Helena and BS: Two Travellers between the Genera Drosophila and Zaprionus

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Abstract

The frequency of horizontal transfers of transposable elements (HTTs) varies among the types of elements according to the transposition mode and the geographical and temporal overlap of the species involved in the transfer. The drosophilid species of the genus Zaprionus and those of the melanogaster, obscura, repleta, and virilis groups of the genus Drosophila investigated in this study shared space and time at some point in their evolutionary history. This is particularly true of the subgenus Zaprionus and the melanogaster subgroup, which overlapped both geographically and temporally in Tropical Africa during their period of origin and diversification. Here, we tested the hypothesis that this overlap may have facilitated the transfer of retrotransposons without long terminal repeats (non-LTRs) between these species. We estimated the HTT frequency of the non-LTRs BS and Helena at the genome-wide scale by using a phylogenetic framework and a vertical and horizontal inheritance consistence analysis (VHICA). An excessively low synonymous divergence among distantly related species and incongruities between the transposable element and species phylogenies allowed us to propose at least four relatively recent HTT events of Helena and BS involving ancestors of the subgroup melanogaster and ancestors of the subgenus Zaprionus during their concomitant diversification in Tropical Africa, along with older possible events between species of the subgenera Drosophila and Sophophora. This study provides the first evidence for HTT of non-LTRs retrotransposons between Drosophila and Zaprionus, including an in-depth reconstruction of the time frame and geography of these events.

Key words: transposable elements, horizontal transfer, non-LTR retrotransposons, drosophilids.

Introduction

The connections among all living entities are determined by the flow of genetic information through the generations, which occurs predominantly through the reproductive process and is termed vertical transfer (VT). However, other nonvertical forms of inheritance have been demonstrated that are generically termed horizontal transfer (HT), by which genes and other genetic sequences are transferred between species through nonsexual means (Schaack et al. 2010). Horizontal transfer of transposable elements (HTT) was long believed to be restricted to prokaryotes (Andersson 2005). Until the 1990s, only a small number of HTTs among eukaryotes had been reported (e.g., Daniels et al. 1984; Anxolabéhére et al. 1988; Daniels et al. 1990; Clark et al. 1994; Robertson and Zumpano 1997; Kordis and Gubensek 1998), but in the last two decades, the number of HTTs described in invertebrates, vertebrates and plants, and between species from different higher taxa or realms has increased considerably (e.g., Silva and Kidwell 2000; De Almeida and Carareto 2006; Diao et al. 2006; Ludwig et al. 2008; Pace et al. 2008; Ray et al. 2008; de Setta et al. 2009; Dias and Carareto 2012; Thomas et al. 2010; Gilbert et al. 2010; de Setta et al. 2011; El Baidouri et al. 2014; Dias et al. 2015; Kofler et al. 2015; Suh et al. 2016; Peccoud et al. 2017; Gao et al. 2018).

Among the genetic elements involved in HTs in eukaryotes, transposable elements (TEs) are the most frequently reported
(Modolo et al. 2014). TEs are repetitive sequences of DNA that have the ability to mobilize in the genome, and due to this mobility, they stand out in HTT studies. The number of identified HTT events increased markedly in the last decade, from 156 cases described prior to 2007 to 2,855 at the time of this query (December 2, 2018), as shown in the database HTT-DB (Horizontally Transferred Transposable Elements DataBase, http://pfa.saogabriel.unipampa.edu.br:8080/HTTdatabase; last accessed December 2016). This database, as well as the results of previous studies (reviewed in Schaack et al. 2010; Carareto 2011; Peccoud et al. 2017), showed that the frequency of HTT is not constant among the different types of elements. A greater number of HTTs involve DNA transposons (2,178) rather than long terminal repeat (LTR) retrotransposons (353) and non-LTR retrotransposons (324). The difference in the frequency of HTTs can be explained by both the structural characteristics and the mechanisms of transposition of the different types of TEs, along with the functionality of the enzymatic machinery necessary to perform the initial transposition events. The presence of a TE as a free DNA molecule in the cell facilitates the transfer out of the cell and the insertion into another host genome, particularly for DNA transposons. The simplicity of the structure of DNA transposons, such as those of the Tc1-Mariner superfamily, could facilitate their transport in a vector and increase the probability of HTT (Schaack et al. 2010). Moreover, the success of an HTT event may be related to the ability of the promoter to drive gene expression in a new genetic and genomic environment, as demonstrated by Palazzo et al. (2017) using the Bari family of DNA transposons. HTT may also be facilitated for retrotransposons such as the Gypsy superfamily LTRs. Some Gypsy elements, such as those belonging to the clades Gypsy and 17.6 in Drosophila, and in the lepidopteran Trichoplusia ni, have an additional open reading frame (ORF) that encodes a retroviral envelope protein (http://www.gydb.org/index.php/Ty3/Gypsy; last accessed September 2017; Friesen and Nissen 1990; Péllisson et al. 1994; Song et al. 1994). The gypsyDM of D. melanogaster, for example, produces a fully functional protein that provides it with both infectious ability and the possibility to be horizontally transferred by contact or feeding when individuals of an “empty” stock are raised on medium containing ground pupae of the stock possessing transposable elements (Kim et al. 1994). These results raise the possibility of Gypsy being both a retrotransposon and a facultative retrovirus, expressing an infectious form only under special circumstances (Song et al. 1994).

The frequency of HTT also differs among groups of organisms. Thus far, there has been an observable bias towards Drosophila/Insecta in the literature for documented HTTs. For example, of the 330 HTT cases published by 2012, 178 were described among Drosophila species. This disproportinate number of HTTs in Drosophila may be a historical deviation, as the pioneer studies of HTT in TEs were performed with this model organism (Carareto 2011; Wallau et al. 2012). Among the events described as HTTs in Drosophila, we previously proposed the transfer of LTR retrotransposons between two groups of species: the melanogaster subgroup of the melanogaster group (subgenus Sophophora, genus Drosophila, Drosophilidae) and the subgenus Zaprionus (genus Zaprionus, Drosophilidae) (De Setta et al. 2009, 2011). The genus Zaprionus, which is divided into two subgenera—Anapronius, of Oriental region distribution, and Zaprionus, of Tropical Africa distribution from 7 Mya (Okada and Carson 1983; Yassin et al. 2008)—originated in the middle and late Miocene in the Eastern biogeographic region (Yassin et al. 2008). The melanogaster subgroup, one of the seven subgroups that form the melanogaster group of the genus Drosophila, also originated in Tropical Africa from a founding lineage that reached the African continent between 17 and 20 Mya from the Eastern region when the faunal interchange between Africa and Eurasia first became possible (Jeffs et al. 1994; Lachaise and Silvain 2004). It has been proposed that three speciation centers in the African continent were responsible for the formation of three species complexes of the melanogaster subgroup: the erecta, the yakuba, and the melanogaster complexes. The erecta (D. erecta and D. orena) and yakuba (D. yakuba, D. teissieri, and D. santomea) complexes evolved in the western Africa from the common ancestral melanogaster lineage, with the first complex between 13 and 15 Mya and the second between 8 and 15 Mya (Lachaise and Silvain 2004) or even later, approximately 6 Mya (Russo et al. 1995). In the melanogaster complex (D. melanogaster, D. simulans, D. sechellia, and D. mauritiana), the dating of the divergence between D. melanogaster and D. simulans from an ancestral lineage in Tropical Africa remains controversial but may have occurred between 3 and 4 Mya (Tamura et al. 2004a, 2004b; Cutter 2008). Later, the ancestor of the subcomplex simulans (D. simulans, D. sechellia, and D. mauritiana) arose during the colonization of Madagascar and the islands of the Indian Ocean, approximately 0.4 Mya (Lachaise and Silvain 2004). The above-proposed evolutionary scenario is shown in figure 1.

