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Phantom, a New Subclass of *Mutator* DNA Transposons Found in Insect Viruses and Widely Distributed in Animals

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ABSTRACT

Transposons of the *Mutator* (*Mu*) superfamily have been shown to play a critical role in the evolution of plant genomes. However, the identification of *Mutator* transposons in other eukaryotes has been quite limited. Here we describe a previously uncharacterized group of DNA transposons designated *Phantom* identified in the genomes of a wide range of eukaryotic taxa, including many animals, and provide evidence for its inclusion within the *Mutator* superfamily. Interestingly three *Phantom* proteins were also identified in two insect viruses and phylogenetic analysis suggests horizontal movement from insect to virus, providing a new line of evidence for the role of viruses in the horizontal transfer of DNA transposons in animals. Many of the *Phantom* transposases are predicted to harbor a FLYWCH domain in the amino terminus, which displays a WRKY–GCM1 fold characteristic of the DNA binding domain (DBD) of *Mutator* transposases and of several transcription factors. While some *Phantom* elements have terminal inverted repeats similar in length and structure to *Mutator* elements, some display subterminal inverted repeats (sub-TIRs) and others have more complex termini reminiscent of so-called *Foldback* (FB) transposons. The structural plasticity of *Phantom* and the distant relationship of its encoded protein to known transposases may have impeded the discovery of this group of transposons and it suggests that structure in itself is not a reliable character for transposon classification.

TRANSPOSABLE elements (TEs) are mobile pieces of parasitic DNA that can replicate and move around in the host genome and are classified on the basis of their transposition intermediate (Craig *et al.* 2002). Class 1 transposable elements are mobilized via an RNA intermediate while class 2, or DNA transposons, mobilize via a DNA intermediate. TEs can be found in bacteria, archaea, and eukaryotes. Indeed, members of some superfamilies of cut and paste DNA transposons are common to all three domains of life, suggesting either their existence prior to the diversification of the three domains from a common ancestor or frequent interdomain horizontal transfer (HT) (Feschotte and Pritham 2007). Cut and paste transposons display a relatively simple structure where autonomous copies carry a single transposase gene flanked on either side by transposase binding sites (often the binding sites are embedded in terminal inverted repeats, TIRs) (Craig *et al.* 2002; Feschotte and Pritham 2007). Nonautonomous copies typically do not carry any transposase gene but instead carry just the binding sites and thus have the ability to move in *trans* utilizing the transposase encoded by an autonomous copy located elsewhere in the genome (Craig *et al.* 2002; Feschotte and Pritham 2007).

One class 2 TE superfamily, *Mutator* (*Mu*)/IS256, is common in bacteria, archaea, and plants but has been described in few other eukaryotes (Talbert and Chandler 1988; Lisch 2002; Chalvet *et al.* 2003; Xu *et al.* 2004; Pritham *et al.* 2005; Hua-Van and Capy 2008). The *Mu* system was first described by Donald Robertson in a line of *Zea mays* that exhibited increased mutation rates (50- to 100-fold) as compared to wild-type stocks (Walbot and Rudenko 2002). It was later discovered that these mutant stocks contained a 1.5-kb DNA insertion in the first intron of the *Adh-1* gene, causing changes in gene expression (Strommer 1982; Bennetzen *et al.* 1984). This insertion was sequenced and identified as a cut and paste DNA transposon called, *Mu1*, with TIRs and 9-bp target site duplications (TSDs) (Barker *et al.* 1984). *Mu* elements identified in maize have conserved TIRs ~220 bp in length, induce an 8- to 9-bp TSD, and have variable internal sequences (Lisch 2002). *Mutator* TEs have been described as the most mutagenic plant transposon (Lisch 2002). Fairly early on, a relationship was noted between the maize *Mutator* transposase with the transposase encoded by IS256 prokaryotic mobile elements (Byrne *et al.* 1989, 1990; Eisen *et al.* 1994). In addition, both *Mutator* TEs and IS256 insertions were shown to be flanked by 8- to 10-bp TSDs, which likely reflected a conserved feature of

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transposase function and further supported a common origin (Eisen *et al.* 1994).

Most of the work on *Mutator* has been done in plants where they have been shown to have a variety of impacts on the evolution of the genomes they invade. They not only cause an increase in mutation rates and changes in gene expression (Lisch 2002; Walbot and Rudenko 2002), but they have also been known to shuffle genomic sequences, including genes, in rice, *Arabidopsis thaliana*, and *Lotus japonicus* (Jiang *et al.* 2004; Hoen *et al.* 2006; Holligan *et al.* 2006; van Leeuwen *et al.* 2007). The transposons that have picked up gene fragments are called Pack-MULEs and typically do not carry a transposase (Jiang *et al.* 2004). *Mutator*-like (MULE) transposases have also been noted for their propensity to become domesticated by the genome and to have given rise to several key genes involved in light sensing in plants (Hudson *et al.* 2003; Cowan *et al.* 2005; Babu *et al.* 2006; Lin *et al.* 2007; Saccaro *et al.* 2007). In addition, *Mutator* elements have been reported to move via horizontal transfer between grass species (Diao *et al.* 2006).

MULEs have been described in various grasses (Yoshida *et al.* 1998; Mao *et al.* 2000; Lisch *et al.* 2001; Saccaro *et al.* 2007), in *A. thaliana* (Yu *et al.* 2000; Singer *et al.* 2001), and other eudicot plants (Holligan *et al.* 2006; van Leeuwen *et al.* 2007), and in two fungi (*Fusarium oxysporum* (Chalvet *et al.* 2003) and *Yarrowia lipolytica* (Neuveglise *et al.* 2005), the parabasalid, *Trichomonas vaginalis* (Lopes *et al.* 2009), and Entamoeba (Pritham *et al.* 2005). In addition to classic *Mutator* TEs, sequences distantly related to *Mutators*, but not complete transposable elements were described from *Entamoeba invadens* and *E. moshkovskii* (Pritham *et al.* 2005). The putative transposases from Entamoeba display only weak sequence identity to the pfam00872 *Mutator* transposase and were called *Phantom* (Pritham *et al.* 2005).

Here we present a comprehensive computational analysis of *Phantom* sequences and their distribution across the eukaryotic tree of life. Detailed structural analysis of these sequences reveals that they are bonafide class 2 TEs, which share structural and coding characteristics with *Mutator* TEs. The taxonomic distribution of *Phantom* illustrates that these elements are widespread in animals, found in a few distantly related protists and two insect viruses.

