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Complete List of Authors:	Ben Amara, Wiem; University of Tunis El Manar Faculty of Sciences of Tunis, Laboratory of Biochemistry and Biotechnology (LR01ES05) Djebbi, Salma; University of Tunis El Manar Faculty of Sciences of Tunis, Laboratory of Biochemistry and Biotechnology (LR01ES05) Ben Lazhar-Ajrout, Wafa; University of Tunis El Manar Faculty of Sciences of Tunis, Laboratory of Biochemistry and Biotechnology (LR01ES05) Naccache, Chahnez; University of Tunis El Manar Mezghani, Maha; University of Tunis El Manar Faculty of Sciences of Tunis, Laboratory of Biochemistry and Biotechnology (LR01ES05)
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Insights on *mauritiana*-like Elements Diversity in *Mayetiola destructor* and *M. hordei* (Diptera: Cecidomyiidae)

Wiem Ben Amara^{1,2}, Salma Djebbi¹, Wafa Ben Lazhar-Ajrourd¹, Chahnez Naccache¹ and Maha Mezghani-Khemakhem^{1*}

¹Laboratory of Biochemistry and Biotechnology (LR01ES05), Faculty of Sciences of Tunis, University of Tunis El Manar, Tunisia

² Sciences Department, CANOPUS International School of Tunis, Tunisia (CIST)

*Corresponding Author: Maha Mezghani-Khemakhem

Abstract:

Mariner-like elements (*MLEs*) are class II transposons belonging to the *Tc1-mariner* family, that have successfully invaded many insect genomes. In the current study, the availability of the Hessian fly *Mayetiola destructor* genome has enabled us to perform *in silico* analysis of *MLEs* using as query the previously described *mariner* element (*Desmar1*) belonging to *mauritiana* subfamily. Eighteen *mauritiana*-like elements were detected and were clustered into three main groups named *Desmar1*-like, *MauCons1* and *MauCons2*. Subsequently, *in vitro* analysis was carried out to investigate *mauritiana*-like elements in *M. destructor* as well as in *Mayetiola hordei* using primers designed from TIRs of the previously identified *MLEs*. PCR amplifications were successful and a total of 12 and 17 *mauritiana*-like elements were discovered in *M. destructor* and *M. hordei*, respectively. Sequence analyses of *mauritiana*-like elements obtained *in silico* and *in vitro* have showed that *MauCons1* and *MauCons2* elements share low similarity with *Desmar1* ranging from 50% to 55% suggesting different groups under *mauritiana* subfamily have invaded the genomes of *M. destructor* and *M. hordei*. These groups are likely inherited by vertical transmission that subsequently underwent different evolutionary histories. This work describes new *mauritiana*-like elements in *M. destructor* that are distinct from the previously discovered *Desmar1* and provides the first evidence of *MLEs* belonging to *mauritiana* subfamily in *M. hordei*.

Keywords: *Mayetiola destructor*, *Mayetiola hordei*, transposable elements, *MLEs*, *mauritiana*, diversity

51 Introduction

52 Transposable elements (TEs) are repetitive DNA sequences able to move from one
53 chromosome to another within the host genome (Mackay, 1989). They are present in the
54 genome of most living organisms with a large range of proportions varying from 1% in
55 prokaryotic genomes to 90% in some plant genomes (Touchon and Rocha, 2007, Guio and
56 Gonzalez, 2019). TEs activity leads to the generation of various mutations and chromosomal
57 rearrangements including insertions, deletions, inversions, duplications, and translocations.
58 These mutations can be deleterious to the host organism but can also be beneficial by providing
59 new advantageous host functions through domestication or gene regulation. (Kidwell and Lisch,
60 1997, Chenais et al., 2012, Bourgeois and Boissinot, 2019, Klai et al., 2020).

61 TEs are classified according to their transposition intermediates into Class I elements or
62 retrotransposons which transpose through “Copy-Paste” mechanism and Class II elements or
63 DNA transposons that use the “Cut and Paste” mechanism for transposition (Wicker et al.,
64 2007, Kapitonov and Jurka, 2008). Additionally, TEs can be autonomous, able to move
65 independently using their own transposition enzymes, or non-autonomous elements, which
66 require the enzymatic machinery of autonomous elements to be mobilized (Feschotte and
67 Pritham, 2007).

