Genome size evolution in the beetle genus *Diabrotica*.

Dimpal Lata[1], Brad S Coates[2], Kimberly K O Walden[3], Hugh M Robertson[3] and Nicholas J Miller[1*]

1. Department of Biological Sciences, Illinois Institute of Technology, Chicago, Illinois, USA.

2. USDA-ARS, Corn Insects & Crop Genetics Research Unit, Ames, Iowa, USA.

3. Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, Illinois USA.

*Correspondence: Nicholas J Miller, 298 R A Pritzker Science Center 3101 S Dearborn St, Chicago, Illinois 60616, nmiller11@iit.edu, +1 312 567 3288.

Abstract

Diabrocite corn rootworms are one of the most economically significant pests of maize in the United States and Europe and an emerging model for insect-plant interactions. Genome sizes of several species in the genus *Diabrotica* were estimated using flow cytometry along with that of *Acalymma vittatum* as an outgroup. Within the *Diabrotica* subgroups fucata and virgifera, genome sizes ranged between 1.59 - 1.68 gigabase pairs (Gb) and between 2.31- 2.65 Gb, respectively, and the *Acalymma vittatum* genome size was around 1.69 Gb. This result indicated that a substantial increase in genome size occurred in the ancestor of the virgifera group. Further analysis of fucata group and virgifera group genome sequencing reads indicated that the genome size difference between the *Diabrotica* subgroups could be attributed to a higher content of transposable elements, mostly miniature inverted-transposable elements (MITEs) and LTR gypsy-like elements.

Introduction

The family Chrysomelidae is one of the largest families of phytophagous beetles (Order: Coleoptera), with nearly 40,000 species. A large number of species are important agricultural and forestry pests causing negative economic impacts (Reid, 1995; Nie et al., 2020). A subtribe of Chrysomelidae, Diabroticina includes important agricultural pests from the genera *Acalymma, Cerotoma,* and *Diabrotica* (Toepfer et al., 2009). Many species of the genus *Acalymma* are specialists on Cucurbitaceae, with *Acalymma vittatum*, the striped cucumber beetle, being one of the key pests of cucurbits in the northeastern United States (Lewis et al., 1990). The bean leaf beetle, *Cerotoma trifurcata,* is an important pest of leguminous crops such as peas and soybeans throughout the eastern USA (Koch et al., 2004). *Diabrotica*, the most diverse genus (Eben & Espinosa de los Monteros, 2013), is among the most destructive insect pests impacting US agriculture. *Diabrotica spp.* are divided into three groups: signifera, fucata, and virgifera, with the latter two containing recognized pest species (Krysan, 1986). The species in the fucata group are multivoltine and polyphagous, while species in the virgifera group are univoltine and oligophagous (Branson & Krysan, 1981; Krysan, 1982). *Diabrotica undecimpunctata* (southern corn rootworm) within the fucata group is a generalist feeder that feeds on several crops, including cucurbits, peanuts, and maize in the southern USA (Jackson et al., 2005). *Diabrotica virgifera virgifera* (western corn rootworm), *Diabrotica barberi* (northern corn rootworm), and *Diabrotica virgifera zeae* (Mexican corn rootworm) from the virgifera group are specialist feeders and are pests of maize. *Diabrotica virgifera virgifera* is most abundant in the US Corn Belt, but is found throughout much of the United States as well as parts of Canada and Mexico. *Diabrotica virgifera virgifera* and *D.barberi* are sympatric in the northern part of the US Corn Belt, while *D. v. zeae,* is sympatric with *D. v. virgifera* over part of their range in Texas, Arizona, and Mexico (Bragard et al., 2019). Diabroticina species show a wide range of host breadth, from

monophagous to polyphagous. It has been found that the ancestors of Diabroticina were monophagous. The genus *Acalymma* has retained the ancestral monophagous habit, and the genus *Cerotoma* evolved oligophagy while the genus *Diabrotica* underwent an increase in diet breadth, shifting from monophagy to polyphagy. The fucata subgroup has retained the polyphagous characteristic, the virgifera subgroup, on the other hand, has become secondarily oligophagous (Eben & Espinosa de los Monteros, 2013).

As the name “corn rootworm” suggests, these insects cause substantial economic damage to maize. The western corn rootworm, *Diabrotica virgifera virgifera*, is considered one of the most destructive pests of maize throughout the US Corn Belt. More than $1 billion is spent on its control and lost yield every year (Sappington et al., 2006). The species has also been introduced into Europe and has become widespread because of a combination of transatlantic introductions and intra-continental movement (Ciosi et al., 2008; Miller et al., 2010; Ciosi et al., 2011). *Diabrotica barberi* is a serious maize pest but is less widespread than *D. v. virgifera* (Capinera, 2008). Although *D.undecimpunctata* is more widely distributed it is unable to survive the winter temperatures of the US Corn Belt, and it is considered an occasional pest of maize.

