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Cchobo, a *hobo*-related sequence in *Ceratitis capitata*

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Abstract

A *hobo*-related sequence, *Cchobo*, with high similarity to the *Drosophila melanogaster* HFL1 and $hobo_{108}$ elements was isolated from the medfly. Thirteen PCR-derived clones, which share 97.9–100% DNA identity, were sequenced, seven of which do not show frame-shift or stop codon mutations in their conceptual translations. The consensus sequence has 99.7% DNA identity with the *D. melanogaster hobo* element HFL1. In a phylogenetic analysis with other *hobo*-related elements, *Cchobo* clusters with the HFL1 and $hobo_{108}$ elements from *D. melanogaster* and *hobo*-related elements from *D. simulans, D. mauritiana* and *Mamestra brassicae*. These elements may have undergone horizontal transfer in the recent past. The genomic distribution of *Cchobo* was studied by FISH to mitotic and polytene chromosomes, which revealed that *Cchobo* is distributed within both the heterochromatin and euchromatin. Intra- and interstrain polymorphisms were detected both at euchromatic and heterochromatic sites. These findings suggest that active copies of the element may be present in the medfly genome.

Introduction

In the medfly, *Ceratitis capitata* (Diptera, Tephritidae), there is genetic and molecular evidence that different endogenous families of TEs are still actively transposing (Gomulski et al., 1997, 2004; Torti et al., 1998, 2000). Genetic information on the mobilisation of TEs in the medfly genome was provided by the discovery of a hybrid dysgenesis syndrome (Torti et al., 1994) reminiscent of those described in *D. melanogaster*. Unlike *Drosophila*, intrastrain dysgenesis is frequently observed in the medfly (Torti et al., 1997). The regulation of dysgenesis is dependent on the inter-strain combinations and, as in the *D. melanogaster* dysgenic systems including that induced by *hobo* (Yannopoulos et al., 1987), is frequently temperature dependent. Molecular evidence, in the form of Southern blot hybridisations, indicated that several different *hobo*-related elements exist in the medfly genome and a 438 bp fragment, CcHRE, with 72.4% nucleotide identity to *hobo* has been isolated (Handler & Gomez, 1996). Moreover, embryonic excision assays using *hobo* from *D. melanogaster,* revealed that the genome possesses a permissive state for *hobo* mobility and endogenous systems capable of mobilizing *hobo* (Handler & Gomez, 1996). These findings suggest that active *hobo*-related elements are present in the medfly genome.

hobo-related elements are Class II elements in that they use a DNA-mediated mode of

transposition (Finnegan, 1989) and are characterised by inverted terminal repeats flanking a single gene that encodes a transposase. They are further classified in the *hAT* family of transposable elements which derives its name from the original *hobo* from *D. melanogaster*, and two plant transposons *Activator* from *Zea mays* and *Tam*3 from *Antirrhinum majus*. *hobo*-related elements are typically 3–5 kb in length with a single ORF and short, conserved inverted terminal repeats. On insertion they generate an 8 bp target site duplication (Atkinson & James, 2002).

In this paper we report the isolation of a *C. capitata hobo*-like sequence, *Cchobo,* with very high DNA identity to the canonical *D. melanogaster* HFL1 *hobo* element (Calvi et al., 1991). The euchromatic and heterochromatic distribution of the *Cchobo* sequence, both on mitotic and polytene chromosomes of different strains, was investigated by FISH analysis. A high degree of inter- and intrastrain polymorphism for the chromosomal distribution of *Cchobo* signals has been ascertained. We suggest that *Cchobo* was recently acquired by horizontal transfer and that active copies of the element may be present in the genome. Apart from its evolutionary interest, this information is important for the potential use of *Cchobo* as a gene transfer vector and other types of genetic manipulations including gene tagging. Moreover, the successful transformation of the medfly using *Hermes* (Michel et al., 2001), a *hobo*-related element isolated from *Musca domestica* (Atkinson, Warren & O'Brochter, 1993; Warren et al., 1994), raises questions about the possibility of *Cchobo*/*Hermes* reciprocal cross mobilisation and about the stability of transformed systems.

Materials and methods

Medfly samples

Samples of flies from two wild populations and six laboratory strains were used in this study. The wild samples were: (1) the native Kenyan population, received in our laboratory as pupae collected from coffee berries in November, 1999, at Ruiru, near Nairobi; (2) the new Guatemala population, received in September, 1997.

The six laboratory strains include two independently derived *white eye* strains which are associated with two mutant alleles of the *white* gene, w^1 and w^2 (Gomulski et al., 2001), two *Hermes* transformed lines (Michel et al., 2001), and two other laboratory strains. All strains are maintained in our laboratory at 24°C and 65% relative humidity.

white eye (w^2): a strain associated with the w^2 allele at the *white* locus (Gomulski et al., 2001) which displays intrastrain hybrid dysgenesis-like phenomena (Torti et al., 1994; 1997).

white eye(w^1): a strain associated with the mutant w^1 allele at the *white* locus (Gomulski et al., 2001). It is the host strain used in the creation of *Hermes*-mediated transgenic lines 7M-6f *re* and 7M-6f *ye* (Michel et al., 2001). It has been maintained in our laboratory since July 2000.

7M-6f *re* and 7M-6f *ye*: subcultures established in our laboratory with samples of the two original lines at F_{15} generation which we received from the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Vienna, Austria, in November 1999. They were derived from a single *Hermes* *Ccwhite*$^+$ transformed female from the *white eye* (w^1) recipient strain (Michel et al., 2001).

Réunion Strain: a 6-year-old strain derived from a wild Réunion population, it was obtained from CIRAD (Réunion) in March 2000.

M325: a line established in 1997, involving flies from different geographic origins.

