Transposable elements in individual genotypes of *Drosophila simulans*

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Abstract

Transposable elements are mobile DNA sequences that are able to copy themselves within a host's genome. Within insects they often make up a substantial proportion of the genome. While they are the subject of intense research, often times when copy number is estimated it is estimated only at the population level, or in a limited number of individuals within a population. However, an important aspect of transposable element spread is the variance between individuals in activity. Do transposable elements accumulate at different rates in different genetic backgrounds? Using two populations of *Drosophila simulans* from California and Africa I estimated transposable element copy number in individual genotypes. Some active transposable elements seem to be a property of the species, while others of the populations. I find that in addition to population level differences in transposable element load certain genotypes accumulate transposable elements at a much higher rate than others. Most likely active transposable elements are fairly rare, and were inherited only by specific genotypes that were used to create the inbred lines. Whether or not this reflects dynamics in natural populations, where transposable elements may accumulate in specific genotypes and maintain themselves in the population rather than being active at low levels population wide, is an open question.

Introduction

Transposable elements are mobile DNA sequences that are able to move around the genome, potentially increasing their copy number in the process. They are abundant and contribute substantially to genetic variation in populations, influencing genome evolution (Biémont *et al.*, 1999; Bennetzen, 2000; Feschotte, 2008). The number and location of transposable element insertions can vary substantially between species, populations, and individuals (Vieira *et al.*, 1999; Vieira & Biémont, 2004; C Vieira, 2008; Kofler & Schlötterer, 2015; Kofler *et al.*, 2015b; Jakšić *et al.*, 2017; Kofler *et al.*, 2018). Introgression of transposable elements between species is also common, and has been reported frequently (Kofler *et al.*, 2015a; Hill & Betancourt, 2018). In addition, they make up a substantial proportion of the genome in many invertebrates and plants (Bennetzen, 2000; Tenaillon *et al.*, 2010; Kofler *et al.*, 2015b). Transposable elements are divided into three categories: long terminal repeat retrotransposons, short interspersed nuclear element retrotransposons, and transposons. The latter moves through DNA excision and repair, while the former two transpose via an RNA intermediate.

The most well studied transposable element population in insects is that of *Drosophila melanogaster*. It has been reported that in *D. melanogaster* total copy number of transposable elements may be similar between individuals, but the location of insertions can vary considerably (Barrón *et al.*, 2014). Overall copy number is thought to be maintained by transposition-selection balance, though new transposable elements may initially have high transposition rates as the host machinery evolves new defenses (Yang & Nuzhdin, 2003; Pasyukova, 2004; Slotkin & Martienssen, 2007; Johnson, 2010; Lee & Langley, 2012; Romero-Soriano & Garcia Guerreiro, 2016). Transposable elements also accumulate in heterochromatic regions, potentially because these regions are thought to be ‘transcriptionally silent’, and thus the deleterious effects of their

insertion are mitigated (Dimitri, 2003). However, they can also contribute to variation in quantitative traits, differences in fitness, and changes in gene expression (Mackay 1984; Mackay 1989, Shrimpton et al. 1990, Mackay et al. 1992, Long et al. 2000) . Some transposable elements have been associated with increases in fitness due to changes in gene regulation (Aminetzach *et al.* 2005; Mateo *et al.*, 2014)).

The recent explosion of population level genomic data has greatly increased our ability to estimate transposable element load in populations. Much of this work on transposable element copy number has occurred in *Drosophila*, where the existence of multiple sequenced inbred panels lend themselves to estimating copy number and insertion site frequency . However, differences in the number of transposable element insertions are thought to accumulate in inbred laboratory lines because active copies of transposable elements are rare and will be inherited only in some lines (Nuzhdin *et al.*, 1997). This is potentially mirroring what occurs in natural populations, where transposable element site heterogeneity may potentially be due to frequent transpositions in rare flies rather than low levels of transposition population-wide (Nuzhdin, 2000). Many studies of transposable element insertions estimate population-level insertion rate using Pool-seq. While Pool-seq is an effective tool for estimating population-level frequency, understanding the dynamics of transposable element spread requires the ability to estimate the variance between genotypes in transposable element copy number, in addition to population-level variation. Here I address this question by using previously sequenced lines of *D. simulans* from two populations to directly estimate transposable element copy number in individual genotypes. I estimate variance in transposable element copy number between inbred genotypes, differences between wild and inbred lines, and differences between the populations in the mean and variance of transposable element copy number.

92

93 **Methods**

94 *Fly lines*

95 21 African *D. simulans* isofemale lines were collected by William Ballard in 2002 from
96 Madagascar and Peter Andolfatto in 2006 from Kenya (Jackson *et al.*, 2017). They were inbred
97 in the lab for nine generations, during which time five were lost and DNA was collected from the
98 initial stocks. These will be used as an estimate of 'wild' *Drosophila* transposable element load,
99 compared to inbred lines. The raw reads are 90 bp paired end illumina sequencing and they were
100 downloaded from SRA PRJEB7673 (Jackson *et al.*, 2017). The first read from each pair was
101 used for mapping. The California lines were collected from the Zuma Organic Orchard in Los
102 Angeles, CA on two consecutive weekends of February 2012, from a pile of rotting strawberries
103 (Signor *et al.*, 2017; Signor & Nuzhdin, 2018; 2019). Reads were single end 100 bp and this
104 project has been deposited at the SRA under accession SRP075682.

