# High-Throughput Sequencing of Transposable Elements Insertions Provides Evidence for Adaptive Evolution of the Invasive Asian Tiger Mosquito Towards Temperate Environments

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**Keywords:** Invasive species, *Aedes albopictus*, local adaptation, genome scan, Transposable Elements, diapause

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21 **Running title:** Molecular adaptation in *Ae. albopictus*

# Abstract

Invasive species represent unique opportunities to evaluate the role of local adaptation during colonization of new environments. Among these, the Asian tiger mosquito, *Aedes albopictus*, is a threatening vector of several human viral diseases, including dengue, chikungunya and the emerging Zika fevers. Its broad presence in both temperate and tropical environments has sometimes been considered as the reflect of a great "ecological plasticity". However, no study has been conducted to assess the role of adaptive evolution in the ecological success of *Ae. albopictus* at the molecular level. In the present study we performed a genomic scan to search for potential signatures of selection leading to local adaptation in a hundred of field collected mosquitoes from native populations of Vietnam and temperate invasive populations of Europe. High throughput genotyping of transposable element insertions generated more than 120 000 polymorphic loci, which in their great majority revealed a virtual absence of structure between bio-geographic areas. Nevertheless, 92 outlier loci show a high level of differentiation between temperate and tropical populations. The majority of these loci segregates at high insertion frequencies among European populations, indicating that this pattern could have been caused by recent events of adaptive evolution in temperate areas. Six outliers were located near putative diapause effector genes, suggesting fine-tuning of this critical pathway during

42 local adaptation.

43 # Introduction

44 Biological invasions represent unique opportunities to study fast evolutionary changes
45 such as adaptive evolution. Indeed, settlement in a novel area represents a biological
46 challenge that invasive species have successfully overcome. The underlying processes
47 could be studied at the molecular level, particularly to gather empirical knowledge about
48 the genetics of invasions, a field of study that has produced extensive theoretical
49 predictions, but for which there is still little evidence in nature (Colautti & Lau 2015).
50 Some of the main concerns are to disentangle the effects of neutral processes during
51 colonization, such as founder events or allele surfing at the migration front, from
52 adaptive evolution (i.e. local adaptation, Lande 2015; Peischl & Excoffier 2015; Colautti
53 & Lau 2015).

54 Adaptation can arise either through the appearance and spread of a new beneficial
55 mutation, the spread of a favorable allele from standing genetic variation, or from
56 hybridization in the introduction area (Handley *et al.* 2011; Colautti & Lau 2015; Bock *et*
57 *al.* 2015). Detection of the footprint of natural selection is however dependent on the
58 availability of informative genetic markers, which should provide a substantial coverage
59 of the genome to allow selection scans and be easily and confidently scored across
60 many individuals. Unfortunately, invasive organisms are rarely model species, making
61 the development of a reliable and efficient marker challenging.

62 The Asian tiger mosquito, *Aedes* (*Stegomya*) *albopictus* (Diptera:Culicidae) is currently
63 one of the most threatening invasive species (Invasive Species Specialist Group).

64 Originating from South-Eastern Asia, this species is one of the primary vectors of
65 Dengue and Chikungunya viruses, and is also involved in the transmission of other
66 threatening arboviruses (Paupy *et al.* 2009), in particular the newly emerging Zika virus
67 (Grard *et al.* 2014; Marcondes & Ximenes 2015; Chouin-Carneiro *et al.* 2016). *Ae.*
68 *albopictus* has now settled in every continent except Antarctica, and is found both under
69 tropical and temperate climates (Bonizzoni *et al.* 2013). While this species is supposed
70 to originate from rain forests of South-Eastern Asia (Hawley 1988), the native area of
71 *Ae. albopictus* encompasses contrasted environments including temperate regions of
72 Japan and China, offering a large potential of fit towards newly colonized environments.
73 For example, the induction of photoperiodic diapause in temperate areas, that has a
74 genetic basis in *Ae. albopictus* (Hawley *et al.* 1987; Hanson & Craig 1994; Urbanski *et*
75 *al.* 2010), is decisive to ensure invasive success in Europe or Northern America. Indeed
76 it allows the susceptible populations to survive through winter at the larval stage into the
77 eggs. Such a trait appears governed by a “genetic toolkit” involving numerous genes
78 and metabolic networks, for which however the genetic polymorphism between
79 diapausing and non-diapausing strains remains to be elucidated (Poelchau *et al.*
80 2013b). In addition, the colonization of new areas that look similar at first glance can still
81 involve *de novo* adaptation: indeed, even environment sharing climatic variables are not
82 necessarily similar regarding edaphic and biotic interactions (Colautti & Lau 2015).
83 Hence, this suggests that whatever are the native and settled environments, it might be
84 possible to find evidence of adaptive evolution in invasive populations of *Ae. albopictus*.

85 To better understand the invasive success of this species, we genotyped 140 field

86 individuals, collected from three Vietnamese (native tropical area) and five European
87 (invasive temperate area) populations, aiming to identify genomic regions involved in
88 local adaptation. To do so, we developed new genetic markers, based on high
89 throughput genotyping of the insertion of Transposable Elements (TEs) that represent at
90 least one third of the genome of *Ae. albopictus* and include recently active families that
91 could reach thousands of copies in one genome (Goubert *et al.* 2015).

92 Amplification of TE insertions is particularly efficient to obtain a large number of genetic
93 markers throughout one genome (Bonin *et al.* 2008), especially if few genomic
94 resources are available (Monden *et al.* 2014), which was case until recently for the
95 Asian tiger mosquito. In addition, such markers represent a seducing alternative to other
96 methods of diversity reduction, such as RAD-sequencing (Miller et al. 2007), that could
97 be less efficient in species with high TE load (Davey et al. 2012) and did not produce
98 satisfying results in *Ae. albopictus* (Goubert *et al.* 2016). In mosquitoes, TEs have been
99 shown to be powerful markers both for population structure analysis (Biedler *et al.* 2003;
100 Boulesteix *et al.* 2007; Santolamazza *et al.* 2008; Esnault *et al.* 2008) and genome
101 scans (Bonin *et al.* 2009). We hypothesized that some TE insertion sites could be
102 located at the neighborhood of targets of natural selection and thus could reach high
103 level of differentiation between native and invasive populations if selective sweeps
104 occurred during local adaptation. In addition, some TEs could also insert near or inside
105 coding regions and thus could be directly involved in environmental adaptation
106 (Casacuberta & González 2013), eventually contributing to the success of invasive
107 species (Stapley *et al.* 2015).

