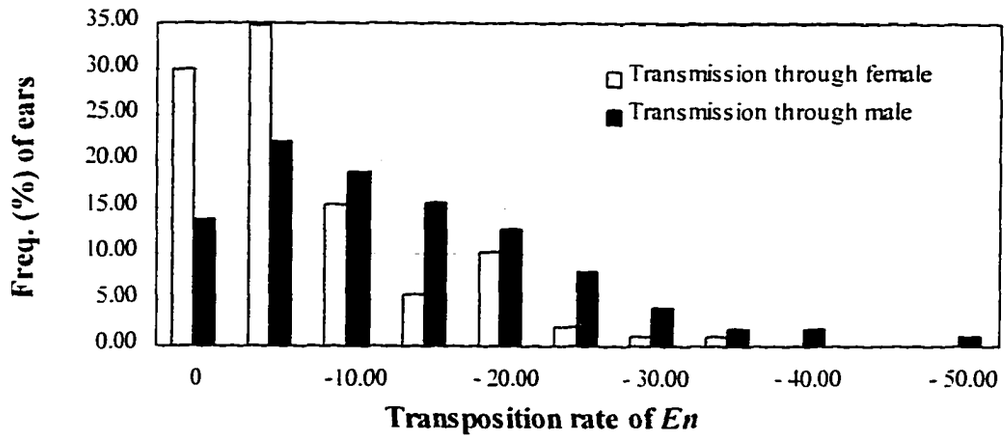


Figure 2. Comparison of transposition rates of *En* when transmitted through female and male. More than 60% of the ears rescued when *En* was transmitted through female showed a transposition rate of none or less than 5%, while most of the ears obtained when *En* was crossed as male showed a range of transposition rate from none up to 25%.

loss or inactivation of *En* after transposition before replication if not during embryogenesis (Figure 3B).

The ear of Figure 3C from Cross 3A is divided into two unequal sections: one quarter has *a1-m(Au)* type, *a1-m(r)* type, and colorless kernels, and the remaining three-quarters includes *a1-m(r)* type and colorless kernels. The reciprocal ear of the Figure 3C from Cross 3B segregates a total of 244 kernels in the following ratio: 44 (18.03%) *a1-m(Au)* type, 54 (22.13%) *a1-m(r)* type, and 146 (59.84%) colorless kernels. Following the same rationale as the explanation provided for the ear of Figure 3A, this ear [from the original *a1-m(Au)/a1-m(r)* genotype] can result from two transposition events in the early stage of ear development: **one** transposition (event 1) occurred of *En* from *a1-m(Au)* in one of the two initial cells and produced one half of the ear with *a1-m(r)* type and colorless kernels only in half of the ear, and **the other** transposition (event 2) occurred of *En* from *a1-m(Au)* in the

Figure 3. Cross: *a1-m(Au)/a1-m(r)* x *a1-m(r)/a1-m(r).*

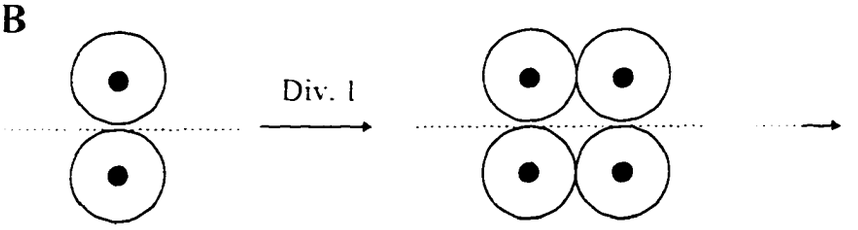
A. Ear with twin halves, one half of the *a1-m(Au)* allele and the other resulting from excision and/or the loss of activity of *En* of this allele.

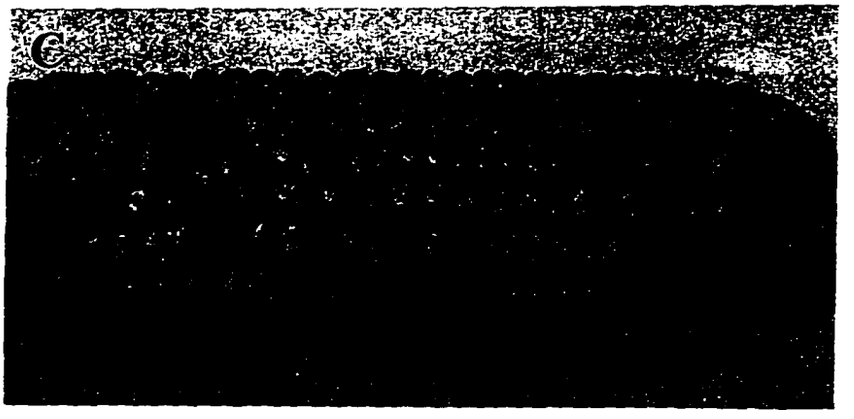
B. Genetic illustration of the transposition of *En* during cob development, leading to the formation of the ear of (A). Each circle represents a cell. Two initial cells (left) are expected to be those formed during embryogenesis. The transposition of *En* before replication (Div. 1) in one the two would be expected to produce the ear of (A).

C. Ear of *a1-m(Au)* type, *a1-m(r)* type and colorless kernels with a quarter of *a1-m(Au)* type and colorless kernels.

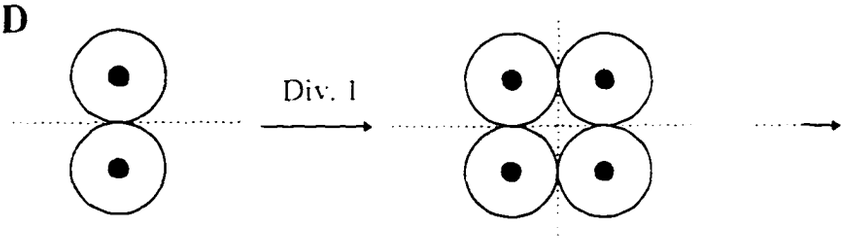
D. Genetic illustration of the transposition of *En* during cob development, leading to the formation of the ear of (C). Two transpositions are expected to occur: one as in (B) and the other after replication in the other cell during the first mitotic division (as illustrated) or before replication following one mitotic division (not illustrated).

B





D



other initial cell and produced the other half divided into two halves: one half of *a1-m(Au)* type, *a1-m(r)* type, and colorless kernels and the other of *a1-m(r)* type and colorless kernels. In event 1, the timing is the same as for the ear of Figure 3A, but the transposed *En* functions and transactivates the *I* of *a1-m(r)*. In event 2, *En* transposed either after replication or before replication in one of the two cells after one mitotic cell division (Figure 3D).

