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1 The transposable elements of the *Drosophila serrata* reference panel

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21

22 **Abstract:** Transposable elements are an important element of the complex genomic ecosystem,
23 proving to be both adaptive and deleterious - repressed by the piRNA system and fixed by
24 selection. Transposable element insertion also appears to be bursty – either due to invasion of
25 new transposable elements that are not yet repressed, de-repression due to instability of
26 organismal defense systems, stress, or genetic variation in hosts. Here, we characterize the
27 transposable element landscape in an important model *Drosophila*, *D. serrata*, and investigate
28 variation in transposable element copy number between genotypes and in the population at large.
29 We find that a subset of transposable elements are clearly related to elements annotated in *D.*
30 *melanogaster* and *D. simulans*, suggesting they spread between species more recently than other
31 transposable elements. We also find that transposable elements do proliferate in particular
32 genotypes, and that often if an individual is host to a proliferating transposable element, it is host
33 to more than one proliferating transposable element. In addition, if a transposable element is
34 active in a genotype, it is often active in more than one genotype. This suggests that there is an
35 interaction between the host and the transposable element, such as a permissive genetic
36 background and the presence of potentially active transposable element copies. In natural
37 populations an active transposable element and a permissive background would not be held in
38 association as in inbred lines, suggesting the magnitude of the burst would be much lower. Yet
39 many of the inbred lines have actively proliferating transposable elements suggesting this is an
40 important mechanism by which transposable elements maintain themselves in populations.

41

42 **Keywords**: Transposable elements, *Drosophila serrata*, copy number, inbred lines

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43 **Introduction**

44 Transposable elements (TEs) are short sequences of DNA that multiply within genomes despite
45 potential deleterious impacts to the host (McClintock 1950). TEs are widespread across the tree
46 of life, often making up a significant portion of the genome (Piegu et al. 2006; Schnable et al.
47 2009; Lee and Langley 2012). TEs also impose a severe mutational load on their hosts by
48 producing insertions that disrupt functional sequences and mediate ectopic recombination
49 (McGinnis et al. 1983; Levis et al. 1984; Lim 1988). TEs can spread through horizontal transfer
50 between non-hybridizing species, allowing them to colonize new host genomes (Kidwell 1983;
51 Kofler et al. 2015; Peccoud et al. 2017). For example, the spread of the *P*-element was
52 documented in *D. melanogaster* from *D. willistoni* in the 1950's, and its subsequent spread into
53 *D. simulans* around 2010 (Daniels et al. 1990b; Kofler et al. 2015).

54 TEs have also been implicated in adaptation. In *Drosophila*, insertion of TEs has been
55 linked to resistance to pesticides and viral infection (Wilson 1993; Daborn et al. 2002;
56 Aminetzach et al. 2005; Magwire et al. 2011; Mateo et al. 2014). In ants and *Capsella rubella*,
57 TEs provide genetic diversity in invading populations which are generally depleted of variation,
58 facilitating adaptation to novel environments (Schrader et al. 2019; Niu et al. 2019). In fission
59 yeast, TE activity was increased in response to stress and TE insertions were associated with
60 stress response genes, supporting the supposition that TEs provide a system to modify the
61 genome in response to stress (Esnault et al. 2019). There is also evidence from vertebrates that
62 TEs provide the raw material for assembling new protein architectures through capture of their
63 transposase domains (Cosby et al. 2020). In summary there is extensive evidence that TEs
64 provide genetic material for adaptation through a variety of mechanisms.

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65 Despite the evidence for an adaptive role for TEs, most TE insertions are thought to be
66 deleterious, and the host has a dedicated defense mechanism termed the PIWI-interacting
67 (piRNA) system. piRNAs bind to PIWI-clade proteins, such as *Argonaute 3* in *D. melanogaster*,
68 and suppress transposon activity transcriptionally and post-transcriptionally (Brennecke et al.
69 2007). The majority of these piRNAs originate from genomic regions which are enriched for TE
70 fragments, termed piRNA clusters (Brennecke et al. 2007; Malone et al. 2009). These piRNA
71 clusters are large, and there is at some evidence that insertion of a TE into a piRNA cluster is
72 enough to initiate piRNA mediated silencing of the TE (Josse et al. 2007; Zanni et al. 2013).
73 Therefore a newly invading TE would proliferate in the host until a copy jumps into a piRNA
74 cluster, which then triggers piRNA silencing of the TE (Bergman et al. 2006; Malone and
75 Hannon 2009; Zanni et al. 2013; Goriaux et al. 2014; Yamanaka et al. 2014; Ozata et al. 2019).
76 These piRNA clusters are preferentially located in heterochromatic regions and usually have low
77 recombination rates (Brennecke et al. 2007). This reduces the efficacy of purifying selection and
78 may serve as 'safe harbors' for TEs to accumulate and develop into piRNA clusters (Brennecke
79 et al. 2007; Kofler 2019; Zhang and Kelleher 2019). piRNA evolution is thought to be rapid
80 enough that adaptation to a novel TE could occur within the lifetime of an individual (Khurana et
81 al. 2011). The transposition rate of TEs is also controlled by other mechanisms, including
82 regulation of promotor activity, chromatin structure, and splicing (Guerreiro 2019). In some
83 cases the mechanism is unknown, such as the accumulation of *copia* in the genomes of inbred *D.*
84 *melanogaster*, suggesting that there is still more to know about the regulation of TE copy number
85 (Pasyukova 2004).

