Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11674–11678, November 1994
Evolution

Molecular evolution of *magellan*, a maize Ty3/*gypsy*-like retrotransposon

(transposable element/interspecific hybridization/*Zea*/*Tripsacum*/waxy allele)

MICHAEL D. PURUGGANAN*† AND SUSAN R. WESSLER*‡§

Departments of *Botany and ‡Genetics, University of Georgia, Athens, GA 30602

Communicated by Wyatt W. Anderson, July 28, 1994

ABSTRACT **The *magellan* transposable element is responsible for a spontaneous 5.7-kb insertion in the maize *wx-M* allele. This element has the sequence and structural characteristics of a Ty3/*gypsy*-like retrotransposon. The *magellan* element is present in all *Zea* species and *Tripsacum andersonii*; it is absent, however, in the genomes of all other *Tripsacum* species analyzed. The genetic distances between *magellan* elements suggest that this retrotransposon is evolving faster than other *Zea* nuclear loci. The phylogeny of *magellan* within *Zea* and *T. andersonii* also reveals a pattern of interspecies transfers, resulting in the movement of *magellan* subfamilies between different species genomes. Interspecific hybridization may be a major mechanism by which this retrotransposon invades and establishes itself in new taxa.**

Retrotransposons are a class of transposable elements that encode reverse transcriptase and transpose via an RNA intermediate (1). The elements are widely distributed and comprise a significant fraction of eukaryotic genomes. In *Drosophila melanogaster*, retrotransposons such as *copia*, *gypsy*, and *412* are believed to comprise nearly 2% of the genome (1) and are responsible for as much as 80% of spontaneous mutations (2). Retrotransposons have been described in 11 plant taxa; most of these elements were discovered as restriction fragment length polymorphisms or as preexisting insertions within nuclear loci (3, 36). Reverse transcriptase sequences have also been isolated by PCR from 68 plant species, including bryophytes, pteridophytes, gymnosperms, and angiosperms (4, 5). These reverse transcriptase sequences are presumably associated with yet unidentified retroelements (5).

Although numerous retrotransposons have been isolated and characterized, relatively little is known about the molecular evolution of these mobile sequences. The mode and tempo of retrotransposon evolution are largely undefined, and the forces that shape retrotransposon sequence evolution are poorly understood (4, 5, 37). It has been suggested by several workers that transposable elements evolve differently from conventional nuclear loci, since transposons can move within genomes and contribute to genetic variation (6–10). Evolutionary rates between retrotransposon sequences may also differ significantly from the evolutionary rates of other nuclear genes (10). Several plant species, for example, are known to harbor highly divergent reverse transcriptase sequences within their genomes (5, 6); the heterogeneity of these sequences is attributed in part to the high mutation rates associated with retrotransposition (5). There is also evidence that retrotransposons may possess the ability to move horizontally across reproductive barriers that isolate species (5, 6, 11).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Understanding the evolutionary biology of retrotransposons requires data on the extent and patterns of molecular variation within element families. In this report, we describe a low-copy-number retrotransposon whose presence within the phylogenetically well-defined genus *Zea* makes it an excellent subject for the study of retrotransposon evolution. This Ty3/*gypsy*-like retrotransposon, which we have named *magellan*, is responsible for a 5.7-kb insertion in a spontaneous mutant allele of the maize waxy (*wx*) locus. The *magellan* element is also found in *Tripsacum andersonii* but is absent in all other *Tripsacum* species tested. We find that this retrotransposon is evolving faster than other nuclear loci and that interspecies transfer of *magellan* elements appears to have occurred within the genus *Zea*.¶

MATERIALS AND METHODS

Strains and General Techniques. Strains carrying the *wx-M* allele were obtained from O. Nelson (University of Wisconsin). Seeds and genomic DNA of *Zea* (from J. Doebley, University of Minnesota) and *Tripsacum* (from S. White, University of Georgia) species were obtained as indicated. Genomic DNA from 14-day-old maize seedlings was isolated by the method of Shure *et al.* (12).

