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A NINE-BASE PAIR DELETION DISTINGUISHES TWO *EN/SPM* TRANSPOSON ALLELES IN MAIZE:THEIR GENETIC ACTIVITY AND MOLECULAR DESCRIPTION

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Two *En/Spm*-transposable element alleles of the *A1* locus in maize *(Zea mays)* are described. One of the alleles is *al-m (papu)*, (PETERSON, 1961). The distinctive phenotype of this allele is characterized with pale and purple sectoring amidst large areas of no sectoring. The other allele, *al-m (Au),* appears full colored but is heavily mutating and expresses large colorless areas. These two alleles differ in the frequency of derivative products [*al-m(papu)*-colorless and pale exceptions vs *al-m(Au)*-mostly colorless exceptions]. A molecular description is provided in an attempt to explain these differences in phenotypes and derivative products. A nine-base-pair deficiency in Exon 2 of the *A1* locus of the *a1-m (papu*) allele originated following the origin of this allele and this

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deficiency is likely responsible for the differential phenotypes. The possible origin of this nine-base-pair deletion is discussed.

Key word: *Al* locus; *En/Spm* unstable alleles; induced deletions. transposable, transposable elements

INTRODUCTION

Gene sequences containing inserts, deficiencies or other alterations that are determined by comparing sequences of the same gene and adjacent sequences from diverse sources are no longer a surprise to the molecular geneticist (KENNEDY, 2007; PENNISI, 2007). Yet, these insert sequences and deletions persist and are maintained despite potential impairment of excision events (BERG and HOWE, 1989). These alterations are a common observation when Southern blots are examined as well as when nucleotide sequences are determined (BENNETZEN and RAMAKRISHNA, 2002; FU and DOONER 2003; SONG and MESSING, 2003; PENNISI, 2007). SCHWARZ-SOMMER *et al*. (1984) in an examination of the *wx-m8* allele insert found two additional inserts, a smaller one, 250 bp, and a second one that was more than 6 kb, each of which were unrelated to the mutation associated with the original *wx-m8* mutant. This chromosome 9 with additional inserts has since been identified with the W23 *Wx* containing chromosome (PURUGANAN *et al*. 1991). In the early days of sequence "hunting", unexpected inserts were identified. Now, (2008) with sequencing procedures vastly improved, these changes are frequently found.

WESSLER *et al* (1986) in a survey of waxy alleles, found variant forms that also included a number of unexpected inserts. In the examination of the *A1* gene, SCHWARZ-SOMMER *et al* (1987) uncovered a retrotransposon-like *Cin4* element in a sequence adjacent to the *A1* locus. Whether these variant forms were a consequence of transposable element (**TE**) involvement cannot be determined because the original parental allele is not available though these inserts had all the trademarks of TE's. Yet with the *En*-containing *a1-m (papu)* and *a1-m (Au)* alleles (PETERSON, 1961, 1970, 1978), this determination can be made because these two phenotypically differentiated alleles are derived from an original insert (PETERSON, 1956; 1961) and the two alleles do show sequence differences (MENSSEN, 1989).

The *En* transposable element originated as an unstable pale-green mutable allele (*pg-m*) (PETERSON, 1953, 1960) from material exposed to the Bikini Atoll atomic bomb tests (ANDERSON, 1948). This *pg-m* allele has subsequently been identified as the *g2* locus and has been sequenced (RUSSINI *et al,* 2001, CRIBB *et al*, 2001). In later experiments following the original *pg-m* study (PETERSON, 1960), it was demonstrated that the *En* element transposed to the *A1* locus on the same chromosome identified following the appearance as a colored sectored tassel in the *pg-m* material in the 1952 genetics nursery in Urbana, Illinois. In crosses of pollen from this tassel, the resulting progeny included an *a1-m (dense)* allele (PETERSON, 1956, 1961) and also a chlorophyll allele (W^{13}) yielding *w-m-13* (PETERSON, 1966) also on the same chromosome. Subsequently, many derivative alleles were

uncovered from the original *a1-m (dense)* allele, two of which have been studied and identified as *a1-m(Au)* and *a1-m(papu)* (PETERSON, 1970; NOWICK and PETERSON, 1981).