DNA preparation

DNA was extracted from individual flies of the Kenya and Guatemala populations and from the *white eye* (w^2) strain using the method of Baruffi et al. (1995). Following treatment with RNase A, the DNA was extracted with phenol/chloroform, precipitated with ethanol and resuspended in TE (10 mM Tris-HCl pH 8, 1 mM EDTA). The DNA concentration was quantified using a Hoefer TKO 100 Fluorometer.

Hybridisation analyses

Pools of genomic DNA (2 μg) from individual flies of the *white eye* (w^2) strain were digested with *Xho*I and electrophoresed on a 20 × 14 cm 0.8% agarose gel in 1 × TBE and transferred to a positively charged nylon membrane according to Southern (1975). Filters were hybridised at 62 °C with 200 ng of the *Drosophila* $hobo_{108}$ element insert as

probe labelled with the Gene Images AlkPhos Direct labelling system (Amersham Life Science) using the random primed method. The hybridisation and detection protocols were those described in the AlkPhos Direct labelling and detection kit. Post-hybridisation stringency washes were performed at 55°C as suggested in the manual. These represent moderately high stringency conditions. Signal detection was performed using CDP-star followed by exposure to autoradiographic film (X-OMAT AR, Kodak).

PCR amplification

The *Cchobo* sequences were amplified from individuals of the *white eye* (w^2) strain and from the two wild samples from Kenya and Guatemala, using two sets of oligonucleotide primers. The primers were named according to the nucleotide numbering system of HFL1 proposed by Calvi et al. (1991). The first set of primers, HRE-995 (5′ gcracsrtvgaywtgtggac 3′) and HRE-1428 (5′ gtagttggagttccatctagt 3′), correspond to the T(V/M)DMWT amino acid consensus sequence of *hobo*-related and *Ac* elements and to the TRWSN region of HFL1, respectively. The second set of primers was based on the HFL1 sequence outside the TVDMWT-TRWSN region; HFL1-866 (5′ acgtcgatgacttactacctgat 3′) and HFL1-1600 (5′ tagagatggatggcaatacgaa 3′).

PCRs were performed in 25 µl reaction volumes using ~2 ng DNA, 1.5 mM $MgCl_2$, reaction buffer (10 mM Tris, 50 mM KCl; pH 8.3), 0.2 mM dNTP mixture, 10 pmol of each primer and 1 unit of *Taq* DNA Polymerase (Pharmacia). Each reaction was topped by ~50 µl mineral oil (Sigma). Amplification was achieved on a Hybaid OmniGene Temperature Cycler. The amplification conditions were: initial denaturation at 94°C for 1 min followed by 35 cycles of 1 min at 94 °C for denaturation, 1 min at 60°C for annealing, and 2 min at 72°C for extension, with a final extension of 10 min. PCR products were resolved by electrophoresis on 1% agarose gels (SeaKem GTG, FMC) with 1 × TBE buffer containing 0.5 µg/ml of ethidium bromide. Every effort was made to avoid contamination of the PCRs including the use of barrier pipette tips, aliquoted reagents and no positive control reactions. A negative control containing all the reagents except target DNA was used in every set of amplifications. If the negative control resulted in an amplification product, the entire set of amplifications was discarded.

PCR products of the expected sizes were eluted from gels using the Roche Gel Extraction Kit. The purified DNA fragments were ligated into the pCR 2.1 vector (Invitrogen). Positive colonies were selected and the size of the insert checked by *Eco*RI digestion. Sequencing of the clones was performed using an Applied Biosystem model ABI 310 DNA Genetic Analyzer with the ABI PRISM Big Dye Terminator Cycle Sequencing Kit, using appropriate primers within the vector.

Cchobo *phylogenetic analysis*

Sequence comparisons of the *Cchobo* sequences with other known *hobo*-related sequences were performed using the BLAST (v.2.1.1) family of programs from NCBI (Altschul et al., 1990). The partial amino acid sequences were aligned using the multiple sequence alignment program Praline (Heringa, 1999) on the Integrative Bioinformatics Institute, University of Amsterdam server (http://ibivu.cs.vu.nl/programs/pralinewww/) using the default alignment parameters with ten iterations of pre-profile global processing and with minimal subsequent manual optimisation. The phylogenetic tree was inferred by maximum parsimony using the branch and bound search option in PAUP*4b10 (Swofford, 1999). To assess the robustness of the tree, a bootstrap test of 1000 replications was performed.

In-situ *hybridisation*

Metaphase chromosomes from the recipient *white eye* strain, the two *Hermes* transformed lines and the Réunion and M325 strains were prepared from the neuroblast cells of third instar larvae using the air-dry method of Willhoeft and Franz (1996). Polytene chromosomes from *white eye* (w^2) and Réunion strains were obtained from the salivary glands using the squash technique of Bedo (1986). Due to the difficulty of rearing wild flies under laboratory conditions we did not succeed in obtaining sufficient F_1 larval progeny from the wild Kenyan and Guatemala flies in order to perform complete *in-situ* analyses.

Approximately 100 ng of the whole plasmid containing the 750 bp *Cchobo* insert derived from the wild Kenyan population sample were labelled

with digoxigenin-11-dUTP by random priming using the DNA labelling kit (Roche). In each FISH experiment, as an internal control, a hybridisation using the single copy gene *white* (Zwiebel et al., 1995) as probe was performed on polytene and mitotic chromosome preparations.

Hybridisation was performed in a sealed moist chamber at 37°C for 12–14 hours in 50% formamide/2× SSC. After post-hybridisation washes (see Torti et al., 2000) the slides were incubated at 37°C for 30 min with 50 µl of rhodamin anti-digoxigenin conjugate sheep antibody (5 µg/ml) (Boehringer, Mannheim). Amplification of the signals was obtained with two additional incubations with the appropriate antibodies. The slides were counterstained with DAPI (4′-6-diamino-2-phenylindole, 10 ng/ml) in 4× SSC and mounted in DABCO (1,4-diazabicyclo 2.2.2 octane) antifade.

