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

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SUMMARY

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 of the possible causes underlying this phenomenon could be related to the high content of transposable elements (TE) in the Y chromosome (toxic Y hypothesis). TE activity is normally repressed by epigenetic mechanisms, but this regulation weakens with age. Since the Y chromosome is rich in TEs, age-related TE activity should be more pronounced in old males than in old females, likely affecting longevity patterns. In this work, we studied the natural variation in SGL in wild-type populations of three different Drosophila species that vary in their TE content: *Drosophila melanogaster*, *Drosophila simulans*, and *Drosophila suzukii*. Transcriptomic data revealed increased copy-specific TE expression in *D. melanogaster* and *D. suzukii* older flies. Moreover, we observed a higher number of upregulated TEs in old males compared to old 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 contributes to a better understanding of the genomic differences that lead to variation in longevity patterns between sexes in several species, and emphasizes the importance of studying the role of TEs in male longevity.

KEYWORDS

Transposable elements, longevity, Drosophila, chimeric transcripts, Y chromosome.

INTRODUCTION

In most animal species, males and females display distinct mortality patterns leading to differences in their longevity[1,2]. 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[1,3,4]. 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 course hypothesis) have been extensively studied[1,3,5-9]. Sex chromosomes have also been proposed to play an important role in SGL since the heterogametic sex generally has the shortest lifespan[1,10-17]. 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[18,19]. 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[13,15,16,20]. Y (or W) chromosomes often display reduced rates of recombination, resulting in the accumulation of repeated sequences (mainly TEs) on these chromosomes[21].

TEs are genomic fragments with the ability to move along the genome, being major components of eukaryotic genomes[22]. Due to their mobile and repetitive nature, they are considered a source of mutations and genetic polymorphisms[22]. As such, TE activity is normally repressed by epigenetic regulation that involves DNA methylation, histone modifications and/or small RNAs[23,24]. However, with age, these silencing mechanisms are disrupted causing a general TE activity[25-27]. 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.

Age-dependent activation of TEs has been suggested to negatively impact lifespan in Drosophila[13,15,25,28-36], termites[37,38], nematodes[39], mice[40,41], and humans[42-45.] 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[13]. 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[46,47]. Moreover, putatively Y-linked TEs disproportionately

81 lose heterochromatin enrichment as individuals age, suggesting that male-specific TEs are
82 particularly prone to de-repression with age[13]. In addition, longevity assays performed in flies
83 with aberrant sex chromosome configurations (XO, XXY, and XYY) showed that the number
84 of Y chromosomes could influence organismal survival[13].
85 In other studies, these results have been challenged. For instance, the generation of *D.*
86 *melanogaster* Y chromosomes with different sizes using CRISPR/Cas9 suggested that the size
87 of this chromosome, and thus, the amount of heterochromatin (and TEs), did not contribute to
88 sex-specific differences in longevity[48]. In *D. pseudoobscura*, variations in Y chromosome size
89 and Y-linked TEs showed that larger Y chromosomes lead to slightly increased TE expression,
90 especially in old males, but these differences do not result in faster ageing[49]. Therefore, the role
91 of the Y chromosome in reducing male longevity through an increased TE expression is still
92 under debate.
93 In this work, we studied the SGL natural variation across three Drosophila species: *D.*
94 *melanogaster*, *D. simulans*, and *D. suzukii*. Additionally, we analysed copy-level TE
95 expression changes associated with longevity in the same species, with particular emphasis on
96 sex-specific differences. Finally, we replaced the Y chromosome among *D. melanogaster*
97 strains to investigate the impact of this chromosome on SGL.
98

RESULTS

SGL varies between populations and species

To study the natural variability 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 (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[50] (see Methods) (Supplementary Table 2).

In all species, as expected, we found that the vast majority of strains (82%) have negative SGL values meaning that males have reduced longevity (Supplementary Fig. 1). The average difference of lifespan was 10.5, 12.5 and 9 days in *D. melanogaster*, *D. simulans*, and *D. suzukii*, respectively. We analyzed the variance in the SGL variable across different hierarchical levels (population and species) using a Bayesian approach (see Methods). The analysis revealed that population contributed significantly to the observed differences in SGL (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. 1A 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. 1A and Supplementary 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. 1A and Supplementary 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. 1B and Supplementary Fig. 1).

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

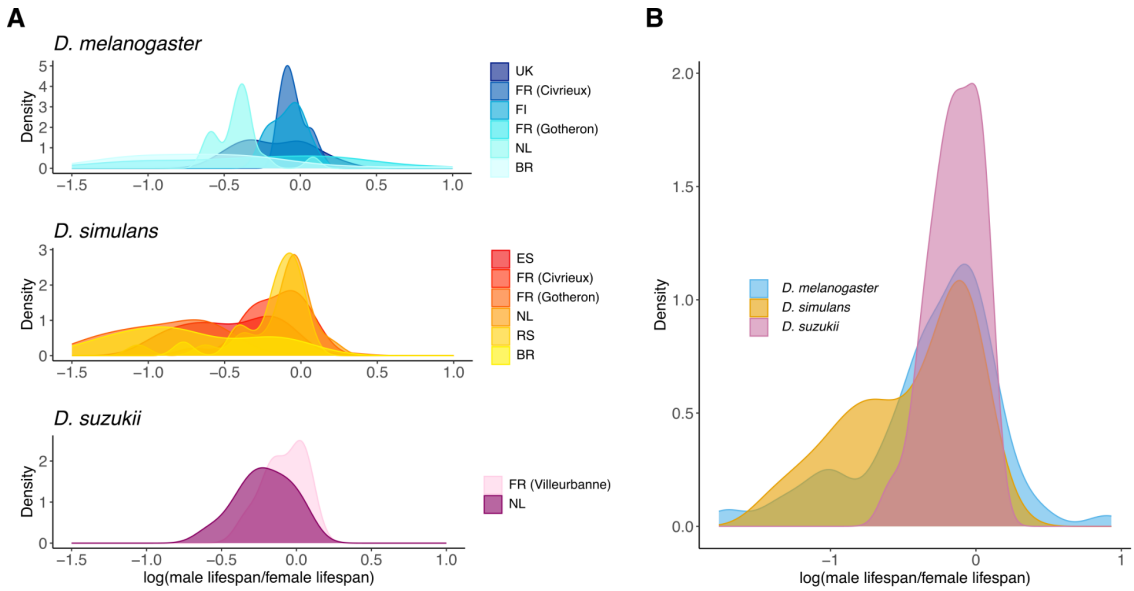


Figure 1. **Natural variability in SGL in different Drosophila species.** Histograms displaying the distribution of SGL values calculated as the logarithm of the ratio between male and female median lifespan. **A**) Histogram displaying the distribution of SGL values per population in *D. melanogaster*, *D. simulans*, and *D. suzukii*. UK: United Kingdom, FR: France, FI: Finland, NL: the Netherlands, BR: Brazil, ES: Spain, RS: Serbia. **B**) Histogram displaying the distribution of SGL values per species.

