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The inveterate wanderer: study of *Enhancer* wandering on chromosome 3 in maize

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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 three 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*

Introduction

It has been well-established that maize transposable elements move from one chromosome position to another, or even onto different chromosomes. The studies illustrating this 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; Chen et al. 1987, 1992). In these studies the unique feature of the negative dosage effect of *Ac* (McClintock 1950, 1951) combined with the *P-vv* allele was utilized to isolate germinal events and examine transposition patterns associated with chromosome replication. Several studies have shown that *Ac* and its receptor element *Ds* mostly transpose 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; Healy et al. 1993; Osborne et al. 1991).

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

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preferences in both directions. In a study at the *wx-m8* allele (Schwarz-Sommer et al. 1985), the frequency of excision events was estimated to range from 10% to 20%. In *Arabidopsis* the 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 one reported by Nowick and Peterson (1981); it is thus termed 'vagabond' *En* (*En^{vag}*). Coincident with the origin of *En^{vag}* is the discovery 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) and 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, thereby 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 (Figure 2D). Further tests with other reporter alleles (Fig. 1) showed that a strong-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*, 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+× a1 sh2/a1 sh2*; cross 2 *a1-m(r) Sh2 En/a1-m1 sh2+×a1 et1/a1 et1*; cross 3 *a1-m(r) Sh2 En/a1 sh2+×a1-m1 sh2/a1-m1 sh2*; cross 4 *a1-m1 sh2 En/a1 Sh2+× a1-m1 sh2/a1-m1 sh2*.

Determination of transposition by chi-square (χ^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 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 the most deviant lines one by one (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 it 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 to 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 dataset 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 dataset with a high transposition frequency. One should realize that the chi-square test cannot detect a certain degree of short distant transposition events. It can only confirm two distantly spaced *En* elements that originated either by conservative or replicative transpositions.

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 case 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 ambigu-