Diabroticina pests have repeatedly evolved behavioral and physiological adaptations to several management strategies like chemical control, crop rotation, and transgenic maize expressing *Bt* proteins (Levine & Oloumi-Sadeghi, 1991; Toepfer et al., 2009; Miller et al., 2009; Eben & Espinosa de los Monteros, 2013; Devos et al., 2013; Gassmann et al., 2014). *Diabrotica virgifera virgifera* evolved resistance to cyclodiene insecticides during the late 1950s (Ball & Weekman, 1962; Sappington et al., 2006; Miller et al., 2009), and later to carbamates, the organophosphate methyl-parathion (Meinke et al., 1998; Miller et al., 2009), and most recently to pyrethroids (Pereira et al., 2017; Souza et al., 2019; Souza et al., 2020). Both *D. v. virgifera* and *D. barberi* have overcome crop rotation strategies through oviposition behavior (O’Neal et

al., 2002) and extended diapause (Krysan et al., 1984), respectively. Resistance to transgenic maize expressing Cry3Bb1 and Cry34/35Ab1 proteins has also evolved in *D. v. virgifera* and *D. barberi* (Gassmann et al., 2014; Calles-Torrez et al., 2019). *Diabrotica virgifera virgifera* has also been shown to be capable of evolving resistance to RNA interference (RNAi) in the laboratory (Khajuria et al., 2018).

Diabroticites have not only evolved resistance to control tactics but have also evolved tolerance to plant-derived secondary metabolites used by plants to confer a degree of protection against several insect herbivores. The benzoxazinoid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is one of the major secondary metabolites produced by maize plants (Sasai et al., 2009). Cucurbitacins are another group of bitter secondary metabolites found in the plants of the family Cucurbitaceae (Rehm & Wessels, 1957). Studies have shown that *D. undecimpunctata* and *D. v. virgifera* from the fucata and virgifera groups of *Diabrotica* can tolerate and sequester the toxic secondary metabolites of plants such as cucurbitacins (Metcalf, 1986; Tallamy et al., 1998) and DIMBOA (Robert et al., 2017) respectively. Although it has been found that both these species can tolerate DIMBOA, they respond distinctly to this metabolite. It was found *D. v. virgifera* gained significantly more dry weight when fed wild-type plants for 10 days compared to being fed mutant plants, deficient for benzoxazinoid biosynthesis. However, *D. undecimpunctata* performed equally well when fed on both types of plants (Alouw & Miller, 2015). The enhanced performance of specialist *D. v. virgifera* may be related to its ability to use DIMBOA as a signal to locate nutritious parts of roots, while the generalist from the fucata group does not (Robert et al., 2012). Further, RNA-Seq studies showed transcripts encoding for a CYP9-like cytochrome P450 monooxygenase were expressed in *D. v. virgifera* larvae feeding on wild type plants but not in

larvae feeding on benzoxazinoid-deficient mutant plants (Miller & Zhao, 2015), suggesting a cytochrome P450 mediated adaptation to benzoxazinoids in *D. v. virgifera*.

As several species in the *Diabrotica* genus can cause damage to crops and adapt to control measures, there has been considerable interest in obtaining sequences of their genomes and understanding genetic mechanisms for adaptations. Most *Diabrotica* genetics and genomics research so far has been concentrated on *D. v. virgifera* (Gray et al., 2009; Miller et al., 2010). The genome of *D. v. virgifera* is one of the larger genomes among beetles and is estimated to be around 2.58 Gb (Coates et al., 2012) whereas the average genome size for Coleoptera is 0.76Gb (Schoville et al., 2018; Gregory, 2021). Sequencing the *D. v. virgifera* genome has posed a challenge. Increased sizes of eukaryotic genomes are generally attributed to corresponding increased numbers of repetitive DNA elements (Kidwell, 2002), where a large proportion of repeats are composed of different transposable element (TE) sequences (Kojima, 2019). Eukaryotic transposons are divided into retroelements that propagate by an RNA intermediate (class I) and DNA elements (class II) that mobilize by a “cut-and-paste” mechanism (Finnegan, 1989; Wicker et al., 2007).

Studies also suggest that the *D. v. virgifera* genome contains a high proportion of repetitive elements (Coates et al., 2012, 2014). The cadherin gene of *D. v. virgifera* is approximately 13.3 fold larger than the *Tribolium castaneum* ortholog due to much larger introns. The presence of numerous MITE-like elements within the cadherin gene of *D. v. virgifera* indicates that the difference in the gene size is due to the insertion of transposable elements in the *D. v. virgifera* introns (Coates et al., 2012). Class I BEL-like long terminal repeat (LTR) retrotransposons have been also found in the *D. v. virgifera* genome (Coates et al., 2014).