MATERIALS AND METHODS

Data mining and identification of elements: Candidate *Phantom* elements were identified using the *E. invadens Phantom* translated ORF [accession no. AANW02000107.1, coordinates 4912–7645 (complete element) and 5257–7012 (ORFs)] as a query in TblastN searches at National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), beginning January 2007 until the final preparation and submission of this article. Additional blast searches were conducted (primarily BlastN and TBlastN) using default parameters and without filtering for simple and complex repeats to identify copies of *Phantom* in different taxa. Searches were conducted against various GenBank databases including whole genome shotgun reads (WGS), nucleotide collection (NR), high throughput genomic sequences (HTGS), genome survey sequences (GSS), and expressed sequence tags (EST) databases. Accession numbers, reading frame, and nucleotide coordinates of all significant hits were annotated for further evaluation. A hit was considered significant when the *e*-value was $<10^{-4}$. TIRs were identified by pairwise comparisons taking 3000 bp upstream and downstream of each contig using Blast. TSDs were identified by aligning 100 bp upstream and downstream from the TIRs of the elements. To maximize the probability of identifying all probable *Phantom* elements, newly identified elements and putative proteins were used as queries using Blast against the WGS and NR database. Autonomous Phantom elements were used to identify related nonautonomous elements. The nonautonomous elements share the same TIRs but do not contain a transposase gene. Majority rule consensus sequences for *Phantom* were generated by constructing majority rule alignments using Clustal (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and MacVector 7.2.2. TE copy numbers for each genome were estimated on the basis of the results of TBlastN and BlastN using consensus sequences to search against the WGS and NR databases in GenBank. Hits with *e*-values lower than 10^{-4} were considered significant. Consensus sequences for all multi-element families can be found in supporting information, File S1.

Identification of open reading frames and conserved domains: Both the Translate (www.expasy.org/tools/dna.html) and ORF Finder tools (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) were utilized to identify open reading frames (ORFs) within *Phantom* elements through conceptual translations. When necessary, frameshifts were judiciously introduced according to nucleotide alignments of closely related sequences. The function of hypothetical *Phantom* proteins was predicted by homology to proteins of known function and by the presence of conserved domains identified through a conserved domain database (CDD) search (Marchler-Bauer *et al.* 2009).

Identification of paralogous "empty" sites: To illustrate the mobility of *Phantom* elements paralogous sites (empty sites) not containing a *Phantom* insertion were analyzed. Empty sites were identified by homology searches utilizing BlastN (word size 7, expectancy 1000) with a query constructed from the sequences directly flanking the insertion site containing the unduplicated target site. The chimeric query sequence (~100 bp in length) was created by extracting the flanking sequence (~50 bp) upstream from the element insertion containing the target site duplication and extracting the flanking sequence (~50 bp) downstream from the element insertion (lacking the element and target site duplication). Paralogous empty sites are defined as duplicated regions in the host genome homologous to the region where a *Phantom* insertion is found but that lacks the *Phantom* insertion.

Alignments and phylogenetic analysis: Alignments of *Phantom*, *Jittery*, *Hop*, *MuDR*, and *IS256/6120* putative catalytic core domains (~200 amino acids) were constructed using ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and MUSCLE (http://www.ebi.ac.uk/Tools/muscle/) using default parameters and visually refined using GeneDoc v3. Phylogenetic trees were created with Mr. Bayes 3.14 (Ronquist and Huelsenbeck 2003) using the amino acid model with a discrete γ-distribution with four rate categories and random starting trees. Two independent runs with four Markov chains each operating for one million generations with a sampling frequency set to 100 were utilized. Convergence was considered for the two runs when the standard deviation split frequencies was <0.001. The temperature difference between the "cold"

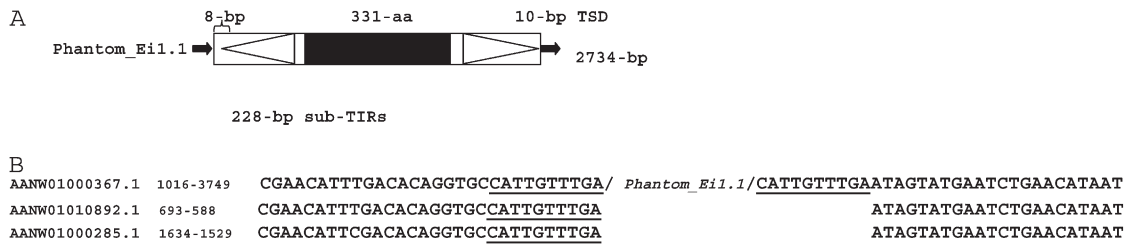


FIGURE 1.—*Phantom* transposable elements in *Entamoeba invadens*. (A) Schematic representation of a complete *Phantom* element in *E. invadens*. Small solid arrows represent target site duplications, subterminal inverted repeats are represented by the large open arrows, solid box represents the open reading frame (ORF). (B) Target site duplications (TSD) created upon *Phantom_Ei* insertions. TSDs are underlined.

chain and the "heated" chain was set to default parameters (temp = 0.2) to improve the chain swap. The first 200 trees recovered in these searches were discarded as burn, on the basis of stabilization of likelihood scores.

RESULTS

***Phantom* proteins from Entamoeba are encoded by bonafide transposable elements:** Sequences encoding putative proteins with weak similarity to *Mutator* transposase and designated *Phantom* have been previously identified in the genomes of *E. invadens* and *E. moshkovskii* (PRITHAM *et al.* 2005). However, whether these proteins were part of bonafide TEs and represent a new lineage of the *Mutator* TEs had not been further investigated. To characterize these putative TEs, the Entamoeba transposases were used as queries in TBlastN searches against the *E. invadens* genome shotgun sequence. This search yielded 49 significant hits. These contigs were subjected to pairwise alignment to reveal the boundaries of sequence identity and evaluated for the presence of structural features typical of DNA transposons, such as potential TIRs and TSD. Discrete units of ~2730 bp in length containing a single uninterrupted ORF (330–400 AA), bracketed by long (194–228 bp) and imperfect (82–100%) subterminal inverted repeats (sub-TIRs) flanked by a 7- to 12-bp TSD (Figure 1A) were identified. The proper boundaries of the TE were confirmed by the identification of paralogous (empty) sites containing the unduplicated target site (Figure 1B). These findings allowed us to show that Entamoeba *Phantom* proteins were carried by bonafide TEs, which display the structural features typical of DNA transposons (Figure 1). Together these data reveal that Entamoeba species harbor a novel lineage of DNA transposons distantly related to previously described TEs of the *Mutator* superfamily, in addition to an entirely distinct grouping of *Mutators* TEs previously described and called *EMULEs* (PRITHAM *et al.* 2005).

Identification of *Phantom* in other taxa: To identify other related TEs, queries representing the *E. invadens* *Phantom*-translated ORF were used in TBlastN searches against all the species with sequences deposited in the GenBank databases. Related ORFs were identified in 31 different species from various eukaryotic taxa including the planarian *Schmidtea mediterranea*, the annelid *Helobdella robusta*, a wide variety of nematodes and insects, the sea urchin *Strongylocentrotus purpuratus*, the ascidians *Ciona intestinalis* and *C. savigny*, several mammals (but not other vertebrates), one species of Candida yeast, three species of Phytophthora (oomycetes), and the Trichomonad *T. vaginalis* (Table 1). Much to our surprise *Phantom*-like transposases were also identified in two insect viruses, *Chelonus bracovirus* and *Glypta fumiferanae ichnovirus*.