108 To distinguish between neutral demographic effects and adaptive evolution, we first
109 performed population genetic analyses to reveal the global genetic structure of the
110 studied populations. We then performed a genomic scan for selection and identified 92
111 candidate loci under directional selection, among which several can be located in the
112 neighborhood of diapause related genes.

# 113 Material and Methods

### 114 Biological samples

115 A total number of 140 flying adult females *Ae. albopictus* were collected in the field at
116 eight sampling sites in Europe and Vietnam during the summers of 2012 and 2013
117 (Figure 1 and Table S1). Individuals were either sampled using a single trap or using
118 aspirators through the sampling site within a 50 meters radius. When traps were used,
119 live mosquitoes were collected after a maximum of two days.

### 120 High throughput Transposon Display (TD) genotyping.

121 Insertion polymorphism of five transposable elements families: I Loner Ele1 (IL1), Loa
122 Ele2B (L2B), RTE4, RTE5 and Lian 1 identified by Goubert *et al.* (2015) in *Ae.*
123 *albopictus* was characterized using Transposon Display (TD), a TE insertion specific
124 PCR method, combined with Illumina sequencing of all TD amplification products
125 (Figure S1). These TE families were chosen according to their high estimate of copy
126 number (from 513 to 4203 copies), high identity between copies, and a “copy and paste”
127 mode of transposition (all these TEs are non-LTR Class I retrotransposons).

128 **DNA Extraction and TD adapter ligation.** Total DNA was extracted from whole adult
129 bodies following the Phenol-Chloroform protocol described by Minard *et al.* (2015). TD
130 was then set up combining methods from previous studies (Munroe *et al.* 1994; Roy *et*
131 *al.* 1999; Akkouche *et al.* 2012; Carnelossi *et al.* 2014). First, individual extracted DNA
132 (≈ 75ng) was used for enzymatic digestion in a total volume of 20 *µ*L, with HindIII
133 enzyme (10U/*µ*L) and buffer R (Thermo Scientific) for 3 hours at 37C. The enzyme was
134 inactivated at 80C for 20 minutes. TD adapters were then build hybridizing Hindlink with
135 MSEB oligonucleotides (100*µ*M, see Table S2) in 20X SSC and 1M Tris in a total
136 volume of 333*µ*L after 5mn of initial denaturation at 92C and 1h at room temperature for
137 hybridization of the two parts. Once ready, TD adapters were then ligated to 20 *µ*L of the
138 digested DNA mixing 2*µ*L of TD adapter with 10U T4 ligase and 5X buffer (Fermentas)
139 in a final volume of 50*µ*L for 3 hours at 23C.
140

141 **Library construction.** For each individual, and for each of the five TE families, TE
142 insertions were amplified by PCR (PCR 1) in a Biorad Thermal Cycler (either C1000 or
143 S1000), in a final volume of 25*µ*L. Mixture contained 2*µ*L of digested-ligated DNA with
144 1*µ*L dNTPs (10mM), 0.5*µ*L TD-adapter specific primer (LNP, 10*µ*M, see S3 Table) and
145 0.5 *µ*L of TE specific primer (10*µ*M), 1U AccuTaq polymerase (5U/*µ*L) with 10X buffer
146 and Dimethyl-Sulfoxyde (Sigma). Amplification was performed as follows: denaturation
147 at 98C for 30 seconds then 30 cycles including 94C for 15 seconds, hybridization at 60C
148 for 20 seconds and elongation at 68C for 1 minute; final elongation was performed for 5
149 minutes at 68C. For L2B and RTE5 TEs, a nested PCR was performed in order to

150 increase specificity in the same PCR conditions using internal forward TE primers and
151 LNP (Table S2). PCR 1 primers include a shared tag sequence that was used for
152 hybridization of the individual indexes by PCR 2.

153 For each TE, three independents PCR 1 were performed from the same digestion
154 product. PCR 1 products (3 PCR * 5 TE per individual) were then purified using volume-
155 to-volume Agencout AMPure XP beads (20*µ*L PCR 1 + 20*µ*L beads) and eluted in 30*µ*L
156 Resuspension buffer. After nanodrop quantification, equimolar pools containing the 3*5
157 PCR products per individual were made using Tecan EVO200 robot. Individual pools
158 were then size selected for fragment ranging from 300 to 600 bp using Agencout
159 AMPure XP beads as follow: first magnetic beads were diluted in H2O with a ratio of
160 1:0.68 then add to 0.625 X PCR products in order to exclude long fragments. A second
161 purification was performed using a non-diluted bead: DNA ratio of 1:8.3 to exclude small
162 fragments.
163 Samples' multiplexing was performed using home made 6 bp index (included in SRA
164 individual name), which were added to the R primer (Table S2) during a second PCR
165 (PCR 2) with 12 cycles in ABI 2720 Thermal Cycler. Mixture contained 15ng PCR
166 products, 1*µ*l of dNTPs (10mM), 0.5*µ*l MTP Taq DNA Polymerase (5U/*µ*l, Sigma), 5*µ*l
167 10X MTP Taq Buffer and 1.25*µ*l of each tagged-primer (20*µ*m) in a final volume of 50*µ*l.
168 Amplification was performed as follows: denaturation at 94C for 60 seconds then 12
169 cycles including denaturation at 94C for 60 seconds, hybridization at 65C for 60 seconds
170 and elongation at 72C for 60 seconds; final elongation was performed for 10 minutes at
171 72C. PCR 2 products were purified using Agencout AMPure XP beads: DNA ratio of

172 1:1.25 to obtain libraries. Finally, TD products were paired-end sequenced on an
173 Illumina Hiseq 2000 (1 lane) at the GeT-PlaGe core facility (Genome and
174 Transcriptome, Toulouse) using TruSeq PE Cluster Kit v3 (2x100 bp) and TruSeq SBS
175 Kit v3.