Older *D. melanogaster* and *D. suzukii* flies have a higher abundance of expressed TE copies

To study TE expression changes associated with longevity, we used three Drosophila species with varying TE content: *D. melanogaster* (20%), *D. simulans* (12%), and *D. suzukii* (47%)[51,52]. RNA-seq was performed on young and old individuals from two inbred strains per species: dmgoth101 and dmgoth63 for *D. melanogaster*, dsgoth31 and dsgoth613 for *D. simulans*, and MT47 and S29 for *D. suzukii*[53] (see Methods) (Supplementary Table 1, 5-7 and Supplementary Fig. 2). A substantial proportion of genes associated with ageing were differentially expressed in the older flies, indicating that the age at which we extracted the RNA was sufficient to classify them as old (see Methods) (Supplementary Fig. 3 and 4).

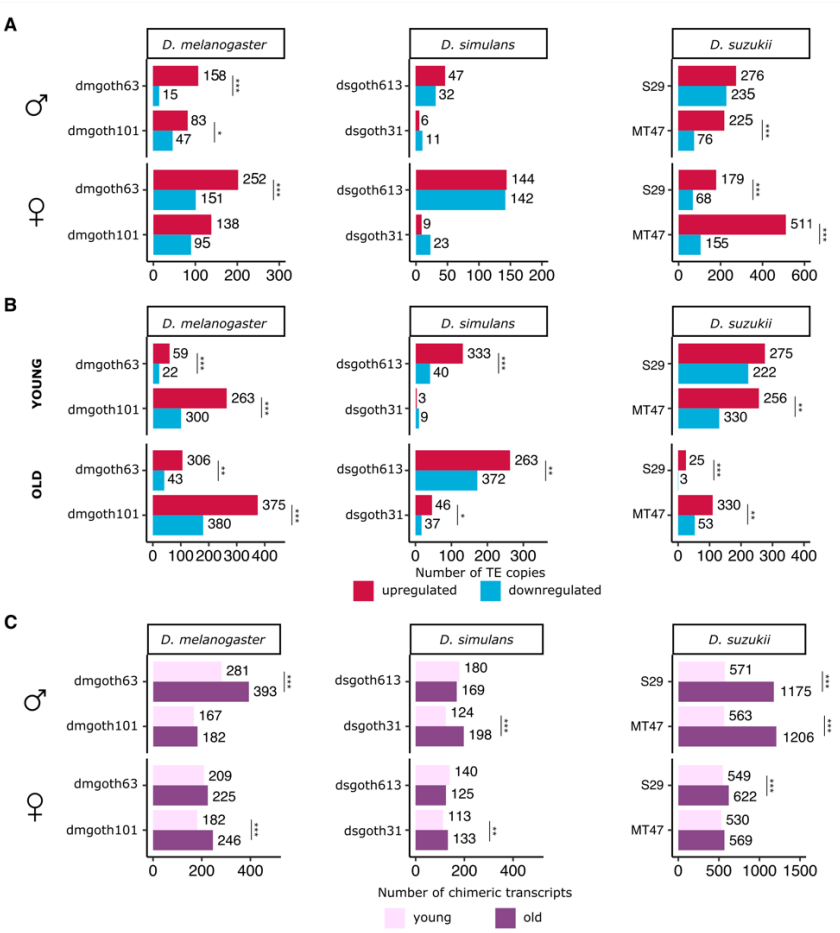
We then used Telescope to precisely measure TE expression at specific genomic locations and assess the impact of age and sex on individual TE copies (see Methods)[54] (Supplementary Tables 9-14). This approach allowed us to measure TE transcript levels without differentiating between TE activation and release of control, thus not measuring transposition rate. These distinctions will not be addressed further in the text.

On the one hand, we found a general trend towards more upregulated than downregulated TE copies with age in both males and females of *D. melanogaster* and *D. suzukii* (Fig. 2A). These results were statistically significant (chi-square, p values < 0.05), except for the *D. melanogaster* females of the dmgoth101 strain and the *D. suzukii* males of the S29 strain (chi-square, p values > 0.05) (Fig. 2A and Supplementary Fig. 5). These findings suggest that TEs

152 can be expressed during ageing as it was observed in other studies from *D. melanogaster* and
153 other species, such as humans[13,15,28-36,42-45].
154 On the other hand, in *D. simulans*, the number of upregulated TE copies did not statistically
155 differ from the number of downregulated ones, likely due to its lower TE content in the genome
156 (Fig. 2A). Moreover, as expected based on the TE content, *D. suzukii* showed an increased
157 number of differentially expressed TE copies (DETEs) in both males and females compared to
158 the other species (Fig. 2A and Supplementary Fig. 5).
159 We also tested whether male and female flies from the same strain shared some of the DETEs.
160 Indeed, we found that some of the upregulated DETEs in males were shared with females
161 (Supplementary Fig. 6). These shared DETEs are thus not present in the Y chromosome,
162 meaning that a proportion of the activated TEs might be not Y specific, at least for some strains.
163 Overall, we observed an increased number of upregulated TE copies in older flies of *D.*
164 *melanogaster* and *D. suzukii*. These findings are in line with the fact that ageing might be
165 associated with the disruption of the epigenetic mechanisms that silence TEs favouring their
166 activation.
167
168 **More TE copies are expressed in old males compared to old females**
169 According to the toxic Y hypothesis, the abundance of TEs on the Y chromosome may lead to
170 higher TE expression in ageing males when compared to ageing females. As expected, we
171 detected more upregulated TE copies than downregulated ones in old male flies compared to
172 old female flies in all the strains of the three species tested (chi-square, p values < 0.05) (Fig.
173 2B and Supplementary Fig. 7). These differences are already observed at young ages (chi-
174 square, p values < 0.05), although it is not the case for the *D. simulans* dsgoth31 and the *D.*
175 *suzukii* S29 strains (chi-square, $p = 0.399$ and $p = 0.105$, respectively) (Fig. 2B). In contrast to
176 the other two species, *D. suzukii* showed a greater number of DETEs between sexes in young
177 flies than in old flies (Fig. 2B).
178 We also found that the upregulated TE insertions observed between old males and females are
179 present in all chromosome arms, with an expected concentration in chromosome centromeres
180 and in the dot chromosome (Supplementary Fig. 8). In all strains, full-length TE copies, which
181 are more likely to transpose, were significantly overrepresented among upregulated TEs
182 compared to their overall genome proportion (chi-square, $p < 0.001$). This suggests that age-
183 dependent TE expression in males may lead to new insertions; however further experiments
184 are needed to confirm this.