86 This apparent contradiction, between the existence of a dedicated repression machinery,
87 and an apparent important role for TEs in adaptation, also complicates inferences about the

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88 tempo and mode of TE transposition. For a long time, active TEs variants were thought to be rare
89 in natural populations (Kaplan et al. 1985; Ronsseray et al. 1991; Brookfield 1991; 1996;
90 Nuzhdin et al. 1997). Or, it was not active TEs which are rare but individuals with 'permissive'
91 genetic backgrounds, such that TEs would remain inactive until encountering a permissive
92 genetic background and then proliferate (Nuzhdin 2000). Either way, these models assumed a
93 transposition – selection balance such that TEs proliferated at approximately the rate that they
94 were removed by selection. Since then, TEs have been observed to undergo bursts of activity,
95 which could occur for multiple reasons such as colonization, hybridization, and stress (Vieira et
96 al. 1999; Romero-Soriano and Garcia Guerreiro 2016; Guerreiro 2019). These bursts are
97 documented in *Drosophila*, rice, fish, and other systems (Vieira et al. 1999; Piegu et al. 2006; de
98 Boer et al. 2007; Bourgeois and Boissinot 2019; Signor 2020). In most cases, transposition bursts
99 in *Drosophila* include few individuals and TEs (Biémont et al. 1987; 1990; Nuzhdin et al. 1997;
100 Yang et al. 2006). The underlying explanation for this burstiness is unclear, including the
101 potential role of burstiness in adaptation. Bursts of transposition would be expected upon
102 invasion of a new TE, prior to silencing by the piRNA system, however TEs also appear to
103 become reactivated in response to stress, or potentially variation in the host suppression system.

104 Recently an inbred panel of 110 genotypes was created for *D. serrata*, a member of the
105 *montium* subgroup (Reddiex et al. 2018). The *montium* group contains 98 species and represents
106 a significant fraction of known *Drosophila* species (Lemeunier et al. 1986; Reddiex et al. 2018).
107 The *D. serrata* panel was sampled from a single large population within its endemic distribution
108 in Australia (Reddiex et al. 2018). *D. serrata* is a model system for understanding latitudinal
109 clines and the evolution of species boundaries (Blows 1993; Jenkins and Hoffmann 1999; Hallas
110 et al. 2002; Hoffmann and Shirriffs 2002; Liefting et al. 2009). While the development of a panel

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111 represents a new opportunity for genomic investigation in the group, such as GWAS, very little
112 work has been done understanding the landscape of repetitive elements in this group. For
113 example, *D. serrata* was found to contain a domesticated *P*-element, though no evidence of
114 active *P*-elements was noted (Nouaud and Anxolabéhère 1997; Nouaud et al. 1999). Screens for
115 the presence of the *Drosophila hobo* element in the *montium* group were mixed, and
116 inconclusive for *D. serrata* (Daniels et al. 1990a). *copia* and *412* were not detected in *D. serrata*,
117 though the DNA transposon *Bari-1* was (Biémont and Cizeron 1999), and evidence for the
118 presence of the *mariner* element is equivocal (Maruyama and Hartl 1991; Brunet et al. 1994).
119 Here we will characterize the TE landscape in the *Drosophila serrata* Genetic Reference panel.
120 This will have two goals: 1) To understand the TE content of *D. serrata* and its relationship to
121 existing TE annotations 2) To understand variability in TE content between individuals in the
122 population and how this relates to the tempo and mode of TE movement. This will provide the
123 groundwork for understanding the role of TEs in evolution in *D. serrata*, as well as provide
124 another investigation into the proliferation of TEs in individual genetic backgrounds.

125 **Methods**

126 *Fly lines and data*

127 110 genotypes of *D. serrata* were collected from a wild population in Brisbane Australia in 2011
128 and inbred for 20 generations (Reddiex et al. 2018). The libraries were sequenced using 100 bp
129 paired-end reads on an Illumina Hi-seq 2000. The raw reads were downloaded from NCBI SRA
130 PRJNA410238. 104 genotypes were used for analysis. 4 genotypes were excluded based on
131 unusually high relatedness, as described in (Reddiex et al. 2018), while the remaining 2
132 genotypes were excluded based on library quality issues.