Hybridisation signals and DAPI fluorescence were viewed through appropriate filters using an Axioplan (Zeiss) microscope equipped with a cooled charge coupled device (CCD) camera (Digital Pixel Inc., Brighton). In all cases CCD camera exposure times were 0.5 s for rhodamine and 0.2 s for DAPI. Digital images were pseudocoloured and merged, allowing simultaneous detection of hybridisation signals and DAPI chromosome counterstaining. IPLab Spectrum version 3.1.1 software, with FISH Capture extension (Digital Scientific) was used to normalise and enhance the images. For each strain, two to three larvae were screened. For each individual ten complete chromosome spreads were examined for both mitotic and polytene chromosome analyses. For mitotic chromosome analyses only the signals that were present in all the metaphases of the same individual were considered. Signals were scored as homozygous when present presumably in the same position on both the homologues, heterozygous when present in only one of the two homologues. The insertion site number was computed as the number of homozygous signals per haploid genome plus the number of heterozygous signals. The localizations of the *Cchobo* insertion sites on polytene chromosomes were determined using the photographic maps of Zacharopoulou (1990). The number of insertion sites were determined on all chromosome arms (2L, 2R, 3L, 3R, 4L, 4R, 5L, 5R, 6L, 6R) and their sum gave the total number of insertion sites per diploid genome.

Double FISH

Double FISH using the *Ccwhite* and *Cchobo* probes was applied to polytene chromosomes from the 7M-6f *re* and 7M-6f *ye* transgenic lines at F_{24}. Labelling of the two probes was performed using digoxigenin for *Ccwhite* and biotin for *Cchobo*, respectively. *Ccwhite* signal detection was as described above, while the *Cchobo* signals were rendered visible with three antibody incubations: avidin–FITC conjugated, anti-avidin and avidin–FITC conjugated antibodies. The fluorescent rhodamin *Ccwhite*, FITC *Cchobo* signals and DAPI staining were recorded separately, pseudocoloured and merged allowing the simultaneous detection of *Ccwhite* (in red) and *Cchobo* (in green) hybridization signals and of DAPI counterstained chromosomes.

Results

Identification and characterisation of a hobo*-like sequence in the medfly*

The *D. melanogaster hobo* element, $hobo_{108}$, was hybridised to a pool of *Xho*I-digested DNA from the dysgenic *white eye* (w^2) strain. This restriction enzyme cuts twice within the $hobo_{108}$ and HFL1 elements, 286 bp from the 5′ and 110 bp from the 3′ end, resulting in fragments of 2849 bp and 2906 bp, respectively. The hybridisation produced a band of ~3 kb and another high molecular weight band (~11 kb) (Figure 1).

PCR using the HRE 995 and HRE 1428 primers on genomic DNA from four different individuals of the *white eye* (w^2) strain produced fragments of 430 bp. These PCR fragments were cloned and ten positive clones were sequenced, three each from two individuals and two each from another two individuals. BLAST searches of the entire GenBank database indicated the highest DNA sequence identity with HFL1 and $hobo_{108}$ (99 and 98.9% identity, respectively). High identities were also found with other *hobo*-related elements: 94.8% with an element from *Mamestra brassicae* (AF487501, Borsatti et al., 2003), 71.9% with CcHRE previously isolated from *C. capitata* (Handler & Gomez, 1996), 60.7% with *Hermes* from *Musca domestica* (Warren et al., 1994), and 39.1% with *homer* from *Bactrocera tryoni* (Pinkerton et al., 1999).

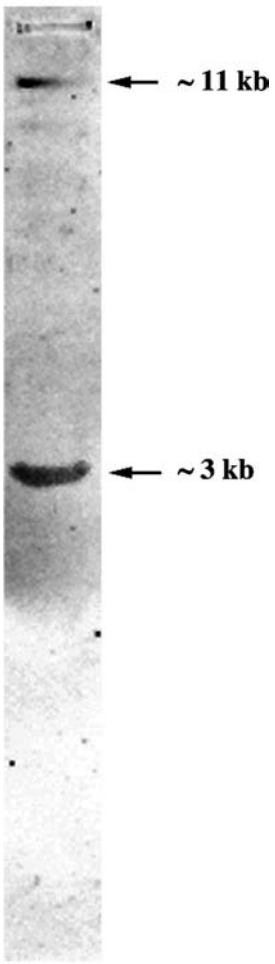

Figure 1. An example of Southern blot hybridisation of genomic DNA from the *white eye* strain. The genomic DNA was digested with *Xho*I and probed with $hobo_{108}$ from *D. melanogaster*.

