

Insertion of miniature subterminal inverted repeat-like elements in diapause-regulated genes in the Colorado potato beetle, *Leptinotarsa decemlineata** (Coleoptera: Chrysomelidae)

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Abstract. Determining the genomic structure of *diapause-associated transcripts* (*DAT*) -2 and -3 led to the isolation of four novel miniature subterminal inverted repeat-like elements (MSITE): *Mild-1*, -2, -3 and -4. *Mild-1a* is inserted within the first intron of *diapause protein-1*. *Mild-1a* is 284 bp in length, has a 14 bp target site duplication and three sets of subterminal inverted repeats. The second element, *Mild-2a*, is inserted within the 3' terminus of *Mild-1a*. *Mild-2a* is 29 bp long with a 3 bp target site duplication and one set of subterminal inverted repeats. Using primers based on *Mild-1*, genomic clones were developed leading to the isolation of *Mild-3a*. *Mild-3a* shares 60% identity with *Mild-1a*, is 253 bp long, has a 9 bp target site duplication and has one set of subterminal inverted repeats. *Mild-4a* is inserted within the first intron of *DAT-2* and is 227 bp in length with a 12 bp target site duplication. *Mild-4a* appears to be an intermediate form between a miniature inverted repeat transposable element (MITE) and a MSITE because the 5' inverted repeat is terminal (i.e., adjacent to the target site duplication) as in MITEs, but the 3' inverted repeat is separated (in this case, by 33 bp) from the 3' target site duplication as in MSITEs. The target site duplications of *Mild-1*, -3 and -4 families share a common conserved core of AATTT. All of the transposable elements are AT rich and are able to form hairpin structures. Within the promoter region of *DAT-3* is a 163 bp sequence (*Mild-1b*) that shares 77% identity to the 3' terminus of *Mild-1a*. *Mild-4a* has identity to 25 and 53 bp regions within the promoter of the *juvenile hormone esterase B* gene. Southern blot analysis revealed the presence of *Mild-1* and -3 elements in both *Leptinotarsa decemlineata* and *Leptinotarsa juncta* indicating that these elements are ancestral to the *L. decemlineata*, *L. juncta* separation.

INTRODUCTION

The Colorado potato beetle (CPB) *Leptinotarsa decemlineata* is the major pest of potato (Ferro, 1985; Hare, 1990; Cloutier et al., 1996) and also attacks tomato (Schalk & Stoner, 1979) and eggplant (Cotty & Lashomb, 1982; Hamilton & Lashomb, 1996). CPB reared under short day photoperiod will feed for about 10 days after emerging as adults, and by day 20 postemergence, most of the beetles will be in diapause (de Wilde et al., 1959; Yocum et al., 2009a, b).

To further clarify the molecular basis of diapause-gene regulation in CPB, the genomic structures of selected genes and their promoter regions are being determined. *Diapause-associated transcripts* (*DAT*) -1, -2 and -3 are expressed prior to day 10 postemergence in diapause-programmed CPB (Yocum, 2003). Sequence analysis of genomic clones of the diapause-regulated genes *DAT-2* and -3 led to the discovery of novel miniature subterminal inverted repeat transposable-like elements (MSITEs). MSITEs bear strong structural similarity to miniature inverted repeat transposable elements (MITEs) and may in some cases be an intermediate in the development of new MITEs (Tu & Orphanidis, 2001). MITEs are small class II DNA-mediated transposable elements (TEs) that are found in organisms ranging from the nematode *Cae-*

norhabditis elegans to human. MITEs as a class share a number of structural features such as their small size (<1600 bp), terminal or subterminal inverted repeats, AT rich composition, lack of open reading frames, and the ability of most to form secondary structures (reviewed, Feschotte et al., 2002a). We report here the isolation of four genomic elements bearing strong similarities to MSITEs. Southern blot analyses revealed that two of these elements are present in high copy number in both *L. decemlineata* and *Leptinotarsa juncta* (false potato beetle).