133 *Classification of TEs*

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134 TEs are a diverse group, and the taxonomy of TEs is contentious and still developing (Wicker et
135 al. 2007; Kapitonov and Jurka 2008; Platt et al. 2016). Here, we will rely only on broad
136 classifications in Class I and Class II elements, including *Helitrons* and miniature inverted-repeat
137 TEs (MITES). Class I elements are retrotransposons which use an RNA intermediate in their
138 'copy and paste' transposition. Class I can be divided into long terminal repeat (LTR) and those
139 that lack LTRs (SINEs and LINEs) (Okada et al. 1997; Havecker et al. 2004; Wicker et al. 2007;
140 Kramerov and Vassetzky 2011; 2019). However, here we will only focus on LTRs in Class I, as
141 benchmarking of software designed to detect non-LTRs is unreliable (Ou et al. 2019). Within
142 the LTRs, there are two major superfamilies – *copia* and *gypsy* – which have distinct terminal
143 sequences (Marlor et al. 1986). Class II elements are known as DNA transposons, or terminal
144 inverted repeat transposons (TIR), and use DNA intermediates in a 'cut and paste' mechanism
145 of transposition (McClintock 1984). Among the TIRs are also non-autonomous small DNA
146 transposons such as miniature inverted-repeat TEs (MITES) (Fattash et al. 2013; Makałowski et
147 al. 2019). These can belong to any of the described TIR superfamilies, but they lack coding
148 potential and rely on other autonomous DNA transposons for transposition. Lastly, the *Helitron*
149 TIR was discovered in 2001 and has a different mechanism of transposition, referred to as a
150 rolling circle, which frequently captures nearby genes or portions of them in the process
151 (Kapitonov and Jurka 2001; Kapitonov and Jurka 2007).

152 *Mapping and copy number estimation*

153 The *D. serrata* 1.0 assembly available from the Chenoweth lab was used for genomic mapping
154 and TE identification (http://www.chenowethlab.org/resources.html) (Allen et al. 2017). The TE
155 library was constructed using the Extensive de-novo TE Annotator pipeline (EDTA) (Ou et al.
156 2019). This pipeline is intended to create a high quality non-redundant TE library based off of a

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157 reference genome. Reads from the *D. serrata* reference panel were mapped to the genome and
158 the TE library using bwa mem version 0.7.15 (Figure 1; Li 2015). Bam files were sorted and
159 indexed with samtools v.1.9 and optical duplicates were removed using picard MarkDuplicates
160 (http://picard.sourceforge.net) (Li et al. 2009; McKenna et al. 2010). Reads with a mapping
161 quality of below 15 were removed (this removes reads which map equally well to more than one
162 location). Using read coverage to determine copy number has been compared to other methods
163 and is neither permissive nor conservative (Srivastav and Kelleher 2017). TE copy number was
164 estimated using the average counts of reads mapping to the TE sequences and the genome with
165 bedtools counts (Quinlan and Hall 2010; Hill et al. 2015). Then, copy number of the TEs could
166 be normalized using the average counts from a 7 MB contig from *D. serrata* which corresponds
167 to a portion of *D. melanogaster* 3L. This is one of the largest contigs in the *D. serrata* assembly.

168 *SNPs and summary statistics*

169 We called SNPs within the TEs using GATK Haplotypecaller (McKenna et al. 2010). SNPs were
170 not filtered for missing calls given that not all individuals will share insertions. However, they
171 were filtered for a coverage of at least 4 reads to be called in an individual. The site frequency
172 spectrum (SFS) of SNPs in the TEs was estimated with VCFtools as the frequency of each SNP
173 in the population, and then the frequency of the SNP frequencies was estimated in R (Danecek et
174 al. 2011). The SFS was then folded in R, replacing any frequency *i* over 0.5 with 1-*i*. This was
175 done because we could not determine the derived allele.

176 *Relationship to TEs in the EMBL TE library*

177 The TE library from *D. serrata* was compared to TEs from the EMBL library using DFAM
178 (Hubley et al. 2016). Hits were required to have a bit score of greater than 350. Multiple hits to
179 the same TE were considered as a single hit, and if more than one EMBL TE was listed the best

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180 bit score was retained. In general there were no TEs from the *D. serrata* library that had similar
181 bit scores between different EMBL TEs.