Determining the coding potential and structural features of these novel TEs: To determine the structural characteristics and identify TIRs and TSD common to *Phantom* TEs pairwise comparisons of upstream and downstream flanking sequences (up to 3000 bp if available) were carried out (Figure 2A). To ensure that complete *Phantom* elements were properly delineated, searches for paralogous (empty) sites in the genome were performed (Figure 2B). Majority rule consensus sequences were constructed for each family of elements identified. These analyses allowed the identification of 77 complete *Phantom* TEs in 21 different species from various eukaryotic taxa representing 38 families (Table 1). The full-length copy numbers of *Phantom* in these taxa are generally low (one to three full-length copies) except for *Phantom* elements in *S. mediterranea* (eight full-length copies, 600 total copies), *T. vaginalis* (nine full-length copies, 780 total copies) and 2 Phytophthora species (approximately eight full-length copies, >300 total copies). Interestingly, some of the elements identified in *P. infestans* and *P. sojae* share high sequence identity (>99%) as compared to the consensus sequence, suggesting that these TEs have been recently active. Together these analyses expand the distribution of TEs related to *Phantom* to three of the five eukaryotic supergroups (as described by KEELING *et al.* 2005) including the Excavates and Chromalveolates, as well as the Unikonts.

Conceptual translations were used to identify ORFs that were annotated on the basis of homology to known proteins and domains present in the NCBI protein and CDD. These analyses indicate that *Phantom* TEs generally encode a single putative protein (300–700 AA) with multiple conserved domains (MARCHLER-BAUER *et al.*

TABLE 1

Distribution of the *Phantom* clade of the *Mutator* superfamily

Taxa	Abb.	Accession	DB	CD	CC	FLCC
Archamoebae						
Entamoeba dispar[a]	Ed	AANV01001934.1	WGS	MULE		
E. invadens[b]	Ei	AANW01000293.1	WGS		200	2
Cnidarians (Hydrozoa)						
Hydra magnipapillata[c]	Hm	XM002166453	NR		60	
Planarians						
Schmidtea mediterranea[b]	Sm	AAWT01029681.1	WGS	FLYWCH	600	8
Annelids						
Helobdella robusta[b]	Hr	AC171129.2	NR	FLYWCH	1	1
Mollusca						
Aplysia californica[c]	Ac	AASC01110148.1	WGS	MULE	30	1
Nematodes						
Caenorhabditis briggsae[c]	Cb	XM_001664648.1	NR		20	
C. elegans[c]	Ce	U37429.1	NR	MULE	30	7
Heterodera glycines[a]	Hg	CB934986.1	EST			
Meloidogyne chitwoodi[a]	Mc	CB830714.1	EST			
M. hapla[c]	Mh	ABLG01001649.1	WGS		17	
M. incognita[c]	Mi	CZ172697.1	GSS		20	
Trichinella spiralis[b]	Ts	AC188123.1	NR	MULE	700	2
Insects						
Acyrthosiphon pisum[c]	Ap	AC202214.3	NR	MULE	900	
Aedes aegypti strain[b]	Aa	AAGE02008886.1	WGS		>1000	3
Apis mellifera[a]	Am	AADG05002861.1	WGS			
Chironomus tentans[a]	Ct	CAC37683.1	NR	MULE		
C. pallidivittatus[a]	Chp	CAC37681.1	NR	MULE		
Culex pipiens quinquefasciatus[b]	Cp	AAWU01011212.1	WGS	MULE	>1000	1
Drosophila ananassae[b]	Da	AAPP01015916.1	WGS	MULE	>1000	1
D. yakuba[c]	Dy	AAEU02002585.1	WGS	MULE	7	
Ixodes scapularis[b]	Is	ABJB010984717.1	WGS		300	1
Nasonia vitripennis[b]	Nv	AAZX01008412.1	WGS	FLYWCH	17	1
Tribolium castaneum[b]	Tc	AAJJ01003811.1	WGS	FLYWCH	>1000	3
Echinoida						
Strongylocentrotus purpuratus[b]	Sp	AAGJ02149146.1	WGS		40	1
Ascidians (Urochordata)						
Ciona intestinalis[b]	Ci	AABS01001273	NR	FLYWCH	7	2
Ciona savignyi[b]	Cs	AACT01008187.1	NR	FLYWCH	>1000	1
Leptocardii (Cephalochordata)						
Branchiostoma floridae[c]	Bf	DE195457.1	GSS	MULE	44	
Vertebrata (Chordata)						
Homo sapiens[b]	Hs	AADC01162133.1	WGS		1	
Pan troglodytes[c]	Pt	AC200913.3	NR		>1000	
Canis familiaris[a]	Cf	AACN010093066	NR			
Equus caballus[b]	Ec	AAWR01022474	WGS	FLYWCH	300	1
Monodelphis domestica[a]	Md	AAFR03063600.1	WGS			
Rattus norvegicus[a]	Rn	AAHX01085823.1	WGS			
Fungi (Ascomycetes)						
Candida glabrata[a]	Cg	CR380951.1	NR			
Stramenopiles (Oomycetes)						
Phytophthora infestans[b]	Pi	AATU01012134.1	WGS	MULE	240	8
P. sojae[b]	Ps	AAQY01002515.1	WGS	MULE	100	13
P. ramorum[b]	Pr	AAQX01003219.1	WGS	MULE	30	3
Parabasalids (Trichomonads)						
Trichomonas vaginalis[b]	Tv	NW_001580983.1	WGS	MULE	780	9
Viruses (dsDNA)						
Chelonus inanitus bracovirus[c]	Cib	CAC82100.3	NR	MULE		
Glypta fumiferanae ichnovirus[b]	Gfi	AB289994.1	NR	MULE	1	1

(*continued*)

TABLE 1

(Continued)

Taxa	Abb.	Accession	DB	CD	CC	FLCC
Yeasts						
Kluyveromyces lactis[a]	Kl	CR382123.1	NR			
Yarrowia lipolytica[a]	Yl	CR382128.1	NR			

Abb., species abbreviation; accession, accession number for one representative hit; DB, database where the hit is deposited (GEN, genomic sequences; EST, expressed sequence tag; WGS, whole-genome sequencing; GSS, genomic survey sequence; NR, nucleotide sequences); CD, conserved domain; CC, copy number (tBlastn and Blastn $> e^{-04}$); FLCC, full-length copy number.

[a] Significant hit to query ($e = 10^{-4}$).

[b] Full-length copy number of *Phantom*.

[c] Protein only.

