1 **A quantitative, genome-wide analysis in *Drosophila*** 2 **reveals transposable elements' influence on gene** 3 **expression is species-specific.**

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23 **Running title:** TEs' impact on gene expression is species-specific

24 Abstract

25 Transposable elements (TEs) are parasite DNA sequences that are able to move and multiply along the
 26 chromosomes of all genomes. They are controlled by the host through the targeting of silencing epigenetic
 27 marks, which may affect the chromatin structure of neighboring sequences, including genes. In this study, we
 28 used transcriptomic and epigenomic high-throughput data produced from ovarian samples of *Drosophila*
 29 *melanogaster* and *Drosophila simulans* wild-type strains, in order to finely quantify the influence of TE
 30 insertions on gene RNA levels and histone marks (H3K4me3 and H3K9me3) enrichments. We find general,
 31 negative impacts of TE insertions on gene RNA levels and H3K4me3 enrichments, which intensity varies
 32 according to the localization of the TE insertion. We also uncover that TE insertions within exons are
 33 associated with a reduction in H3K9me3 enrichments, which we propose is associated with TE splicing out.
 34 Overall, we find that gene RNA and histone mark levels show contrasted dependencies on TE insertions
 35 regarding the species. The fold-decrease in TE-carrying gene proportions between extreme expression
 36 classes is 2.6 in *D. melanogaster* and 1.8 in *D. simulans*, while it is strongly contrasted between extreme
 37 classes of H3K4me3 enrichments: 4.4 in *D. melanogaster* and 19.2 in *D. simulans*. This provide a new light
 38 on the considerable natural variability provided by TEs, which may be associated with contrasted adaptive
 39 and evolutionary potentials.

40

41 Introduction

42 Transposable elements (TEs) are parasite DNA sequences that are able to move and multiply along the
 43 chromosomes of all genomes (Wells and Feschotte 2020). As such, they are source of mutations and even
 44 genome instability if uncontrolled (Biémont and Vieira 2006; Malone and Hannon 2009; Senti and
 45 Brennecke 2010). Such control generally consists in the targeting of silencing epigenetic marks at TE
 46 copies, which may spread to neighboring sequences and impact gene expression. In this regard, few attempts
 47 were made to finely analyze and quantify TEs' influence at the whole genome scale (Cridland et al. 2015;
 48 Hollister and Gaut 2009; Lee and Karpen 2017; Uzunović et al. 2019). However, TE contents, activities and

control pathways vary a lot in nature (Akkouche et al. 2012, 2013; Baduel et al. 2021; Mérel et al. 2021, 2020). The present study therefore sheds new light on this question integrating natural variability at the intra- and inter-specific levels in a *Drosophila* system. Specifically, we assessed the transcriptomic and epigenetic status of genes in the ovaries of wild-type strains of *D. melanogaster* and *D. simulans* in relation with TE contents.

Previous studies focusing on a few TE families in wild-type strains of *D. simulans* and *D. melanogaster* uncovered between-species differences in histone mark landscapes (Rebollo et al. 2012). Lee and Karpen (Lee and Karpen 2017) previously provided an analysis on the repressive histone mark H3K9me2 (Histone 3 Lysine 9 dimethylation) around TEs from two *Drosophila* Genetic Reference Panel (DGRP) strains (*D. melanogaster*), and concluded to pervasive epigenetic effects of TEs. Here, we focused on H3K9me3 (Histone 3 Lysine 9 trimethylation, repressive) and H3K4me3 (Histone 3 Lysine 4 trimethylation, permissive) marks, which proved to be particularly relevant in TE epigenetic dynamics (Ozata et al. 2019), and we produced genome-wide data from four wild-type strains of *D. melanogaster* and four wild-type strains of *D. simulans*, which allows us to provide a quantitative assessment of the contribution of TEs to gene expression, in a comparative genomics perspective (Fig. 1).

Previous research already described the effects of TE insertions on gene expression using collections of strains of *D. melanogaster* (Cridland et al. 2015; Everett et al. 2020; Osada et al. 2017; Zhang et al. 2020). The novelties of ours is 1) to bring together TE insertion polymorphism, RNA-seq, ChIP-seq on two histone marks, and small RNA-seq data on the exact same strains, 2) to focus on ovaries, which is the organ where strong selective pressures act on TE control (and not on whole bodies or mix of tissues), 3) to provide a new, quantitative approach based on linear models, and 4) to reach a larger evolutionary scale thanks to the inclusion of data not only from *D. melanogaster* but also from its sister species *D. simulans*. We investigated the impacts of TEs on gene expression at the whole genome scale using eight previously characterized, wild-type strains of *D. melanogaster* and *D. simulans* (Mohamed et al. 2020). These strains derived from samples collected in France and Brazil, two strains per location and per species. Using the Oxford Nanopore long read sequencing technology, we previously produced high quality genome assemblies at the chromosome resolution for each of the strains (Mohamed et al. 2020).

Henceforth, we reveal that TE insertions within genes (exons and introns) have stronger negative impacts on gene RNA levels and H3K4me3 enrichments compared to TE insertions in the gene flanking regions, and that the proportion of genes carrying TE insertions decreases when RNA levels and H3K4me3 increase, and increases with H3K9me3 enrichments. We also uncover a surprising result regarding TE insertions within exons: they are associated with a reduction in H3K9me3 enrichments. Considering that H3K9me3 is involved in the definition of splice sites, we speculate that TE insertions within exons are not purged by natural selection provided they are skipped from the corresponding transcripts, which may be achieved through reductions in H3K9me3 marks. Regarding orthologous genes across strains, the more genes display different RNA and histone mark levels, the more likely they have different numbers of TE insertions (especially regarding RNA levels in *D. melanogaster*, and H3K4me3 enrichments in *D. simulans*). These results participate in the accurate, quantitative understanding of TEs' impacts in genomes. Moreover, our major finding in this research work is that, across genes, RNA and histone mark levels show contrasted dependencies on TE insertions regarding the species, *D. melanogaster* or *D. simulans*. This provide a new light on the considerable natural variability provided by TEs, which may be associated with contrasted adaptive and evolutionary potentials, all the more sensible in a rapidly changing environment (Baduel et al. 2021; Fablet and Vieira 2011; Mérel et al. 2021).

