Evidence for the recent horizontal transfer of long terminal repeat retrotransposon

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The evolutionary dynamics existing between transposable elements (TEs) and their host genomes have been likened to an ''arms race.'' The selfish drive of TEs to replicate, in turn, elicits the evolution of host-mediated regulatory mechanisms aimed at repressing transpositional activity. It has been postulated that horizontal (cross-species) transfer may be one effective strategy by which TEs and other selfish genes can escape host-mediated silencing mechanisms over evolutionary time; however, to date, the most definitive evidence that TEs horizontally transfer between species has been limited to class II or DNA-type elements. Evidence that the more numerous and widely distributed retroelements may also be horizontally transferred between species has been more ambiguous. In this paper, we report definitive evidence for a recent horizontal transfer of the *copia* long terminal repeat retrotransposon between *Drosophila melanogaster* and *Drosophila willistoni*.

Drosophila | evolution

Long terminal repeat (LTR) retrotransposons are a class of repetitive, mobile DNA sequences that transpose via the reverse transcription of an RNA intermediate. LTR retrotransposons are a primary source of spontaneous mutations that have major phenotypic effects (1) and are hypothesized to be of special evolutionary significance (2–4). Despite the fact that many LTR retrotransposons have been characterized, there have been few detailed studies on the naturally occurring variation existing among these elements within and between populations and species (5–8).

One outstanding issue concerning LTR retrotransposon evolution is the possibility of the horizontal transfer of elements between species. There is strong evidence that DNA elements have been transferred horizontally across species boundaries. The best examples of this are the *P* and *mariner* transposable elements of *Drosophila* (9, 10). Although evidence has been presented that LTR retrotransposons may have also experienced horizontal transfer in their evolution, these data have been less conclusive. This is largely because the suspected horizontal transfers occurred too long ago (11, 12) or between such closely related species (5) that alternative vertical transmission hypotheses cannot be completely eliminated. In this paper, we report that a *copia* LTR retrotransposon isolated from *Drosophila willistoni* is virtually identical in sequence to *copia* retrotransposons present within *Drosophila melanogaster* despite the fact that these two species have been separated from a common ancestor by ≈50 million years (13). Although *copia* is abundant in all *melanogaster* group species, it displays a patchy distribution in *D. willistoni.* Collectively, our findings indicate a recent horizontal transfer of the *copia* LTR retrotransposon from *D. melanogaster* to *D. willistoni*.

Materials and Methods

***Drosophila* Strains.** *D. melanogaster* Iquitos, Peru was provided by Jean R. David (Centre National de la Recherche Scientifique, Gif-sur-Yvette, France). Chris Babcock and Margaret Kidwell (University of Arizona, Tucson, AZ) provided *D. willistoni* natural populations collected from Florida, Grenada, St. Vincent, and St. Croix. *Drosophila simulans* (Australia; #0251.4), *D. willistoni* (Royal Palm Park, Miami, FL; #0811.2), and *D. willistoni* (Santa Maria, de Ostuna, Nicaragua; #0811.0) were obtained from the National *Drosophila* Species Stock Center (Bowling Green, OH). The lab strain w1118 (U-85012) was obtained from the Umea Stock Center (Umea, Sweden). The Oregon R strain (B-2380) was obtained from the Bloomington Stock Center (Bloomington, IN).

PCR. Genomic DNA was prepared from adult flies as described (14). The following primers were used in the PCRs. All *copia* and *Adh* primers were synthesized at the Molecular Genetics Instrumentation Facility at the University of Georgia (Athens, GA). Christian Schlotterer (University of Veterinary Medicine, Vienna, Austria) provided the rDNA primers.

Copia primers: Cop Ltr (5′-CTATTCAACCTACAAAAATA-ACG-3′), Cop Gag (5′-CCTTCTCTGCCACAGTGGTGACA-3′), Cop Pcs (5′-ATTACGTTTAGCCTTGTCCAT-3′), Cop XhoapaI (5′-CTCGAGGGGCCCAGTCCATGCCTAATA-3′).