Discordant phenotypes between parent and reciprocal progeny

Out of 203 reciprocal crosses (plants from *a1-m(Au)* type kernels - Crosses 3A and B), seven reciprocal progeny did not segregate *a1-m(Au)* type kernels: three progeny (from Group A, Group B, and Group E, respectively) produced both ears of only *a1-m(r)* type and colorless kernels (**the independent *En* class**), and four progeny (one from Group A, one from Group D, and two from Group E) produced both ears of only colorless kernels (**the *En*-loss class**). Thus, *a1-m(Au)* type kernels were planted (endosperm phenotype), but the parental embryo genotype lacked the *a1-m(Au)* allele. These seven crosses therefore produced progeny of discordant phenotypes with the parental phenotype of *a1-m(Au)* type.

If the embryo genotype was originally *a1-m(r)/a1-m(Au)* from Cross 1, this discordance of the progeny phenotype with the parental phenotype should result from transposition events before cell differentiation to ear and tassel during embryogenesis or in all the initial cells destined in the embryo for the ear and tassel development. This rationale may ideally require multiple transposition events to occur in different cells, leaving no *a1-m(Au)* allele transmitted to progeny. In this scenario, it is possible that a reciprocal cross may result in two ears with discordant phenotypes distinguishable from each other (for example, one ear with expected phenotypes - *a1-m(Au)* type, *a1-m(r)* type, and colorless kernels and the other without *a1-m(Au)* type kernels); however, no such crosses were observed.

Alternatively, the discordant phenotypes between parent and progeny can result from either heterofertilization or transposition of *En* during late microsporogenesis. The independent *En* class is expected to result from a transposition during microsporogenesis, and the *En*-loss class can originate either from heterofertilization or from the loss of *En* activity.

Heterofertilization refers to fertilization of a single embryo sac by two pollen grains, in which the polar and egg nuclei are fertilized by sperms of different pollen sources, respectively.

From Cross 1, where *a1-m(Au)* type kernels were selected, heterofertilization is expected to produce kernels with the endosperm genotype of *a1-m(r)/a1-m(r)/a1-m(Au)* and the embryo genotype of *a1-m(r)/a1*. In Cross 3, these kernels will not produce the nonconcordant progeny of the independent *En* class but only that of the *En*-loss class. The possibility of heterofertilization by two pollens [one carrying *a1* and the other carrying *a1* and the transposed *En* from *a1-m(Au)* or a newly activated *En*] is very remote: each selected group had none or seven *a1-m(r)* type kernels from Cross 1 (Table 2); all *a1-m(r)* type kernels from Cross 3 were very clear, showing no sign of a newly activated *En*. Whether the *En*-loss class resulted from either heterofertilization or loss of *En* activity cannot be concluded in this study.

Discussion

Epigenetic changes of *a1-m(Au)* types

The spontaneous changes in the genetic activity of a transposable element are well correlated with a degree of methylation. It has been observed that *Ac* elements of *wx-m7* and *wx-m9* can undergo spontaneous changes in their genetic activity and the phase variation between activity and inactivity (Schwartz and Dennis 1986; Chomet *et al.* 1987). Epigenetic changes of *En* have also been well documented (Schläppi *et al.* 1993 and references therein). Earlier, these were considered phase changes (Peterson 1966).

Off-types resulted from phase changes of *En* of *a1-m(Au)* during kernel development. Given the previously known correlation of methylation with such epigenetic activity of transposable elements, off-types can be interpreted as resulting from epigenetic modification of *En* in association with methylation. Some of these observations in the current study could be due to the tissue-specific differences in the degree of methylation of *En*s between the embryo and endosperm. The observed aleurone color variegation comes from the activity of *En*s in the endosperm in the current generation, whereas the aleurone variegation in the next generation represents the heritability of the *En* genotype in the embryo.

Transposition of *En* of *a1-m(Au)*

Following detection of the transposition mechanism hypothesis (see Materials and Methods), a clear sector(s) as a probable indication of somatic transpositions before or after replication during cob development were not detected, except on those ears of Figure 3. The random distribution of transposition events (i.e., *a1-m(r)* type kernels) on the ear, where *En* was transmitted as female, indicates that *En* transposes late during ear development. This suggests that transpositions are independent in most cases, if not all: very late transpositions, especially after replication, during cob development are difficult to detect. Cases that transpositions occurred at the last cell division of cob development to produce two sib kernels (e.g., one *a1-m(r)* type and the other *a1-m(Au)* type or colorless) sitting side by side, would not affect the estimated frequencies in this study (Tables 3 and 4). The calculated transposition rates are not different from or higher than previous studies: ~20% (Nowick and Peterson 1981), 10%-20% (Schwarz-Sommer *et al.* 1985), and 7.5% (Cardon *et al.* 1993).

Transposition after replication, when observed in individual kernels, is expected to produce twinned kernels with *a1-m(r)* type and *a1-m(Au)* type, as noticed by Fedoroff (1983). However, such twinned kernels were very infrequently observed. Given all the observations, we believe that **most** individual transposition events occur before replication, and **sometimes after** replication. Cases of transposition after replication observed include ears of Figure 3 and ears showing phenotypes disconcordant with parent. Previously, Nowick and Peterson (1981) found that approximately 20% of the progeny carried two or more *En*s in detecting secondary transposition sites. Dash and Peterson (1994) interpreted post-excision loss events due to transpositions after replication. Seo and Peterson (1996) accounted for approximately 5% of the progeny by the transpositions after replication.

Biased transposition frequencies as male versus female

En of *a1-m(Au)* transmitted at a higher frequency as male than as female (Table 4). A *dSpm* allele, *bz-m13*, showed mutation rates of 71% and 53% when transmitted as male and as female, respectively (RABOY *et al.* 1989). A higher *Mu* activity when transmitted as female was observed (Brown and Sundaresan 1992; Lisch *et al.* 1995), while more *Mu*

insertions into the *y1* gene were isolated when *Mu* was used as male (Robertson and Stinard 1993).

One possible explanation for such biased mutation frequency may come from the difference in the production of male and female gametophytes. In brief, both a stamen (male) and a pistil (female) begin as a lobe, respectively (Kiesselbach 1949). Each lobe gives rise to a spikelet with two flowers. In the female, only one of the two flowers commonly persists; it differentiates to form first one pistil and then the megaspore, so that eventually each spikelet normally produces one kernel of corn. An ear produces hundreds of kernels. In the male, each flower develops three stamens that are observed as anthers when pollen is shed; each anther has four loculi, in each of which the cells of sporogenous tissue divide until a large number of microspore mother cells are formed, and each mother cell differentiates into four three-celled pollen grains. A tassel produces millions of pollen grains. In such developmental difference, one mutation would be revealed as a single event in the female and as multiple events in the male. In this series of events, more chances of mutation provided through male can make higher transposition frequencies than through female. Alternatively, the differential frequency may come from the difference in timing of transposition relative to gametophyte production in the male versus the female lineage.