68 The *Tc1/mariner* transposons are the most widespread superfamily of DNA transposons among
69 eukaryotes (Yuan and Wessler, 2011). TEs of *Tc1/mariner* superfamily are classified into at
70 least eight families according to the DDE/D signature variants, namely: DD34E/*Tc1*-Like
71 Elements (*TLEs*) (Emmons et al., 1983), DD34D/*Mariner*-Like Elements (*MLEs*) (Jacobson et
72 al., 1986), DD35E/TR (Zong et al., 2020), DD36E/IC (Sang et al., 2019), DD37D/maT (Zhang
73 et al., 2016), DD37E/TRT (Zhang et al. 2016), DD41D (Gomulski et al., 2001) and DDxD/pogo
74 (Shao and Tu, 2001, Dupeyron et al., 2020, Wang et al., 2021). Among these various families
75 of the *Tc1/mariner* superfamily, *MLEs* have been well studied in several eukaryotic organisms

especially insects where they were identified for the first time (Jacobson et al., 1986, Robertson, 1993).

A full length *MLE* exhibit a simple structure of approximately 1300 bp in length consisting of an intronless gene encoding a transposase flanked by two terminal inverted repeats (TIRs) of 28–30 bp with typical TA dinucleotides serving as target site duplication (TSD). The *MLE* transposase is characterized by a N-terminal DNA-binding domain of Helix-Turn-Helix (HTH) type and a C-terminal catalytic domain with the conserved motif DD(34)D (Plasterk et al., 1999, Brillet et al., 2007, Yuan and Wessler, 2011).

At least eleven *MLE* subfamilies have been described according to the similarities of the transposase catalytic core amino acid sequences (Robertson, 1997, Green and Frommer, 2001, Carr, 2008, Bui et al., 2007, Wallau et al., 2014, Zhang et al., 2015). However, five subfamilies are considered as the main references namely: *cecropia*, *elegans/briggsae*, *irritans*, *mauritiana*, *mellifera* (Robertson and MacLeod, 1993).

As all TEs, the *MLEs* have a life cycle of three fundamental stages including invasion, maturity, and senescence (Kidwell and Lisch, 2001, Plasterk and van Luenen, 2002). The invasion stage consisting on the introduction of a TE into a virgin genome either by horizontal transfer (HT) mediated by introgression or vectors (Finnegan, 1985, Daniels et al., 1990).

During the maturity stage, the host genome will develop different mechanisms to reduce the *MLE* activities and limit its deleterious proliferation. Mechanisms include Over Production Inhibition (OPI) (Lohe and Hartl, 1996) as well as epigenetic regulation by methylation and post-transcriptional regulation through piwiRNA (piRNA) or microRNA (miRNA), considered as candidates for silencing mechanisms (Hamilton et al., 2002, Xie et al., 2004, Abrusán and Krambeck, 2006, Piriyaongsa and Jordan, 2007).

During the senescence stage, a great loss of *MLE* copies will happen and some of them could be extinct by vertical inactivation or stochastic loss (Le Rouzic and Capy, 2005, Lohe et al., 1995). To escape this selective pressure, some *MLEs* tend to invade new genomes by HT to

initiate a new cycle and perpetuate. Horizontal transfer of *MLEs* was proposed for the first time in insects then it was reported in different taxa (Lampe et al., 2003, Robertson et al., 2002, Oliveira et al., 2012, Robertson and Lampe, 1995).

The Cecidomyiidae are one of the largest families of Diptera affecting cereals in different parts of the world. The Hessian fly, *Mayetiola destructor* (Say, 1817) and the barley gall midge, *Mayetiola hordei* (Kieffer, 1909) cause considerable damage to wheat and barley cultures particularly in the Mediterranean region, in Europe and in different states of USA (Gagné et al., 1991, Ratcliffe et al., 2000, Cherif et al., 2020, El Bouhssini et al., 2013). For more than half a century, *M. destructor* has been considered as an ideal model to study the plant-insect gene interactions (Harris et al., 2003, Stuart et al., 2012, Hatchett and Gallun, 1970, Flor, 1971).