Drosophila Adh primers: Adh-e2.1 (5′-CTGGACTTCTGG-GACAAGCG-3′), Adh-e2.2 (5′-TGCAACATTGGATCCGT-CACT-3′), Adh-i2 (5′-TTGTTTTTTCTTGAAAACTTT-GCGTT-3′), Adh-e3 (5′-TAGATGCCCGAGTCCCAGTG-3′).

Drosophila rDNA internal transcribed spacer (ITS) primers: CS249 (5′-TCGTAACAAGGTTTCCG-3′), CS 250 [5′-GTT (A/G) GTTTCTTTTCCTC-3′].

The following conditions were used in all PCR reactions: 1× PCR buffer (Fisher), 200 μM dNTPs (Pharmacia), 3 mM magnesium chloride (Fisher), 0.5 μM each primer, 2.5 units of *Taq* polymerase (Fisher), and 100 ng of genomic DNA. Thermal cycler conditions were as follows: 5 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C followed by a final 5 min at 72°C. The *D. willistoni copia* PCR product was then subcloned into pCR2.1 by using the TA cloning method (Invitrogen).

Southern Hybridization. Genomic DNA was isolated as described (14). For each of the populations assayed, 5 μg of genomic DNA was digested to completion with *Eco*RI and *Apa*I according to the manufacturer's instructions (Promega). Digested DNA was fractionated through 1% agarose gel and transferred to Hybond-N nylon membrane (Amersham Pharmacia). The membrane was prehybridized for 4 h at 66°C in 0.5 M sodium phosphate buffer, pH 7.6, 7% SDS. A 5′ *copia* probe was generated with PCR by using the *D. willistoni copia* clone sequenced here as a template with primers XhoapaI and Cop Gag. The *copia* probe was labeled by using [^{32}P]dATP (ICN) and

Abbreviations: TE, transposable element; LTR, long terminal repeat; ULR, untranslated leader region; ITS, internal transcribed spacer; *Adh*, alcohol dehydrogenase gene.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF175766).

See commentary on page 12211.

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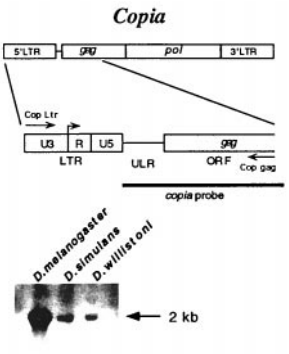

Fig. 1. *Copia* is an LTR retrotransposon. The genomic structure of *copia* consists of two LTRs that flank a single ORF with homology to the *gag* and *pol* loci of retroviruses. The 5′ region of *copia* was PCR amplified from *D. willistoni* genomic DNA by using the Cop Ltr and Cop gag primers. Southern hybridization of genomic DNA with a *copia* probe under conditions of high stringency confirmed the presence of *copia* in *D. melanogaster*, *D. simulans*, and *D. willistoni*.

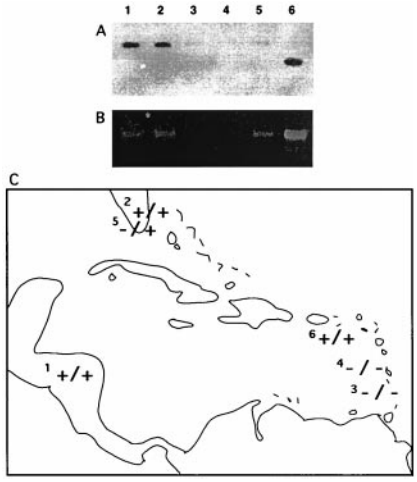

Fig. 2. Southern hybridization (*A*) and PCR (*B*) survey for the presence of *copia* in various *D. willistoni* populations: 1, Santa Maria, de Ostuna, Nicaragua; 2, Royal Palm Park; 3, Grenada; 4, St. Vincent, Grenadines; 5, South Florida; 6, St. Croix. We attribute the slightly smaller band in the St. Croix sample to a deletion within the *copia gag* region flanked by the *Apa*I–*Eco*RI restriction sites. The PCR results revealed no such intraspecific size polymorphism within the *copia* ITR–ULR. (*C*) The first + or − represents the results of Southern hybridization followed by the second + or − that represents PCR results.

the High Prime DNA Labeling Kit (Boehringer Mannheim). The probe was added to the prehybridization buffer and hybridization proceeded for 18 h at 66°C. The membrane was washed under the following high-stringency conditions: 10 min each at 64°C, 3 times the SSC, 0.1% SDS; 2× SSC, 0.1% SDS; 1× SSC, 0.1% SDS; and finally 0.1 × SSC, 0.1% SDS for 10 min at room temperature.