185 We finally performed a permutation test to check whether DETEs found in old male flies are
186 enriched nearby (+-1kb) differentially expressed genes (DEGs) (see Methods). Overall, we
187 found no differences (p-values > 0.05) or statistically significant depletion (p-values < 0.05) in
188 both upregulated and downregulated TE insertions nearby genes (Supplementary Table S15).
189 In summary, using a copy-specific approach, comparisons between old male and old female
190 flies revealed an increased number of expressed TE copies than repressed.
191
192 **Ageing increases the number of TE chimeric transcripts in *D. suzukii* males**
193 TEs can influence nearby genes by altering their expression and structure, potentially
194 generating chimeric transcripts with both gene and TE sequences[55-57]. To investigate if age-
195 dependent TE activation is linked to increased chimeric transcripts, we used the ChimeraTE
196 pipeline to quantify gene-TE chimeras in RNA-seq data from young and old flies of *D.*
197 *melanogaster*, *D. simulans*, and *D. suzukii*[56]. We define the number of gene-TE chimeric
198 transcripts as the count of distinct chimeric transcripts (isoforms) found in the dataset, rather
199 than the number of reads mapping to these chimeric transcripts.
200 Older flies showed a significant increase in chimeric transcripts compared to younger flies in
201 some samples (chi-square, p values < 0.001): dmgoth63 males, dmgoth101 females, dsgoth31
202 males and females, S29 males and females, and MT47 males (Fig. 2C). Remarkably, in *D.*
203 *suzukii*, males exhibited over twice as many chimeric transcripts in older flies compared to
204 younger ones (Fig. 2C). Moreover, within each strain, there is only a small proportion of shared
205 chimeric transcripts between different sexes or age groups (Supplementary Fig. 9).
206 In all species, there was a moderate positive correlation between chimeric transcript generation
207 and TE family copy number (Pearson correlation p values < 0.05) (Supplementary Fig. 10). In
208 *D. melanogaster*, the TE families *roo*, *INE-1*, and *copia* were most frequently associated with
209 chimeric transcripts, with *roo* and INE-1 being abundant in both young and old flies, and *copia*
210 showing increased chimeric transcript formation in older flies[56,57] (Supplementary Fig. 10 and
211 11). In *D. simulans*, the most prevalent families producing chimeric transcripts were *roo*, *INE-*
212 *1*, *P-elements*, and *GATE* elements, whereas in *D. suzukii*, it was predominantly *Jockey-3* and
213 *TART* elements (Supplementary Fig. 12 and 13).
214 Finally, we quantified the proportion of genes producing chimeric transcripts in old flies that
215 were found differentially expressed. In *D. melanogaster* males, an average of 4.34% of
216 chimeric genes showed age-related expression changes, rising to 13.94% in females. Similarly,
217 in *D. simulans*, we found an average of 2.10% and 12.45% in males and females, respectively.
218 In contrast, minimal discrepancies between sexes were observed in *D. suzukii*, with males and

219 females displaying average proportions of 7.62% and 5.64%, respectively. These proportions
220 were higher than expected in male samples (chi-square, p values < 0.01) except for the *D.*
221 *melanogaster* dmgoth101 (chi-square, p = 0.052), and the *D. simulans* dmgoth31 strains (chi-
222 square, p = 0.132). In females, only *D. melanogaster* strains had an enrichment of DEGs among
223 the ones generating chimeric transcripts (chi-square, p = 0.009 and p < 0.001 for dmgoth63 and
224 dmgoth101, respectively).
225 Overall, we observed an increase in chimeric transcripts with age in some samples, particularly
226 in *D. suzukii* males. While these changes may relate to age-dependent TE activation, they could
227 also result from increased gene expression or reduced splicing efficiency with age[33,58].
228



229
230
231 **Figure 2**. **Ageing increases the number of activated TE copies in different Drosophila species. A**) Barplots showing the
232 number of upregulated (in red) and downregulated (in blue) TE copies with ageing for both males (upper panel) and females
233 (bottom panel) in each strain. **B**) Barplots showing the number of upregulated (in red) and downregulated (in blue) TE copies
234 in young males compared to young females (upper panel) and in old males compared to old females (bottom panel) in each
235 strain. **C**) Ageing increases the generation of chimeric transcripts in some samples. Barplots showing the number of identified
236 chimeric transcripts per strain as a sum of chimeric transcripts supported only by paired-end reads that have mapped in both
237 transcripts and TE sequences ("chimreads_evidence"), chimeric transcripts supported only by the transcriptome assembly
238 ("transcriptome_evidence"), and chimeric transcripts for which both previous methods have predicted the same chimeric
239 transcripts ("double_evidence"). Asterisks indicate level of statistical significance of chi-square tests (* $p \leq 0.05$, ** $p \leq 0.01$,
240 ***$p \leq 0.001$).
241

Increased TE expression in aged males is 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.

As expected, males showed reduced lifespan in *D. melanogaster* and *D. simulans* (Log Rank p values <0.001), with negative SGL values (Fig. 3A-D, and Supplementary Table 3). 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. 3E and 3F and Supplementary Table 3). Thus, increased TE expression in aged males compared to aged females (Fig. 2B) might be associated with reduced lifespan in *D. melanogaster* and *D. simulans*, but not clearly in *D. suzukii* (Fig. 3).

Finally, when analyzing each strain individually, we found no correlation between male lifespan or SGL values and their overall TE content (Pearson correlation p values < 0.05).