105 *Mapping and copy number estimation*

106 Reads were mapped using bwa mem version 0.7.15 to the *D. simulans* 2.02 assembly and the 179
107 consensus transposable element sequences from EMBL, downloaded from Flybase.org (Li
108 2015). Of these 128 were used for the analysis, removing those from non-*D. melanogaster*
109 species that did not have a presence in *D. simulans*. Bam files were sorted and index with
110 samtools v.1.9 and optical duplicates were removed using picard MarkDuplicates
111 (http://picard.sourceforge.net) (Li *et al.*, 2009; McKenna *et al.*, 2010). Reads with a mapping
112 quality of below 15 were removed (this removes reads which map equally well to more than one
113 location). Using read coverage to determine copy number has been compared to other methods
114 and is neither permissive nor conservative (Srivastav & Kelleher, 2017). Transposable element

115 copy number was estimated per family by comparing average coverage of the transposable
116 sequence to average coverage of chromosome arm 2L in R, and computing population averages
117 and individual copy number (Hill *et al.*, 2015). P-values of comparisons between means and
118 variances were corrected for multiple testing using Bonferroni correction.

119 *SNPs and summary statistics*

120 I called SNPs within the transposable elements and the genomes using GATK Haplotypecaller
121 (McKenna *et al.*, 2010). SNPs were filtered for a minimum depth of four. SNPs were not filtered
122 for missing calls given that not at all individuals will share insertions. Tajima's *D* was estimated
123 in windows of 1 kb using VCFtools, and prior to estimation indels and SNPs with more than two
124 alleles were removed (Danecek *et al.*, 2011). The site frequency spectrum of SNPs was
125 calculated in R to better understand insertion dynamics of transposable elements in *D. simulans*.

126

127 **Results and Discussion**

128 *Population-level variation*

129 Of the 128 elements examined in the population, 85 have different mean numbers of insertions
130 between the two populations (t-test, Bonferroni corrected $p = .05/128$). Of those, only 17 are
131 higher in the African populations, suggesting that overall the CA population has more
132 transposable element insertion sites. Indeed, overall Californian *D. simulans* have an average of
133 1,797 insertions per genotype, while African *D. simulans* have 1,496. The five elements with the
134 largest difference in copy number in Californian *D. simulans* compared to African are the *INE-1*,
135 *Tc1*, *transib2*, *1360*, and *Cr1a*. These are present on average in 37 more copies in Californian *D.*
136 *simulans*. The elements more common in Africa include *baggins*, *X-element*, *R1A1-element*,
137 *diver2*, *Rt1c*, *Rt1b*, *jockey*, *ninja*, *Osvaldo*, *gypsy*, *Bari1*, *gypsy8*, *Tirant*, *mariner*, *BS3*, *G5A*, and

138 *aurora-element*. *G5A*, *gypsy8*, *X-element*, *baggins*, *R1A1-element*, *copia*, *G7*, *invader6*, *diver*,
139 *hobo*, *invader3*, *Rt1b*, *Rt1c*, *Bari1*, and *Tirant* also have significantly different (and higher)
140 variance compared to Californian *D. simulans* (F-test, Bonferroni corrected $p = 0.05/128$). 20
141 elements have different and larger variance in the Californian *D. simulans* compared to African
142 (F-test, Bonferroni corrected $p = 0.05/128$). This includes *Stalker4*, *Tc3*, *frogger*, *F-element*, *412*,
143 *accord*, *G4*, *rover*, *17.6*, *HMS-Beagle*, *gypsy6*. The most abundant transposable elements in the
144 California population were the highly abundant *INE-1*, *Cr1a*, *G6*, *1360*, *transib2*, *Tc1*, *baggins*,
145 and *roo*. In the African populations this is similar, *INE-1*, *Cr1a*, *G6*, *baggins*, *R1A1-element*,
146 *diver2*, *X-element*, and *HB*, followed by *1316* and *roo*.

147 *Stalker4, Stalker*, *Bari2*, *Tc3*, *G7*, and *Tart-C* were never present as more than a fraction
148 of an element in any individual, and in general coverage was limited to small regions within
149 these elements. This suggests that if these elements are present they are old and degraded. *G3*
150 and *hopper2* are estimated as being present in ~1 copy per individual in both populations,
151 however closer inspection reveals that all reads are mapping to the start and end of the elements.
152 This suggests that the only copy or copies present have internal deletions. For the *G-element* all
153 but a small fraction of reads map to one 140 bp sequence, but they map abundantly to that
154 particular sequence, such that the copy number is estimated as close to 6 per individual. Again,
155 reads which map equally well to more than one location were filtered out, thus this does not
156 represent non-specific mapping to repetitive elements. These patterns are not seen in the other
157 transposable elements, where coverage is even across the length of the element.