Figure 1. Graphic summary of the study.

Eight wild-type strains from *D. melanogaster* and *D. simulans* were included in the study. The present datasets are RNA-seq and ChIP-seq for H3K4me3 and H3K9me3 marks, and were prepared from ovarian

samples. They were analyzed in parallel with already published data produced from the same *Drosophila* strains: ovarian piRNA repertoires and genome assemblies based on Oxford Nanopore long read sequencing (Mohamed et al. 2020). For RNA-seq and ChIP-seq, TE-derived reads were analyzed at the TE family level, and gene-derived reads were analyzed in relation to TE insertions inside or nearby the genes (therefore restricted to the TE insertions included within the gray bubbles).

Results

TE expression and epigenetic targeting in *Drosophila* ovaries

We first considered TE-derived RNA-seq reads from all our sequence files, which we analyzed at the TE-family level (Fig. 1). TEs account for 0.6% to 1.2%, and 0.5% to 0.7%, of read counts corresponding to annotated sequences (genes and TEs) within the ovarian transcriptomes of our strains of *D. melanogaster* and *D. simulans*, respectively (Fig. 2A). We find significant positive correlations between per TE family RNA counts and family sequence occupancy (total number of bp spanned by each TE family along the genome) (Spearman correlations, $\rho = 0.20$ to 0.31 , and 0.28 to 0.35 , in *D. melanogaster* and *D. simulans*, respectively; Supplemental_Fig_S2A). We also find significant positive correlations between per TE family RNA counts and piRNA counts (Spearman correlations, $\rho = 0.65$ to 0.70 , and 0.64 to 0.68 , in *D. melanogaster* and *D. simulans*, respectively; Supplemental_Fig_S2B), as already described for wild-type strains (Lerat et al. 2017).

We assessed the contribution of histone mark enrichments to TE RNA amounts considering the following linear model on log-transformed normalized read counts: $\text{RNA} \sim \text{H3K4me3} + \text{H3K9me3} + \text{input}$. These models led to adjusted r^2 as high as 0.48 to 0.64 , and 0.45 to 0.60 , in *D. melanogaster* and *D. simulans* strains, respectively, suggesting that this approach captures a significant portion of TE RNA amount variation. We find that TE RNA amounts are positively correlated with H3K4me3 and negatively correlated with H3K9me3 amounts (Fig. 2B), as expected considering that H3K4me3 and H3K9me3 are activating and silencing marks, respectively. We used a similar approach to analyze piRNA amounts, and considered the following linear model on log-transformed read counts: $\text{piRNA} \sim \text{H3K4me3} + \text{H3K9me3} + \text{input}$. We

obtained even higher adjusted r^2 values, from 0.71 to 0.75, and 0.64 to 0.68, in *D. melanogaster* and *D. simulans* strains, respectively. We find that TE-derived piRNA amounts are positively correlated both with permissive H3K4me3 and repressive H3K9me9 enrichments (Fig. 2C).

TE insertions within or nearby genes

In the following sections, we focus on gene-derived reads from all our sequence files, which we analyzed in relation with the presence of TE insertions within or nearby genes. Based on gene annotations, we distinguished the different functional regions of genes, *i.e.* exons, introns, 5' UTRs, 3' UTRs, upstream, or downstream sequences (5 kb flanking regions). In the present study, we considered a set of 17,417 annotated genes for *D. melanogaster*, and 15,251 for *D. simulans* (see Material and Methods). We quantified the number of TE insertions within genes (Fig. 2D), and found that they only account for ~10% of the total number of TE insertions per genome. The majority of genes (93%) are devoid of TE insertions, and 6% have exactly one TE insertion (Fig. 2E, Supplemental_Fig_S1). 92% to 94% of genes are devoid of TE insertions in their upstream flanking regions (over 5 kb), and 6% to 8% display exactly one insertion. The proportions are the same regarding the downstream flanking regions.

138

139 **Figure 2.** (A) Proportions of TE read counts in RNA-seq data over read counts corresponding to genes and
 140 TEs. For each strain, two biological replicates are shown. (B) Contributions of H3K4me3 and H3K9me3

141 enrichments to TE-derived RNA read counts (according to the model $\text{RNA} \sim \text{H3K4me3} + \text{H3K9me3} + \text{input}$
 142 calculated on log transformed read count numbers, at the TE family level). Plain-colored bars: p-values
 143 < 0.01 , light-colored bars: p-values in $[0.01; 0.05]$, empty bars: p-values > 0.05 . (C) Contributions of
 144 H3K4me3 and H3K9me3 enrichments to TE-derived piRNA read counts (according to the model $\text{piRNA} \sim$
 145 $\text{H3K4me3} + \text{H3K9me3} + \text{input}$ calculated on log transformed read count numbers, at the TE family level).
 146 Plain-colored bars: p-values < 0.01 , light-colored bars: p-values in $[0.01; 0.05]$, empty bars: p-values > 0.05 .
 147 (D) Numbers of TE insertions per functional region per strain. Upstream and downstream regions are 5 kb
 148 sequences directly flanking transcription units 5' and 3', respectively. (E) Numbers of genes for each value of
 149 TE insertion numbers. dmgoth101 and dsgoth31 are shown as examples; all strains can be found in
 150 Supplemental_Fig_S1.