176 **Bioinformatic treatment of TD sequencing.**

177 The different steps of the informatics treatment from the raw sequencing dataset to
178 population binary matrices for presence/absence of TE insertions per individual are
179 described in Figure S2. First, the paired-end reads of each individual were quality
180 checked and trimmed using UrQt v. 1.0.17 (Modolo & Lerat 2015) with standard
181 parameters and a *t* quality threshold of 10. Reads pairs were then checked and trimmed
182 for Illumina adapter contamination using cutadapt (Martin 2011). Specific amplification of
183 TE insertions was controlled by checking for the expected 3' TE sequence on the R1
184 read using Blat (Altschul *et al.* 1990) with an identity threshold of 0.90. Only reads with
185 an alignment-length/read-length ratio ≥ 0.90 were then retained. R2 reads for which
186 the R1 mate passed this filter were then selected for the insertion loci construction, after
187 the removal of the TD adapter on the 5' start using cutadapt and the removal of reads
188 under 30 bp. Selected reads were separated in each individual according to the TE
189 families for loci construction.

190 In order to correct the inter-individual coverage variations, we performed a sampling of
191 the cleaned reads. First, for each TE family, distribution of the number of read per
192 individual was drown, and individuals with less reads than the first decile of this
193 distribution were removed; then cleaned reads of the remaining individuals were

194 randomly sampled at the value of the first decile of coverage (this value varies among
195 TE families). For each TE, the sampled reads of each retained individual were clustered
196 together using the CD-HIT-EST program (Li & Godzik 2006) to recover insertion loci.
197 During this all-to-all reads comparison, the alignments must had a minimum of 90
198 percent identity, and the shortest sequence should be 95% length of the longest, global
199 identity was used and each read was assigned at its best cluster (instead of the first that
200 meet the threshold). In a second step, the reference reads of each locus within
201 individual, given by CD-HIT-EST, were clustered with all the reference reads of all
202 individuals, using the same threshold, in order to build the locus catalog including list of
203 loci of all individuals and the coverage for each locus in each individual. After this step,
204 insertion loci that matched known repeats of the Asian tiger mosquito (Goubert *et al.*
205 2015) were discarded; alignments were performed with Blastn using default parameters.

206 Since the quality control removed a substantial number of reads for the construction of
207 TE insertions catalog, the raw R2 reads (with their TD adapter removed), that could
208 have been discarded in a first attempt were then mapped over the catalog in order to
209 increase the scoring sensibility. Before mapping, the raw R2 reads were also sampled at
210 the first decile of individual coverage (as described previously). At this step, individuals
211 that have been removed from at least two TE families for loci construction were
212 definitively removed from the whole analysis. Mapping was performed over all insertion
213 loci of all TE families in a single run in order to prevent multiple hits. Blat was used with
214 an identity threshold of 90 percent. Visual inspection of alignment quality over 30
215 sampled loci per TE family was performed in order to ensure the quality of scoring.

216 In order to check if the sampling procedure would affect our results, the read sampling
217 procedures and subsequent analysis were performed independently 3 times (replicates
218 M1, M2 and M3).

219

220 **Genetic analyses and Genomic scan.**

221 Population structure analyses were performed independently for each TE family.
222 Principal Coordinate Analysis (PCoAs) were performed to identify genetic clusters using
223 the ade4 package (Dray & Dufour 2007) of R 3.2.1 (R development core team 2015). S7
224 coefficient of Gower and Legendre was used as a genetic distance since it gives more
225 weight to shared insertions. Shared absences were not used because they do not give
226 information about the genetic distance between individuals. Pairwise populations $F_{ST}$
227 were computed using Arlequin 3.5 (Excoffier & Lischer 2010); significance of the index
228 was assessed over 1,000 permutations using a significance threshold of 0.05.

229 The genomic scan was performed in two steps for each of the sampling replicates of
230 each TE. First, Bayescan 2.1 (Foll & Gaggiotti 2008) was used to test for each locus
231 deviation from neutrality. Bayescan consider a fission/island model where all
232 subpopulations derive from a unique ancestral population. In this model, variance in
233 allele frequencies between subpopulations is expected to be due either to the genetic
234 drift that occurred independently in each subpopulation or to selection that is a locus-
235 specific parameter. The differentiation at each locus in each subpopulation from the
236 ancestral population is thus decomposed into a $\beta$ component (shared by all loci in a

237 subpopulation) and is related to genetic drift, and a $\alpha$ component (shared for a locus by
238 all subpopulations) due to selection. Using a Bayesian framework, Bayescan tests for
239 each locus the significance of the $\alpha$ component. Rejection of the neutral model at one
240 locus is done using posterior Bayesian probabilities and controlled for multiple testing
241 using false discovery rate. In addition, Bayescan manage uncertainty about allele
242 frequency from dominant data such as the TD polymorphism, leaving the $F_{IS}$ to freely
243 vary during the estimation of parameters. Bayescan was used with default values except
244 for the prior odds that were set to 100 (more compatible with datasets with a large
245 number of loci, see Bayescan manual), and a significance *q*-value threshold of 0.05 was
246 used to retain outlier loci. In a second step, only outliers suggesting divergent directional
247 selection between, Europe and Vietnam were considered. To identify them, locus-by-
248 locus Analyses of Molecular Variance (AMOVAs) were performed using Arlequin 3.5 for
249 each TE family. Significance of the $F_{CT}$ (inter group differentiation) between Vietnamese
250 and European populations was assessed performing 10,000 permutations with a
251 significance threshold of 0.05. For each dataset, Bayescan outliers were crossed with
252 significant $F_{CT}$ loci to retain candidate loci.
253 To identify the genomic environment of the candidate loci, the outlier sequences
254 (reference R2 read) were mapped onto the assembled genome of *Ae. albopictus* (Chen
255 *et al.* 2015) using Blastn. Blastn alignments were performed with default parameters and
256 sorted according to alignment score and after visual inspection of each alignment.
257 Outlier loci with multiple identical hits were discarded. To identify genes surrounding the
258 mapped outliers, the complete transcriptome of *Ae. albopictus* (including eggs, larvae
259 and adult females, downloaded at www.albopictusexpression.org Armbruster *et al.*) was

260 mapped over the reference genome using blat with default parameters; after alignment,
261 one best hit was retained per transcript according to the best alignment score. When a
262 transcript had multiple best hits, all positions for the transcript were considered.