There were two options relative to the phenotypic differences in the two phenotypes. Were the differences changes in *En* or was the cause of the differential phenotypes in the *A1* sequence.

Because of the striking phenotypic difference in pattern appearance (Figures 1, 2) and high frequency of derivative products (PETERSON, 1970; 1978; 1981) between the two alleles as well as the differences in the distribution and type of derivatives, a molecular investigation was initiated to examine the nucleotide sequence of the element inserts in each of the two *A1* alleles. It was determined that a major feature causing these pattern differences resides in the flanking sequences of the *A1* gene and the origin of this difference sheds some light on the possible means by which mobile elements create DNA sequence changes.

MATERIAL AND METHODS

The two alleles, *a1-m (Au)* and *a1-m (papu)*, were isolated as derivative, distinguishable phenotypes from the original *a1-m(dense)* insert at the *A1* locus (PETERSON, 1956). The recurrent backcross parent, *a1 sh2*, represents a close linkage of the *a1* and *sh2* (shrunken 2) genes (.25 units).

The isolation of plant DNA: DNA was isolated as described by SCHWARZ-SOMMEr *et al.*, 1984.

The allele, *al-m(Au)* was cloned as Mbol partial digestion fragment into the lambda vector EMBL-4 as previously described by SOMMER *et al.* 1990. *A1* and *al-m(papu)* have been isolated by O'REILLy et al. (1985).

The 5'end of *En* and flanking *al-m(papu)* and *al-m(Au)* sequences were subcloned into *pUCp* vector (VIEIRA AND MESSING, 1982) as *XmmI/SalI* fragments, the respective 3' ends as *SspI/SalI* fragments (Figure 3).

Sequencing was according to MAXAM and GILBERT (1980).

All other methods for the studies in this report were performed as published earlier (MENSSEN *et al.* 1990).

RESULTS

The phenotypes:

a1-m (papu): This allele's distinctive appearance is readily distinguished from the *a1-m(Au)* allele. The *a1-m (papu* allele has large colorless and pale areas interspersed with dark colored sectors (Figure 1), thus, leading to the nomenclature of the allele pale (pa) and purple (pu). The purple sectors are much later occurring and in an aleurone that is colorless non-mutating (arrow, Figure 1). This coloration or lack of coloration on individual kernels (Figure 1) is correlated with the incidence and frequency of colorless and pale germinal derivatives and to a much

lesser extent full purple colored types among the progeny of test crosses (Table 1). It has previously been demonstrated that these colorless and pale types are an indication that an *En* has excised, most of the excisions are in a linkage distance from the insertion site (PETERSON, 1970; NOWICK and PETERSON, 1981).

Figure 1. *al-m (papu)* allele.
Arrow indicates the adjacent-colorless, pale, and purple-like colored sectors.

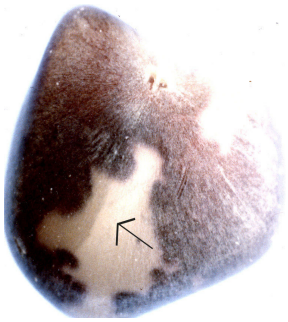

Fig.2 *a1,(Au)*/ The large colorless sector
The large colorless sectors give the appearance of a colored *A1* mutating to colorless *al*.
However, these colorless sectors are mutations to non-responsive (nr) types (Arrow indicates colorless, non-mutating sector).

a1-m (Au): On first examination, the full-colored appearance of the kernels that are accentuated with large colorless sectors (Figure 2, arrow), resemble the phenotypes of the *a1-m (papu)* kernels except for the pale sectors that are so prevalent with the *a1-m(papu)* allele. Because of this full-colored appearance, the allele was initially thought to be an unstable *A1* allele and thus called *a1-m (Au)*, (*A1* unstable).

