Exploring the role of transposable elements to sex gap in longevity in *Drosophila* species

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ABSTRACT

In *Drosophila*, like in many other animal species, females tend to live longer than males, a phenomenon known as sex gap in longevity (SGL). One possible explanation for this phenomenon could be related to the activity of transposable elements (TE), which may be present at higher levels in the heterogametic sex. TE activity is normally repressed by epigenetic mechanisms, but this regulation weakens with age. Sex chromosomes, such as the Y chromosome, are enriched in TEs, and age-related TE activity may therefore be more pronounced in older males than in older females, likely affecting longevity patterns. Using three *Drosophila* species, we show that SGL varies naturally among wild-type populations, reflecting both intra- and inter-species differences. Transcriptomic data revealed increased age-related TE expression in *D. melanogaster* and *D. suzukii* flies, but not in *D. simulans*. Moreover, we observed a higher number of upregulated TE copies in older males compared to older females across all the three species tested. Additionally, we detected an increase in TE-chimeric transcript generation in some aged samples, particularly in *D. suzukii* males. Finally, the replacement of the Y chromosome between strains with different SGL led to a progressive reduction in male lifespan and increased TE transcriptional release over generations, suggesting a Y chromosome important role in male longevity. Our work highlights the importance of investigating the role of TEs to better understand differences in longevity between sexes and across different species.

INTRODUCTION

In most animal species, males and females display distinct mortality patterns leading to differences in their longevity (Marais, et al. 2018; Lemaître, Ronget and Gaillard 2020). This phenomenon is known as sex gap in longevity (SGL) and several hypotheses have been proposed to explain its evolution across the tree of life (Brooks and Garratt 2017; Marais, et al. 2018; Bronikowski, et al. 2022). Among these hypotheses, the putative role played by sex-specific allocation to sexual competition (sexual selection hypothesis) or the asymmetric inheritance of cytoplasmic genomes (mother's curse hypothesis) have been extensively studied (Frank and Hurst 1996; Vinogradov 1998; Maklakov, et al. 2008; Camus, et al. 2012; Brooks and Garratt 2017; Marais, et al. 2018; Cayuela, et al. 2023). Sex chromosomes have also been proposed to play an important role in SGL since the heterogametic sex generally has the shortest lifespan (Pipoly, et al. 2015; Carazo, et al. 2016; Marais, et al. 2018; Brown, et al. 2020b; Xirocostas, et al. 2020; Nguyen and Bachtrog 2021; Peona, et al. 2021; Marais and Lemaître 2022; Sultanova, et al. 2023). On the one hand, the hemizygosity of the X (in XX/XY taxa) or Z (in ZW/ZZ taxa) chromosome suggests that the shortened lifespan of the heterogametic sex might be due to the unconditional expression of recessive deleterious alleles on its single X (or Z) chromosome (unguarded X effect). However, if the unguarded X effect contributes to sex differences in lifespan is still under debate (Brengdahl, et al. 2018; Connallon, et al. 2022). Alternatively, the toxic Y effect presents another possible hypothesis underlying SGL, postulating that the elevated transposable element (TE) abundance of this chromosome (or W chromosome) compared to other chromosomes may have detrimental consequences for the heterogametic sex (Brown, et al. 2020b; Nguyen and Bachtrog 2021; Peona, et al. 2021; Warmuth, et al. 2022). Y (or W) chromosomes often display reduced rates of recombination, resulting in the accumulation of repeated sequences (mainly TEs) on these chromosomes (Erlandsson, et al. 2000).

TEs are genomic fragments with the ability to move along the genome, being major components of eukaryotic genomes (Bourque, et al. 2018). Due to their mobile and repetitive nature, they are considered a source of mutations and genetic polymorphisms (Bourque, et al. 2018). As such, TE activity is normally repressed by epigenetic regulation that involves DNA methylation, histone modifications and/or small RNAs (Slotkin and Martienssen 2007; Merenciano, et al. 2024). However, with age, these silencing mechanisms are disrupted causing a general increase of TE activity (Wood and Helfand 2013; Copley and Shorter 2023; Yushkova and Moskalev 2023). The toxic Y effect thus proposes that because of the high abundance of TEs on the Y chromosome, more TEs may become active in old males than in

old females, which might contribute to sex-differences in ageing and in the reduced male lifespan. Because TEs on the Y chromosome are mostly truncated and non-functional, the impact of TEs on longevity is likely a global effect of the mobilome rather than being driven by Y-linked insertions specifically.

Age-dependent activation of TEs has been suggested to negatively impact lifespan in *Drosophila* (Driver and McKechnie 1992; Nikitin and Woodruff 1995; Woodruff and Nikitin 1995; Li, et al. 2013; Wood and Helfand 2013; Chen, et al. 2016; Wood, et al. 2016; Brown, et al. 2020b; Fabian, et al. 2021; Nguyen and Bachtrog 2021; Rigal, et al. 2022; Yang, et al. 2022), termites (Elsner, et al. 2018; Post, et al. 2023), nematodes (Sturm, et al. 2023), mice (De Cecco, et al. 2019; Ricci, et al. 2023), and humans (Van Meter, et al. 2014; Bogu, et al. 2019; Teoli, et al. 2024; Tsai, et al. 2024). More specifically, in *Drosophila melanogaster*, it has been suggested that aged males underwent a general loss of H3K9me2 histone modification more rapidly than females, resulting in a more pronounced TE activation during ageing in males (Brown, et al. 2020b). Indeed, in this species, it was described that the Y chromosome, and TEs in general, affect chromatin formation genome-wide, in agreement with the heterochromatin sink model (Gatti and Pimpinelli 1992; Brown, et al. 2020a). In addition, longevity assays performed in flies with aberrant sex chromosome configurations (XO, XXY, and XYY) showed that the number of Y chromosomes could influence organismal survival (Brown, et al. 2020b).

In other studies, these results have been challenged. For instance, the generation of *D. melanogaster* Y chromosomes with different sizes using CRISPR/Cas9 suggested that the size of this chromosome, and thus, the amount of heterochromatin (and TEs), did not contribute to sex-specific differences in longevity (Delanoue, et al. 2023). In *D. pseudoobscura*, variations in Y chromosome size and Y-linked TEs showed that larger Y chromosomes lead to slightly increased TE expression, especially in old males, but these differences do not result in faster ageing (Nguyen, et al. 2022). Therefore, the impact of the Y chromosome on male longevity through TE abundance and increased TE expression is still under debate.

In this work, we studied the SGL natural variation across three *Drosophila* species: *D. melanogaster*, *D. simulans*, and *D. suzukii*. Additionally, we analysed TE expression changes associated with longevity in the same species, with particular emphasis on sex-specific differences. Finally, we replaced the Y chromosome among *D. melanogaster* strains to investigate its impact on SGL and lifespan.

RESULTS

SGL varies between populations and species

To study the natural variation in SGL, we performed longevity assays in three *Drosophila* species: *D. melanogaster*, *D. simulans*, and *D. suzukii.* For each species, we analyzed between two and six wild-type populations, totaling 28-132 strains, with 100 flies per strain (Fig. 1 and Supplementary Table 1). SGL was measured as the logarithm of the ratio between male and female median lifespan for each strain, a metric previously used in mammals (Lemaître, Ronget, Tidière, et al. 2020) (see Methods) (Supplementary Table 2).

In agreement with the literature, the vast majority of strains across all species (82%) exhibited negative SGL values, indicating reduced male longevity (Fig. 1). Specifically, statistically significant negative SGL values were observed in 58% of *D. melanogaster* strains, 65% of *D. simulans* strains, and 35% of *D. suzukii* strains (Fig. 1 and Supplementary Table 2). On average, male lifespan was shorter by 10.5 days in *D. melanogaster*, 12.5 days in *D. simulans*, and nine days in *D. suzukii*.

We then analyzed the variance in the SGL variable across different hierarchical levels (population and species) using a Bayesian approach (see Methods). The analysis revealed that the population of origin contributed significantly to the observed differences in SGL (Supplementary Fig. 1A). Notably, it explained 38.2% (95% CI [16.2, 57.5]) of the total variance suggesting substantial within-species variability attributable to population-level factors (Fig. 1 and Supplementary Fig. 1). In *D. melanogaster*, the coefficient of variation in SGL values ranged from 0.42 to 1.99, while in *D. simulans*, it ranged from 0.45 to 1.80 (Fig. 1). For *D. suzukii*, the coefficients ranged from 0.87 to 1.68, indicating that, although we could analyze only two populations, this species may have the lowest variation in SGL (Fig. 1). The species effect explained 8.6% (95% CI [0.1, 20.8]) of the total variance, also suggesting differences in SGL across species (Fig. 1 and Supplementary Fig. 1B).

In summary, our extensive analysis of SGL showed that males have an overall reduced lifespan compared to females in all three *Drosophila* species tested. Moreover, we found significant within- and between-species variability of SGL values in the wild.