151

152 **TEs contribute to RNA level variability across genes within** 153 **genomes**

154 One of the objectives of the present work was to quantify the association of TE insertions with the
 155 differences in expression levels that we observe across the set of genes of a genome. We focused on the
 156 subset of genes that we found expressed in the ovaries (see Material and Methods). They account for 8,028
 157 to 8,209 genes depending on the strains, and 6,149 to 6,431 genes, in *D. melanogaster* and *D. simulans*,
 158 respectively. We first quantified TE insertion contribution to gene RNA levels using the following linear
 159 models built on log-transformed TPM (Transcript Per Million): $\text{TPM} \sim \text{gene} + \text{upstream} + \text{downstream}$,
 160 where these variables correspond to the numbers of TE insertions in gene body, 5 kb upstream, and 5 kb
 161 downstream, respectively. We found that the strongest effect on gene RNA amounts is a negative correlation
 162 with the number of TE insertions in gene bodies. TE insertions that are located upstream of genes are not
 163 correlated to gene transcript amounts on average. On the contrary, TE insertions that are located downstream
 164 of genes are associated with increased gene transcript amounts, however only in *D. simulans* strains (Fig.
 165 3A).

166 We investigated the impacts of TE insertions within genes into more details using the following model: log-
 167 transformed $\text{TPM} \sim \text{exon} + \text{intron} + \text{utr5} + \text{utr3} + \text{upstream} + \text{downstream}$, which allowed us to refine the

168 correlations between expression and each category of functional region. We found that TE insertions in all
169 internal regions display a general tendency to reduce gene transcript amounts (Supplemental_Fig_S3A).
170 Then, considering each genome individually, we defined 10 TPM classes, and calculated the proportion of
171 genes with at least one TE insertion in each class. This procedure allowed us to bring out clear negative
172 correlations between the average TPM and the proportion of such genes (Fig. 3B). This indicates that the
173 more the genes are transcribed, the lower the probability that they carry TE insertions. Depending on the
174 strain, we observe a 2.2 to 2.9-fold decrease in the proportions of such genes between the two extreme
175 expression classes in *D. melanogaster*, and 1.5 to 2.2 in *D. simulans*. Using the model including more
176 detailed characterization of functional regions (exon, intron, 5' UTR, 3' UTR, upstream, downstream), we
177 found similar negative correlations in *D. melanogaster*, especially for exons and introns, and also, but more
178 mildly, for 5' UTRs and 3' UTRs. These trends are much less pronounced in *D. simulans*. In addition, in
179 both species, the expression classes do not shown differences in the proportions of TE-carrying genes within
180 the 5 kb upstream or downstream flanking regions (Supplemental_Fig_S4).

181

182 **TEs contribute to histone enrichment variability across genes** 183 **within genomes**

184 We used a similar approach to analyze H3K4me3 and H3K9me3 enrichments (*i.e.* we aligned ChIP-seq reads
185 against whole gene sequences and computed corresponding read counts). Similar to what we found for RNA
186 levels, TE insertions within genes are significantly negatively correlated with H3K4me3 amounts (Fig. 3C).
187 On the contrary, TE insertions in the flanking downstream regions have a mild, positive impact, although
188 detected in *D. melanogaster* only. We could not detect impacts from insertions in upstream regions.

189 When we detailed the functional regions within genes, we detected a robust, negative impact of TE insertions
190 within introns on gene H3K4me3 levels in *D. melanogaster* (Supplemental_Fig_S3B). Similarly, we find
191 mild, negative impacts of TE insertions within 5' UTRs.

192 Considering H3K9me3 amounts, the impacts of TE insertions are more significant and more widespread
193 across strains. Using the model $H3K9me3 \sim \text{input} + \text{gene} + \text{upstream} + \text{downstream}$, we detect significant,

positive contributions of TE insertions in all regions (gene, upstream, downstream) (Fig. 3E). These results are in agreement with TEs being the preferential targets for H3K9me3 deposition, which then spreads to neighboring regions. Using the detailed model $H3K9me3 \sim \text{input} + \text{exon} + \text{intron} + \text{utr5} + \text{utr3} + \text{upstream} + \text{downstream}$, we find negative contributions of TE insertions within introns and flanking regions (both upstream and downstream). Unexpectedly however, TE insertions within exons have a clear, negative impact on gene H3K9me3 amounts (Supplemental_Fig_S3C).

Then, similar to the approach we used for RNA level analyses, we classified genes according to their histone mark enrichment levels, and computed the proportions of TE-carrying genes for each of these classes (Fig. 2D,F). We find significant, negative correlations between these proportions and H3K4me3 enrichment levels, which are stronger for *D. simulans* strains compared to *D. melanogaster*. The reduction in the proportion of TE-carrying genes is 3.1 to 5.7-fold, and 17.1 to 20.5-fold between the extreme histone classes, in *D. melanogaster* and *D. simulans*, respectively. When considering detailed functional regions, we found that this effect holds on for exons, introns, and 5' UTRs (Supplemental_Fig_S5). No significant effects are detected for 3' UTRs, and flanking regions.

Regarding H3K9me3, we found significant, strong positive correlations between the proportion of TE-carrying genes and histone levels in *D. simulans* genomes: the proportion increases 8.3 to 11.7-fold between the extreme histone classes. Surprisingly, correlations are weaker in *D. melanogaster* genomes. However, we detected strong, positive correlations between the proportions of genes carrying TE insertions within flanking regions (both upstream and downstream) and H3K9me3 amounts, consistently across all eight genomes.

