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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

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Abstract Transposition studies of the transposon, *En*/*Spm*, have dealt with general aspects of the timing of the excision event with regard to DNA replication and plant development, but without describing details of the process. By following the excision events of an *En* transposon inserted at the *a1* locus [*a1-m*(*Au*)], several features of this process can be elucidated. In progenies from reciprocal crosses between the *a1-m*(*Au*) allele containing an *En* insert, and a nonautonomous *En* allele, [*a1-m*(*r*) is a deficiency derivative of *En*], several features of the *En* at the *a1-m*(*Au*) allele can be observed taking place during ear development and during microsporogenesis. First, it has long been known that the distribution of mutant kernel phenotypes on an ear indicates that *En* transposes late in most of the events during ear development. Second, the phase change of *En* (presence and absence of activity) is observed during cob development. Third, discordant kernel phenotypes of two ears, reported herein, resulting from a reciprocal cross with the parental phenotype can be deduced to arise from the transposition of *En* during microsporogenesis and subsequent fertilization, leading to a discordant genotype between endosperm and embryo. The phase change and discordance lead us to conclude that these events can arise from transposition after host DNA replication. It can also be concluded that the activity of the *En* inserted in this *a1-m*(*Au*) allele is not limited to a specific stage or timing during plant development. Further, this study illustrates the power of genetic analysis in the determination of cellular events.

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Introduction

There are more than ten transposable element systems in maize, including among others, *Activator* (*Ac*), *Enhancer/Suppressor-mutator* (*En*/*Spm*), and *Mutator* (*Mu*). Transposable elements are expressed as variegation which represents transposition (excision) of elements from genes with observable phenotypes. This transposition process, by which a transposable element excises from a donor site and re-inserts into another site, is considered a cut-and-paste process (Saedler and Nevers 1985) and 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). In the *Bg* system, Montanelli et al. (1984) concluded that *Bg*-induced reversion events take place in postmeiotic mitotic divisions during gametogenesis. However, it is not clear whether transpositions of such elements occur at the same time during plant development or with regard to host DNA replication.

The timing and mechanism of transposition of *Activator* (*Ac*) from the mutable pericarp (*P-vv*) locus during cob (ear of maize) development has been well understood with the advantage of two features: (1) the *P-vv* allele controlling the phenotype of the cob and pericarp affects maternally inherited tissue (pericarp and cob), and (2) a negative *Ac* dosage effect that defines the relevant dosage phenotype (Brink and Nilan 1952; van Schaik and Brink 1959; Greenblatt and Brink 1962; Greenblatt 1984; Chen et al. 1987, 1992). This production of distinguishable twin sectors has been explained by *Ac* transposition from its original position after chromosome replication (Greenblatt 1984) and this has been proven molecularly (Chen et al. 1987, 1992).

Mu elements transpose but maintain their copies at the original sites (Alleman and Freeling 1986; Lisch et

al. 1995). This observation led Lisch et al. (1995) to propose a duplicative transposition model. 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).

A demonstrated parallel to 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 the *Spm-c* element (*Spm* used to identify dosage of the element, McClintock 1971). Whether *En* transposition is associated with DNA replication could not be determined from transposition studies of *En* by applying statistical methods to confirm the sites of transposed elements (Peterson 1970; Nowick and Peterson 1981).

En transposition associated with chromosome replication was first genetically demonstrated by somatic post-excision loss of *En* (Dash and Peterson 1994). Cell lineage continuity in the endosperm enabled Dash and Peterson (1994) to observe the transposition of *En* from the autonomous *En*-containing allele *wx-844* in the endosperm and the fate of the transposed *En* in the aleurone. They proposed that the transposition of *En* **after replication** explains all of the observations.

Further support of the transposition of *En* after replication was shown with genetic experiments by Seo and Peterson (1996). Following analysis of segregation ratios that lead to different linkage values between the two pairs, Seo and Peterson (1996) explained this aberrant segregation as the consequence of *En* transposition after replication and subsequent recombination among the original and transposed *En*s.

The present study includes the genetic analyses of reciprocal crosses between the *En*-containing *a1-m*(*Au*) allele, and *a1-m*(*r*) (the non-suppressible defective *En* allele) (Cross 1). Several features were noticed in the reciprocal crosses. First, the distribution of kernel phenotypes on a progeny ear, when a heterozygous *En* parent, *a1-m*(*Au*) *Sh2*/*a1 sh2*, was crossed as female by *a1-m*(*r*), illustrated that *En* transposes late in most of the events during ear development. Second, the phase change of *En* (presence and absence of *En* activity) is observed during early cob development. Third, several pairs of two reciprocal ears showed an unexpected phenotype, in that both ears from a reciprocal cross had the same exceptional kernel phenotypes but lacked the parental phenotype. Such discordant phenotypes upon analysis are considered to arise from transposition of *En* during microsporogenesis and subsequent fertilization that can result in genotypes that are discordant between endosperm and embryo. The phase change and discordance are hypothesized to result from transposition after host DNA replication. These observations suggest that *En* also occasionally transposes after chromosome replication.