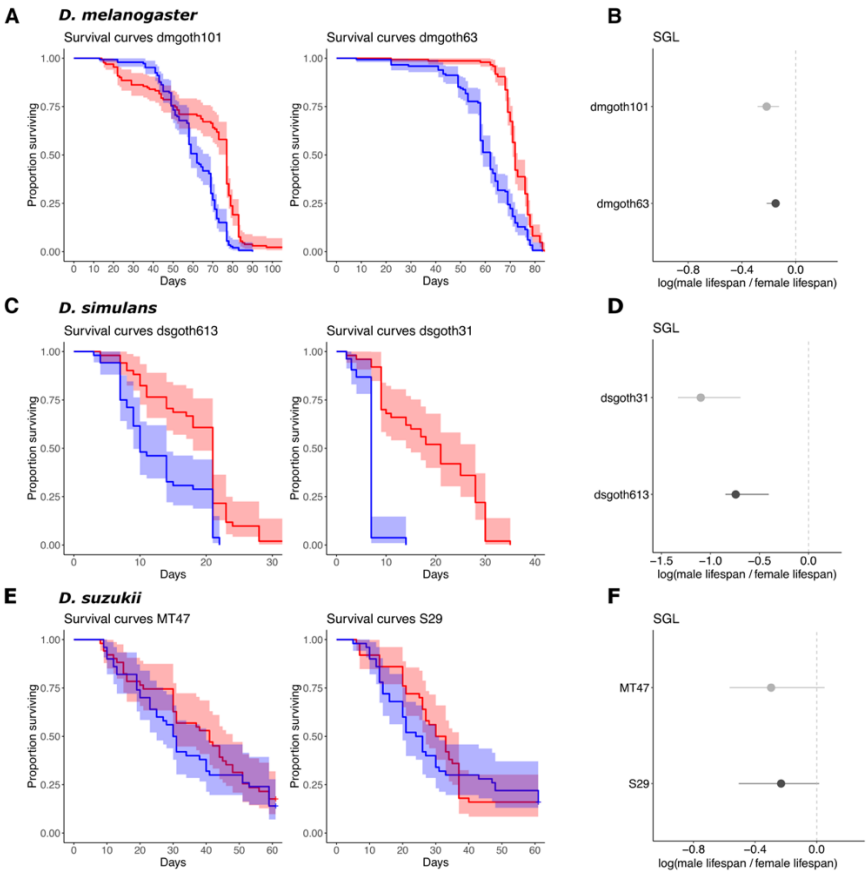


Figure 3. *D. melanogaster* and *D. simulans* males have reduced lifespan. Kaplan–Meier survivorship curves for males (blue) and females (red) in **A**) *D. melanogaster*, **C**) *D. simulans,* and **E**) *D. suzukii* with the shaded region indicating the upper and lower 95% confidence interval calculated 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. 4A and Supplementary Table 4). 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. 4A). 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. 4A). In both reciprocal crosses, males exhibited a progressive reduction in lifespan across all generations, unlike females, whose lifespan showed less variation across generations (standard deviation of mean lifespan in A crosses: 22.21 for males and 12.13 for females; B crosses: 14.42 for males and 10.06 for females) (Fig. 4B and 4C). Repeating the experiment with strains that did not show SGL differences (dmgoth101 and dmgoth63) did not result in significant SGL value increases across generations (Supplementary Fig. 14 and Supplementary Table 4).

In summary, we found that the replacement of the Y chromosome between strains with different SGL values is associated with a progressive increase in SGL due to a reduced male lifespan. If SGL values were directly linked to the Y chromosome's TE content, we might expect the replacement of the Y chromosome to result in an SGL value matching that of the donor strain across generations. However, this was not observed since SGL values increased in both reciprocal crosses when replacing the Y chromosome between strains with different SGL values. While further experiments are needed, these findings may indicate that the modification of the TE composition and distribution on the Y chromosome may have a negative impact in male lifespan.

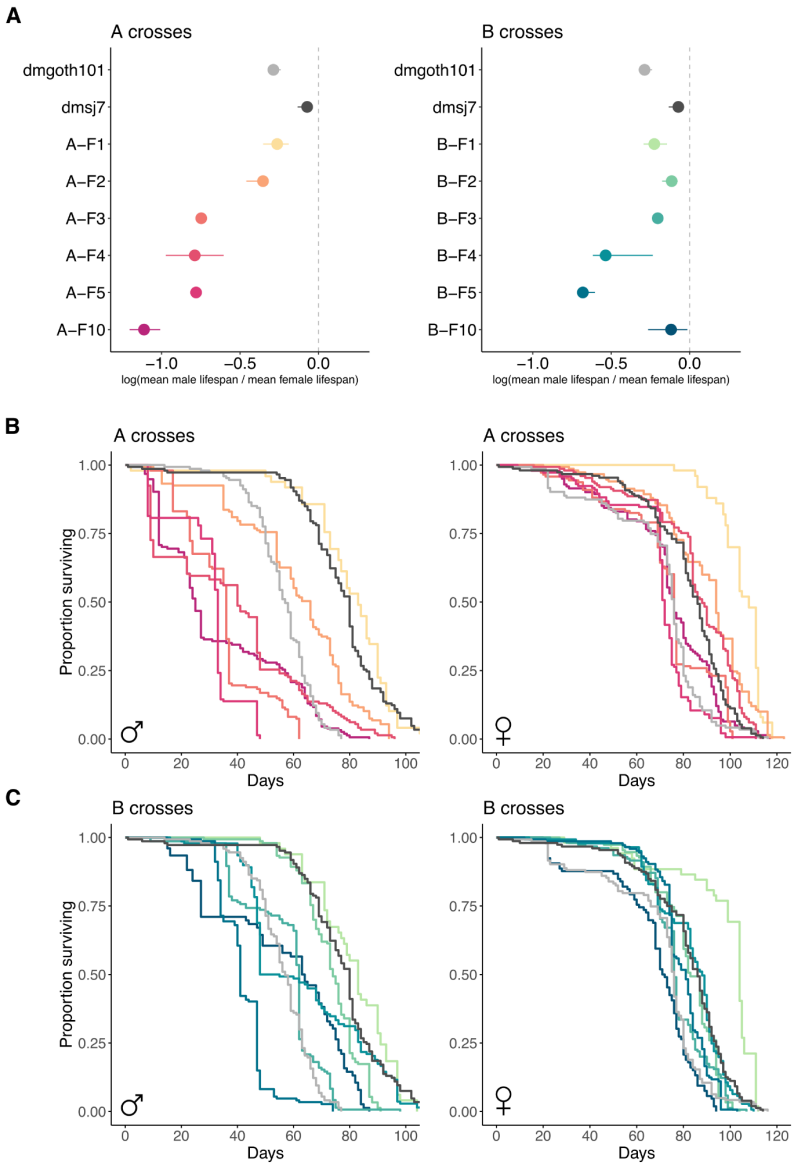


Figure 4. Y chromosome replacement is associated with reduced male lifespan. A) SGL values measured as the logarithm of the ratio between male and female median lifespan are shown for A reciprocal crosses (left panel) and B reciprocal crosses (right panel) of *D. melanogaster*. Results for maternal strains 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[59] (Supplementary Table 8).

We assessed the effects of reciprocal cross (A or B), generation (F1 or F10), sex, and their interactions on TE reads percentage (see Methods). F10 flies had more TE 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. 5). In addition, males had more TE reads than females in both generations (GLM, estimate 0.295, $p < 0.001$), but the interaction between generation and sex was not statistically significant (GLM, estimate 0.077, $p = 0.249$) (Fig. 5). 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 reads increased in F10 compared to F1 in both sexes and reciprocal crosses.

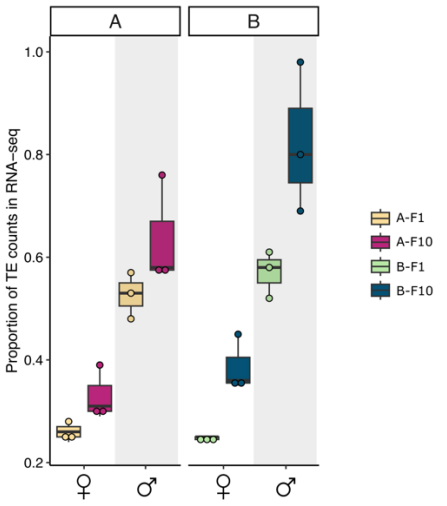


Figure 5. Y chromosome replacement is associated with increased TE expression. Boxplots showing the percentage of reads mapping to TE sequences in the RNA-seq data in F1 and F10 generations of introgression in both A and B reciprocal crosses. Boxplots show the median (horizontal line), first and third quartiles (lower and upper bounds, respectively), and minimum and maximum values (lower and upper whiskers, respectively). Each dot represents an independent biological replicate.