263

264 **PCR validation and Outlier analyses.**

265 Pairs of primers were designed for each outlier locus in order to be used in standardized
266 conditions. Forward primer was located in the TE end of the concerned family and
267 reverse primer was set from the outlier locus (pairs of primers which successfully
268 amplified insertions are provided in Table S2). All primer pairs were first tested on a set
269 of 10 individuals in order to assess their specificity using 1/50 dilution of starting DNA
270 from the TD experiment. Validated primers were then used to check the insertions
271 polymorphism in 47 representative individuals from the 8 populations studied in the TD
272 experiment using 1/50 dilutions of the starting DNA (not all individuals could be used
273 because of DNA limitations). All PCRs were conducted in a final volume of 25*µ*L using
274 0.5*µ*L of diluted DNA, 0.5*µ*L of each primer (10*µ*M), 1*µ*l of dNTPs (10mM) and 1U of
275 DreamTaq Polymerase with 1X green buffer (ThermoFisher Scientific). Amplification
276 was performed as follows: denaturation at 94C for 2 minutes then 34 cycles including
277 denaturation at 94C for 30 seconds, hybridization at 60C for 45 seconds and elongation
278 at 72C for 45 seconds; final elongation was performed for 10 minutes at 72C. After 45
279 minutes migration of the PCR product on 1X electrophoresis agarose gel, CG and MB
280 assessed insertion polymorphism independently.

281 # Results

282 **High throughput TE insertion genotyping.**

283 Sequencing produced a total of 102,319,300 paired-end reads (2x101bp). After quality
284 and specificity filtering 24,332,715 reads were suitable for analyses. On average, a total
285 number of 128,491 polymorphic insertion loci were available for each of the three
286 sampling replicates. The mean number of loci per individual and per TE family ranged
287 from 1025 ± 290 s.d. (IL1 family, mean and s.d. averaged over the three replicates) to
288 3266 ± 766 s.d. (RTE5 family). Details are given in Table S1. While our read sampling
289 procedure could have artificially lowered the mean insertion frequency of the loci, this
290 effect should be small because in our final datasets, the TE insertion frequencies (*i. e.*
291 the number of individuals that share an insertion) are not correlated with the mean
292 number of read per individual at the considered locus (Figure S3).

293

294 **Population structure.**

295 Principal Coordinate Analyses (PcoAs) were performed independently for each of the
296 five TEs (Figure 2). Among the three main Principal Coordinates (PCs), individuals tend
297 to be grouped according to their respective populations with little overlap between
298 groups. However, the three main PCs represent only a small fraction of the total
299 variance (< 10%), suggesting a weak genetic structuring between the populations.
300 Overall, individuals from Vietnamese populations (HCM, TA, VT) tend to be grouped

301 together in a single cluster, at the exception of 13 to 14 individuals from HCM when
302 using L2B and RTE5 TE families (S2 Figure), along with six individuals of VT with the
303 RTE4 TE family (Figure 1) that can not be clearly distinguished from European samples.
304 BCN individuals (Spain) represent the most homogeneous group, well differentiated
305 from Vietnamese and French individuals (SP, CGN, NCE and PLV).
306 In agreement with PCoAs, Analyses of Molecular Variance (AMOVAs) attributed very
307 few genetic variances among groups (Vietnam-Europe) and between populations within
308 groups (Table 1). In the studied populations, most of the genetic variance was
309 distributed among individuals within groups.

310 Measures of genetic differentiation among pairs of populations were consistent with
311 PCoAs and AMOVAs (File S1): the BCN population shows the highest $F_{ST}$ values with
312 the other populations for each of the five TEs ($0.051 < F_{ST} < 0.148$), while Vietnamese
313 populations were the most closely related ($0.011 < F_{ST} < 0.032$). While VT is located
314 100 km away from TA and HCM (both sampled in the same city, Hô Chi Minh, Vietnam)
315 the $F_{ST}$ values are very similar between the three Vietnamese populations, suggesting
316 no influence of geography at this scale. CGN and NCE, sampled in the same urban area
317 (Nice agglomeration), are also little or not significantly differentiated depending on the
318 TE family. The previously identified intermediate pattern of HCM with some European
319 populations at L2B and RTE5 loci (PCoAs analyses) is also found at the $F_{ST}$ level,
320 especially regarding the low differentiation with the PLV population for these markers
321 ($0.011 < F_{ST} < 0.020$).

322

323 **Genomic scan.**

324 Research of outlier loci for both selection signature using Bayescan (non-hierarchical
325 island model), and for significant $F_{CT}$ (between Europe-Vietnam group differentiation)
326 identified 92 candidate insertion loci (Figure 3). Most of these insertions are found in
327 both areas (no private allele), except for RTE4_6 and RTE4_7 that were not found in
328 Vietnam. In addition, a majority of outliers corresponds to high frequency insertions in
329 Europe, while the same trend is not observed at 92 randomly chosen loci among those
330 having the same minimum insertion frequency (≥ 20 individuals/locus) between Europe
331 and Vietnam (Figure 4). PCR amplification of the outlier loci were carried out on a
332 representative panel of 47 individuals to validate the insertion pattern detected by TD
333 (see Material and Method). For loci where the amplification was successful, the insertion
334 pattern observed by PCR always confirmed the results from TD (Figure S5).

335 From 92 outlier loci, 21 could be attributed to a unique position on the *Ae. albopictus*
336 genome. Annotation and distance to surrounding genes are reported on S2 File. We
337 found that six outliers (S2 File, sheet 3, highlighted) loci are located on contigs that
338 harbor genes previously identified in *Ae. albopictus* as being differentially expressed
339 between diapause-induced and non diapause-induced samples , and for which
340 orthologs in *Drosophila melanogaster* are known to be part of well-identified functional
341 networks (Poelchau *et al.* 2013b). All these six loci are found to be outliers because of
342 their high insertion frequencies in Europe compared with Vietnam.

343 # Discussion

344 The goal of our study was to identify genomic regions involved in adaptive evolution of
345 *Ae. albopictus* thanks to the development of new genetic markers. Through high-
346 throughput genotyping of five TE families' insertion polymorphisms, we identified up to
347 128,617 polymorphic loci among a hundred of individuals from eight sampling sites. The
348 estimated genome size of *Ae. albopictus* exceeds one billion base-pairs (Goubert *et al.*
349 2015; Dritsou *et al.* 2015; Chen *et al.* 2015). Accordingly, the amount of markers scored
350 in this study offers a comfortable genomic density of one marker every 10 kb.