The high number of HTTs among species of the subgroup melanogaster and the subgenus Zaprionus could be explained by the presence of shared putative vectors such as viruses, bacteria and microparasitoids that would have facilitated transfers of genetic material between those species that overlapped geographically and temporally during their origin and diversification (De Setta et al. 2009, 2011). A small effective population size (Ne) during the species origin could be another factor facilitating HTT, since permissiveness to the fixation of TEs in the genome (in general, including those introduced by HTTs) can be increased in newly formed species due to small Ne, which reduces the efficacy of natural selection against invasive DNA (reviewed in Carareto 2011). However, how many and which of these factors contributed to the high exchange rate of genetic material in TEs between these two species groups remains unknown.
This study is the first to investigate the occurrence and evolutionary relationships of two families of non-LTR retrotransposons *Helena* (Petrov et al. 1995) and *BS* (Udomkit et al. 1995) in species of the genus *Zaprionus* and to perform comparative analyses with the sequences of both families in species of the genus *Drosophila*. *Helena* and *BS* belong to the Jockey superfamily of the LINE order (Wicker et al. 2007). *Helena* has a 25 bp poly-A tail and two overlapping ORFs (ORF1 and ORF2). The first ORF is 1,737 bp long, encodes a 579 aa protein that has high similarity to the gag protein of other LINE-like elements and contains a domain PRE_C2H2 (associated with zinc fingers). The second ORF, which starts on the last base of ORF1, is 2,721 bp, encodes a 907 aa protein corresponding to the pol gene and contains the apyrimidinic endonuclease and exonuclease (ENDO_EXO) domains and the reverse transcriptase (RTASE) domain (Rebollo et al. 2008).

Both elements show discontinuous distribution within the genus *Drosophila*, varying in terms of structure, sequence conservation, number of copies and transcriptional activity (Petrov et al. 1998; Rebollo et al. 2008; Granzotto et al. 2009, 2011). The element *Helena* has been identified in six species of the melanogaster subgroup of the melanogaster group (*D. melanogaster*, *D. simulans*, *D. sechellia*, *D. yakuba*, *D. erecta*, and *D. ananassae*), three species of the obscura group (*D. pseudoobscura* and *D. persimilis*) and *D. mojavensis* (Granzotto et al. 2011). *BS* has also been reported in six species of the melanogaster subgroup (*D. yakuba*, *D. erecta*, *D. simulans*, *D. melanogaster*, *D. sechellia*, and *D. ananassae*), two species of the obscura group (*D. pseudoobscura* and *D. persimilis*), and *D. mojavensis* (Granzotto et al. 2011). *BS* was characterized recently in a

**Fig. 1.**—Evolutionary scenario and historical biogeography of drosophilid in the Old World with emphasis on the subgroup melanogaster (melanogaster group, Sophophora subgenus, genus *Drosophila*) and the subgenus Zaprionus (Zaprionus genus). The ages (numbers in My) of the African continent colonization of the melanogaster subgroup and Zaprionus subgenus, migrations (arrows) and lineages diversification in each region are indicated (Okada and Carson 1983, Jeffs et al. 1994, Russo et al. 1995, Yassin et al. 2008, Lachaise and Silvain 2004).
larger number of species—D. melanogaster, D. erecta, and D. mojavensis—with complete ORFs and transcriptional activity (Granzotto et al. 2011).

Our study shows evidence of HTTs of the Helena and the BS elements that could have occurred concomitantly with the HTTs of LTR retrotransposons (De Setta et al. 2009, 2011). We were able to detect at least four HTT events that took place in Tropical Africa between the ancestral species of the melano- gaster subgroup and the ancestral ZapriOnus species, along with other putative transfers of both elements between species of the subgenera Drosophila and Sophophora with an Oriental origin.

**Material and Methods**

**Biological Material and Sequencing of Elements**

The occurrence of the Helena and BS elements were investigated through PCR and Sanger sequencing in 11 species of the genus ZapriOnus (supplementary table S1, Supplementary Material online): ten species of the subgenus ZapriOnus (Z. indianus, Z. gabonicus, Z. afric anus, Z. ornat us, Z. cameroonensis, Z. davidii, Z. tuberculatus, Z. inermis, Z. nigranus and Z. sepsoides) and one species of the subgenus Anaprionus (Z. bogoriensis). Genomic DNA was extracted from the ovaries of 20 individuals of each species using the phenol-chloroform method (Lowett 1986).

For amplification of the Helena element, a pair of primers (DsechF: 5’ AGG ATT TGT CAT GCC AGG CT 3’ and DsechR: 5’TGT TTG GTG CCA TGT GT 3’) that amplifies a 640 bp sequence of the gene of the complete element of D. sechellia were used following the following PCR conditions: 200 ng of genomic DNA, each dNTP 0.5 μM at 0.625 mM, 0.75 μM at 0.8 mM MgC2, each primer at 0.5 μM at 10 mM, and 1 U of Platinum Taq (Invitrogen) in 1× buffer, in a final volume of 25 μl. The amplification reaction was performed with the following parameters: 95 °C for 5 min, 24 cycles of 95 °C for 1 min, 62 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. BS element amplifications were performed with a pair of primers (DmelF: 5’ TGA AGA GAG CCC TGA ATC GT 3’ and DmelR: 5’ GTG AGT AAG CAG GGA TTG ATG GT 3’) that amplifies a 774 bp sequence of the gene of the complete element of D. melanogaster. The amplification reaction was performed under the same conditions described above, with cycling as follows: 95 °C for 5 min, 35 cycles of 95 °C for 2 min, 62 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR fragments were purified with an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) and cloned with a TOPO TA Cloning Kit (Invitrogen) according to the manufacturer’s instructions. Three to ten clones were randomly selected and sequenced in an ABI 3730 xl DNA Analyzer (Applied Biosystems), at the Center for Biological Resources and Genomic Biology (CREBIO,UnESP, Jaboticabal, Brazil), using the M13 primers. The RT sequences generated have been deposited in GenBank under accession numbers MH047863 to MH047945.

**Identification of Elements in silico**

Sequences of the Helena and BS retrotransposons were also obtained both from public databases and from 26 genomes of Drosophila (Sessegolo et al. 2016) and three genomes of Zaprionus made available by A. Haudry (supplementary tables S2, S3 and S4, Supplementary Material online). Raw reads were automatically filtered for quality using UrQt (Modolo and Lerat 2015). TEs were identified by de novo assembly and annotation in dnaPipeTE (Goubert et al. 2015) from random samples corresponding to 0.25× coverage.

To identify the TEs in the publicly available genomes, we searched for sequence similarity with the reference sequences of the Helena element of D. simulans (4,912 bp; Rebollo et al. 2008) and the BS element of D. melanogaster (5,124 bp; Udomkit et al. 1995) using BLASTn (Altschul et al. 1990) with a cut-off value ≤1e^-10. The three best hits were extended by 3 kb in the 5’ and 3’ flanking regions to allow identification of the ends of the elements. The most conserved and most complete sequence obtained by BLASTn was considered as the reference sequence for each element in each genome. With the reference sequence of each species, the BLASTn search was performed a second time, using as parameters length ≥300 bp and identity ≥80%. Finally, each putative TE sequence was analyzed for the presence and integrity of the PRE_C2H2 (ORF1), ENDO_EXO and RTASE (ORF2) of the Helena and BS coding sequences using a BLAST CD-search against the Conserved Domain Database (CDD) (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi; last accessed July 2016).