332 **DISCUSSION**

333 In this work, we characterized SGL variability across wild-type populations in three Drosophila
334 species, finding greater population-level variance and an overall reduced male lifespan
335 compared to females, consistent with previous studies[60].

336 Sex-differences in lifespan have been associated with TE expression in some organisms such
337 as *D. melanogaster* and *D. miranda*[13,49]. Here, using a copy-specific approach, we found a
338 general age-related increase in TE copies in both males and females of *D. melanogaster* and
339 *D. suzukii*. This result suggests that there might be an age-related derepression of TEs, likely
340 due to a general loss of heterochromatin in repetitive regions in aged flies, as it was previously
341 observed in *D. melanogaster*[13]. Specifically, male flies from the same species have been shown
342 to undergo a more rapid heterochromatin mark loss producing a more pronounced derepression
343 of TEs in old males compared to old females[13].

344 Previous studies in a *D. melanogaster* laboratory strain showed some differences in the total
345 fraction of repetitive reads that increased during ageing between males (4.2%) and females
346 (2.6%)[13]. Although we did not follow the same approach, differential expression analysis
347 between old males and old females did reveal more upregulated specific TE copies in old males
348 compared to old females in all the species tested. These results suggested that, although TE
349 expression should be seen as an indirect evidence of genomic instability, male genome integrity
350 might be more susceptible to ageing effects compared to females, in agreement with the toxic
351 Y hypothesis.

352 Indeed, particularly in *D. melanogaster* and *D. simulans* species, we found that increased TE
353 expression in aged males compared to aged females might be associated with reduced lifespan.
354 However, we could not find a significant correlation between male lifespan or SGL values and
355 overall TE content. This paradox has been explored in long-lived species, which often exhibit
356 an overall reduction in both TE abundance and expression[37,39,41,61]. However, exceptions exist
357 where TEs might play a beneficial role in ageing[62-64]. For instance, in blind mole rats (*Spalax*
358 *galili*), TE activation can act as a tumour suppressor by triggering the innate immune system[63].
359 In the Greenland shark (*Somniosus microcephalus*), the longest-lived vertebrate, TEs constitute
360 the 70.6% of the genome, the highest proportion described in sharks[64]. In this case, TE
361 expansion may be linked to the expansion of DNA repair genes, which in turn may help to
362 tolerate higher levels of TE activity[64]. Therefore, unraveling the complex mechanisms of
363 delayed aging requires careful consideration of species-specific features, including the
364 abundance, composition, and distribution of TE in the genomes[27].

365 Besides TE expression, the toxic Y hypothesis also proposes that age increases TE
366 transposition, leading to a greater number of somatic TE insertions that impact genomic
367 stability and cellular homeostasis, ultimately contributing to a decreased lifespan. In *D.*
368 *melanogaster*, it has been shown that lifespan is decreased as somatic genetic damage produced
369 by *P element*, *mariner*, and *mdg4* insertions[29-31,33,65]. Yet, some recent studies found that the
370 number of new TE insertions do not seem to correlate with age, suggesting that TE expression
371 instead could have a global impact in transcriptional dysfunction during ageing and affecting
372 lifespan[35,36,44,66,67]. Here, we observed that ageing increases the generation of TE-chimeric
373 transcripts in some samples, and specially in *D. suzukii* males. Thus, our findings suggest that
374 age-related TE expression could influence transcript structure by increasing the presence of
375 TE-chimeric transcripts in *D. suzukii* males*,* hinting that the impact on fly lifespan may not
376 solely be attributed to TE somatic transposition.
377 Our results also revealed that, in *D. melanogaster,* we can manipulate SGL values. The
378 replacement of the Y chromosome between strains exhibiting differences in SGL values and
379 male lifespan was associated with a progressively decline in male survival, leading to a more
380 pronounced SGL. Further analysis will be needed to determine whether Y chromosome size
381 differences (associated with a different Y-linked TE abundance) or differences in TE
382 distribution are responsible for the observed effects. In fact, two recent works indicate that male
383 survival is not linked to Y chromosome size in both *D. melanogaster* and *D.*
384 *pseudoobscura*[48,49]. Hence, based on our introgression experiments, we hypothesize that
385 differences in TE composition among Y chromosomes (proportion of full-length copies or
386 average enrichment in repressive histone marks) may offer a more plausible explanation for
387 variances in male lifespan compared to Y chromosome sizes[68,69]. However, it is also
388 conceivable that other genomic regions other than TEs on the Y chromosome are contributing
389 to reduced lifespan. Thus, while TEs are strong candidates, we cannot conclude beyond the
390 fact that some factor on the Y chromosome is linked to reduced lifespan.
391 In summary, this work reveals that SGL is a variable trait in the wild, and emphasizes the
392 importance of studying the role of TEs in the evolution of sex differences in lifespan across
393 different species.
394
395

RESOURCE AVAILABILITY

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Cristina Vieira (**:** cristina.heddi@univ-lyon1.fr).

This study did not generate new unique reagents.

Sequencing data have been deposited at NCBI SRA repository under the BioProject accession number PRJNA1159534 and are publicly available as of the date of publication.

This paper does not report original code.

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

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: MM, PG, JMG, JFL, GABM, MF, MB, CV

Performed the experiments: MM, SJ, CMB, NB, VRR, VR

Analyzed the data: MM, VR

Contributed material / analysis tools: VR

Wrote the paper: MM

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION TITLES AND LEGENDS

Document S1. Figures S1–S14.

Document S2. Tables S1–S16.

FIGURE LEGENDS

Figure 1. **Natural variability in SGL in different *Drosophila* species**. Histograms displaying the distribution of SGL values calculated as the logarithm of the ratio between male and female