MATERIAL AND METHODS

Insects

Leptinotarsa decemlineata is in culture at the USDA-ARS, Red River Valley Agricultural Research Center, Fargo, ND (Yocum, 2001). *Leptinotarsa juncta* was obtained from cultures maintained at the USDA-ARS, Insect Biocontrol Laboratory, Beltsville, MD. *Leptinotarsa texana* was collected by J. Patt, USDA-ARS, Weslaco, TX. *Zygogramma exclamationis* (sunflower beetle) was collected near Fargo, ND. *Zygogramma bicolorata* (Mexican beetle) was obtained from India by P. Burange. All beetles were frozen at -80°C until needed.

Clone development

The promoter region of the *DAT-3* gene was isolated using gene-specific primers, the proof-reading Pfu ultra DNA

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polymerase (Stratagene, La Jolla, USA) and the Universal Genome Walker Kit (Clontech, Mountain View, USA). Genomic clones of transposable elements were generated using primers based on *Mild-1a* and Pfu ultra DNA polymerase and the Universal Genome Walker Kit. Adenine overhang was added by incubating the PCR reaction with 1.25U of platinum® Taq DNA polymerase for 2 min at 94°C and 10 min at 72°C (Invitrogen, Carlsbad, USA). All amplicons were cloned using the TOPO TA Cloning™ kit (Invitrogen).

Southern analysis

DNA was extracted from frozen beetles by placing them onto dry ice and removing the legs and elytrons. Ten beetles were then transferred to a prechilled mortar and pestle and ground to a fine powder in liquid nitrogen. The powder was transferred to a 50 ml conical tube containing 20 ml of DNA extraction buffer [10 mM Tris-HCl (pH 8.0), 0.1 M EDTA (pH 8.0), 0.5% SDS]. Twenty microliters of RNase A (10 µg/µl) were added to the extraction buffer and incubated at 37°C for 30 min. Following the RNase A digestion, 100 µl of Proteinase K (20 µg/µl) were added to the DNA solution and incubated at 50°C for 3 h. The DNA was then extracted using an equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1). The DNA was spooled by adding 0.1 volume of 3 M ammonium acetate (pH 5.2) and 1.8 volume of ice-cold 95% ETOH to the aqueous phase. After drying, the DNA pellets were dissolved in 0.1 to 1 ml 1X TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)]. DNA concentration and quality were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, USA) and agarose gel analysis using 1 Kb+ plus and high mass DNA ladders (Invitrogen).

Twenty micrograms of DNA were incubated for 2 h at 4°C in 140 µl of the appropriate restriction buffer with frequent mixing. Five microliters of EcoRI (10 U/µl, Invitrogen), Fsp I (5 U/µl, New England BioLabs, Ipswich, USA), or Xho I (20 U/µl, New England BioLabs) were then added to the appropriate reaction mixture and incubated at 37°C for 30 min. After 30 min an additional 5 µl of enzyme were added to each digestion and incubated at 37°C overnight. The digested DNA samples were precipitated using 0.1 volume of 3 M sodium acetate and 2.5 volume of 95% ETOH. The DNA pellets were resuspended in 15 µl of 1X TE and separated on a 0.7% TAE (25 mM Tris-acetate, 1 mM EDTA) agarose gel. Following separation, the gels were incubated in denaturing buffer (0.5 M NaOH, 1.5 M NaCl) for 30 min, neutralization buffer [0.5 M Tris-HCl (pH 7), 1.5 M NaCl] for 30 min then in 20X SSC (3 M NaCl, 0.3 M Na citrate) for 30 min. Using a TurboBlotter™ (Whatman, Clifton, USA), the DNA was transferred overnight onto positively charged nylon membrane (Roche, Indianapolis, USA) using 20X SSC as the transfer buffer. Following the transfer, the filters were UV cross-linked (12,000 µJ/cm²) and stored at -20°C until needed.