214

215 **Private and shared TE insertions are associated with similar** 216 **patterns**

It has already been described that TE impacts differ across young (*i.e.* polymorphic) and old (*i.e.* fixed) TE copies; indeed, the pool of old TE insertions has been purged from deleterious insertions by natural selection. Indeed, Uzunovic *et al.* (Uzunović *et al.* 2019) showed in the plant *Capsella* that young TE insertions had a

220 negative effect on gene expression while old insertions were more likely to increase expression. In this view,
 221 we performed the same analyses as above separately on TE insertions that are unique to one genome
 222 (private) and those that are shared by all four strains of the species (common). The majority of the TE
 223 insertions (>80%) that we included in the analysis in the first place fall in this last category, leading to
 224 differences in statistical power between both sets of insertions. Despite this bias, the observed patterns are
 225 highly similar (Supplemental_Table_S1). Since our approach is gene-centered, it is very likely that our
 226 primary set of TE insertions is already biased: when deleterious, insertions within or near genes have such a
 227 negative impact that we are not able to catch them from natural samples. Therefore, our primary set of TE
 228 insertions may already correspond to copies that have passed the filter of natural selection.

229

230 **Figure 3. (A)** Contribution of TE insertion numbers (within genes or in the 5 kb upstream or downstream
 231 sequences) to gene-derived RNA read counts. Plain-colored bars: p-values < 0.01, light-colored bars: p-

values in [0.01; 0.05], empty bars: p-values > 0.05. (B) Proportions of genes carrying at least one TE insertion according to gene expression classes. We plotted confidence intervals for the proportion of TE-carrying genes and we fitted a linear regression line with mean TPM per class (Pearson correlation coefficients and the corresponding p-values are indicated). (C) Contribution of TE insertion numbers (within genes or in the 5 kb upstream or downstream sequences) to gene H3K4me3 enrichments. Plain-colored bars: p-values < 0.01, light-colored bars: p-values in [0.01; 0.05], empty bars: p-values > 0.05. (D) Proportions of genes carrying at least one TE insertion according to gene H3K4me3 enrichment classes. We plotted confidence intervals for the proportion of TE-carrying genes and we fitted a linear regression line with mean histone enrichment per class (Pearson correlation coefficients and the corresponding p-values are indicated). (E) Contribution of TE insertion numbers (within genes or in the 5 kb upstream or downstream sequences) to gene H3K9me3 enrichments. Plain-colored bars: p-values < 0.01, light-colored bars: p-values in [0.01; 0.05], empty bars: p-values > 0.05. (F) Proportions of genes carrying at least one TE insertion according to gene H3K9me3 enrichment classes. We plotted confidence intervals for the proportion of TE-carrying genes and we fitted a linear regression line with mean histone enrichment per class (Pearson correlation coefficients and the corresponding p-values are indicated).

247

TE insertions are associated with orthologous gene expression variability across genomes

Next, we used our experimental dataset to infer the contribution of TE insertions at the inter-genomic level, *i.e.* we compared expression levels of orthologous genes across genomes. We performed pairwise comparisons and calculated the coefficient of variation (CV, square root of the variance divided by the mean) of TPM for genes that are transcribed in our ovarian samples. We defined 10 classes based on CV values, and computed the proportion of genes that displayed different numbers of TE insertions within each class. The general trend is positive correlation between these proportions and the mean CVs of each class, and the adjustment is generally better using the square root of mean CV. This indicates that the more the genes show differences in expression levels, the greater the probability that they have different numbers of TE insertions

(Fig. 4A, Supplemental_Fig_S7, Supplemental_Fig_S8). The effect appears to be stronger in *D. melanogaster* compared to *D. simulans*.

We applied the same approach to ChIP-seq data: for each gene and each pair of strains, we considered the model $H3K9me3(i \text{ stands for } 4 \text{ or } 9) \sim \text{input} + \text{strain}$, and retrieved the absolute value of the effect of the strain factor. We defined 10 classes of genes based on the values of this variable (square root transformed), and, within each class, we computed the proportion of genes that carried different numbers of TE insertions across strains. Considering H3K4me3, results are not significant for *D. melanogaster*, but there are clear positive correlations between these proportions and the difference in histone mark enrichment for *D. simulans* (Fig. 4B). In other terms, the more the genes show differences in H3K4me3 enrichments, the greater the probability that they carry different numbers of TE insertions. This trend is essentially pronounced for TE insertions located in introns: we observed a 2.0 to 4.0-fold increase in the proportions of genes with different numbers of TE insertions, depending on the considered pairs of strains (Supplemental_Fig_S9, Supplemental_Fig_S10).

Considering H3K9me3, we find the same trends, *i.e.* positive correlations between the proportions of genes that display different numbers of TE insertions and differences in H3K9me3 enrichments, and essentially due to TE insertions in introns (Fig. 4C, Supplemental_Fig_S11, Supplemental_Fig_S12). However, the majority of these correlations are not significant at the 0.05 threshold.

Even if we observe substantial variability across pairwise comparisons, altogether, these results indicate that differences in TE insertion numbers between strains lead to clear differences in RNA levels, and weak differences in histone mark enrichments, at least for those that we tested here.

278

A RNA levels

B H3K4me3 enrichments

C H3K9me3 enrichments

279

280 **Figure 4. Pairwise comparisons across strains of TE insertion numbers and RNA levels or histone**
 281 **mark enrichments.** For each pair of strains, proportions of orthologous genes carrying different numbers of

TE insertions according to (A) differences in gene RNA levels (computed based on variation coefficient), (B) size of the strain effect for H3K4me3 enrichments, (C) and H3K9me3 enrichments. We plotted confidence intervals for the proportion of genes carrying different numbers of TEs and we fitted a linear regression line with square root of mean CV per class (A) or square root of mean histone enrichment effect per class (B, C) (Pearson correlation coefficients and the corresponding p-values are indicated).