Germinal derivatives: Origin of colorless, **n**on-**r**esponsive (*nr*) alleles

In testcrosses of each of the alleles (Cross 1)

$$\frac{a1\text{-}m\ (papu)\ Sh2}{a1\text{-}o\quad\ sh2} \ or\ \frac{a1\text{-}m\ (Au)\ Sh2}{al\text{-}o\quad sh2} \times \frac{a1\text{-}o\ sh2}{a1\text{-}o\ sh2} \qquad \textbf{Cross 1}$$

a large number of round (non-shrunken) colorless and pale and occasional colored (Table 1) derivative types arise. Such a distribution was previously reported (PETERSON, 1970). These stable colorless and pale derivatives are tested for the presence and relocation of the excised *En* by crossing with an *En* reporter [*a1-m(r)]* allele (Cross 2 and Figure 5).

a1-m (pale-nr) Sh2	x	*a1-m(r) Sh2*	**Cross 2**
a1-o sh2		*a1-m(r) Sh2*	

These derivatives are referred to as *nr* because they are stable and *non-responsive* to *En*. In this cross, an *En* is detected and often on the same chromosome as the originating site (PETERSON, 1970; NOWICK and PETERSON, 1980).

The frequency of the colorless derivatives from *al-m (papu*) is 28.4% (Table 1) and that of the pale derivatives is 16.9% (Table 1). The frequencies of colorless are more than the pales. (There is one full color derivative in ear 4). This is in agreement with the earlier study (NOWICK and PETERSON, 1981) where the pale derivatives occurred at a lesser frequency than the colorless derivatives. This unequal occurrence of the two types became explainable following the analysis of the nucleotide sequences.

Table 1 Round colorless and pale Derivatives from the cross a1-m(papu)/a1sh2 x a1sh2/a1sh2

ear	*a1m(papu*)	%	colorless	%	pale	%	purple	Total
1	84	56.0	31	20.6	35	23.2	0	150
2	77	55.7	41	29.7	20	14.4	0	138
3	104	55.0	64	33.8	21	11.1	0	189
4	30	42.8	23	32.8	16	23.1	1	69
Ave.				26,4		16.9		546

Adapted from Peterson, 1970 Table 1.

The *a1-m(Au)* allele also gives rise to a large number of colorless derivatives (Avg. 29.6%) (Table 2). In tests of a number of the colorless derivative products, the excised *En* was located residing most of the time in a nearby site on chromosome 3 (PETERSON, 1970; NOWICK and PETERSON, 1981). The derivatives represent a mixed class. Though some represent the excision of *En*, a number are fractured *En*'s whereby the derivative very dark pale (near purple) has lost some functional components of *En*, but has retained its terminal inverted repeats because some of these derivatives inserts do respond to *En* (PETERSON, 1989, 1995). However, the combined colorless and pales of *a1-m(papu)* represent a higher value to that of *a1-m(Au)* though there is ear to ear variation in derivative products (PETERSON, 1970). This would be consistent when the molecular findings are considered. One colored derivative from *a1-m(Au)* was a responding allele (PETERSON, 1995).

Nevertheless, though these two alleles originated separately from the same *a1-m* allele (PETERSON, 1961) they do have different phenotypes, frequencies and types of different derivative products. In order to uncover the basis of these phenotypic differences between the two alleles, a molecular analysis of the two alleles was initiated.

Table 2 Round colorless derivatives from cross a1m(Au) Sh/a1 sh2 xa1 sh2/a1 sh2

ear	Colored	*a1-m(Au)*	%	colorless	%	total
1	37	45	40.2	30	26.7	112
2	40	159	70.0	27	12	226
3	67	117	54.2	32	14.8	216
Ave.				29.6		551

Molecular Analysis of the a1-m(papu) and a1-m(Au) alleles

One *al-m (papu)* and four *al-m(Au)* clones were used in Southern Blot Analysis. The *al-m(papu)*-phage clone (110 *En*) and the four *al-m(Au)* phage clones (75, 58, 27, 16, 7) (Figure 3) were used to illustrate the position of the elements in Exon 2. The blot in Fig. 4. supports the contention that the two alleles are in Exon 2 of the A_1 gene, the same position 20 bases from the 5^0 of the exon (Fig.5.)