351 We provide here a new and cost efficient method to quickly generate a large amount of
352 polymorphic markers without extensive knowledge about one species genome.
353 Specifically, this strategy appears extremely valuable for species with a large genome
354 size, where TE density could severely compromise the development of more classical
355 approaches, such as the very popular RAD-sequencing.

356 The genetic structure of the studied populations showed strong consistency between
357 sampling replicates of individuals' reads, demonstrating the robustness of the method in
358 spite of an initial substantial coverage variation among individuals. Population genetics
359 analyses revealed a very low level of genetic structuring between European and
360 Vietnamese populations. Among the studied populations, AMOVAs showed that most of
361 the genetic variation is distributed between individuals within populations (> 90%), and
362 as suggested by pairwise $F_{ST}$ and PCoAs, only a small part (< 10%) of the genetic
363 variance is due to differentiation between populations. The genetic differentiation we

364 measured is indeed as high among European populations as it is between populations
365 from Europe and Vietnam.

366 This singular population structure is in agreement with previous results gathered in *Ae.*
367 *albopictus* using different collections of allozymes, mtDNA or microsatellites markers
368 (Black *et al.* 1988; Kambhampati *et al.* 1991; Zhong *et al.* 2013; Gupta & Preet 2014;
369 Manni *et al.* 2015). Moreover, a recent analysis performed with a set of 11
370 microsatellites on individuals from the same populations (at the exception of BCN)
371 showed a similar distribution of genetic variation among hierarchical levels (Minard *et al.*
372 2015). These results demonstrate the reliability of our markers and confirm that a non-
373 hierarchical island model can likely fit the global genetic structure. The genetic diversity
374 observed in Europe is compatible with a scenario of multiple and independent
375 introductions, as already suggested for *Ae. albopictus* (Urbanelli *et al.* 2000; Birungi &
376 Munstermann 2002; Takumi *et al.* 2009; Becker *et al.* 2013). However, as previously
377 suggested, this pattern could also be the result of founder events that may occur during
378 colonization and/or a restriction of gene flow between populations consecutive to their
379 introduction. Answering such a question would require an extended sampling all over
380 the native area.

381 Outlier analysis revealed 92 loci with high posterior probabilities of being under positive
382 selection between European and Vietnamese populations. When possible, the PCR
383 amplification of the outlier loci, using a set of representative individuals, always
384 confirmed a shift of insertion frequencies toward either the European or the Vietnamese
385 sampling sites. This suggests that in spite of a reduced coverage, introduced by

386 sampling in the dataset, the scored insertion polymorphisms are reliable. In addition, our
387 method of analysis is likely to be conservative: the Bayescan outliers were selected for
388 their consistency with a significant $F_{CT}$ between European temperate and Vietnamese
389 tropical populations, which avoid retaining outliers that we were not looking for, for
390 example those due to a population specific event.

391 We were able to assign a unique position for 21 of the outlier loci on the *Ae. albopictus*
392 genome. As expected by the *Ae. albopictus* genomic composition (Goubert *et al.* 2015;
393 Dritsou *et al.* 2015; Chen *et al.* 2015), an important part of the other outlier loci were
394 located in repeated regions (44,6% of total), despite our efforts to remove *a priori* loci
395 occurring in known transposable elements. Since the *Ae albopictus* genome publication
396 is very recent, no gene set or other genome annotation are currently available. We thus
397 took advantage of the *Ae. albopictus* transcriptome data (Poelchau *et al.* 2013a) to
398 annotate regions surrounding the detected ouliers. We found six outliers located in
399 contigs which also harbor genes differentially expressed between individuals induced for
400 diapause and controls (Poelchau *et al.* 2013b), two of them, being located either in an
401 intron (RTE4_7442) or within 3kb (RTE4_17015) of these candidate genes. It is worth
402 mentioning that these two genes belong to the same functional group (GO:0005576
403 extracellular region). Diapause is a critical developmental stage found only in temperate
404 populations of the Asian tiger mosquito. Interestingly, this functional pathway has been
405 shown to benefit from fast adjustments thanks to local adaptation. For instance,
406 Urbanski *et al.* (2012) showed that invasive American populations originating from
407 Japan have rapidly evolved a new adaptive clinal response to diapause induction,

408 independent from that observed in the native area. Thus, adaption in the temperate
409 regions could have led to several selective sweeps on gene or regulatory sequences
410 involved in this critical pathway, allowing the settlement of the mosquito in new
411 temperate areas.

412 Interestingly, and as it is the case for these six outliers, we found significantly more
413 outlier loci with a high frequency in Europe and low frequency in Vietnam than the
414 opposite pattern. This was unexpected regarding our initial assumptions: a favored allele
415 selected in one or another environment has *a priori* no reason to be more often
416 associated with the presence or the absence of a TE insertion at linked sites. However,
417 we found that the majority of the sequenced TE insertions segregates at low frequencies
418 (around 10% of all individuals). When considering the linked region of one polymorphic
419 TE insertion, if a favorable mutation appears in an individual where the insertion is
420 absent, the increase of frequency of this “absence” haplotype will thus, most of the time,
421 have a modest effect on the genetic differentiation at this marker, since it is already
422 segregating at high frequency. By contrast, if a favorable mutation appears in a TE
423 “presence” haplotype, the increase in frequency of the linked TE insertion would lead to
424 high differentiation ($F_{CT}$) values. In absence of an alternative explanation, our outlier loci
425 could thus indicate in which subset of populations the adaptive mutation occurred, and
426 in the present case, this would have happened more frequently in the temperate
427 populations.

428 Two scenarios, not mutually exclusive, could be invoked in the light of our data. A simple
429 case would be a direct adaptive evolution in European invasive population that

430 originated from tropical regions of the native area. A second hypothesis, could be that
431 invasive temperate populations came from Northernmost territories of the native area
432 such as northern China or Japan where *Ae. albopictus* populations are already cold-
433 adapted. It would be thus interesting to know whether the observed signature of
434 selection results from more “ancient” adaptations in the native area, or if it originates
435 from more recent fine tuning of cold-related traits in the invasive areas. A recent study
436 (Porretta *et al.* 2012) suggested, using new variable *COI* mtDNA sequences and
437 historical species range modeling, that Northern areas of the native range of *Ae.*
438 *albopictus* would be the latest to have been colonized after a range expansion from
439 Southern refugia following the last glacial around 21,000 years ago. The authors
440 suggested that *Ae. albopictus* may have followed the human populations during their
441 expansion from South to North in this area, that began approximately 15,000 years ago.
442 Thus wherever the origin of the invasive individuals sampled in Europe, it is likely that
443 they are representatives of populations that had recently undergone a shift of selective
444 pressure from tropical to temperate climatic conditions. This could explain why so many
445 outliers are associated with high insertion frequency in Europe, and that candidate
446 genes in the diapause pathway are found in the neighborhood of some of these outliers.
447 An easy way to distinguish between these possibilities would be to search if the same
448 outlier insertions are present in several temperate populations from the native area.