Discussion

The common-held view is that, as parasites that are fought against by the genomes, TEs have a general negative impact on gene expression (Cridland et al., 2015; Lee, 2015; Lee & Karpen, 2017). Our present findings are in agreement with this idea. However, the originality of this research work is to provide an unprecedented quantitative view, which allows to precisely decipher TE impacts, integrating intra- and inter-species data from two closely related drosophila species. In addition, this study combines genomic, transcriptomic, and epigenetic high-throughput sequencing data, all produced from the same strains and tissues from natural strains, which allows to draw actual connections between the different nucleic repertoires of the cells. Moreover, the chosen tissue is ovary, which is known to be the place of the strongest epigenetic control of TE sequences, through the piRNA pathway (Malone and Hannon 2009; Senti and Brennecke 2010). This is therefore where we are to expect the strongest impacts of TEs on genes.

Expression and epigenetic marks of TE sequences

Our results uncovered a lower contribution of TEs to the *D. simulans* transcriptome as compared to *D. melanogaster*. This is in agreement with the lowest contribution of TEs in the genomes of *D. simulans*, in terms of sequence occupancy and copy numbers (Mohamed et al. 2020; Vieira et al. 1999). In both species, we found that H3K9me3 marks on TE sequences were associated with a decrease in TE-derived RNA amounts, and the opposite for H3K4me3 marks. On the contrary, we observed that both histone marks are positively correlated with TE-derived piRNA amounts, which is congruent with the piRNA-targeted deposition of H3K9me3 marks at transcriptionally active TE copies (Czech et al. 2018).

308

309 **TE impact on gene expression and histone marks : intragenomic** 310 **approach**

311 As expected due to the spreading of TE epigenetic marks to flanking sequences, our analyses revealed that
312 TE insertions within genes induce a general decrease in gene RNA levels and H3K4me3 enrichments, and an
313 increase in H3K9me3 enrichments. While we found that TE insertions in flanking regions equally induce
314 H3K9me3 enrichments, we found that the impacts of TE insertions on RNA levels and H3K4me3
315 enrichments are stronger for TE insertions within genes compared to TE insertions in flanking regions. This
316 had already been observed by Cridland *et al.* (Cridland et al. 2015), who found a stronger correlation with
317 extreme gene expression for TEs inserted into genes compared to TEs upstream of genes. This may be due to
318 differences in deposition patterns for these marks, which result in difference in spreading abilities. Indeed,
319 contrary to H3K9me3, H3K4me3 marks are known to appear as peaks. In addition, we observe that TE
320 insertions in flanking regions are associated with increases in gene RNA levels and H3K4me3 enrichments.
321 This may be related to TEs bringing transcription factor binding sites or behaving as enhancers, as was
322 already described (Sundaram et al. 2014; Villanueva-Cañas et al. 2019; Moschetti et al. 2020).

323 While the detailed analyses of gene functional compartments (exon, intron, 5' UTR, 3' UTR) generally
324 revealed the same patterns as the complete gene sequence, we were surprised to uncover a negative influence
325 of TE insertions in exons on H3K9me3 enrichments. This finding may be explained by the influence of
326 H3K9me3 on the splicing machinery: it was shown in mammals that H3K9me3 favors the inclusion of
327 alternative exons while demethylation is associated with exon skipping (Bieberstein et al. 2016; Saint-André
328 et al. 2011). We propose that the TE insertions in exons that are maintained and not purged by natural
329 selection, are those that are spliced out from transcripts, so that the coding sequence of the gene is not
330 impaired. This specific splicing would be possible for TE insertions that are associated with a reduction in
331 H3K9 methylation, which allows the splicing out of the TE.

332

333 **TE impacts on genes are different between *D. melanogaster* and** 334 ***D. simulans***

335 Although less abundant in the genome, the epigenetic impacts of TEs are more pronounced in *D. simulans*.
336 Indeed, we found that the probability that a gene carries at least one TE insertion is more strongly correlated
337 with its histone enrichment in *D. simulans* compared to *D. melanogaster* (Fig. 5). Similarly, although using
338 a different, more indirect genome-wide approach, Lee and Karpen (Lee and Karpen 2017) also came to the
339 conclusion that, at the same time, *D. simulans* displays a lower TE content and stronger epigenetic effects of
340 TE control, compared to *D. melanogaster*. Again, it sustains the idea that *D. simulans* epigenetic machinery
341 is less efficient since a strong pattern in H3K9me3 and/or H3K4me3 results in a weak pattern at the RNA
342 levels, or in other words, since weak histone patterns in *D. melanogaster* are enough to produce a strong
343 RNA response. Alternatively, upstream of the regulatory effectors, it may suggest that the targeting of TE
344 sequences for histone mark deposition is more efficient in *D. simulans*, maybe because the colonization of
345 the *D. simulans* genome by TEs started more recently (as suggested by our previous results (Mohamed et al.
346 2020)). We expect that the strength of the epigenetic control of TEs should decrease after the colonization, as
347 the equilibrium between the genome and its TEs is reached. These contrasted impacts of TE insertions on
348 genes through epigenetic marks across the species provide an additional demonstration of the considerable
349 natural variability due to TEs. We predict that this leads to contrasted adaptive and evolutionary potentials,
350 all the more sensible in a rapidly changing environment (Baduel et al. 2021; Fablet and Vieira 2011; Mérel
351 et al. 2021).

352

D. simulans

D. melanogaster

353

354 **Figure 5. Graphic summary.** The probability that a gene carries at least one TE insertion is more strongly
 355 correlated with its histone enrichment in *D. simulans* compared to *D. melanogaster*. On the contrary, this
 356 probability is more strongly correlated with gene RNA levels in *D. melanogaster*.