Element Isolation and Sequence Analysis. The *wx-M* insertion was previously localized by Southern blot analysis of genomic DNA (13). This insertion was cloned on a 7.8-kb *Sal* I fragment following ligation of size-fractionated genomic DNA into the λ vector ZAPII (Stratagene) and screening of recombinant phage with radioactively labeled *wx* probes. A 1.4 kb *Sst* I and a 5.2-kb *Kpn* I fragment containing the 3′ and 5′ ends of the insertion, respectively, were subcloned into pUC119 and the insertion termini were sequenced (14).

Both the 5′ *magellan* termini and reverse transcriptase sequences were analyzed separately by PCR amplification. The *magellan* 5′-terminal primer sequences are 5′-TGTCAGGAGACTGACGCAGC-3′ (primer 1) and 5′-GGGTCGTTGCGGTCTACTGC-3′ (primer 2). The reverse transcriptase sequences are 5′-CTAAGCTTTAYCAYCARHTNMGNAT-3′ (primer 3) and 5′-TCGAATTCTGNCCNARNMDYTYNAC-3′ (primer 4). Primers 3 and 4 were designed to recognize conserved coding regions in Ty3/*gypsy*-like elements (15). PCR conditions were as described (13); annealing temperatures as low as 52°C were used in screening for the presence of *magellan* long terminal repeat (LTR) sequences. PCR amplification of *magellan* 5′-terminal sequences was conducted with genomic DNAs from *Zea mays* ssp. *mays*, *Z. mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis*, *Z. mays* ssp. *huehuetenangensis*, *Z. luxurians*, *Z. diploperennis*, *Tripsac-*

Abbreviation: LTR, long terminal repeat.
†Present address: Department of Biology, 0116, University of California at San Diego, La Jolla, CA 92093.
§To whom reprint requests should be addressed.
¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U03916–U03968).

um andersonii, T. dactyloides, T. australe, T. peruvianum, and *T. cundinamarce* as templates. The reverse transcriptase sequence was amplified from *wx-M*-containing clones. PCR-amplified DNAs were cloned in pCRII by the TA cloning procedure (Invitrogen) and sequenced by either the dideoxy sequencing method (Sequenase; United States Biochemical) or the automated sequencing facility of the University of Georgia Molecular Genetics Instrumentation Facility.

Phylogenetic Analyses. *magellan* sequences were aligned contiguously beginning at nucleotide 30 of the 5′ LTR, and gaps were added by visual inspection to maximize similarity. Phylogenetic trees were inferred under maximum parsimony by using the heuristic search algorithm of the PAUP 3.0 program (16). *magellan* subfamilies were determined by inferring within-species element phylogenies and identifying monophyletic element groups. These subfamilies contain *magellan* copies that share at least 97% sequence similarity. *magellan* copies whose sequence were closest to the element consensus for a subfamily were chosen as representatives for the groups; representative members from each subfamily were then used in all-*Zea/Tripsacum* analysis. The PHYLIP 3.3 package (17) was utilized in computing pairwise Kimura two-parameter DNA distances (18) under a transition/transversion ratio of 2. Both sequence and distance data were also used for tree building under the DNAPARS and neighbor-joining PHYLIP algorithms.

RESULTS

Structure of the Retrotransposon Insertion in *wx-M*. The *wx-M* allele was previously shown to contain a 5.7-kb insertion in a *Sal* I fragment that spans exons 1–9 of the 14 exons of the maize *wx* gene (19). The *wx-M* insertion was isolated and found to be located within *wx* exon 3 just 2 bp downstream of the intron 2/exon 3 splice junction. The insertion has some of the structural features of a retrotransposon (see Fig. 1), including (*i*) a 341-bp LTR at the 3′ end; (*ii*) short inverted repeats at the termini of the LTR, with TG. . .CA as the terminal dinucleotides; (*iii*) a 24-bp $tRNA^{Met}$ primer binding sequence adjacent to a truncated 5′ LTR sequence; and (*iv*) a 13-bp polypurine tract adjacent to the 3′ LTR. We have named this retrotransposon *magellan*. Although the *magellan* insertion within the *wx-M* allele contains a 3′ LTR, it appears to lack an intact 5′ LTR due to a deletion that removes much of the 5′ LTR as well as 25 bp of *wx* sequence (Fig. 1). The absence of an intact 5′ LTR in the *wx-M* insertion suggests that this *magellan* copy may be unable to further retrotranspose. PCR amplification of maize genomic DNA demonstrates that *magellan* sequences containing intact 5′ termini are present in other locations within the maize genome (see Fig. 3).