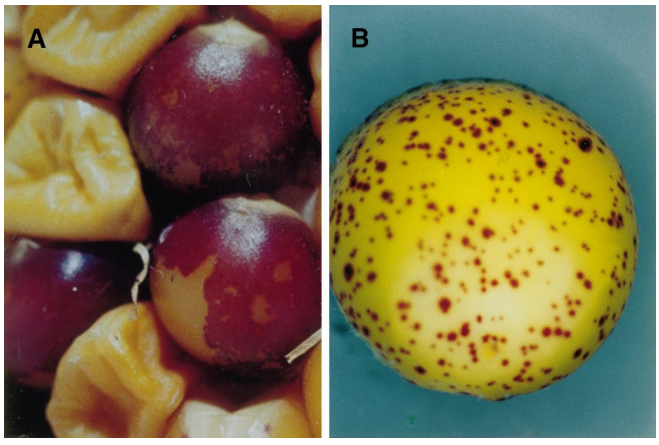

Fig. 1 **A** *a1-m*(*Au*) type kernels (almost colored kernels with colorless sectors) from the cross *a1-m*(*Au*) *Sh2*/*a1 sh2*×*a1 sh2*/*a1 sh2*. The colorless-shrunken kernels are the *a1 sh2*/*a1 sh2* segregates. *a1-m*(*Au*) is an autonomous *En* allele at *a1*. **B** An *a1-m*(*r*)-type kernel (highly variegated kernel) from the cross *a1-m*(*r*) *Sh2*/*a1 sh2*, *En*×*a1 sh2*/*a1 sh2*. *a1-m*(*r*) is a deletion derivative of *En* and responds to the trans-signal of *En*

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 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: Fig. 1A] (Nowick and Peterson 1981). Though the *a1-m*(*Au*)-type appears full-colored, progeny tests of these kernels segregate the *a1-m*(*Au*) phenotype (Fig. 1A), verifying that the selected kernels were *a1-m*(*Au*). The allele *a1-m*(*r*), originally isolated in *pale green mutable* stocks (Peterson 1956), is a deletion derivative of *a1-m*(*Au*) and serves as a reporter for *En*/*Spm*. The kernels of this *En*-deletion derivative *I* element of *a1-m*(*r*) (Cuypers et al. 1988) are colorless; however, in the presence of *En*/*Spm* transposases provided *in trans*, it can be excised giving rise to variegated kernels [***a1-m*(*r*)-type** kernel: Fig. 1B].

Rationale for these experiments: detection of the transposition mechanism hypothesis

Following the *P-vv* twin-sector studies, Greenblatt (1984) presented a hypothesis for the transposition of *Ac* after chromosome replication at the *P-vv* locus. With *a1-m*(*Au*), a parallel to the *P-vv* twin sector can be established from Cross 2A (see next section – Genetic crosses) in which *a1-m*(*Au*) is used as female. The parallel in this study is deduced from the distribution of definitive kernel phenotypes on a full ear (see Fig. 3A and C). The *a1-m(Au)* allele produces almost fully colored kernels with occasional colorless sectors when used as female (Fig. 1A and see Fig. 3A). The kernels of the *a1-m*(*r*) allele are colorless without *En* but variegated with *En* (Fig. 1B). Thus the working **transposition-mechanism hypothesis** is as follows: (1) with *no transposition*, the ear contains only *a1-m*(*Au*)-type and colorless kernels (no sector), (2) *transposition before chromosome replication* at a certain cell stage during early cob development results in an ear with a defined sector of *a1-m*(*r*)-type and colorless kernels only within the same continuous cell lineage, and (3) *transposition after chromosome replication* would result in a ear with a twin sector, in which one part of the twin has the three phenotypes, [*a1-m*(*Au*)-type, *a1-*

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

Entry no.	No. (%) of			Goodness-of-fit χ^2 test[a]		
	al-m(*Au*)-type	*al-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] $\chi^2=\sum[(O-E)^2/E]$ (O=observed value, E=expected value). Tabular values with two d.f. are 5.99 at $\alpha=0.05$, 9.21 at $\alpha=0.01$

[b] When standardized values (% values in parenthesis) 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

m(*r*)-type, and colorless kernels (see Fig. 3C, bottom portion of ear)] and the other part has the two phenotypes, *a1-m*(*r*)-type and colorless kernels (see Fig. 3, top portion). The boundary of the twin sector may not be as definitive as that of the *P-vv* twin sectors (Greenblatt and Brink 1962) if *En* transposes late during cob development. However, in this study, the identified twin-sector is large enough to be very definitive and comparable in definition to the *P-vv* twin-sector studies.