357

358

359 **Methods**

360 ***Drosophila* strains**

361 The strains under study in the present work were previously described in Mohamed et al. (Mohamed et al.
362 2020). The eight samples of *D. melanogaster* and *D. simulans* wild-type strains were collected using fruit
363 baits in France (Gotheron, 44°56'0"N 04°53'30"E - "goth" strains) and Brazil (São Jose do Rio Preto
364 20°41'04.3"S 49°21'26.1"W – "sj" strains) in June 2014. Two isofemale lines per species and geographical
365 origin were established directly from gravid females from the field (French *D. melanogaster*: dmgoth63,
366 dmgoth101; Brazilian *D. melanogaster*: dmsj23, dmsj7; French *D. simulans*: dsgoth613, dsgoth31; Brazilian
367 *D. simulans*: dssj27, dssj9). Brothers and sisters were then mated for 30 generations to obtain inbred strains
368 with very low intra-line genetic variability. Strains were kept at 24°C in standard laboratory conditions on
369 cornmeal–sugar–yeast–agar medium.

370 **Genome annotation**

371 Genome assemblies were produced in (Mohamed et al. 2020) and have been deposited in the European
372 Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB50024
373 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB50024>).

374 We used the genome assemblies produced for each of the eight strains using the Oxford Nanopore
375 sequencing technology, and previously published (Mohamed et al. 2020). Throughout the present analysis,
376 we kept scaffolds corresponding to complete chromosomes 2L, 2R, 3L, 3R, 4, and X.

377 TE annotation: We used RepeatMasker 4.1.0 (<http://repeatmasker.org/>) -species *Drosophila* in order to
378 identify TE sequences in the assemblies, followed by OneCodeToFindThemAll (Bailly-Bechet et al. 2014)
379 with default parameters, in order to parse RepeatMasker results.

380 Gene annotation: For *D. melanogaster*, we retrieved dmel-all-exon-r6.16.fasta, dmel-all-intron-r6.16.fasta,
381 dmel-all-five_prime_UTR-r6.16.fasta, dmel-all-five_prime_UTR-r6.16.fasta, and dmel-all-gene-r6.16.fasta,
382 which are available at ftp.flybase.net/genomes/Drosophila_melanogaster/dmel_r6.16_FB2017_03/fasta/. For
383 *D. simulans*, we retrieved dsim-all-exon-r2.02.fasta, dsim-all-intron-r2.02.fasta, dsim-all-five_prime_UTR-

384 r2.02.fasta, dsim-all-three_prime_UTR-r2.02.fasta, and dsim-all-gene-r2.02.fasta, which are available at
 385 ftp.flybase.net/genomes/Drosophila_simulans/dsim_r2.02_FB2017_04/fasta/. These different sequence sets
 386 were aligned to our assemblies using BLAT (Kent 2002) and pslReps from the BLAT suite, in order to keep
 387 the best hit (-singleHit). We filtered out genes with 0 hit and genes with multiple hits. For each gene, we also
 388 checked that the positions of the hits for each of the internal regions (*i.e.* exon, intron, 5' UTR, and 3' UTR)
 389 were included in the positions of the complete gene hit. We excluded regions that did not fulfill these criteria.
 390 We also excluded hits that were not on the same chromosome as the corresponding gene on the reference
 391 genome. Then, we used the GenomicRanges R package (version 1.38.0) (Lawrence et al. 2013) and the
 392 subsetByOverlaps function to cross gene and TE annotations.

393 TE genomic sequence occupancy (bp) was computed using OneCodeToFindThemAll (Bailly-Bechet et al.
 394 2014). Data corresponding to the *DNAREP1* transposon (also known as *INE-1*) were removed because it is a
 395 highly abundant element displaying mainly fixed insertions in the *melanogaster* complex of species
 396 (Chakraborty et al. 2021).

397 In order to determine which TE insertions were common (shared) to the four strains of a species or unique
 398 (private) to one strain, we performed pairwise comparisons of TE gff using Liftoff (Shumate and Salzberg
 399 2020). Private insertions to one strain are those that appear in the unmapped output of the three other strains.
 400 Common insertions are those that are found in all pairwise comparisons.

401 **Small RNA extraction, sequencing, and analyses**

402 Small RNA extraction, sequencing, and analyses had already been performed and described in Mohamed et
 403 al. (Mohamed et al. 2020). Sequence files had been deposited in NCBI SRA under the accession number
 404 PRJNA644327.

405 **RNA-seq preparation**

406 RNA was extracted from ovaries of 30 3-to-5 day-old females. Two replicates per strain were produced.
 407 RNA extraction was carried out using RNeasy Plus (Qiagen) kit following manufacturer's instructions. After
 408 DNase treatment (Ambion), quality control was performed using an Agilent Bioanalyzer. Libraries were

constructed from mRNA using the Illumina TruSeq RNA Sample Prep Kit following manufacturer's recommendations. Libraries were sequenced on Illumina HiSeq 3000 with paired-end 150 nt reads.

RNA-seq analysis

TE read counts were computed at the family level using the TEcount module of TETools (Lerat et al. 2017) and the list of TE sequences available at <ftp://pbil.univ-lyon1.fr/pub/datasets/Roy2019/>. In agreement with other research works (Chakraborty et al. 2021), we removed the *DNAREP1* family from the analyses because it is a highly abundant element displaying mainly fixed insertions in the *melanogaster* complex of species (it is also known as *INE-1*, and corresponding to DNAREP1_DM, DNAREP1_DSim, and DNAREP2_DSim entries from our TE sequence list).