Due to the high identities of the ten medfly clones with HFL1, a second set of primers (HFL1-866 and HFL1-1600) based on the HFL1 sequence enabled the amplification and cloning of three additional fragments of 750 bp, one each from the *white eye* (w^2) strain and from the wild Guatemalan and Kenyan samples. The DNA identities between all 13 clones in the region between HRE 995 and HRE 1428 vary from 97.9 to 100% (three clones from the *white eye* (w^2) strain and the two clones from Kenya and Guatemala were identical). These five identical clones may represent the same copy of the *Cchobo* sequence in the genome. Seven of the 13 clones, five from the *white eye* (w^2) strain (including the three identical clones) and those from the wild Kenyan and Guatemalan populations, encode potentially functional transposases as they do not contain any frame-shift or stop codon mutations. As the 750 bp clones differed only within the HRE-995/HRE-1428 region, it was possible to construct a consensus sequence that we named *C. capitata hobo*, or *Cchobo*. The identical clones had no influence on the resolution of ambiguous positions in the construction of the consensus sequence. The consensus *Cchobo*, excluding the HFL1-866/HFL1-1600 primer sequences, is 711 bp in length and encodes 237 amino acids without frame-shift mutations or stop codons (Figure 2). *Cchobo* and HFL1 share 99.72% DNA identity, differing at only two positions, 574 and 669 in Figure 2. The *Cchobo* sequence has a transversion at both positions: at 574 an adenosine in HFL1 is replaced by cytidine in *Cchobo* and at 669 a guanosine in HFL1 is replaced by a thymidine in *Cchobo*. The transversion at position 574 results in the replacement of a lysine (K) with a threonine (T) in *Cchobo*, whereas the transversion at position 669 results in the replacement of an aspartate (D) with a tyrosine (Y) in the *Cchobo* conceptual translation. Unfortunately the *Cchobo* sequences do not extend as far as the sequence coding the variable tandemly repeated threonine–proline–glutamic acid (TPE) motif which appears to play an important role in *hobo* activity in *D. melanogaster* (Souames et al., 2003).

Phylogenetic analysis

A search of the sequence databases using the BLASTP algorithm revealed significant amino acid identities/similarities of the consensus *Cchobo* with other *hobo*-related elements from tephritid fruit flies: *Anastrepha suspensa* AsHRE (U514551, 26.4/50.9%), *Bactrocera dorsalis* BdHRE (U51453, 31.9/51.9%), *Bactrocera cucurbitae* BcHRE (U51452, 62.3/78.8%) and *C. capitata* CcHRE (U51454, 74.7/87.0%, Handler & Gomez, 1996). High identities/similarities were also found with *D. melanogaster* HFL1 (M69216, 99.2/99.2%, Calvi et al., 1991) and $hobo_{108}$ (X04705, 99.2/99.2%, Streck, MacGaffey & Beckendorf, 1986), a *hobo*-related element from *Mamestra brassicae* (AF487501, 92.0/92.8, Borsatti et al., 2003), *Musca domestica Hermes* (L34807, 61.3/77.5%,

Warren et al., 1994), *Hermes* (U36211, 61.7/77.9%, O'Brochta et al., 1996) and *Musca vetustissima Hector* (U17152, 58.2/75.0%, Warren et al., 1995).

The analysis of the phylogenetic relationships between *Cchobo* and these *hobo*-related elements was based on their amino acid sequences. As most of the considered elements are incomplete, the phylogenetic analysis was limited to an alignment of 170 amino acids in length, corresponding to the conserved T(V/M)DMWT/TRWNSN region. The phylogenetic relationships were inferred by maximum parsimony, using the branch and bound search option. The resulting tree, shown in Figure 3 is rooted with the *hAT* element *Activator* from *Zea mays* (Calvi et al., 1991). The tree is one of two equally parsimonious trees of length 591 with a consistency index of 0.880. The *Cchobo* consensus clusters with the *hobo* elements from *D. melanogaster*, *D. simulans*, *D. mauritiana* (Simmons, 1992) and *M. brassicae* (Borsatti et al., 2003) elements supported by a 97% bootstrap value. The previously characterised *C. capitata* CcHRE element is clearly distinct from this cluster. The other tephritid *hobo*-related elements appear to be more differentiated. BcHRE clusters with *Hector* from *M. vetustissima* while BdHRE and AsHRE form a distinct cluster.

Chromosomal distribution and abundance

The chromosomal distribution of the *Cchobo* sequence was studied by FISH using the *Cchobo* 750 bp fragment derived from the Kenyan population as a probe to mitotic chromosomes from six laboratory strains with different origins: *white eye* (w^2), Réunion, M325, two *Hermes* transformed

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ACGTCGATGACTTACTACCTGATCCAACAACATTAAGTCGGAAGGCCAAATCGGATGCAG  60
   V  D  D  L  L  P  D  P  T  T  L  S  R  K  A  K  S  D  A   19

AAGAGAAGAGGAGTCTAATCTCGTCCGAGATAAAAAAAAGCTGTGGATAGCGGAAGAGCAA 120
E  E  K  R  S  L  I  S  S  E  I  K  K  A  V  D  S  G  R  A   39

GTGCGACCGTCGACATGTGGACTGACCAGTATGTCCAAAGAAACTTTTTGGGCATCACTT 180
S  A  T  V  D  M  W  T  D  Q  Y  V  Q  R  N  F  L  G  I  T   59

TCCATTACGAAAAAGAATTTAAACTTTGTGACATGATTTTGGGACTAAAATCGATGAATT 240
F  H  Y  E  K  E  F  K  L  C  D  M  I  L  G  L  K  S  M  N   79

TTCAAAAATCGACTGCCGAAAACATTTTAATGAAAATTAAAGGTTTATTTTCGGAATTCA 300
F  Q  K  S  T  A  E  N  I  L  M  K  I  K  G  L  F  S  E  F   99

ATGTTGAGAACATTGATAATGTTAAGTTTGTGACTGACAGGGGAGCAAATATAAAAAAGG 360
N  V  E  N  I  D  N  V  K  F  V  T  D  R  G  A  N  I  K  K  119

CTTTAGAGGGCAATACCCGTTTAAATTGTAGCAGTCACCTGTTGTCAAATGTTTTAGAAA 420
A  L  E  G  N  T  R  L  N  C  S  S  H  L  L  S  N  V  L  E  139

AATCGTTTAACGAGGCCAATGAACTCAAAAAAATTGTGAAATCATGCAAAAAAATCGTGA 480
K  S  F  N  E  A  N  E  L  K  K  I  V  K  S  C  K  K  I  V  159

AGTACTGCAAAAAATCAAATTTGCAGCATACTCTAGAAACCACTTTGAAAAGCGCCTGTC 540
K  Y  C  K  K  S  N  L  Q  H  T  L  E  T  T  L  K  S  A  C  179