**Phylogenetic Inferences**

From all Helena and BS sequences obtained, the RT sequences were extracted and aligned with the RT-amplified sequences of ZapriOnus species using MAFFT software (Katoh et al. 2017). The phylogenies were reconstructed using the maximum likelihood (ML) method with 1,000 bootstrap replicates to validate the robustness of the phylogeny (Felsenstein 1985). Additionally, Bayesian inferences of phylogeny (BI) were performed with the program BEAST v16.1 (Drummond et al. 2012) with an a posteriori phylogenetic support test, which involved the sampling of 100,000 trees with 10% burn-in. The evolutionary model of substitution that best fit the data was determined by the Find Best DNA Model (MEGA7, Kumar et al. 2016). The sequences of elements belonging to the Jockey clade to which Helena and BS belong (Metcalfe and Casane 2014), Jockey1 (zind_LINE_comp657, zafr_LINE_comp2868, divir_LINE_comp1826) and Jockey2 (dan_LINE_comp1636, dbip_LINE_comp1936) obtained from the dnaPipeTE data set, were used as an outgroup. Sequences of the elements Doc...
gabonicus and Z. indianus performed with MEGA 7 using the Tamura-3-parameter as \((\text{EF458322.1})\) as the query. The phylogenetic inference was obtained from GenBank for most of the species \((\text{supplementary table S5, Supplementary Material online})\). For which was obtained from the HTT-DB \((\text{http://lpa.saogabriel.unipampa.edu.br:8080/httdatabase; last accessed December 2016})\) whereas those of the species Z. africanus, Z. gabonicus and Z. indianus were obtained through searching directly the genomes. The orthology of these genes was verified using OrthoDB—The Hierarchical Catalog of Orthologs v9.1 \((\text{http://www.orthodb.org/; last accessed February 2017})\). All genes were single-copy, except for the CG4386 gene of D. melanogaster (a peptidase S1 gene family), which presents two paralogs in eight species (D. melanogaster, D. ananassae, D. erecta, D. simulans, D. yakuba, D. persimilis, D. pseudoobscura, and D. grimshawi). In this case, phylogenetic analysis identified the orthologous sequences, and only one of the two sets of orthologs were used in the VHICA analysis. The same 30 genes were used for reconstructing a phylogeny required for interpreting the HTT signals \((\text{supplementary table S6, Supplementary Material online})\). For both TEs, a consensus was used when the sequences from each genome had <10% nucleotide divergence, as recommended in the VHICA tool. In this analysis, it was possible to use only the sequence of the BS element found in D. yakuba genome, which was not used in the phylogenetic analysis because of a deletion comprising a large part of the reverse transcriptase gene.

### Results

**Distribution of the Elements Helena and BS in the Genomes of Zaprionus and Drosophila**

In addition to the 11 species of Drosophila in which the element Helena had been previously identified \((\text{Petrov et al. 1995; Grazzotto et al. 2009})\), we showed its presence in four other species of the melanogaster group, three species of the melanogaster subgroup (D. teissieri, D. mauritiana, and D. orena) and one species of the ananassae subgroup (D. bipectinata), all sequences found by genome sequence search. However, Helena was not found in the genomes of the nine Oriental species of the melanogaster group \((D. malerkotliana, D. suzukii, D. biarmipes, D. elegans, D. eugracilis, D. ficushila, D. kikkawai, D. rhopaloa, and D. takahashii)\) among the 11 Oriental species whose genomes were investigated \((\text{supplementary table S2, Supplementary Material online})\). The element Helena was found in 11 of the Zaprionus species studied, except for the Oriental Z. bogoriensis, which was the only species of the subgenus Anaprionus studied \((\text{supplementary tables S1 and S3, Supplementary Material online})\). Analysis of the occurrence and integrity of the domains PRE_C2H2 (ORF1), ENDO_EXO and RTASE (ORF2) in all insertions of the Z. africanus, Z. gabonicus, and Z. indianus genomes showed that a single copy of Helena from Z.

Vertical and Horizontal Inheritance Consistence Analysis (VHICA)

The VHICA approach \((\text{Wallau et al. 2016})\) was used to corroborate the phylogenetic inferences of vertical and horizontal transfer by providing statistical support. The method is based on discrepancies between the rate of evolution in synonymous sites (\(dS\)) and the preferential use of codons (ENC) between pairs of TE sequences and vertically transferred orthologous genes. Statistical support for the HTT inferences is given by a linear regression between the distribution of ENC and \(dS\) values \((\text{with the Bonferroni correction, } P < 0.01)\). For each pair of species, the correlation between ENC and \(dS\) is calculated, and the residuals of a linear regression ENC = \(a \cdot dS + b\) among reference genes, assumed to be vertically transmitted, and those of the TEs are plotted in a graph. Statistically significant deviation is interpreted as indicative of HTT. Thirty orthologous genes from 20 species were used. The gene sequences of D. melanogaster, D. sechellia, D. simulans, D. yakuba, D. erecta, D. ananassae, D. biarmipes, D. bipectinata, D. elegans, D. ficushila, D. takahashii, D. kikkawai, D. grimshawi, D. persimilis, D. pseudoobscura, D. mojavensis, and D. virilis were obtained from the HTT-DB \((\text{http://lpa.saogabriel.unipampa.edu.br:8080/httdatabase; last accessed December 2016})\) whereas those of the species Z. africanus, Z. gabonicus and Z. indianus were obtained through searching directly the genomes. The orthology of these genes was verified using OrthoDB—The Hierarchical Catalog of Orthologs v9.1 \((\text{http://www.orthodb.org/; last accessed February 2017})\). All genes were single-copy, except for the CG4386 gene of D. melanogaster (a peptidase S1 gene family), which presents two paralogs in eight species (D. melanogaster, D. ananassae, D. erecta, D. simulans, D. yakuba, D. persimilis, D. pseudoobscura, and D. grimshawi). In this case, phylogenetic analysis identified the orthologous sequences, and only one of the two sets of orthologs were used in the VHICA analysis. The same 30 genes were used for reconstructing a phylogeny required for interpreting the HTT signals \((\text{supplementary table S6, Supplementary Material online})\). For both TEs, a consensus was used when the sequences from each genome had <10% nucleotide divergence, as recommended in the VHICA tool. In this analysis, it was possible to use only the sequence of the BS element found in D. yakuba genome, which was not used in the phylogenetic analysis because of a deletion comprising a large part of the reverse transcriptase gene.

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D. simulans had the three domains, but two of them—ENDO_EXO and RTASE—were incomplete. In Z. gabonicus, only two domains were predicted, but both were incomplete (supplementary fig. S1, Supplementary Material online).

The BS element had been previously identified in nine species of Drosophila (Udomkit et al. 1995; Granzotto et al. 2011). We were able to show its presence in only two other species of the melanogaster group (D. ficsuphila and D. bipectinata), which were found by genome sequence searching, both of which are of Oriental origin (supplementary table S4, Supplementary Material online). In Zaprionus, BS was found in six of the 11 species investigated (supplementary tables S1 and S3, Supplementary Material online): five species of the vittiger group (Z. indians, Z. africans, Z. gabonicus, Z. davidii, and Z. ornatus) and one species of the inermis group (Z. sepsoides). Analyses of the genomes of Z. africans, Z. gabonicus, and Z. indians showed that at least three copies of Z. indians and one copy of Z. gabonicus present the domains PRE_C2H2, ENDO_EXO and RTASE, indicating they are putative full-length insertions (supplementary fig. S2, Supplementary Material online).