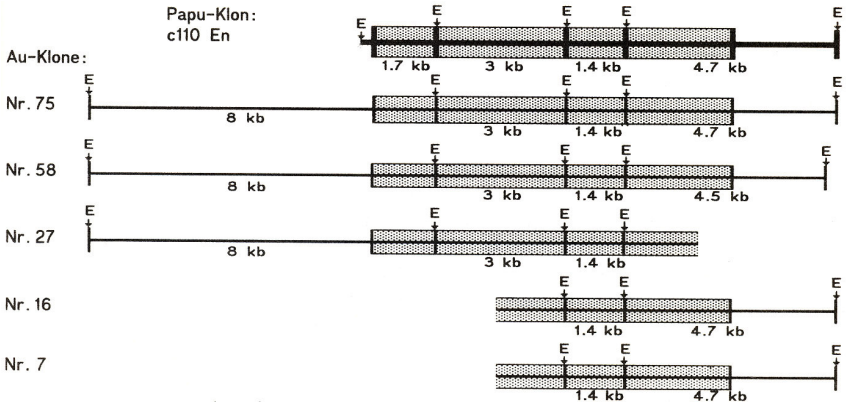

Figure 3. The *a1m(papu*) and *a1m(Au)* elements are the shaded areas. The dark line extending from E to E (E's on the end) is the *A1* gene

Fig. 4 Southern blot analysis (EcoR1) of the isolated clones were hybridized with Av1-Sal 1 fragment of the *A1* gene.

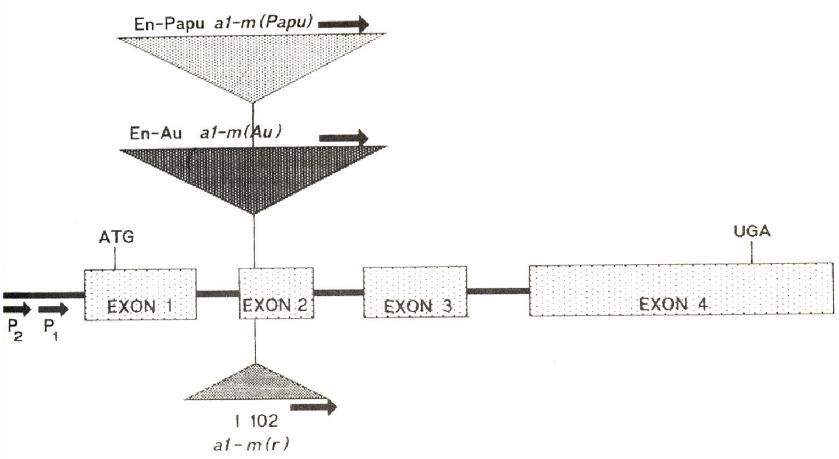

Figure 5. The positions of the *al m (papu)* and *al-m (Au)* alleles in exon 2 of *Al*, 20 bp from 5' of exon 2. The derivative, *al-(mr)-102)* is also at this site. This is a reporter allele used in Cross 2.

Analysis of the two elements: The 5' end (260bp) and the 3'end (850bp) (Figure 6) of the two elements *al-m(Au)* and *al-m(papu)* were sequenced. A T-C transition of base pair 7872 is seen in the *al-m(papu)* allele (Figure 7) changing a TTG codon to TCG. This point mutation lies in a non-translated region (Figure 7).

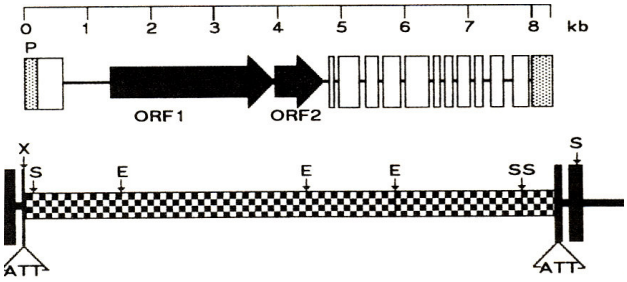

Fig. 6 The two figures; 260 bp from the 5' and 850 bp from the 3' of *En* in Exon 2 of the A1 gene (see Fig.5). The upper figure is the *En* element and the checked boxes in the lower figure shows the cuts in the subclones (not shown)

```
0                  7720                  7740
.                  .                     .                     .                     .
GGCGGCTCCAATGACCCACCAACAGAATGAATTAATATGGAGGCTTGTGTGGAACTT
-------+---------+---------+---------+---------+---------+---------
CCGCCGAGGTTACTGGGTGGTTGTCTTACTTAATTATACCTCCGAACACACCTTGAA

0                  7780                  7800
.                  .                     .                     .                     .
ATGATTGCGTTTTGTATGGACTTTAACTTGTTTTAGATGGATTTGAACTTCTTTCGT
-------+---------+---------+---------+---------+---------+---------
TACTAACGCAAAACATACCTGAAATTGAACAAAATCTACCTAAACTTGAAGAAAGCA

0                  7840                  7860
.                  .                     .                     .                  C  .
GACTTGAACTTGTATGAATATTGAATATGGTGCTTGTGTTATGTTATGTTTGAATATG
-------+---------+---------+---------+---------+---------+---------
CTGAACTTGAACATACTTATAACTTATACCACGAACACAATACAATACAACTTATAC

0                  7900
.                  .                     .
CTTGTGTTGTGATATATTGAATGTTGTG
-------+---------+---------+
GAACACAACACTATATAACTTACAACAC
```