Genome and gene annotations: The genomes of *D. melanogaster* and *D. simulans* were downloaded from flybase (<http://flybase.org/>) along with GTF gene annotations. *D. melanogaster*: ftp://ftp.flybase.net/genomes/Drosophila_melanogaster/dmel_r6.16_FB2017_03/fasta/dmel-all-chromosome-r6.16.fasta.gz; ftp://ftp.flybase.net/genomes/Drosophila_melanogaster/dmel_r6.16_FB2017_03/gtf/dmel-all-r6.16.gtf.gz. *D. simulans*: ftp://ftp.flybase.net/releases/FB2017_03/dsim_r2.02/fasta/dsim-all-chromosome-r2.02.fasta.gz; ftp://ftp.flybase.net/releases/FB2017_03/dsim_r2.02/gtf/dsim-all-r2.02.gtf.gz.

Reads mapping: The alignment of all read samples on their corresponding reference genome was performed with Hisat2 version 2.1.0 (Kim et al. 2019) using the following command line options: hisat2-build \$Genome \$GenomeIndex to build an index of the genome; hisat2 -p 7 -k 2 --dta -x \$GenomeIndex -1 \$Sample_1.fq.gz -2 \$Sample_2.fq.gz -S \$Sample.sam to align each sample on the reference genome. The --dta option is used as recommended in the New tuxedo pipeline to perform an assembly of the transcripts (Pertea et al. 2016). The -k 2 option allows to report a maximum of two alignments per sequencing read. As we wanted to exclude potentially expressed TEs from the assembly, we discarded multimapped reads using the following command line: samtools view -h -@ 7 -f 0x2 \$Sample.bam |awk '{if(substr(\$1, 0, 1)=="@" || \$5==60){print \$0}}' >\$Sample.sam. The option -f 0x2 extracts the properly aligned paired reads from the bam. The awk command also keeps the header section of the SAM file as well as the reads with a MAPQ=60. In Hisat2, a MAPQ=60 means that the read has been uniquely aligned regardless of the

435 mismatches / indels number. The filtered SAM file is finally converted to BAM and sorted: samtools view -h
436 -@ 7 -b -S \$Sample.sam >\$Sample_filtered.bam && samtools sort -@ 7 \$Sample_filtered.bam sorted.bam
437 && mv sorted.bam \$Sample_filtered.bam.

438 Transcripts assembly: We used StringTie (Pertea et al. 2015) to assemble the transcripts using the genomic
439 coordinates of the aligned reads, for each sample: stringtie -p 7 -o \$Sample_assemb.gtf -j 2
440 \$Sample_filtered.bam && echo "\$Sample\$ _assemb.gtf" >> mergelist.txt. The -j option specifies that at least
441 two reads are needed to support an exon-exon junction and create a new transcript. This is thus more
442 stringent than the default value of 1 used by StringTie. One assembly per sample is created; we used the
443 following command line to merge these assemblies: stringtie --merge -o merged.gtf mergelist.txt.

444 Transcript expression quantification: Stringtie was also used to quantify transcript expression in each sample:
445 stringtie -e -B -p 7 -G merged.gtf -o \$Sample/\$Sample.gtf \$Sample_filtered.bam. -e option is used to
446 estimate the expression of transcripts of the GTF file given to the -G option. -B option is used to create an
447 output needed to run ballgown (Frazee et al. 2015) in the next step.

448 Count matrix generation: We used the prepDE.py
449 (<https://github.com/gpertea/stringtie/blob/master/prepDE.py>) script from StringTie (Pertea et al. 2015) to
450 generate the count matrix from the output of the previous step.

451 Gene expression level quantification: We used the ballgown R package (Frazee et al. 2015) to estimate the
452 TPM (Transcript Per Million) expression of each gene or transcript. Data are loaded with ballgown from the
453 output of StringTie -e -B. Transcripts with low expression are filtered out as recommended in the new tuxedo
454 procedure (Pertea et al. 2016) using: subset(data,"rowMeans(texpr(data))>0.5", genomesubset=TRUE).
455 FPKM were then calculated using texpr function from ballgown. TPM where finally computed from this
456 table by normalizing the sum of each sample to 1e6.

457 Comparison to gene annotation: Genes and transcripts reconstructed by StringTie were compared to the
458 published gene annotation of the genome using gffcompare
459 (<https://ccb.jhu.edu/software/stringtie/gffcompare.shtml>). Each new transcript was assigned a class code
460 depending on its similarity to a reference transcript, as described in gffcompare documentation.

Quantification of the effects of TE insertions on gene transcript levels: considering only genes expressed in ovaries, we computed mean TPMs across replicates and used the following linear models after log transformation: $TPM \sim \text{gene} + \text{upstream} + \text{downstream}$, where “gene”, “upstream”, and “downstream” are the numbers of TE insertions in gene body, 5 kb upstream sequences, and 5 kb downstream sequences, respectively. Size effects for each of these factors were then recorded. In order to go into more details regarding the locations of TE insertions, we also used $TPM \sim \text{exon} + \text{intron} + \text{utr5} + \text{utr3} + \text{upstream} + \text{downstream}$. In parallel, for each strain, we defined 10 classes of genes based on mean TPM values, and computed the proportion of genes carrying at least one TE insertion in each of the 10 classes.

For the intergenomic analysis, we considered all pairs of strains within species, and for each pair of strains and each gene, we computed the difference in TE insertion numbers on one hand and the coefficient of variation (CV, square root of variance divided by mean) for TPM on the other hand. We then defined 10 classes of genes based on CV values and, for each of these classes, we computed the proportions of genes carrying different numbers of TEs.