182 *Relationship between TEs annotated by EDTA*

183 Potentially related TEs from the EDTA library were identified using ncbi blastn 2.8, with the
184 minimum criteria being an alignment of greater than 400 bp for LTRs and TIRs, and 200 bp for
185 MITEs (Camacho et al. 2009). The sequences were aligned and oriented using the R package
186 DECIPHER (Wright 2016). The fasta alignments were converted to nexus format, and indels
187 were coded as binary characters, using the perl script 2matrix (Salinas and Little 2014). Trees
188 were made if there were four or more related TEs using MrBayes 3.2.7 (Ronquist et al. 2012).
189 The trees were built using a GTR substitution model and gamma distributed rate variation across
190 sites. The markov chain monte carlo chains were run until the standard deviation of split
191 frequencies was below .01. The consensus trees were generated using sumt conformat=simple.
192 The resulting trees were displayed with the R package ape (Paradis et al. 2004).

193

194 **Results**

195

196 *EDTA*

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197 EDTA identified 676 TEs in the *D. serrata* reference genome. The sequences of these TEs are
198 available at https://github.com/signor-molevol/serrata_transposable. The classification of the TEs
199 into superfamilies is broadly correct, and in many cases there is no clear relationship to an
200 existing TE. However, some errors are evident, for example, element *444* is classified as *copia*,
201 but aligns quite well with the *297* element in *D. melanogaster*, which is a member of the *17.6*
202 clade/*gypsy* superfamily. In addition, some unknown elements such as *69* align well with existing *D. melanogaster* annotations, in this case *17.6*. In all 6 elements that were classified as unknown or *copia* align well with members of the *gypsy* superfamily from *D. melanogaster.* Therefore classification below the superfamily level is generally ambiguous, though MITEs, *Helitrons*, and TIRs are distinguishable. This may be due to deletion of canonical sequences, nested insertions, or other ambiguities of TEs.

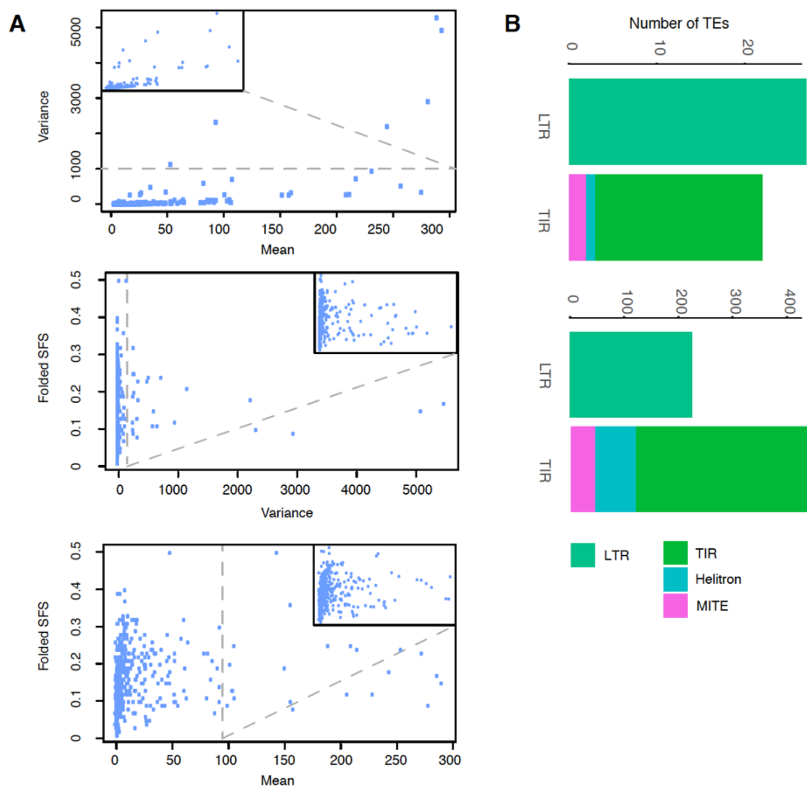

Figure 1: A. Mean and variance of copy number as well as folded SFS in transposable elements of Australian *D. serrata*. On the top, the majority of identified transposable elements have low mean copy number and variance within the population. The top three most variable elements are excluded from all graphs so as not to compress the y-axis. B. The 52 most variable transposable elements (top) with variance greater than 50 versus all of the transposable elements (bottom). Overall the number of LTRs represent fewer of the total identified TEs, however of those that are highly variable they are equally likely to be LTRs or TIRs.