Figure 7. Point mutation in Exon 11 *al-m (papu)* indicated by the T to C transition.

Nine-base pair deletion: In a comparison of the flanking sequences of the A_1 gene of the two alleles, it was discovered that there is a nine base pair deletion, in *al-m(papu)* relative to *al-m(Au)*. This nine base pair deletion is in Exon 2, forty-five base pairs distal to the site of the *En* insert (Fig 8).

```
AACGTTGGGAAGACGAAGCCATTGATGGACCTTCCCGGAGCAACGGAGCGCCTGTCCATA
---------+---------+---------+---------+---------+---------+
TTGCAACCCTTCTGCTTCGGTAACTACCTGGAAGGGCCTCGTTGCCTCGCGGACAGGTAT

TGGAAAGCCGACCTGGCGGAGGAAGGCAGCTTCCACGACGCCATCAGGGGCTGCACCG
---------+---------+---------+---------+---------+--------
ACCTTTCGGCTGGACCGCCTCCTTCCGTCGAAGGTGCTGCGGTAGTCCCCGACGTGGC
```

Figure 8. The site of the nine-base-pair deletion in the *al-m (papu)* alleles-45 bp 3' of the *al-m (papu)* insert.

Comparison of Exon 2 of the A_1 gene from Zea mays (LC), Zea mays asp parviglumis (Teosinte), Antirrhinum majus, and Hordeum vulgare.

A comparison of the four species shows conservation of the sequences in teosinte (Figure 9). The last line shows the nucleotides (large letters) that are conserved between the four species (Fig. 9). The sequences on either side of the 9bp deletion are conserved (Fig. 10).

```
AACGTTGGGA AGACGAAGCC ATTGATGGAC CTTCCCGGAG CAACGGAGCG LC
AACGTTGGGA AGACGAAGCC ATTGATGGAC CTTCCCGGAG CAACGGAGCG T
AATATGAAGA AGGTAAAACA CCTAATAGAA CTGCCAAAAG CAGACACGAA Am
GACCCCAAGA AAACGTTGCA CCTGCAGGCC CTGGAAGGAG CCAAGGAGAG H
aAcgtt..GA AgacgaagC. ..TgatgGac CT.cc.ggAG Caa.ggaG.g

CCTGTCCATA TGGAAAGCCG ACCTGGCGGA GGAAGGCAGC TTCCACGACG
CCTGTCCATA TGGAAAGCCG ACCTGGCGGA GGAAGGCAGC TTCCACGACG
CTTGACATTG TGGAAGGCAG ACATGACAGT AGAAGGAAGC TTCGACGAAG
GCTGCACTTG TTCAAAGCAA GCTTGTTGGA AGAAGGCACT TTCGATTCTG
ccTGtcc.T. TggAAaGC.g aCcTGgcgGa .GAAGGcAgc TTC.AcgacG

CCATCAGGGG CTGCACC
CCATCAGGGG CTGCACC
CAATTCAAGG TTGCGAA
CCATCGCCGG CTGTGAC
CcATcaggGG cTGc..c
```

Figure 9. Comparision of the nucleotide sequences of exon 2 of LC (line C-(*Zea mays*), T (Teosinte); AM *(Antirrhinum majus)* and H *(Hordeum)*. The site of the insert is indicated by a line over the nucleotides. The last line illustrates the conserved sequences between the 4 species. The conserved base pairs are indicated by cap letters, small letters indicate changes.

```
NVGKTKPLMD LPGATERLSI WKADLAEEGS FHDAIRGCT
NVGKTKPLMD LPGATERLSI WKADLAEEGS FHDAIRGCT
NMKKVKHLIE LPKADTNLTL WKADMTVEGS FDEAIQGCE
DPKKTLHLQA LEGAKERLHL FKASLLEEGT FDSAIAGCD
nv.Ktk.Lmd LpgAterLs. wKAdlaeEGs F.dAIrGCt
```

Figure 10. Amino acid sequences of the four species of Figure 9. The deletion of *al-m(papu)* is shaded.