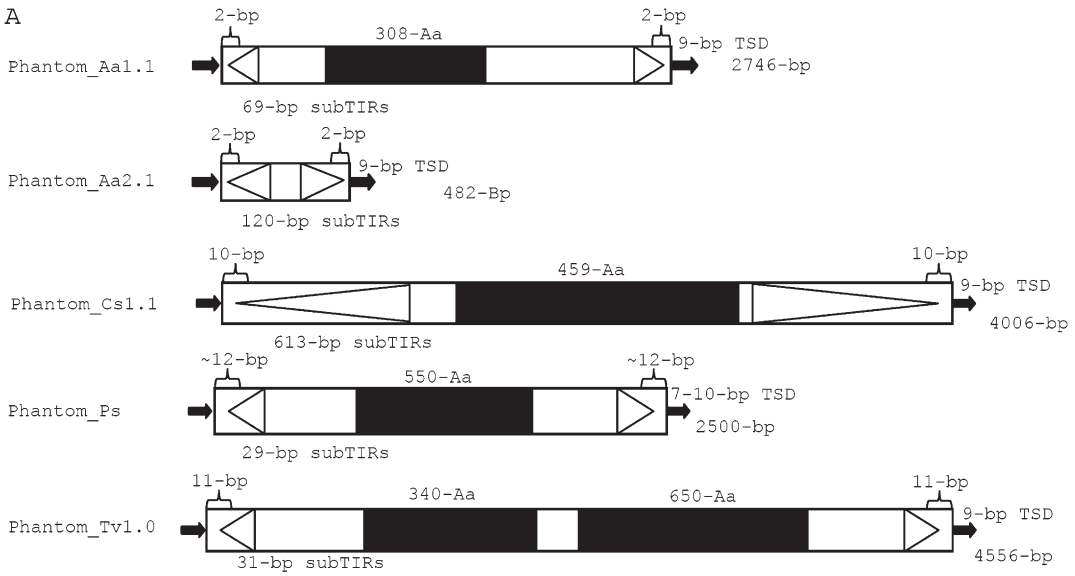
2009). A pfam00872 MULE transposase domain (*e*-values range 10^{-8}–10^{-1}) was readily identifiable in several of the translated *Phantom* ORFs (Table 1, Figure 3) therefore we refer to this putative protein as the transposase.

Further analyses using multiple sequence alignments of *Phantom* proteins and other previously identified *Mutators* and bacterial and archaeal *IS* sequences revealed a region of ~200 aa in *Phantom* that is homologous to the previously identified *Mutators* including *MULEs*, *Jittery*, *Hop*, and some bacterial and archaeal *IS* sequences (Robertson 1978; Barker *et al.* 1984; Yu *et al.* 2000; Chalvet *et al.* 2003; Xu *et al.* 2004; Pritham *et al.* 2005), presumably the catalytic core of the transposase. Multiple sequence alignments containing *Phantom*, *Jittery*, *Hop*, *MuDR*, and related *IS256* sequences were constructed using MUSCLE. The alignments were edited by removing regions of low sequence conservation, which resulted in an ~200-aa conserved region located in the C terminus of the proteins. This region is marked by the DDCHE motif (Figure 4). This region was previously identified in *Mutator* elements including *IS256*, the MURA protein in *MuDRs* and *TvMULEs* (Lisch 2002; Hua-Van and Capy 2008; Lopes *et al.* 2009).

Mutator transposases frequently harbor an N-terminal DNA binding domain (DBD) and a C-terminal catalytic domain. To determine what kind of domains are detected in *Phantom* transposases, the proteins were used to query the CDD at NCBI (Marchler-Bauer *et al.* 2009). A *Mutator* TPASE domain (pfam00872) and/or a FLYWCH domain (pfam04500) was detected in many of the transposase proteins (Figure 4). Cellular proteins that harbor the FLYWCH domain are all involved in transcriptional regulation and have been identified in the genome of *Drosophila melanogaster*, *Homo sapiens*, and *Caenorhabditis elegans* (Babu *et al.* 2006). The presence of a FLYWCH domain in a *Mutator* transposase has not to our knowledge been previously reported. However, the FLYWCH domain bears a WRKY–GCM1 fold also found in the DNA binding domain of other MULE transposases (Babu *et al.* 2006). This suggests that DNA binding domains displaying a WRKY–GCM1 fold are an ancient component of all *Mutator* transposases and therefore, the presence in *Phantom* transposases supports *Phantom* as a bonafide member of the *Mutator* superfamily.

***Phantom* elements belong to three different structural variant groups:** *Phantom* elements range in size from 2 to 5 kb and with few exceptions belong to three different structural variant groups. The elements in the first group are characterized by TIRs that are between 200 and 800 bp in length and are reminiscent of those typically associated with the *MuDR* elements of the *Mutator* superfamily (Lisch 2002). The second and most prevalent group of elements has sub-TIRs, characterized by inverted repeats between 10 and 880 bp in length and located 2–15 bp downstream from the termini (Figure 2A). Sub-TIRs are not specific to *Phantoms* and have been previously described for *Jittery* (Xu *et al.* 2004) elements of the *Mutator* superfamily, as well as other elements belonging to different superfamilies, some examples including *Microuli* (Tu and Orphanidis 2001) and *Microns* (Akagi *et al.* 2001). The third and final group includes those elements with TIRs of variable length (60–624 bp) characterized by repeated internal units (9–16 bp in length) reminiscent of the TIRs that characterize *Foldback*-like (*FB*-like) elements (Bingham and Zachar 1989). *Phantom* elements with *Foldback*-like TIRs are found in *Aedes aegypti*, *Ciona intestinalis*, *C. savigny*, and *Culex pipiens* (Figure 5). *Foldback*-like TIRs have not been previously reported in other *Mutator* elements.

Nonautonomous *Phantoms* were identified in the mosquitoes, *A. aegypti* and *C. pipiens* and *D. ananassae* and *Tribolium castaneum*. The nonautonomous elements identified in *A. aegypti* can be classified as MITEs as they have reached high copy numbers and are fairly homogenous in size. The elements identified in *D. ananassae* and *T. castaneum* have not reached high copy numbers and are likely old as they have incurred other insertions. These elements are flanked by 8- to 9-bp TSDs and their TIRs share a strong similarity to the autonomous *Phantoms* identified, indicating that these elements are likely moved in *trans* by the transposases encoded by autonomous *Phantoms* in these species.