An open reading frame adjacent to the *magellan* 3′ LTR encodes an integrase domain present in the *pol*-like genes of other retroelements. The *magellan* integrase domain shares 45% amino acid identity with the yeast Ty*3* and *Lilium henryi del* integrase region (20) (Fig. 2*A*). By PCR amplification, a 310-bp sequence that encodes the *magellan* reverse transcriptase was isolated from *wx-M*-containing clones. This reverse transcriptase shares 43% and 48% amino acid identity with homologous sequences in Ty*3* and *del*, respectively (Fig. 2*B*).

FIG. 2. Alignment of the integrase (*A*) and reverse transcriptase (*B*) domains of *magellan*, yeast Ty*3*, and *Lilium del* retrotransposons. Dashes indicate gaps. Amino acid residues which are identical between *magellan* and the adjacent sequences are indicated by dots. *Ty3* and *del* sequences are from refs. 15 and 20.

***magellan* Sequences Within the Genera *Zea* and *Tripsacum*.** The presence of *magellan* within the genera *Zea* and *Tripsacum* was assayed in a PCR survey using primers that recognize the *magellan* 5′ LTR and an internal *magellan* sequence (Fig. 3*A*). These primers were located ≈590 bp apart. A fragment of approximately this size was detected following PCR amplification of genomic DNA from *Zea* species and *T. andersonii*. However, no PCR product was evident when other *Tripsacum* species were surveyed, even when a reduced annealing temperature was used in the reaction. DNA blot analysis with a *magellan* LTR sequence as the probe showed that this retrotransposon was present in four to eight copies in *Zea* and *T. andersonii* genomes (unpublished observations).

To investigate the molecular evolution of *magellan* in greater detail, the PCR-amplified *magellan* 5′-terminal regions were cloned and 7–10 independent clones from each species were sequenced. A total of 490 nucleotide sites were used in the analyses; this region includes most of the *magellan* 5′ LTR, the primer binding site, and internal sequences adjacent to the LTR. Of the 490 nucleotide sites examined, 230 sites were polymorphic between *magellan* copies within *Zea* and *T. andersonii*. Overall, the proportion of polymorphic sites was 0.47, which is higher than has been found for any other *Zea* nuclear loci investigated thus far (21, 22).

A large proportion of these site differences were nucleotide substitutions, although 27 insertion/deletion polymorphisms ranging in size from 1 to 4 bp were also observed. The distribution of polymorphisms among the isolated sequences reveals that *magellan* copies residing in *Zea* and *T. andersonii* genomes are organized into 14 distinct subfamilies (Table 1). One to three *magellan* subfamilies are found within each of the *Zea* genomes. The levels of within-species

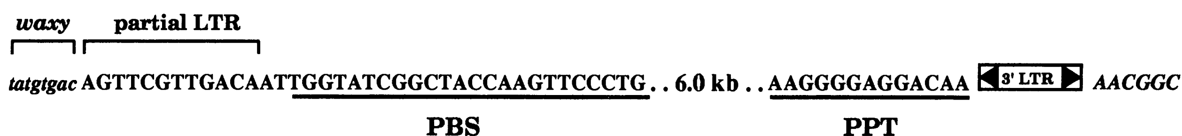

FIG. 1. Structure of the *wx-M* allele. The presumptive primer binding site (PBS) and polypurine tract (PPT) are indicated. The *wx* sequence is italicized and intron sequences are in lowercase. The *wx* intron 2 sequence and partial *magellan* 5′ LTR are also shown.