Genetic crosses

Cross 1 is a testcross in which each *a1-m*(*Au*) line was crossed as a male on an *a1-m*(*r*) plant. (*In the display of the crosses, females are conventionally placed on the left and males on the right of the illustrative crosses*). In the F_1 ears from Cross 1, three kernel phenotypes appeared: standard *a1-m*(*Au*) (Fig. 1A), spotted *a1-m*(*r*) (Fig. 1B), and the unexpected, off-type kernels (data not shown). The spotted *a1-m*(*r*) type kernels (Fig. 1B) are a phenotype resulting from a transposed *En* from the *a1-m*(*Au*) allele followed by transactivation of the *I* element of the *al-m*(*r*) allele. The standard *a1-m*(*Au*)-type kernels were used in reciprocal crosses with *a1-m*(*r*) to assay the transposition of *En* (Crosses 2A and B).

The off-type kernels of variegated phenotypes refer to any type that belongs neither to the *a1-m*(*Au*) type nor to the *a1-m*(*r*)-type kernels. Progeny test of off-type kernels indicated they resulted from epigenetic changes of the *a1-m*(*Au*)*En*. Epigenetic modification of *En* has been documented in correlation with a degree of methylation (Schwartz and Dennis 1986; Chomet et al. 1987; Schläppi et al. 1993 and references therein). The off-type kernels were not considered in this study.

Cross 1: *a1-m*(*r*) *Sh2*/*a1-m*(*r*) *Sh2*×*a1-m*(*Au*) *Sh2*/*a1 sh2** (*designated the "sample population")

[Standard ***a1-m*(*Au*)-type** kernels are advanced to Crosses 2A and B]

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

Cross 2B: *a1-m*(*r*) *Sh2*/*a1-m*(*r*) *Sh2*× ***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 2A and B) to test whether the segregation ratio in one set (of progeny of 2A) fits into the ratio in the other (of progeny of 2B), 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 percentages so that they are at 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 the goodness-of-fit χ^2 test in comparing the transposition rates with two standardized sets of data in a reciprocal cross. The test was made twice; first, one set of standardized data was used as observed values and the other set of standardized data was used as expected values, and second, vice versa. Only when both calculated values showed significance, were the transposition rates of an *En* considered different in a reciprocal cross.

What follows is the rationale that observed/standardized data can be used as expected values, which are normally theoretical values that originate from an expected or theoretical ratio. It is common that the transposition frequency should be equal whether *En* is crossed as a female or as a male, and vice versa. This general assumption leads to the following: (1) that one set of data can be reasonably used as expected values when it is tested to fit into the other set of data by a goodness-of-fit χ^2 test, and (2) that a χ^2 test of independence can be excluded, in which no theoretical segregation ratio is required. Further, an independence χ^2 test is less stringent in this study because it tests whether sets of observed values are independent, rather than whether they are homogeneous.

Results

In the original experiment designed to examine the transposition of *En* from the *a1-m*(*Au*) allele, 150 plants with the *a1-m*(*Au*)/*a1* genotype [*a1-m*(*Au*)] type as a sample population were crossed to *a1-m*(*r*) plants (Cross 1). There were 140 crosses with progeny containing more than 100 kernels, which were used in the analysis.

Estimation of transposition frequency

Following the rationale of these experiments, 140 crosses of the sample population (Cross 1) were made, and from these crosses 133 had one or more *a1-m*(*r*) type

Table 2 Segregation of different progeny types from Cross 1 [*a1-m*(*r*)/*a1-m*(*r*)×*a1-m*(*Au*)/*a1*] of selected families

Family	Plant no.	*a1-m*(*Au*)-type	*a1-m*(*r*)-type	Colorless	No. progeny[a]		
					(1)	(2)	(3)
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
Total					203	4	23