158 The *D. melanogaster pogo* and *Helitron* elements were not present in these populations,
159 which has been previously noted, suggesting that these transposable elements are not present in
160 *D. simulans* (Kofler *et al.*, 2015b). A full-length version of *Quasimodo* (two copies) and *gypsy6*

161 (one copy) were present in one individual. In other individuals reads map to very specific small
162 regions of the transposable elements, suggesting old and degraded copies. *Stalker3* is also
163 present in one genotype as a full-length copy. There is no mapping in the African populations
164 and very little in Californian populations, suggesting that old or degraded copies are not present
165 in the rest of the genotypes sampled.

166 *Site frequency spectrum*

167 I examined the site frequency spectrum of each transposable element in African and Californian
168 *D. simulans* (Table 2). In some cases there are no polymorphisms (*Dmau\mariner*) or very few
169 (*Dmel\p-element*) therefore this is uninformative. Furthermore, overall the Californian
170 population has more intermediate frequency polymorphisms (measured using Tajima's *D*; Signor
171 et al. 2017) compared to the African population (Supplementary Figure 1), which may be
172 expected to affect the site frequency spectrum overall. There are different amounts of
173 information in these estimates, thus they must be interpreted along with Table 1 – for example
174 *Quasimodo* is really on present in two full length copies in a single individual, thus this
175 estimation of the site frequency spectrum is not informative with regards to the spread of
176 *Quasimodo* in the population.

177 Elements with site frequency spectrum heavily biased towards low frequency SNPs in
178 Californian *D. simulans* include *G6*, *flea*, and *Juan* (Figure 1, Table 2). In African *D. simulans*
179 this includes *Tabor*, *Transpac*, *flea*, *Juan*, *Bari1*, *G6*, and *accord* (Figure 1, Table 2). Thus in
180 both populations *G6*, *flea*, *Bari1*, and *Juan* likely have recent activity. This is consistent with
181 other work on *Juan*, which suggests it is actively transposing in the species (Kofler *et al.*,
182 2015b). The larger number of transposable elements with low frequency SNPs in African

183 populations may be due to the overall difference in the site frequency spectrum between
184 populations (Supplementary Figure 1; Signor *et al.* 2017).
185 *The p-element*
186 The *p-element* recently invaded *D. simulans* from *D. melanogaster* as described in (Kofler *et al.*,
187 2015a), however Pool-seq cannot tie *p-element* insertions to specific individuals, only determine
188 the average number of insertions. What was reported previously was 0.4 insertions in Florida
189 populations, and 29 in South Africa (Kofler *et al.*, 2015a). What we see in the California
190 population is an average of 2 insertions, however that is because the majority of individuals do
191 not have any insertions (137 individuals have less than .3 estimated copies, Figure 1). The
192 remaining individuals have between .5 and 39 copies. We recovered the single reported SNP in
193 the *p-element*, a G to A transition at position 2040. It is interesting that it is not invading
194 genotypes in the population at the same rate, but rather reaching high copy number in some
195 genotypes and not others (Nuzhdin 2000). However, given the observed 'outliers' (see next
196 section), one genotype for a given transposable element with high copy number, this may be
197 reflective of how some transposable elements invade populations. It is also possible that *p-*
198 *elements* are just proliferating in strains that contained an active copy prior to collection
199 (Nuzhdin & Mackay 1997). This was observed previously in lab strains of *D. melanogaster*,
200 though contamination and introgression may also have played a role (Rahman *et al.* 2015).
201 *Transposable elements in individual genomes*
202 Some transposable elements have considerably higher copy number in particular genotypes
203 compared to the population average. For example, in one genotype *Dsim\ninja* is present in 29
204 copies, compared to the population mean of three. *Dsim\ninja* has 10 fixed differences and 27
205 polymorphisms in this strain from the California population, and the population average is 7.5

206 fixed differences and 264 polymorphisms (Supplementary Figure 4 & 5). This suggests that
207 *Dsim\ninja* was recently active in this genome. This is true of several transposable elements
208 which have outliers in the population. *Stalker2* has an outlier with 17 fixed SNPs and 8
209 polymorphisms, compared to a population average of 14 fixed SNPs and 43 polymorphisms. The
210 *gypsy10* element has a single outlier individual with 203 fixed differences compared to *D.*
211 *melanogaster*, and 77 polymorphisms. The population mean is 158 fixed differences and 222
212 polymorphisms. Other transposable elements with large outliers in the California population
213 include *opus*, *blood*, *GATE*, *diver*, *Tabor*, *INE-1*, *diver2*, *idefix*, *1731*, *412*, and *297*.
214 Sampling of the African populations was much more limited thus less genotype-specific
215 variation is sampled, and indeed only two transposable elements had large outliers, both in the
216 same individual from Madagascar: *copia*, and *diver*. This individual had 11 fixed differences in
217 *copia*, and 20 polymorphisms, while the population average is 10 fixed differences and 78
218 polymorphisms (and 20 copies compared to 4-11 for the rest of the population). For *diver* this
219 individual had 20 fixed differences and 84 polymorphisms, compared to a population average of
220 12 fixed differences and 215 polymorphisms (and 30 copies compared to 4-10 for the rest of the
221 population). The outlier individual is one that was inbred in the lab. In general being inbred in
222 the lab is not affecting overall transposable element copy number however, as comparing
223 between lines that were sequenced directly upon collection and those that there inbred, there is
224 no significant difference between the mean number of transposable elements for any
225 transposable element family. All of this is to suggest that transposable element load varies
226 significantly between genotypes within a population, though which transposable elements are
227 able to 'wake up' and multiply may be shared between genotypes in different populations
228 (*diver*). That is to say that some recent transposable element activity is shared across the species