Discordant phenotypes between parent and reciprocal progeny

Most of the ears examined from Cross 1 (*a1-m(r)/ a1-m(r)* x *a1-m(Au)/a1*) inherit the activity of *a1-m(Au)* into the progeny, randomly distributed together with *a1-m(r)* type and colorless kernels (with some exceptions; see the two ears in Figure 3). It was previously noted that the random distribution of *a1-m(r)* type kernels, when *a1-m(Au)* was crossed as female, results from single independent transpositions occurring late in ear development in a mitotic cell that is in a cell lineage to a single megasporocyte prior to fertilization. This is possible for most transposition events observed when *a1-m(Au)* was crossed as male. In support of this, it is contended that discordant phenotypes between parent and reciprocal progeny do originate from discordant genotypes between endosperm and embryo.

To the exclusion of the possibility that multiple transposition events during early plant development and heterofertilization were involved in the formation of discordant progeny of the independent *En* class (see Results), the parental embryo of the independent *En* class would carry a transposed *En*. Likewise, it is possible that the embryo of the *En*-loss class would have no *En* activity as results of loss or inactivation. According to this scenario, the embryo genotypes of the independent *En* class with *a1-m(r)* type and colorless kernels would be *a1-m(r)/a1**, *En*, and those of the *En*-loss class with colorless kernels only would be *a1-m(r)/a1**, *En** (*a1**: recessive *a1* gene after transposition, *En**: lost or inactivated *En* after transposition) (Figure 4A).

The different constructs of endosperm and embryo may result from transposition after replication during the second mitosis during Cross 1. One sperm nucleus of an intact *a1-m(Au)* uniting with polar nuclei and the other of either *a1**, *En* (the independent *En* class), or *a1**, *En** (the *En*-loss class) uniting with an egg would give rise to the different genotypes between endosperm and embryo (Figure 4B). Without further transposition, reciprocal crosses of these disconcordant genotypes will segregate only different embryo and endosperm phenotypes from the parent. Such nonconcordance of endosperm and embryo was also reported in the study by Walker *et al.* (1997) of a new element *PIF* (*P Instability Factor*) at the *r* gene.

Alternate events could also occur; that is, a selected kernel with a colorless endosperm would produce *a1-m(Au)* type progeny if transposition occurred in the first mitosis of megasporogenesis resulting in two polar haploid nuclei without an active *En* and an egg cell with an intact *a1-m(Au)*. This condition would necessitate a test of colorless kernels, so it could not be seen from the genotypes of Cross 1. However, transposition events late during ear development mentioned previously and during microsporogenesis seem to be compatible with the assumption that *En* of *a1-m(Au)* transposes mostly during gametogenesis. Single events were preferred to be interpreted to arise in a mitotic cell that leads to a single megasporocyte or during meiosis (Walker *et al.* 1997).

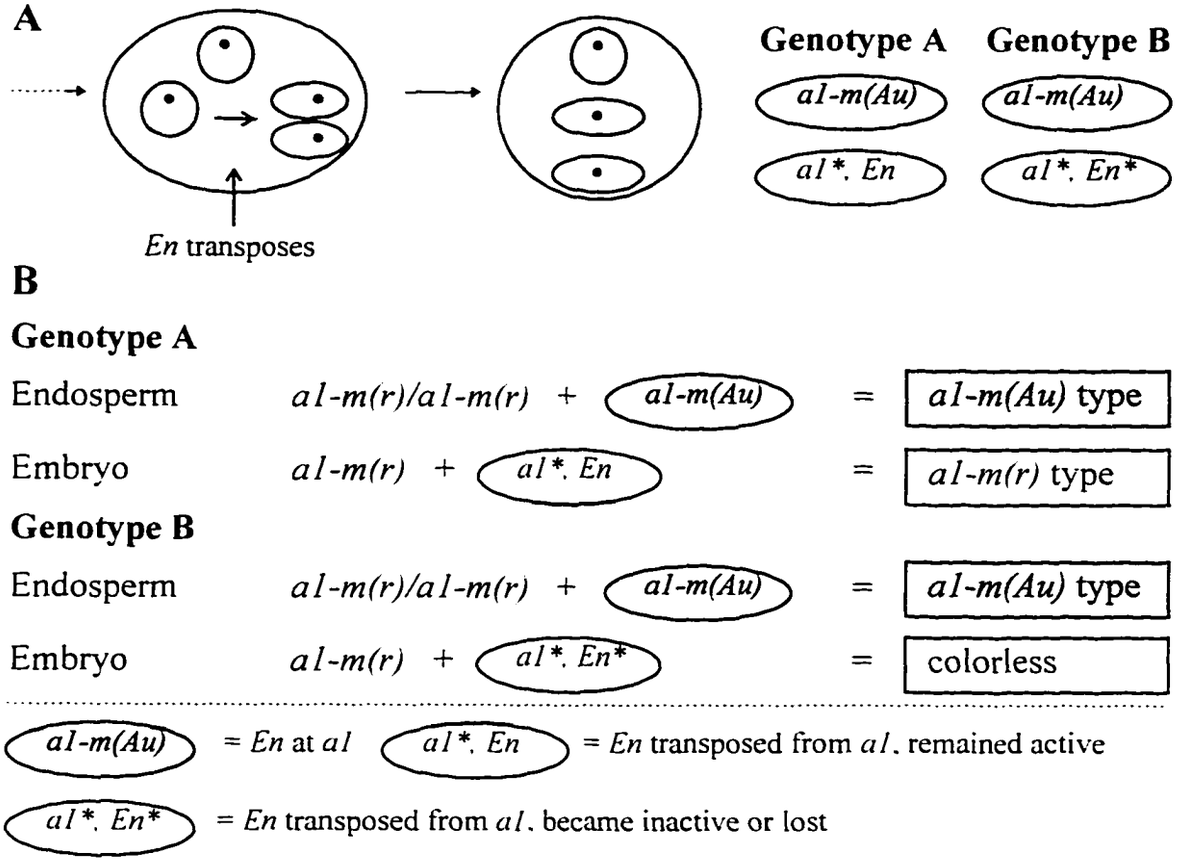


Figure 4. A. Late microsporogenesis: formation of two sperm cells via the second mitotic division. Two sperm cells carry different genotypes as a result of the transposition of *En*, likely after replication during the second mitotic division. In genotype A, *En* is active after transposition from the *al* gene. In genotype B, the transposed *En* (*En**) was lost or inactive. The *al* gene left behind after transposition became non-functional (*al**).