B

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AAGE02029677.1 21366-24111    CCTCAACAGTGTGATAAAATGCACAGAGATCAAGAA/Phantom_Aa1.1/GATCAAGAACCTACTGTCAGAAAGGGGATCTATCTG
AAGE02025264.1 17845-17946    CCTCAACAGTGTGATAAAATGCACAGAGATCAAGAA                        CCTACTGTCAGAAAGGGGATCTATCTG
AAGE02029677.1 21315-24171    CCTCAACAGTGTGATAAAATGCACAGAGATCAAGAA                        CCTACTGTCAGAAAGGGAATCTATCTG

AAGE02008886.1 3810-4291      GCTTTGTTAGCTCGGATGCTGTCATAGATGAATCGC/Phantom_Aa2.1/ATGAATCGCACTACAGGATGTTATCAGTTGATTGCC
AAGE02000379.1 106110-106210  GCTTTGTTAGCTCGGATGCTGTCATAGATGAATCGC                        ACTACAGGATGTTATCAGTTGATTGCC
AAGE02000783.1 6757-6857      GCTTTGTTAGCTCGGATGATGTCATAGATGAATCGC                        ACTACAGGATGTTATCAGTTGATTGCC

AACT01010384.1 15670-19675    TTATATTCCTTTTTTGCTGAGAAGGTTGAAGCGGTG/Phantom_Cs1.1/GAAGCGGTGTGTTACACCGGATAATTCTTGTGTGTG
AACT01066705.1 8539-8430      TAATCATCCTTTTT-GCTGAGAGGGTTGAAGCAGTG                        TGTTACACCGGATAATTCTTGTGTGTG

AAQY01001616.1 8596-6203      GCCCACGAGCCCAGGCGCCACCTTGACCACGGGTCC/Phantom_Ps1.8/ACGGGTCCGAATGCCGACATGCTCACGAACCCGGAC
AAQY01001485.1 19286-19177    GCCCACGAGCCCAGACGCCACCTTGACCACGGGTCC                      GAATGCCGACATGCTCACGAACCCGGAC
AAQY01001585.1 226640-226531  GCCCACGAGCCCAGACGCCACCTTGACCACGGGTCC                      GAATGCCGACATGCTCACGAACCCGGAC

AAHC01000630   15347-20686    TAAAGTTTAATTGCCAAGGTTCATCTAATTTTTTGA/Phantom_Tv1.2/ATTTTTTGATTTTTAATGTTTTATGAATTTTATTTA
AAHC01000082.1 67607-67716    TAAAGTTTAATTGCCAAGGTTCATCTAATTTTTTGA                        TTTTTAATGTTTTATGAATTTTATTTA
AAHC01000116.1 57713-57604    TAAAGTTTAATTGCCAAGGTTCATCTAATTTTTTGA                        TTTTTAATGTTTTATGAATTTTATTTA
AAHC01000133.1 992-883        TAAAGTTTAATTGCCAAGGTTCATCTAATTTTTTGA                        TTTTTAATGTTTTATGAATTTTATTTA
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Figure 2.—Schematic representation of *Phantom* elements. TSDs are represented by solid arrows, open arrows represent sub-TIRs, and ORFs are represented by solid boxes. (B) TSDs created upon *Pantom_Aa*, *Phantom_Cs*, *Phantom_Ps*, and *Phantom_Tv* insertions. TSDs are underlined. Aa, *Aedes aegypti*; Cs, *Ciona savigny*; Ps, *Phytophthora sojae*; and Tv, *Trichomonas vaginalis*.

***Phantoms* form a well-supported clade with *Mutator/IS256* elements:** *Mutator* elements were previously described in plants, fungi, Entamoeba, and *T. vaginalis* (Taylor and Walbot 1987; Mao *et al.* 2000; Lisch *et al.* 2001; Lisch 2002; Chalvet *et al.* 2003; Xu *et al.* 2004; Pritham *et al.* 2005; Hua-Van and Capy 2008; Lopes *et al.* 2009). A phylogenetic analysis was generated from the (200 aa) catalytic domain alignment using a Bayesian method (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The Bayesian tree revealed five distinct well-supported groups including *Phantom*, *MuDR*/IS256, *Jittery*, *TvMULES*, and *EMULEs* (Figure 6). The branching pattern supports *Phantom* in forming a unique group affiliated to the *Mutator* superfamily.

DISCUSSION

A novel group of DNA transposons found in many eukaryotic genomes: A lineage of TEs identified in diverse eukaryotes with genome sequences present in the database were described and are called *Phantom*. Most families of *Phantom* elements display common features including a single putative transposase gene flanked by terminal or subterminal inverted repeats and a TSD variable in sequence usually 9 bp in length, but ranges from 7 to 12 bp, which is consistent with previously identified *Mutators* including *Pack MULEs*, *CUMULEs*, *MULEs* in *A. thaliana*, *Hop*, and *IS256* sequences (Eisen *et al.* 1994; Yu *et al.* 2000; Chalvet *et al.* 2003; Jiang *et al.* 2004; van Leeuwen *et al.* 2007). The size, structure, and organization of *Phantom* elements and the TSD are consistent with the *Mutator* superfamily (Feschotte and Pritham 2007).

Structural features of *Phantom* elements: Three structural variants (TIR, sub-TIR, and FB) typify *Phantom* elements. The first category (TIR) encompasses the elements, which display a structure typical of *MuDR* elements including inverted repeats (200–800 bp in length) located precisely on either flank of the element.