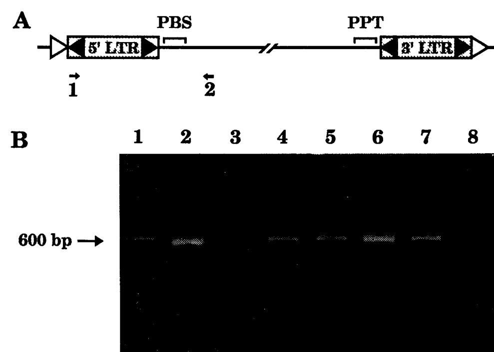

FIG. 3. PCR survey of *magellan* in the genus *Zea* and *Tripsacum*. (*A*) Structure of *magellan* giving approximate locations of PCR primers 1 and 2. The region between primers includes the 5′ LTR, the primer binding site, and ≈220 bp of internal sequence. Open triangles flanking LTRs indicate host-sequence direct repeats. (*B*) PCR-amplified 590-bp bands indicate presence of *magellan* in all *Zea* species and *T. andersonii*. Lanes: 1, 100-bp DNA ladder; 2, *Z. mays* ssp. *mays*; 3, *Z. mays* ssp. *parviglumis*; 4, *Z. mays* ssp. *huehuetenangensis*; 5, *Z. mays* ssp. *mexicana*; 6, *Z. diploperennis*; 7, *Z. luxurians*; 8, *T. andersonii*.

sequence polymorphisms are considerably higher than nucleotide variation levels within individual subfamilies (Table 1). The higher levels of within-species variation are due to a large number of fixed nucleotide differences that exist between different *magellan* subfamilies resident within a species genome.

To compare the evolutionary rate of *magellan* with that of other nuclear genes, interspecific two-parameter Kimura distances (18) were calculated for *magellan* sequences as well as a portion of the *Zea* nuclear gene *Adh2* (23) (Table 2). In every case, *magellan* has a higher interspecific nucleotide substitution value than *Adh2*. The mean ratio of interspecific *magellan* distance to the corresponding interspecific *Adh2* distance is 2.3. When only noncoding *Adh2* sequences are considered, the mean distance ratio drops to 2.

Phylogeny of *magellan*. Using parsimony analysis, we determined the phylogenetic relationships with *Zea* and *Tripsacum* (Fig. 4). The inferred tree was concordant with phylogenetic trees derived from a neighbor-joining analysis, as well as an alternative parsimony analysis using the DNAPARS algorithm of PHYLIP (unpublished observations). The *magellan* phylogeny was compared with a host *Zea* phylogeny previously constructed by using morphological, isozyme, and chloroplast DNA markers (24, 25). The comparison reveals two patterns of phylogenetic organization for this retrotransposon within the genus *Zea*. In one group of *magellan* subfamilies, shown without arrowheads in Fig. 4, element phylogeny agrees with the phylogeny of the host species. Within this group, *magellan* subfamilies from different *Z. mays* subspecies form a clade distinct from elements present in the *Z. diploperennis* and *Z. luxurians* genomes.

The phylogeny of a second group of *magellan* subfamilies, however, is incongruent with the *Zea* phylogeny; these subfamilies, indicated by arrowheads in Fig. 4, include (*i*) two *Z. mays* ssp. *mays* and ssp. *mexicana* subfamilies (mays2 and mex2) which are closely related to *Z. diploperennis* elements and (*ii*) a *Z. luxurians* element contained in a clade that includes *Z. mays* ssp. *parviglumis* and ssp. *huehuetenangensis magellan* elements. The anomalous location of these subfamilies is strongly supported by bootstrapping. All bootstrap replicates place the anomalous ssp. *mays* and ssp. *mexicana* subfamilies in the *Z. diploperennis*/*Z. luxurians* clade and group a *Z. luxurians* element within the *Z. mays* clade.