**Phylogenetic Inferences**

The evolutionary relationships of the elements Helena and BS were evaluated by the maximum likelihood (ML) and Bayesian inference (BI) methods and produced similar results. The main differences between the ML and the BI trees were in the robustness values of the clades (ML trees not shown). Sixty-six Helena sequences were used: 39 from the subgenus Zaprionus and 28 from the genus Drosophila (fig. 2). All the sequences identified as Helena form a monophyletic group, corroborating the identification method used. Several phylogenetic incongruities can be identified among the sequences of the Helena elements of the Drosophila species from the melanogaster, repleta, and viniis groups and those of the subgenus Zaprionus species. The two clades that diverged approximately 9.5 Mya are incongruent with the species phylogeny (mirroring the Helena tree in fig. 2) despite strong posterior probability support (PP = 1). The first clade contains sequences from Oriental species of the group melanogaster (D. bipectinata and D. ananassae) and from Nearctic and Neotropical species of the repleta (D. mojavensis, D. koepferae, and D. buzzatii) and viniis (D. viniis) groups, sharing a common ancestral sequence dated at 7.4 Mya. The second clade contains Helena sequences of Tropical African species belonging to the sub-group melanogaster and the subgenus Zaprionus, which share a common ancestral sequence dated at 5.5 Mya. In this clade, the sequences of the melanogaster complex (D. melanogaster, D. simulans, D. sechellia, and D. mauritiana) are more closely related to those of the subgenus Zaprionus (node PP = 0.91) than to the other species of its subgroup (erecta complex: D. erecta, D. orena; yakuba complex: D. yakuba and D. teissierii), and they share a common ancestral sequence dated at 4.1 Mya. Finally, the Z. ornatus sequences are grouped with D. sechellia sequences (node PP: 0.95), with the time of divergence from an ancestral sequence dated at 0.2 Mya. Apart from Z. ornatus, the sequences of the species of each complex of the subgenus Zaprionus grouped as expected according to the phylogeny of the species proposed by Yassin et al. (2008), as expected by vertical inheritance.

We observed a smaller divergence between Helena sequences of distantly related species than between closely related species. For example, the mean divergence between the Helena sequences of the melanogaster complex and the Zaprionus genus is lower (6.7%) than that between the Helena sequences of this complex and the yakuba and the erecta complexes (10.5% and 10.2%, respectively), which form the melanogaster subgroup (supplementary table S7, Supplementary Material online). More interestingly, Helena sequences of Z. ornatus are more similar to the sequences of D. sechellia (2%) than to the Helena sequences of the other Zaprionus species (7%). However, although they are grouped in the phylogeny, the sequences of the Oriental melanogaster group (D. bipectinata and D. ananassae) are as divergent from those of the repleta and viniis groups (25%) as they are from those of the melanogaster complex (25%) and the Zaprionus subgenus (28%). These degrees of divergence are incongruent with the phylogenetic relationships between these species.

The phylogenetic analyses for the element BS corroborated our method of identification for this element because the BS sequences are grouped in a clade with high posterior probability (PP = 1) (fig. 3). Such as the Helena element, phylogenetic incongruities involving the BS sequences were observed when compared with the species phylogeny (mirrored the BS tree). BS sequences of the Oriental species of the group melanogaster, D. ficsuphila, and D. bipectinata, are positioned basally in the BS clade. The other clade contains all the other sequences, and the Nearctic species of the group repleta of the genus Drosophila (D. mojavensis) and the obscura group of the subgenus Sophophora (D. persimilis and D. pseudoobscura) occupy a basal position. Interestingly, as with the element Helena, the sequences of the melanogaster species complex (D. melanogaster, D. simulans, and D. sechellia) are more closely related to the sequences of the Zaprionus species than to the sequences of D. erecta (mela- nogaster subgroup), with high posterior probability (PP = 1). The origin of the clade of sequences from species in the melanogaster complex and the Zaprionus subgenus is dated at 2.7 Mya, whereas that of the clade that includes the sequences of erecta and the melanogaster complexes and Zaprionus sequences (PP = 1) is dated at 7.3 Mya. The divergence of this clade from the one possessing sequences of the species D. mojavensis, D. persimilis, and D. pseudoobscura is dated at 12.2 Mya (PP = 1). Finally, the unexpected clustering of a sequence of Z. africans (Z. afr_9) with the clade of Z. sepsoides (PP = 1) is incongruent with the phylogenetic relationships between the two species.
The divergence estimated between the BS sequences of the different clades highlights incongruences with the species phylogeny (supplementary table S8, Supplementary Material online). Mean divergence between the BS sequences of the melanogaster complex and the subgenus Zaprionus (8%) is lower than the divergence between the sequences of this complex and that of D. erecta (18.5%). Additionally, the BS sequences of the species of the Oriental melanogaster group (D. bipectinata and D. ficusphila) are less divergent from those included in the clad that groups the sequences of the obscura and repleta groups (21%) than from those of the melanogaster (34%) and Zaprionus (33%) complexes.

Network Analysis

The network analyses corroborated the results of the phylogenies showing that the BS and Helena sequences from species belonging to the melanogaster complex are more closely related to the sequences from species of the subgenus Zaprionus (figs. 4 and 5).

The Helena sequences of the Zaprionus subgenus and the melanogaster subgroup are very similar, separated by short branches that correspond to substitutions of fewer than ten amino acids. In contrast, the sequences of Zaprionus and the Tropical African species of the melanogaster group are separated from the sequences of the Oriental species of the melanogaster group by long branches corresponding to substitutions of at least 30 amino acids. The center of the network is occupied by many median vectors, without establishing direct relationships among the sequences of the species of the Zaprionus subgenus and the melanogaster subgroup. The sequences of the melanogaster species group from the Oriental region are also bound by median vectors to the sequences of species of the subgenus Drosophila (virilis and repleta groups), which might represent either unsampled sequences or ancestral states of the sequences that were
One exception is the direct relationship between the sequences of D. sechellia and the sequences of Z. ornatus. This suggestion of direct ancestry is concordant with the phylogenetic reconstruction (fig. 4).

Similar to Helena, the BS sequences are closely related to those of the melanogaster complex (fig. 5). Their sequences are separated by branches corresponding to substitutions of an average of 13 amino acids, fewer than those of the melanogaster group species that diverged in the Oriental region, which showed substitutions of at least 146 amino acids, or 59 amino acids substitutions relative to the sequences of species of the groups obscura (D. pseudoobscura, D. persimilis) or repleta (D. mojavensis). For the Helena sequences, the central median vectors may represent ancestral sequences not sampled, which preclude direct inference of the relationship between the sequences of the species of Zaprionus and those of the subgroup melanogaster. However, these vectors reinforce the separated clustering of the BS sequences from the groups vittiger and inermis, along with the clustering of Z_afr9 with the sequences of Z. sepsoides.