The first described *MLEs* in *M. destructor*, were identified using a PCR-based method and consisted of partial elements belonging to *irritans* subfamily (Russell & Shukle, 1995). Subsequently, a full-length copy of *MLE* with intact ORF and perfect TIRs belonging to *mauritiana* subfamily were identified experimentally and named *Desmar1* (Russell and Shukle, 1997, Behura et al., 2010). More recently, *irritans MLEs* from TIR to TIR were identified in *M. destructor* and *M. hordei* using complementary approaches combining *in silico* analyses and *in vitro* experiments (Ben Amara et al., 2017). In *M. hordei*, *MLEs* of *mauritiana* elements have not yet been reported. In this work, we explore the diversity of *mauritiana MLEs* in both species of *Mayetiola* using experimental and homology-based methods.

Materials and Methods

Insect Sampling and Identification

Total DNA was extracted from individual insects of *M. destructor* and *M. hordei* using the salting-out protocol (Sunnucks and Hales, 1996) then conserved at -20°C in sterile water. Species identification was carried out by PCR-RFLP of the cytochrome b gene method described by Mezghani Khemakhem et al. (2002).

Data sources

The *M. destructor* genome of 153 Mb available in NCBI BioProject PRJNA45867 was used to identify *MLEs mauritiana*-like elements. This genome was assembled into 36,371 contigs with a 14 kb contig N50 length and 24,475 scaffolds with a 756 kb N50 length. In addition, the transcriptome shotgun assembly (TSA) of the orange wheat blossom midge *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) was screened to identify similar *mauritiana*-like elements. This TSA consists of 24,383 complementary DNA (cDNA) contigs and is available in GenBank (PRJNA192921).

In silico investigation of *mauritiana*-like elements

The *M. destructor* whole genome sequence (WGS) was investigated to identify and characterize the *MLEs* of *mauritiana* subfamily. For this purpose, the Basic Local Alignment Search Tool:BLAST (Altschul et al., 1990) was applied with the default parameters using the whole *Desmar1* element (from TIR to TIR) as a query (Accession number: U24436.1) to search and extract nucleotide sequences using the BLAST-N program. Genomic scaffolds exhibiting similarities with queries (E-value < E-10) were selected and complete *mauritiana*-like elements were extracted.

PCR amplifications

A series of PCR has been performed to amplify *mauritiana*-like elements in *M. destructor* and *M. hordei* using primers listed in **Table 1**. These primers were designed from TIRs ends and from conserved domains of internal regions of *MLEs*. PCR program consisted of an initial cycle at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 15s, annealing step at varying temperatures depending on the sequences of the primers used and extension at 72°C for 2 min and a final extension at 72°C for 10 min. PCR amplification products were visualized on 1% agarose gel stained with ethidium bromide then cloned and sequenced as described by Ben Amara et al. (2017).

152 Inverse and nested PCR amplifications

153 Genomic DNA (500 ng) were individually extracted from *M. destructor* and *M. hordei* and
 154 digested with 5U of restriction endonuclease EcoRI or DraI or HindIII (Promega®) at 37°C for
 155 1h then circularized in 150 µl volume reaction containing 100 U of T4 DNA Ligase (New
 156 England BioLabs) and 30 µl of reaction buffer. After incubation for 5 hours at 37°C, a volume
 157 of 10 µl of circularized products is used to perform iPCR and nPCR using primers reported in
 158 **Table 1** and conditions described by (Ben Lazhar-Ajrout et al., 2016). The products of iPCR
 159 and nPCR were subsequently purified, cloned and sequenced on both strands following the
 160 experimental protocol of Kharrat et al. (2015).

161 Sequences and Phylogenetic analyses

162 . TIRs were checked out using the online tool einverted (Durbin and Rice, 1999) and then
 163 aligned and visualized by CLC Genomics Workbench 9.5.3 program
 164 (<https://www.qiagenbioinformatics.com/>).

165 Alignment of the nucleotide and protein sequences of *MLEs* was performed by MAFFT
 166 program using the default parameters (gop 1.53;
 167 gep 0.123) then visualized on Genedoc (Nicholas and Nicholas, 1997, Long et al., 2016). The
 168 HTH domains of the transposases were predicted on the PRABI platform (Combet et al., 2000)
 169 and the NLS were predefined by the online program SeqNLS (Lin and Hu, 2013) using the
 170 transposase reference patterns described by Augé-Gouillou et al. (2001).