CGACTAGATGGAACTCCAACTACAAAATGATGACGTCCATTCTGGATAACTGGCGTAGTG 600
P  T  R  W  N  S  N  Y  K  M  M  T  S  I  L  D  N  W  R  S  199

TGGATAAAATATTAGGTGAAGCTGATATCCATGTAGATTTTAATAAATCATCTTTAAAAG 660
V  D  K  I  L  G  E  A  D  I  H  V  D  F  N  K  S  S  L  K  219

TTGTGGTATATATTCTAGGAGACTTTGAACGAATATTTAAGAAGTTGCAAACATCTAGCT 720
V  V  V  Y  I  L  G  D  F  E  R  I  F  K  K  L  Q  T  S  S  239

CACCATCTATATGCTTCGTATTGCCATCCATCTCTA                         756
S  P  S  I  C  F  V  L  P  S  I  S                           251
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Figure 2. Nucleotide sequence and conceptual translation of *Cchobo* consensus derived from the 13 sequenced clones (GenBank Acc. No. AY848833-AY848845). The DNA and amino acid replacements with respect to HFL1 are underlined.

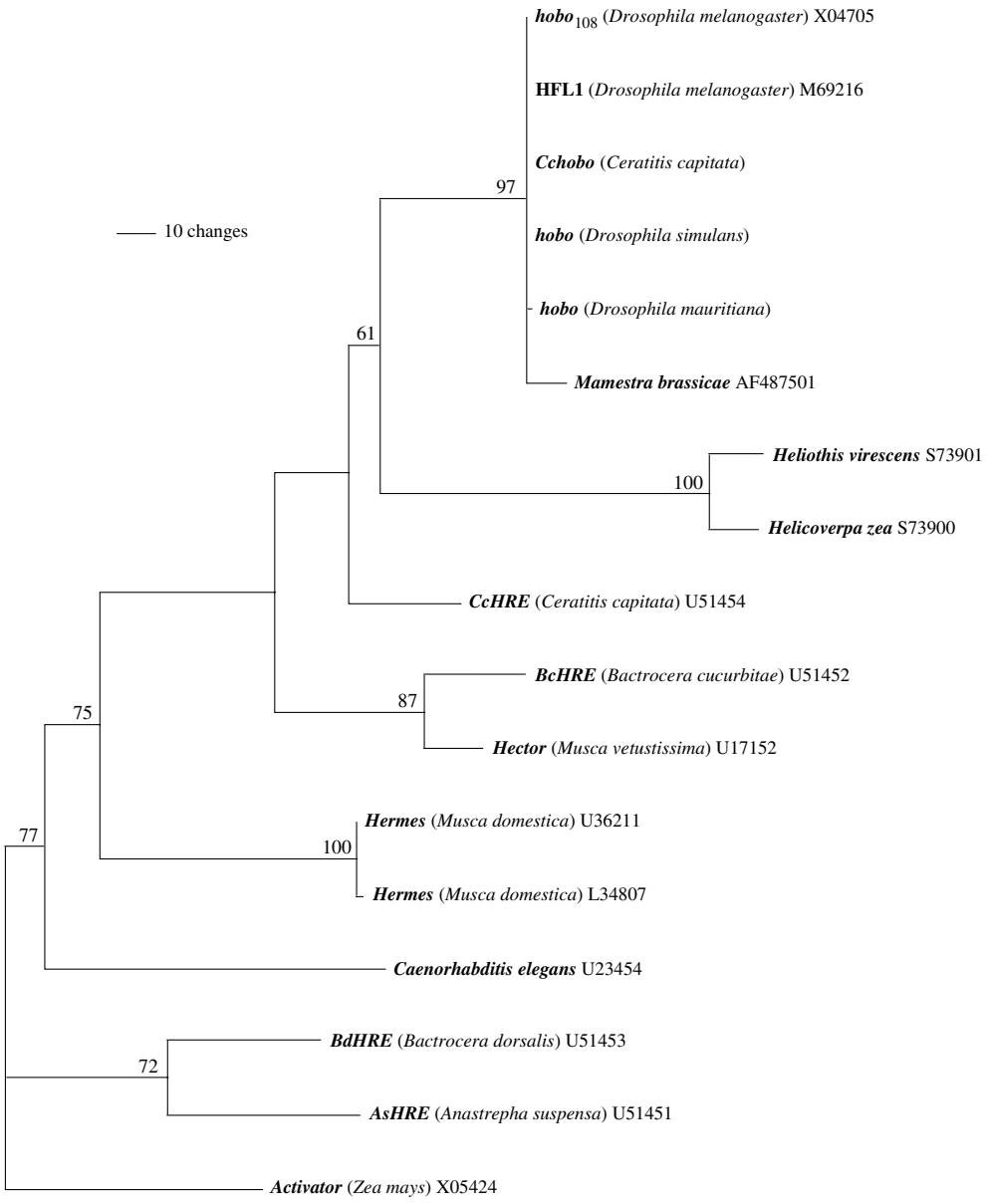

Figure 3. Phylogenetic relationships between the *Cchobo* consensus and other *hobo*-related elements based on their amino acid sequences. The *Activator* element from *Z. mays* was used as an outgroup. The tree was obtained using maximum parsimony with the branch and bound search option. The tree, one of two equally parsimonious trees, has length 591 and consistency index of 0.880. Bootstrap values greater than 60% are indicated.

lines, 7M-6f *ye* F_{19}, 7M-6f *re* F_{19}, and the recipient *white eye* strain (w^1) used in their construction. The DAPI counterstaining of mitotic chromosomes after FISH allowed the localization of *Cchobo* signals with respect to the DAPI− (euchromatic) and DAPI+ (heterochromatic) regions (Torti et al., 2000).