Although the genome size of *D. v. virgifera* has been reported, no genome size data have been obtained for the other species in the genus *Diabrotica* and related genera. Since the

genome size of *D. v. virgifera* is relatively large, we hypothesized that there has been a recent expansion in genome size in the lineage leading to it. We tested this hypothesis by estimating and comparing the genome sizes of *D. v. virgifera* with those of several *Diabrotica* species and an outgroup species, *A. vittatum.* As a high proportion of repetitive elements were found in the cadherin gene of *D. v. virgifera,* we further hypothesized that genome size expansion in the lineage leading to *D.v. virgifera* was due to a general increase in repetitive elements. To test this hypothesis we looked at the nature and quantity of repetitive elements in the virgifera group and compared it with the fucata group.

Materials and Methods

Sample Collection

Specimens of *D. v. virgifera* and *A. vittatum* were collected from a maize field in Illinois in 2017, while *D. barberi* were collected from Wisconsin by Tracy Schilder, Wisconsin Department of Agriculture. Specimens of *D. v. zeae* were collected from Texas by Thomas Sappington, US Department of Agriculture Agricultural Research Service and *D. balteata* were provided by Blair Siegfried and Heather McAuslane, University of Florida, from a laboratory colony. *Diabrotica undecimpunctata* were obtained from Crop Characteristics (Farmington, Minnesota, USA). Adult *Periplaneta americana,* which were used as an external reference (Guo et al., 2015; He et al., 2016) for flow cytometric measurement, were obtained from Carolina Biological Supply (Burlington, North Carolina, USA). All samples were flash-frozen in liquid nitrogen and preserved at -80°C.

Sample Preparation

Genome size estimates were generated for eight individuals from five species of *Diabrotica* and one species of *Acalymma*. Preparations of nuclei were based on the method of Hare and Johnston (2012). The heads of single individuals were homogenized in 1ml of cold Galbraith buffer (4.26 g of $MgCl_2$, 8.84 g of sodium citrate, 4.2g of MOPS, 1ml of Triton-x, and 1mg of boiled ribonuclease A into 1 liter of ddH_20) placed in a 7ml Kontes Dounce. The homogenate was filtered through a 20 µm nylon mesh in a 1.5ml Eppendorf tube. Nuclei were stained with propidium iodide at 50 µg/ml, in the dark at 4°C for an hour. In addition to the test sample, the brain tissue of *P. americana* was used as a standard (Hanrahan & Johnston, 2011). The brain tissue of *P. americana* was dissected out, and the nuclear suspension was prepared and stained as described above.

Flow Cytometric Analysis

Stained nuclei were analyzed using an Attune NxT Flow Cytometer (Thermo Fisher Scientific, Waltham, Massachusetts). The propidium iodide-stained nuclei were excited by exposing them to the 488 nm blue laser. Red fluorescence from the propidium iodide was collected using a 610/20 emission filter in the YL2 detector channel. The calibration of the flow cytometer was performed using a standard manufacturer's protocol before use. The samples were inverted and mixed several times to resuspend and then loaded onto the cytometer for analysis. During each sample run, the linearity of the fluorescence measurement was confirmed by checking that the mean channel number of the 4C nuclei (G2 phase) was double that of 2C nuclei (G1 phase). At least 1000 nuclear events were collected under each unknown and standard 2C peak. The nuclei peak (PI fluorescence histogram) and coefficient of variation (CV) for each peak of interest (sample and standard) were obtained using the gating function in the Attune Software. The coefficient of variation (CV) was evaluated and confirmed to be less than 5 percent which is

considered appropriate for accurate genome size estimates (Dolezel et al., 2007; Tomaszewska et al., 2021). The known genome size of the standard (3.41Gb, Harahan & Johnson, 2011) and the relative fluorescence obtained from the sample and standard were then used to estimate the genome size using the following formula:

Sample 1C DNA content = [(sample 2C mean peak position) / (standards 2C mean peak position)] * standards 1C.

Genome size variations were analyzed using analysis of variance (Anova) and Tukey's HSD post-hoc analyses using R statistical software (version: 4.10) (*R Foundation for Statistical Computing, Vienna, Austria*., 2020). Letters were assigned showing significance based on Tukey HSD post-hoc test using R statistical software (version: 4.10) (R Core Team 2021).