229 (*G6*), populations (*p-element*) and some is specific to genotypes within a population. Those that
230 'wake up' in individual lines appears to be due to sampling of individuals that are permissive or
231 contain active transposable elements, rather than an overall increase in transposable element
232 activity in inbred lines.

233 *Comparison to other studies*

234 Previously *HMS Beagle*, *blood*, *flea*, *opus*, *Stalker*, and *F-element* were described as
235 being present in 0-1 copies in most populations from a large survey, with some populations
236 having a significant number of insertions (Biémont *et al.*, 1999). In our populations all of these
237 elements with the exception of *Stalker* were present in on average more than six insertions. Of
238 these only *flea* has more low frequency polymorphisms suggesting that it has spread within the
239 genome recently. Vieira *et al.*, 1999 detected *17.6* at one insertion site in *D. simulans*, and here it
240 was present on average in 1 (CA) to 0.5 (AF) copies in these populations, which is more frequent
241 than recent papers such as Kofler *et al.*, 2015b which detected four copies in the population of
242 ~800 which was sampled. Vieira *et al.*, 1998 estimated 0-30 copies of *412* and 20-100 copies of
243 *roo* per genome including heterochromatin. We observed 5-42 copies of *412* in both populations,
244 with a single outlier at 73. For *roo* we observed 20-75 copies in both populations. Thus our
245 estimates are consistent with previous estimates for these elements that include heterochromatin.
246 It has also been suggested that *412* is recently active (Biémont *et al.*, 1999).

247 *Tirant* has previously been reported as having higher copy number in African *D.*
248 *simulans*, potentially due to a recent mobilization of the element (Fablet *et al.*, 2006). We find
249 that pattern here, including a higher variance in the African populations where copy number
250 ranges from 2 to 6.68, compared to 2-3.8 in California (Fablet *et al.*, 2006). Viera et al. 1999
251 sampled diverse *D. simulans* populations and found ~1 *Tirant* element per genome, though this

252 was limited to euchromatic regions and older *Tirant* insertions may potentially have accumulated
253 in heterochromatic regions. The *Dmau\mariner* element has a higher copy number in Africa than
254 in the Californian *D. simulans*, from 0-5 with an average of 2.33, compared to 0-3 with an
255 average of 1.22. *Dmau\mariner* also contains no polymorphisms, which is consistent with
256 previous work which found sequence identify between *mariner* copies of ~99% (Capy et al.
257 1990; Capy et al. 1992). This is also consistent with a recent spread of *Dmau\mariner* in *D.*
258 *simulans* (Capy et al. 1990; Capy et al. 1992). For *mariner2* we find copy number to be
259 relatively stable in both populations (9.6 in California, 7.3 in Africa), while in Kofler et al. 2015
260 *mariner2* is only reported in six copies in the entire population of ~800, though again this is
261 limited to euchromatic regions.

262 The *G6* element has a large difference from previously reported values, with an average
263 of 66 insertions in Californian *D. simulans* and 69 in African. However, only 37 insertions where
264 reported total for a previously estimated population of ~800 *D. simulans* isofemale lines (Kofler
265 *et al.*, 2015b). Only 18% of reads in Kofler et al. (2015) map to euchromatin, but this is not a
266 large enough fraction to explain the difference. In addition, in the populations reported here the
267 *G6* element has primarily low frequency polymorphisms (Table 2), suggesting that this is a
268 recent expansion of copy number. The population examined in Kofler et al. (2015) is from South
269 African in 2013, a later collection date than either population here, though it is possible that the
270 *G6* activation is geographically limited to more northern populations. Overall our estimates are
271 higher than the work of Kofler et al. 2015, which only estimates more than one insertion per line
272 for four transposable elements (*1360*, *hobo*, *roo* and *Tc-2*). This is conservative compared to
273 other estimates of euchromatic insertions, for example Vieira et al. 1999 use *in situ* hybridization
274 to estimate copy number and found more than one insertion on average for nineteen elements (of

36 measured, *412*, *blood*, *burdock*, *copia*, *coral*, *flea*, *gypsy*, *HMS beagle*, *mdg3*, *opus*, *roo*, *Tirant*, *dor*, *F-element*, *Helena*, *I-element*, *jockey*, *Bari1*, and *hobo*). There is other evidence this work may be conservative, for example Kaminker *et al.*, 2002 estimated 146 *roo* insertions in the euchromatin of the *D. melanogaster* reference genome, while Kofler *et al.*, 2015b estimates 1966 total for a population of 554 (approximately 3.6 per individual). This may also be due to inherent variability between populations.