Representative examples of the hybridisation patterns in these strains are shown in Figure 4. It appears evident that in all the considered strains *Cchobo* signals are spread both on the sex chromosomes and on the five autosomes. On each of the five autosomes in the six considered strains, the signals may be distributed along the chromosomal

arms, at the chromosomal tips and/or in the centromeric regions. However the chromosomal distribution in terms of euchromatic and heterochromatic regions and number of signals differ between and within the strains. On the long arms of the X and Y chromosomes the signals may be present at different locations on the DAPI + (heterochromatic) bands and/or on the DAPI– (euchromatic) bands. The *Cchobo* chromosomal distribution and signal intensities also differ between the two *Hermes* transformed lines and their recipient *white eye* (w^1) strain, despite their recent common origin. On the X chromosome in the 7M-6f *ye* line a strong hybridisation signal covered the entire short arm while in the 7M-6f *re* line, a signal with a reduced intensity was observed.

Estimates of the mean numbers of *Cchobo* signals, the mean number of insertion sites and the number of the heterozygous sites per individual in the six considered strains are reported in Table 1. Although they represent only approximations, due to the low resolution of mitotic chromosomes, these estimates allow us to portray the differences in *Cchobo* distributions between these strains. It appears evident that the strains are heterogeneous for the mean number and proportion of heterozygous *Cchobo* insertion sites per individual. The most heterozygous strain appears to be the *Hermes* transformed 7M-6F *ye* line with 9.5 ± 0.7 (SD) heterozygous autosomal sites, whereas the most homozygous strain appears to be the old established Réunion strain with only 3.5 ± 2.1 heterozygous autosomal sites per individual.

Distribution of Cchobo *insertion sites on polytene chromosomes*

FISH to polytene chromosomes was applied to the *white eye* (w^2) and Réunion strains. Polytene chromosomes allow higher resolution of *Cchobo* signals but do not permit the assessment of the homozygous/heterozygous state and hence heterozygosity estimates. This higher resolution permitted the estimation of the mean number of insertion sites per individual as 18.5 ± 2.1 (SD) and 27 ± 1.4 in w^2 and the Réunion strain,

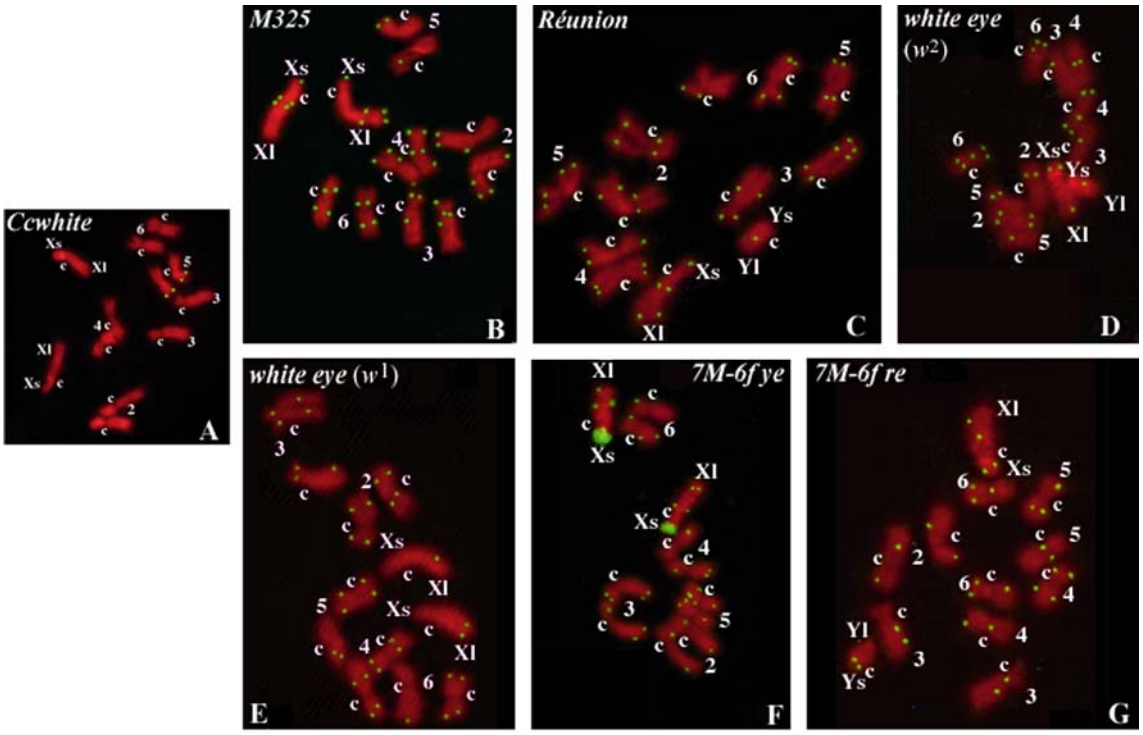

Figure 4. Fluorescent *in-situ* hybridisation (FISH) using *Cchobo* to mitotic chromosomes from laboratory strains with different origins. The chromosome numbering is described in Bedo (1986) and Zacharopoulou (1987). The positions of the centromeres (c) are indicated. In panel A a control hybridisation, using the *white* gene as a probe, is presented.