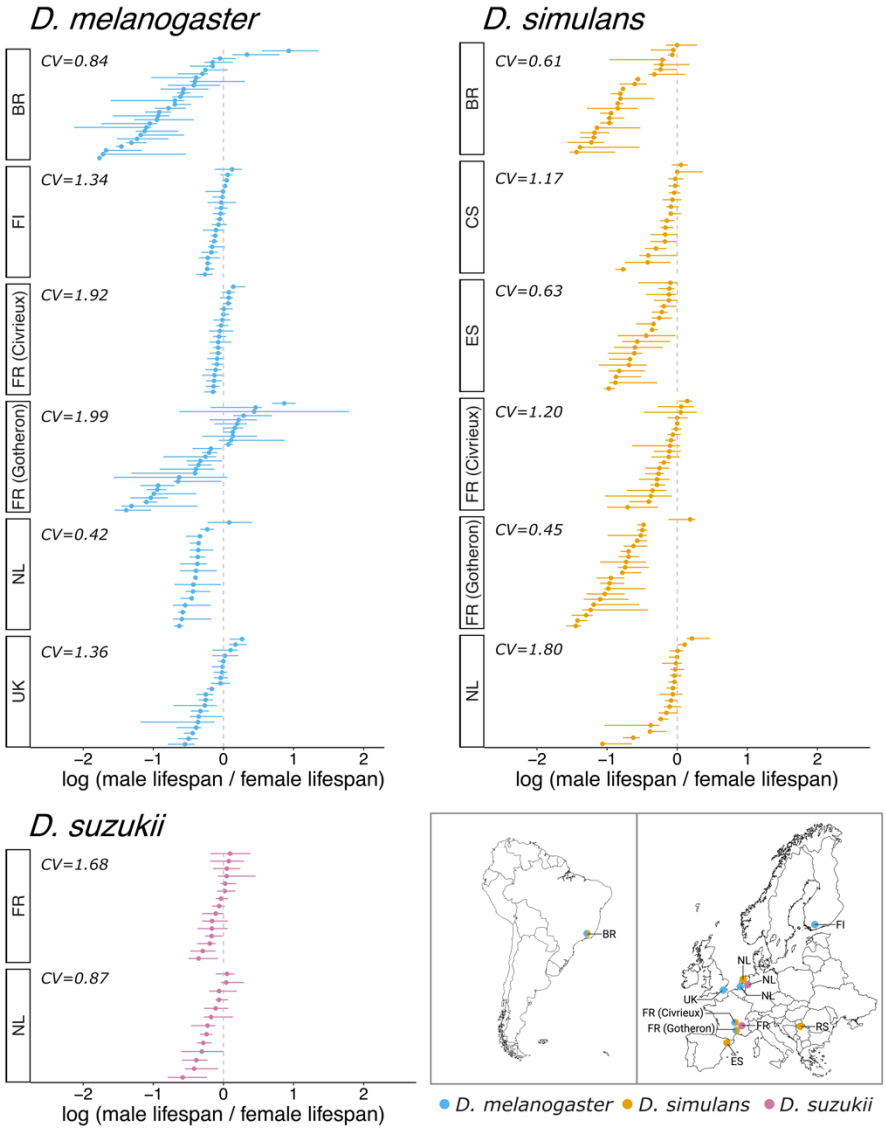


Figure 1. SGL values per strain and geographical distribution of all the populations tested from *D. melanogaster*, *D. simulans*, and *D. suzukii*. Dots show mean SGL values calculated as the logarithm of the ratio between male and female median lifespan and lower and upper whiskers indicate the upper and lower 95% confidence intervals. Coefficients of variation (CV) were calculated for every population. UK: United Kingdom, FR: France, FI: Finland, NL: the Netherlands, BR: Brazil, ES: Spain, RS: Serbia.

***D. melanogaster* and *D. suzukii* flies show higher abundance of expressed TE families with age**

To study TE expression changes associated with age, we used three *Drosophila* species with varying TE content: *D. melanogaster* (20%), *D. simulans* (12%), and *D. suzukii* (47%) (Mérel, et al. 2020; Mérel, et al. 2021). RNA-seq was performed on young and old individuals from two inbred strains per species, which were previously sequenced with long-read techniques: dmgoth101 and dmgoth63 for *D. melanogaster*, dsgoth31 and dsgoth613 for *D. simulans*, and MT47 and S29 for *D. suzukii* (Mohamed, et al. 2020) (see Methods) (Supplementary Table 1

159 and Supplementary Fig. 2). A substantial proportion of genes associated with ageing were
160 differentially expressed in the older flies, indicating that the age at which we extracted the RNA
161 was sufficient to classify them as old (see Methods) (Supplementary Fig. 3 and 4).
162 We used TEtools to assess whether TE expression varies with age, irrespective of sex (see
163 Methods) (Supplementary Tables 3 and 4) (Lerat, et al. 2017). To do that, we first looked at
164 the percentage of reads mapping to TE sequences in our RNA-seq data. Using a generalized
165 linear model (GLM) analysis, we found that the percentage of TE-derived reads increase during
166 ageing in all the species tested (estimate 0.314, $p < 0.001$) (Fig. 2A). In particular, *D.*
167 *melanogaster* showed a greater difference between young and old flies compared to the other
168 species (Fig. 2A). Furthermore, comparing to *D. melanogaster*, *D. simulans* exhibited less TE-
169 derived reads (estimate -0.652, $p < 0.001$), whereas *D. suzukii* showed more (estimate 0.725, p
170 < 0.001), consistent with their respective TE contents (Fig. 2A).
171 Since certain TE families might contribute more to the age-related increase in expression, we
172 also analyzed TE expression at the family level. In both *D. melanogaster* and *D. suzukii*, we
173 observed a greater number of upregulated TE families compared to downregulated ones in
174 older flies across all strains (chi-square, all $p < 0.05$), except for males from the *D.*
175 *melanogaster* dmgoth101 strain (chi-square, $p = 0.999$) (Fig. 2B). Similar results were found
176 for both males and females, suggesting that TEs can be activated in ageing as it was observed
177 in other studies from *D. melanogaster* and other species, such as humans (Fig. 2B) (Driver and
178 McKechnie 1992; Nikitin and Woodruff 1995; Woodruff and Nikitin 1995; Li, et al. 2013; Van
179 Meter, et al. 2014; Chen, et al. 2016; Wood, et al. 2016; Bogu, et al. 2019; Brown, et al. 2020b;
180 Fabian, et al. 2021; Nguyen and Bachtrog 2021; Rigal, et al. 2022; Yang, et al. 2022; Teoli, et
181 al. 2024; Tsai, et al. 2024). However, in *D. simulans*, the number of upregulated TE families
182 did not statistically differ from the number of downregulated ones in older flies (chi-square, all
183 $p > 0.05$), probably due to its reduced TE content. Nonetheless, there was a tendency towards
184 a higher number of upregulated TE families (Fig. 2B). Although the number of upregulated TE
185 families generally increases with age, these TE families showed little to no overlap between
186 sexes or between strains of the same species (Supplementary Fig. 5). This result suggested that
187 TE expression during ageing is not family specific.
188 Overall, we observed an increased number of upregulated TE families in older flies of *D.*
189 *melanogaster* and *D. suzukii*. In *D. simulans*, there was a similar tendency, although it was not
190 statistically significant, likely attributed to its reduced TE content. These findings are in line
191 with the fact that ageing might be associated with the disruption of the epigenetic mechanisms
192 that silence TEs favouring their activation.