431 median lifespan. A) Histogram displaying the distribution of SGL values per population in *D.*
432 *melanogaster*, *D. simulans*, and *D. suzukii*. UK: United Kingdom, FR: France, FI: Finland, NL:
433 the Netherlands, BR: Brazil, ES: Spain, RS: Serbia. B) Histogram displaying the distribution
434 of SGL values per species.
435
436 **Figure 2**. **Ageing increases the number of activated TE copies in different Drosophila**
437 **species.** A) Barplots showing the number of upregulated (in red) and downregulated (in blue)
438 TE copies with ageing for both males (upper panel) and females (bottom panel) in each strain.
439 B) Barplots showing the number of upregulated (in red) and downregulated (in blue) TE copies
440 in young males compared to young females (upper panel) and in old males compared to old
441 females (bottom panel) in each strain. C) Ageing increases the generation of chimeric
442 transcripts in some samples. Barplots showing the number of identified chimeric transcripts
443 per strain as a sum of chimeric transcripts supported only by paired-end reads that have mapped
444 in both transcripts and TE sequences ("chimreads_evidence"), chimeric transcripts supported
445 only by the transcriptome assembly ("transcriptome_evidence"), and chimeric transcripts for
446 which both previous methods have predicted the same chimeric transcripts
447 ("double_evidence"). Asterisks indicate level of statistical significance of chi-square tests (* p
448 ≤ 0.05, ** $p \leq 0.01$, ***$p \leq 0.001$).
449
450 **Figure 3. *D. melanogaster* and *D. simulans* males have reduced lifespan**. Kaplan–Meier
451 survivorship curves for males (blue) and females (red) in A) *D. melanogaster*, C) *D. simulans,*
452 and E) *D. suzukii* with the shaded region indicating the upper and lower 95% confidence
453 interval calculated from the Kaplan–Meier curves. SGL values measured as the logarithm of
454 the ratio between male and female median lifespan are shown for each strain of B) *D.*
455 *melanogaster*, D) *D. simulans,* and F) *D. suzukii*. Dots represent mean SGL values and lower
456 and upper whiskers indicate the upper and lower 95% confidence intervals.
457
458 **Figure 4. Y chromosome replacement is associated with reduced male lifespan.** A) SGL
459 values measured as the logarithm of the ratio between male and female median lifespan are
460 shown for A reciprocal crosses (left panel) and B reciprocal crosses (right panel) of *D.*
461 *melanogaster*. Results for maternal strains are shown in grey (dmgoth101) and black (dmsj7)
462 while different colours represent generations of introgression from F1 to F10. Dots show mean
463 SGL values and lower and upper whiskers indicate the upper and lower 95% confidence
464 intervals. B) Kaplan–Meier survivorship curves for males (left panel, n=150) and females (right

465 panel, n=150) in A reciprocal crosses. C) Kaplan–Meier survivorship curves for males (left
466 panel, n=150) and females (right panel, n=150) in B reciprocal crosses. Male lifespan between
467 dmgoth101 and dmgoth101 was statistically significantly different (Log-Rank test $p < 0.001$).
468
469 **Figure 5. Y chromosome replacement is associated with increased TE expression.**
470 Boxplots showing the percentage of reads mapping to TE sequences in the RNA-seq data in F1
471 and F10 generations of introgression in both A and B reciprocal crosses. Boxplots show the
472 median (horizontal line), first and third quartiles (lower and upper bounds, respectively), and
473 minimum and maximum values (lower and upper whiskers, respectively). Each dot represents
474 an independent biological replicate.
475
476

477 **METHODS**

478 **Fly strains and rearing**

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

487

488 **Longevity assays**

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

490 50 recently (less than 8 hours) emerged males and 50 virgin females from each strain were
491 placed in groups of 10 in tubes containing nutritive medium ("LM" for *D. melanogaster* and
492 *D. simulans* and "Dalton" for *D. suzukii*). Each tube was considered as an independent
493 biological replicate thus having five replicates per sex and strain. Flies were separated by sex
494 without anaesthesia using an aspirator. Dead flies in every vial were counted every day, except
495 for weekends, until 80% of the flies in the tube were dead. Alive flies were placed in new tubes
496 with fresh medium every week. Longevity assays were performed at 25ºC and at constant
497 humidity of 60%.

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

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

509 *Longevity assays in the Y introgressed strains*

510 150 recently (less than 8 hours) emerged males and 150 virgin females from each
511 strain/generation of introgression were placed in groups of 10 in tubes containing "LM"
512 medium. Again, each tube was considered as an independent biological replicate thus having
513 15 replicates per sex and strain/generation of introgression. Flies were separated by sex without
514 anaesthesia using an aspirator. Dead flies were counted every day, except for weekends, until
515 all the flies in the tubes were dead. Alive flies were placed in new tubes with fresh medium
516 every week. Longevity assays were performed at 25ºC and at constant humidity of 60%.
517
518 **SGL values calculation**
519 We estimated sex-specific median adult lifespan (in days) for each strain/generation of
520 introgression in our dataset (Supplementary Tables 2-4). The median adult lifespan
521 corresponds to the time when 50% of the individuals in a particular strain are dead. To estimate
522 the confidence interval for both males and females of each strain, we implemented a
523 bootstrapping procedure. We performed 100,000 bootstrap iterations to calculate the median
524 adult lifespan for each sample and then we calculated the logarithm ratio of median longevity
525 between males and females (SGL values)[50]. Confidence intervals for the difference in median
526 longevity between males and females was computed using quantiles of the bootstrap of the
527 logarithm ratios.
528 For the analysis of SGL natural variation, we used a Bayesian approach implemented in the
529 MCMCglmm package in R to partition the variance and identify the contribution of species,
530 population, and sampling variation to the observed differences in SGL. We fitted a hierarchical
531 regression model including species as a fixed effect and population as a random effect, with
532 SE weighting for the longevity measurements. The priors were set as follows: for the residual
533 variance, we used an inverse-Wishart prior with nu = 0.002; for the population random effect,
534 we used an inverse-Wishart prior with nu = 1 and a large variance (a = 1000) for the prior mean
535 (diff_median_longevity~species+(1|population)+idh(SE):units).
536 We also performed survival analysis using the Kaplan-Meier method and Cox proportional
537 hazard models. The survival analysis was conducted separately for male and female flies within
538 each strain/generation of introgression. We used the *survfit* function in R to estimate the
539 survival probabilities over time based on the event status (dead or alive). Additionally, we
540 generated survival curves using the *ggsurvplot* function from the *survminer* R package. These
541 curves represent the estimated survival probabilities over time using a Kaplan-Meier
542 estimation. Finally, we conducted pairwise comparisons to evaluate the significance of
543 difference in survival distributions among various samples. This analysis was performed using