Finally, three distinct *T. andersonii magellan* subfamilies are found in several places within the retrotransposon phylogeny. One *T. andersonii* subfamily is closely related to *Z. luxurians* elements. Two other *T. andersonii magellan* subfamilies are in the *Z. mays* clade. One of these subfamilies groups with *Z. mays* ssp. *parviglumis* and ssp. *huehuetenangensis* elements.

Table 1. Nucleotide polymorphisms in *magellan* 5′-terminal regions

Species	No. of sequence polymorphisms			
	Ts	Tv	Indels	Total
Z. mays				
ssp. *mays*	30	17	8	55
mays1	5	9	3	17
mays2	5	0	1	6
ssp. *mexicana*	35	6	6	47
mex1	8	3	0	11
mex2	NA	NA	NA	NA
ssp. *parviglumis*	14	6	3	23
ssp. *huehue*	14	6	1	21
Z. diploperennis	28	11	4	44
dip1	10	6	3	19
dip2	4	0	2	6
Z. luxurians	51	7	5	67
lux1	7	0	2	9
lux2	32	8	3	43
lux3	NA	NA	NA	NA
T. andersonii	51	8	10	68
and1	3	2	0	5
and2	10	1	5	16
and3	NA	NA	NA	NA

Sequence polymorphisms within species and within subfamilies are shown. The within-species values include all polymorphisms found in comparing all sequences from a given species. NA, not applicable since only a single member of a subfamily was sequenced; Ts, transitions; Tv, transversions; indels, insertion/deletions.

DISCUSSION

Structure and Evolution of the *magellan* Retrotransposon. A maize retrotransposon is responsible for the large insertion found within the mutant *wx* allele *wx-M*. This retrotransposon, which we have named *magellan*, is also responsible for a spontaneous insertion in exon 1 of the maize *Pl* gene (P. Cooper, B. Kent, and K. Cone, personal communication). The sequence and location of both the integrase and reverse

Table 2. Comparison of interspecific distances between *magellan* and *Adh2*

	mays	*mex*	*par*	*dip*	*lux*
Z. mays mays	—	0.024 (3.5)	0.042 (1.6)	0.053 (2.5)	0.065 (2.8)
Z. mays mex	0.007	—	0.032 (NA)	0.043 (1.9)	0.071 (NA)
Z. mays par	0.026	NA	—	0.038 (1.1)	0.044 (1.3)
Z. dip	0.021	0.022	0.035	—	0.057 (4.1)
Z. lux	0.023	NA	0.035	0.014	—

magellan and *Adh2* distances are above and below the diagonal, respectively. All distances are in substitutions per site. *magellan*/*Adh2* distance ratios are shown in parentheses. NA, no interspecific comparison is available; *mex, mexicana; par, parviglumis; dip, diploperennis; lux, luxurians.*

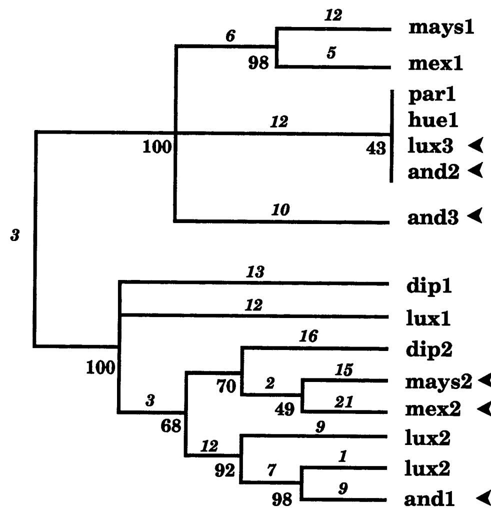

FIG. 4. Phylogenetic tree of *magellan* subfamilies within *Zea* and *Tripsacum*. The phylogeny was generated under maximum parsimony by using PAUP. The tree is an unrooted majority-rule tree with total length of 238 steps and a consistency index of 0.702. Numbers next to the nodes give percentage of bootstrap values that support the node. Italicized numbers above the branches give the mean number of changes along the branch. The *magellan* subfamilies whose placement in the tree is inconsistent with the *Zea/Tripsacum* phylogeny are indicated by arrowheads.

transcriptase domains encoded by *magellan* reveal that it belongs to the Ty*3/gypsy*-like element class (38). Only two other Ty*3/gypsy*-like retrotransposons have so far been reported in plant genomes: the *del* element of *Lilium henryi* (20) and the *IFG7* element of *Pinus radiata* (15). The *Lycopersicon* genome also contains several copies of *gypsy*-like sequences (3). The *magellan* element, however, is the only plant Ty*3/gypsy*-like element that has shown evidence of recent transposition.