Sequencing Data

Data from Illumina whole-genome shotgun sequencing were used to analyze the repetitive DNA content of *D. barberi, D.undecimpunctata,* and *D. v. virgifera*. Adult *D. barberi* (*n* = 71) and *D. undecimpunctata* (*n* = 50) were collected from maize fields near Ames, Iowa, and Monmouth, Illinois, respectively. Each sample was pooled by species, flash-frozen in liquid nitrogen and ground in liquid nitrogen, and then DNA extracted from ~3.0 mg of tissue using the Qiagen DNeasy Blood and Tissue Extraction kit, with modifications as described (Coates et al., 2014). Two micrograms of extracted DNA was submitted to the Iowa State University DNA Facility (Ames, IA, USA) from which ~500bp insert indexed sequencing libraries were generated using the Illumina TruSeq v2 Library Construction Kit (Illumina, San Diego, CA). Single-end 100-bp reads were generated from *D. barberi* and *D. undecimpunctata* libraries in separate lanes of an Illumina HiSeq2500. Raw reads were submitted to the Biotechnology Information (NCBI) Short

Read Archive (SRA) under accessions SRR13363759 and SRR13364002 for *D. barberi* and *D. undecimpunctata*, respectively.

Diabrotica virgifera virgifera adult females of inbred line Ped12-6-A-3 were flash-frozen prior to genomic DNA isolation. Briefly, whole beetles were homogenized in an SDS-based cell lysis solution followed by overnight incubation with Proteinase K at 55C. Cellular debris was pelleted and RNA was digested with RNaseA. The homogenate was mixed with a high-salt solution and incubated overnight at 4°C. The DNA in the supernatant was precipitated overnight with ethanol at -20°C. DNA was quantified on an Invitrogen Qubit. A paired-end short-insert genomic DNA library was prepared at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign using an Illumina TruSeq DNAseq Sample Prep kit. Reads were sequenced to 100bp with the Illumina TruSeq SBS sequencing kit version 3 on an Illumina HiSeq 2000 instrument using Casava 1.8 for basecalling.

Sequencing generated 90 million single-end reads of 100bp for *D. barberi,* 118 million single-end reads of 100bp for *D.undecimpunctata*, and 116 million 100-bp paired-end reads for *D. v. virgifera*.

Annotation and Quantification of Repeat Content

Raw reads were quality-filtered using fastp software (version 0.20.1) with a minimum 20 average Phred score. Repetitive elements in the genomes of *D.undecimpunctata, D. barberi,* and *D. v. virgifera* were assembled and quantified using dnaPipeTE v1.3 (Goubert et al., 2015) and annotated using the DeepTE tool (Yan et al., 2020). To quantify the proportion of TEs, dnaPipeTE uses samples of sequence reads instead of genome assemblies, making this pipeline applicable for genomes with lower sequencing depth. The pipeline performed assembly of repetitive reads into contigs from low coverage sampling of raw reads using Trinity (Grabherr et al., 2011) and annotated them using RepeatMasker (Smit et al., 1996-2004) with built-in

Repbase libraries (Bao et al., 2015, version 2017-01-27). Quantification was done by mapping a random sample of reads onto the assembled repeats. The parameters set as the benchmark for repeat content analysis for genomes greater than 500Mb (Goubert et al., 2015), including the coverage parameter, were used to run dnaPipeTE. The pipeline was run for all three species using 0.1x coverage. Additionally, 0.1x coverage was chosen based on the high N50 metric and plateauing point of transposable elements i.e, increasing the coverage beyond 0.1x only marginally increased the proportion of transposable elements for all three species. The dnaPipeTE pipeline does not annotate novel repeats that do not match an entry in the included Repbase library. A high proportion of repeats from each of the three beetle species were not annotated by dnaPipeTE. DeepTE, a deep learning method based on convolutional neural networks, was used to classify and annotate the unknown TEs. DeepTE uses eight trained models to classify TEs into superfamilies and orders. All the TE contigs assembled by dnaPipeTE were analyzed using DeepTE, whether or not they had been previously classified by dnaPipeTE. Combining the results of the assembly and quantification by dnaPipeTE with the classification results from DeepTE allowed the abundance of repeat families in the genomes of all three species to be determined.

For comparison with the dnaPipeTE *de-novo* assembly of repetitive elements, the percentage of repetitive elements in the *D. v. virgifera* genome assembly (NCBI RefSeq accession GCF_003013835.1) was analyzed with RepeatModeler version 2.0.1 (Flynn et al., 2019). Repeatmodeler is a *de-novo* transposable element identification package that uses three repeat finding programs (RECON, RepeatScout, and LtrHarvest/Ltr_retriever) to discover repetitive DNA sequences in the genome. These repetitive DNA sequences were annotated by repeatClassifier based on the similarity to RepBase and Dfam databases. The annotated library

produced was used as input to RepeatMasker to detect and mask repeats in the genome. Default parameters were used to run RepeatModeler.