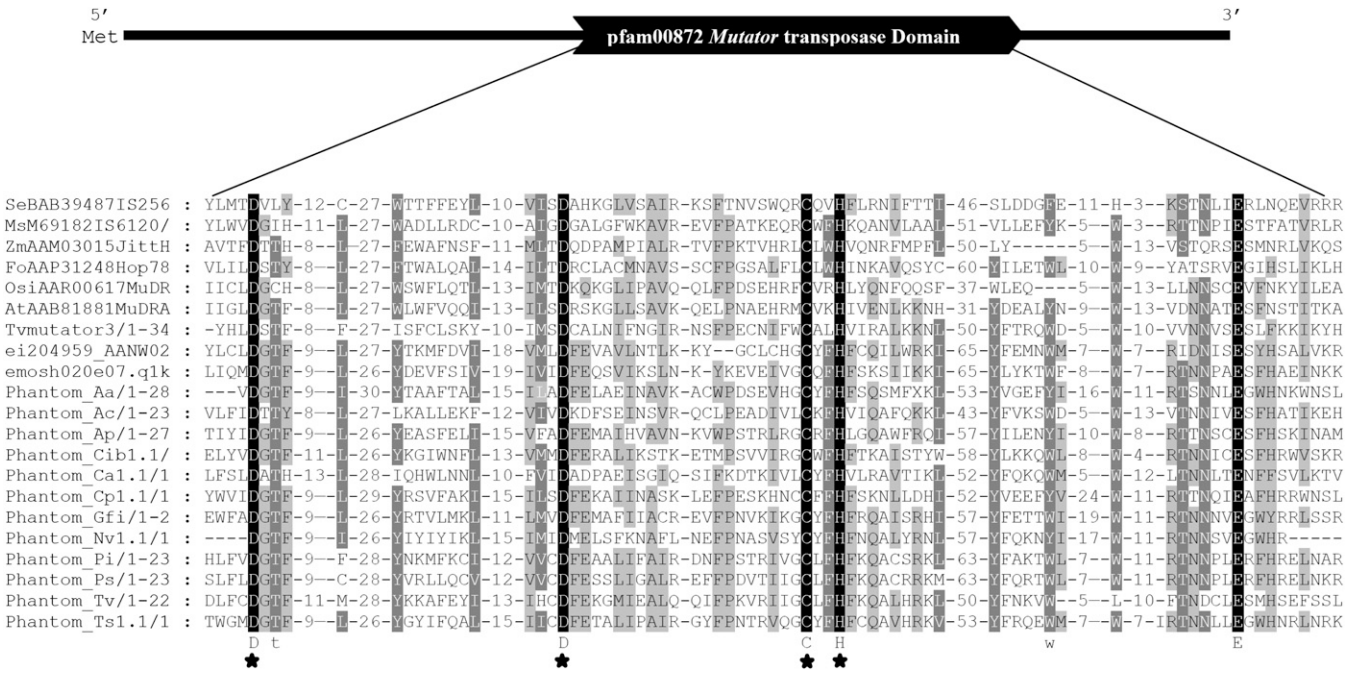

FIGURE 3.—MUSCLE alignment of the conserved domain found in transposases from the *IS256/Mutator* superfamily corresponding to the pfam00872. Solid residues have related physical or chemical properties and occurred in every sequence. Shaded residues occur in some of the sequences. The star symbol below the residues D, D, C, H refers to conserved residues previously described by HUA-VAN and CAPY (2008) and LISCH (2002). Aa, *Aedes aegypti*; Ac, *Aplysia californica*; Ap, *Acyrothosiphon pisum*; At, *Arabidopsis thaliana*; Ce, *Caenorhabditis briggsae*; Cib, *Chelonus inanitus bracovirus*; Ca, *Candida albicans*; Cp, *Culex pipiens quinquefasciatus*; Ei, *Entamoeba invadens*; Em, *Entamoeba moshkovskii*; Gfi, *Glypta funiferanae ichnovirus*; Ms, *Mycobacterium smegmatis*; Nv, *Nasonia vitripennis*; Os, *Oryza sativa*; Pi, *Phytophthora infestans*; Ps, *Phytophthora sojae*; Se, *Staphylococcus epidermis*; Tv, *Trichomonas vaginalis*; Ts, *Trichinella spiralis*; Zm, *Zea mays*; Se, IS256; and Ms, Pfam0082.

The second category (sub-TIR) are quite untraditional in their structure in that the termini of the element are not part of the inverted repeat; instead, they are characterized by a nonrepetitive region of 2–15 bp flanking the inverted repeat. The noncanonical sub-TIR structure made it difficult to properly demarcate these elements. This structure was validated by the identification of paralagous empty sites, which illustrate mobility in the past. Elements in the third structural category (FB) display long complicated TIRs characterized by highly repeated subunits reminiscent of TEs previously classified as *Foldback* elements (Figure 5). Our phylogenetic analysis also revealed that *FARE2*, a TE from *A. thaliana* described as a *Foldback* element, forms a clade (pp = 98) with the transposases encoded by *MuDR* TEs from plants (Figure 6). Therefore, FARE2 can be considered as a *Mutator* and not a member of a distinct *Foldback* superfamily of TEs. No relationship between the structure of the TE and the domains present in the transposase could be detected.

The labiality in structural morphologies is not unique to *Phantom* elements; recent studies have shown that the FB element, *Galileo*, is a member of the *P*-element superfamily where the canonical families display short TIRs (MARZO *et al.* 2008). Since the TIRs typically contain the transposase binding sites required for the cleavage and integration of DNA transposons (CRAIG *et al.* 2002), we propose that structural variation might evolve to avoid recognition by the host. It is possible that the repetitive structure inherent to the TIRs may be detected by the host genome and become the target of silencing. For example, it has been shown that the TIRs of *Mu* elements in maize are methylated, resulting in transcriptional silencing (LISCH 2002). A TE without TIRs, might avoid transcriptional silencing and might successfully outcompete TEs with TIRs. Another strategy to outcompete TEs with a simple TIR structure might be to increase the number of transposase binding sites within the TIR. It has been proposed that the tandemly repeated sequences in FB TIRs increase the chances of transposition by increasing DNA binding sites for the transposases (POTTER 1982). The co-occurrence of structural variability within the *P*-element superfamily suggests that the structure of DNA transposons and in particular the transposase binding sites may be under selective pressure to be flexible, perhaps in response to host genome defense and should not be relied upon for classification. Therefore, FB structure does not signify an alliance to a FB superfamily as has been previously reported.

Most of the *Phantom* elements have coding capacity for a protein that ranges in size between 350 and 700 aa. Many of the proteins contain a conserved DDCHE motif (Figure 3) and a pfam00872–*MULE* transposase domain, further suggesting an allegiance to the *Mutator*

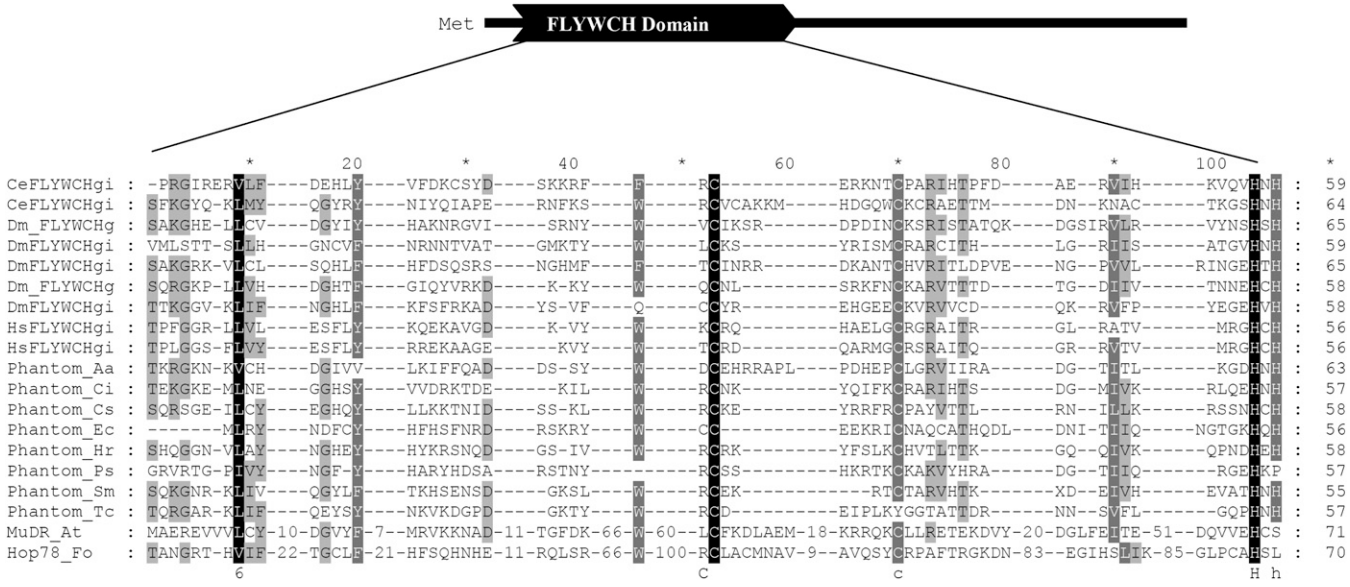

Figure 4.—MUSCLE alignment of the conserved FLYWCH domain found in *Phantoms*, *MuDR*, and *Hop78*. Solid residues have related physical or chemical properties and occurred in every sequence. Shaded residues occur in some of the sequences. Aa, *Aedes aegypti*; At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Ci, *Ciona intestinalis*; Cs, *Ciona savigny*; Dm, *Drosophila melanogaster*; Ec, *Equus caballus*; Fo, *Fusarium oxysporum*; Hr, *Helobdella robusta*; Hs, *Homo sapiens*; Ps, *Phytophthora sojae*; Sm, *Schmidtea mediterranea*; and Tc, *Triboleum castaneum*.