Genomic Library. A genomic library was constructed from Royal Palm Park *D. willistoni* DNA. Approximately 2 μg of genomic DNA was partially digested with *Bam*HI (Promega) according to the manufacturer's instructions. This DNA was then ligated into 1 μg of lambda arms of the replacement vector Lambda-DASH II (Stratagene).

The genomic library was screened by using a full-length *copia* element (Dm5002) as a probe. Supported nitrocellulose membranes (Schleicher & Schuell) containing plaque lifts were screened under the conditions described above. Plugs of isolated plaques were placed in 500 μl of SM. Lambda DNA was isolated by using the Qiagen (Chatsworth, CA) lambda kit.

Sequence Analysis. Automated sequencing was done at the University of Georgia Molecular Genetics Instrumentation Facility (Athens, GA). The *D. willistoni copia* subclone was sequenced in both directions by using T7 and M13 reverse primers. The resulting chromatograms were aligned and compared by using the SEQED program (Applied Biosystems) to resolve any sequence ambiguities. *Copia* sequences correspond to the following accession numbers: *D. melanogaster* c1-no. M11240, *D. melanogaster* c2-no. X04456, *D. simulans* c1-no. D10880, and *D. willistoni* c4-no. AF175766.

Results

Polymorphism for the Presence of *Copia* Exists Among Geographic Populations of *D. willistoni*. *Copia* is an LTR retrotransposon that is widely distributed among *Drosophila* species (12). Southern hybridization of *D. willistoni* with a *copia* probe under conditions of high stringency confirmed earlier reports (15) that the element is present in *D. willistoni* (Fig. 1). A Southern hybridization survey of recently established strains representing six populations of *D. willistoni* demonstrated that there is geographic polymorphism for the presence or absence of *copia* among *D. willistoni* (Fig. 2).

PCR was also used to survey the above strains for the presence of *copia*. Primers that flank an ≈1-kb region of *copia*, which includes the 5′ LTR, the adjacent untranslated leader region (ULR) and *gag* encoding sequence, were used in an attempt to amplify *copia* sequences from *D. willistoni* genomic DNA (Fig. 1). The results of the PCR experiments were consistent with the Southern hybridization results in all cases except one (Fig. 2). PCR of genomic DNA from one of the South Florida strains that tested negative in our hybridization screen did nevertheless yield a *copia* PCR product. We interpret this observation to be the result of the high sensitivity of the PCR technique.

A *Copia* LTR Retrotransposon Isolated from a *D. willistoni* Florida Population Is >99% Identical to *D. melanogaster Copia* LTR Retrotransposons. A *copia* PCR product isolated from the second South Florida population (Royal Palm Park) that tested positive for the presence of *copia* via both hybridization and PCR was subcloned and sequenced. Alignment of this *D. willistoni copia* sequence with published sequences of *copia* elements isolated from *D. melanogaster* and *D. simulans* demonstrated sequence similarities of >99% (Fig. 3). Previous results from a survey of *melanogaster* subgroup *copia* LTR-ULR variation revealed substantially lower sequence identities of ≈90% between *copia* sequences isolated from the sibling species *D. melanogaster* and *D. simulans* (5). Remarkably, the *D. willistoni copia* sequence isolated here was identical to that of a published *D. melanogaster copia* sequence (16) isolated in another laboratory. This identity of *copia* elements isolated from *D. melanogaster* and *D. willistoni* is particularly striking given the fact that these two species are estimated to have last shared a common ancestor ≈50 million years ago (13).