449 It is important to note that the results presented here are only restricted to a subset of
450 the Asian tiger mosquito populations located in temperate and tropical environments. It
451 is thus probable that some of the outliers detected could be specific to this particular

452 comparison and do not reflect the global pattern of differentiation between tropical and
453 temperate populations. Research of the same outliers between other tropical and
454 temperate populations from the native and non-native areas would be extremely
455 valuable to extrapolate our results at a larger scale. Should the same outlier insertions
456 be found at high frequencies in temperate locations – such as in USA, Japan or China –,
457 extended investigations about the origin of invasive populations would help clarify if
458 those similarities are due to an ancestral sweep or parallel sweeps that occurred
459 independently in several populations. This study already provides for some candidate
460 loci a set of functional primers that could be directly used to answer this question in any
461 DNA sample of *Ae. albopictus*.

462 We report here the first leads supporting adaptive evolution at the molecular level in the
463 Asian tiger mosquito. Progress in the annotation of published genomes, and the looming
464 availability of supplementary genomic resources will allow gaining the most from these
465 results. We hope that this work will contribute to unravel the implication of adaptive
466 processes during the invasion of disease vectors.

467

468 # Acknowledgements

469 We thank Van Tran-Van, Christophe Bellet, Grégory Lambert, Huynh Kim Ly Khanh and
470 Trang Huynh who made possible and contributed to the samplings in France and
471 Vietnam. We are grateful to Valèria Romero Soriano and her family for their help during
472 sampling in Sant Cugat dèl Vallès. Library construction and sequencing was made in

473 collaboration with Clémence Genthon and Olivier Bouchez. We thank Manon Vigneron
474 for PCR validation experiments. We also thank Rita Rebollo who provided insightful
475 comments and English revision of the manuscript. This work was performed using the
476 computing facilities of the CC LBBE/PRABI. C.G. received a grant from the French
477 Ministry of Superior Education. This work was supported by the Centre National de la
478 Recherche Scientifique, the Institut Universitaire de France, and preliminary
479 experiments benefited from of grant of the Federation de Recherche 41 "Bio-
480 Environnement et Santé". ". Funding for mosquito sampling in Vietnam was provided by
481 grants from EC2CO CNRS and occurred within the framework of GDRI "Biodiversity and
482 Infectious Diseases in Southeast Asia". Original maps used to describe sampling were
483 freely downloaded from http://d-maps.com/carte.php?num_car=4719&lang=en and
484 http://d-maps.com/carte.php?num_car=708&lang=en
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639

640 # Data Accessibility

641 Paired-end raw sequences are available through SRA at NCBI under SRP070185

642 (Bioproject PRJNA312147)

643 Final presence/absence matrices (including replicates) are available at Dryad

644 (doi:10.5061/dryad.9p925) at http://datadryad.org/review?doi=doi:10.5061/dryad.9p925

# 645 Author contributions

646 CG, CV and MB conceived the experiments and conducted the analyses. CG and HH

647 developed and performed the molecular experiments. GM, CVM and PM conducted the

648 sampling in France and Vietnam. All authors contributed to the final version of the

649 manuscript.