Results

Genome size from flow cytometry:

The genome sizes of *D. v. zeae*, *D. v. virgifera,* and *D. barberi* from the virgifera group were estimated to be 2.65Gb ± 0.01, 2.64 Gb ± 0.02, and 2.31Gb ± 0.04, respectively. The genomes of *D. balteata* and *D.undecimpunctata* from the fucata group were estimated at 1.68Gb ± 0.01 and 1.59 Gb ± 0.00. The outgroup species *A. vittatum* genome size is estimated to be 1.69 Gb ± 0.01. An analysis of variance (ANOVA) showed a significant difference ($p < 2 \times 10^{-16}$) in the genome sizes of the species under study. A subsequent Tukey HSD test showed that there were no significant differences in genome size between *D. v. virgifera* and *D. v. zeae*, between *D. balteata* and *D.undecimpunctata,* or between *D. balteata* and *A. vittatum*. The estimated genome size for each species with their phylogenetic relationships is shown in Figure1.

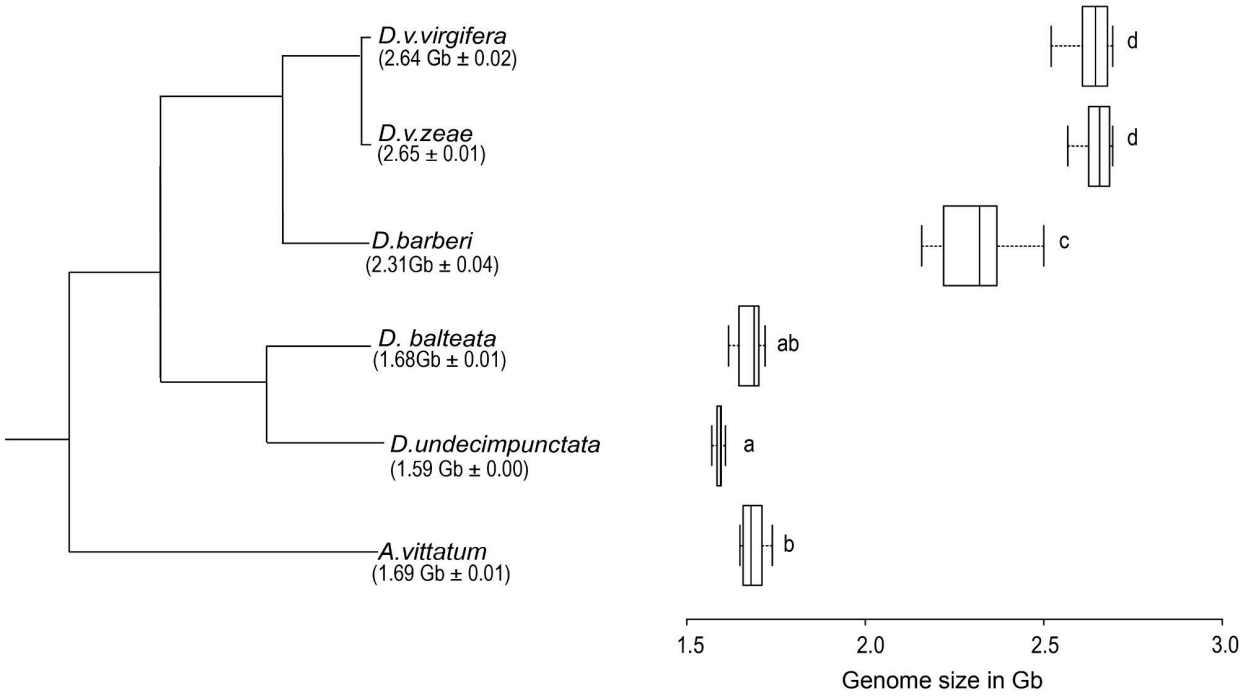

Figure1: Genome size evolution within the genus *Diabrotica.* Phylogeny of *Diabrotica* and outgroup *Acalymma vittatum* is based on figure 2 of Eben & Espinosa de los Monteros, 2013. Letters a - d indicate groups with no significant difference in mean 1C-value (Tukey HSD; α = 0.05).

Repeat Content analysis:

The repeatomes of *D.v.virgifera, D. barberi,* and *D.undecimpunctata* comprised 72.5%, 70.4%, and 52.7% of their genomes, respectively. The repeat content obtained via Repeat Modeler for the draft *D. v. virgifera* genome was 57.5%. As the assembly of the *D.v. virgifera* genome was based on short-reads, TEs were expected to be under-represented because of the difficulty of assembling individual copies. TE-rich large genomes are difficult to assemble and often end up with high levels of fragmentation around repetitive regions leading to underestimation of TE content (Green, 2002).