171 Phylogenetic relationships were established between *MLEs* of the two *Mayetiola* species and
 172 the *S. mosellana* as well as other *mariner* subgroups belonging to other insect species retrieved
 173 from GenBank and arbitrarily chosen. They were constructed by the Maximum Likelihood
 174 method according to the HKY85 model (Hasegawa et al., 1985) which was arbitrarily chosen with
 175 a bootstrap of 1000 replication. Trees were obtained by Mega10 software (Kumar et al., 2018)
 176 then visualized and edited by the iTOL v3 program (Letunic and Bork, 2016).

The classification of MLE sequences from *M. destructor*, *M. hordei* and *S. mosellana* was carried out using the Unweighted Pair Group Method with Variation of Metric (UPGM-VM) which is an ascending hierarchical classification analogous to the classical UPGMA with some methodological differences (Rouault et al., 2009, Filee et al., 2015). The classification was implemented among a set of 449 transposable elements belonging to the *Tc1/mariner* superfamily.

Results

In silico identification of *mauritiana*-like elements in *M. destructor* genome

The BLASTN using *Desmar1* as query allowed the identification of 17 contigs containing 18 sequences named *Mdes1* to *Mdes18*. These elements were sized from 1202 to 1321 bp and exhibited TIRs of 22 to 45 bp with the TA target site duplication (**Table 2**). Apart from *Mdes1* element, all identified *MLEs* have one or two imperfect TIRs flanking ORFs with stop, frameshift and/or indel mutations that encode putative transposases sizing from 324 to 368aa. The phylogram inferred from the alignment of the 18 *mauritiana*-like elements with *Desmar1* and the other references of *MLEs* sub-families showed two clades; the first one included *Desmar1* and the 13 *mauritiana*-like elements (*Mdes1* to *Mdes13*) while the second contained the 5 remaining *mauritiana*-like elements that are subdivided into two groups (*Mdes15* to *Mdes18*) and (*Mdes14*) (**Figure 1**).

In vitro search for *mauritiana*-like elements

The *mauritiana*-like elements were amplified in *M. destructor* and *M. hordei* samples using the three primers *DES*, *MauCons1*, *MauCons2* designed from TIRs of *Desmar1*, *Mdes15-Mdes18* and *Mdes14*, respectively (**Figure 1**). The resulted PCRs generated bands sized between 1100 and 1300 bp corresponding to the typical sizes of *MLEs* in both species (**Figure 2**).

- **Characterization of *Desmar1*-like elements amplified by *DES* primer**

201 The *Desmar1*-like elements amplified by *DES* primer in *M. destructor* and *M. hordei* were
 202 designed according to the nomenclature proposed by Robertson and Asplund (1996). A total of
 203 five elements were identified in *M. destructor* and seven elements in *M. hordei* named
 204 *Mdesmar1.1* to *Mdesmar1.5* and *Mhormar1.1* to *Mhormar1.7*, respectively. These elements are
 205 available under the GenBank accession numbers MW093693 to MW093697 and MW093705 to
 206 MW093711. Nucleotide sequence analyses showed a strong similarity between the *Desmar1*-
 207 like elements amplified in the two species ranging from 98% up to 100%.

208 Like *Desmar1*, all predicted protein sequences exhibited a 347 aa transposase bordered by the
 209 MEN motif at the start and the YF/LE motif at the end except for *Mdesmar1.4* which has a
 210 transposase of 330 aa (**Figure 3a**).

211 Transposase analyses showed that the two signature motifs WVPHEL and YSPDLAP were
 212 conserved except for *Mdesmar1.5* which presents a substitution changing the Tryptophan (W)
 213 residue into Glycine (G) in the first motif (**Figure 3a**). In addition, domains of the DD(34)D
 214 catalytic triad are maintained intact in all *MLEs* except for the element *Mdesmar1.4* which has
 215 a deletion of 17 amino acid in the C-terminal domain. This deletion extends from residue 146
 216 to residue 162 covering the TGDEKW motif of the catalytic triad.

217 Further analyses revealed a TGA stop codon at position 200 in the *Mdesmar1.1* element due to
 218 a transition from G to A of nucleotide 764. Meanwhile, the four elements *Mhormar1.1* to
 219 *Mhormar1.4* showed a stop codon at position 173 due to a transversion from T to A of
 220 nucleotide 686.

221 In both species of *Mayetiola*, *MLEs* have TIRs displaying mirror and palindrome motifs centred
 222 around positions 14 and 15. TIRs are perfect in *M. destructor* and imperfect in *M. hordei*
 223 containing four substitutions at the positions 17, 18, 23 and 24 at the 5' TIRs and only 2
 224 substitutions at positions 23 and 27 at the 3' TIRs (**Figure 3b**).