The DNA sequences of the *magellan* 5′-terminal region within *Zea* species possess high levels of nucleotide polymorphisms (Table 1). The *magellan* interspecific pairwise distances are also more than twice as great as the interspecific distances for the *Adh2* gene (Table 2), suggesting that this retrotransposon is evolving at 2–3 times the rate of other, conventional nuclear loci. The elevated *magellan* substitution rates are not surprising, since retrotransposon mutation rates are affected by two replication mechanisms: (*i*) cellular DNA replication, which utilizes DNA polymerase, and (*ii*) reverse transcriptase-mediated replication during retrotransposition. RNA-dependent cDNA synthesis using reverse transcriptase is an error-prone mechanism, and mutation rates for this process are typically several orders of magnitude higher than those for DNA polymerase-replicated nuclear genes (26). Retrotransposon evolution may thus be governed both by slow accumulation of mutations through cellular replication and by bursts of substitutions associated with occasional retrotransposition.

Patterns of *magellan* Distribution: Direct Vertical Transmission vs. Interspecies Transfer. Transposable-element distribution patterns appear to be governed by two mechanisms: (*i*) direct vertical inheritance through an evolutionary lineage and (*ii*) interspecies transfer mechanisms. Vertically transmitted mobile elements behave like conventional nuclear multigene families; transposons present in a species' genome are directly inherited from an ancestral species. Interspecies transfer involves the movement of elements between taxa and may occur through a sexual mechanism (interspecific hybridization) or a nonsexual mode (horizontal transfer). The distribution of *Drosophila P* and *mariner* elements as well as several plant retrotransposons may be explained by either of these interspecies transfer pathways (4, 5, 27, 28). Most horizontal transfer events require vector mechanisms to facilitate transmission. In the case of *P* elements, for example, the mite *Proctolaelaps regalis* is believed to have participated in the movement of the element from *Drosophila willistoni* to *D. melanogaster* (28). For many systems, however, it is difficult to determine the precise mechanisms that permit interspecies transfer to occur (11).

Comparison of the *magellan* and host *Zea* phylogenies suggest that the element distribution pattern within the genus *Zea* results from both direct vertical and interspecies transfer mechanisms. Most *magellan* subfamilies appear to be vertically inherited within the genus *Zea*; support for vertical transmission comes from the correspondence between the phylogeny of these subfamilies and their host species. However, several *magellan* subfamilies within *Z. mays* ssp. *mays*, *Z. mays* ssp. *mexicana*, and *Z. luxurians* have phylogenetic relationships that are incongruent with the accepted *Zea* phylogeny. The retention of ancient *magellan* subfamilies in present-day species genomes could account for the anomalous placement of these subfamilies. This appears unlikely, however, since a number of element establishment and extinction events within the genus *Zea* would be required to explain the *magellan* phylogeny. A more probable explanation for this phylogenetic incongruence is that *magellan* interspecies transfer has occurred within the genus *Zea*. The reconstructed phylogeny provides evidence for the occurrence of at least two *magellan* interspecies transfer events: (*i*) a retrotransposon subfamily in *Z. diploperennis* moving into the genome of a common ancestor of *Z. mays* ssp. *mays* and ssp. *mexicana* and (*ii*) a *Z. mays* ssp. *parviglumis* and/or ssp. *huehuetenangensis* element transferring into the *Z. luxurians* lineage.