Conclusions

The general picture that one draws from this summary is that non-African *D. simulans* have a higher insertion number than African populations. *D. simulans* is currently being invaded by transposable elements, and this spread is likely occurring concordant with the world-wide colonization of *D. simulans*, as has been posited by previous studies (Lachaise et al., 1988; Vieira et al., 1999; Biémont et al., 2003). However, African populations have their own transposable element dynamics, with some transposable elements seeming to share activity between populations (*G6*) and others being more active in African *D. simulans* (*baggins*, *Bari1*, etc.).

Transposable element load is an attribute of species, populations, and individual genotypes. In inbred laboratory lines active transposable element copies may be inherited by some lines and not others, and active transposable elements can accumulate over time (Nuzhdin *et al.*, 1997). This can cause differences over time in the number of insertions within a line and large differences between lines in transposable element copy number (Nuzhdin *et al.*, 1997). This may also be reflective of natural patterns in which transposable elements proliferate in particular genotypes rather than at low levels in the population as a whole (Nuzhdin, 2000). Overall,

looking at variance between individuals is an important part of understanding the ways in which transposable elements maintain themselves in populations.

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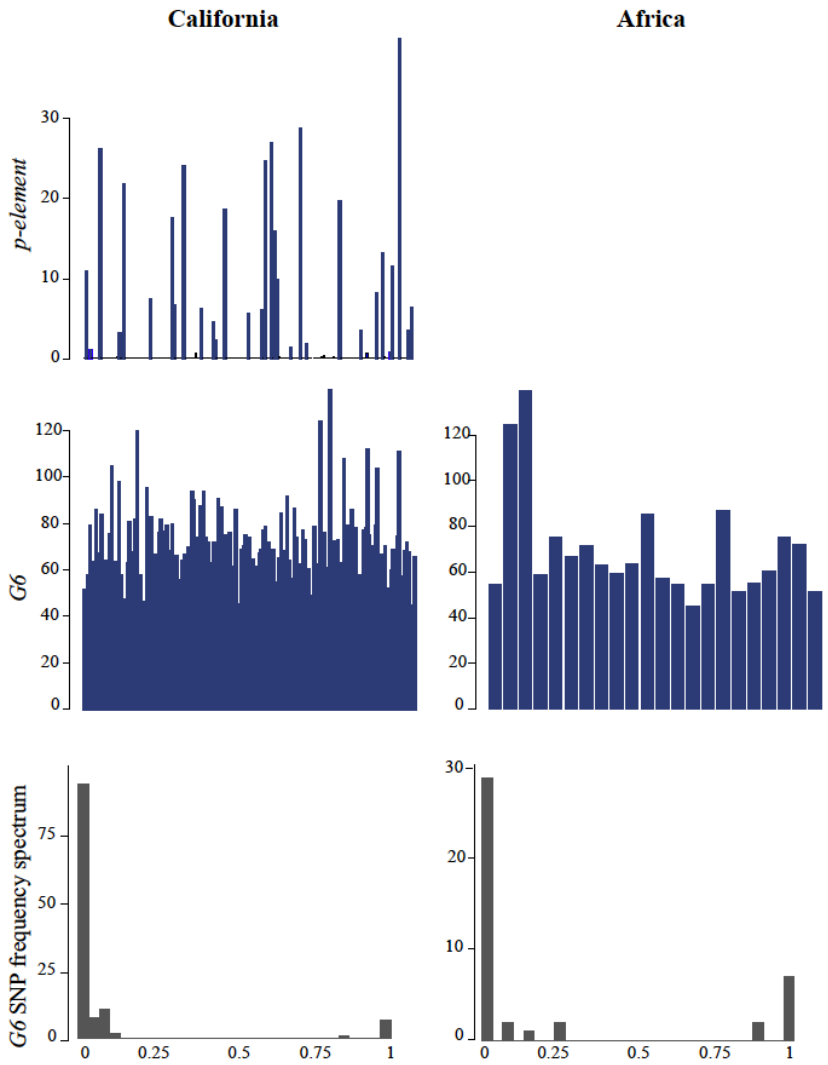

306 Figure 1: Estimated copy number for the *p-element* and *G6* in Californian and African *D.*
307 *simulans*. Each bar represents an individual from the population. As expected, the *p-element* was
308 not found in the African population sampled in the early 2000's, but by 2012 when the
309 Californian *D. simulans* was sampled it had invaded. *G6* has a high copy number in both
310 populations of *D. simulans*, which wasn't recorded in previous studies on African *D. simulans*.
311 The site frequency spectrum in the last row also suggests recent spread of the *G6* element in *D.*
312 *simulans*, as there are primarily low frequency SNPs. Note that while fixed SNPs are included in
313 this graph to illustrate divergence from D. melanogaster they are not included in the estimation
314 of average site frequency spectrum shown in Table 2.
315