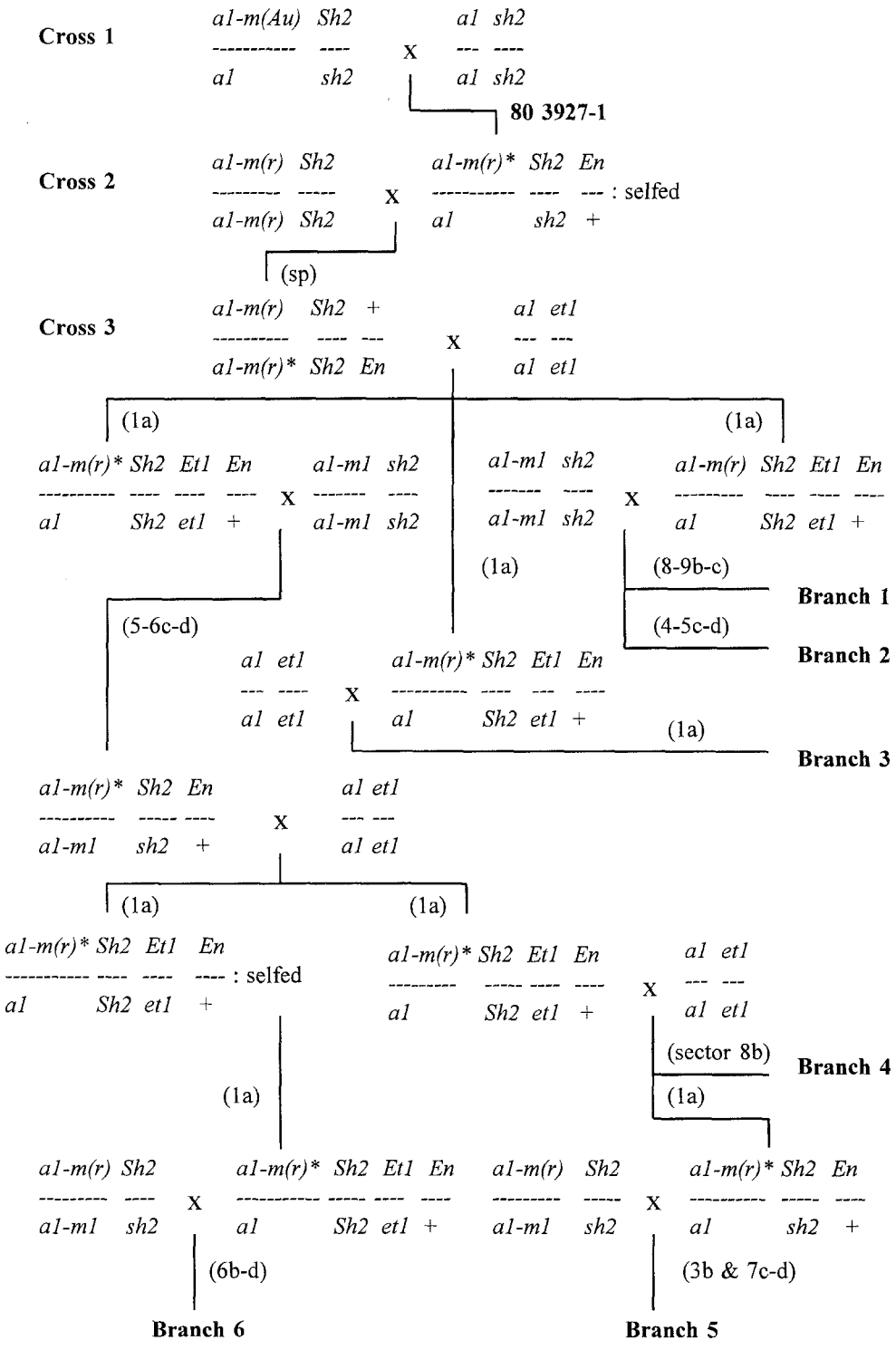

Fig. 1 Origin of *a1-m(r)3927-1* and En^{vag}. Series of crosses that were carried out to isolate *a1-m(r)3927-1* and En^{vag} and to generate the materials used in this experiment. *a1-m(r)3927–1* is marked with an asterisk (*) to distinguish it from *a1-m(r)*. Parental phenotype or mutability pattern 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 spots 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

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

Plant no.	Segregation			χ^2-test: α=0.05, d.f.=1, value=3.84)		
	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*: 1, linked; 2, independent; 3 extra *En*'s (see Materials and methods)

Fig. 2A–D Heritable phenotypes of *a1* alleles in the presence of *En*. **A**, *a1-m(Au)*, an autonomous allele, produces kernels that are almost completely covered with colorless sectors from the cross *a1-m(Au) Sh2/a1 sh2* × *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* + × *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* + × *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* + × *a1 sh2/a1 sh2*: this new allele produces one or two spots (1a-b), or no spot

ous 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 will therefore 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 a recombination of 45.96% (90 1650-23) was determined to be independent of *a1*.

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*×*a1 sh2/a1 sh2* (cross 1 in Fig. 1) were colorless and 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 pres-

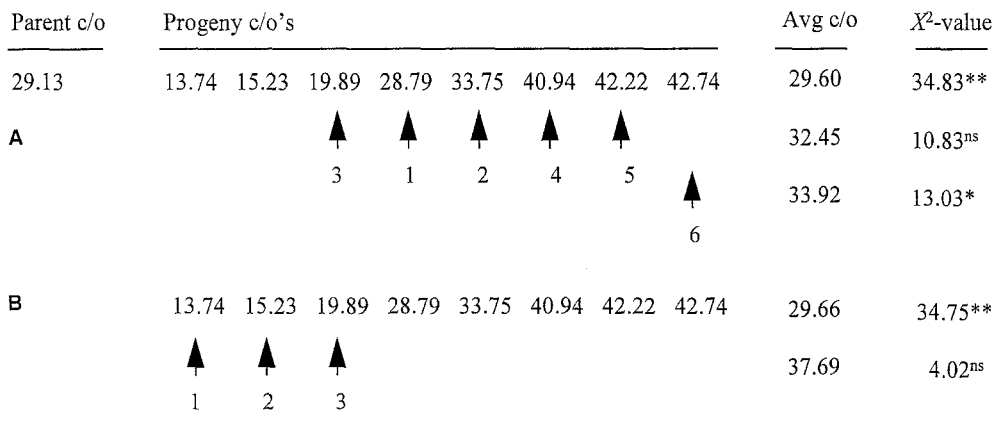

Figure 3A, B Comparison of chi-square (χ^2) test procedures: the inclusion chi-square test (**A**) developed for this study and the exclusion chi-square test (**B**) used in the previous study by Nowick and Peterson (1981). $\chi^2=\Sigma[(O-E)^2/E]$ (O=observed c/o's for individual ears, E=expected value, equals the average c/o of all observed c/o's). *Significant. **Highly significant. ^{ns}Not significant. Part of the dataset 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

ence of a transposed *En* and selfed them as well 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)*, densely 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_1s by crossing to *a1 et1* (cross 3 in Fig. 1), the kernels of a noticeable number of the progeny colorless, plump and very lightly spotted (Figure 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 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).

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 many frequent transpositions as selection of the former. Whether DNA modification such as methylation or rearrangement occurred in these *En*'s is a pos-

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

Year	Number of ears considered				% of ears		Ratio (%)
	Total	Trans-positions	Inde-pendent	Linked	Trans-positions	Inde-pendent	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

sibility, 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).