[a] Number of progeny ears recovered from a selection of 50 *a1-m*(*Au*)-type kernels in each family, based on the reciprocal cross Cross 2A [*a1-m*(*Au*)/*a1-m*(*r*)×*a1-m*(*r*)/*a1-m*(*r*)] and B [*a1-m*(*r*)/*a1-m*(*r*)×*a1-m*(*Au*)/*a1-m*(*r*)]. The order is the number of reciprocal crosses: (1) in which both ears were harvested, (2) only one ear was rescued when *En* was crossed as female, and (3) only one ear was rescued when *En* crossed as male, respectively

Table 3 Overall transposition frequencies of *En* illustrating the progeny types from the crosses in this study

Item	Sample[a]	Family A	Family B	Family C	Family D	Family E
a1-m(*Au*)-type	15431	10284	13102	12140	10593	14250
a1-m(*r*)-type	4450	2151	847	1102	808	1972
Total[b]	19881	12435	13949	13242	11401	16222
Colorless	16047	11396	13625	13196	11905	14770
Trans. freq. (%)[c]	22.38	17.30	6.07	8.32	7.09	12.16

[a] Sample population from Cross 1 in which *a1-m*(*Au*) was used as a male
[b] Total=*a1-m*(*Au*)-type+*a1-m*(*r*)-type
[c] Transposition frequency=(*a1-m*(*r*)-type/Total)×100

kernels (Fig. 1B) with a frequency among individual ears ranging from 0.32% up to 51.23% (data not shown). The *a1-m*(*r*)-type kernels result when the transposed *En* of *a1-m*(*Au*) transactivated the *I* element of the *a1-m*(*r*) allele. Each *a1-m*(*r*)-type kernel is a case of an individual transposed *En* from *a1-m*(*Au*). To further test the transposition frequency of *En*, five progeny ears from the 140 crosses were selected and designated as five families, A to E (each ear represents one family; Table 2). These five families fell into two classes. In the one class, three families (A, B and E) segregated some *a1-m*(*r*) type kernels, while the other two families (C and D) segregated no *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. Fifty standard *a1-m*(*Au*)-type kernels from each of the five families (A–E) were randomly selected, and reciprocal crosses were made with the *a1-m*(*r*) line (Crosses 2A and B).

These various crosses with the five families led to two features of transposition: the transposition mechanism, and the reciprocal differences in the frequency of *En* transposition. As to the transposition mechanism, examination of the kernel phenotypes on individual ears obtained from Cross 2A (see Materials and methods), revealed that *a1-m*(*r*)-type kernels are scattered randomly over the ear. No clear sectors of a group of *a1-m*(*r*)-type kernels were detected as a probable indication of somatic transpositions before or after replication during cob development. Thus, the random distribution of *a1-m*(*r*)-type kernels, where *En* was transmitted as a female, indicates that *En* transpositions are generally, if not always, independent events. These independent transposition events on each ear can, therefore, be interpreted to occur late during the ontogeny of ear development, specifically in a mitotic cell leading to a single megasporocyte through meiosis and prior to the development of the embryo sac. These studies reported here provide confirmation of independent excision events alluded to in previous *En* transposition studies (Peterson 1970; Nowick and Peterson 1981; Seo and Peterson 1996).

In the crosses illustrated (Cross 1), two phenotypes are expected: the *al-m*(*Au)*-type and the *a1-*(*mr*)-type from the transposed *En*s. Thus, 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 [the latter is expected to result from transpositions of *En* of the *a1-m*(*Au*)]. Thereby, transposition rates were estimated with the combined data from Crosses 2A and 2B for each family (Table 3). The estimated frequencies ranged, and averaged, approximately from 6 to 17%. Families A and E showed higher transposition-rates than the other families (B, C and D). All the families have lower transposition-rates than that of the sample population, whose rate was estimated with the data from Cross 1, in which *a1-m*(*Au*) was used only as a male. The higher transposition-rate of the sample population could be due to several factors: a higher transposition of *En* when transmitted as male, an overall rate of the different crosses, the presence of extra *En*s in some sample individuals, or possibly a combination of these factors.

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

Item	Family A		Family B		Family C		Family D		Family E	
	F	M	F	M	F	M	F	M	F	M
a1-m(*Au*)-type	4874	5410	6838	6264	6314	5826	5287	5306	7917	6333
a1-m(*r*)-type	943	1208	333	514	317	785	261	547	882	1090
Total[a]	5817	6618	7171	6778	6631	6611	5548	5853	8799	7423
Colorless	5155	6241	6955	6670	6400	6796	5768	6137	7830	6940
Trans. freq. (%)[b]	16.21	18.25	4.64	7.58	4.78	11.87	4.70	9.35	10.02	14.68

[a] Total=*a1-m*(*Au*)-type+*a1-m*(*r*)-type
[b] Transposition frequency=(*a1-m*(*r*)-type/Total)×100