Prehybridization and hybridization were carried out at 42°C in Dig Easy Hyb buffer (Roche, Indianapolis, USA). Filters were screened with the following oligo probes: Mild-1a: 5'-CATGTGTCCCGAGGTGTTGAAAATGACGGT-3' or Mild-3a: 5'-CCAAACCGTTATTTCACACCTCGACACGT-3'. Probes were labeled using Dig oligonucleotide 3'-end labeling kit and were detected using the Dig High Prime DNA labeling and Detection Start Kit II as described in the manufacturers protocols (Roche).

Bioinformatics

The Blastn and Blastx programs (Altschul et al., 1997) were used to search the GenBank sequence repository for sequence identity. Sequence alignments and percent identity calculations

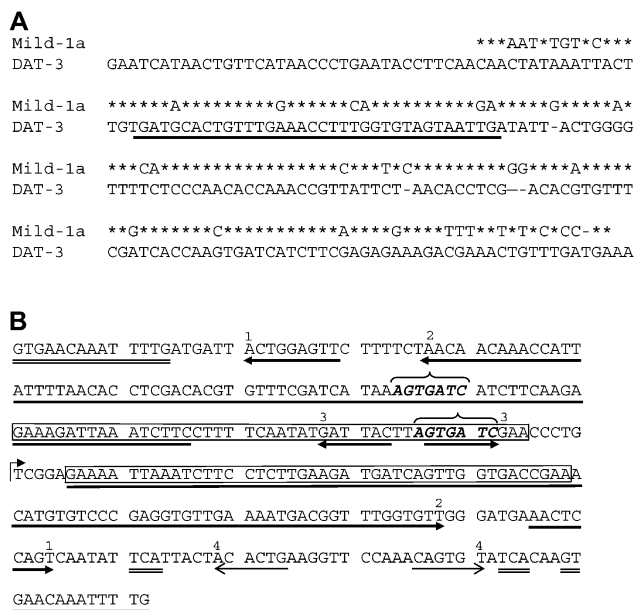


Fig. 1. Sequence alignment of *Mild-1a* (with embedded *Mild-2a*) with the promoter region of *DAT-3* and the genomic structure of *Mild-1a* with embedded *Mild-2a*. (A) Alignment of *Mild-1a* and *Mild-2a* with *DAT-3* promoter region; underline highlights *Mild-2a*; (*) identical nucleotide; (-) insertion or deletion. (B) Genomic structure of *Mild-1a* and -2a; the target site duplications are indicated by double underlines; *Mild-1a* is longer than *Mild-2a*. The subterminal inverted repeats are indicated by arrows, corresponding inverted repeats are labeled with the same number: heavy arrows for *Mild-1a*, and thin arrows for *Mild-2a*. A seven bp internal direct repeat is in bold italic under brackets and the 45 bp internal direct repeat is boxed. The bent arrow indicates the region of similarity between *Mild-1a* and the promoter region of *DAT-3*.

were carried out using ContigExpress and AlignX (Invitrogen). MSITE secondary structure analysis was performed using Mfold (Zuker et al., 1999; Zuker, 2003).

Phylogenetic analysis

DNA from individual insects was amplified using standard PCR conditions described previously (Roehrdanz et al., 2010). Primers were LCO-1490: 5'-TTTCTACAAATCATAAAGATA TTGG-3' and HCO-2198: 5'-TGATTTTGTGTCACCCCTGA AGTTTA-3' which flank the mitochondrial *cox1* barcode region. PCR products were sequenced by the Iowa State University DNA sequencing facility in Ames, IA. GenBank accession numbers for the sequences are: *Leptinotarsa decemlineata*, HQ605768 (CPB3704), HQ605769 (CPB3948); *Leptinotarsa juncta*, HQ605770 (FPB-A), HQ605771 (FPB4053); *Leptinotarsa texana*, HQ605774 (LTX3854), HQ605775 (LTX3855); *Zygogramma exclamationis*, HQ605776 (SFB3809), HQ605777 (SFB3852), HQ605778 (SFB3853); *Zygogramma bicolorata*, HQ605772 (ZBC3846), HQ605773 (ZBC3847). Additional GenBank sequences used in analysis were EU498301 (*Diabrotica virgifera*) and AJ312413 (*Tribolium castaneum*).