The movement of *magellan* between *Zea* species could occur through several different mechanisms, including horizontal transfer via insect, viral, or bacterial vectors (11). Interspecific hybridization, however, may be the primary mode by which *magellan* moves between species genomes. Several independent reasons support hybridization as the mechanism responsible for *magellan* interspecies transfer. All *Zea* species are interfertile and are capable of producing viable progeny (29). Moreover, all *Zea* species are in biogeographical contact in Mexico and Central America; different species can occasionally be found growing together in this region (25). Finally, the occurrence of low-level introgressive hybridization within this genus is supported by both isozyme (29) and molecular (22, 30) data.

Interspecies hybridization is considered a major factor in the evolution of flowering plants (31). Natural hybridization has several genetic consequences, including introgression of nuclear and organellar genes into parental species populations. Our findings suggest that hybridization may also facilitate the invasion and establishment of plant transposable elements in genomes of recipient species. Given the large number of plant species capable of interspecific hybridization, it may be that transposable elements are able to move more easily between plant species genomes than between animal genomes. Moreover, since transposition provides mobile elements with a built-in genetic drive mechanism, elements could move across hybrid zones more readily than conventional, nuclear loci.

Retrotransposons as Phylogenetic Markers. The *magellan* phylogeny provides information that may assist in addressing outstanding phylogenetic questions within *Zea* and *Tripsacum*. There has been interest, for example, in discovering the precise origin of *T. andersonii*. Based on cytogenetic and molecular evidence, *T. andersonii* is believed to be an intergeneric hybrid between unknown *Zea* and *Tripsacum* progenitors (32, 33). The *magellan* phylogeny reveals that *T. andersonii* elements are closely related to both *Z. luxurians*

and *Z. mays* subfamilies (Fig. 4). Based on the retrotransposon phylogeny, either *Z. luxurians* or *Z. mays* (or both) is the *Zea* progenitor of *T. andersonii*. Interspecies transfer of *magellan* is probably responsible for obscuring the precise ancestral relationship of *T. andersonii* elements. It is probable that *Z. luxurians* contributed the *Zea* genome to *T. andersonii*; biogeographical as well as rDNA data also support a *Z. luxurians* ancestor (32, 34). The *Z. mays*-like *magellan* elements may thus have hitchhiked into the *T. andersonii* genome via a *Z. luxurians* intermediate.

Transposable-element relationships could also aid systematists in determining the origin of domesticated maize. The chloroplast DNA phylogeny of *Zea* is ambiguous with regard to the maize progenitor, although isozyme analysis shows that *Z. mays* ssp. *parviglumis* is genetically closest to ssp. *mays* (24, 25). The *magellan* phylogeny, however, implies that the teosinte ancestor of maize is *Z. mays* ssp. *mexicana*. Morphological evidence supports *Z. mays* ssp. *mexicana* as the most likely candidate for the ancestor of *Z. mays* ssp. *mays* (33). However, the possibility of interspecies transfer, lineage sorting effects (35), and differing rates of element-copy evolution caution against drawing any strong conclusions on *Zea* evolution on the basis of retrotransposon phylogenies. In general, the rapid evolution of retrotransposon sequences compared with other nuclear genes may be of value in molecular systematic investigation at the infrageneric level. The presence of multiple elements within species genomes and the possibility of interspecies transfer of elements suggest that element sequences should be used in systematic investigation only in conjunction with other molecular and classical phylogenetic markers.

We are grateful to J. Doebley, M. Arnold, J. F. McDonald, A. Jaworski, R. Malmberg, L. Gunter, and E. Friar for stimulating discussions. M. J. Varagona isolated and characterized the initial *wx-M* clones. This work was supported by a grant from the National Institutes of Health to S.R.W.