Noticeably, all the TIRs contain the conserved sequence 5'YYAGRT3' involved in the cleavage of the TIRs ends described by Bigot et al. (2005) with A replaced by G nucleotide in the third position in the two species of *Mayetiola*.

- **Characterization of *Desmar1*-like elements flanking regions by iPCR**

Analyses of fragments obtained from iPCR allowed the identification of TIRs, the dinucleotide TA as well as the flanking regions of *Desmar1*-like elements in *M. hordei*. The 5' and 3' sides are 231 bp and 237 bp in length, respectively and have a protein similarity of 53% with the helicase PIF1 5'-3' ATP dependent in the hexapod *Folsomia candida* which inhibits telomere elongation by inactivation of telomerases.

- **Characterization of the *mauritiana*-like elements amplified by *MauCons1* primer**

The *in vitro* investigation of *mauritiana*-like elements using the *MauCons1* primer generated four elements from *M. destructor* and five elements from *M. hordei* which were designated *Mdesmar1.6* to *Mdesmar1.9* and *Mhormar1.8* to *Mhormar1.12*, respectively. These elements are available under the GenBank accession numbers MW093698 to MW093701 and MW093712 to MW093716. The *MauCons1*-amplified elements in *M. destructor* sized from 1138 to 1226 pb while those amplified in *M. hordei* sized from 1205 to 1214 bp.

The *MauCons1* transposases shared protein similarities ranging from 45% to 55% with *Desmar1* and other transposases from Drosophilidae and from the two hymenopteran species *Tapinoma nigerrimum* and *Messor bouvieri*. Moreover, the *MauCons1* transposases present high similarities between elements isolated from the two species of *Mayetiola* as revealed by high protein similarity between *Mhormar1.8*, *Mhormar1.9*, *Mhormar1.10* and *Mdesmar1.7* elements ranging from 95% to 99%.

Alignment of putative transposases identified *in vitro* and *in silico* showed patterns slightly different from *Desmar1*-like elements. The first signature motif WVPHQL displayed an E instead of Q amino acid and the motifs flanking the transposase are modified (**Figure 5**). In

addition, these transposases exhibited stop codons, and deletions spanning different signature motifs, catalytic triad as well as NLS domains. These mutations resulted in the shift of the stop codon to the 3'-TIRs in *Mdesmar1.7* to *Mdesmar1.9*. In both species, the 5'YYAGRT3' motif is conserved in all TIRs but with a difference at the last nucleotide which was replaced by G compared to *Desmar1*-like elements (**Figure S1**).

- **Characterization of the *mauritiana*-like elements amplified by *MauCons2* primer**

Three *mauritiana*-like elements were amplified in *M. destructor* and named *Mdesmar1.10* to *Mdesmar1.12* (GenBank accession numbers: MW093702 to MW093704). These elements displayed total length ranging from 1229 to 1284 bp and share 53% of protein identity with *Desmar1*. In *M. hordei*, five sequences were isolated and named *Mhormar1.13* to *Mhormar1.17* (GenBank accession numbers: MW093717 to MW093721). Identified elements have size varying between 1285 to 1299 bp and share 47% of protein similarity with *Desmar1*. The alignment of putative *MauCons2* transposases has shown two alignment blocks that differ by species (**Figure 6**). These transposases displayed WVPHQL motif and a YIG end like *MauCons1* elements. Besides, different stop codons and mutations altering the signature motifs and the DD34D triad domain were detected in all transposases.

In both species, TIRs analyses showed the modification of the 5'YYAGRT3' sequence generating the 5'YYGGRAA3' motif. Moreover, all TIRs are imperfect with substitution and ins/del mutations modifying the organization of the palindromic and mirror patterns (**Figure S2**).

Otherwise, the BLASTN performed on the TSA of *S. mosellana* using the consensus sequences generated from the three *mauritiana*-like elements' groups revealed four transcripts. Two partial transcripts (GAKJ01010239.1 and GAKJ01010238.1) exhibited 78% of nucleotide similarity with *Desmar1*-like elements and covered only the start of transposase up to the WVPHEL signature motif. The third partial transcript (GAKJ01006734.1) exhibited a nucleotide similarity of 71% with *MauCons1* and *MauCons2* elements and spreads from the

start of transposase to the motif WVPHQL. As for the last transcript (GBRL01006532.1), it was found to span all of the signature motifs as well as the catalytic domains with 77% of nucleotide identity (**Figure 7**).