The evolutionary history of the chrysomelids and *Tribolium* was inferred using the Minimum Evolution method (Rzhetsky & Nei, 1992). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next

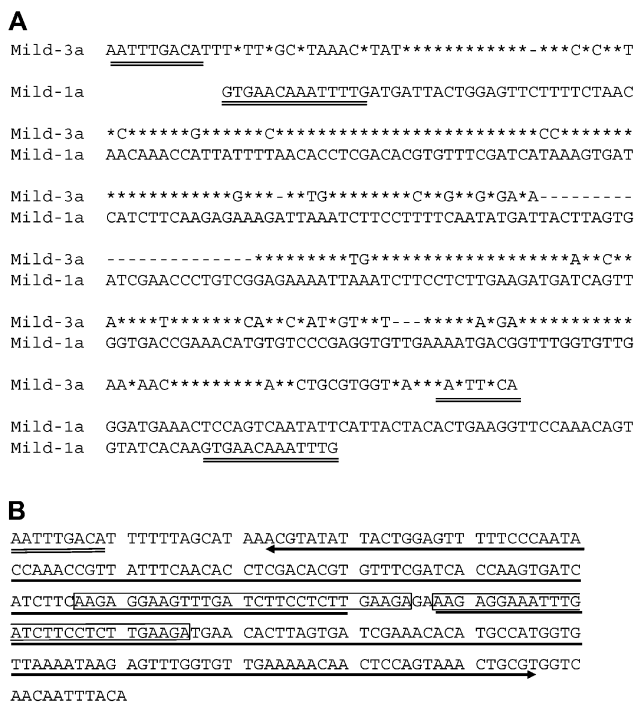


Fig. 2. Sequence alignment of *Mild-1a* (*Mild-2a* removed) with *Mild-3a* and the genomic structure of *Mild-3a*. (A) Alignment of *Mild-1a* and *Mild-3a*; (*) identical nucleotide; (–) insertion or deletion. (B) Genomic structure of *Mild-3a*; the target site duplications are indicated by double underlines. The subterminal inverted repeats are indicated by arrows. The boxed sequences are the internal direct repeats.

to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. There were a total of 658 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

RESULTS

Miniature inverted repeat transposable element

BlastN analysis of the *DAT-3* promoter region (GenBank accession no. [DQ011146](#)) revealed a 163 bp region 1411 bp upstream of the *DAT-3* deduced TATA box with 77% identity to the intron of *diapause protein-1* (*DP-1*) from *L. decemlineata* (Koopmanschap et al., 1995, GenBank accession no. [X86074](#)) (Fig. 1A). Inspection of the region of the *DP-1* gene with identity to the *DAT-3* promoter region revealed the presence of a transposable element (TEL)-like sequence embedded in the first intron of *DP-1* at nucleotides 1204 to 1487 (Fig. 1B). The 284 bp TEL-like sequence has three sets of imperfect subterminal inverted repeats (SIRs) 9, 83 and 6 bp in length with 89, 83 and 83% identity respectively between the 5' and 3' counterparts (Fig. 1B). The TEL is AT rich (63%) and is flanked by perfect 14 bp (GTGAACAAATTTTG) target site duplications (TSD). Mfold analysis of this sequence revealed that it would form a hairpin structure with a ΔG of -69.5 kcal/mol (Zuker et al., 1999). This 284 bp TEL-like sequence bears strong similarities to MSITEs. Because of its size, its ability to form a secondary struc-