Population frequency of TEs

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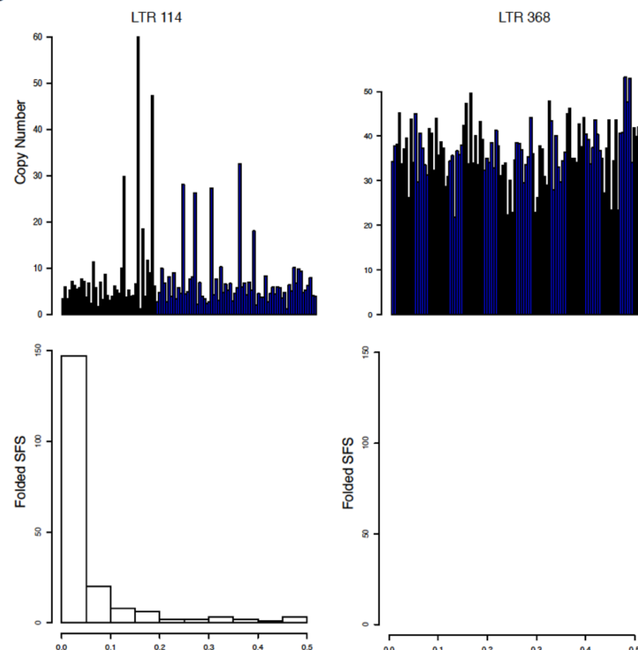
219 An average of 17% of reads from individual *D. serrata* lines mapped to TE sequences. The
220 average number of TEs per genome in this population of *D. serrata* is 19,909, however almost
221 50% of that total (9,036) are from a single repetitive uncharacterized sequence (Supplemental
222 File 1). This sequence is classified as an LTR, though it does not share sequence similarity to
223 other well characterized LTRs. This element shares a 36 bp segment with *D. melanogaster INE-*
224 *1*, and may be misclassified given that *INE-1*s are generally very abundant. The next closest in
225 copy number is a TIR with 541 copies, thus
226 this is a significant outlier. 6 TEs identified in
227 the reference are likely not present in this
228 population. 2 of these are present as partial
229 copies in a subset of individuals. Overall
230 among the elements identified by EDTA
231 approximately twice as many are TIRs
232 compared to LTRs (Figure 1). However, the
233 majority of the identified TEs have low copy
234 number and variance. 390 of the identified
235 elements have an average copy number of
236 less than 3, and all but 2 of those have a
237 variance of less than 1 (Figure 1,
238 Supplemental file 1; the other two have
239 variances of 3 and 4). Of those remaining,
240 148 have a variance of 3 or less (Figure 1).
241 Therefore the vast majority of TEs in this



Element	Average	Variance	SFS
217 TIR	58.38	226.59	NA
368 LTR	36.63	41.26	NA
389 TIR	17.87	6.42	0.03
411 LTR Gypsy	102.80	155.98	NA
436 TIR	30.83	57.81	0.05
459 TIR	28.33	49.28	0.04
610 LTR Gypsy	5.89	4.50	NA
614 MITE	4.05	2.24	0.02
624 TIR	7.18	5.08	NA
638 LTR Gypsy	107.17	176.07	NA

Figure 2: A. A list of transposable elements with variance of at least 2 and either no SNPs or and SFS of 0.05 or less. B. Variation in copy number in the population for two transposable elements (top) and the folded SFS (bottom).

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242 population vary little in copy number (Figure 1). However, among those that do vary
243 considerably, 52 elements have a variance in the population greater than 50. These represent a
244 very different subset of TEs than those identified overall – an approximately equal number are
245 LTRs and TIRs (Figure 1). This suggests that LTRs are more active in the population, which is
246 consistent with other work on transposable elements that found that LTR insertions were
247 generally of more recent origin than TIRs (Kofler et al. 2015).

248 *Folded SFS*

249 Overall ~6% of SNPs in TEs have a frequency of higher than 60% in this population. Rather than
250 determine the ancestral state by adding an outgroup, we chose to fold the allele frequency
251 spectrum. Overall the folded SFS is low (average of 0.13), however SNPs that are not in TEs
252 also have excess of low frequency variants genome-wide according to genome-wide Tajima's *D*
253 (Reddiex et al. 2018). There is not a clear relationship between the folded SFS and
254 mean/variance (Figure 1A). A folded SFS of greater than 0.2 is associated with lower copy
255 number and variance overall (Figure 1A). 16 elements have a folded SFS of 0.3 or higher, and
256 the majority of these have low variance in copy number (2 or less) suggesting that they are not
257 active and have been diverging. There are two obvious types of TEs that have likely been
258 spreading recently in the population as a whole – those with no SNPs or low SFS, and
259 with high variance and/or copy number. 5 TEs have no SNPs and therefore no folded SFS can
260 be calculated (*217*, *411*, *610*, *624*, *638*, Table 1, Figure 2). This includes *610*, a *gypsy* element
261 which aligns to the internal sequence of *Dsim\ninja* suggesting it is distantly related, but more
262 recently moved into *D. serrata* than TEs with no obvious relatives in related species. As with the
263 population of TEs as a whole, most TEs with a low SFS (lower than .05) also have low copy
264 number (less than 3) and low variance (less than 1). Of these 71 elements, 4 are an exception and