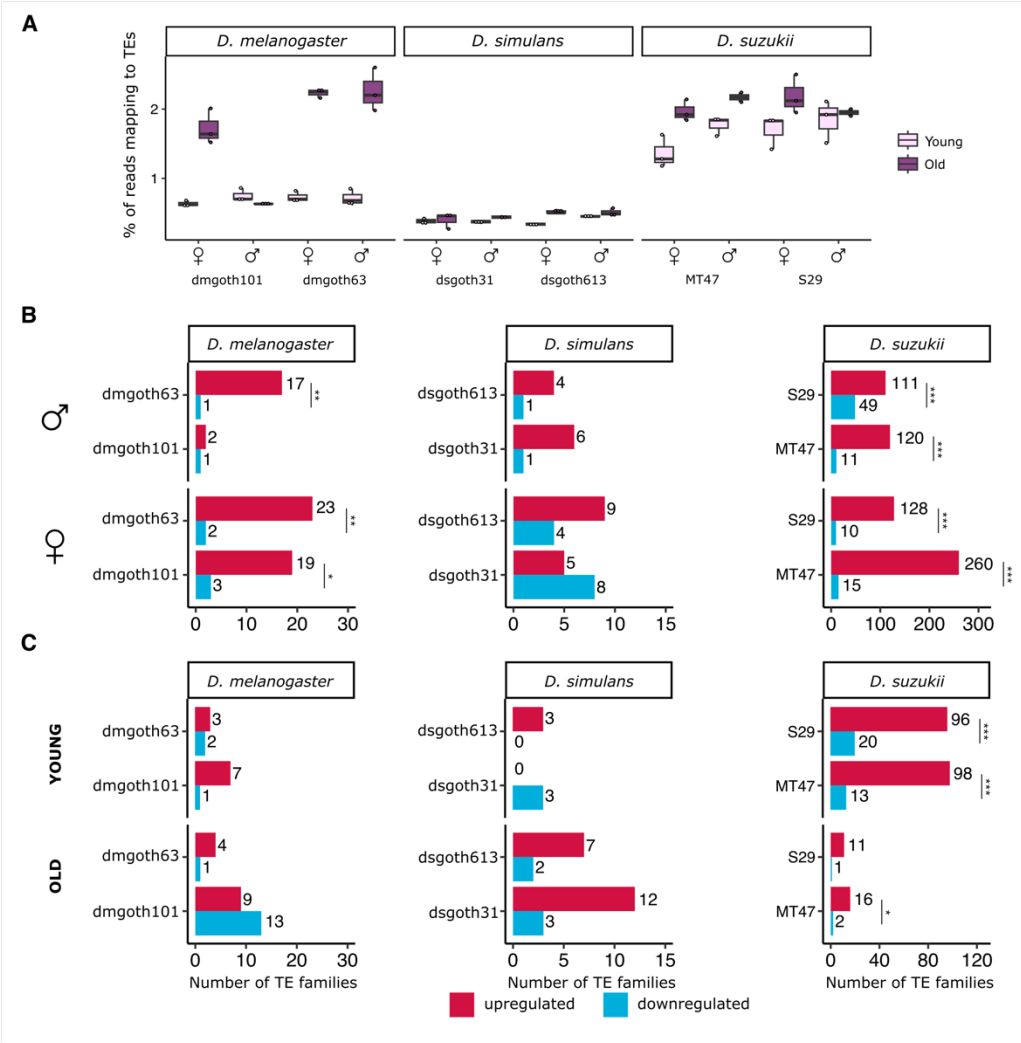


Figure 2. **Ageing increases the number of TE transcripts and upregulated TE families in different *Drosophila* species.** **A**) Boxplots showing the percentage of reads mapping to TE sequences in the RNA-seq data in young and old male and female flies of each strain. Boxplots show the median (horizontal line), first and third quartiles (lower and upper bounds, respectively), and minimum and maximum values (lower and upper whiskers, respectively). Each dot represents an independent biological replicate. **B**) Barplots showing the number of upregulated (in red) and downregulated (in blue) TE families with ageing for both males (upper panel) and females (bottom panel) in each strain. **C**) Barplots showing the number of upregulated (in red) and downregulated (in blue) TE families in young males compared to young females (upper panel) and in old males compared to old females (bottom panel) in each strain. Asterisks indicate level of statistical significance of chi-square tests (* $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$).

The age-related increase in TE-derived reads and expressed TE families is not influenced by sex

As flies age, the high TE content on the Y chromosome may contribute to higher TE expression in older males compared to females. We investigated this by comparing the percentage of TE-derived reads in older males and females.

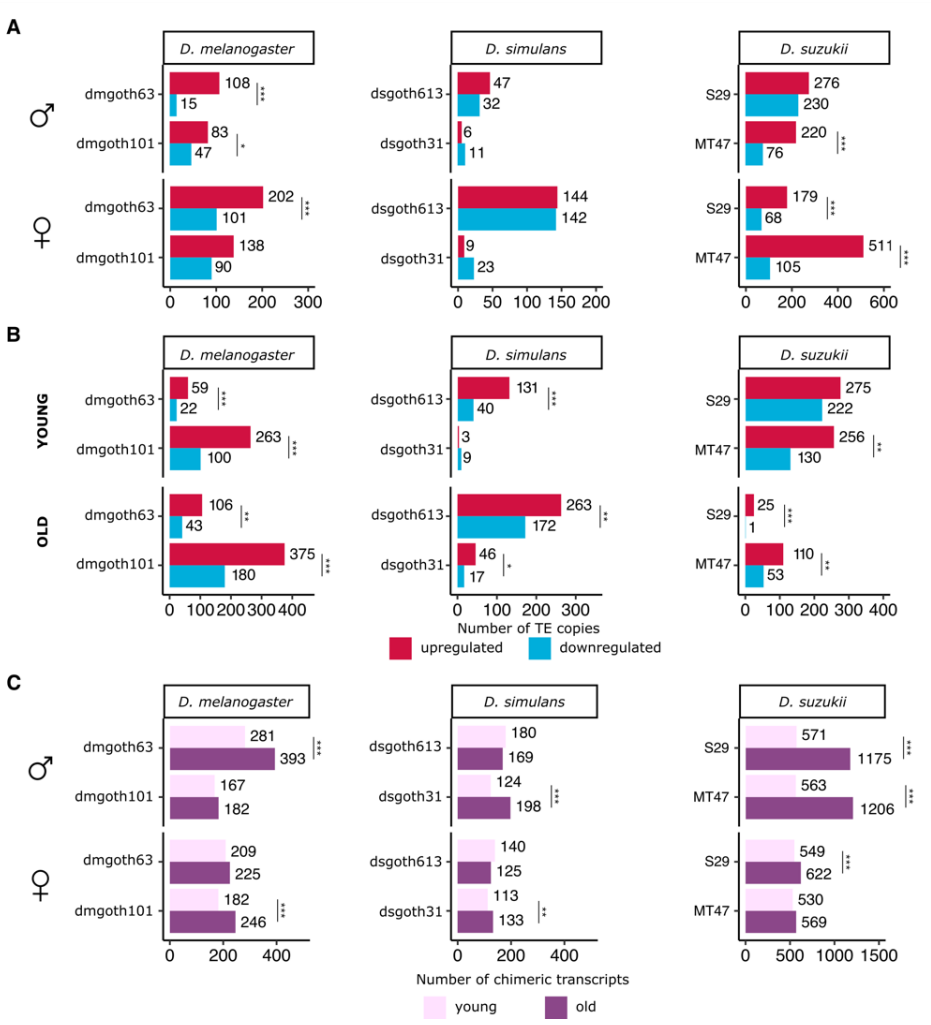
Performing a GLM analysis, we found no statistically significant effects of sex (estimate 0.056, $p = 0.398$) or the interaction between sex and age (estimate -0.140, $p = 0.271$) on the percentage

212 of TE-derived reads (Fig. 2A). Similarly, at the TE family level, we found no statistically
213 significant differences in the number of upregulated and downregulated TE families between
214 older males and older females, except in the *D. suzukii* MT47 strain (chi-square, $p = 0.029$)
215 (Fig. 2C). However, there was a general trend toward finding more upregulated TE families
216 (Fig. 2C). Again, little overlap between differentially expressed TE families were found
217 between strains from the same species, suggesting that there are no particular TE families
218 leading to the sex differences in TE expression of older flies (Supplementary Fig. 5). When
219 comparing the expression of TE families between young male and female flies, we also
220 observed no statistically significant differences between the number of upregulated and
221 downregulated TE families in *D. melanogaster* and *D. simulans* (chi-square, p values > 0.05)
222 (Fig. 2C). Conversely, in *D. suzukii*, we did observe more upregulated TE families in young
223 males compared to young female flies (chi-square, p values < 0.001) (Fig. 2C).
224 Altogether, we found no significant sex differences in the overall age-related increase of TE
225 transcripts, nor in the number of TE families up- or downregulated in older males versus older
226 females.
227
228 ***D. melanogaster* and *D. suzukii* flies show higher abundance of expressed TE copies with**
229 **age**
230 We previously observed that TE-derived reads, as well as some TE families, increase with age,
231 without apparent sex-specific effects. However, our family-level approach might mask
232 differences in the expression of individual TE copies, which could be selectively activated or
233 released due to locus-specific chromatin relaxation. To address this, we used Telescope to
234 precisely measure TE expression at specific genomic locations and assess the impact of age
235 and sex on individual TE copies (see Methods) (Bendall, et al. 2019) (Supplementary Tables 3
236 and 4). Telescope is a computational tool that resolves ambiguous mapped reads by
237 probabilistically assigning them to their most likely source transcript using a Bayesian model
238 (Bendall, et al. 2019). We performed this analysis with three biological replicates to ensure the
239 robustness and reliability of our results.
240 On the one hand, we found a general trend towards more upregulated than downregulated TE
241 copies with age in both males and females of *D. melanogaster* and *D. suzukii* (Fig. 3A). These
242 results were statistically significant (chi-square, all $p < 0.05$), except for the *D. melanogaster*
243 females of the dmgoth101 strain and the *D. suzukii* males of the S29 strain (chi-square, all $p >$
244 0.05) (Fig. 3A). On the other hand, in *D. simulans*, the number of upregulated TE copies did
245 not statistically differ from the number of downregulated ones (Fig. 3A). These findings are in

246 line with our previous analysis on TE-derived reads analysed with TEtools software (Fig. 2A).
247 Moreover, as expected based on the TE content, *D. suzukii* showed an increased number of
248 differentially expressed TE copies (DETEs) in both males and females compared to the other
249 species (Fig. 3A).
250 We also tested whether male and female flies from the same strain shared some of the DETEs.
251 Indeed, we found that some of the upregulated DETEs in males were shared with females
252 (Supplementary Fig. 6). These shared DETEs are therefore absent from the Y chromosome,
253 indicating that a subset of activated TEs might not be present in the Y chromosome, at least in
254 some strains, suggesting a broader, global influence of the Y chromosome on TE expression.
255 Overall, we observed an increased number of upregulated TE copies in older flies of *D.*
256 *melanogaster* and *D. suzukii*.
257
258 **More specific TE copies are expressed in older males compared to older females**
259 Using a copy-specific TE expression approach, we tested whether the higher TE abundance in
260 males, driven by their TE-rich Y chromosome, results in a greater number of expressed TE
261 copies in aging males compared to aging females.
262 We detected more upregulated TE copies than downregulated ones in old male flies compared
263 to old female flies in all the strains of the three species tested (chi-square, all $p < 0.05$) (Fig.
264 3B). These differences are already observed at young ages (chi-square, all $p < 0.05$), although
265 it is not the case for the *D. simulans* dsgoth31 and the *D. suzukii* S29 strains (chi-square, $p =$
266 0.399 and $p = 0.105$, respectively) (Fig. 3B). In contrast to the other two species, *D. suzukii*
267 showed a greater number of DETEs between sexes in young flies than in old flies (Fig. 3B).
268 We also found that the upregulated TE copies observed between old males and females are
269 present in all chromosome arms, with an expected concentration in chromosome centromeres
270 and in the dot chromosome (Supplementary Fig. 7). In all strains, full-length TE copies, which
271 are more likely to transpose, were significantly overrepresented among upregulated TEs
272 compared to their overall genome proportion (chi-square, $p < 0.001$). This suggested that age-
273 dependent TE expression may lead to new insertions; however further experiments are needed
274 to confirm this.
275 We finally performed a permutation test to check whether DETEs found in old male flies are
276 enriched nearby (+-1kb) differentially expressed genes (DEGs) (see Methods). Overall, we
277 found no differences (p-values > 0.05) or statistically significant depletion (p-values < 0.05) in
278 both upregulated and downregulated TE insertions nearby genes (Supplementary Table 5).