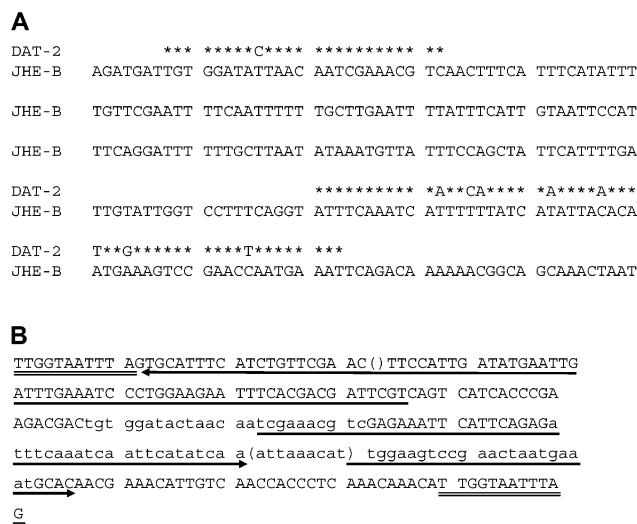


Fig. 3. Regions of sequence identity between *DAT-2* intron, the promoter region of *JHEB* and the genomic structure of *Mild-4a*. (A) Identity between *Mild-4a* and *JHEB* promoter region; (*) identical nucleotide. (B) Genomic structure of *Mild-4a*; the target site duplications are indicated by double underlines. The inverted repeats are indicated by arrows. Lower case letters indicates sequences in common between *Mild-4a* and the *JHEB* promoter region. Brackets indicate position of the indel.

ture, and its structural similarity to MSITEs, we are designating this element *Mild-1a* (miniature subterminal inverted repeat transposable-like element *Leptinotarsa decemlineata*). *Mild-1a* also has two internal direct repeats: a perfect 7 bp repeat and an imperfect 45 bp repeat (82% identity) (Fig. 1B). *Mild-1a* is not structurally symmetrical in that the distance between the 5' TSD and SIR is only 6 bp whereas there are 44 bp between the 3' SIR and TSD. Upon close inspection it appears that a 29 bp MSITE-like sequence is inserted in the 3' region of *Mild-1a*. The inserted MSITE (*Mild-2a*) has a 3 bp TSD (TCA) and a 6 bp SIR and is AT rich (62%) (Fig. 1B) and can form a hairpin with a ΔG of -4.6 kcal/mol. Alignment of the 163 bp promoter region of *DAT-3* with *Mild-1a* reveals that this region has high similarity to *Mild-1a*, containing all of *Mild-2a* and the 3' half of *Mild-1a*. The 3' TSD is degraded but still discernable (Fig. 1A). This element inserted within the promoter region of *DAT-3* is being designated as *Mild-1b*.

Other genomic clones developed as a part this project led to the isolation of a 708 bp clone with 67% identity to *Mild-1a* without the *Mild-2a* insertion (TEL sequence shown, Fig. 2A). The clone contains a copy of another complete MSITE-like sequence (Fig 2B). This element is 253 bp in length, 60% AT, and has an imperfect 9 bp TSD (AATTTG/-ACA), a 29 bp imperfect internal direct repeat (97% identity) and one set of SIRs 109 bp in length (81% identity) that can form a hairpin structure with a ΔG of -72.9 kcal/mol (Fig. 2B). This element was designated *Mild-3a* (GenBank accession no. [DQ011149](#)).

Blastn analysis of a genomic clone of the first intron of the *L. decemlineata* *DAT-2* gene (Yocum, 2003) revealed two regions, a 25 and a 53 bp stretch with 96 and 85%

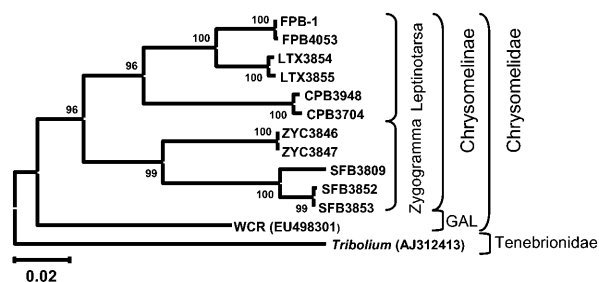


Fig. 4. Minimum evolution tree using mitochondrial *cox1* sequences of Chrysomelids (*Leptinotarsa*, *Zygogramma*, *Diabrotica*) and a Tenebrionid (*Tribolium*). GAL, Galerucinae. Scale is the number of nucleotide substitutions per site.