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265 have a low SFS, higher copy number, and higher variance. This includes a MITE element and
266 three TIRs. A lack of SNPs, or low SFS, along with higher copy number and variance, suggests
267 that the TEs have been spreading recently in the population (Table 1).
268 However, TEs may also have been in the population for long enough to accumulate
269 SNPs, but experience bursts of activity. Among the 52 TEs with a variance higher than 50, 17
270 TEs have an SFS of greater than 0.2 suggesting they have been in the population for longer but
271 have had active transposition of divergent copies (Supplemental Table 1). This group consists of
272 15 LTRs, one MITE, and one TIR. This could be due to the presence of older copies
273 accumulating SNPs compared to younger active copies, or divergence between different active
274 copies in the population, or both.
275

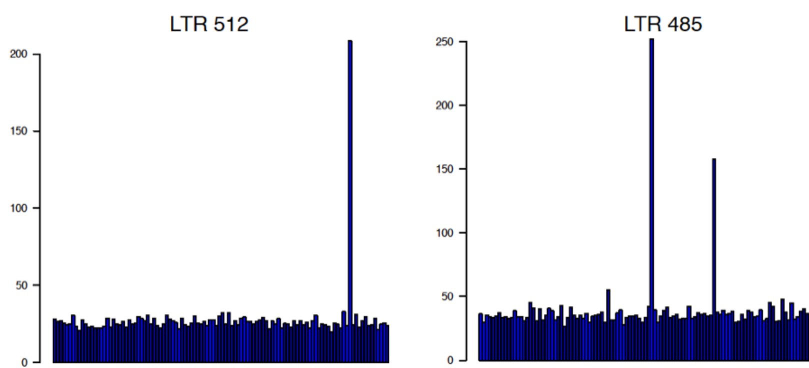

Figure 3: An example of genotypes with an accumulation of transposable elements. In both panels the population average is 20-30, while individual genotypes have in excess of 150 copies.

Outliers in individual genotypes

281 TEs tend to proliferate in particular inbred genotypes. Out of 104 genotypes, 73 have no TEs with a number of insertions that classify them as
284 outliers. 12 genotypes contain a single TE with a copy number that is considered an outlier, and
285 the remaining 19 contain 2 or more outliers. This includes 2 genotypes with 13 and 8 TEs with a
286 copy number that is considered an outlier. This also tends to group by TE, as only 36 TEs have at
287 least 1 genotype in which they are an outlier, however for 18 of these this is only in 1 genotype.

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288 For 5 genotypes, 5 TEs are shared as being outliers, with an additional 2 genotypes which share
289 outliers for 4 of the 5. Many of these outliers are large, for example for element *512* the majority
290 of the population has 20-30 copies, while a single individual has > 200 (Figure 3).

291 *Relationship to existing TE annotations*

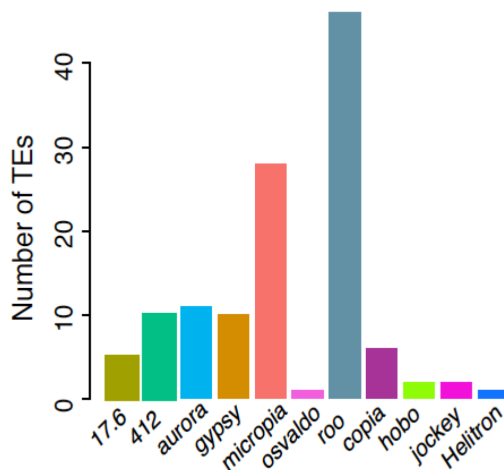

Figure 4: The classification of transposable elements into clades which could be tied to annotated *D. melanogaster* elements. The two *Helitron* elements potentially related to those from *Heliconius* are not included.

123 of the 676 identified elements have a well-supported relationship to existing DFAM TE annotations (Figure 4). This includes, for example, 27 elements that are related to the *D. melanogaster Max-Element* and 10 elements that are related to the *D. simulans ninja* element. One of these is also among the most variable TEs (variance greater than 50), and is most closely related to the *Circe* element (*Osvaldo* family). These are likely to be TEs that moved between species more recently, and they are
302 almost exclusively LTRs. The exception being two TIRs from the *hobo* family, one *Helitron*
303 from *D. melanogaster*, and two *Helitrons* most closely related to elements from *Heliconius*. No
304 evidence of *P*-elements were found in the population of identified TEs. In addition, *jockey*
305 elements (non-LTR retrotransposons) are not intended to be identified as a part of this pipeline
306 but do appear to be the identity of two transposons. The overall phylogeny of the TEs is not what
307 we wish to emphasize here, as the structure of TE classification changes frequently (for example
308 whether something is a clade or a family, etc.). In *Drosophila* there is evidence that *gypsy*
309 elements are infectious, as they can be transferred among strains through exposure or

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310 microinjection (Song et al. 1994; Kim et al. 1994). This makes them more likely to spread

311 between species.