279 In summary, using a copy-specific approach, comparisons between older male and older female
280 flies revealed an increased number of expressed TE copies than repressed. The copy-specific
281 TE expression analysis revealed sex differences that were likely masked in family-level
282 analysis.
283
284 **Ageing increases the number of TE chimeric transcripts in *D. suzukii* males**
285 TEs can influence nearby genes by altering their expression and structure, potentially
286 generating chimeric transcripts with both gene and TE sequences (Casacuberta and González
287 2013; Coronado-Zamora and González 2023; Oliveira, et al. 2023). To investigate if age-
288 dependent TE activation is linked to increased chimeric transcripts, we used the ChimeraTE
289 pipeline to quantify gene-TE chimeric transcripts in RNA-seq data from young and old flies of
290 *D. melanogaster*, *D. simulans*, and *D. suzukii* (Oliveira, et al. 2023). We define the number of
291 gene-TE chimeric transcripts as the count of distinct chimeric transcripts (isoforms) found in
292 the dataset, rather than the number of reads mapping to these chimeric transcripts.
293 Older flies showed a significant increase in chimeric transcripts compared to younger flies in
294 some samples: dmgoth63 males, dmgoth101 females, dsgoth31 males and females, S29 males
295 and females, and MT47 males (chi-square, all $p < 0.001$) (Fig. 3C). Remarkably, in *D. suzukii*,
296 males exhibited over twice as many chimeric transcripts in older flies compared to younger
297 ones (Fig. 3C). Moreover, within each strain, there is only a small proportion of shared chimeric
298 transcripts between different sexes or age groups (Supplementary Fig. 8).
299 In all species, there was a moderate positive correlation between chimeric transcript generation
300 and TE family copy number (Pearson correlation all $p < 0.05$) (Supplementary Fig. 9). In *D.*
301 *melanogaster*, the TE families *roo*, *INE-1*, and *copia* were most frequently associated with
302 chimeric transcripts, with *roo* and INE-1 being abundant in both young and old flies, and *copia*
303 showing increased chimeric transcript formation in older flies (Coronado-Zamora and
304 González 2023; Oliveira, et al. 2023) (Supplementary Fig. 10). In *D. simulans*, the most
305 prevalent families producing chimeric transcripts were *roo*, *INE-1*, *P-elements*, and *GATE*
306 elements, whereas in *D. suzukii*, it was predominantly *Jockey-3* and *TART* elements
307 (Supplementary Fig. 11 and 12).
308 Finally, we quantified the proportion of genes producing chimeric transcripts that were found
309 differentially expressed with age. The contribution of these genes was very low: in *D.*
310 *melanogaster* we found on average 2.92% in males and 5.34% in females; in *D. simulans*,
311 1.64% in males and 6.21% in females; and in *D. suzukii*, 4.75% in males and 3.56% in females.
312 Moreover, there was no enrichment of DEGs among those generating chimeric transcripts in

313 any sample (chi-square tests, all p > 0.05). Gene-TE chimeric transcripts could interfere with
314 TE quantification. If a highly expressed host gene produces a chimeric transcript containing a
315 TE sequence, part of the reads from that transcript could be misassigned to the TE, thereby
316 artificially inflating its estimated expression level. However, although gene–TE chimeric
317 transcripts could in theory inflate TE expression, their contribution in our data is very limited,
318 and we do not expect them to affect our overall results.
319 Altogether, we observed an increase in gene-TE chimeric transcripts with age in some samples,
320 particularly in *D. suzukii* males. While these changes may relate to age-dependent TE
321 activation, they could also result from increased gene expression or reduced splicing efficiency
322 with age (Wood, et al. 2016; Bhadra, et al. 2020).
323



324
325
326
327 **Figure 3**. **Ageing increases the number of activated TE copies in different *Drosophila* species. A**) Barplots showing the
328 number of upregulated (in red) and downregulated (in blue) TE copies with ageing for both males (upper panel) and females
329 (bottom panel) in each strain. **B**) Barplots showing the number of upregulated (in red) and downregulated (in blue) TE copies
330 in young males compared to young females (upper panel) and in old males compared to old females (bottom panel) in each
331 strain. **C**) Ageing increases the generation of chimeric transcripts in some samples. Barplots showing the number of identified
332 chimeric transcripts per strain as a sum of chimeric transcripts supported only by paired-end reads that have mapped in both

transcripts and TE sequences ("chimreads_evidence"), chimeric transcripts supported only by the transcriptome assembly ("transcriptome_evidence"), and chimeric transcripts for which both previous methods have predicted the same chimeric transcripts ("double_evidence"). Asterisks indicate level of statistical significance of chi-square tests (* $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$).

Increased TE expression in aged males could be associated with reduced lifespan both *D. melanogaster* and *D. simulans*

To investigate whether the increased expression of some specific TE copies in aged males compared to aged females correlates with sex lifespan differences, we performed longevity assays across all strains from our transcriptome analysis.

Males showed reduced lifespan in *D. melanogaster* and *D. simulans* (Log Rank all $p < 0.001$), with negative SGL values (Fig. 4A-D, and Supplementary Table 6). Nevertheless, in *D. suzukii*, differences in lifespan between males and females were not statistically significant (Log Rank $p = 0.400$ and $p = 0.500$ for MT47 and S29, respectively), though negative SGL values suggest some variance in ageing associated with sex (Fig. 4E and 4F and Supplementary Table 6). Thus, the increased number of DETEs in aged males compared to aged females (Fig. 3B) might be associated with reduced lifespan in *D. melanogaster* and *D. simulans*, but not clearly in *D. suzukii* (Fig. 4).

Finally, when analyzing each strain individually, we found no correlation between male lifespan or SGL values and their overall TE content (Pearson correlation all $p < 0.05$). Hence, overall TE content does not appear to be directly linked to reduced lifespan, as previously suggested for *D. melanogaster* and other species like the Greenland shark (Delanoue, et al. 2023; Sahm, et al. 2024).

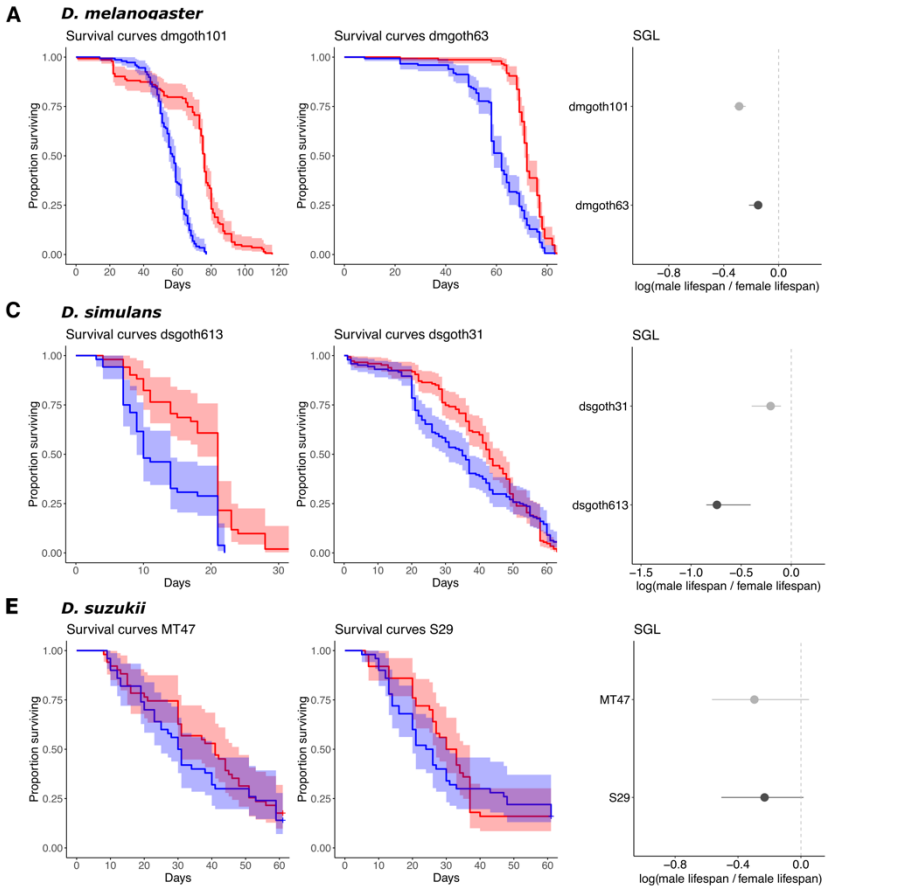


Figure 4. *D. melanogaster* and *D. simulans* males have reduced lifespan. Kaplan–Meier survivorship curves for males (blue) and females (red) in **A**) *D. melanogaster*, **C**) *D. simulans,* and **E**) *D. suzukii* with the shaded region indicating the upper and lower 95% confidence interval calculated from the Kaplan–Meier curves. SGL values measured as the logarithm of the ratio between male and female median lifespan are shown for each strain of **B**) *D. melanogaster*, **D**) *D. simulans,* and **F**) *D. suzukii*. Dots represent mean SGL values and lower and upper whiskers indicate the upper and lower 95% confidence intervals.