544 the *pairwise_survdiff* function, applying the Benjamini-Hochberg correction method for
545 multiple comparisons.
546
547 **DNA extraction, nanopore sequencing and genome assemblies**
548 Genome assemblies for *D. melanogaster* dmgoth101 and dmgoth63 and for *D. simulans* strains
549 dsgoth31 and dsgoth613 were obtained from Mohamed et al., 2020[53]. To obtain genome
550 assemblies of *D. suzukii*, we sequenced with Oxford Nanopore Technologies (ONT) two inbred
551 strains: S29 and MT47. DNA was extracted using phenol chloroform from a pool of 10 adult
552 females. The quantity and quality of genomic DNA were assessed using a NanoDrop™ One
553 UV-Vis spectrophotometer (Thermo Fisher Scientific) and a Qubit® 1.0 Fluorometer
554 (Invitrogen). Three micrograms of DNA were then repaired with the NEBNext FFPE DNA
555 Repair Mix (NEB M6630). End repair and dA-tailing were carried out using the NEBNext End
556 Repair/dA-Tailing Module (E7546, NEB). Ligation was evaluated with the Ligation
557 Sequencing Kit 1D. MinION sequencing was conducted according to the manufacturer's
558 instructions using R9.4.1 flow cells (FLO-MIN106, ONT) and a Nanopore MinION Mk1b
559 sequencer (ONT), operated by ONT MinKNOW (v.18.3.1). Following sequencing, base
560 calling was performed using *Guppy* (v.4.0.5) in high-accuracy mode.
561 Quality control of Nanopore reads was conducted using *NanoPlot* (v.1.10.2)
562 (https://github.com/wdecoster/NanoPlot). Reads with a quality score below 7 were excluded
563 from further analysis. Contig assembly was performed with *Flye* (v.2.8)[70] using default
564 parameters, except for the –plasmids option. All contigs were aligned with *minimap2* (v2.16)[71]
565 using the –x map-ont option, followed by four rounds of polishing with *RACON* (v1.3.2)[72]
566 under default settings. Assembly quality metrics were evaluated using *Assembly-Stats* (v1.0.1)
567 (https://github.com/sanger-pathogens/assembly-stats). Any assembly inconsistencies were
568 manually reviewed with *D-genies* (v1.2.0)[73] and corrected using *samtools* (v1.9.0) with the
569 *faidx* function, and *Gepard* (v1.4.0) was used to identify breaking points. Super scaffolding of
570 all corrected assemblies was carried out with *RaGOO* (v.1.1), using the –s and –t 4 parameters,
571 based on the reference genome assembly[74]. The resulting chromosome-scale assemblies were
572 then analyzed using Benchmarking Universal Single-Copy Orthologs (BUSCO) (v.5.4.3)[75]
573 with the diptera_odb10 lineage (-l parameter). Gene annotation for the Nanopore genomes was
574 completed with *Liftoff* (v.1.6.3), referencing the WT3_2.0 *D. suzukii* genome[74].
575
576 **RNA-seq extraction and sequencing**

577 RNA was extracted from six inbred strains, comprising two strains for species: *D.*
578 *melanogaster* (dmgoth101 and dmgoth63), *D. simulans* (dsgoth31 and dsgoth613) and *D.*
579 *suzukii* (MT47 and S29). For young samples, male and female flies of each strain were
580 separately placed in several tubes with nutritive medium in groups of 20-25. When flies were
581 5 day-old, 30 flies per sex were dissected and ovaries and testes were removed from female
582 and male flies, respectively. For old samples, male and female flies of each strain were also
583 separately placed in several tubes with nutritive medium in groups of 20-25. 30 flies per sex
584 were dissected and ovaries and testes were removed from female and male flies, respectively,
585 when at least 25% of the flies in the tube were dead. Consequently, older samples may exhibit
586 varying chronological ages across strains. Three replicates per strain, age and sex were
587 performed. Then, for both young and old samples RNA was extracted from the obtained
588 carcasses. For the Y chromosome replacement experiments, RNA was extracted from both F1
589 and F10 male and female flies from the two reciprocal crosses. Samples were obtained as
590 mentioned before when flies were 5 days-old.
591 RNA extraction was carried out using the RNeasy Mini kit (Qiagen) following manufacturer's
592 instructions and then a DNase treatment (Invitrogen) was performed. mRNA libraries were
593 prepared with the Illumina TruSeq RNA Sample Prep Kit, following the manufacturer's
594 guidelines. Libraries were sequenced on the Illumina HiSeq 4000 platform with paired-end 100
595 nt reads. The sequence raw files have been deposited in NCBI SRA under the BioProject
596 accession number PRJNA1159534.
597
598 **RNA-seq mapping and gene quantification**
599 Quality of the *fastq* sequencing files was assessed with FastQC (v.0.11.9). Then, *fastp*
600 (v.0.23.4) was used to filter out bad reads, adapter removal and trimming[76]. Trimmed reads
601 from *D. melanogaster*, *D. simulans,* and *D. suzukii* were then mapped against the Flybase *D.*
602 *melanogaster* (r6.16)[77], the *D. simulans* (r2.02)[77], and *D. suzukii* WT3_2.0[74] reference
603 genomes, respectively with *STAR* (v.2.7.11a)[78] using default parameters. On average, 92.7%
604 of the reads were uniquely mapped to the corresponding genomes. To quantify gene expression,
605 we used *htseq* (v.2.0.3)[79], with parameter *-t exon*, which uses the sum of the reads aligned in
606 all exons to assign counts for each gene (Supplementary Tables 5-7).
607
608 **TE annotation**
609 All the six strains used in the transcriptomic analysis (dmgoth101, dmgoth63, dsgoth31,
610 dsgoth613, MT47, and S29) have been sequenced with long-read sequencing technologies and

genome assemblies were available or generated[52,53]. TE insertions were identified in the genome assemblies using *RepeatMasker* (v.4.1.2), which masks the genome where there are fragments homologous to consensus TE sequences. Parameters used were: *-cutoff 250, -a, -s, -norna*. Consensus TE sequences used for *D. melanogaster* was obtained by using the parameter *-species drosophila.* However, consensus sequences for *D. simulans* were obtained from https://github.com/bergmanlab/drosophila-transposons/, while for *D. suzukii* from https://github.com/vmerel/DSu-TE/tree/main/TE_db. Finally, *bed* files containing the coordinates of each annotated TE were created for every genome.

Differential expression analysis of genes

Differential expression analyses of genes were conducted using the R package *DESeq2* (v.1.40.2)[80]. Raw read counts were merged into a unified matrix for each strain and subsequently normalized using default parameters (Supplementary Tables 5-8). Differential expression of genes was assessed between old and young samples in each strain separately. The log2 fold-change was adjusted using the *apeglm* (v.1.22.1) algorithm from the *lfcshrink* function to minimize noise and preserve significant differences[81]. Genes were considered as significantly differentially expressed when the adjusted p-value was < 0.05 and the log2 fold-change $> |1|$.

Age-related genes found differentially expressed in old flies

Genes previously associated with ageing in *D. melanogaster* were obtained from the Animal Ageing and Longevity Database (AnAge) (https://genomics.senescence.info/genes/search.php?search.php?show=1&sort=1&page=1&organism=Drosophila%20melanogaster). From this list, only genes with a described "increased" lifespan effect were selected. Then, gene symbols were converted to FlyBase IDs with the *Batch Download* online tool (https://flybase.org/batchdownload). We finally looked for overlap of the age-associated genes and the DEGs found in the young versus old samples comparisons. For *D. simulans*, we used the gene ortholog table available in FlyBase to look for correspondences in gene IDs. We could analyze a total of 149, 152, and 120 age-associated genes in *D. melanogaster, D. simulans*, and *D. suzukii*, respectively.