312

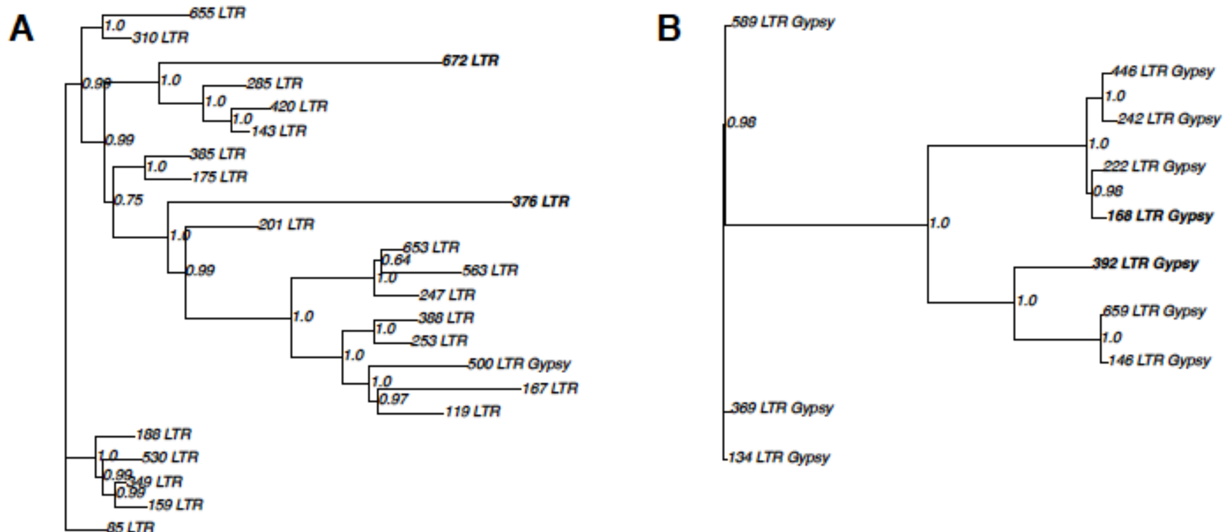

Figure 5: Relatedness between groups of TEs annotated by EDTA. Posterior probabilities of each division is shown, however branch lengths are not meaningful given that TEs do not follow a standard substitution model. This does not represent only the degradation of old copies of TEs, different members of the TE families continue to likely be active, shown in bold.

313 *Relationship between TEs annotated by EDTA*

314 40 groups of 170 TEs annotated by EDTA are clearly related to one another (Supplemental File

315 3). For example, 8 TEs (*52, 60, 276, 346, 367, 424, 539, 601*) share sequence similarity for the

316 entirety of their length, but are separated by 39 deletions spread across the TE. In the largest

317 related group of TEs, 23, most of the versions of this TE have low copy number and variance

318 (Figure 4A, average copy number 2.3, average variance < 1). However, two members of the

319 group are likely still active and have relatively high copy number and variance (*376* and *672*,

320 copy number 27, 79; variance 10, 102). Note that the more active TEs do not group together

321 (shown in bold), however because TEs cannot be assumed to follow a standard substitution

322 model the branch lengths are not meaningful (Figure 4A). In another case, 3 members of the

323 group are more distantly related, while 7 members are more closely related and form 2 clear

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324 groups of origin (Figure 4B). Yet again, those which are active in the population, as evidenced
325 by higher copy number and variance, are not the most closely related (Figure 4B, shown in bold)
326 The distance between TEs cannot be understand in the same way as genomic DNA,
327 however in some cases the TEs are clearly related by a simple tree of SNPs and indels (as
328 above), and in others it is more complicated. For example, in a group of 4 TEs (*237, 225, 358,*
329 *468*) the long terminal sequence of the TEs is nearly identical and allies them with *Max*
330 elements, however member *468* appears to also carry an unshared insertion of a portion of a *297*
331 element, among other complicated indels. *468* has a copy number of 5, indicating it continued to
332 be active while carrying a portion of a *297* element, though it has low variance between
333 individuals. Interestingly, in another case these relationships appear to describe the origin of 3
334 MITEs (*399, 405, 472*) from a parental TIR (*660*). The parental TIR has a high copy number in
335 the population, with an average of 286 and a variance of more than 6,000. This is not intended to
336 be an exhaustive accounting of relationships between these TEs, for example at some point all
337 members of the *roo* clade shared an ancestor. Rather, this is intended to describe recent
338 divergence between members of a group within this species.
339
340 Discussion
341 There is an abundance of evidence from inbred lines that genotypes can vary considerably in
342 copy number. The question remains – is it due to differences in the permissiveness of the genetic
343 background, or inheritance of active TEs that segregate at low frequency in the population? In
344 the former scenario, genes segregating in natural populations modify transcription and the rate of
345 transposition of specific TEs, including polymorphisms in genes such as *Argonaute 3* and
346 variation in the integration of TEs into piRNA clusters (Birchler et al. 1989; Pélisson et al. 1994;
347 Csink et al. 1994; Lee and Langley 2010; 2012; Zhang and Kelleher 2019). Indeed, variation in