- **Evolutionary dynamics of *mauritiana*-like elements**

Phylogenetic analyses of the *mauritiana*-like elements identified in the two *Mayetiola* species and *S. mosellana* by experimental and bioinformatic approaches allowed the construction of the phylogram shown in **Figure 8**. The branch lengths reflect the evolutionary time between sequences enabling the estimation of distances between *mauritiana*-like elements in *M. destructor*, *M. hordei* and *S. mosellana* and so the estimation of the transposition events having occurred over time. The tree is divided in two major clades: the *Desmar 1*-like clade and the *MauCons1/Maucons2* clade. Elements of *Desmar 1*-like clade showed several subgroups with different branch lengths reflecting different events of transposition followed by accumulation of inactivating mutations in *M. destructor*. Noticeably, a burst of a recent group of *Desmar 1*-like elements in *M. destructor* and *M. hordei* is observed. This group shares a single common ancestor with *S. mosellana* elements. The *MauCons1/Maucons2* clade showed a divergence of *S. mosellana* sequences from those of *Mayetiola* species. Sequences of *Mayetiola* split into two subclades with close branch lengths: the *MauCons1* and the *MauCons2* showing a species-related evolution.

Otherwise, all MLEs of *Mayetiola* and *Sitodiplosis mosellana* species were inferred in a phylogenetic tree to study their classification and distribution among known MLEs. The classification was performed using the UPGM-VM method from 449 nucleotide sequences. The phylogenetic analyses showed that all identified elements were clustered in a monophyletic group of *mauritiana* subfamily group (**Figure 9**).

Discussion and conclusion

The *mariner*-like elements (MLEs) have been known to invade a big variety of arthropods and especially insects (Robertson, 1993, Yamada et al., 2015). They represent an important driver of evolution in their host genome. In the current work, we focused on the identification and characterization of MLEs belonging to the *mauritiana* subfamily in the two

economically important pests *M. destructor* and *M. hordei* damaging respectively wheat and barley.

The combination of bioinformatic and experimental approaches allowed the identification of three *mauritiana* groups in the two species of *Mayetiola*. The first group contains elements similar to *Desmar1* previously reported by Russell and Shukle (1997) whereas the two other groups contain newly identified *mauritiana*-like elements *MauCons1* and *MauCons2* which are divergent from *Desmar1*. Invasions of different lineages of the same *MLE* subfamilies have also been described in different insects including Drosophilidae (Wallau et al., 2014).

Although two *MLE* subfamilies were identified in *M. destructor* by Shukle and Russell (1995), the current study allowed the identification for the first time of *MLEs* belonging to *mauritiana* subfamily in *M. hordei* in which only *irritans*-like elements were reported (Ben Amara et al., 2017). Most of the *Desmar1*-like elements identified in *M. destructor* and *M. hordei* exhibited intact transposases displaying few mutations suggesting their recent invasions in bursts leading to the spread of several copies. This is supported by the short branches of the *Desmar1*-like clade in both *Mayetiola* species. Similar observations were reported in diatoms and vertebrates involving *Tc1/mariner* elements (Hermann et al., 2014, Zong et al., 2020).

The high similarity observed between the *Desmar1*-like sequences (> 90%) suggests a possible HT event between the two *Mayetiola* species. Indeed, the *MLEs* dynamic is often characterized by recurrent extinction followed by HT from one species to another to ensure their persistence (Lohe et al., 1995, Hartl et al., 1997a, Wallau et al., 2014). Alternatively, the high percentage of identity between *Desmar1*-like elements could be explained by a constant turnover of *MLEs* with transposition rapidly followed by loss through host purifying selection leading to a limited sequence variation (Pereira, 2004).

Transposase sequence analyses of *Desmar1*-like elements in *M. hordei* showed conserved transversion having generated a stop codon. This kind of substitutions could be a

host genome mechanism to inactivate recently transposed elements by vertical inactivation (Lohe et al., 1995, Hartl et al., 1997b), given that transversions are less frequent but more detrimental than transitions (Lyons and Lauring, 2017).