superfamily. Phylogenetic analysis based on an alignment of the transposase domain with *Phantom* and selected *Mutator* transposases reveals that *Phantom* forms a well-supported group separate from previously described *Mutators*. In addition, *Phantom* elements identified in *A. aegypti*, *C. intestinalis*, *C. savigny*, *E. caballus*, *H. robusta*, *P. sojae*, *S. mediterranea*, and *T. castaneum* contain a conserved FLYWCH DNA binding domain (Figure 4). FLYWCH is a DNA binding domain classified under the WRKY–GCM1 superfamily of DBDs (Babu *et al.* 2006). The WRKY–GCM1 DBDs are a common feature of some *MULE* and plant *MuDR* transposases (Babu *et al.* 2006).

Our results reveal that *Phantom* transposases harbor a FLYWCH domain that is a member of the WRKY–GCM1 superfamily. The cellular proteins that harbor the FLYWCH domain are limited to animals, while *Phantom* transposases have a broader phylogenetic distribution, suggesting that the transposases were the source of these DNA binding domains. This pattern is consistent with the hypothesis that *MuDR* and *MULE* transposases are the progenitor of the DNA binding domain, found in all WRKY–GCM1 transcription factor proteins (Babu *et al.* 2006).

Distribution of *Phantom* elements in eukaryotes: TEs of the *Mutator* superfamily are widespread in plants (supergroup Plantae) but previously were reported in few other eukaryotes including Entamoeba and various fungi (supergroup Unikont) (Talbert and Chandler 1988; Lisch 2002; Chalvet *et al.* 2003; Xu *et al.* 2004; Pritham *et al.* 2005) and in the genome of *T. vaginalis* (supergroup Excavate) (Hua-Van and Capy 2008; Lopes *et al.* 2009). This study broadens the distribution of the *Mutator* superfamily by revealing the widespread occurrence in animal genomes including human, as well in the genomes of three Phytophthora species, which are part of the Chromalveolate supergroup. In addition, related transposases were also identified in two insect viruses, *C. bracovirus* and *Gf. ichnovirus*. It is interesting to point out that *Phantom* elements have a broader phylogenetic

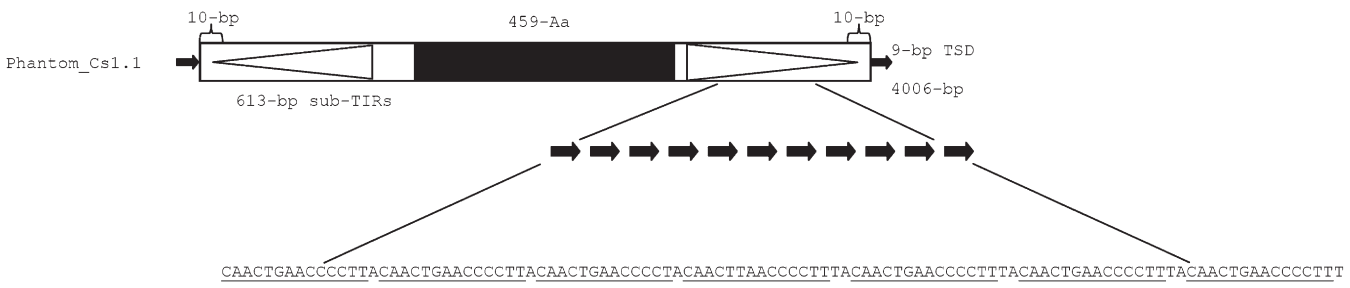

Figure 5.—Schematic representation of *Foldback*-like TIRs occurring in some *Phantom* elements. TSDs are represented by solid arrows, open arrows represent sub-TIRs, and ORFs are represented by solid boxes. Tandemly repeated sequences within the sub-TIRs are represented by small solid arrows. Repeated sequence motifs are underlined.