DISCUSSION

The *En* insertions in the distinguishable *a1-m(papu)* and *a1-m(Au)* alleles reside at the same site in exon 2 of the *A1* gene. Their phenotypic difference (Figures 1 and 2) can be ascribed to the nine-base pair deletion in the distal side (3') of the insert, 45 bases away from the site of the insert. Each *En* of the two alleles excises approximately at the same rate from the *A1* locus. The elements themselves show no alterations in the nucleotide sequences (except for the T to C in exon 11 that have been sequenced or in restriction patterns).

al-m (papu): The nine base pair deletion maintained the reading frame of this allele and as such, provides a basic pale. This explains the phenotypic expression of the mutability as pale spotting (Figure 1) allele phenotype. The rare origin of purple colored spots or colored germinal derivatives (PETERSON, 1970) will be considered in a later paragraph. This is a rare event that has not been readily seen [(Figure 1 (PETERSON, 1970)].

The most frequent event is the origin of colorless germinal derivatives leading to a non-functioning allele. A large number of these have been tested (NOWICK and PETERSON, 1981; PETERSON, 1981 and unpublished) and found to be caused by transpositions from the exon 2 site and most of them to a nearby linked site. (Thus, the element has excised). Their frequency is symptomatic of deletion forming excisions following copying errors (COEN *et al*, 1989) as they represent a lack of gene action and these are non-responsive. The pales on the other hand could represent correct excisions, thus restoring the correct reading frame and the nine base pair deletion exposing the pale phenotype.

The difference in frequencies between the two germinal derivative types *al-m(papu)* (pale and colorless) is noteworthy. If the pales represent a correct excision event that maintains the reading frame, then it would follow that that frequency would represent the number of times that an excision event would occur that does not lead to an alteration at the insertion site. Since the pales are approximately one-third of the total recovered excisions, this would represent the recovery of the original sequence or an in-frame reading. This assumes that there is considerable flexibility in terms of the transcription unit leading to a functional protein. This correct recovery of unmutated excision sites is more frequent than

the excisions for *wx-m8* where less than 10% were a correct reading frame (SCHWARZ-SOMMER *et al*, 1985).

Original al-m allele: The original allele was a dense type [*a-1m(dense)*], identified by a large number of medium late spots (Figure 2b, PETERSON 1961). The two alleles, *a1-m(Au)*, and *a1-m (papu)* are derived exceptions, that were distinguishable by their phenotype (There were many others that have not been analyzed molecularly). Because the deletion is found with the *a1-m (papu)* allele, it would follow that the nine base pair deletion represents a post insertion event of the originating *a1-m* allele (PETERSON, 1956, 1961). In fact, this deletion occurred following the origin of *a1-m(Au)* and of course, the originating insertion of *En* in *Al*. Because both alleles appeared on different ears, the *a1-m (Au)* allele's flanking sequence represents the parental type and the *a1-m (papu)* is a derived type. [The molecular differences between *al-m(dense)* and *al-m(Au)* could not be determined as *al-m(dense*) is not available].

Origin of the 9-base pair deletion. How the deletion occurred is not known since no detectable change occurred with the element (though the complete sequence of 8000+ bases had not been determined). Nevertheless, if enough exceptions were pursued via molecular analysis, a number of alterations might be anticipated in the vicinity of insertion sites. Whether there is homology of the element with flanking sequences that would lead to deletion formation by exchanges remains a possibility. It is only because this was a nine-base pair deletion that maintained the reading frame could such an exception be recovered. Others, with out-of-frame changes would maintain the element but mutability might not be expressed due to a lack of message fidelity and consequently, anthocyanin coloration.