To eliminate the possibility that our results may have occurred because of contamination during the PCR process, we instituted a series of rigorous controls. Southern hybridization of *D. willistoni* DNA under conditions of high stringency eliminated the possibility that our PCR data result from contamination of our genomic DNA samples with a minute amount of *copia*-cloned sequence.

An additional series of PCR controls were performed to eliminate the possibility of contamination during the PCR process. Every PCR was performed with a double-distilled water negative-control template to guard against contamination of the

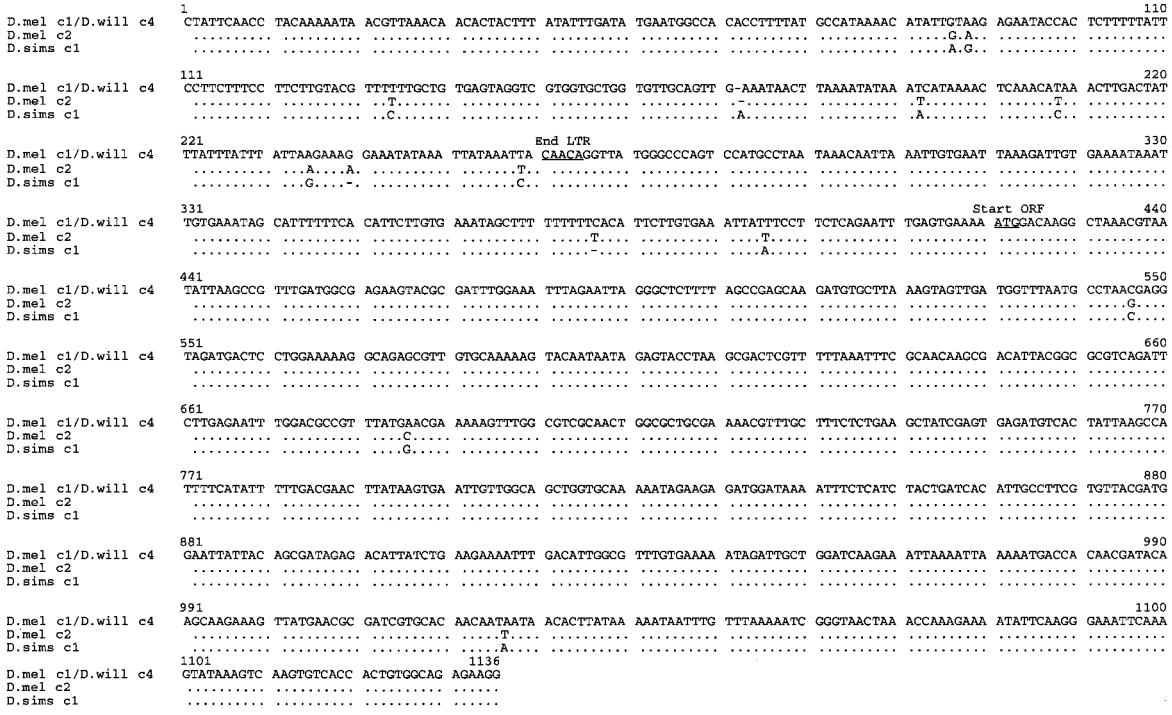

Fig. 3. Sequence alignment of the 5′ region of *copia* consisting of the 5′ LTR (1–265), ULR (266–430), and part of the *gag* locus (431–1,136) of the ORF. Dots in the alignment represent identity and dashes represent a gap in the sequence. The *D. melanogaster* no. M11240 (D. mel c1) and *D. willistoni* (Royal Palm Park; D. will c4) sequences are 100% identical. Polymorphic sites present in two other *copia* sequences isolated from *D. melanogaster* (D. mel c2) no. X04456 and *D. simulans* no. D10880 (D. sims c1) are shown below the *D. mel* c1/*D. will* c4 consensus sequence.