**Identification of Vertical and Horizontal Transfers**

To evaluate whether the phylogenetic incongruities observed for the Helena and BS elements can be explained by HTT events, we used the VHICA method, a recently proposed strategy, to differentiate HTT and VT events involving TEs among related species (Wallau et al. 2016). In this study, VHICA was used only for comparisons with the species of the subgenus Zaprionus with sequenced genomes—Z. indiarius, Z. gabonicus and Z. africanus—which allowed access to sequences of genes orthologous to those of Drosophila.
species (see supplementary table S6, Supplementary Material online). For the Drosophila species, this analysis only used sequences from species belonging to the melanogaster subgroup because the use of VHICA is not recommended when the sequences exhibit divergence (pairwise distance) higher than 30%.

The linear regression analyses performed by VHICA showed a scenario in which the sequences of the Helena and the BS elements are outside the limit of variance for VT in several comparisons between species (fig. 6 and supplementary figs. S3–S6, Supplementary Material online). For example, in comparisons of D. simulans versus Z. indianus and D. sechellia versus Z. gabonicus, the sequences have a low rate of evolution in synonymous sites (dS) and a high effective number of codons (ENC), values that are significantly different from the estimated variance limit for the orthologous genes in the linear regression, thus supporting the inference of HTT. However, there is no evidence of HTT events among species of the melanogaster subgroup, as exemplified by the comparisons between D. yakuba and D. simulans, in which the dS and ENC values of the two elements are within the range of estimated values for the orthologous genes. In the heatmaps, which present the results of the linear regressions, we can observe strong signals of HTT between the sequences of the Helena and the BS elements of the Drosophila species of the subgroup melanogaster (D. mauritiana, D. sechellia, D. melanogaster, D. yakuba, D. tei, D. teissieri, D. ore, D. orena, D. ere, D. erecta, D. bik, D. bipectinata, D. ana, D. ananassae, D. vir, D. virlis, D. moj, D. mojavensis, D. buzz, D. buzzati, D. koe, D. koeperae). In this analysis, 86 codons and 65 sequences were used.

**Discussion**

Three requirements must be considered when proposing HTTs: 1) the high similarity between sequences of TEs from
distantly related species, 2) inconsistencies between species and TEs phylogenies, and 3) the discontinuous distribution of TEs in a group of species (reviewed in Loreto et al. 2008; Carareto 2011; Wallau et al. 2012). It is also important to consider whether the elements are amenable to mobilization due to the conservation of their structure and whether the lifestyle and geographical distribution of host species corroborate the molecular results (Loreto et al. 2008). In addition to those factors, the mechanism of transposition has been used to justify the differential frequency of HT among types of TEs. Peccoud et al. (2017) used bioinformatics analyses to identify 206 cases of HTT of non-LTR retrotransposons in insects. Combined with the HTT events published before 2018, 302 HTT cases are reported at http://lpa.saogabriel.unipampa.edu.br:8080/httdatabase/resultado/resultado.jsp?organism; last accessed December 2016, but in most cases, the TEs involved are identified only at the superfamily level. Among them, 18 HTT cases deserve special mention because they exemplify the ability of specific LINE families to travel across genomes from species belonging to the same genus to higher taxa. Of these cases, 16 events involved the transfers of five LINE families in Drosophila: two events involved Jockey (Mizrokhi and Mazo 1990; Sánchez-Gracia et al. 2005), one event involved the F element, one event involved Doc (Sánchez-Gracia et al. 2005), one event involved the I element (Kidwell 1983; Brégliano and Kidwell 1983; Bucheton et al. 1984) and 11 events involved Penelope (Evgen’ev et al. 2000; Lyozin et al. 2001; Morales-Hojas et al. 2006). Additionally, the transfer of two RTE elements between species of different classes of vertebrates were reported, as Bov-B was transferred between snakes and ruminants probably by reptile ticks (Kordis and Gubensek 1998; Walsh et al. 2013) and two waves of transfers of AviRTE occurred between birds and parasitic nematodes (Suh et al. 2016). The results presented herein with the Helena and BS elements contribute to the enrichment of our knowledge of the HTT of non-LTR retrotransposons by increasing the number of events, TEs and species evaluated.

The three main requirements above-mentioned to propose HTTs are met in our study: high similarity between sequences of the Helena and BS from distantly related species, inconsistencies between the species and the element phylogenies, and their discontinuous distribution (absence
of Helena and BS in Z. bogoriensis, which belongs to the Anaprionus subgenus, and of BS in four species of the Zaprionus subgenus). The discontinuous distribution scenario could be explained by two hypotheses. The first hypothesis assumes the presence of Helena and BS in the ancestor of the two subgenera of the genus Zaprionus and later losses of both elements in the subgenus Anaprionus, and do also in some species of the subgenus Zaprionus. These losses could have occurred gradually due to either the accumulation of mutations, which would indicate a process of extinction of the TE, or genetic drift. Because the only member of the subgenus Anaprionus available for analysis was Z. bogoriensis, we could not test the first hypothesis. The second hypothesis assumes the complete absence of Helena and BS in the ancestral of the genus Zaprionus and recent introduction in the subgenus Zaprionus by HTT. In the case of HTT into the subgenus Zaprionus, the introduction of both elements would have occurred concomitantly with the divergence of the species belonging to this subgenus in Africa. However, we did not discard the possibility that the Asian species carry very divergent Helena and BS sequences, but unfortunately, except for Z. bogoriensis the Asian species were not available for analysis. Even though, this does not invalidate our second hypothesis, because it was formulated for the sharing of similar Helena and BS sequences between species of Zaprionus and those of the subgroup melanogaster. We tested this hypothesis using phylogenetic reconstruction, networks and the VHICA method and investigated whether both non-LTR retroposons were inserted into the ancestor of the subgenus Zaprionus by one or more independent HTT events.

The Helena Element

The Helena element was identified in all species of the African Zaprionus subgenus; however, it was not identified in Z. bogoriensis, the only species analyzed from the Oriental Anaprionus subgenus. As shown in the phylogeny, the clustering of all the Helena sequences sampled in species of the subgenus Zaprionus within the clade of the African species of the melanogaster group, on one hand, and that of Helena sequences of Z. ornatus together with those of D. sechellia, on the other hand, indicate that more than one HTT event occurred among the Tropical African drosophilids.

The divergence of the Drosophila and Zaprionus genera is estimated to have occurred between 40 and 60 Mya (Russo et al. 1995; Yassin et al. 2008). As illustrated in figure 1, the genus Zaprionus diversified on the Oriental region during the Quaternary period at approximately 7 Mya and from there, a lineage would have migrated to the islands of the Indian Ocean and to the African continent, where the diversification of the Zaprionus subgenus complexes is proposed to have occurred at approximately 4 Mya (Yassin et al. 2008). The clade that aggregates all the sequences of Helena from Zaprionus was estimated to be 4.1 My old (fig. 2), values consistent with the diversification of the subgenus in tropical Africa. Estimates of the origin of the element Helena are
therefore consistent with the period of diversification in the African continent of the subgenus Zaprionus, along with the divergence of the melanogaster complex (Lachaise and Silvain 2004; Tamura et al. 2004a, 2004b; Cutter 2008). The clustering of Z. ornatus with D. mauritiana and D. sechellia is also incongruent with the divergence time of the species included in this clade, whose Helena ancestral sequence was estimated to originate 0.4 Mya. The dating of this clade is similar to the divergence time of D. mauritiana and D. sechellia (0.4 Mya, Lachaise and Silvain 2004) and is much more recent than the diversification of the Z. ornatus species complex (4.4 Mya, Yassin et al. 2008).