There have been numerous studies that illustrate the alterations at the site of excision (SCHWARZ-SOMMER *et al*, 1984; WESSLER, *et al*. 1986; MENSSEN *et al*., 1990) however here is a case where the element was retained and a nearby flanking sequence was altered. This would suggest that transposable elements could increase the local mutation rate without excising from the original position. How the nearby transposon could induce changes (in this case 45 bp away) is not known but an 8.4 kb transposon could be "*awkward*" in a replicating chromosome.

There is a possible explanation of the origin of the nine base pair deletion linked to the *a1-m (papu)* allele. If an excision took place from the sister chromosome of the *a1-m(papu)* allele, and the excised *En* transposed to the nearby site (46 bp away) and this was followed by a secondary excision from the 46 bp site, the deletion could have occurred at this site. The 46 bp site at the time of reception of the transposed *En* would be unreplicated. Following replication, an *En* would have been linked to *a1-m(papu)*. Subsequent transposition and accompanying excision error would leave the *a1-m(papu)* chromosome with a nine base pair deletion (Figure 8). This scenario is supported by the GREENBLATT (1984) model and the imposition of a deficiency by the *wx*-derived deletions from *wx-m8* (SCHWARZ-SOMMER *et al*., 1985), and the *wx-m9* cases (WESSLER *et al*., 1986).

Alternatively, it could be that the deletion was not caused by *En* but formation by another transposon residing in the original *al-m dense* material. This could be a transposon residing in the genome in the vicinity or approximately at that position in Exon 2 and excised again.

The purple spots in a1-m (papu): With the 9-base-pair deletion and the hypothesis that this is the pale-inducing factor, the appearance of purple spots presents a problem. There are several options to explain these purple spots.

(1) Could this be a second *A* allele expressing? Not likely, as there would be more purple spots and independent of the presence of the *a1-m (papu):*allele.

(2) Could it be a second-site mutation in the *A1* gene that suppresses the pale phenotype as has been shown for the tryptophane synthetase locus in E. coli (YANOFSKY and CRAWFORD, 1959)? Again here, the frequency is too high compared to the expected based on the Yanofsky and Crawford events.

(3) The reviewers suggested an alternaiive. Element excision followed by gap repair. This would require using the homologous chromosome as a template and this would restore the functional allele. This mechanism was demonstrated by ENGELS(1993) and JOHNSON-SCHLITZ, ENGELS (1993).

(4) The reviewers suggested an alternaiive. Element excision followed by gap repair. This would require using the homologous chromosome as a template and this would restore the functional allele. This mechanism was demonstrated by Engels(1993) and Johnson-Schlitz, Engels (1993).

Notes:

(1) In Table 1, the 16.9% pale are excisions that return the site from the original sequence. This leaves 28.4% colorless with the aborted repair.

(2) In Table 2, the 29.6% colorless are in the same category as 28.4% colorless of *al-m (papu).* As the colored in Table 2 could be mislabeled, some could be true revertants and some *al-m(Au).*

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DELECIJA DUŽINE DEVET BAZNIH PAROVA JE UZROK RAZLIKE *EN/SPM* TRANSPOZON ALELA KOD KUKURUZA*:* GENETIČKA AKTIVNOST I DESKRIPCIJA

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I z v o d

U radu su opisana dva alela transpozon elemenata *En/Spm* u *A1* lokusu kukuruza (*Zea mays)*. Jedan od tih alela je *A1-m* (*papu*); (Peterson, 1961). Dva različita fenotipa su karakteristična: svetli i crveni sektori su okruženi velikom površinom aleurona bez sektora. Drugi alel, *a1-m* (*Au*) daje potpuno obojen aleuron sa vrlo izraženim prisustvom mutacija koje se eksprimiraju kao bezbojni areali. Ova dva alela se razlikuju po učestalosti produkata derivata |*a1-m(papu)*- bezbojan i svetli delovi nasuprot *a1-m(Au)- većim delom bezbojni sektori*|. Izvršen je opis na molekularnom nivou da bi se objasnile razlike fenotipova i produkata derivata. Deficijencija devet baznih parova u Exonu 2 *A1* lokusa *a1-m (papu)* alela ima poreklo u tom alelu i delecija je verovatno odgovorna za pojavu različitih fenotipova. Diskutovano je i moguće poreklo delecije od devet baznih parova.

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