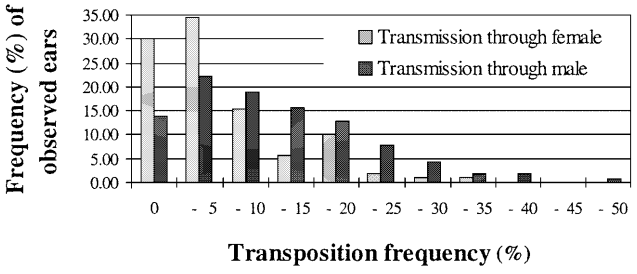

Fig. 2 Comparison of transposition frequencies of *En* when transmitted through female and male. The X-axis illustrates the transposition frequency of *En* observed on individual ears and the Y-axis shows the frequency of ears that displayed the transposition frequency indicated. For example, for *En* transmitted as a female, a large number of ears (35%) occur in the 5% transposition frequency column. However, for *En* transmitted as a male, only 22% of the ears are in the 5% column. More than 60% of the ears obtained when *En* was transmitted through the female showed transposition frequencies of zero or less than 5%, while most of the ears obtained when *En* was transmitted through the male showed a range of transposition frequencies from zero up to 25%

Reciprocal difference in the transposition frequency of *En*

A comparison was further made on the reciprocal differences of transposition rates of *En* when transmitted as female and male within each family. The transmission frequencies vary among the five families (Table 4). For *En* as a female, the range was approximately from 4.6% (family B) to 16.2% (family A). For *En* used as a male, the range was approximately from 7.6% (family B) to 18.2% (family A). Transposition frequencies in each family were consistently higher when *En* is transmitted as male than as female. Considering the overall transmission frequencies, more than 60% of the ears from Cross 2A (*En* as female) showed zero or less than 5%, while most of ears from Cross 2B (*En* as male) ranged up to 25% (Fig. 2).

Each reciprocal cross (of two ears from Cross 2A and Cross 2B, 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 (Table 2) from the five families, 24 reciprocal crosses revealed a highly significant difference at α=0.01 (three in A, five in B, eight in C, six in D, and two in E), and additionally 22 reciprocal crosses showed a significant difference at α=0.05 (five in A, three in B, five in C, two in D, and seven in E). Out of these 46 (24+22) reciprocal crosses, seven showed higher transposition frequencies when *En* was transmitted as female, indicating that the higher transposition of *En* as a male in a cross is expected to be preferable but was not always the case.

Analysis of sectored ears

From the sectored ears obtained from Cross 2A [*a1-m*(*Au*)/*a1-m*(*r*)×*a1-m*(*r*)] two were of special interest. The ear in Fig. 3A has a phenotype that is equally divided into two halves; one half includes the *a1-m*(*Au*)-type and colorless kernels, and the other half only colorless kernels. The other ear (Fig. 3C) has a phenotype that is divided into two unequal sections: one quarter contains three phenotypes [*a1-m*(*Au*)-type, *a1-m*(*r*)-type, and colorless kernels], and the remaining three-quarters includes two phenotypes [*a1-m*(*r*)-type and colorless kernels].

The colorless half of the ears in Fig. 3A most likely arose from some change in the transposed *En*, either from a change of state, such as a deletion within *En*, or an epigenetic change of phase of *En* after transposition. What is clear is that either of these events occurred **before** chromosome replication in one of the two initial cells giving rise to the ear. [It has been documented that the different cell lineages destined to give rise to the ear (2–4 cells) and tassel (2–4 cells) are pre-determined in the dormant embryo (Regiroli and Gavazzi 1976; Coe and Neuffer 1978; Johri and Coe 1983).] Transposition could occur after chromosome replication in the parent cell of the two initial cells. The putative diagrammatic sequence of cell division involving transposition with two initial cells is illustrated in Fig. 3B. Whether the change of state was involved in this ear can be resolved either by testcrossing with an autonomous *En* or by molecular analysis; however, no further tests were made.