The above-mentioned incongruities are also observable in the network in which the Helena sequences of the subgroup melanogaster and Zaprionus form a separate group from the other sequences of species that did not share an evolutionary period in Africa. However, due to the large number of median vectors between the sequences, the direction of the HTT cannot be clearly inferred, except for the Helena sequences of D. sechellia and Z. ornatus, for which a direct relationship of ancestry-descent exists (D. sechellia to Z. ornatus). In summary, our results suggest the occurrence of two HTT events of Helena among the species of the subgenus Zaprionus and the subgroup melanogaster. The first event would have occurred at approximately 4 Mya between the ancestor of the melanogaster complex and the ancestor of the subgenus Zaprionus, and the second would have occurred at <0.5 Mya and involved the transfer of Helena from D. sechellia to Z. ornatus.

The sequences of Helena of the Oriental species of the melanogaster group, D. bipectinata and D. ananassae, also suggest a relationship inconsistent with the species phylogeny of the melanogaster subgroup, as they clustered with sequences of the species belonging to the repleta and virilis groups. Although the species of the repleta group in which Helena was sampled are currently native to the Nearctic region, the ancestor of the group evolved in Asia, from whence it migrated to the Americas at approximately 30 Mya (Throckmorton 1982), as shown in figure 1. This grouping suggests another HTT event between ancestors of subgroups of the melanogaster, virilis and repleta groups.

The BS Element

Similar to Helena, BS presented a discontinuous distribution pattern: it was found in six of the 11 species of Zaprionus (belonging to the complexes sespioides, davidi and ornatus) and in 14 of the 21 Drosophila species tested, including only two of the 11 Oriental species of the melanogaster group. Two basal incongruities call attention to the phylogenetic tree of BS. The first is due to the BS sequences of the melanogaster complex clustering more closely with the sequences of the Zaprionus subgenus than with those of D. erecta, which belongs to the same subgroup (fig. 3). The clade that combines these sequences is dated at 2.6 Mya, which is near the period of diversification of the species of the complex melanogaster (Lachaise and Silvain 2004; Tamura et al. 2004a, 2004b; Cutter 2008). This phylogenetic incongruence, the distance values, the network and the statistical results provided by the VHICA analysis support our hypothesis of the occurrence of one or more HTT events of the element BS between the ancestor of the melanogaster complex and ancestors of the complexes belonging to the inermis and vittiger groups, which evolved from 3.9 Mya in the Indian Ocean Islands and from 4.4 Mya in Central Africa, respectively (Yassin et al. 2008; Yassin and David 2010). In addition, the polyphyly inside the clade Zaprionus and the relatively short time of species divergence makes incomplete lineage sorting an equally probable explanation for the phylogenetic incongruities within this clade.

Two additional BS HTT events were detected in our phylogenetic analyses. The sequences of the species D. persimilis and D. pseudoobscura of the group obscura (subgenus Sophophora) are grouped more closely with those of D. mojavensis (subgenus Drosophila). These sequences form a clade with high support that are grouped with the sequences of the melanogaster-Zaprionus subgroup dated at 12.5 Mya. The sequences of the Oriental species D. bipectinata and D. ficusphila of the melanogaster group are grouped basally to this clade. These species belong to three subgenera and five different species groups (subgenus Sophophora: melanogaster and obscura groups; subgenus Drosophila: repleta group; and subgenus Zaprionus: vittiger and inermis groups) that share a recent common ancestral BS sequence dated at 18.4 Mya. This date coincides with that of the migration of the proto-melanogaster founder population to Africa (between 17 and 20 Mya) from the Eastern region (Lachaise and Silvain 2004). Although Drosophila species of the pseudoobscura subgroup and the repleta group are native to the New World, their ancestors are of Asian origin. The obscura group would have been subdivided into obscura and pseudoobscura subgroups while still in Asia, and the latter would have been introduced in the Late Miocene in the Americas, at approximately 13 Mya (Russo et al. 1995). The HTTs of BS among the ancestral strains of one or more subgroups of the melanogaster group to the ancestor of the pseudoobscura subgroup could have occurred, and from this, HTT could have subsequently occurred to species of the repleta group (here represented by D. mojavensis) in the Americas (fig. 1).

Genomic Promiscuity between the melanogaster Complex and the Subgenus Zaprionus

We present robust evidence supporting the hypothesis of the occurrence of several HTT events involving the non-LTR retrotransposons Helena and BS between species of the melanogaster complex and species of the subgenus Zaprionus. Helena and BS were present in the melanogaster group.
before the divergence of the African subgroup. Because these species have evolved in Tropical Africa over the past 10 My, several opportunities for HTT must have occurred, allowing the transfer of TEs with a low probability of invasion of new genomes.

Importantly, the non-LTR retrotransposons examined in this study were not the only participants in HTTs between these species in this period. Some studies previously showed evidence of HTTs between species of the melanogaster and Zaprinus involving the LTR retrotransposons Tom, 297, 17.6, rover (Vidal et al. 2009), Gypsy (Herédia et al. 2004; De Setta et al. 2009), Microopia (De Setta et al. 2009), Copia (De Setta et al. 2011), and DNA transposons Mariner (Maruyama and Hartl 1991), Mos1-Like (Brunet et al. 1999) and hAT (Deprà et al. 2010). In most of these studies, only one species of Zaprinus (Z. indianus) and few TEs were studied, and the direction of the HTT was unclear. We previously proposed that three Gypsy variants and one Microopia variant, both LTR retrotransposons, invaded the genomes of species of the subgenus Zaprinus in waves of HTT from the ancestor of the melanogaster group between 0.6 and 10 Mya (De Setta et al. 2009). Earlier still, at approximately 11 Mya, an invasion of the LTR retrotransposon Copia occurred in the genus Zaprinus from an ancestor of the group melanogaster (De Setta et al. 2011). This element is even present in Z. bographicus (subgenus Anaprinus), which does not have Helena, BS, Gypsy, or Microopia, reinforcing HTT at an earlier point in the evolution of these drosophilids.

The results of the above-cited studies showed that after invasion, the LTR retrotransposons maintained their transposition activity and participated in some HTT events within the subgenus Zaprinus. In this study, we also identified putatively complete copies of at least BS in the subgenus Zaprinus through analysis of the integrity of the protein domains. Because the number of sequenced Zaprinus genomes remains very low, we cannot exclude the possibility that BS and Helena elements are still transpositionally active in this subgenus or that they had been active until recently. Together, the data presented herein reveal a panorama of extensive genetic material exchange between species of the subgroup melanogaster and those of the subgenus Zaprinus during the origin and diversification of the species in Tropical Africa between 4 and 1 Mya. This findings also support the hypothesis of permissiveness to the fixation of transferred TEs in the genomes of newly diversified species, mainly associated with small effective population sizes, which could reduce the efficacy of natural selection against invasive DNA (reviewed in Carareto 2011).

Conclusions and Perspectives
The importance of this study goes beyond stating whether the HT of TEs occurred between species of the subgenera Sophophora and Zaprinus. The in-depth reconstruction of the timeframe and geography of these events allowed us to reaffirm a historical scenario for the evolution of the genomes of these groups of species. For the first time, we identified the HTT of non-LTR retrotransposon in Zaprinus. The growth of genomic data for nonmodel species and high-throughput genomic analysis, which were combined in this study, corroborate recent data indicating that the HTT of non-LTR retrotransposons is not as rare as previously thought. Knowledge of the period of species diversification, the life history and the environment in which they evolved facilitates an understanding of not only population history but also the processes of diversification for one of the most important known sources of genetic diversity: transposable elements.