Y chromosome replacement leads to increased SGL by reducing male lifespan

To check whether the Y chromosome is associated with changes in SGL, we performed introgression experiments to replace the Y chromosome between *D. melanogaster* strains with different SGL values (see Methods).

Introgression crosses were made between strains dmgoth101 (-0.29 SGL, 95% CI [-0.32, -0.24]) and dmsj7 (-0.07 SGL, 95% CI [-0.13, -0.04]), with longevity assays conducted over ten generations. "A crosses" started with dmgoth101 females and dmsj7 males, while "B crosses" were the reciprocal. Both crosses showed an increase in SGL values across generations, primarily due to a reduction in male lifespan (Fig. 5A and Supplementary Table 7). In A crosses, SGL rose from -0.26 (95% CI [-0.35, -0.19]) to -1.11 (95% CI [-1.20, -1.01]) from generation F1 to F10 (Fig. 5A). Similarly, B crosses showed an initial rise from -0.23 (95% CI [-0.29, -0.14] to -0.68 (95% CI [-0.71, -0.60]) by F5 but decreased by F10 (95% CI [-0.27, -0.01])) (Fig. 5A). In both reciprocal crosses, males exhibited a progressive reduction in lifespan

380 across all generations, unlike females, whose lifespan showed less variation across generations
381 (standard deviation of mean lifespan in A crosses: 22.21 for males and 12.13 for females; B
382 crosses: 14.42 for males and 10.06 for females) (Fig. 5B and 5C). Repeating the experiment
383 with strains that did not show SGL differences (dmgoth101 and dmgoth63) did not result in
384 significant SGL value increases across generations (Supplementary Fig. 13 and Supplementary
385 Table 7).
386 In summary, we found that the replacement of the Y chromosome between strains with
387 different SGL values is associated with a progressive increase in SGL due to a reduced male
388 lifespan. If SGL values were directly linked to the Y chromosome's TE content, we might
389 expect the replacement of the Y chromosome to result in an SGL value matching that of the
390 donor strain across generations. However, this was not observed since SGL values increased
391 in both reciprocal crosses when replacing the Y chromosome between strains with different
392 SGL values. While further experiments are needed, these findings indicate that the
393 modification of the TE composition and distribution on the Y chromosome and/or the Y
394 chromosome as a whole may have a negative impact in male lifespan.
395

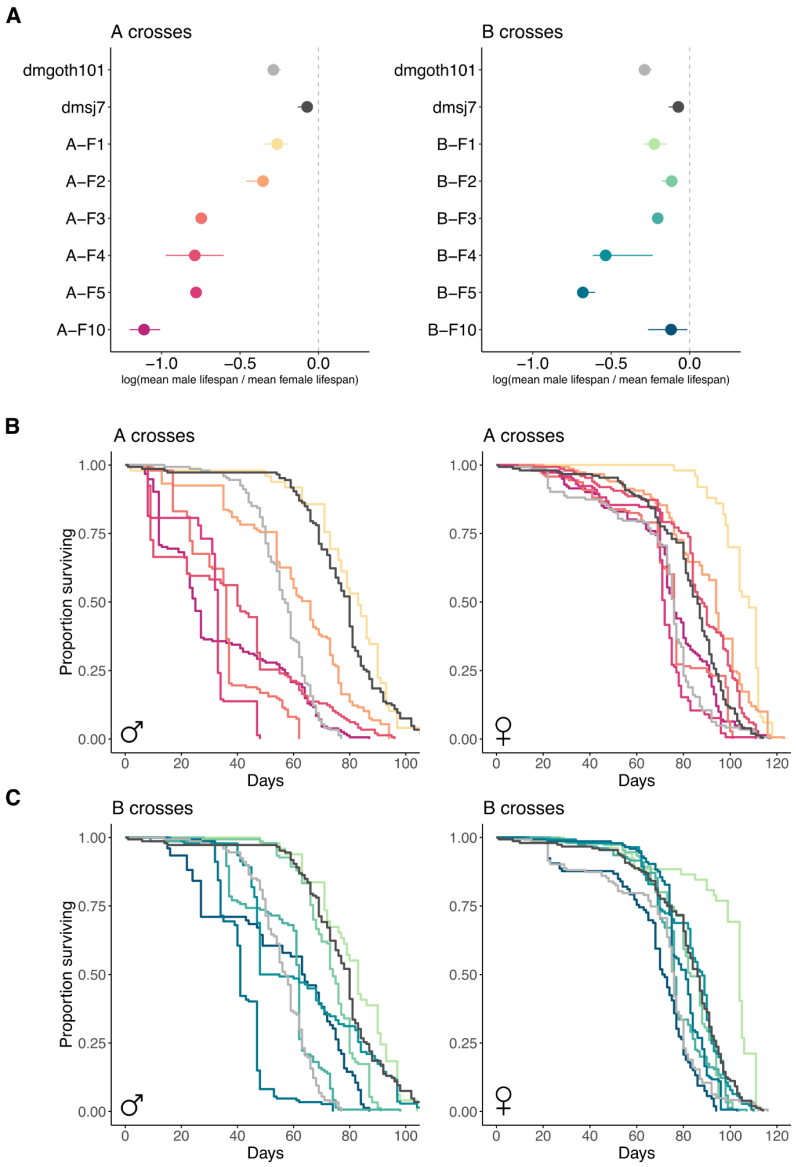


Figure 5. Y chromosome replacement is associated with reduced male lifespan. A) SGL values measured as the logarithm of the ratio between male and female median lifespan are shown for A reciprocal crosses (left panel) and B reciprocal crosses (right panel) of *D. melanogaster*. Results for maternal strains are shown in grey (dmgoth101) and black (dmsj7) while different colours represent generations of introgression from F1 to F10. Dots show mean SGL values and lower and upper whiskers indicate the upper and lower 95% confidence intervals. **B**) Kaplan–Meier survivorship curves for males (left panel, n=150) and females (right panel, n=150) in A reciprocal crosses. **C**) Kaplan–Meier survivorship curves for males (left panel, n=150) and females (right panel, n=150) in B reciprocal crosses. Male lifespan between dmgoth101 and dmgoth101 was statistically significantly different (Log-Rank test $p < 0.001$).

Y chromosome replacement is associated with an increase TE expression

To assess the impact of Y chromosome replacement on TE expression, we extracted RNA from 5-7 days old male and female *D. melanogaster* flies at generations F1 and F10 in reciprocal introgression crosses between dmgoth101 and dmsj7 (see Methods). TE expression was measured using TEtools (Lerat, et al. 2017).

We assessed the effects of reciprocal cross (A or B), generation (F1 or F10), sex, and their interactions on TE-derived reads percentage (see Methods). F10 flies had more TE-derived

reads than F1 in both sexes (GLM, estimate 0.105, $p = 0.033$), with differences more pronounced in B crosses (GLM, estimate 0.068, $p = 0.048$) (Fig. 6). In addition, males had more TE-derived reads than females in both generations (GLM, estimate 0.295, $p < 0.001$), and the interaction between generation and sex was not statistically significant (GLM, estimate 0.077, $p = 0.249$) (Fig. 6). These findings suggested that the Y replacement might be associated with increased TE transcripts across generations.

Overall, Y chromosome replacement between strains with different SGL values was associated with increased TE expression in males compared to females already in the first introgression cross (F1). The percentage of TE-derived reads increased in F10 compared to F1 in both sexes and reciprocal crosses.

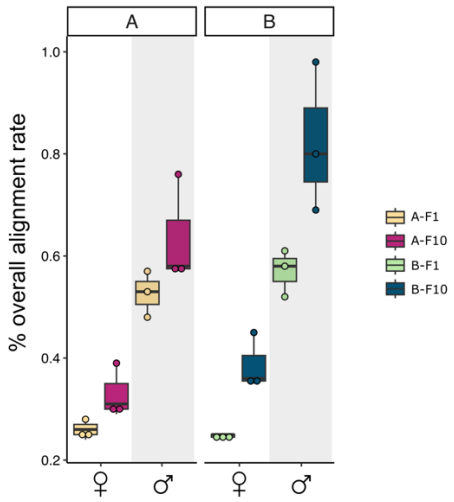


Figure 6. Y chromosome replacement is associated with increased TE expression. Boxplots showing the percentage of reads mapping to TE sequences in the RNA-seq data in F1 and F10 generations of introgression in both A and B reciprocal crosses. Boxplots show the median (horizontal line), first and third quartiles (lower and upper bounds, respectively), and minimum and maximum values (lower and upper whiskers, respectively). Each dot represents an independent biological replicate.