ChIP-seq preparation

Chromatin immunoprecipitation was performed using 50 ovary pairs dissected from three to five day old females. Ovaries were re-suspended in A1 buffer containing 60mM KCl, 15mM NaCl, 15mM Hepes, 0,5% Triton and 10mM Sodium butyrate. Formaldehyde (Sigma) was added to a final concentration of 1.8% for secondary cross-linking for 10 min at room temperature. Formaldehyde was quenched using glycine (0.125 M). Cross-linked cells were washed and pelleted twice with buffer A1, once with cell lysis buffer (140mM NaCl, 15mM Hepes, 1mM EDTA, 0.5mM EGTA, 1% Triton X100, 0.1% Sodium deoxycholate, 10mM sodium butyrate) , followed by lysis in buffer containing ,140mM NaCl, 15mM Hepes, 1mM EDTA, 0.5mMEGTA, 1% Triton X100, 0.5% SDS, 0.5% N-Laurosylysarcosine, 0.1% sodium deoxycholate, 10mM sodium butyrate for 120 min at 4°C. Lysates were sonicated in Bioruptor sonicator to reach a fragment size window of 200-600 bp.

Chromatin was incubated overnight at 4°C with the following antibodies: for H3K9me3 ChIP using α -H3K9me3 (actif motif #39161, 3 μ g/IP) and for H3K4me3 using α -H3K4me3 (millipore #07-473, 3 μ g/IP)

antibodies. The Magna ChIP™ A/G Chromatin Immunoprecipitation Kit (cat# 17-10085) was used following manufacturer's instructions. Final DNA recovery was performed by classic phenol/chloroform DNA precipitation method using MExtract high density tubes to maximize DNA recovery. DNA fragments were then sequenced on an Illumina HiSeq 4000 apparatus, with paired-end 100 nt reads. Due to technical issues, only one replicate could be used for dsgoth31 input.

ChIP-seq quality check: Validation of H3K4me3 enrichment around promoters.

Raw reads were trimmed using trim_galore (<https://zenodo.org/record/5127899#.YbnMs73MLDc>) with default parameters along with --paired, --clip_R1 9, --clip_R2 9, and --max_n 0. Mapping was performed using Bowtie2 ((Langmead and Salzberg 2012)) with --sensitive-local against the *D. melanogaster* r6.16 and *D. simulans* r2.02 genomes. Samtools was used to convert SAM to coordinated sorted BAM files, while sambamba ((Tarasov et al. 2015)) was used to filter for uniquely mapping reads and to remove duplicates (sambamba view -h -t 2 -f bam -F "[XS] == null and not unmapped and not duplicate"). For *D. melanogaster* datasets, we filtered available blacklisted regions ((Amemiya et al. 2019)) with bedtools. Finally coverage files containing reads per genome coverage (RPGC) were obtained with DeepTools (Ramírez et al. 2016) bamCoverage with --extendReads, --effectiveGenomeSize 129789873 for *D. melanogaster* available from the Deeptools suite, and --effectiveGenomeSize 121102921 computed with [unique-kmers.py](https://github.com/dib-lab/khmer) from khmer (<https://github.com/dib-lab/khmer>). Promoter regions were obtained with gencode_regions (https://github.com/saketkc/gencode_regions) and along with coverage files were used in DeepTools computeMatrix and plotProfile to build the average coverage of H3K4me3 and H3K9me3 around transcription start sites in both species. The corresponding profiles looked as expected (Supplemental_Fig_S13).

510 ChIP-seq analysis

511 For each of the immunoprecipitated samples (H3K4me3, H3K9me3, input), TE read counts were computed
512 at the family level using the TEcount module of TETools (Lerat et al., 2017) and the list of TE sequences
513 available at <ftp://pbil.univ-lyon1.fr/pub/datasets/Roy2019/>.

514 ChIP-seq counts were normalized across samples of the same species using the counts(normalize=T)
515 function of DESeq2 1.26.0 (Love et al. 2014). This was done independently for each of the
516 immunoprecipitated samples (H3K4me3, H3K9me3, input). We then performed a log-transformation using
517 the rlogTransformation function of DESeq2, and subsequently considered mean values across replicates. We
518 only kept genes expressed in ovaries.

519 In order to quantify the effects of TE insertions on histone marks enrichments, we used the following linear
520 models on log transformed read counts: histone mark (either H3K4me3 or H3K9me3) ~ input + gene +
521 upstream + downstream, where “gene”, “upstream”, and “downstream” are the numbers of TE insertions in
522 gene body, 5 kb upstream sequences, and 5 kb downstream sequences, respectively. Size effects for each of
523 these three factors were then recorded. In order to go into more details regarding the locations of TE
524 insertions, we also used histone mark (either H3K4me3 or H3K9me3) ~ input + exon + intron + utr5 + utr3 +
525 upstream + downstream.

526 In parallel, for each strain, we defined 10 classes of genes based on histone mark enrichment, built upon the
527 values of {log transformed histone mark read counts (either H3K4me3 or H3K9me3) – log transformed input
528 read counts}, calculated for each gene. We computed the proportion of genes carrying at least one TE
529 insertion in each of the 10 classes.

530 For the intergenomic analysis, for each pair of strains and each ovary-expressed gene, we considered the
531 model H3Kime3 (i stands for 4 or 9) ~ input + strain, and retrieved the absolute value of the effect of the
532 strain factor. We defined 10 classes of genes based on the values of this variable (square root transformed),
533 and computed the proportion of genes that carried different numbers of TE insertions within each class.

534

535 **Data access**

536 The RNA-seq data generated in this study have been submitted to the NCBI BioProject database
537 (<https://www.ncbi.nlm.nih.gov/bioproject/>) under accession number PRJNA795668. The ChIP-seq
538 data generated in this study have been submitted to the NCBI BioProject database under accession
539 number PRJNA796157. TE and gene annotations have been deposited to Zenodo doi:
540 10.5281/zenodo.5844405.

541

542 **Competing interest statement**

543 The authors declare no competing interests.

544

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