B. Double fertilization with *al-m(r)* as female results in disconcordant genotypes between embryo and endosperm. The fertilization of the pollen of genotype A with *al-m(r)* produced the *al-m(Au)* type endosperm but produced *al-m(r)* type kernels in the next generation of the cross with *al-m(r)* (the independent *En* class). The fertilization of the pollen of genotype B with *al-m(r)* produced the *al-m(Au)* type kernel but produced colorless kernels in the next generation of the cross with *al-m(r)* (the *En*-loss class). The endosperm or aleurone color of the current generation is the phenotypic expression of the genotype of the embryo of the previous generation.

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CHAPTER 6. THE UNILATERAL APPEARANCE OF THE EFFECT OF A MODIFIER IN THE *Enhancer/Suppressor-mutator* (*En/Spm*) SYSTEM IN MAIZE: FEMALE EXPRESSION

A paper published in Maydica[1,2]

Beom-Seok Seo and Peter A Peterson[3]

Abstract

Modifiers in the *En/Spm* system in maize have previously been described to affect either the timing or frequency of mutation events of several autonomous and nonautonomous alleles. In this study we describe another serendipitously discovered *Modifier* (*Mdf*), with the advantage of reciprocal crosses, that mediates to increase the mutability of an *En/Spm* responsive allele *a1-m(r)*. Interestingly, in reciprocal crosses the *Mdf* effect is observed only when *Mdf* is transmitted by the female side of the cross. This unilateral appearance of the *Mdf* effect is accounted for by the two-to-one interaction hypothesis, in which at least two copies of *Mdf* and at least one copy of *En/Spm* are required in the endosperm for the manifestation of the *Mdf* effect.

Key words *En/Spm* transposon; Modifier; Unilateral appearance; Two-to-one interaction

Introduction

Modifiers in the *En/Spm-I/dSpm* transposable element system have previously been described (McClintock 1957, 1958; Peterson 1976). These modifiers have been identified by their effects on mutability pattern expression including excision timing and frequency of En/Spm autonomous and nonautonomous alleles. Because of this, a modifier affecting

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[2] A tribute to J. Smith for his fruitful career in genetics from the Ames laboratory where he began. This tribute from his mentor (PAP) and a later student (B.-S. S.) from the same laboratory that launched JD's career.

[3] Corresponding author

transposon expression can be defined as a complementary component that changes in either a positive or negative way the mutable patterns of the autonomous and nonautonomous elements, but has no suppressor or mutator function itself.

McClintock (1957) earlier isolated a single kernel exhibiting a mutability higher than that of a particular state of the suppressible *a1-m1* allele under study from a cross of *a1-m1 Sh2/a1 sh2, En/+* x *a1 sh2/a1 sh2*. With subsequent tests of the progeny of this kernel by selfing and outcrosses on another state of *a1-m1* and several *a1 sh2* testers, a *Modifier* (*Mod*) was uncovered and verified to be responsible for the increased mutability. Further, this *Mod* does not affect the timing but rather the frequency of mutations of the *a1-m1* allele. McClintock (1958) further studied the activity of *Mod* with four different states of the *a1-m1* allele: first, that of a few, relatively late-occurring mutations; second, that of more mutations but most of which occur relatively late in development; third, that of very many mutations, all occurring late in development; and fourth, that of many mutations, some of which occur early in development. *Mod* affects that first and second states only, elevating the mutability pattern up to those of the second and the third, respectively. This *Mod* was mapped on the *a1 sh2*-carrying chromosome 3 and the *wx*-carrying chromosome 9. McClintock also observed that two distinct mutability patterns of *a1-m1* induced by *Spm* and *Spm-w*, respectively, cannot be distinguished under the presence of *Mod*.

Another modifier Restrainer (*Rst*) described by Peterson (1976) modifies the timing and frequency of the mutability of the autonomous (*En*-containing) *c1* mutable allele, *c1-m5 5292*, changing its standard coarse (early mutability events) pattern to a fine pattern (late mutability patterns), thus delaying excision events. Peterson (1976) further verified that *Rst* spontaneously arises at a low frequency among several *c1* mutable alleles producing coarse patterns (Reddy and Peterson 1983).

Unlike the above-mentioned modifiers, *Mediator* (*Med*) described by Muszynski *et al.* (1993) is an essential third component in inducing the mutability of *Irma*, the 3.3 kb complex, *En*-related insert of a *c2-mutable* allele *c2-m881058Y*. *Irma* has no homology with *En/Spm* in its internal 1.7 kb region and in addition includes 11-14% nucleotide changes within the 12 bp TNPA (one of the *En/Spm* transposases) binding motifs. Muszynski *et al.*

(1993) hypothesized that *Med* probably provides a helper function for TNPA either by stabilizing weak binding or by aligning a displaced binding of TNPA to the altered subterminal binding motifs. *Med* does not have any functional effect on any type of *En* alleles tested, except that the allele, *En-low*, conditions low mutability of *Irma*. Based on its suggested function, *Med* can also be classified as a modifier in that it complements the interaction between *En* and *Irma* in a positive way.

In the course of an *En* transposition study (Seo and Peterson 1997), a modifier, *Mdf*, was identified in the *En/Spm* transposable element system. *Mdf*, when coupled with *En/Spm*, induces a higher excision frequency of the responsive allele, *a1-m(r)*. Unlike previously identified modifiers described above, the *Mdf*-mediated effect, the enhanced mutability of *a1-m(r)*, only appears when transmitted by the female. This unilateral transmission is explained with a two-to-one interaction hypothesis, in which at least two copies of *Mdf* and at least one copy of *En* are required for the manifestation of the *Mdf* effect. Thus, getting two copies via male transmission would not be possible when a single copy of *Mdf* is present (except from a B-chromosome translocation arrangement).

Materials and Methods

The autonomous allele *a1-m(Au)* includes a fully functioning *En* insert into the second exon of the *a1* gene (Menssen 1988). This *En* in *a1-m(Au)* can self-transpose and produces almost fully colored kernels with a few colorless sectors (in Peterson 1961, Fig. 2A: Nowick and Peterson 1981). The *En/Spm* reporter allele *a1-m(r)* is a derivative of the *a1-m(Au)* allele and was isolated in *pale green mutable* stocks (Peterson 1956) and has been maintained in a heterozygous state with another reporter allele, *a1-ml* (Fig. 1). The phenotype of this *En*-deletion derivative *I* element insert is colorless in that the insert interferes with the transcription of the *a1* gene, but can be excised when *En/Spm* transposases are provided in trans, resulting in variegated kernels (Fig. 2A).