431 **DISCUSSION**

432 In this work, we characterized SGL variability across wild-type populations in three
433 *Drosophila* species, finding greater population-level variance and an overall reduced male
434 lifespan compared to females, consistent with previous studies (Yoon and Gagen 1990).
435 Sex-differences in lifespan have been associated with TE expression in some organisms such
436 as *D. melanogaster* and *D. miranda* (Brown, et al. 2020b; Nguyen, et al. 2022). Here, using a
437 copy-specific approach, we found a general age-related increase in TE copies in both males
438 and females of *D. melanogaster* and *D. suzukii*. This result suggests that there might be an age-
439 related derepression of TEs, likely due to a general loss of heterochromatin in repetitive regions
440 in aged flies, as it was previously observed in *D. melanogaster* (Brown, et al. 2020b).
441 Specifically, male flies from the same species have been shown to undergo a more rapid
442 heterochromatin mark loss producing a more pronounced derepression of TEs in older males
443 compared to older females (Brown, et al. 2020b).
444 Previous studies in a *D. melanogaster* laboratory strain showed some differences in the total
445 fraction of repetitive reads that increased during ageing between males (4.2%) and females
446 (2.6%) (Brown, et al. 2020b). Here, we could not detect statistically significant effects of sex
447 or the interaction between sex and age on the percentage of TE-derived reads, indicating that
448 age is associated with a similar increase in TE transcript levels in both males and females. In
449 this work, the difference in fraction of TE-derived reads between old and young samples was
450 1.47% in males and 1.37% in females from dmgoth63; and 0.07% in males and 0.01% in
451 females from dsgoth31. Moreover, it was previously found in a laboratory strain that 32 TE
452 families showed a significant increase in expression during ageing in males while only six were
453 found in females (Brown, et al. 2020b). Although we did not follow the same approach,
454 differential expression analysis between older males and older females did reveal more
455 upregulated specific TE copies in older males in all the species tested. The TE expression
456 analysis at the copy-level allowed to uncover sex differences that were likely diluted in family-
457 level assessments. These results suggested that, although TE expression should be seen as an
458 indirect evidence of genomic instability, male genome integrity might be more susceptible to
459 ageing effects compared to females.
460 Indeed, particularly in *D. melanogaster* and *D. simulans* species, we found that increased TE
461 copy expression in aged males compared to aged females might be associated with reduced
462 lifespan. However, we could not find a significant correlation between male lifespan or SGL
463 values and overall TE content. This paradox has been explored in long-lived species, which

464 often exhibit an overall reduction in both TE abundance and expression (Elsner, et al. 2018;
465 Ricci, et al. 2023; Sturm, et al. 2023; Teefy, et al. 2023). However, exceptions exist where TEs
466 might play a beneficial role in ageing (Sulak, et al. 2016; Zhao, et al. 2021; Sahm, et al. 2024).
467 For instance, in blind mole rats (*Spalax galili*), TE activation can act as a tumour suppressor
468 by triggering the innate immune system (Zhao, et al. 2021). In the Greenland shark (*Somniosus*
469 *microcephalus*), the longest-lived vertebrate, TEs constitute the 70.6% of the genome, the
470 highest proportion described in sharks (Sahm, et al. 2024). In this case, TE expansion may be
471 linked to the expansion of DNA repair genes, which in turn may help to tolerate higher levels
472 of TE activity (Sahm, et al. 2024). Therefore, unraveling the complex mechanisms of delayed
473 aging requires careful consideration of species-specific features, including the abundance,
474 composition, and distribution of TE in the genomes (Yushkova and Moskalev 2023).
475 Besides TE expression, it has been proposed that age might increase TE transposition, leading
476 to a greater number of somatic TE insertions that impact genomic stability and cellular
477 homeostasis, ultimately contributing to a decreased lifespan. In *D. melanogaster*, it has been
478 shown that lifespan is decreased as somatic genetic damage produced by *P element*, *mariner*,
479 and *mdg4* insertions (Nikitin and Woodruff 1995; Woodruff and Nikitin 1995; Li, et al. 2013;
480 Wood, et al. 2016; Chang and Dubnau 2019). Yet, some recent studies found that the number
481 of new TE insertions do not seem to correlate with age, suggesting that TE expression instead
482 could have a global impact in transcriptional dysfunction during ageing and affecting lifespan
483 (Treiber and Waddell 2017; Rigal, et al. 2022; Yang, et al. 2022; Pabis, et al. 2024; Tsai, et al.
484 2024). Here, we observed that ageing increases the generation of TE-chimeric transcripts in
485 some samples, and specially in *D. suzukii* males. Thus, our findings suggest that age-related
486 TE expression could influence transcript structure by increasing the presence of TE-chimeric
487 transcripts in *D. suzukii* males*,* hinting that the impact on fly lifespan may not solely be
488 attributed to TE somatic transposition.
489 Our results also revealed that, in *D. melanogaster,* we can manipulate SGL values. The
490 replacement of the Y chromosome between strains exhibiting differences in SGL values and
491 male lifespan was associated with a progressively decline in male survival, leading to a more
492 pronounced SGL. Further analysis will be needed to determine whether Y chromosome size
493 differences (associated with a different Y-linked TE abundance) or differences in TE
494 distribution are responsible for the observed effects. In fact, two recent works indicate that male
495 survival is not linked to Y chromosome size in both *D. melanogaster* and *D. pseudoobscura*
496 (Nguyen, et al. 2022; Delanoue, et al. 2023). Hence, based on our introgression experiments,
497 we hypothesize that differences in TE composition among Y chromosomes (proportion of full-

length copies or average enrichment in repressive histone marks) may offer a more plausible explanation for variances in male lifespan compared to Y chromosome sizes or TE abundance (Lemos, et al. 2010; Griffin, et al. 2015). However, it is also conceivable that other genomic regions other than TEs on the Y chromosome are contributing to reduced lifespan, as well as mitochondrial genome – Y chromosome interactions (Ågren, et al. 2020). Thus, while TEs are strong candidates, we cannot conclude beyond the fact that some factor on the Y chromosome is linked to reduced lifespan.

In summary, this work reveals that SGL is a variable trait in the wild, and emphasizes the importance of studying the role of TEs in the evolution of sex differences in lifespan across different species.

510 **METHODS**

511 **Fly strains and rearing**

512 Samples of wild-type populations of *D. melanogaster*, *D. simulans,* and *D. suzukii* were
513 collected, and different isofemale strains were established for each species and geographical
514 origin directly from gravid females from the field. A comprehensive list of all populations
515 along with collection information is available in Supplementary Table 1. *D. melanogaster* and
516 *D. simulans* fly strains were reared in vials with a nutritive "LM" medium containing agar,
517 maize flour, yeast, Nipagine, ethanol and water, while *D. suzukii* strains were reared in vials
518 with "Dalton" medium containing agar, cornmeal, yeast, sugar, Nipagine, ethanol and water.
519 Fly strains were kept in a 12:12 hour light/dark cycle at 25 °C.

520

521 **Longevity assays**

522 *Longevity assays for the analysis of SGL natural variation in different Drosophila species*

523 50 recently (less than 8 hours) emerged males and 50 virgin females from each isofemale strain
524 were placed in groups of 10 in tubes containing nutritive medium ("LM" for *D. melanogaster*
525 and *D. simulans* and "Dalton" for *D. suzukii*). Each tube was considered as an independent
526 biological replicate thus having five replicates per sex and strain. Flies were separated by sex
527 without anaesthesia using an aspirator. Dead flies in every vial were counted every day, except
528 for weekends, until 80% of the flies in the tube were dead. Alive flies were placed in new tubes
529 with fresh medium every week. Longevity assays were performed at 25ºC and at constant
530 humidity of 60%.

531 *Longevity assays in the inbred strains included in the transcriptome analysis*

532 For *D. melanogaster* strains, 150 recently (less than 8 hours) emerged males and 150 virgin
533 females from each strain were placed in groups of 10 in tubes containing "LM" medium. Each
534 tube was considered as an independent biological replicate thus having 15 replicates per sex
535 and strain. For *D. simulans* and *D. suzukii* strains, 50 recently (less than 8 hours) emerged males
536 and 50 virgin females from each strain were placed in groups of 10 in tubes containing nutritive
537 medium ("LM" for *D. simulans* and "Dalton" for *D. suzukii*). Each tube was considered as an
538 independent biological replicate thus having five replicates per sex and strain. Dead flies in
539 every vial were counted every day, except for weekends, until 80% of the flies in the tube were
540 dead. Alive flies were placed in new tubes with fresh medium every week. Longevity assays
541 were performed at 25ºC and at constant humidity of 60%.