PCR reagents. In the first series of internal PCR controls, primers that amplify the internal transcribed spacer (ITS) regions of the multicopy *Drosophila* rDNA genes were employed. ITS regions are variable and show species-specific size polymorphisms (17). Primers homologous to highly conserved sequences within the 18S and 28S rDNA coding regions that flank the ITS-1, 5.8S rDNA and ITS-2 regions were used to amplify genomic DNA from *D. melanogaster* and *D. simulans* as well as that from the *D. willistoni* sample (Royal Palm Park) from which we amplified the *copia* sequence. Each species sample resulted in PCR products of unique size (Fig. 4). No evidence of contamination was found in any of these reactions despite the fact that we separately established, by using intentional contamination of genomic DNA samples, that as little as 100 pg of contaminating *D. melanogaster* DNA could be detected in our assays.

A second series of internal PCR controls utilized primers homologous to sequences within the *Drosophila* alcohol dehydrogenase gene (*Adh*). *Adh* genes present in *melanogaster* group species contain two introns, the second of which is missing in *willistoni* group species (18). In our control experiments, we utilized primers homologous to conserved sequences within the second and third exons of *Drosophila Adh*. PCR products obtained by using *melanogaster* group species DNA as a template are predicted to be ≈70 bp larger than those obtained by using *willistoni* group species DNA as a template because of the missing intron in *D. willistoni Adh*. Consistent with our previous controls, the *D. willistoni* genomic DNA sample resulted in only the smaller *Adh* product with no evidence of DNA contamination from any of the *melanogaster* group species (Fig. 4).

To further ensure that the *D. willistoni* genomic DNA preparations were free from contaminating *D. melanogaster* DNA, we performed a final PCR control utilizing primers homologous to sequences in the second exon and second intron of the *D. melanogaster Adh* gene. The second exon primer was designed with two polymorphisms at the 3′ end unique to *D. melanogaster Adh*. The second intron primer is homologous only to *D. melanogaster Adh* sequences because this intron is missing in the *D. willistoni Adh* gene; therefore, this primer pair can only amplify an *Adh* product from *D. melanogaster* DNA and not *D. willistoni* DNA. This primer pair was able to amplify a product of the expected size from as little as 5 ng of *D. melanogaster* genomic DNA. Samples of *D. willistoni* DNA up to 200 ng did not yield any PCR product by using the same primers; thus, the results of this control experiment also indicate that the *D. willistoni* DNA preparations do not contain any contaminating *D. melanogaster* genomic DNA.

As a final control to eliminate any possibility that our results are attributable to contamination during the PCR process, we isolated and partially sequenced a *copia* positive clone from a *D. willistoni* genomic library. This library was constructed by using DNA isolated from flies representing the Royal Palm Park population. The 441-bp LTR-ULR sequence contained within the *D. willistoni* genomic clone is 99.5% identical to that of the *D. willistoni copia* PCR product described above. Based on these controls, we conclude that the sequence identity between the *D. willistoni* and *D. melanogaster copia* elements reported here is not an artifact of experimental contamination.

Discussion

The coding regions of the highly conserved *Adh* genes of *D. willistoni* and *D. melanogaster* display less than 80% sequence identity, which reflects the fact that these two species diverged from a common ancestor ≈50 million years ago (19). It seems unlikely that 100% sequence identity could be selectively or otherwise maintained between retrotransposons separated from a common ancestor for this length of time; moreover, the LTR and ULR are noncoding regions that are expected to evolve even