identity within the promoter of *L. decemlineata* juvenile hormone esterase *B* gene (*JHEB*) (Vermunt et al., 1998, GenBank accession no. [AF39135](#)) (Fig. 3A). Examining these regions led to identification of another complete MSITE-like TEL in the first intron of *DAT-2*. The TEL is 227 bp in length with perfect 12 bp TSD (TTGGTAATTAG) and is AT rich (64%). This TEL has an odd arrangement of inverted repeats. The 5' inverted repeat is terminal, being adjacent to the TSD, while the 3' inverted repeat is separated by 33 bp from the 3' TSD (Fig. 3B). Aligning the 5' inverted repeat with the two 3' inverted repeats including the 9 bp indel yielded identity of 88% (Fig. 3B). Mfold analysis of this sequence revealed that it would form a hairpin structure with a ΔG of -58.4 kcal/mol. This new MSITE-like sequence is being designated as *Mild-4a* (GenBank accession no. [EF121856](#)). The TSDs of *Mild-1a*, *Mild-3a* and *Mild-4a* have a common conserved core of AATTT. A Blastn search restricted to *Tribolium castaneum* genome using *Mild-1*, *-2*, *-3* and *-4* yielded no significant results. Comparison of mitochondrial *cox1* sequences indicates a genetic distance between *L. decemlineata* and *T. Castaneum* of 0.214 while the distance between *L. decemlineata* and *L. juncta* is 0.102.

Southern analysis

Probes designed against *Mild-1a* and *Mild-3* revealed that these two elements are throughout the genome of both *L. decemlineata* and *L. juncta* (Fig. 5, Only *Mild-3* results are shown, *Mild-1a* gave similar results to *Mild-3*). The heavy smearing on the southern blots indicates that *Mild-1a* and *Mild-3* elements are common throughout the genomes in these two beetle species. The lesser genetic distance between *L. juncta* and *L. texana* (0.040) suggests that the latter species could also have these elements.

CONCLUSIONS AND DISCUSSION

Transposable elements are major component of eukaryotic genomes examined to date. Transposons are grouped into broad classes based on the means in which they move within the genome. Class 1 transposons move through a RNA intermediate involving several different enzymes. Class 2 transposons' means of transposition is by a "cut and paste" reaction facilitated by a transposase. Transposons can be further subdivided above the family

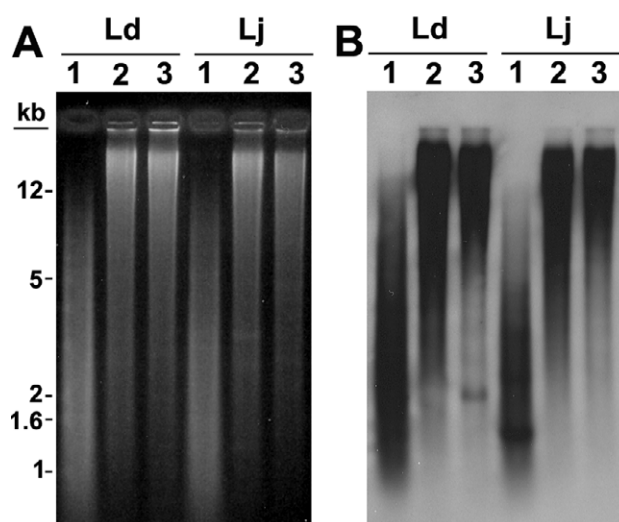


Fig. 5. Southern blot analysis of the putative transposable element *Mild-3a* in *Leptinotarsa decemlineata* (Ld) and *Leptinotarsa juncta* (Lj). Twenty micrograms of genomic DNA were digested with *EcoR* 1 (1), *Fsp* 1 (2), or *Xho* 1 (3) and separated on a 0.7% agarose gel. Ethidium bromide stained agarose gel (A), southern filter probe with a digoxigenin-labeled probe (B). Probe against *Mild-1a* gave results similar to *Mild-3a* but at a somewhat lower level of hybridization (data not shown).