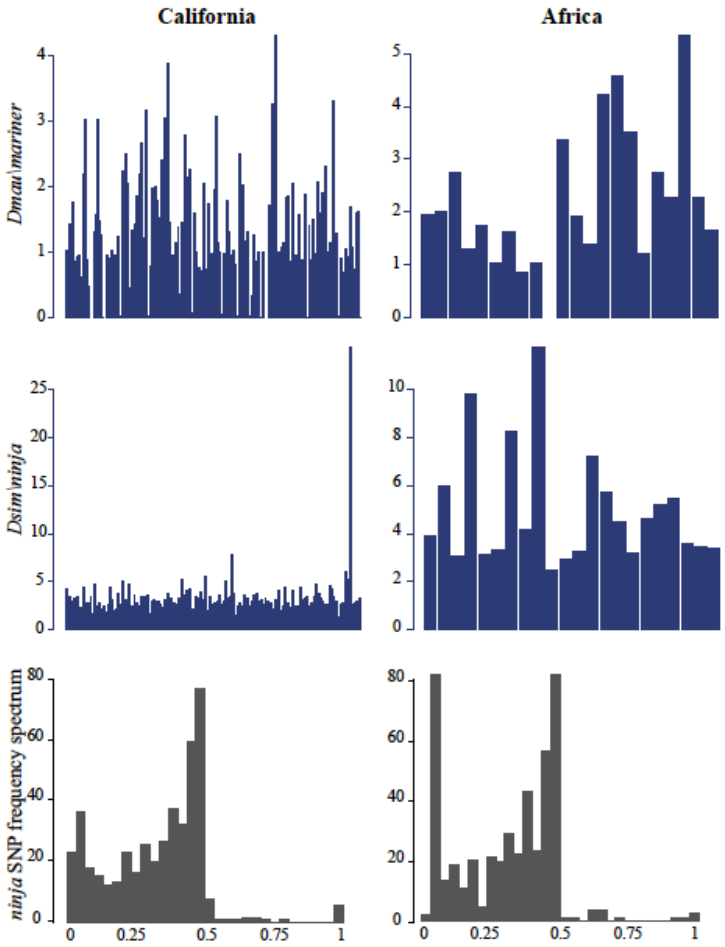

Figure 2: Estimated copy number for the two non-*D. melanogaster* transposable elements included here, *Dsim\ninja* and *Dmau\mariner*. Variance between individual genomes is large for transposable elements, for example in California *Dsim\ninja* varies from 1-29, and in Africa from 2-12. The individual with 29 copies of *Dsim\ninja* has 10 fixed SNPs within the element and 27 polymorphisms compared to other population members (for example, 239 polymorphisms and 5 fixed SNPs), suggesting that it has been recently active within that genome. The site frequency spectrum of *D. sim\ninja* is broad, suggesting that outside of the genome with an

active copy of *D.sim\ninja* this element has been diverging within this species for some time. In contrast, no SNPs were called in *Dmau\mariner*, suggesting recent colonization in *D. simulans*.