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348 the integration of TEs into piRNA clusters appears to be quite common, as Zhang and Kelleher
349 (2019) documented 80 unique independent insertions of *P-elements* into piRNA clusters in the
350 *Drosophila* Genetic Reference Panel (Mackay et al. 2012). If laboratory lines differ in these
351 alleles, this can cause between line variability in transposition rates. In the latter scenario,
352 different lines may have inherited copies of TEs with differences in the propensity to transpose
353 (Ronsseray et al. 1991; Kim et al. 1994; Nuzhdin et al. 1997; Nuzhdin 2000).

354 While we cannot measure the likelihood of individual genotypes inheriting multiple
355 active copies of TEs while fellow members of the population inherit none, the fact that multiple
356 TEs are proliferating in individual genotypes supports the idea that these individuals have
357 polymorphisms in genes or other repressive structures that are more permissive to TE
358 transposition. Were the genotypes with clear TE proliferation different for every TE family this
359 would not support either scenario, however it does seem more likely that these genotypes have a
360 polymorphism which fails to repress more than one type of TE, rather than that they
361 preferentially inherited multiple active copies. We cannot at this time directly look for
362 polymorphisms in repressive genes or complexes. Currently we are unable to establish clear
363 homologs of the *D. melanogaster* genes known to affect piRNA silencing in *D. serrata*, but as
364 the *D. serrata* assembly improves this may be possible. In addition, the methods developed
365 recently be Zhang and Kelleher (2019) to measure differences in piRNA cluster integration using
366 small RNA libraries shows promise for determining whether we can detect polymorphisms in
367 these individual genotypes for repressive alleles.

368 However, the fact that the TEs which are proliferating do not appear to be a unique
369 population suggests that there is interaction between potentially active TEs and genetic
370 background – not all TEs are potentially active in all potentially permissive backgrounds. This

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371 suggests that the transposition rate of TEs in natural populations will be complex, depending
372 upon differences in the inherited TE population and variation in the host genome. There is
373 already a lot of evidence that there are multiple pathways and factors that control transposition in
374 *Drosophila*. For example, in *D. melanogaster* strain *iso-1* the piRNA pathway produces normal
375 *hobo* and *I*-element specific piRNAs, yet there is a high level of *hobo* and *I*-element transposition
376 (Zakharenko et al. 2007; Shpiz et al. 2014). In *D. simulans*, there is large amounts of variation in
377 piRNA pathway genes (Fablet et al. 2014). Therefore there is abundant opportunity for variation
378 in the host ability to suppress a TE and the ability of the TE to transpose.

379 Since the discovery of the piRNA repression system for TEs, the lifecycle of a TE in a
380 host has been envisioned as three steps. First, the TE invades a novel population or species and
381 amplifies unencumbered. TE proliferation is then slowed by segregating insertions in piRNA
382 clusters, and finally inactivated by fixation of piRNA cluster insertions (Kofler 2019). However,
383 clearly bursts, or activity, continues at some level within the population as many of the
384 potentially active TEs in *D. serrata* have a high SFS. This indicates that the TEs have been in the
385 population long enough to accumulate SNPs, potentially including copies with different SNPs
386 continuing to proliferate in the population. It is true that suppression by piRNA cluster insertion
387 may be unstable, but exactly why that is or how important it is for TE survival is not clear.

388 The accumulation of TEs in laboratory lines should be associated with fitness declines,
389 and be eliminated by selection (Nuzhdin et al. 1997). However, accumulation of TE insertions in
390 individual genotypes, or overall, in genotypes kept in small mass cultures appears to be the rule
391 rather than the exception (Pasyukova 2004; Rahman et al. 2015; Signor 2020). Muller's rachet
392 may be responsible for the accumulation of insertions, even if they are deleterious (Muller 1932;
393 1964). What is clear is that TEs are important sources of spontaneous mutations in *Drosophila*,

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394 and that in laboratory lines, over time, they may make up a large fraction of the total number of
395 mutations in particular genotypes.

396 Availability of data and materials
397 All raw data is available at NCBI SRA PRJNA410238.

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401

402 Competing interests
403 We declare that we have no competing interests.
404

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408 Authors' contributions
409 S.S. conceived of the study, performed bioinformatics, and drafted portions of the manuscript. Z.T.
410 performed bioinformatics, interpreted the data, and contributed to the manuscript draft.
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