Two transposition events could explain the phenotype of the ear in Fig. 3C. **One** transposition event from *a1-m*(*Au*) occurred **before** chromosome replication in one of the two initial cells and this gave rise to half of the ear resulting in one half of the ear with *a1-m*(*r*)-type and colorless kernels [Fig. 3C (top half)]. This could occur after chromosome replication in the parent cell of the two initial cells. **The second** transposition occurred after chromosome replication in the other initial cell or before chromosome replication in one of the two cells after one mitotic cell division and produced the other half that is equally divided into two parts: one part includes *a1-m*(*Au*)-type, *a1-m*(*r*)-type, and colorless kernels; and

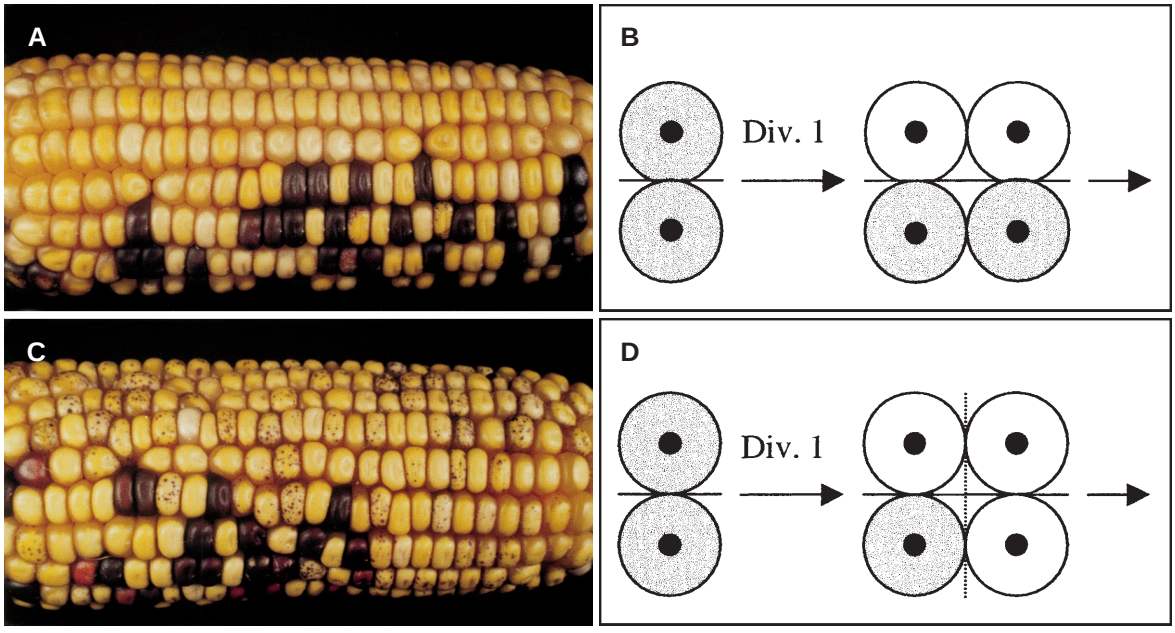

Fig. 3A–D Cross: *a1-m*(*Au*)/*a1-m*(*r*)×*a1-m*(*r*)/*a1-m*(*r*). **A** The ear illustrates two half sectors: one half includes the *a1-m*(*Au*) type and colorless kernels, and the other half only colorless kernels. **B** Diagramatic illustration of the transposition of *En* during cob development, leading to the formation of the ear of **A**. Each *circle* represents a cell. The two initial cells (*left*) are expected to be those formed during embryogenesis. With the *En* reporter *a1-m*(*r*) tester used, the colorless half most likely came either from a change of state, such as a deletion, within the *En* or the loss of *En* activity (epigenetic change of phase) after transposition. Either of these events occurred before chromosome replication (*Div. 1*) in one of the two initial cells. It could occur after chromosome replication in the parent cell of the two initial cells (data not shown). **C** The ear shows the phenotype that is divided into two unequal sectors: one quarter contains *a1-m*(*Au*)-type, *a1-m*(*r*)-type, and colorless kernels, and the remaining three-quarters includes *a1-m*(*r*)-type and colorless kernels. **D** Diagramatic 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** transposition before chromosome replication in one of the two initial cells gave rise to one half of the ear with *a1-m*(*r*)-type and colorless kernels. This could occur after chromosome replication in the parent cell of the two initial cells (data not shown). The **other** transposition after chromosome replication in the other initial cell produced the other half that is equally divided into two parts: one part of *a1-m*(*Au*)-type, *a1-m*(*r*)-type, and colorless kernels, and the other of *a1-m*(*r*)-type and colorless kernels. This could occur before chromosome replication in one of the two cells after one mitotic cell division (data not shown)

the other, *a1-m*(*r*)-type and colorless kernels. The putative diagrammatic sequence of cell division involving two transpositions at the stage of two initial cells is illustrated in Fig. 3D.