Genetic crosses made during this study are as follows:

parent 1 = *a1 Sh2/a1-m(r) Sh2, En*; parent 2 = *a1-m(r) Sh2/a1-m(r) Sh2*

Cross 1: *a1-m(r) Sh2/a1-m(r) Sh2, Mdf* x *a1-m(Au) Sh2/a1 sh2*.

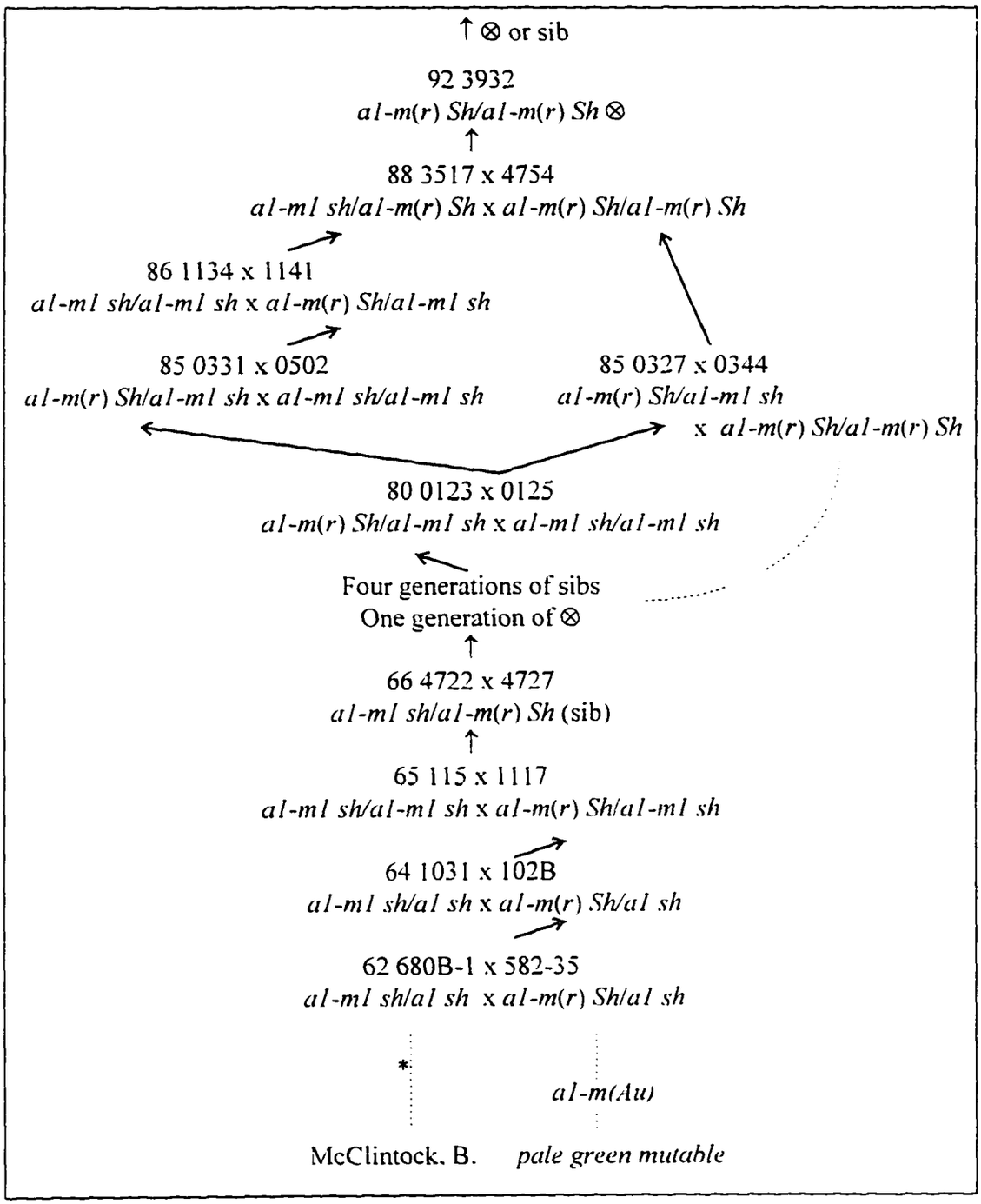

Figure 1. The pedigree of the origin of the *En/Spm* reporter allele, *al-m(r)*. *al-m(r)* is a deletion derivative of *al-m(Au)* and is located in the second exon of *al* (Menssen 1988). *[Sent by B. McClintock to Peterson Lab (Ames) in 1961]

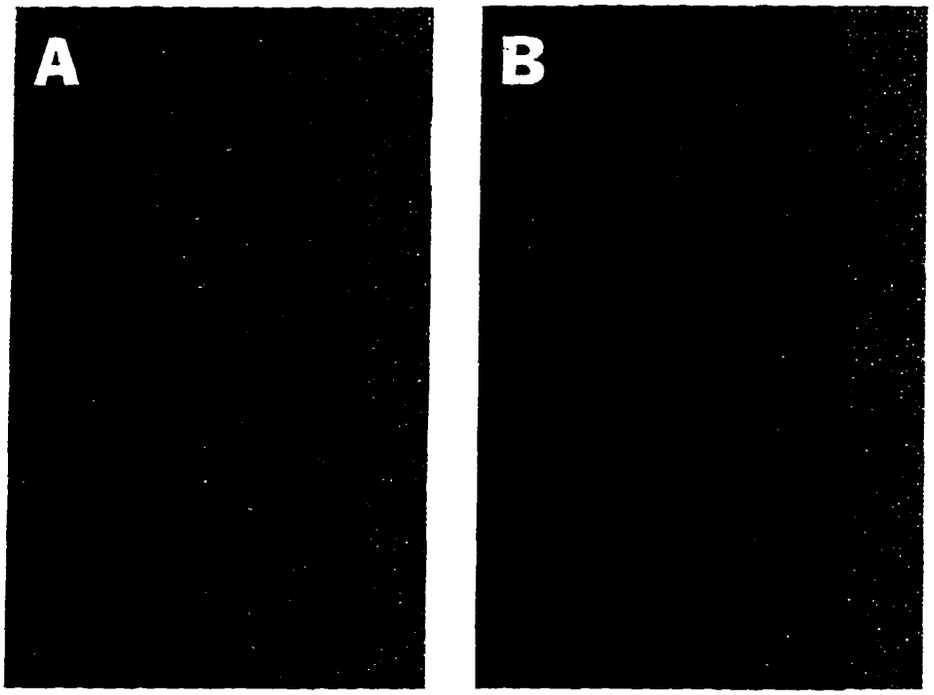

Figure 2. A reciprocal half-sib family ('96 1439E-1) illustrating the unilateral appearance of the *Mdf* effect.