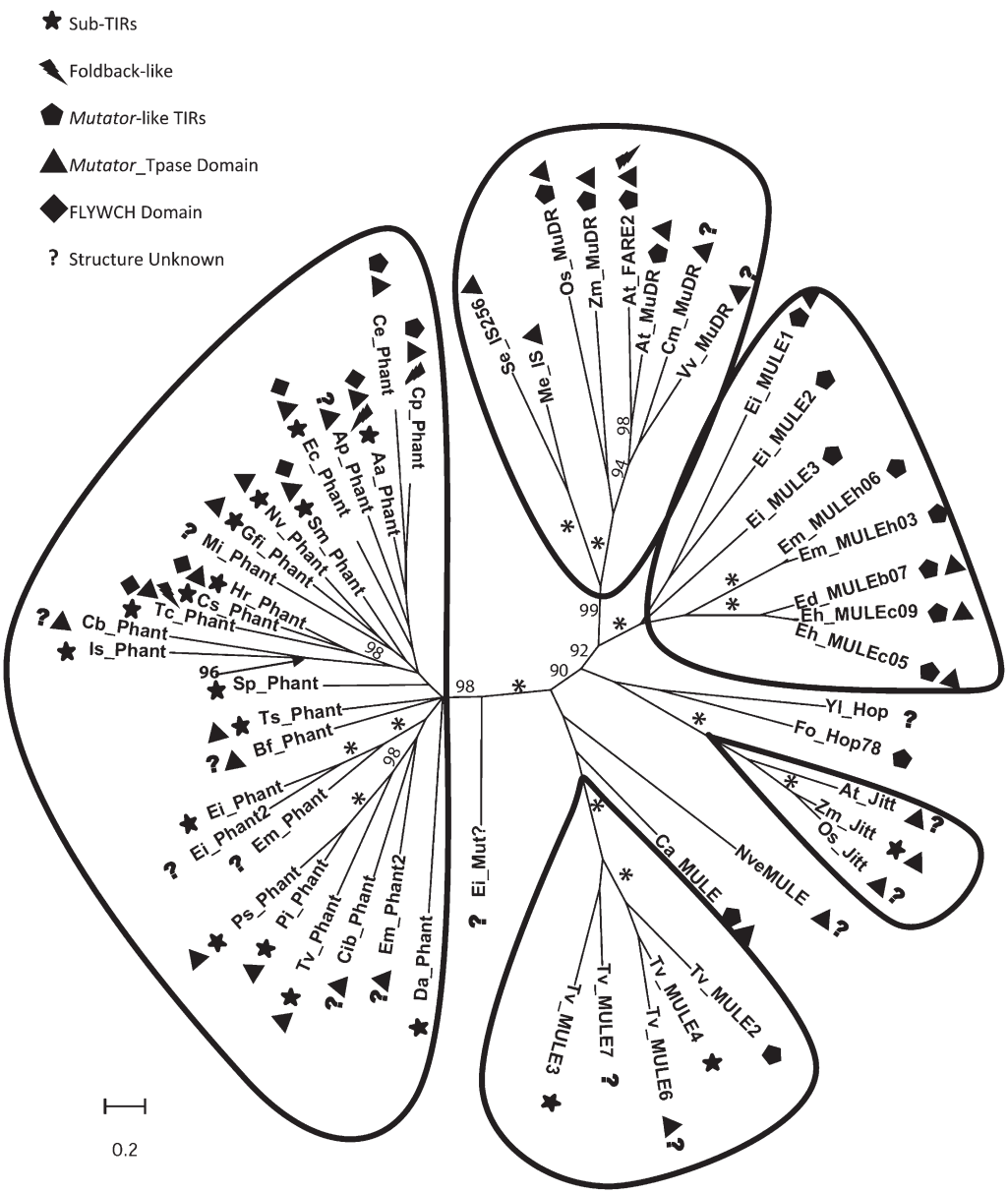

FIGURE 6.—Comparative phylogenetic analysis of *Phantom* proteins and the conserved domains of TvMULEs, CaMULEs, MuDRs, Jittery, Hop, EMULEs, FARE2, and IS256 transposases. Bayesian tree constructed using Mr. Bayes v3.14 from an alignment portion of the *Phantom* transposase corresponding to the conserved DDCHE motif from representative species and rooted with IS256 proteins from bacteria and archaea. GenBank accession numbers for previously identified sequences in parenthesis. Aa, *Aedes aegypti*; Ap, *Acyrthosiphon pisum*; At, *Arabidopsis thaliana*; Bf, *Branchiostoma floridae*; Ca, *Candida albicans*; Cb, *Caenorhabditis briggsae*; Ce, *Caenorhabditis elegans*; Cib, *Chelonus inanitus bracovirus*; Cm, *Cucumis melo*; Cp, *Culex pipiens*; Cs, *Ciona savigny*; Da, *Drosophila ananassae*; Ec, *Equus caballus*; Ed, *Entamoeba dispar*; Eh, *Entamoeba histolytica*; Ei, *Entamoeba invadens*; Emosh, *Entamoeba moshkovskii*; Fo, *Fusarium oxysporum*; Gfi, *Glypta fumiferanae ichnovirus*; Hr, *Helobdella robusta*; Is, *Ixodes scapularis*; Mi, *Meloidogyne incognita*; mosh, *Entamoeba moshkovskii*; Ms, *Mycobacterium smegmatis*; Nv, *Nassonia vitripennis*; Nve, *Nematostella vectensis*; Os, *Oryza sativa*; Pi, *Phytophthora infestans*; Ps, *Phytopthora sojae*; Se, *Staphylococcus epidermidis*; Sm, *Schmidtea mediterranea*; Sp, *Strongylocentrotus purpuratus*; Tc, *Tribolium castaneum*; Ts, *Trichinella spiralis*; Tv, *Trichomonas vaginalis*; Vv, *Vitis vinifera*; Yl, *Yarrowia lipolytica*; and Zm, *Zea mays*. TvMULEs used are the majority rule consensus sequences from the *T. vaginalis* genome. The corresponding accession numbers for MULEs, MuDRs, Jittery, Hop, FARE2, and IS transposases are as follows: Se_IS256: BAB394871; Me_IS: NP634554; Os_MuDR: NP918808; Zm_MuDR: M76978.1; At_FARE2: AAD15518; At_MuDR: NP178710; Cm_MuDR: RepBase:AY582736; Vv_MuDR: CAAP02002575.1; Ei_MULE1: 205732; Ei_MULE2: 206048; Em_MULE3: 206038; Em_MULEh06: 131h06; Em_MULEh03: 010h03; Ed_MULEb07: 98816116b07; Eh_MULEc09: 2841599c09; Yl_Hop: XP504344; Fo_HOP78: AAP31248; At_Jitt: AAD51282; Zm_Jitt: AF247646.1; Os_Jitt: XP475123; NveMULE: MuDR-2_Nv; Ca_MULE: AACQ01000039.1; and Ei_Mut: AANW02000228.1.

distribution than other lineages of the *Mutator* superfamily. This observation suggests that the *Phantom* lineage is the most ancient clade of the *Mutator* superfamily and that *Hop*, *Jittery*, and *MuDR* may be viewed as more derived clades or alternatively that *Phantom* elements may be subject to horizontal transfer more readily than other *Mutators*.

Horizontal transfer of *Phantom*: Numerous examples of related TEs occurring in distantly related animal genomes have been documented and can only be explained by invoking horizontal transfer, although the mechanism remains a mystery (DANIELS *et al.* 1990; ROBERTSON 2002; PACE *et al.* 2008). It has been hypothesized that viruses may make good vectors as they are known to frequently pickup host genes and are infectious. Indeed, TEs have been previously identified in viral genomes. For example, *piggybac* and *TED* were identified when they passed from the Lepidopteran host to the

infectious baculovirus (Lerch and Friesen 1992; Wang and Fraser 1993). These HT events were caught in the act during experiments in the laboratory. More recently, a reptilian SINE was identified bioinformatically in the genome of the taterapox virus that infects mammals, revealing that HT of TEs to viruses occurs in nature (Wang and Fraser 1993; Ozers and Friesen 1996; Piskurek and Okada 2007). Interestingly, we have identified *Phantom* transposases and/or complete *Phantom* elements in two double-stranded polydsDNA viruses, *Gf. ichnovirus* (*Phantom_Gfi*) and *C. bracovirus* (*Phantom_Cib*) that are known to infect wasps (Table 1). The host species of these viruses has not been sequenced; however, phylogenetic analysis based on the putative transposases encoded by these elements reveals a monophyletic clade between *Phantom_Gfi* and the *Phantom* transposase from the wasp *Nasonia vitripennis* (pp = 69). This clade is nestled within a well-supported clade (pp = 97) of other insect and invertebrate transposases, which lends support to the HT occurring from the insect to the virus rather than vice versa. *Phantom* elements found in dsDNA viruses adds to the growing body of evidence (Fraser *et al.* 1983; Friesen and Nissen 1990; Jehle *et al.* 1998; Lerch and Friesen 1992; Piskurek and Okada 2007; Xu *et al.* 2006) that dsDNA viruses may act as vectors for horizontal movement of TEs between eukaryotes.

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Phantom, a New Subclass of *Mutator* DNA Transposons Found in Insect Viruses and Widely Distributed in Animals

Claudia P. Marquez and Ellen J. Pritham

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File S1

Consensus sequences for all mult-element families

File S1 is available for download as an Excel file at http://www.genetics.org/cgi/content/full/genetics.110.116673/DC1.