# Tables and Figures

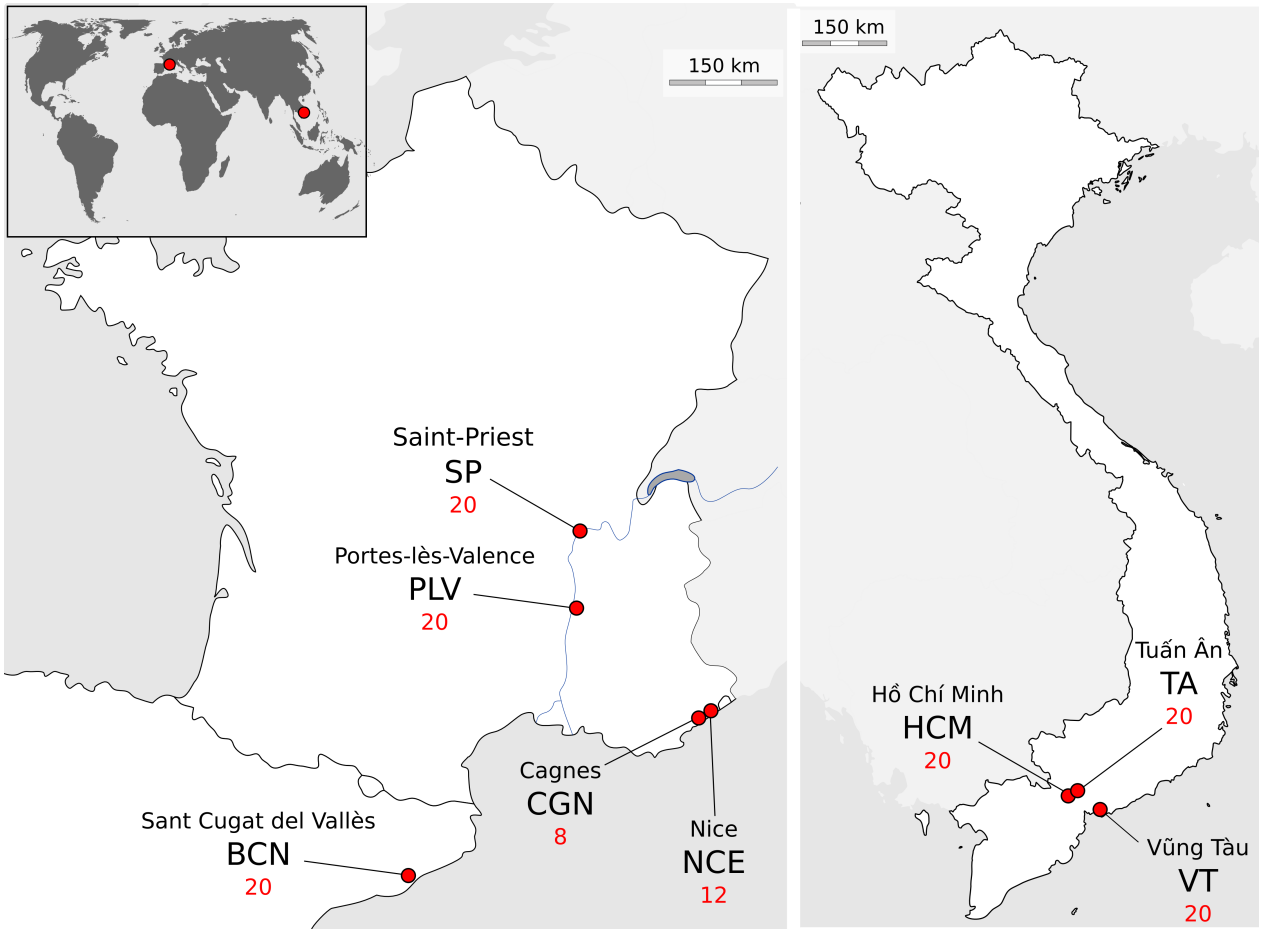


**Figure 1.** Sampling sites (with abbreviations) of *Ae. albopictus* in Europe and Vietnam. Red numbers correspond to the total number of individual sampled. Supporting information about samples are available in Table S1.

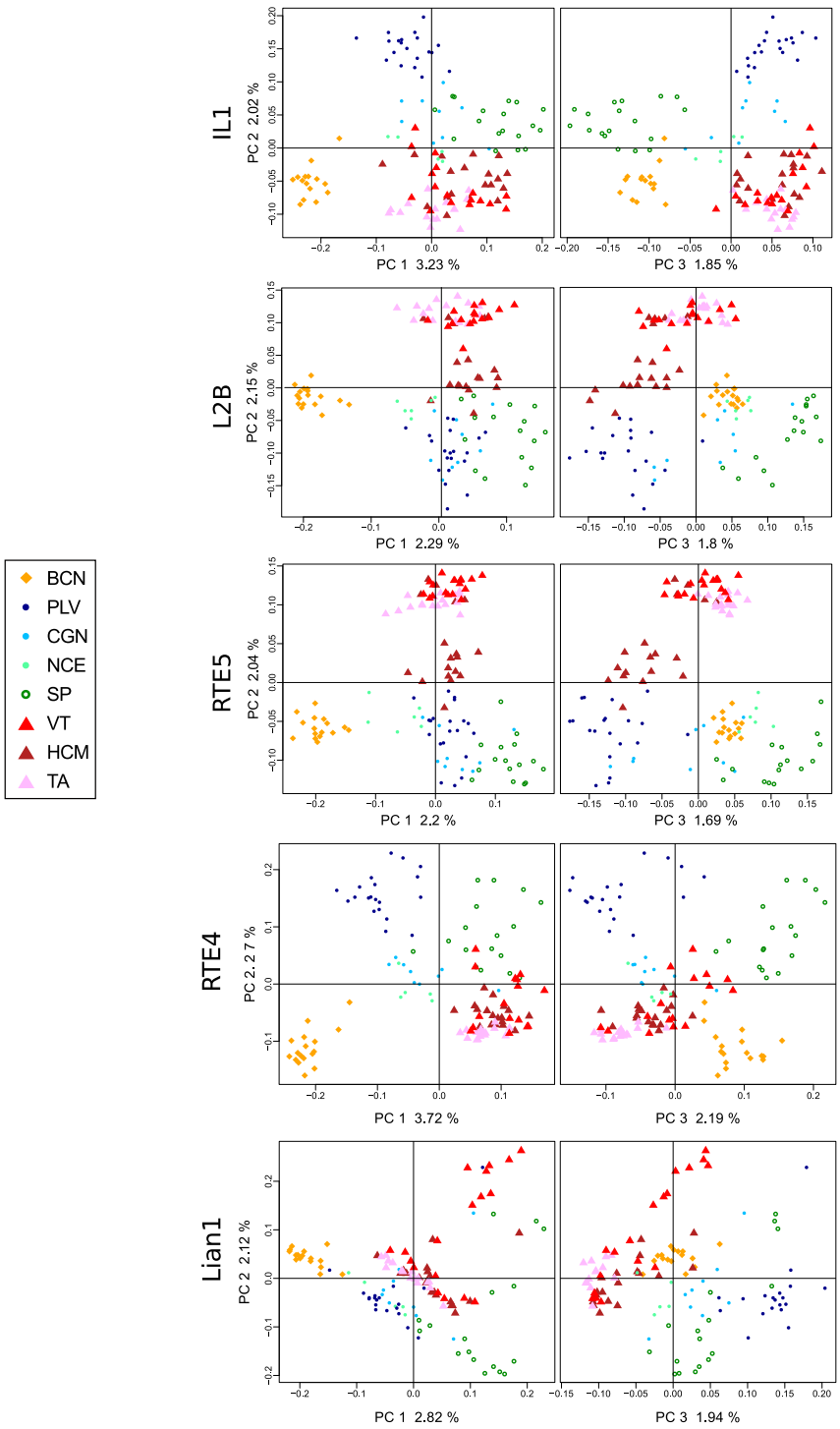


**Figure 2. Principal Coordinate Analyses (PCoAs).** Projection of individuals over the three first principal coordinates (PC) of PCoAs for each of the 5 TE families and for the first replicate (M1, see Material and Methods). Proportion of inertia represented by each axes is noted in %. circles: European populations; triangles: Vietnamese populations. Results for other sampling replicate can be found in Figure S4.