A. An ear from Cross 2A [*a1 Sh2/a1-m(r) Sh2, En* (parent 1) x *a1-m(r) Sh2/a1-m(r) Sh2* (parent 2)] segregates the *a1-m(r)* phenotype kernels with *En* (~50%) and colorless kernels without *En* (~50%).

B. The reciprocal ear from Cross 2B [*a1-m(r) Sh2/a1-m(r) Sh2, Mdf* (parent 2) x *a1 Sh2/a1-m(r) Sh2, En* (parent 1)] segregates the high *a1-m(r)* phenotypes with *En* and two *Mdf*s in the endosperm (~25%), *a1-m(r)* phenotypes with *En* (~25%) and colorless kernels (~50%).

Cross 2A: *a1 Sh2/a1-m(r) Sh2, En* (parent 1) x *a1-m(r) Sh2/a1-m(r) Sh2* (parent 2)

Cross 2B: *a1-m(r) Sh2/a1-m(r) Sh2* (parent 2) x *a1 Sh2/a1-m(r) Sh2, En* (parent 1)

During Cross 1, the presence of *Mdf* was not known, and randomly chosen *a1-m(r)* plants were propagated by sib-mating and entered as parent 2 in Crosses 2A and B. Crosses 2A and B include reciprocal crosses. The presence of *Mdf* in parents 1 and 2 of Cross 2 depends on

where the *Mdf* effect is expressed. For example, if the *Mdf* effect is observed on only the ear of parent 1, then *Mdf* is present in parent 1. In the Results and Discussion sections, *En* is always present when mutability is observed, only the *Mdf* is variable. This will be clarified in the Results section.

Results

From Cross 1, kernels of the standard *a1-m(r)* phenotype (Fig. 2A) have the expected expression of the *a1-m(r)/a1, En/+* genotype following only the transposition of *En* from the *a1-m(Au)* allele. Some of these *a1-m(r)* phenotypes obtained from Cross 1 were further tested to determine whether these phenotypes were heritable and truly arose from the interaction of *a1-m(r)* with a transposed *En* from *a1-m(Au)*. This was done by making reciprocal crosses with *a1-m(r)* (Crosses 2A and B). In these crosses, most of the selections produced the expected *a1-m(r)* phenotype, but in several reciprocal crosses, two different kernel phenotypes appeared: one, typical of the [*a1-m(r)* with *En*] phenotype (Fig. 2A); the other, a more highly variegated type (Fig. 2B). Compared to the standard [*a1-m(r)* with *En*] phenotype, this unexpected highly variegated phenotype appears to result from more frequent excisions of the *I* element of *a1-m(r)* but with the similar timing of excision. Hereby it is hypothesized that the increased mutability of *a1-m(r)*, the high *a1-m(r)* phenotype, is caused by a modifier, named *Mdf*, that appears to affect the excision frequency of the *I* insert of *a1-m(r)*.

But in reciprocal crosses, an interesting feature was observed with *Mdf*. The *Mdf*-induced phenotype only appears in the progeny of only one of the crosses, namely, in the progeny of the cross where the female parent was carrying *Mdf*. Thus, there is only a unilateral appearance of the high *a1-m(r)* phenotype. That is, the *Mdf* effect is only transmitted on the female ear in this comparison of reciprocal crosses. (Note, in the development of the embryo sac, the polar nuclei contributing to the endosperm have two nuclei.) Figure 2 provides an example of the unilateral appearance of the high *a1-m(r)* phenotypes on only one of two ears in a reciprocal cross. This unilateral appearance of the *Mdf* effect seems to exclude the idea that the interaction between one *Mdf* and one *En* mediates the increased mutability of *a1-m(r)*. Because the high mutability (Fig. 2B) is

expressed only when the *Mdf*-containing parent is the female in the cross. it is likely that two or more copies of *Mdf* are required for its phenotypic manifestation.

This unilateral appearance of the *Mdf* effect on only the female ear in a reciprocal cross (Crosses 2A and B) and the requirement of two or more copies of *Mdf* for the manifestation of the high *a1-m(r)* phenotype lead us to the hypothesis of the obligate interaction between **two *Mdf*s** and **one *En*. the two-to-one interaction.** in the endosperm. The genotypic basis for the *Mdf* expression is shown in Table 1A and B.

Table 1. The basis for the *Mdf* expression in a reciprocal cross: **the two-to-one interaction**.

A. The endosperm genotypes and phenotypes from the cross of *a1/a1. En/+. Mdf/+* x *a1-m(r)/a1-m(r)*. Only the copy number of *En* and *Mdf* is indicated. because *a1-m(r)* is always present.

Gametes (F\M[1])	+. +	Phenotype
En. Mdf	*En/En/+. Mdf/Mdf/+*	High[2]
En. +	*En/En/+. +/+/+*	Standard[3]
+. Mdf	*+/+/+. Mdf/Mdf/+*	-[4]
+. +	*+/+/+. +/+/+*	-[4]

B. The endosperm genotypes and phenotypes from the cross of *a1-m(r)/a1-m(r)* x *a1/a1. En/+. Mdf/+*. Only the copy number of *En* and *Mdf* is indicated. because *a1-m(r)* is always present.

Gametes (F\M1)	*En. Mdf*	*En. +*	*+. Mdf*	*+. +*
+. +	*+/+/En. +/+/Mdf*	*+/+/En. +/+/+*	*+/+/+. +/+/Mdf*	*+/+/+. +/+/+*
Phenotype	Standard[3]	Standard[3]	-[4]	-[4]

[1]Segregation of *En* and *Mdf* in female (F) and Male (M) gametes. respectively (+ = no element). [2]The high *a1-m(r)* phenotype (the *Mdf* effect. see Fig. 2B). [3]The standard *a1-m(r)* phenotype (Fig. 2A). [4]Only colorless kernels.

The source of *Mdf* is not known, but the analysis of the reciprocal crosses showing the unilateral appearance of the high *a1-m(r)* phenotype indicated the segregation of *Mdf* among the *a1-m(r)* testers (Fig. 1) and some Cross 1 progenies (parent of Cross 2). This leads us to favor the belief that the *a1-m(r)* tester is the likely source of *Mdf*. But the *a1-m(Au)* line has never previously been recombined with any reporter allele in successive generations until this study, so a modifier, if any, could not have been previously identified.