1. Bingham, P. M. & Zachar, Z. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 485–502.
2. Green, M. M. (1988) in *Eukaryotic Transposable Elements as Mutagenic Agents*, eds. Lambert, M. E., McDonald, J. F. & Weinstein, I. B. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 41–50.
3. Grandbastien, M. A. (1992) *Trends Genet.* **8**, 103–108.
4. Flavell, A. J., Dunbar, E., Anderson, R., Pearce, S., Hartley, R. & Kumar, A. (1992) *Nucleic Acids Res.* **20**, 3639–3644.
5. Voytas, D., Cummings, M., Konieczny, A., Ausubel, F. & Rodermel, S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7124–7128.
6. McDonald, J. F. (1990) *BioScience* **40**, 183–191.
7. Maruyama, K. & Hartl, D. (1991) *Genetics* **128**, 319–329.
8. Charlesworth, B. & Langley, C. H. (1989) *Annu. Rev. Genet.* **23**, 251–287.
9. Vanderwiel, P. L., Voytas, D. F. & Wendel, J. F. (1993) *J. Mol. Evol.* **36**, 429–447.
10. Purugganan, M. D. (1993) *Trends Ecol. Evol.* **8**, 239–243.
11. Kidwell, M. (1992) *Genetica* **86**, 275–286.
12. Shure, M., Wessler, S. R. & Fedoroff, N. V. (1983) *Cell* **35**, 225–233.
13. Wessler, S. R. & Varagona, M. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4177–4181.
14. Vieira, J. & Messing, J. (1987) *Methods Enzymol.* **153**, 3–11.
15. Xiong, Y. & Eickbush, T. H. (1990) *EMBO J.* **9**, 3353–3362.
16. Swofford, D. (1991) *Phylogenetic Analysis Using Parsimony* (Illinois Natural History Survey, Champaign).
17. Felsenstein, J. (1991) *PHYLIP: Phylogenetic Inference Package Version 3.3* (University of Washington, Seattle).
18. Kimura, M. (1980) *J. Mol. Evol.* **16**, 111–120.
19. Varagona, M., Purugganan, M. D. & Wessler, S. R. (1992) *Plant Cell* **4**, 811–820.
20. Smyth, D. R., Kalitsis, P., Joseph, J. & Sentry, J. W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5015–5019.
21. Shattuck-Eidens, D., Bell, R. N., Neuhausen, S. L. & Helentjaris, T. (1990) *Genetics* **126**, 207–217.
22. Gaut, B. & Clegg, M. T. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 5095–5099.
23. Goloubinoff, P., Paabo, S. & Wilson, A. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 1997–2001.
24. Doebley, J., Renfroe, W. & Blanton, A. (1987) *Genetics* **117**, 139–147.
25. Doebley, J. (1990) *Maydica* **35**, 143–150.
26. Holland, J., Spindler, K., Horodyski, F., Grabau, E., Nichol, S. & Vandepol, S. (1982) *Science* **215**, 1577–1585.
27. Robertson, H. (1993) *Nature (London)* **362**, 241–245.
28. Houck, M. A., Clark, J. B., Peterson, K. & Kidwell, M. G. (1991) *Science* **253**, 1125–1129.
29. Doebley, J. F. (1983) *Ann. Mo. Bot. Gard.* **70**, 32–70.
30. Doebley, J. (1989) *Evolution* **43**, 1555–1559.
31. Arnold, M. L. (1992) *Annu. Rev. Ecol. Syst.* **23**, 237–261.
32. deWet, J., Fletcher, G., Hilu, K. & Harlan, J. R. (1983) *Am. J. Bot.* **70**, 706–711.
33. Wilkes, H. G. (1967) *Teosinte: The Closest Relative of Maize* (Busse Institute, Harvard Univ., Cambridge, MA).
34. Talbert, L., Doebley, J. F., Chandler, V. & Larson, S. (1990) *Am. J. Bot.* **77**, 722–726.
35. Avise, J. C. (1989) *Evolution* **43**, 1192–1197.
36. Voytas, D., Konieczny, A., Cummings, M. P. & Ausubel, F. (1990) *Genetics* **126**, 713–721.
37. MacRae, A. & Clegg, M. T. (1992) *Genetica* **86**, 55–66.
38. Boeke, J. & Corces, V. G. (1989) *Annu. Rev. Microbiol.* **43**, 403–434.