level by if they are autonomous or nonautonomous elements. Nonautonomous elements lack the open reading frames needed to encode the enzymes necessary for transposition (Feschotte et al., 2002b). Nonautonomous elements are formed from internal deletions of autonomous transposons. Individual MITE families have evolved from either the IS630-Tc1-mariner or PIF-Harbinger of autonomous transposons (Feschotte et al., 2002b; Turcotte & Bureau, 2002; Feschotte et al., 2005; Menzel et al., 2006). Both of these superfamilies of transposons are found in insects (Tu, 2005).

MITEs were first isolated from maize, *Zea mays* (Bureau & Wessler, 1992). Though most extensively investigated in plants and insects, MITEs have also been isolated from the fungus *Neurospora crassa* (Yeadon & Catcheside, 1995), the frogs *Xenopus borealis* and *Xenopus leavis* (Unsal & Morgan, 1995), the nematode *Caenorhabditis elegans* (Oosumi et al., 1996), the fish *Ctenopharyngodon idellus*, *Cyprinus carpio*, *Danio aequipinnatus*, *Danio albolineatus*, *Danio latipes*, *Danio rerio* and *Hypophthalmichthys molitrix* (Izsvak et al., 1999) and humans, *Homo sapiens* (Morgan, 1995; Smit & Riggs, 1996). The copy number of an individual MITE can vary greatly from a few (8 copies of *mpING* in the Indica cultivar 93-1 of rice, *Oryza sativa*) (Jiang et al., 2003; Yu et al., 2002) to over 6,000 copies of *mPIF* in maize (Zhang et al., 2001). In plants screened to date, MITEs as a class are a major component of the total TEL population within their genome. In *Arabidopsis* the MITE composition is 25% of total TELs, and MITEs make up approximately 75% of all TELs in the rice genome, with over 90,000 copies of individual MITEs (see Fig. 1 in Jiang et al., 2004). The species distribution of individual MITE families can vary greatly: *Ditto*, *Wanderer*,

Explorer, *Snap*, *Crackle* and *Pop* are restricted to rice (Bureau et al., 1996; Song et al., 1998; Yang et al., 2001), and *Stowaway* is found in both monocots and dicots (Bureau & Wessler, 1994b). *Tourist*-like MITEs are found in grasses, *Arabidopsis*, *C. elegans* and insects (Bureau & Wessler, 1992, 1994a; Le et al., 2000, 2001; Tu, 2005). A transposable element is classified as a MITE based on seven normally seen structural characteristics and not on its sequence identity (reviewed, Feschotte et al., 2002a). (1) MITEs are characterized by their small size varying from 70 to 1,600 bp. The putative MSITEs described here are 29 to 284 bp. At 29 bp, *Mild-2a* would be the smallest MSITE reported in the literature. (2) Most MITEs have TIRs, although a few families such as *Mint1* (Feschotte & Mouches, 2000), *Pop* and *Crackle* (Song et al., 1998) and *Microuli* (Tu & Orphanidis, 2001) lack TIRs and have SIRs instead. *Mild-1*, -2, -3 and -4 clearly fall in the small group of MITEs lacking TIRs and having SIRs instead. *Mild-4a* appears to be an intermediate between a true MITE and a MSITE, in that its inverted repeat is terminal only on one side and it has a 33 bp insert between the inverted repeat and the TSD on the other side. Tu & Orphanidis (2001) proposed that MSITEs may serve as an intermediate for development of some novel MITEs either through mutation or recombination events: *Mild-4a*'s structure gives credence to this theory. (3) Many MITEs have the ability to form a hairpin-like secondary structure, a characteristic shared by *Mild-1a*, -2a, -3a and -4a. (4) MITEs are nonautonomous elements lacking an open reading frame. A Blastx search (March 25, 2010) failed to find identity to any known entries in GenBank for *Mild-1a*, -2a, -3a or -4a. (5) MITEs have preferred target sites from 2 to 9 bp in length that are duplicated upon insertion so that the MITE is flanked by direct repeats (TSD). The direct repeats flanking the *L. decemlineata* Mild-elements vary between 3 and 14 bp in length with *Mild-1*, -3 and -4 sharing the common core sequence of AATTT. (6) The AT composition of the Mild elements varies between 60 and 64%, a range commonly seen in other MITEs. (7) Individual members of a MITE family can vary in size.