Considering the possibility that a modifier can also transpose (McClintock 1958), the *Mdf* copy number can be one or more in the parents of Cross 2. Supporting this, both ears from some reciprocal crosses (Table 2: 96 1344A-7) were observed to express the *Mdf* effect, in which approximately a third or a half of the high *a1-m(r)* phenotypes are segregating due to the presence of two copies of *Mdf* in the genome. Table 2 includes the *En* and *Mdf* genotypic designations of all the exceptional cases and indicates the probable number of elements segregating in both parents. Current data indicate no linkage among all segregating *En*s and *Mdf*s.

Table 2. Segregation of the *Mdf* effect [the high *a1-m(r)* phenotype (in Fig. 2B)] in reciprocal crosses from Cross 2A: *a1 Sh2/a1-m(r) Sh2, En* (parent 1) x *a1-m(r) Sh2/a1-m(r) Sh2* (parent 2) and cross 2B: *a1-m(r) Sh2/a1-m(r) Sh2* (parent 2) x *a1 Sh2/a1-m(r) Sh2, En* (parent 1). Crosses are indicated in parentheses.

	No. of kernels				%	No. of *Mdf* and *En*[1]	
1996 Plant No	*En.Mdf*	*En*	Colorless	Total	*En.Mdf*	Parent 1	Parent 2
1341B-1(2A)	96	87	197	380	25.26	1 *Mdf*, 1 *En*	None
(2B)	0	175	181	356			None
1344B-2(2A)	0	208	255	463		0 *Mdf*, 1 *En*	ND[2]
(2B)	61	50	136	247	24.70		1 *Mdf*
1344A-7(2A)	166	70	78	314	52.87	1 *Mdf*, 2 *En*s	2 *Mdf*s[3]
(2B)	148	129	117	394	37.56		1 *Mdf*

[1]Copy number of *Mdf* and *En* elements in the parents 1 and 2, estimated under the hypothesis of the interaction between two *Mdf*s and one *En*. [2]ND not determined. [3]Two copies segregating independently.

This normally unilateral appearance of the *Mdf* effect (exception in Table 2) is always accompanied by the presence of 25% of the high *a1-m(r)* phenotypes on only one of the progenies (Table 2). Under the two-to-one interaction hypothesis, the unilateral appearance of approximately 25% high *a1-m(r)* phenotypes only on the female ear (Fig. 2B) can be accounted for only with one *Mdf* in either the parent 1 or the parent 2 and one *En* in the parent 1. The appearance of the *Mdf* effect on only the ear from the Cross 2A is described with the cross between one *Mdf* and one *En* in parent 1 and no elements in parent 2 (Table 3A), and that from Cross 2B is with the cross between no *Mdf* and one *En* in parent 1 and one *Mdf* in parent 2 (Table 3B). Other combinations, as in the reciprocal family number 1344-7 in Table 2, are expected to reveal the *Mdf* effect on both ears.

Table 3. Segregation of the copu number of *Mdf* and *En* int he endosperm based on the two-to-one interaction hypothesis of the unilateral appearance of the *Mdf* effect, in which the interaction between two *Mdf*s and one *En* induces the high *a1-m(r)* phenotype (dark kernels in Fig. 2B). Proportion of high *a1-m(r)* phenotypes is boldfaced. Cross 2A: *a1 Sh2/a1-m(r) Sh2, En/+, Mdf/+* (parent 1) x *a1-m(r) Sh2/a1-m(r) Sh2* (parent 2); Cross 2B: *a1-m(r) Sh2/a1-m(r) Sh2, Mdf/+* (parent 2) x *a1 Sh2/a1-m(r) Sh2, En/+* (parent 1).

A. The unilateral appearance of the *Mdf* effect only on the ear of Cross 2A.

Parent 1 (1 *Mdf*, 1 *En*)		Parent 2 (no *Mdf*, no *En*)	
Ear from Cross 2A	**1/4 (2 *Mdf*s, 2 *En*s)**	Ears from Cross 2B	1/4 (1 *Mdf*, 1 *En*)
	1/4 (2 *Mdf*s, no *En*)		1/4 (1 *Mdf*, no *En*)
	1/4 (no *Mdf*, 2 *En*s)		1/4 (no *Mdf*, 1 *En*)
	1/4 (no *Mdf*, no *En*)		1/4 (no *Mdf*, no *En*)

B. The unilateral appearance of the *Mdf* effect only on the ear of Cross 2B.

Parent 1 (no *Mdf*, 1 *En*)		Parent 2 (1 *Mdf*, no *En*)	
Ear from Cross 2A	1/4 (1 *Mdf*, 2 *En*s)	Ears from Cross 2B	**1/4 (2 *Mdf*s, 1 *En*)**
	1/4 (no *Mdf*, 2 *En*s)		1/4 (2 *Mdf*s, no *En*)
	1/4 (1 *Mdf*, no *En*)		1/4 (no *Mdf*, 1 *En*)
	1/4 (no *Mdf*, no *En*)		1/4 (no *Mdf*, no *En*)

Discussion

Mdf expression depends on two doses

In the current study, the unilateral appearance of the *Mdf* effect and the two-to-one interaction hypothesis required that one *Mdf* needs to be transmitted through the female, so that two copies are present in the endosperm genotype. However, when two or more copies of *Mdf* are present, the *Mdf* effect is observed on both ears. The requirement of two copies of *Mdf* seems to imply that the amount of the *Mdf* product is a limiting factor. Contrary to *Mdf*, one copy of *Mod* (McClintock 1958) or *Rst* (Peterson 1976) is adequate to modify the mutable patterns of alleles under study, respectively.

Other possible hypotheses for the unilateral appearance of the *Mdf* effect may include parental imprinting (for review, see Peterson and Sapienza 1993) and nontransmission of *Mdf* via pollen. However, these mechanisms can be excluded by the appearance of the *Mdf* effect and segregation ratio in both ears of the reciprocal family '96 1344A-7 (Table 2). Possible involvement of these mechanisms, however, is beyond the observed phenotype in the *Mdf* expression: only the two-to-one interaction hypothesis can account for the segregation - one-fourth - of the *Mdf* effect when only one *Mdf* and one *En* are present in the parent.

Mdf origin

The *Mdf* effect was observed on the progeny ears of Cross 2, whose parent 1 is the standard *a1-m(r)* phenotypes from Cross 1, and *Mdf* is observed to segregate in both parents of Cross 2. The presence of *Mdf* in parent 2 does not support the origin of *Mdf* in Cross 1 generation but rather the segregation of *Mdf* in the *a1-m(r)* line. *Rst* was observed to arise spontaneously, as a single event, and segregate independently of the autonomous component *En* in stocks that produce coarse patterns (Peterson 1976). *Mod* was also isolated as a single event and not found in *a1-m1 sh2* testers (McClintock 1958). All *Mod*s and *Spm*s were analyzed to segregate independently.