RNA-seq mapping, quantification and differential expression analysis for specific TE copies

644 Quantification of TE copies was performed using *Telescope*[54]. Briefly, for each strain, trimmed
645 paired-end reads were aligned to the corresponding genome with *bowtie2* (v.2.5.1) using the
646 parameter *-k 100*. Then, again for each strain, we generated a *gtf* file containing all the TE
647 copies present in the strain following the format specified by *Telescope*. After that, the
648 generated *sam* file was converted to *bam* with *samtools view* (v.1.17). Finally, for the locus-
649 specific quantification of TEs, the function *telescope assign* was used taking the already
650 generated *gtf* and *bam* files as inputs (Supplementary Tables 9-14). Differential expression
651 analysis of TE copies was conducted using the R package *DESeq2* (v.1.40.2) as mentioned
652 before[80]. TE copies were considered as significantly differentially expressed when the adjusted
653 p-value was < 0.05 and the | log2 fold-change | > 1.
654
655 **Identification of full-length insertions**
656 Identification of full-length insertion among the upregulated TE copies found between old
657 males and females (adjusted p-value was < 0.05 and | log2 fold-change | > 1) was performed
658 with a generated *python* script that detects as full-length copies the ones that are at least 80%
659 of the length of the consensus sequence and with a Kimura divergence value < 20 (values
660 obtained from the previously generated *RepeatMasker* output files). For *D. melanogaster* and
661 *D. simulans*, TE consensus sequences were obtained from
662 https://github.com/bergmanlab/drosophila-transposons/, while *D. suzukii* consensus sequences
663 were obtained from Mérel et al., 2021[52].
664
665 **Permutation tests**
666 To test whether DETEs found in old male flies were enriched nearby DEGs (+-1kb), we first
667 created *bed* files with the +-1kb flanking regions for these genes in each specific genome. *bed*
668 files with the coordinates of all TE copies were also generated for each strain. Then, we
669 performed permutation tests by intersecting TE positions with gene regions (+-1kb) using the
670 R package *regioneR*[82], parameter *randomization = resampleRegions*, with 1000 random
671 samples. Hence, for every up and downregulated gene set of size *n*, the software determines
672 the count of those containing TEs in the +-1kb flanking regions. Subsequently, it conducts 1000
673 random samples of n genes each to assess if the observed number of genes with TEs deviated
674 from the expected count in a genome-wide context (Supplementary Table 15).
675
676 **Identification of chimeric transcripts**

677 Gene-TE chimeric transcripts were detected with *ChimeraTE* Mode 2 for each strain, sex, and
678 age separately[56]. We used Mode 2 (chimTE_mode2.py), which identifies transcripts without a
679 reference genome, using the prediction of chimeras from fixed and polymorphic TEs because
680 we focused on capturing all the possible new age-related generated chimeric transcripts.
681 Briefly, stranded RNA-seq reads were mapped against its respective annotation of transcripts
682 and TE insertions. We used the transcript annotation from the release 6.54 *dmel-all-transcript-*
683 *r6.54.fasta* for *D. melanogaster*
684 (https://ftp.flybase.net/genomes/Drosophila_melanogaster/dmel_r6.54_FB2023_05/fasta/dme
685 l-all-transcript-r6.54.fasta.gz); from the release 2.02 *dsim-all-transcript-r2.02.fasta* for *D.*
686 *simulans*
687 (https://ftp.flybase.net/genomes/Drosophila_simulans/dsim_r2.02_FB2017_04/fasta/dsim-all-
688 transcript-r2.02.fasta.gz), and the *dsuz-all-transcripts-LBDM_Dsuz_2.1.fasta* file for *D.*
689 *suzukii*[74]. Reference TE file for *D. melanogaster* and *D. simulans* was available online
690 (https://github.com/bergmanlab/drosophila-transposons/tree/master/current), while reference
691 TE file for *D. suzukii* was obtained online (https://github.com/vmerel/DSu-
692 TE/tree/main/TE_db). We used the parameters *–strand rf-stranded* and *–assembly* to search for
693 chimeric transcripts in the *de novo* transcript assembly. For each strain, sex and age, we got
694 three output files: list of chimeric transcripts supported only by paired-end reads that have
695 mapped in both transcripts and TE sequences, list of chimeric transcripts supported only by the
696 transcriptome assembly, and chimeric transcripts supported by both mapped paired-end reads
697 and transcriptome assembly. These three lists were merged and just the name of the gene and
698 the TE family were kept to easily analyze the number of chimeric transcripts in each sample
699 (Supplementary Table 16).
700
701 **Y chromosome replacement crosses**
702 To check whether the Y chromosome is associated with changes in SGL, we performed
703 introgression experiments to replace the Y chromosome between strains with different SGL
704 values. The lack of recombination in Drosophila males enabled the generation of flies that
705 differ only in the Y chromosome. Reciprocal crosses between the *D. melanogaster* strains
706 dmgoth101 and dmsj7 were performed. We assigned the name "A crosses" to crosses initiated
707 by mating a dmgoth101 female with a dmsj7 male, followed by successive backcrossing of
708 male offspring with dmgoth101 females over ten generations. Conversely, "B crosses"
709 represented the reciprocal pairing wherein dmsj7 females were initially mated with dmgoth101
710 males, followed by analogous backcrossing of male offspring with dmsj7 females. Five virgin

711 females and five males aged 4-5 days old were placed in a tube containing a nutritive medium,
712 with five replicates performed for each reciprocal cross. Every 2-3 days, the flies were
713 transferred to new tubes with fresh medium until four tubes were obtained for each reciprocal
714 cross. This ensured enough offspring to carry out longevity assays and facilitated the
715 continuation of introgression crosses. F1 male flies were subsequently crossed with the
716 maternal strain, and the male offspring of each generation was consistently crossed with the
717 maternal strain for 10 generations. In each generation (except for generations F6-F9), longevity
718 assays were carried out as previously described with 10 virgin females and 10 males from each
719 of the five replicated reciprocal crosses (n=150).
720
721 **TE transcript quantification in introgression crosses**
722 TE transcript quantification was performed with *TEtools*, using the TEcount module[59]. One of
723 the inputs of the TEcount module is the *rosette* file, a tabular file containing the names of each
724 TE copy in the genome that easily groups them in TE families (or other criteria if needed)[59].
725 To do that, for each strain (dmgoth101 and dmgoth63), we first build a *fasta* file with all the
726 TE sequences annotated using *bedtools getfasta* and the previously generated *bed* file with TE
727 annotations. Then, we generated a *rosette* file per strain writing in the first column the name of
728 the specific TE copy and in the second column the TE family in which it belongs. After that,
729 we merged the two *rosette* files. A GLM analysis was performed to assess the effects of the
730 reciprocal cross, generation, sex, and the interaction between generation and sex on the
731 percentage of TE reads in R (glm(alignment_rate ~ cross + generation + sex + generation:sex,
732 family = gaussian(link = "identity"). The model showed a good fit (residual deviance = 0.1188,
733 AIC = -47.315).
734
735

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