Unlike *M. destructor*, *M. hordei* exhibited imperfect TIRs in *Desmar1*-like elements with mutations altering mirror and palindromic regions described by Bigot et al. (2005). However, this finding cannot confirm the inactivation of these elements as imperfect TIRs were previously described in naturally active *MLEs* like *Mos1* from *Drosophila mauritiana* and *Mboumar.9* from *Messor bouvieri*. These active elements displayed different affinities for their transposase.

Otherwise, elements of *MauCons1* and *MauCons2* groups seem to be less active compared to *Desmar1*-like elements since they carry several mutations altering specific domains that lead to non-functional transposases. Such elements could be able to evolve as pseudogenes that accumulate mutations neutrally (Hartl et al., 1997b). Many other *mauritiana* *MLEs* were found to be inactive in different species like aphids (Kharrat et al., 2015) and Bruchidae species (Djebbi et al., 2017). More interestingly, some deletions were found to cover the same positions in both *Mayetiola* species. This could be a mechanism contributing to defective *MLEs* or element decay as described in the *irritans* subfamily (Ben Amara et al., 2017, Ben Lazhar-Ajrout et al., 2016).

Furthermore, *Desmar1*, *MauCons1* and *MauCons2*-like elements exhibited high sequence identity between *M. destructor* and *M. hordei* which may indicate an eventual vertical inheritance of these *MLEs* from an intermediate common ancestor. Indeed, *MLEs* have been shown to undergo long-term, vertical inheritance in other dipteran genomes such as the stalk eyed flies (Carr, 2008). Similar studies carried on ants highlighted a vertical transmission with differential evolutionary success and spread into different genomes during species-separation processes (Sanllorente et al., 2020). Other studies have revealed the establishment of *irritans*

subfamily elements in closely related *Bactrocera* species originating from the expansion from a common ancestor (Ben Lazhar-Ajrout et al., 2016) .

Moreover, *mauritiana*-like elements in *Mayetiola* species were found to share a common ancestor with *S. mosellana* whose sequences diverged to undergo their own evolutionary history. Indeed, in *S. mosellana*, the *Desmar1*-like elements would be counter-selected since the presence of fragmented copies of *MLEs* while the *MauCons1*/*MauCons2* elements would evolve independently from *Mayetiola* sequences as one clade. In the two *Mayetiola* species, the clade of *MauCons1* and *MauCons2* elements would split later in two subclades transmitted vertically to *M. destructor* and *M. hordei*. Subsequently, *MauCons1* *MauCons2* evolved in both species separately with possible HT events involving specifically *MauCons1* elements. Nevertheless, an eventual introgression should not be excluded as alternative event to HT inside the *MauCons1* clade due to an old event of hybridization between *M. destructor* and *M. hordei*. Similar hypothesis of introgression was recently suggested for the germline restricted chromosomes (GRCs) in the two Diptera species; Sciaridae and Cecidomyiidae (Hodson et al., 2021)

To sum up, the genomes of *M. destructor* and *M. hordei* hosted three different groups of *mauritiana* subfamily that have exhibited old events of transposition which occurred before the *Mayetiola* speciation. These groups should be inherited by vertical transmission in *M. destructor* and *M. hordei* genomes to undergo different evolutionary histories. According to the scenario suggested by Le Rouzic et al. (2013), the vertical transmission of *mauritiana*-like elements should be followed by proliferation and differentiation with accumulation of different mutations. Further studies should be focusing on the *MLEs* activities and insertion sites to check eventual impact on the genome evolution of these cecidomyiid flies.

Conflicts of interest

The authors declare no competing financial interests.

380

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a.

b.

Figure 1. a. Phylogeny based on the nucleotide sequences of the 18 full length *mauritiana*-like elements identified *in silico* in *M. destructor* **b.** Representation of the primers designated from the ends of the TIRs of the three consensus *DES*, *MauCons1* and *MauCons2*