To further investigate the classes of repeat families that contributed to the genome size variation between the two groups of *Diabrotica*, the DeepTE annotations were coupled with the dnaPipeTE abundance quantification to estimate the abundance of different repeat elements in the genomes of *D. v. virgifera, D.barberi,* and *D.undecimpunctata*. The TEs that accounted for most of the difference in the genome size of the two groups were annotated as class II DNA Tc1-mariner Miniature-repeat Transposable Elements (MITEs) (ClassII_DNA_TcMar_MITE) and class II DNA hAT MITE (ClassII_DNA_hAT_MITE) of transposable elements and class I long terminal repeat (LTR) Gypsy (ClassI_LTR_Gypsy) transposable elements (Figure 2). The *D. v. virgifera* genome contained a large amount of ClassII_DNA_TcMar_MITE(0.609 Gb), ClassII_DNA_hAT_ MITE (0.245 Gb), and ClassI_LTR_Gypsy(0.303 Gb). Similarly, *D. barberi* also had a high amount of ClassII_DNA_TcMar MITE(0.527 Gb), ClassII_DNA_hAT_ MITE

(0.1895 Gb), and ClassI_LTR_Gypsy(0.270 Gb). The *D. undecimpunctata* had a lower amount of ClassII_DNA_TcMar MITE(0.159 Gb), ClassII_DNA_hAT_ MITE (0.057 Gb), and ClassI_LTR_Gypsy(0.177 Gb). Transposable elements from other repeat families such as nLTRS, helitrons, and others from class I and class II DNA elements were not as prominent as those mentioned above. Single-low copy sequences representing the non-repetitive portion of the genomes of *D. v. virgifera*, *D.barberi*, and *D.undecimpunctata* totaled 0.722 Gb, 0.684 Gb, and 0.746 Gb, respectively.

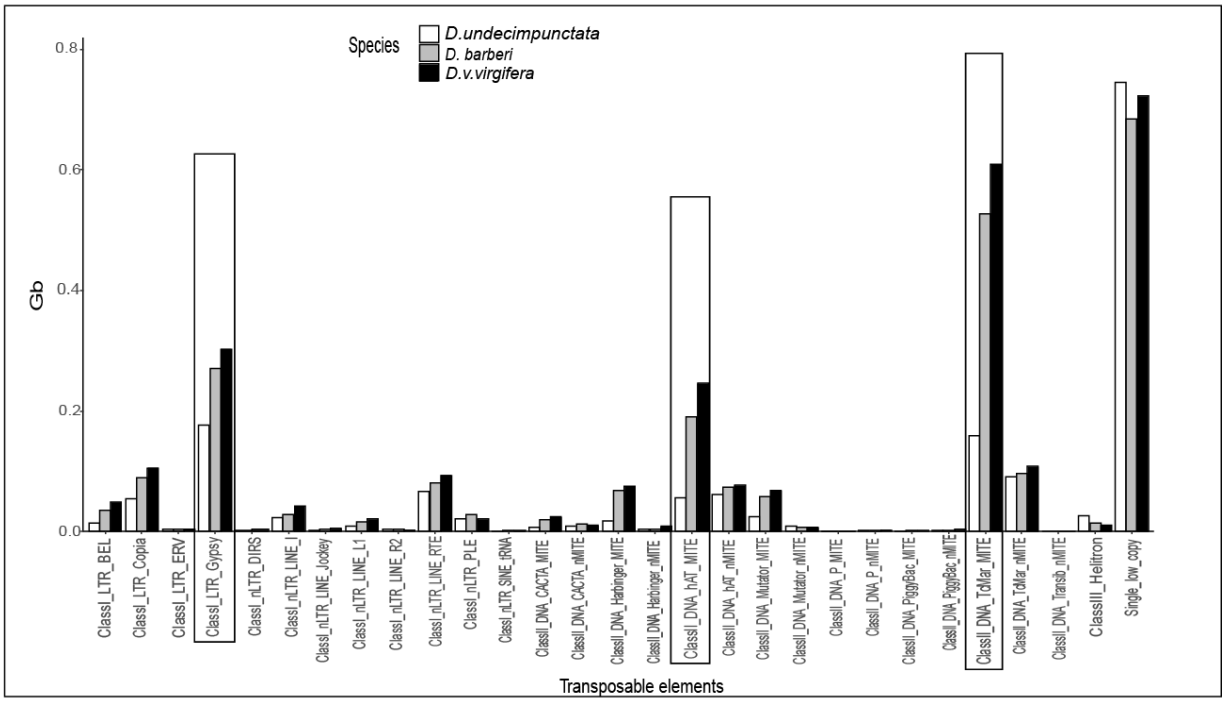

Figure 2: Quantities of transposable elements present in the genomes of three species of *Diabrotica.* The boxed transposable elements are those with high contributions to the genome size variation between the groups of fucata and virgifera.