These modifiers can be derivatives of autonomous *En/Spm* elements, resulting in the retainment of a partial function in binding to the motif sequences in dimerization function, so that the products of modifiers can interfere with the interaction between the DNA sequences

and transposases of *En/Spm* in a positive or negative way. This idea may derive support from the transposition of modifiers, although it is contradictory (see *Ds1*, Sutton *et al.* 1984; *Irma*, Muszynski *et al.* 1993). But it cannot be excluded that modifiers may arise independently of *En/Spm* but their products have a functional domain to interfere with either the sequences or transposases of *En/Spm* elements. This assumed independent origin and the isolation of modifiers as individual events might be a correlated phenomenon. This further may suggest that there could be a cellular factor(s) that participate in *En/Spm* transposition. With this assumption, whether new, unexpected events are due to the loss of control or tolerance of the cells is a matter of speculation.

Mdf mechanism

En/Spm modifiers described thus far have been uncovered by their ability to change in either a positive or negative way the mutable patterns of the autonomous and nonautonomous elements, but they have no Suppressor or Mutator function themselves. Do the products of modifiers affect the mutable patterns of a specific *En/Spm* allele? *Mod* (McClintock 1958) seems to increase the excision frequency, not the timing, of only two of the four *a1-m1* states under study, so that the *Mod* effect can be said to be allele-specific. *Mdf* in the current study also affects the excision frequency in a positive way but does not affect the timing of *a1-m(r)*. On the other hand, *Rst* (Peterson 1976) affects the excision frequency in a positive way but the timing in a negative way in delaying the timing an autonomous *En* element at the *c1* gene. A common phenomenon among these three modifiers is the positive effect on the excision frequency of the interacting *En/Spm* alleles. This may suggest that modifiers facilitate the formation of the transposition intermediate complex, likely via the mediation of more or efficient binding of transposases at the TIR (terminal inverted repeat complex) (Frey *et al.* 1990). However, since none of the modifiers has been isolated molecularly, their origin and method of changing mutable patterns are only speculations.

Concerned with the modifier-mediated enhanced excision frequency of a transposable element, it may be worthwhile to speculate why the excision of an element does not always take place in all the cells on the stage where the element expresses. If a interpretation can be

made that modifier products increase the probability of excision. then a mutable pattern of an element could in turn be a function of a probability that transposases operate to reveal their phenotype in inherent conditions such as the structural composition of the elements of interest and other unknown cellular states at a give time.

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CHAPTER 7. GENERAL CONCLUSIONS

General Discussion and Recommendations for Future Research

The *Uq-ruq* transposable element system

The interaction between an autonomous element and its non-autonomous element occurs in an exclusive manner. The *Uq-ruq* transposable element system is one of the exceptions to such rule, in that the *Uq* system shares the functionality of the *Ac-Ds* system (Caldwell and Peterson 1992) with *Ds1* (*ruq*) as the common element between the two systems (Pisabarro *et al.* 1991). Together with other similarly exceptional systems such as *F-En-c1m(r)888104* with *En/Spm-I/dSpm* (Peterson 1997) and *Spf-r-R#2* with *Fcu-rcu* (Gonella and Peterson 1978), the cloning of *Uq* may reveal an interesting evolutionary connection relative to the diversity and origin of transposable element systems.

The diversity of the functional aspects among *Uq* elements appeared as differential spotting patterns (DSPs) in the interaction with the *Uq* reporter alleles at both the *a1* or *c1* loci. Underlying causes for DSPs may include differential methylation in association with different positions and different responding capabilities among *ruq* elements. Position effects have often been observed with transposable elements (Peterson 1977). The differential responding capability of *ruq* elements could come from point mutations, small insertions, or small deletions at critical sites. However, this is not proven at a molecular level and remains to be proven. The diversity uncovered by Pisabarro *et al.* (1991) suggests the contention that nucleotide alterations may be a basis for these phenotypic differences.

The phenotypic pleiotropy of the *Mn5* gene on endosperm development and pollen tube growth, together with its effect on the agronomic trait of seed size and nutrition, makes the exploration of the allele an attractive point of interest. With this study, *Mn5::Uq* is mapped between *lg1* and *gl2* on chromosome 2S and both *Mn5* and *Uq* can be targeted at the molecular level.

Probing of genomic DNA from *Mn5::Uq*/+ and +/+ lines with an *Ac* probe indicates that *Uq* is a unique element. Since *Mn5::Uq* is located on chromosome 2, the co-segregating band with *Mn5::Uq* can be cloned. Several options are available such as positional cloning

and chromosome walking, chromosome landing approach, and contig mapping. However, these methods are very tedious and costly. Prior to such an undertaking, this co-segregating band can be tested with *Ds1*: the nucleotide relationship between *Uq* and *Ds1* was not yet determined. If some degree of homology is detected with *Ds1*, the use of that segment of the co-segregating band would be the best way to clone and sequence *Uq*. Or, tagging a known gene with *Uq* would be another choice of attack.

The variable penetrance and expressivity due to the presence of a transposon insert is not exceptional but a common observation (Strommer and Ortiz 1989; Ortiz *et al.* 1990; Barkan and Martienssen 1991; Martienssen and Baron 1994; Greene *et al.* 1994). The cloning of *Uq* and *Mn5* will help uncover the bases for such variability of *Mn5::Uq* expression.

The *En/Spm - I/dSpm* transposable element system

The wanderings of an autonomous *En*, En^{vag}, around the *a1* locus on chromosome 3 were observed at a high frequency rate of ~50%. The following of such movements more than three generations demonstrates how much a transposable element can manipulate the maize genome through its transposition of excision and insertion, as reviewed in Literature Review (CHAPTER 1).

Transposition of a transposon at an appropriate timing during plant development can uncover the developmental pattern of a specific organ or tissue. This feature has not been much exploited. Genes that have been studied taking advantage of transposons might not have been appropriate for such purpose. Excision timing of most of transposons is limited to a certain stage, and thus it is not possible to detect the pattern. Or, so many crosses need to be made to rescue events that lead to the exhibition of a pattern of plant development. Out of more than 200 reciprocal crosses (Chapter 5), only a couple of events were discovered that disclose a developmental pattern of ear. Recent inventions to entrap genes or enhancers, taking advantage of selectable markers and transposons (Sundaresan *et al.* 1995), may contribute to finding plant developmental pattern.

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