661 **Table 1. Analyses of Molecular Variance (AMOVAs).** Results for the three replicates
662 (M1, M2, M3) of read sampling for the five TE families (IL1, L2B, RTE5, RTE4, Lian1).
663 Values are given in percentage of the total genetic variance

| | IL1 | L2B | RTE5 | RTE4 | Lian1 |
|---|---|---|---|---|---|
| Among groups | [0.59-0.70] | [1.22-1.29] | [1.08-1.10] | [1.97-2.04] | [0.67-0.74] |
| Among populations within groups | [5.15-5.37] | [3.58-3.63] | [3.36-3.40] | [6.67-6.78] | [4.47-4.56] |
| Within populations | [94.04-94.16] | [95.08-95.18] | [95.51-95.55] | [91.18-91.30] | [94.77-94.81] |

intervals reports min and max values among the 3 sampling replicates

664

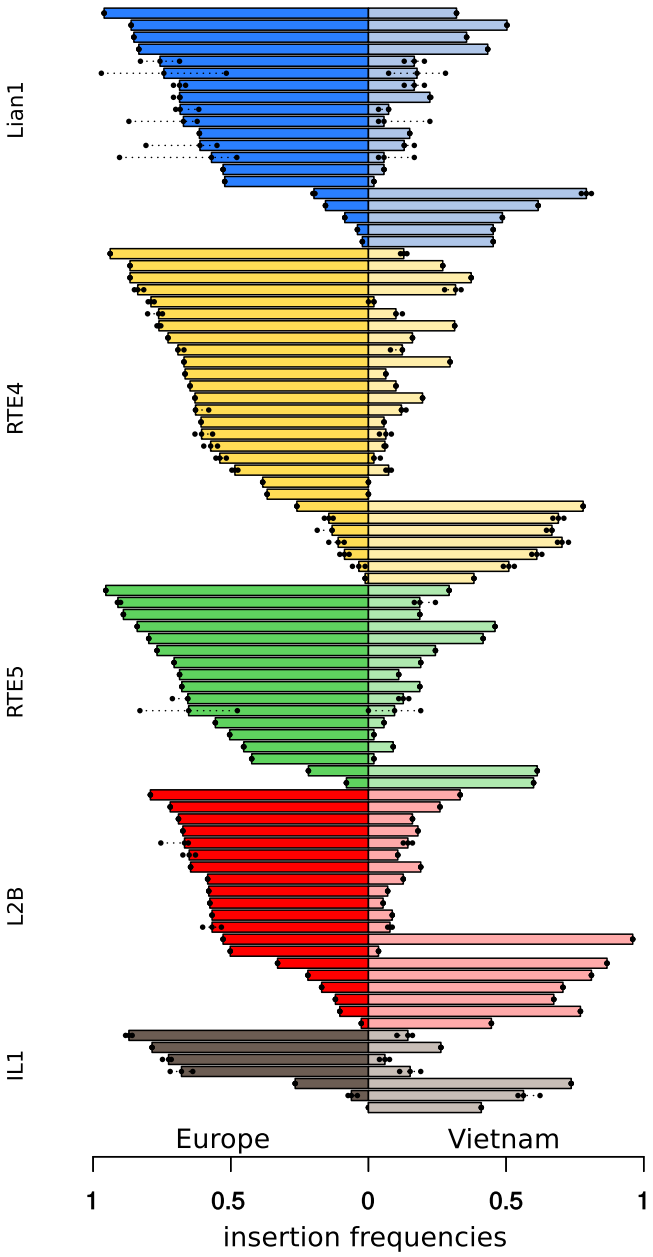


**Figure 3. Insertion frequencies in Europe and Vietnam for the 92 outlier loci**. Bars represent the median value from the three reads sampling replicates and dots the values from the other replicates (if outlier found in replicate). Colors correspond to each of the 5 TE families.

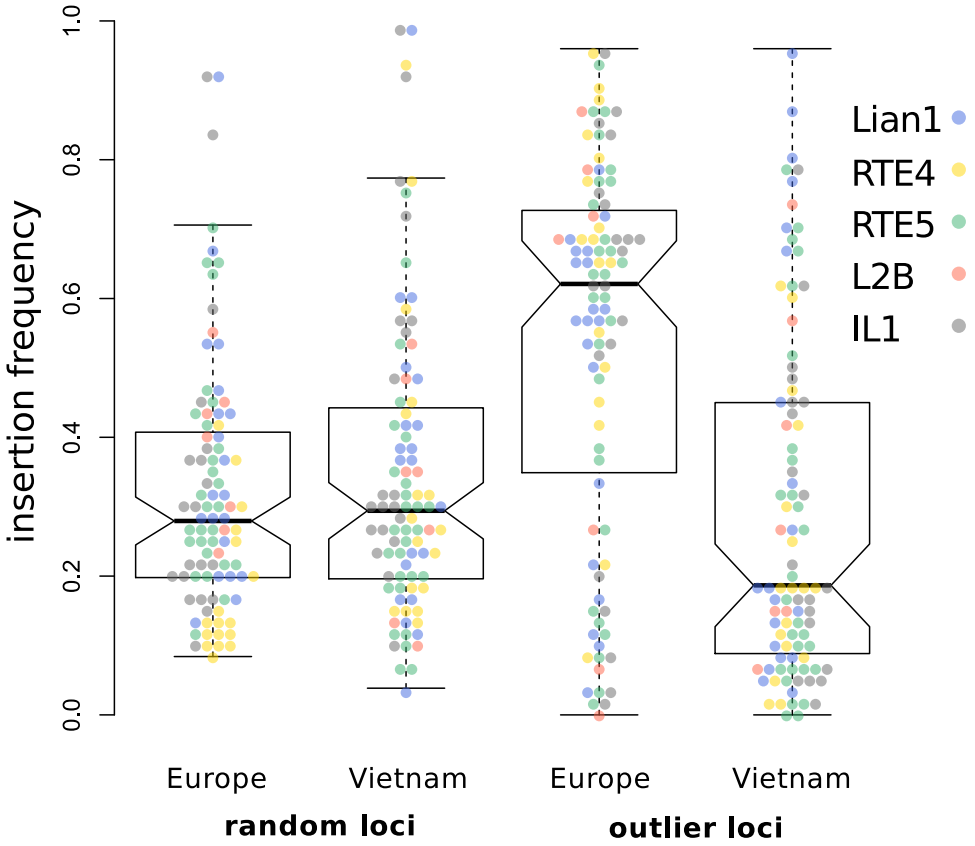


**Figure 4. Comparison of outliers frequencies with randomly selected loci.** Insertion frequencies of 92 randomly chosen loci among those having the same minimum insertion frequency (≥20 individuals) as outliers compared to the 92 outlier loci. Random loci were taken from the first replicate (M1) and values for outliers are median values obtained among the three replicates. Non-overlapping notches indicate a significant difference between the true medians (thick dark horizontal bars).