Discussion:

Diabrotica is the most diverse genus within the subtribe Diabroticina and includes 354 species native to America. Only species from the fucata and virgifera groups occur in the United States (Krysan, 1986), while species from the signifera group are endemic to South America. The economically significant pest species in this genus either belong to the virgifera group or to the fucata group, justifying the need to study them comprehensively. The signifera group species are not of economic importance, and their biology is also mostly unknown (Clark et al., 2001) and so the group is understudied. The expansion and adaptability of the pest species generates a sense of urgency to study them.

Our results demonstrated that the genome size of virgifera group species are approximately 1Gb larger than that of the fucata group and *Acalymma* species. The genome size for *D. v. virgifera* obtained in this study and in a previous study (Coates et al., 2012) are consistent. Our genome size results, when coupled with the species' phylogenetic relationship, indicated that an expansion in genome size occurred in the common ancestor of the virgifera group leading to *D. barberi, D. v. virgifera,* and *D. v. zeae*. There was also a significant difference in the genome sizes of *D. barberi* and *D. virgifera subsp.* suggesting a possible further expansion of the genome size in the common ancestor of *D. v. virgifera* and *D. v. zeae*. The estimated divergence time between *Acalymma* and *Diabrotica* species and between the *Diabrotica* species group was dated at 45 million years ago (Mya) and 31 Mya respectively. Another radiation within the Diabrotica genus took place around 17 Mya resulting in virgifera group (Eben & Espinosa de los Monteros, 2013). Repeat elements of species from fucata and virgifera were studied to understand the basis of genome size expansion in *Diabrotica.* The genomes of the virgifera and fucata group of *Diabrotica* species contained many common TEs, but the abundance of three TE families differed substantially and accounted for much of the difference in genome size between the two groups and also within the virgifera group between

D.barberi and *D. virgifera subsp*. The MITE-like Tc1-mariner and hAT elements and LTR Gypsy retroelements were more abundant in the virgifera group. Miniature-repeat Transposable Elements (MITEs) are short AT-rich (<0.5kb) derivatives of DNA elements whose internal sequence lacks an open reading frame (Lu et al., 2012), contain conserved terminal inverted repeats, are flanked by target site duplications, and are closely associated with euchromatic genes (Kuang et al., 2009). Gypsy elements are one of the most abundant classes of the long terminal repeat (LTR)-retrotransposons superfamily with large numbers of copies found in almost all the plants, animals, and fungi tested (Thomas-Bulle et al., 2018). Generally, class I transposable elements have been reported to be in high abundance in insect genomes, such as *Tribolium castaneum* (Wang et al., 2008), *Drosophila* (Clark et al., 2007)*, Bombyx mori* (Osanai-Futahashi et al., 2008) in comparison to class II elements. However, in *Diabrotica*, we discovered an abundance of class II elements and, to some extent, class I elements.

Genome size varies enormously among eukaryote species (Hidalgo et al., 2017), and among beetles in the Family Chrysomelidae (Hanrahan & Johnston, 2011; Petitpierre et al., 1993). There are studies showing a positive correlation between genome size and phenotypic traits such as body size in groups of invertebrates, including aphids (Finston et al., 1995) and flies (Ferrari & Rai, 1989). Equally, there are studies showing negative correlations between phenotypic traits and genome sizes, such as the relation between body size and genome size for the *Pimelia* and *Phylan* genera in the beetle family Tenebrionidae (Palmer et al., 2003; Palmer & Petitpierre, 1996). Cases of negative correlation of genome size with pupal development have been reported in ladybird beetles and with the overall development rate in *Tribolium* (Carreras et al., 1991; Gregory et al., 2003). It has been previously hypothesized that genome size negatively correlates with the host range in insect herbivores (Zhang et al., 2019). However, there are also studies showing that genome size may be positively correlated with the

host range in insect herbivores (Matsubayashi & Ohshima, 2015; Calatayud et al., 2016). This contradiction in results, along with our data suggests that the genome size may not be related directly to the host range.

Comparative genomic studies in insects have revealed that repeat elements can make large contributions to genome size variation. Variation in TE abundance can be seen both within and among species (Lynch, 2007). Honeybees, with a genome size of 230Mb, show very few repeat elements, representing a case of TE extinction (Weinstock et al., 2006). Similarly, the small genome of *Belgica antarctica,* the Antarctic midge, (99 Mb) is also due to the reduction of repeats in the genome (Kelley et al., 2014). There are also cases of TE proliferations, such as in *Locusta migratoria,* that led to its large genome size of 6.5 Gb (Wang et al., 2014). Cases of increase in genome size as a consequence of transposable elements have also been reported in wood white (Leptidea) butterflies and North American fireflies (Lampyridae) (Talla et al., 2017; Lower et al., 2017). This further suggests that genome size in insects is fairly plastic and largely driven by the loss and gain of transposable elements.