Element	CA Average	Variance	AF Average	Variance	Element	CA Average	Variance	AF Average	Variance	Element	CA Average	Variance	AF Average	Variance
297	18.44	7.87	12.11	8.31	*GATE*	26.98	12.73	28.32	13.07	*NOF*	1.04	0.21	0.97	0.32
412	27.78	31.66	14.77	5.39	*gtwin*	4.85	0.73	4.78	0.87	*opus*	7.64	5.03	6.85	9.54
1360	64.03	37.76	35.07	20.84	*gypsy*	4.97	1.06	5.92	2.02	*Osvaldo*	5.44	0.85	6.96	1.38
1731	6.69	6.86	7.10	3.65	*gypsy10*	8.37	5.13	6.34	3.94	*P-element*	2.36	44.92	-	-
17.6.	1.10	0.13	0.50	0.00	*gypsy11*	3.73	0.29	2.42	0.08	*pogo*	-	-	-	-
3S18	11.89	5.66	12.59	4.38	*gypsy12*	3.97	0.75	4.51	0.86	*Porto1*	10.02	0.65	7.49	0.39
accord	6.05	10.35	2.54	1.17	*gypsy2*	0.95	0.02	0.81	0.01	*Q-element*	0.95	0.03	0.72	0.01
accord2	4.84	0.81	3.23	0.41	*gypsy3*	3.45	0.95	3.08	0.73	*Quasimodo*	0.19	0.03	0.08	0.00
aurora-element	0.70	0.02	0.89	0.02	*gypsy4*	2.07	0.04	1.98	0.08	*R1-2*	1.13	0.05	1.21	0.11
baggins	52.65	15.51	62.04	42.68	*gypsy5*	3.68	1.12	2.26	0.52	*R1A1-element*	34.07	72.24	49.39	301.81
Bari1	4.92	4.58	18.05	30.07	*gypsy6*	0.39	0.01	0.31	0.00	*R2-element*	39.52	65.11	30.05	74.21
Bari2	0.12	0.00	0.11	0.00	*gypsy7*	0.86	0.01	0.64	0.00	*roo*	50.89	66.75	33.38	43.96
blood	12.12	11.30	7.98	10.57	*gypsy8*	4.77	0.69	6.38	1.95	*rooA*	19.29	1.30	18.61	1.19
BS	2.93	0.27	2.94	0.17	*gypsy9*	3.14	0.24	2.46	0.23	*rover*	1.24	0.15	0.58	0.03
BS3	6.41	0.63	6.95	0.51	*HB*	43.04	20.11	40.52	32.24	*Rt1a*	3.61	0.26	3.91	0.38
BS4	3.31	0.13	2.93	0.07	*Helena*	29.20	17.08	22.24	10.19	*Rt1b*	17.43	6.41	24.71	8.67
Burdock	12.73	4.35	9.27	3.04	*Helitron*	-	-	-	-	*Rt1c*	20.60	14.16	27.78	41.37
Circe	6.56	1.79	5.88	2.80	*HeT-A*	6.52	4.70	1.50	1.59	*S-element*	1.41	0.06	0.83	0.05
copia	9.22	4.00	6.52	11.85	*HMS-Beagle*	13.44	2.59	10.49	2.17	*S2*	0.73	0.01	0.57	0.00
Cr1a	125.69	88.15	97.02	39.36	*HMS-Beagle2*	0.82	0.01	0.69	0.00	*springer*	2.10	0.10	2.11	0.09
diver	6.75	8.19	8.14	26.95	*hobo*	32.87	12.41	31.79	32.23	*Stalker*	0.28	0.00	0.19	0.00
diver2	35.58	21.62	44.05	32.27	*hopper*	23.58	16.70	15.88	11.24	*Stalker2*	2.86	3.49	2.31	1.14
Dm88	8.43	0.45	8.34	0.34	*hopper2*	0.77	0.06	0.66	0.05	*Stalker3*	0.08	0.03	-	-
Dmau\mariner	1.22	0.72	2.33	1.54	*I-element*	37.33	35.49	21.37	13.78	*Stalker4*	0.09	0.00	0.06	0.00
Doc	25.20	24.77	23.59	37.24	*Idefix*	3.70	1.91	2.64	0.57	*Tabor*	9.95	44.94	5.81	3.25
Doc2-element	30.43	6.53	28.56	8.46	*INE-1*	193.45	296.57	116.27	104.78	*TAHRE*	3.09	1.10	1.01	1.35
Doc3-element	20.57	1.17	19.21	1.48	*invader1*	4.62	0.11	4.55	0.18	*TART-A*	1.05	0.24	0.54	0.09
Doc4-element	3.68	0.25	2.99	0.07	*invader2*	6.09	1.50	4.29	0.92	*TART-B*	0.94	0.20	0.36	0.15
Dsim\ninja	3.14	4.99	4.93	5.85	*invader3*	6.71	1.93	6.47	5.80	*TART-C*	0.02	0.00	0.01	0.00
F-element	28.41	11.23	19.78	1.48	*invader4*	3.24	0.08	3.07	0.06	*Tc1*	54.48	36.25	31.21	10.81
FB	22.44	20.74	13.75	14.15	*invader5*	0.66	0.01	0.56	0.00	*Tc1-2*	12.45	2.47	8.46	1.85
flea	13.71	10.09	10.85	13.78	*invader6*	5.85	1.55	6.20	3.67	*Tc3*	0.15	0.00	0.06	0.00
frogger	1.05	0.06	0.89	0.01	*Ivk*	14.98	2.26	12.87	2.72	*Tirant*	2.77	0.06	3.58	2.17
Fw2	6.50	0.20	5.64	0.14	*jockey*	8.73	3.10	11.61	4.33	*Tom1*	26.03	6.41	17.00	7.94
Fw3	3.01	0.07	2.89	0.16	*jockey2*	5.55	0.15	4.68	0.04	*transib1*	19.12	8.84	12.41	4.99
G-element	4.22	0.93	4.08	1.26	*Juan*	21.46	27.13	18.68	13.75	*transib2*	55.12	44.06	27.48	39.43
G2	25.99	15.12	23.93	16.78	*looper1*	2.24	0.11	1.56	0.07	*transib3*	2.81	0.07	2.07	0.07
G3	0.91	0.01	0.76	0.01	*mariner2*	9.61	0.54	7.34	0.28	*transib4*	4.14	0.17	2.80	0.16
G4	4.10	0.23	3.52	0.06	*Max-element*	29.78	17.50	32.43	15.97	*Transpac*	8.21	4.81	5.35	4.09
G5	15.05	2.32	15.99	2.77	*McClintock*	13.49	8.00	6.86	2.81	*X-element*	34.10	8.98	41.49	23.77
G5A	10.93	0.66	11.67	2.70	*mdg1*	6.43	1.00	4.95	0.85	*Xanthias*	9.43	3.14	8.90	6.90
G6	66.62	309.64	69.36	532.88	*mdg3*	7.02	4.73	5.94	3.01	*ZAM*	2.92	0.12	2.01	0.23
G7	0.18	0.00	0.20	0.01	*micropia*	8.01	4.05	6.53	2.71					

Table 1: Summary statistics for copy number of transposable elements from the Californian and African *D. simulans*.