Supplementary Material
Supplementary data are available at Genome Biology and Evolution online.

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Literature Cited
De Almeida LM, Carareto CM. 2006. Sequence heterogeneity and phylogenetic relationships between the copia retrotransposon in Drosophila species of the repleta and melanogaster groups. Genet Sel Evol. 38(5):535–550.
Altschul SF, et al. 1990. Basic local alignment search tool. J Mol Biol. 215(3):403–410.
Andersson JO. 2005. Lateral gene transfer in eukaryotes. Cell Mol Life Sci. 62(11):1182–1186.
Ankolabédère D, Kidwell MG, Periquet G. 1988. Molecular characteristics of diverse populations are consistent with the hypothesis of a recent invasion of Drosophila melanogaster by mobile P elements. Mol Biol Evol. 5(3):252–269.
Baidouri ME, et al. 2014. Widespread and frequent horizontal transfers of transposable elements in plants. Genome Res. 24(5):831–838.
Bandelt HJ, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 16(1):37–48.
Bréjiliano J, Kidwell MG. 1983. Hybrid dysgenesis determinants. In: Shapiro J, editor. Mobile genetic elements. New York: Academic Press. p. 363–410.
Brunet F, Godin F, Bazin C, Capy P. 1999. Phylogenetic analysis of Mos1-like transposable elements in the Drosophilidae. J Mol Evol. 49(6):760–768.
Bucheton A, Paro R, Sang HM, Pelisson A, Finnegan D. 1984. The molecular basis of I-R hybrid dysgenesis in Drosophila melanogaster: identification, cloning, and properties of the I factor. Cell 38(1):153–163.
Carareto CM. 2011. Tropical Africa as a cradle for horizontal transfers of transposable elements between species of the genera Drosophila and Zaprinus. Mol Genet Elements 1(3):179–188.
Clark JB, Maddison WP, Kidwell MG. 1994. Phylogenetic analysis supports horizontal transfer of P transposable elements. Mol Biol Evol. 11(1):40–50.
Cordaux R, Hedges DJ, Batzer MA. 2009. Retrotransposition of Alu elements: how many sources? Trends Genet. 25(10):464–467.
Cutter AD. 2008. Divergence times in Caenorhabditis and Drosophila inferred from direct estimates of the neutral mutation rate. Mol Biol Evol. 25(4):778–786.
Daniels SB, Peterson KR, Strausbaugh KMG, Chovnicl A. 1990. Evidence for horizontal transmission of the P transposable element between Drosophila species. Genetics 124:339–355.
Daniels SB, Strausbaugh LD, Ehrman L, Armstrong R. 1984. Sequences homologous to P elements occur in Drosophila simulans. Proc Natl Acad Sci USA. 81(21):6794–6797.
Depri M, Panzera Y, Ludwig A, Valente VLS, Loreto ELS. 2010. hosimarya: a new IAT transposon group in horizontal transfer. Mol Genet Genomics 283(5):451–459.
Diao X, Freeling M, Lisch D. 2006. Horizontal transfer of a plant transposon. PLoS Biol. 4(1):e5.
Dias ES, Carareto CMA. 2012. Ancestral polymorphism and recent invasion of transposable elements in Drosophila species. BMC Evol Biol. 12(1):119.
Dias ES, et al. 2015. Large distribution and high sequence identity of a Copia-type retrotransposon in angiosperm families. Plant Mol Biol. 89(1–2):83–97.
Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUiT and the BEAST 1.7. Mol Biol Evol. 29(8):1969–1973.
Evgen’ev M, Zelentsova H, Mnjohan L, Poluecota H, Kidwell MG. 2000. Invasion of Drosophila viridis by the Penelope transposable element. Chromosoma 109(5):350–357.
Felsenstein J. 1985. Phylogenies and the comparative method. Am Nat. 125(1):1–15.
Friesen PD, Nissen MS. 1990. Gene organization and transcription of TED, a lepidopteran retrotransposon integrated within the baculovirus genome. Mol Cell Biol. 10(6):3067–3077.
Gao D, et al. 2018. Horizontal transfer of non-LTR retrotransposons from arthropods to flowering plants. Mol Biol Evol. 35(2):354–364.
Gilbert C, Schaack S, Pace JK, Brindley PJ, Feschotte C. 2010. A role for host-parasite interactions in the horizontal transfer of transposons across phyla. Nature 464(7293):1347–1350.
Goubert C, et al. 2015. De novo assembly and annotation of the Asian tiger mosquito (Aedes albopictus) genome and drafAETe from raw genomic reads and comparative analysis with the yellow fever mosquito (Aedes aegypti). Genome Biol. 7(4):1192–1205.
Granzotto A, Lopes FR, Lerat E, Vieira C, Carareto CMA. 2009. The evolutionary dynamics of the Helena retrotransposon revealed by sequenced Drosophila genomes. BMC Evol Biol. 9(1):174.
Granzotto A, Lopes FR, Vieira C, Carareto CMA. 2011. Vertical inheritance and bursts of transposition have shaped the evolution of the BS non-LTR retrotransposon in Drosophila. Mol Genet Genomics 286(1):57–66.
Herédia F, Loreto ELS, Valente VLS. 2004. Complex evolution of gypsy in drosophilid species. Mol Biol Evol. 21(10):1831–1842.
Jeffs PS, Holmes EC, Ashburner M. 1994. The molecular evolution of the alcohol dehydrogenase and alcohol dehydrogenase-related genes in the Drosophila melanogaster species subgroup. Mol Biol Evol. 11(2):287–304.
Jowett T. 1986. Preparation of nucleic acids. In: Roberts DB, editor. Drosophila: a practical approach, vol. 335. Washington (DC): Oxford. p. 275–277.
Katoh K, Rozevickj I, Yamada KD. 2017. Article Navigation MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. 18(3):108–108.
Kidwell MG. 1983. Evolution of hybrid dysgenesis determinants in Drosophila melanogaster. Proc Natl Acad Sci USA. 80(6):1655–1659.
Kim AI, et al. 1994. Retroviruses in invertebrates: the gypsy retrotransposon is apparently an infectious retrovirus of Drosophila melanogaster. Proc Natl Acad Sci USA. 91(4):1285–1289.
Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 16(2):111–120.
Koller R, Hill T, Nolte V, Betancourt AJ, Schlötzer C. 2015. The recent invasion of natural Drosophila simulans populations by the P-element. Proc Natl Acad Sci USA. 112(21):6659–6663.
Kords D, Gubensek F. 1998. Unusual horizontal transfer of a long interspersed nuclear element between distant vertebrate classes. Proc Natl Acad Sci USA. 95(18):10704–10709.
Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33(7):1870–1874.
Lachaise D, Silvain JF. 2004. How two Afrotropical endemics made two cosmopolitan human commensals: the Drosophila melanogaster–D. simulans palaeogeographic riddle. Genetics 120(1–3):17–39.
Loreto ELS, Carareto CMA, Capy P. 2008. Revisiting horizontal transfer of transposable elements in Drosophila. Heredity (Edinb) 100(6):545–554.
Ludwig A, Valente VL, Loreto EL. 2008. Multiple invasions of Errantivirus in the genus Drosophila. Insect Mol Biol. 17(2):113–124.
Lyazin GT, et al. 2001. The structure and evolution of Penelope in the viridis species group of Drosophila: an ancient lineage of retroelements. J Mol Biol. 52(5):445–456.