542 *Longevity assays in the Y introgressed strains*

543 150 recently (less than 8 hours) emerged males and 150 virgin females from each
544 strain/generation of introgression were placed in groups of 10 in tubes containing "LM"
545 medium. Again, each tube was considered as an independent biological replicate thus having
546 15 replicates per sex and strain/generation of introgression. Flies were separated by sex without
547 anaesthesia using an aspirator. Dead flies were counted every day, except for weekends, until
548 all the flies in the tubes were dead. Alive flies were placed in new tubes with fresh medium
549 every week. Longevity assays were performed at 25ºC and at constant humidity of 60%.
550
551 **SGL values calculation**
552 We estimated sex-specific median adult lifespan (in days) for each strain/generation of
553 introgression in our dataset (Supplementary Tables 2, 6, and 7). The median adult lifespan
554 corresponds to the time when 50% of the individuals in a particular strain are dead. To estimate
555 the confidence interval for both males and females of each strain, we implemented a
556 bootstrapping procedure. We performed 100,000 bootstrap iterations to calculate the median
557 adult lifespan for each sample and then we calculated the logarithm ratio (ln, base e) of median
558 longevity between males and females (SGL values) (Lemaître, Ronget, Tidière, et al. 2020).
559 Confidence intervals for the difference in median longevity between males and females was
560 computed using quantiles of the bootstrap of the logarithm ratios.
561 For the analysis of SGL natural variation, we used a Bayesian approach implemented in the
562 MCMCglmm package in R to partition the variance and identify the contribution of species,
563 population, and sampling variation to the observed differences in SGL. We fitted a hierarchical
564 regression model including species as a fixed effect and population as a random effect, with
565 SE weighting for the longevity measurements. The priors were set as follows: for the residual
566 variance, we used an inverse-Wishart prior with nu = 0.002; for the population random effect,
567 we used an inverse-Wishart prior with nu = 1 and a large variance (a = 1000) for the prior mean
568 (diff_median_longevity~species+(1|population)+idh(SE):units).
569 We also performed survival analysis using the Kaplan-Meier method and Cox proportional
570 hazard models. The survival analysis was conducted separately for male and female flies within
571 each strain/generation of introgression. We used the *survfit* function in R to estimate the
572 survival probabilities over time based on the event status (dead or alive). Additionally, we
573 generated survival curves using the *ggsurvplot* function from the *survminer* R package. These
574 curves represent the estimated survival probabilities over time using a Kaplan-Meier
575 estimation. Finally, we conducted pairwise comparisons to evaluate the significance of
576 difference in survival distributions among various samples. This analysis was performed using

577 the *pairwise_survdiff* function, applying the Benjamini-Hochberg correction method for
578 multiple comparisons.
579
580 **DNA extraction, nanopore sequencing and genome assemblies**
581 Genome assemblies for *D. melanogaster* dmgoth101 and dmgoth63 and for *D. simulans* strains
582 dsgoth31 and dsgoth613 were obtained from Mohamed et al., 2020 (Mohamed, et al. 2020).
583 To obtain genome assemblies of *D. suzukii*, we sequenced with Oxford Nanopore Technologies
584 (ONT) two inbred strains: S29 and MT47. DNA was extracted using phenol chloroform from
585 a pool of 10 adult females. The quantity and quality of genomic DNA were assessed using a
586 NanoDrop™ One UV-Vis spectrophotometer (Thermo Fisher Scientific) and a Qubit® 1.0
587 Fluorometer (Invitrogen). Three micrograms of DNA were then repaired with the NEBNext
588 FFPE DNA Repair Mix (NEB M6630). End repair and dA-tailing were carried out using the
589 NEBNext End Repair/dA-Tailing Module (E7546, NEB). Ligation was evaluated with the
590 Ligation Sequencing Kit 1D. MinION sequencing was conducted according to the
591 manufacturer's instructions using R9.4.1 flow cells (FLO-MIN106, ONT) and a Nanopore
592 MinION Mk1b sequencer (ONT), operated by ONT MinKNOW (v.18.3.1). Following
593 sequencing, base calling was performed using *Guppy* (v.4.0.5) in high-accuracy mode.
594 Quality control of Nanopore reads was conducted using *NanoPlot* (v.1.10.2)
595 (https://github.com/wdecoster/NanoPlot). Reads with a quality score below 7 were excluded
596 from further analysis. Contig assembly was performed with *Flye* (v.2.8) (Heng Li 2013) using
597 default parameters, except for the –plasmids option. All contigs were aligned with *minimap2*
598 (v2.16) (Li 2018) using the –x map-ont option, followed by four rounds of polishing with
599 *RACON* (v1.3.2) (Vaser, et al. 2017) under default settings. Assembly quality metrics were
600 evaluated using *Assembly-Stats* (v1.0.1) (https://github.com/sanger-pathogens/assembly-stats).
601 Any assembly inconsistencies were manually reviewed with *D-genies* (v1.2.0) (Cabanettes and
602 Klopp 2018) and corrected using *samtools* (v1.9.0) with the *faidx* function, and *Gepard* (v1.4.0)
603 was used to identify breaking points. Super scaffolding of all corrected assemblies was carried
604 out with *RaGOO* (v.1.1), using the –s and –t 4 parameters, based on the reference genome
605 assembly (Paris, et al. 2020). The resulting chromosome-scale assemblies were then analyzed
606 using Benchmarking Universal Single-Copy Orthologs (BUSCO) (v.5.4.3) (Simão, et al. 2015)
607 with the diptera_odb10 lineage (-l parameter). Gene annotation for the Nanopore genomes was
608 completed with *Liftoff* (v.1.6.3), referencing the WT3_2.0 *D. suzukii* genome (Paris, et al.
609 2020).
610

611 **RNA-seq extraction and sequencing**

612 RNA was extracted from six inbred strains, comprising two strains for species: *D.*
613 *melanogaster* (dmgoth101 and dmgoth63), *D. simulans* (dsgoth31 and dsgoth613) and *D.*
614 *suzukii* (MT47 and S29). For young samples, male and female flies of each strain were
615 separately placed in several tubes with nutritive medium in groups of 20-25. When flies were
616 5 day-old, 30 flies per sex were dissected and ovaries and testes were removed from female
617 and male flies, respectively, and only carcasses were kept. For old samples, male and female
618 flies of each strain were also separately placed in several tubes with nutritive medium in groups
619 of 20-25. 30 flies per sex were dissected and ovaries and testes were removed from female and
620 male flies, respectively, and only carcasses were kept when at least 25% of the flies in the tube
621 were dead. Consequently, older samples may exhibit varying chronological ages across strains.
622 Three replicates per strain, age and sex were performed. Then, for both young and old samples
623 RNA was extracted from the obtained carcasses. For the Y chromosome replacement
624 experiments, RNA was extracted from both F1 and F10 male and female flies from the two
625 reciprocal crosses. Samples were obtained as mentioned before when flies were 5 days-old.
626 RNA extraction was carried out using the RNeasy Mini kit (Qiagen) following manufacturer's
627 instructions and then a DNase treatment (Invitrogen) was performed. mRNA libraries were
628 prepared with the Illumina TruSeq RNA Sample Prep Kit, following the manufacturer's
629 guidelines. Libraries were sequenced on the Illumina HiSeq 4000 platform with paired-end 100
630 nt reads. The sequence raw files have been deposited in NCBI SRA under the BioProject
631 accession number PRJNA1159534.

632

633 **RNA-seq mapping and gene quantification**

634 Quality of the *fastq* sequencing files was assessed with FastQC (v.0.11.9). Then, *fastp*
635 (v.0.23.4) was used to filter out bad reads, adapter removal and trimming (Chen 2023).
636 Trimmed reads from *D. melanogaster*, *D. simulans,* and *D. suzukii* were then mapped against
637 the Flybase *D. melanogaster* (r6.16) (Öztürk-Çolak, et al. 2024), the *D. simulans* (r2.02)
638 (Öztürk-Çolak, et al. 2024), and *D. suzukii* WT3_2.0 (Paris, et al. 2020) reference genomes,
639 respectively with *STAR* (v.2.7.11a) (Dobin, et al. 2013) using default parameters. On average,
640 92.7% of the reads were uniquely mapped to the corresponding genomes. To quantify gene
641 expression, we used *htseq* (v.2.0.3) (Anders, et al. 2015), with parameter *-t exon*, which uses
642 the sum of the reads aligned in all exons to assign counts for each gene.

643

644 **TE annotation**

645 All the six strains used in the transcriptomic analysis (dmgoth101, dmgoth63, dsgoth31,
646 dsgoth613, MT47, and S29) have been sequenced with long-read sequencing technologies and
647 genome assemblies were available or generated (Mohamed, et al. 2020; Mérel, et al. 2021). TE
648 insertions were identified in the genome assemblies using *RepeatMasker* (v.4.1.2), which
649 masks the genome where there are fragments homologous to consensus TE sequences.
650 Parameters used were: *-cutoff 250, -a, -s, -norna*. Consensus TE sequences used for *D.*
651 *melanogaster* was obtained by using the parameter *-species drosophila.* However, consensus
652 sequences for *D. simulans* were obtained from https://github.com/bergmanlab/drosophila-
653 transposons/, while for *D. suzukii* from https://github.com/vmerel/DSu-TE/tree/main/TE_db.
654 Finally, *bed* files containing the coordinates of each annotated TE were created for every
655 genome.
656
657 **Differential expression analysis of genes**
658 Differential expression analyses of genes were conducted using the R package *DESeq2*
659 (v.1.40.2) (Love, et al. 2014). Raw read counts were merged into a unified matrix for each
660 strain and subsequently normalized using default parameters (Supplementary Tables 3 and 4).
661 Differential expression of genes was assessed between old and young samples in each strain
662 separately. The log2 fold-change was adjusted using the *apeglm* (v.1.22.1) algorithm from the
663 *lfcshrink* function to minimize noise and preserve significant differences (Zhu, et al. 2019).
664 Genes were considered as significantly differentially expressed when the adjusted p-value was
665 < 0.05 and the log2 fold-change $> |1|$.
666
667 **Age-related genes found differentially expressed in old flies**
668 Genes previously associated with ageing in *D. melanogaster* were obtained from the Animal
669 Ageing and Longevity Database (AnAge)
670 (https://genomics.senescence.info/genes/search.php?search.php?show=1&sort=1&page=1&o
671 rganism=Drosophila%20melanogaster). From this list, only genes with a described "increased"
672 lifespan effect were selected. Then, gene symbols were converted to FlyBase IDs with the
673 *Batch Download* online tool (https://flybase.org/batchdownload). We finally looked for
674 overlap of the age-associated genes and the DEGs found in the young versus old samples
675 comparisons. For *D. simulans*, we used the gene ortholog table available in FlyBase to look for
676 correspondences in gene IDs. We could analyze a total of 149, 152, and 120 age-associated
677 genes in *D. melanogaster, D. simulans*, and *D. suzukii*, respectively.
678