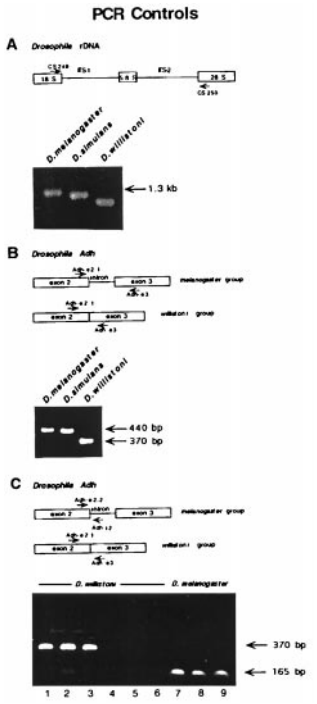

Fig. 4. PCR contamination controls. (*A*) Primers CS 249 and CS 250 were used to PCR amplify genomic DNA representing *D. melanogaster* (Iquitos), *D. simulans* (Australia), and *D. willistoni* (Royal Palm Park) populations. Each species showed ITS PCR products of unique size with no evidence of contamination. (*B*) Primers Adh-e2.1; Adh-e2.2; and Adh-e3 were used to PCR amplify genomic DNA from the same DNA samples used in (*A*). PCR of *D. willistoni* (Royal Palm Park) genomic DNA resulted in only the smaller *Adh* product with no evidence of DNA contamination from any of the *melanogaster* group species. (*C*). Primer Adh-e2.1 and Adh-e3 were used to PCR amplify 10 ng of *D. willistoni* genomic DNA representing the Royal Palm Park (lane 1), Santa Maria, de Ostuna, Nicaragua (lane 2), and South Florida (lane 3) populations. Primer Adh-e2.2 is homologous to a region of the *D. melanogaster Adh* gene located ≈65 bp 5′ of the second intron. Adh-e2.2 differs from the homologous *D. willistoni* sequence in its 3′ end rendering it incapable of amplifying the *D. willistoni Adh* gene. The *D. melanogaster*-specific primers Adh-e2.2 and Adh-i2 were used in an effort to amplify a product from 200 ng of the same *D. willistoni* genomic DNA samples used to generate the products depicted in lanes 1–3. The results (lanes 4–6) demonstrate that there is no contaminating *D. melanogaster* DNA in the *D. willistoni* samples. The *D. melanogaster*-specific primers Adh-2.2 and Adh-i2 were used to PCR amplify 5 ng of genomic DNA representing two *D. melanogaster* lab strains, w1118 (lane 7) and Oregon R (lane 8) and a strain representing the *D. melanogaster* (Iquitos) population (lane 9).

more rapidly than coding regions of the element. Previous surveys of LTR sequence variation among other retrotransposon families indicate that high levels of divergence often exist both within and between host species (20); thus, the most reasonable explanation for our results is that *copia* has been horizontally transferred between *D. willistoni* and *D. melanogaster* in the recent evolutionary past.

D. willistoni is a New World species with a distribution from South America north through Mexico, the Caribbean Islands, and Florida. Because *D. melanogaster* is a cosmopolitan species with a range overlapping that of *D. willistoni*, the physical opportunity for horizontal transfer between these two species exists; however, because *D. melanogaster,* which originated in Africa, has become cosmopolitan in the last 100–200 years (21), there has been only a recent window of opportunity for direct horizontal transfer between these two species. A well-documented horizontal transfer of a *P* element is believed to have occurred from *D. willistoni* to *D. melanogaster* within this short time frame (9). The fact that *copia* is abundant and widespread among *D. melanogaster* subgroup species but scarce with patchy distribution in *D. willistoni* suggests that the direction of transfer of *copia* was from *D. melanogaster* to *D. willistoni*.

The mechanism by which horizontal transfers occur remains obscure; however, there is circumstantial evidence suggesting that parasitic mites may be involved in vectoring DNA between *Drosophila* species (22). In addition, transmission by insect viruses is another likely mechanism by which transposable elements may be vectored between species (23).

Studies addressing the molecular evolution of transposable elements often yield data that appear consistent with the hypothesis of horizontal transfer. As stated above, there is particularly strong evidence indicating that DNA-type elements (*P* and *mariner*) have crossed species boundaries via horizontal transfer (9, 24, 25). Although previous reports have also suggested that LTR retrotransposons may horizontally transfer across species boundaries (8, 9, 23, 24), this evidence has been less definitive (7, 26). Our finding that *copia* elements isolated from two distantly related *Drosophila* species are sequentially identical seems inconsistent with any model of vertical transmission.

It has been postulated that cross-species transfers may be an effective strategy by which DNA-type transposable elements escape inactivation over evolutionary time (27, 28). Because *copia* and other LTR retrotransposons are known to be subject to effective host-mediated repression (6, 14), it is likely that significant selective pressure exists to favor horizontal transfer of LTR retrotransposons as well. There is a growing body of evidence that retrotransposons play a major role in eukaryotic genome organization and evolution (2–4, 29–33). If the horizontal transfer of LTR retrotransposons is found to be widespread, the evolutionary consequences could be significant.

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