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 in one parent class is expected to be equal to that in the other class, and this is also expected for recombinant classes. All of the families listed in Table 3, however, showed an unexpected segregation; the size of the opposite parental or recombinant segregates was 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. However, 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* and 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 used 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 results confirmed and classified each entry in Table 3

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 + × a1-m1 sh2/a1-m1 sh2*

Plant no.	Parent C/O[b]	Segregation[a]				Total	Donor C/O[c]	Trans C/O[d]
		sp pl (P)	Cl pl (R)	Cl sh (P)	sp sh (R)			
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[e]	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
90 5012-12*	13.13	135	1	40	105	281	0.74	72.41
90 5013-2	6.54	146	10	98	42	296	6.41	30.00
90 5013-10	6.54	101	4	53	35	193	3.81	39.77
90 5014-16	6.54	125	13	49	59	246	9.42	54.63

Cross 2: *a1-m1 sh2 +/a1 Sh2 En × a1-m1 sh2/a1-m1 sh2*

Plant no.	Parent C/O[b]	Segregation[a]				Total	Donor C/O[c]	Trans C/O[d]
		sp pl (P)	Cl pl (R)	Cl sh (P)	sp sh (R)			
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] 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 is 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 is 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 a 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 cases of transposition when inferred from the difference between parent c/o and donor c/o (see text for details)

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 chomosome (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).

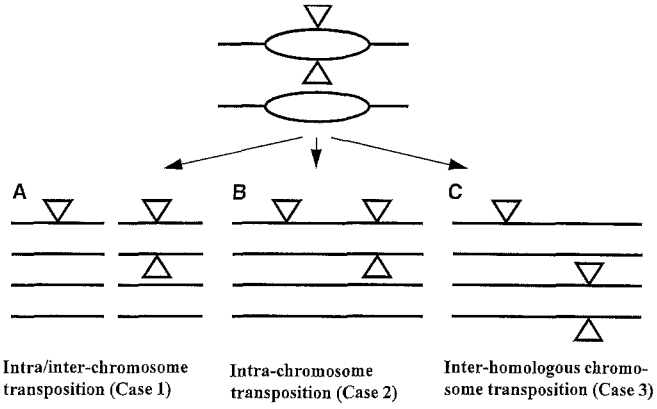

Fig. 4 Replicative transposition models of *En* to explain the aberrant segregation due to extra *En*'s. For each case, refer to Table 4. **A**, Transposition occurs after replication of *En* to an unreplicated site independent of the donor site, leading to a second replication. Whether the insertion 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 possessing one element. This pattern supports case 3 in Table 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 single crossovers between donor c/o and trans c/o (total number of kernels 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 (Fig. 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 had 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, sev-

Table 4 Methods of genetic and chi-square tests to verify aberrant segregation ($df = 3$, $\alpha = 0.05$, 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

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

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

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

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

Phenotype[a]	Plant no. 88 4659-25			
	Observed	Expected	Expected	χ^2-value
sp pl (P)	120	108.39	108.39	1.24
Cl sh (P)	28	108.39	27.10	0.03
Cl pl (R)	4	3.62	3.61	0.04
sp sh (R)	72	3.62	84.90	1.69
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 (Fig. 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 occured to an independent site, independent segregation of the *trEn* turns half the parent Clsh into sp sh (84 = 168/2), and half add to the number of the recombination sp sh (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 [187.21 = 225.48 – 38.27 = (462 × 0.1657/2)], and half (sp sh) add to the recombinant sp sh (43.79 = 5.52 + 38.27)

Case 3 Given that transposition occurred to the homologous chromosome, three-quarters of the gametes carry *En*, allowing a quarter of parent Cl sh to have no *En* thereby remaining Cl sh (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)

Table 5 Chi-square test of families from Table 3 to classify each into a specific case demonstrated in Table 4 ($df = 3$, $\alpha = 0.5$, value = 7.81)

Cross 1: *a1-m(r) Sh2 En/a1 sh2 + × 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
90 5012-12	13.13	0.14	0.00	0.76	0.00	0.90	3	71.68
90 5013-2	6.54	0.40	0.03	0.30	0.13	0.86	2	23.59
90 5013-10	6.54	0.72	0.03	0.45	0.30	1.50	2	35.96
90 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 × a1-m1 sh2/a1-m1 sh2*

Plant no.	Parent C/O[b]	Segregation[a]				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

eral families were not 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 twice as inferred from the difference between parent and donor c/o's. Whereas parent and donor sites of *En*'s in these four families seemed 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* appeared (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 a 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 (Mensen 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 the 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 of the trans c/o's were larger than donor c/o's (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 our study support these observations. Extra *En*'s in the progeny relate to about 5% of the experimental lines and appear as 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 distinguishable from 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 the 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 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 the 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 any explanation or data, that *Ac* excised from the *bz1* locus creates and thus leaves a *Ds* at *bz1*, even though an *Ac* is 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 of 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 thus this mechanism 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 have been 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 the 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 the *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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