Host genomes have employed several mechanisms to suppress transposable element expression and mobility (Bourque et al., 2018) which includes epigenetic silencing either through histone modifications or DNA methylation, targeted mutagenesis, small RNA interference, as well as sequence-specific repressors such as the recently profiled KRAB zinc-finger proteins (Fouché et al., 2020; Maupetit-Mehouas & Vaury, 2020). At the same time, some transposable elements have evolved regulatory sequences controlling their own copy number to autonomously replicate in the genome (Lohe & Hartl, 1996; Saha et al., 2015). TE de-repression is triggered by environmental stimuli, in particular stress (Bundo et al., 2014; Fouché et al., 2020; Voronova et al., 2014) impacting transcription levels and increasing

transpositional activity (Dubin et al., 2018). Additionally, there are other factors influencing TE mobilization for example demethylation and the removal of repressive histone marks during epigenetic reprogramming stages (Russell & LaMarre, 2018)

Transposable elements are a major component of many eukaryotic genomes and have been associated with several mechanisms shaping eukaryotic genome function, structure, and evolution (Charlesworth & Charlesworth, 1983; Chuong et al., 2017). These transposons are capable of changing position within the genome, leading to mutations by inserting themselves into functional regions and causing change by either modifying or eliminating gene expression (Feschotte, 2008; Oliver & Greene, 2009). TE integration and excision can introduce novel variation at the individual and population-scale (McClintock, 1950; Wendel & Wessler, 2000) introducing phenotypic modifications through changes in epigenetic mechanisms and providing genetic motifs (Jiang et al., 2004; Kapitonov & Jurka, 2007; Stuart et al., 2016; Wessler et al., 1995). They may also lead to genomic rearrangement (Maumus et al., 2015; Mat Razali et al., 2019). In particular, MITES and LTR retrotransposon have played an essential role in shaping gene structure and altering gene function (Wessler et al., 1995).

Initially, MITEs were found as key components of plant genomes, as they are frequently associated with genes with high copy numbers indicating a possible role in gene expression and genome evolution (Santiago et al., 2002; Oki et al., 2008). They are also found in animals including mosquitoes, *Drosophila*, fish, and humans (Deprá et al., 2012). Similar to MITES, LTR retrotransposons were also first discovered in plants. They are usually located largely in intergenic regions and are often the single largest component of plant genomes (Feschotte et al., 2002; Kumar & Bennetzen, 1999). Previous studies have also revealed that both MITES and LTR retrotransposons can regulate downstream gene expression by inserting into promoter regions (Wang et al., 2017; Butelli et al., 2012; Liu et al., 2019).

Mostly, TE insertions are presumed to be deleterious or neutral, but some have been shown to be selectively advantageous. There are several studies showing that TE-mediated insertions have led to insecticide resistance. In pink bollworm *Pectinophora gossypiella*, a major pest of cotton (Rostant et al., 2012), several independent TE insertions in the *PgCad1* gene conferred resistance to *Bt* Cry1Ac toxin (Fabrick et al., 2011; Wang et al., 2019). Cases of resistance to *Bacillus thuringiensis* (Bt) toxins have also been reported in *Heliothis virescens* which is caused by disruption of a cadherin-superfamily gene by TE insertion (Gahan, 2001). TE insertions in xenobiotic metabolism related genes such as those encoding cytochrome P450 monooxygenases and glutathione S-transferases in *Helicoverpa armigera* are the cause of resistance to insecticides (Klai et al., 2020). Another example demonstrating that TEs can produce adaptive mutations has been reported in *D. melanogaster* (Rostant et al., 2012; Gilbert et al., 2021). An increased resistance to dichlorodiphenyltrichloroethane (DDT) in *D. melanogaster* has been reported due to Cyp6g1 upregulation caused by insertion of the Accord transposon in the 5′ regulatory region of the Cyp6g1 gene (Chung et al., 2007). Similarly, TE insertion, which truncates the CHKov1 gene in *D. melanogaster*, confers resistance towards organophosphate (Aminetzach, 2005).

The role played by TEs, if any, in Diabroticina evolution, both long term such as speciation and host plant shifts, and in recent evolution, such as their adaptation to pest management, is unknown. However, the ongoing effort of the United States Department of Agriculture Agricultural Research Service (USDA-ARS) Ag100Pest initiative to sequence other species of *Diabrotica* may help us understand and answer these questions.

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