Table 1. Mean number (±SD) of *Cchobo* insertion sites on mitotic chromosomes from the six laboratory strains

Strain	Sex chromosomes		Autosomes		
	Mean number of insertion sites per chromosome (±SD)		Mean number of insertion sites per individual (±SD)	Number ± SD (and proportion ±SD) of heterozygous sites per individual	
	X	Y			
Réunion	2.7 ± 0.6	1.0	11.0 ± 0.0	3.5 ± 2.1	(0.3 ± 0.10)
M325	2.0 ± 1.1	n.d	12.5 ± 0.7	6.6 ± 0.7	(0.5 ± 0.03)
white eye (w^2)	1.5 ± 0.7	1.0 ± 0.0	9.5 ± 0.7	7.5 ± 0.7	(0.7 ± 0.10)
white eye (w^1)	1.0 ± 1.0	1.0	11.5 ± 0.7	6.0 ± 0.0	(0.5 ± 0.02)
7M-6f *re*	1.5 ± 0.7	1.0 ± 0.0	10.5 ± 0.7	7.0 ± 1.4	(0.6 ± 0.20)
7M-6f *ye*	2.5 ± 0.6	n.d	13.5 ± 2.1	9.5 ± 0.7	(0.7 ± 0.05)

The proportion of heterozygous *Cchobo* signals on the five autosomes is also reported, n.d: not determined.

respectively. The higher standard deviation in the w^2 strain indicates a greater level of intrastrain polymorphism than in the Réunion strain. Figure 5 shows examples of the *Cchobo* hybridisation patterns obtained in the two strains. The signals are located both in the pericentric and telomeric sections and along the chromosome arms. The interstrain variable sites are distributed on all the polytene chromosome elements and include also telomeric and pericentric regions.

The double FISH on the two *Hermes* transformed lines, 7M-6f *re* and 7M-6f *ye*, was performed using the *Ccwhite* and *Cchobo* probes. The *Ccwhite* probe hybridises not only to the mini-*white* marker gene within the *Hermes* transgene but also to the genomic copy of the *white* gene on the long arm of chromosome 5 (65C), which thus acts as a positive control. The majority of the *Ccwhite* and *Cchobo* signals did not overlap (Figure 6), indicating that the *Cchobo* sequences and *Hermes*/*Ccwhite* transgene were localised in different sites on the chromosomes.

Discussion

The initial Southern hybridisation using the $hobo_{108}$ element as a probe together with the subsequent PCR-derived clones and *in-situ* hybridisation results show that a *hobo*-related sequence, *Cchobo*, with high identity to *Drosophila hobo* elements, is present in the medfly genome. The presence in the Southern hybridisation of an additional high molecular weight band is analogous to the situation in *D. melanogaster*, where the band has been shown to represent divergent *hobo*-related sequences (Simmons et al., 1998). These, in *Drosophila* are thought to represent relics of earlier *hobo* invasions that occurred more than 10 million years ago in the ancestral genome that gave rise to the melanogaster and montium subgroups (Boussy & Itoh, 2004). We suggest that *Cchobo* is a medfly acquisition by horizontal transfer and that active copies of the element may be present in the genome.

Sequence and phylogenetic analyses revealed that the copies of *Cchobo* are highly homogeneous and are very similar to the *D. melanogaster* HFL1 and $hobo_{108}$ elements. The high identity of *Cchobo* and the HFL1/$hobo_{108}$ elements and the time of divergence of their host species, estimated as 100 ± 20Mya (Kwiatowski et al., 1997), excludes the possibility of *hobo* element acquisition from a common ancestor. Assuming that the *Cchobo* sequences are evolving neutrally, we can tentatively apply the estimated rate of 1% DNA divergence per Myr as estimated in drosophilids (Werman et al., 1990; Powell et al., 1993). At this rate, the *Cchobo* sequences which differ, on average, by 0.625% from the consensus *Cchobo* sequence, have been diverging for ~0.625 Myr. Exclusion of the potentially duplicate *Cchobo* copies from the *white eye* strain results in an estimate of ~0.7 Myr for medfly *Cchobo* acquisition.

C. capitata (Fletcher, 1989) and *D. melanogaster* (David & Capy, 1988) both originated in Africa

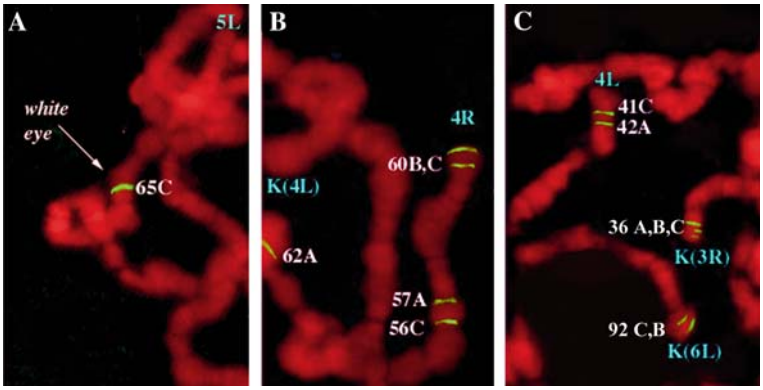

Figure 5. Fluorescent *in-situ* hybridisation using *Cchobo* probe to salivary gland polytene chromosomes of the (B) Réunion and (C) *white eye* (w^2) laboratory strains. The chromosomal arms (L and R) and sections are labelled in blue and white, respectively. The centromeres are labelled (K). In panel A a control hybridisation, using the *white* gene as a probe, is presented.

where they have overlapping distributions. Direct horizontal transfer from *D. melanogaster* to *C. capitata* is improbable as the canonical *hobo* element is thought to have been acquired by the *D. melanogaster* genome during the twentieth century (Boussy & Periquet, 1993; Periquet et al., 1994). Either both species independently acquired the elements by horizontal transfer from a common source or the element was transferred directly or indirectly from the medfly to *D. melanogaster*. The presence of nearly identical *hobo*-related elements in *D. simulans*, *D. mauritiana* and *M. brassicae* support the hypothesis of numerous horizontal transfer events of one or more *hobo* elements into the different species in the recent past. As to the mechanisms involved in the horizontal transfer we have no information, but it has been proposed that possible vectors include viruses, mycoplasm, spiroplasms, bacteria, protozoans, nematodes, fungi and parasitoids (Kidwell, 1994). However, in the case of the *Drosophila* species it has been suggested that rare interspecific hybridisation could have provided the route for the transmission of *hobo* (Simmons, 1992; Boussy & Itoh, 2004).