Discordance between the phenotypes of parent and reciprocal crosses

A discordance relates to the differences between the selected endosperm phenotype of the parent and phenotypes among the tested progeny that would reveal the embryo genotype. Out of 203 reciprocal crosses (see Crosses 2A and B and Table 2), seven had no parental *a1-m*(*Au*)-types among the progeny. Of the seven, there were two classes: (1) **the independent *En* class** – three reciprocal crosses (each from Family A, Family B, and Family E, respectively) produced both ears of only *a1-m*(*r*)-type and colorless kernels; (2) **the *En*-loss class** – four reciprocal crosses (one from Family A, one from Family D, and two from Family E) produced both ears of only colorless kernels. Thus, though the original selection included *a1-m*(*Au*)-type kernels (endosperm phenotype), the parental genotype (embryo) lacked the *a1-m*(*Au*) allele as revealed in the progeny test. These seven reciprocal crosses therefore illustrate a difference between the endosperm phenotype and the embryo genotype as they produced progeny of discordant phenotypes. The events implicated in this discordance are discussed in the next section.

Discussion

Transposition of *En* from the *a1-m*(*Au*) allele indicated two alternative events

Following the criteria of the transposition mechanism hypothesis (see Materials and methods), somatic transposition occurring before or after replication during cob development is an infrequent event. Of the 203 ears examined, only those shown in Figs. 3A and C showed this phenotype. The random distribution of transposition events (as disclosed by the *a1-m*(*r*)-type kernels) on the ear, where *En* was transmitted as a female, indicates that *En* transposes late during ear development and as single events in the cell leading to megasporogenesis. Very late transpositions during cob development affecting adjacent pistillate spikelets (Kiesselbach 1949) would be difficult to detect. Such

cases, where 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 kernels originating from adjacent spikelets], would not affect the estimated frequencies in this study (Tables 3 and 4). The calculated transposition rates are not very different from, or higher than, previous studies; about 20% (Nowick and Peterson 1981), 10–20% (Schwarz-Sommer et al. 1985), and 7.5% (Cardon et al. 1993).

Transposition after chromosome replication, when observed in individual kernels, is expected to produce twinned kernels with *a1-m*(*r*)-type and *a1-m*(*Au*)-type, as observed by Fedoroff (1983). Such twinned kernels, however, were infrequently observed. All *En*-related transposition studies including this one suggest that **most** individual transposition events occur before chromosome replication, though **sometimes after** chromosome replication. Cases of transposition after replication observed include ears of Fig. 3 and ears showing phenotypes discordant with the parent (see the last section of the Discussion).

Biased transposition frequencies in male- versus female-originated crosses

Transposition frequency of *En* from *a1-m*(*Au*) as measured by the occurrence of *al-m*(*r*)-type kernels on a ear is higher when transmitted through the male than through the female (Table 4). There are numerous reports of male versus female differences in transposition frequencies. 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).

Discordant phenotypes between the parent and its reciprocal progeny

Most of the progeny ears examined from Cross 1 [*a1-m*(r)/*a1-m*(*r*)×*a1-m*(*Au*)/*a1*] express the *a1-m*(*Au*) phenotypes, which are randomly distributed together with *a1-m*(*r*)-type and colorless kernels, and with the exceptions shown in the illustrations in Fig. 3. As noted, the general picture of the progeny of *a1-m*(*Au*) crossed as female includes a random distribution of *a1-m*(*r*)-type kernels. This results from single independent transpositions occurring late in ear development in a mitotic cell in the initials of the pistillate spikelet, leading to a single megasporocyte prior to fertilization. These single independent events are possible for most transpositions observed when *al-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.

Discordance of genotypes in individual kernels between endosperm and embryo were observed in seven cases: three cases of the independent *En* class and four cases of the *En*-loss class. If the original embryo genotype from Cross 1 was *a1-m*(*r*)/*a1-m*(*Au*), this discordance of the progeny phenotype with the parental phenotype could be explained as resulting from transposition events **before** cell differentiation to ear and tassel during embryogenesis or in all the initial cells destined in the embryo for 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 the 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 the expected phenotypes – *a1-m*(*Au*)-type, *a1-m*(*r*)-type, and colorless kernels; and the other without *a1-m*(*Au*)-type kernels). No such crosses, however, were observed.

Either hetero-fertilization (fertilization of a single embryo sac by two pollen grains) or transposition of *En* during late microsporogenesis may be offered as an explanation for discordant phenotypes between parent and progeny. For the transposition of *En* during late microsporogenesis, leading to the production of discordant progeny of the independent *En* class, the observation is unambiguous. However, the *En*-loss class can originate either from hetero-fertilization or from the loss of *En* activity. From Cross 1, where *a1-m*(*Au*)-type kernels were selected, hetero-fertilization is expected to produce kernels with an endosperm genotype of *a1-m*(*r*)/*a1-m*(*r*)/*a1-m*(*Au*) and an embryo genotype of *a1-m*(*r*)/*a1*. In Cross 2, these kernels will not produce the discordant progeny of the independent *En* class but only that of the *En*-loss class. Whether the *En*-loss class resulted from either hetero-fertilization or loss of *En* activity cannot be concluded in this study, but can be tested by molecular analysis (see below).