The tree was inferred using the Maximum Likelihood method (model HKY85) with a bootstrap of 1000 replications. The bootstrap values for nodes below 50% were omitted. The reference elements belonging to the 5 sub-families of MLEs, the TLEs (DD34E), the *maT* (DD37D), the Plant MLE (DD39D) and the *rosa* elements (DD41D) were taken from the GenBank database and whose accession numbers are as follows: *M. destructor Desmar1* (U24436.1) *DIv3-Hf* (DQ015886.1), *Tapinoma nigerrimum Tnigmar* (HE577149.1), *Messor bouvieri Mboumar* (AJ781770.1), *Drosophila teissieri Dtesmar1* (AF035567.1), *Drosophila simulans DsmarL14* (AF037055.1), *Drosophila sechellia Dsemar* (AF035569.1), *D.mauritiana Mos1* (M14653.1), *Musca domestica Mdmr1* (AF373028.1), *Ceratitis capitata Ccmar2* (AY155493.1), *Apis mellifera Ammar1* (AY155490.1), *Forficula auricularia Famar1* (AY155492.1), *Girardia tigrina Gtmar1* (X71979.1), *Gtmar-9* (X80894.1), *Attacus atlas Aamar1* (AB006464.1), *Bombyx mori Bmmar2* (D88671.1), *Haematobia irritans Himar1* (U11642.1), *H. sapiens Hsmar2* (U49974.1), *Chrysoperla plorabunda Cpmar1* (U11654.1), *Mantispa pulchella Mpmar1* (U11649.1), *Adoxophyes honmai Ahmar1* (AB020617.1), *Caenorhabditis elegans Cemar1*(AF014939_1), *Cemar2* (Y39A3A_1), *Adineta vaga Avmar1* (DQ138284_1), *Glycine max Soymar1* (AF078934.1), *Beta vulgaris*

Vulmar1 (AJ556159.1), *Ceratitis rosa Crmar1* (AY034623.1), *Bactrocera tryoni Batmar2* (KX931016.1), *Bombyx mori Bmmar1* (U47917.1), *Misgolas hubbardi MhmaT1* (JF779677.1), *Caenorhabditis briggsae TCb1* (X54218.1), *Caenorhabditis elegans Tc1* (X01005.1), *Drosophila virilis Tc1-like* (U26938.1), *Drosophila heteroneura Tc1* (X17356.1).

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Figure 2. Results of PCR amplifications of the *mauritiana*-like elements in *M. destructor* and *M. hordei* obtained by the *DES*, *MauCons1*, *MauCons2* and the internal primers

100 bp and 1 kb denote size markers (Ladder)

Figure 4. iPCR and nPCR results for regions flanking *MLE* obtained from *M. hordei*

The yellow arrow on the gel indicates the excised band. In the sequence obtained, the underlined region corresponds to the DNA flanking the element *Mhormar1*; the region in grey is the one surrounding the 5' side of the element and amplified by the reverse primers while the underlined black region corresponds to the one surrounding the 3' side of the element and amplified by the sense primers. The black primers correspond to iPCR and the primers in purple correspond to nPCR. The EcoRI enzyme restriction site is marked in blue. Element TIRs are boxed and bordered by TA dinucleotide (TSD) and *MLE* sequences are marked in burgundy red.

Figure 5. Alignment of putative transposases of *mauritiana*-like elements amplified by *MauCons1* primer in *M. destructor* and *M. hordei*

Figure 6. Alignment of putative transposases of *mauritiana*-like elements amplified by *MauCons2* primer in *M. destructor* and *M. hordei*

Figure 7. Comparison between the cDNA contigs identified in *S. mosellana* and *MLE* consensus constructed from the *mauritiana*-like elements of *M. destructor* (*Desmar-cons1*, *Desmaucons1* and *Desmaucons2*) and *M. hordei* (*Hormaucons1* and *Hormaucons2*)

Figure 8. Phylogram of identified *mauritiana*-like elements in the two *Mayetiola* species and in *Sitodiplosis mosellana*.

Figure 9. Classification of *Mhormar1* and *Mdesmar1* MLEs using the UPGM-VM method among a set of 449 transposable elements belonging to the *Tc1/mariner* superfamily. The 449 elements were subdivided in 8 groups: MLEs of *mellifera* subfamily (59 sequences), MLEs of *mauritiana* subfamily (87 sequences including 31 *Mdesmar1* elements, 18 *Mhormar1* elements and 4 *S. mosellanae* elements), MLE of *capitata* subfamily (3 sequences), MLE of human *irritans* subfamily (8 sequences), MLE of *cecropia* subfamily (15 sequences), MLEs of *briggsae/elegans* subfamily (18 sequences), MLE of arthropod *irritans* subfamily (136

sequences) and the external group containing 124 other MLEs, TLEs and IS elements indicated with black branches. The reading sense of this circular tree is indicated at right by a dashed arrow

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