The presence in the Southern hybridisation of an additional high molecular weight band is analogous to the situation in *D. melanogaster*, where they have been shown to represent divergent *hobo*-related sequences (Simmons et al., 1998). These, in *Drosophila* are thought to represent relics of earlier *hobo* invasions that occurred more than 10 million years ago in the ancestral genome that gave rise to the melanogaster and montium subgroups (Boussy & Itoh, 2004).

Horizontal transfer may play a significant role in the evolution of transposable elements (Maruyama & Hartl, 1991; Kidwell, 1992; Lohe et al., 1995), allowing them to survive the pressures of vertical inactivation and stochastic loss that would lead to their extinction. An

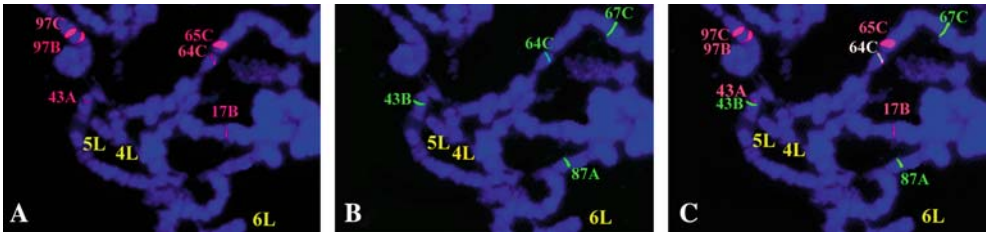

Figure 6. Double FISH using the *Cchobo* and the *Ccwhite* probes on polytene chromosomes from the 7M-6f *re* original line at F_{24}. The *Ccwhite* and *Cchobo* insertion sites are indicated in red and in green, respectively. Sites of co-localization of the two probes are in white. (A) distribution of *Ccwhite* gene (the genomic copy of the *white* gene is visible at position 65C on the long arm of chromosome 5, as are signals corresponding to the *Ccwhite* sequence within the *Hermes* transgene); (B) distribution of the *Cchobo* signal (C) merged image, showing co-localization of the two probes at 64C.

element transmitted to another species by horizontal transfer may quickly invade the genome as long as the genome has not established conditions repressing the activity of the element (Biémont & Cizeron, 1999). Horizontal transfer of *hAT* family elements, to which *hobo* belongs, may be a frequent occurrence as they are widespread in both the animal and plant kingdoms (Calvi et al., 1991; Atkinson, Warren & O'Brochta, 1993; Koga et al., 2000) and are not reliant on host factors (Baker et al., 1986; Handler & Gomez, 1995; O'Brochta et al., 1996; Lozovskaya et al., 1996).In addition to the evidence of *hobo* transfer between *Drosophila* species (Simmons, 1992; Simmons et al., 1998; Boussy & Itoh, 2004), a case of horizontal transfer of another *hAT* element, *Tol2*, into the Medaka fish genome has recently been reported (Koga et al., 2000).

Active copies of Cchobo *may be present in the medfly genome*

FISH data indicate the presence of about 14 *Cchobo* insertion sites in the medfly genome. However, *Cchobo* shows a marked variation among the strains in its chromosomal location and this polymorphism involves both heterochromatic and euchromatic regions. As the considered strains, like the majority of medfly laboratory strains, have complex and diverse origins, the polymorphism observed between these strains may be merely the result of the assortment of ancestral *Cchobo* insertion site polymorphism. Alternatively, the observed polymorphism may be due to the presence of *Cchobo* elements which were activated by previous crosses of different lines in the formation of the strains. The sequence analysis also suggests that active copies of *Cchobo* may be present in the genome; seven of the 13 sequenced clones do not contain frame-shift or stop codons in their conceptual translations. Five of these copies were found in the *white eye* (w^2) strain, which displays a dysgenic syndrome whose aberrant traits are very similar to those induced by *hobo* elements in *D. melanogaster* (Torti et al., 1994, 1997). That similar functional copies are present in other medfly strains and wild populations is supported by the manifestation of hybrid dysgenesis traits when flies from wild populations are crossed with laboratory strains from different origins. Moreover excision assays revealed that functional *hobo*-related transposase is present in the medfly genome (Handler & Gomez, 1996).

New transposition events, rather than the assortment of ancestral polymorphism, almost certainly account for the different *Cchobo* chromosomal distributions in the two *Hermes* transformed lines, which originated from a single transformed female (Michel et al., 2001) and the recipient *white eye* (w^1) strain. That *Cchobo* behaved differently in the two transformed lines is evident from the strong signal on the X chromosomes of the 7M-6f *ye* line. It may represent a cluster of multiple insertions within the short arm, perhaps the result of an interaction between *Cchobo* and *Hermes* such as that demonstrated by Sundararajan, Atkinson and O'Brochta (1999). Such *Cchobo* multiple transpositions may have been induced by the exogenous *Hermes* transposase during the transformation process. Moreover the *Cchobo* cluster would not be eliminated by recombination, as the *C. capitata* sex chromosomes are largely recombinationally isolated (Willhoeft & Franz, 1996). Most interestingly, the presence of this *Cchobo* cluster indicates that the accumulation of this element within discrete heterochromatic regions can occur over a very short time. Similar heterochromatic *hobo* clusters generated by multiple local transpositions are found on the X chromosomes of *D. melanogaster* (Carmena & Gonzalez, 1995; Pimpinelli et al., 1995).

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