Element	CA Average	AF Average	Element	CA Average	AF Average	Element	CA Average	AF Average
297	0.29	0.29	*GATE*	0.28	0.53	*NOF*	0.37	0.37
412	0.31	0.31	*gtwin*	0.35	0.35	*opus*	0.38	0.38
1360	0.26	0.26	*gypsy*	0.35	0.35	*Osvaldo*	0.37	0.37
1731	0.23	0.23	*gypsy10*	0.35	0.35	*P-element*	-	-
17.6.	0.44	0.44	*gypsy11*	0.44	0.44	*pogo*	-	-
3S18	0.16	0.16	*gypsy12*	0.35	0.35	*Porto1*	0.41	0.41
accord	0.08	0.08	*gypsy2*	0.42	0.42	*Q-element*	0.32	0.32
accord2	0.36	0.36	*gypsy3*	0.33	0.33	*Quasimodo*	0.05	0.05
aurora-element	0.31	0.31	*gypsy4*	0.22	0.22	*R1-2*	0.33	0.33
baggins	0.29	0.29	*gypsy5*	0.24	0.24	*R1A1-element*	0.32	0.32
Bari1	0.05	0.05	*gypsy6*	0.33	0.33	*R2-element*	0.35	0.35
Bari2	0.40	0.40	*gypsy7*	0.24	0.24	*roo*	0.14	0.14
blood	0.18	0.18	*gypsy8*	0.36	0.36	*rooA*	0.32	0.32
BS	0.34	0.34	*gypsy9*	0.41	0.41	*rover*	0.42	0.42
BS3	0.42	0.42	*HB*	0.34	0.34	*Rt1a*	0.39	0.39
BS4	0.37	0.37	*Helena*	0.19	0.19	*Rt1b*	0.29	0.29
Burdock	0.22	0.22	*Helitron*	-	-	*Rt1c*	0.35	0.35
Circe	0.28	0.28	*HeT-A*	0.43	0.43	*S-element*	0.42	0.43
copia	0.11	0.11	*HMS-Beagle*	0.32	0.32	*S2*	0.42	0.42
Cr1a	0.24	0.24	*HMS-Beagle2*	0.42	0.42	*springer*	0.35	0.35
diver	0.29	0.29	*hobo*	0.23	0.23	*Stalker*	0.28	0.28
diver2	0.28	0.28	*hopper*	0.33	0.33	*Stalker2*	0.15	0.15
Dm88	0.40	0.40	*hopper2*	0.37	0.37	*Stalker3*	-	-
Dmau\mariner	-	-	*I-element*	0.26	0.26	*Stalker4*	0.25	0.25
Doc	0.15	0.15	*Idefix*	0.38	3.70	*Tabor*	0.08	0.08
Doc2-element	0.20	0.20	*INE-1*	0.31	0.31	*TAHRE*	0.40	0.40
Doc3-element	0.35	0.35	*invader1*	0.39	0.39	*TART-A*	0.31	0.31
Doc4-element	0.37	0.37	*invader2*	0.29	0.29	*TART-B*	0.34	0.34
Dsim\ninja	0.30	0.30	*invader3*	0.20	0.20	*TART-C*	0.10	0.10
F-element	0.22	0.22	*invader4*	0.40	0.40	*Tc1*	0.34	0.34
FB	0.37	0.37	*invader5*	0.44	0.44	*Tc1-2*	0.40	0.40
flea	0.04	0.04	*invader6*	0.14	0.14	*Tc3*	0.08	0.08
frogger	0.17	0.17	*Ivk*	0.34	0.34	*Tirant*	0.35	0.35
Fw2	0.44	0.44	*jockey*	0.10	0.10	*Tom1*	0.29	0.29
Fw3	0.36	0.36	*jockey2*	0.40	0.40	*transib1*	0.30	0.30
G-element	0.53	0.53	*Juan*	0.06	0.06	*transib2*	0.34	0.34
G2	0.35	0.35	*looper1*	0.34	0.34	*transib3*	0.37	0.37
G3	0.50	0.50	*mariner2*	0.42	0.42	*transib4*	0.40	0.40
G4	0.33	0.33	*Max-element*	0.39	0.39	*Transpac*	0.04	0.04
G5	0.34	0.34	*McClintock*	0.19	0.19	*X-element*	0.25	0.27
G5A	0.37	0.37	*mdg1*	0.40	0.40	*Xanthias*	0.25	0.25
G6	0.10	0.10	*mdg3*	0.15	0.15	*ZAM*	0.31	0.31
G7	0.36	0.36	*micropia*	0.24	0.24			

Table 2: Average site frequency spectrum for each element, excluding sites that are fixed relative to *D. melanogaster*. *Dmau\mariner* and the *p-element* are present in at least one population, but have no polymorphic SNPs. Other elements without an estimated site frequency spectrum are not present in the population.

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