679 **Quantification and differential expression analysis for TE families**
680 TE quantification at a family level was performed with *TEtools*, using the TEcount module
681 (Lerat, et al. 2017). One of the inputs of the TEcount module is the *rosette* file, a tabular file
682 containing the names of each TE copy in the genome that easily groups them in TE families
683 (or other criteria if needed) (Lerat, et al. 2017). To do that, for each strain, we first build a *fasta*
684 file with all the TE sequences annotated using *bedtools getfasta* and the previously generated
685 *bed* file with TE annotations. Then, we generated the *rosette* file writing in the first column the
686 name of the specific TE copy and in the second column the TE family in which it belongs. For
687 the TE quantification of the introgressed F1 and F10 generations, a merged *rosette* file between
688 the strains dmgoth101 and dmsj7 was used. Differential expression analysis of TE families was
689 conducted using the R package *DESeq2* (v.1.40.2) as mentioned before, and merging in a single
690 matrix gene and TE family counts (Love, et al. 2014) (Supplementary tables 3 and 4). TE copies
691 were considered as significantly differentially expressed when the adjusted p-value was < 0.05
692 and the | log2 fold-change | > 1.
693
694 **RNA-seq mapping, quantification and differential expression analysis for specific TE**
695 **copies**
696 Quantification of TE copies was performed using *Telescope* (Bendall, et al. 2019). Briefly, for
697 each strain, trimmed paired-end reads were aligned to the corresponding genome with *bowtie2*
698 (v.2.5.1) using the parameter *-k 100*. Then, again for each strain, we generated a *gtf* file
699 containing all the TE copies present in the strain following the format specified by *Telescope*.
700 After that, the generated *sam* file was converted to *bam* with *samtools view* (v.1.17). Finally,
701 for the locus-specific quantification of TEs, the function *telescope assign* was used taking the
702 already generated *gtf* and *bam* files as inputs. Differential expression analysis of TE copies was
703 conducted using the R package *DESeq2* (v.1.40.2) as mentioned before, and merging in a single
704 matrix gene and copy-specific TE counts (Love, et al. 2014) (Supplementary tables 3 and 4).
705 TE copies were considered as significantly differentially expressed when the adjusted p-value
706 was < 0.05 and the | log2 fold-change | > 1.
707
708 **Identification of full-length insertions**
709 Identification of full-length insertion among the upregulated TE copies found between old
710 males and females (adjusted p-value was < 0.05 and | log2 fold-change | > 1) was performed
711 with a generated *python* script that detects as full-length copies the ones that are at least 80%
712 of the length of the consensus sequence and with a Kimura divergence value < 20 (values

713 obtained from the previously generated *RepeatMasker* output files). For *D. melanogaster* and
714 *D. simulans*, TE consensus sequences were obtained from
715 https://github.com/bergmanlab/drosophila-transposons/, while *D. suzukii* consensus sequences
716 were obtained from Mérel et al., 2021 (Mérel, et al. 2021).
717
718 **Permutation tests**
719 To test whether DETEs found in old male flies were enriched nearby DEGs (+-1kb), we first
720 created *bed* files with the +-1kb flanking regions for these genes in each specific genome. *bed*
721 files with the coordinates of all TE copies were also generated for each strain. Then, we
722 performed permutation tests by intersecting TE positions with gene regions (+-1kb) using the
723 R package *regioneR* (Gel, et al. 2016), parameter *randomization* = *resampleRegions*, with 1000
724 random samples. Hence, for every up and downregulated gene set of size *n*, the software
725 determines the count of those containing TEs in the +-1kb flanking regions. Subsequently, it
726 conducts 1000 random samples of n genes each to assess if the observed number of genes with
727 TEs deviated from the expected count in a genome-wide context (Supplementary Table 5).
728
729 **Identification of chimeric transcripts**
730 Gene-TE chimeric transcripts were detected with *ChimeraTE* Mode 2 for each strain, sex, and
731 age separately (Oliveira, et al. 2023). We used Mode 2 (chimTE_mode2.py), which identifies
732 transcripts without a reference genome, using the prediction of chimeras from fixed and
733 polymorphic TEs because we focused on capturing all the possible new age-related generated
734 chimeric transcripts. Briefly, stranded RNA-seq reads were mapped against its respective
735 annotation of transcripts and TE insertions. We used the transcript annotation from the release
736 6.54 *dmel-all-transcript-r6.54.fasta* for *D. melanogaster*
737 (https://ftp.flybase.net/genomes/Drosophila_melanogaster/dmel_r6.54_FB2023_05/fasta/dme
738 l-all-transcript-r6.54.fasta.gz); from the release 2.02 *dsim-all-transcript-r2.02.fasta* for *D.*
739 *simulans*
740 (https://ftp.flybase.net/genomes/Drosophila_simulans/dsim_r2.02_FB2017_04/fasta/dsim-all-
741 transcript-r2.02.fasta.gz), and the *dsuz-all-transcripts-LBDM_Dsuz_2.1.fasta* file for *D.*
742 *suzukii*(Paris, et al. 2020). Reference TE file for *D. melanogaster* and *D. simulans* was available
743 online (https://github.com/bergmanlab/drosophila-transposons/tree/master/current), while
744 reference TE file for *D. suzukii* was obtained online (https://github.com/vmerel/DSu-
745 TE/tree/main/TE_db). We used the parameters *–strand rf-stranded* and *–assembly* to search for
746 chimeric transcripts in the *de novo* transcript assembly. For each strain, sex and age, we got

747 three output files: list of chimeric transcripts supported only by paired-end reads that have
748 mapped in both transcripts and TE sequences, list of chimeric transcripts supported only by the
749 transcriptome assembly, and chimeric transcripts supported by both mapped paired-end reads
750 and transcriptome assembly. These three lists were merged and just the name of the gene and
751 the TE family were kept to easily analyze the number of chimeric transcripts in each sample
752 (Supplementary Table 8).
753
754 **Y chromosome replacement crosses**
755 To check whether the Y chromosome is associated with changes in SGL, we performed
756 introgression experiments to replace the Y chromosome between strains with different SGL
757 values. The lack of recombination in *Drosophila* males enabled the generation of flies that
758 differ only in the Y chromosome. Reciprocal crosses between the *D. melanogaster* strains
759 dmgoth101 and dmsj7 were performed. We assigned the name "A crosses" to crosses initiated
760 by mating a dmgoth101 female with a dmsj7 male, followed by successive backcrossing of
761 male offspring with dmgoth101 females over ten generations. Conversely, "B crosses"
762 represented the reciprocal pairing wherein dmsj7 females were initially mated with dmgoth101
763 males, followed by analogous backcrossing of male offspring with dmsj7 females. Five virgin
764 females and five males aged 4-5 days old were placed in a tube containing a nutritive medium,
765 with five replicates performed for each reciprocal cross. Every 2-3 days, the flies were
766 transferred to new tubes with fresh medium until four tubes were obtained for each reciprocal
767 cross. This ensured enough offspring to carry out longevity assays and facilitated the
768 continuation of introgression crosses. F1 male flies were subsequently crossed with the
769 maternal strain, and the male offspring of each generation was consistently crossed with the
770 maternal strain for 10 generations. In each generation (except for generations F6-F9), longevity
771 assays were carried out as previously described with 10 virgin females and 10 males from each
772 of the five replicated reciprocal crosses (n=150).
773
774 **TE transcript quantification in introgression crosses**
775 TE transcript quantification was performed with TEtools, using the TEcount module (Lerat, et
776 al. 2017). One of the inputs of the TEcount module is the *rosette* file, a tabular file containing
777 the names of each TE copy in the genome that easily groups them in TE families (or other
778 criteria if needed) (Lerat, et al. 2017). To do that, for each strain (dmgoth101 and dmgoth63),
779 we first build a *fasta* file with all the TE sequences annotated using *bedtools getfasta* and the
780 previously generated *bed* file with TE annotations. Then, we generated a *rosette* file per strain

781 writing in the first column the name of the specific TE copy and in the second column the TE
782 family in which it belongs. After that, we merged the two *rosette* files. A GLM analysis was
783 performed to assess the effects of the reciprocal cross, generation, sex, and the interaction
784 between generation and sex on the percentage of TE-derived reads in R (glm(alignment_rate ~
785 cross + generation + sex + generation:sex, family = gaussian(link = "identity"). The model
786 showed a good fit (residual deviance = 0.1188, AIC = -47.315).
787
788

DATA ACCESS

The sequencing data generated in this study has been submitted to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA1159534.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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