In the discordant independent *En* class, the parental embryo would carry a transposed *En*, which led to the formation of discordant progeny (see Results). Likewise, it is possible that the embryo of the *En*-loss class would have no *En* activity as a result of loss or inactivation. Under such a scenario, the embryo genotypes of the two classes would be an independent *En* class with *a1-m*(*r*)-type and colorless kernels, i.e., *a1-m*(*r*)/*a1**, *En*, and those of the *En*-loss class with colorless kernels which would only be *a1-m*(*r*)/*a1**, *En** (*a1**: recessive *a1* gene after transposition, *En**: lost or inactivated *En* after transposition).

Each of these genotypes can result from transposition after replication during the second pollen mitosis that yields two sperm nuclei during Cross 1 [*a1-m*(*Au*) as a male] (Fig. 4A). One sperm nucleus of an intact *a1-m*(*Au*) unites with the polar nuclei, and the other sperm nucleus of either *a1**, *En* (the independent *En* class) or *a1**, *En** (the *En*-loss class) unites with an egg. This results in the different genotypes between endosperm and embryo and these sequential events are dia-

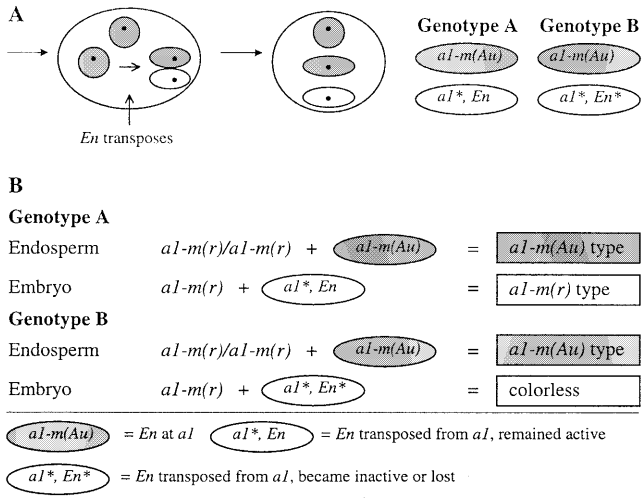

Fig. 4 **A** Late microsporogenesis prior to formation of the pollen grain: formation of two sperm cells via the second mitotic division. Two sperm cells carry different genotypes as a result of the transposition of *En*, most likely after replication during the second mitotic division. In genotype **A**, *En* is active after transposition from the *a1* gene. In genotype **B**, the transposed *En* (*En**) was lost or inactive. The *a1* gene left behind after transposition is nonfunctional [(*a1** and previously referred to as *a1-m*(*nr*), Peterson 1970)]. **B** Double fertilization with *a1-m*(*r*) as female results in discordant genotypes between embryo and endosperm. The fertilization of the pollen of genotype **A** with *a1-m*(*r*) produced the *a1-m*(*Au*)-type endosperm, but produced *a1-m*(*r*)-type kernels in the next generation of the cross with *a1-m*(*r*) (the independent *En* class). The fertilization of the pollen of genotype **B** with *a1-m*(*r*) produced the *a1-m*(*Au*)-type kernel, but produced colorless kernels in the next generation of the cross with *a1-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

grammed in Fig. 4B. Reciprocal crosses of plants grown from such kernels of discordant genotypes between endosperm and embryo (Cross 2) will produce progeny of different phenotypes as observed in those seven cases. A similar discordance of endosperm and embryo was reported in the study by Walker et al. (1997) of a new element *PIF* (*P* instability factor) at the *r* gene.

As mentioned previously, the colorless progeny of the *En*-loss class may either come from hetero-fertilization or transposition and loss of *En*. Hetero-fertilization that gave an *a1-m*(*Au*)-type kernel during Cross 1 is expected to produce the embryo of *a1-m*(*r*)/*a1*, while transposition and loss of *En* produces the embryo of *a1-m*(*r*)/*a1**. Since the genetic test cannot distinguish the parent null *a1* allele from the newly derived null *a1** allele, the molecular analysis of the sequence variation between these two null *a1* alleles is expected to solve the argument of hetero-fertilization versus transposition.

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