Maruyama K, Hartl DL. 1991. Evidence for interspecific transfer of the transposable element manner between Drosophila and Zaprinus. J Mol Evol. 33(6):514–524.
Metcalfe CJ, Casane D. 2014. Modular organization and reticulate evolution of the ORF1 of Jockey superfamily transposable elements. 5:19.
Mizroki LJ, Mazo AM. 1990. Evidence for horizontal transmission of the mobile element jockey between distant Drosophila species. Proc Natl Acad Sci USA. 87(23):9216–9220.
Modolo L, Lerat E. 2015. UrQT: an efficient software for the Unsupervised Quality trimming of NGS data. BMC Bioinformatics 16:137.
Modolo L, Picard F, Lerat E. 2014. A new genome-wide method to track horizontally transferred sequences: application to Drosophila. Genome Biol. Evol. 6(2):416–432.
Morales-Hojas R, Vieira CP, Vieira J. 2006. The evolutionary history of the transposable element Penelope in the Drosophila viridis group of species. J Mol Evol. 62(2):262–273.
Okada T, Carson HL. 1983. The genera Phorticailla DUDA and Zaprinus COQUILLETT (Diptera, Drosophilidae) of the Oriental region and New Guinea. Jpn J Entomol 51:539–553.
Pace JK, II, Gilbert C, Clark MS, Feschotte C. 2008. Repeated horizontal transfer of non-LTR retrotransposons from arthropods to flowering plants. Mol Biol Evol. 35(2):354–364.
Palazzo A, Caizzi R, Viggiano L, Marsano RM. 2017. Does the promoter contribute a barrier in the horizontal transposon transfer
process? Insight from Bari Transposons. Genome Biol. Evol. 9(6):1637–1645.
Pecquod J, Loiseau V, Cordaux R, Gilbert C. 2017. Massive horizontal transfer of transposable elements in insects. Proc Natl Acad Sci USA. 114(18):4721–4726.
Pélisson A, et al. 1994. Gypsy transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the Drosophila flamenco gene. EMBO J. 13:4401–4411.
Petrov DA, Schultz J, Hartl DL, Lozovskaya ER. 1995. Diverse transposable elements are mobilized in hybrid dysgenesis in Drosophila virilis. Proc Natl Acad Sci USA. 92(17):8050–8054.
Petrov DA, Chao Y-C, Stephenson EC, Hartl DL. 1998. Pseudogene evolution in Drosophila suggests a high rate of DNA loss. Mol Biol Evol. 15(11):1562–1567.
Ray DA, et al. 2008. Multiple waves of recent DNA transposon activity in the bat, Myotis lucifugus. Genome Res. 18(5):717–728.
Rebollo R, Lerat E, Kleine LL, Bérimont C, Vieira C. 2008. Losing Helena: the extinction of a Drosophila line-like element. BMC Genomics 9:149–160.
Robertson HM, Zumpano KL. 1997. Molecular evolution of an ancient mariner transposon, Homar1, in the human genome. Gene 205(1–2):203–217.
Romero-Soriano V, Guerreiro MPG. 2016. Expression of the retrotransposon Helena reveals a complex pattern of TE deregulation in Drosophila hybrids. PLoS One 11(1):e0147903.
Russo CAM, Takezaki N, Nei M. 1995. Molecular phylogeny and divergence times of drosophilid species. Mol Biol Evol. 12(3):391–404.
Sánchez-Gracia A, Maside X, Charlesworth B. 2005. High rate of horizontal transfer of transposable elements in Drosophila. Trends Genet. 21(4):200–203.
Schaack S, Gilbert C, Feschotte C. 2010. Promiscuous DNA: horizontal transfer of transposable elements and why it matters for eukaryotic evolution. Trends Ecol Evol. 25(9):537–546.
De Setta N, Van Sluys MA, Capy P, Carareto CM. 2009. Multiple invasions of Gypsy and Micropia retroelements in genus Zaprionus and melanogaster subgroup of the genus Drosophila. BMC Evol. Biol. 9:279–297.
De Setta N, Van Sluys MA, Capy P, Carareto CMA. 2011. Copia retrotransposon in the Zaprionus genus: another case of transposable element sharing with the melanogaster subgroup. J Mol Evol. 72(3):326–338.
Sharp PM, Li WH. 1989. On the rate of DNA sequence evolution in Drosophila. J Mol Evol. 28(5):398–402.
Sessegolo C, Burlet N, Haudry A. 2016. Strong phylogenetic inertia on genome size and transposable element content among 26 species of flies. Bioll Lett. 12(8):20160407.
Silva JC, Kidwell MG. 2000. Horizontal transfer and selection in the evolution of P elements. Mol Biol Evol. 17(10):1542–1557.
Song SU, Gerasimova T, Kurkulos M, Boeke JD, Corces VG. 1994. An env-like protein encoded by a Drosophila retroelement: evidence that gypsy is an infectious retrovirus. Genes Dev. 8(17):2046–2057.
Suh A, et al. 2016. Ancient horizontal transfers of retrotransposons between birds and ancestors of human pathogenic nematodes. Nat Commun. 7:11396.
Tamura K, Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 10(3):512–526.
Tamura K, Nei M, Kumar S. 2004a. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA. 101(30):11030–11035.
Tamura K, Subramanian S, Kumar S. 2004b. Temporal patterns of fruit fly (Drosophila) evolution revealed by mutation clocks. Mol Biol Evol. 21(1):36–44.
Thomas T, et al. 2010. Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. ISME J. 4(12):1557–1567.
Throckmorton LH. 1982. Pathways of evolution in the genus Drosophila and the founding of the Repleta group. In: Barker JSF and Starmer WT (eds). Ecological Genetics and Evolution – The Cactus-Yeast Drosophila Model System. Academic Press, Sydney, p. 33–47.
Udomkit A, Forbes S, Daiglelish G, Finnegan DJ. 1995. BS a novel LINE-like element in Drosophila melanogaster. Nucleic Acids Res. 23(8):1354–1358.
Vidal NM, Ludwig A, Loreto ELS. 2009. Evolution of Tom, 297, 17.6 and rover retrotransposons in Drosophilidae species. Mol Genet Genomics 282(4):351–362.
Wallau GL, Capy P, Loreto E, Rouzic AL, Hua-Van A. 2016. VHICA, a New method to discriminate between vertical and horizontal transposon transfer: application to the mariner family with in Drosophila. Mol Biol Evol. 33(4):1094–1109.
Wallau GL, Ortiz MF, Loreto ELS. 2012. Horizontal transposon transfer in eukarya: detection, bias, and perspectives. Genome Biol Evol. 4(8):689–699.
Walsh AM, Kortschak RD, Gardner MG, Bertozzi T, Adelson DL. 2013. Widespread horizontal transfer of retrotransposons. Proc Natl Acad Sci USA. 110(3):1012–1016.
Wicker T, et al. 2007. A unified classification system for eukaryotic transposable elements. Nat Rev Genet. 8(12):973–982.
Yassin A, et al. 2008. Grafting the molecular phylogenetic tree with morphological branches to reconstruct the evolutionary history of the genus Zaprionus (Diptera: Drosophilidae). Mol Phylogenet Evol. 47(3):903–915.
Yassin A, David JR. 2010. Revision of the Afrotopical species of Zaprionus (Diptera, Drosophilidae), with descriptions of two new species and notes on internal reproductive structures and immature stages. Zookeys 51:33–72.

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