A distinguishing characteristic of *Mild-1a* and -3a is their internal direct repeats. Some of these internal direct repeats are relatively large in relationship to the overall size of the MSITE in which they are found, 45 and 29 bp for *Mild-1a* and -3a respectively. The large internal direct repeats are located just on either side of the hairpin loop. These repeats are substantially longer than the pentameric internal direct repeats found in *Tourist* (Bureau & Wessler, 1992).

Besides these structural similarities, certain propensities of MITEs have emerged in the literature. MITEs target low copy sequences and therefore are commonly inserted near or within the noncoding regions of genes (Jiang et al., 2003, 2004). This propensity of MITEs to insert within the noncoding regions of genes led to the initial identification of *Mild-1* and -2 within the first intron of *DP-1*, and *Mild-4* within the first intron of *DAT-2*. Besides their preference for noncoding regions around

genes, MITEs commonly insert within other MITEs. The insertion of one MITE into another is a common enough occurrence that it has led to the identification of new MITE families (Bureau & Wessler, 1994b; Feschotte & Mouches, 2000; Petersen & Seberg, 2000; Tu, 1997). This nesting behavior led Feschotte & Mouches (2000) to suggest that MITEs may be preferred targets for insertion by other MITEs. The presence of *Mild-2a* within the 3' end of *Mild-1a* is an example of this nesting of MITEs. The heavy smearing observed in the Southern blots would indicate that the elements *Mild-1* and *Mild-3* are present in high copy numbers in these two congeneric species. Southern results of MITEs in other species varies from distinct bands to smears as seen in *L. decemlineata* and *L. juncta* (Bureau & Wessler, 1992; Braquart et al., 1999; Yang et al., 2001; Menzel et al., 2006; Remigereau et al., 2006).

TEs are a major component of most eukaryotic genomes varying from approximately 2% for *C. elegans* (Waterston & Sulston, 1995) to approximately 58% in maize (SanMiguel et al., 1996; Messing et al., 2004). By their sheer number, TEs have a major influence on genome size and architecture, thereby affecting an organism's phenotypic traits by mutagenesis, genomic rearrangement (review: Kidwell & Lisch, 2001, 2002) and nucleotypic effects (review: Gregory & Hebert, 1999). The insertions of MITE-like elements in the promoter regions of *DAT-3* and *JHEB* and in introns of *DAT-2* and *DP-1* would suggest that TEs may have played a role in the evolutionary development and phenotypic expression of diapause in the Colorado potato beetle. This possible role for TEs in *L. decemlineata* diapause is emphasized by the fact that the *JHEB* gene regulates the expression of diapause by controlling juvenile hormone titers (Denlinger et al., 2005). Determining what the exact role, if any, TEs play in the *L. decemlineata* diapause awaits the sequencing of its genome and extensive field studies.

In conclusion: (1) Even with our current limited information about *L. decemlineata* and *L. juncta* genomes, MITE-like elements appear to be common in both. (2) The presence of *Mild-1* and -3 elements in both *L. decemlineata* and *L. juncta* indicate that these elements occurred in their common ancestor. (3) The presence of these elements with the promoter regions and introns of diapause-regulated genes would indicate a possible role of TEs in the evolution and regulation of diapause

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