Identification of a new *hobo* element in the cabbage moth, *Mamestra brassicae* (Lepidoptera)

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Borsatti, F., Azzoni, P. and Mandrioli, M. 2003. Identification of a new *hobo* element in the cabbage moth, *Mamestra brassicae* (Lepidoptera). — *Hereditas 139*: 151–155. Lund, Sweden. ISSN 0018-0661. Received July 24, 2003. Accepted September 25, 2003.

A complete *hobo*-like element, called *Mbhobo*, was identified in the cabbage moth, *Mamestra brassicae*. This element has a high sequence similarity to the HFL1 *hobo* element of *Drosophila melanogaster*. Amplification of *Mbhobo* termini indicated that transposition occurred into a 5′-GTGGGTAC-3′ target sequence that was duplicated upon insertion. This target site conforms to the consensus sequence established for the insertion sites of insect *hAT* elements. *Mbhobo* has a single 1935 bp long ORF with significant homology to the *D. melanogaster HFL1 hobo* transposase. FISH experiments evidenced *Mbhobo* clusters located in heterochromatic regions of Z and W sex chromosomes and in heterochromatic areas of chromosome pair 10.

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A variable portion of both prokaryotic and eukaryotic genome consists of interspersed and moderately repeated sequences that are able to transpose in the host genome.

Most transposons are organized in families of autonomously and non-autonomously transposing elements characterized by their ability to respond to the same transposase. Moreover, transposons of the same family usually share extensive nucleotide similarity at their termini (RUBIN et al. 2001).

The maize element *Activator* (Ac), the *Drosophila melanogaster* element *hobo* and the *Anthirrhinum majus* Tam3 element form a superfamily of eukaryotic transposable elements referred to as the hAT (hobo-Ac-Tam) transposon family (reviewed by RUBIN et al. 2001). Additional elements, such as Hermes and Hector (WARREN et al. 1994, 1995), were also reported to belong to this superfamily of transposable elements (TEs). All these mobile DNAs share a 50 amino acid long domain located in the C termini of the hAT transposase. This domain was shown to be involved in dimerization as well as in additional interaction functions (ESSERS et al. 2000).

A second conserved feature of hAT transposons is that they mediate the formation of an 8 bp host duplication upon insertion.

In the present paper, the isolation and molecular characterization of a *hobo*-like element from *M. brassicae* genome is reported. The overall structure of the mobile DNA described in *M. brassicae* is unique for insect hAT elements both the in the size and arrangement of the terminal repeats and their proximity to the transposase gene.

MATERIAL AND METHODS

Genomic DNA samples were extracted from *M. brassicae* CRL-8003 cells (ATCC, USA) according to MANDRIOLI (2002).

The presence of *hobo* elements in *M. brassicae* genome was evaluated by PCR using the primers F_{HOBO} (5′-TGGACCGATCAATYCGTZAA) and R_{HOBO} (5′-CTTAATCTGTGCTGCAAYCTWGC) corresponding to the central portion of *hobo* transposase. The amplification mix contained 100 ng of *M. brassicae* genomic DNA, 1 μM of each primer, 200 μM dNTPs and 2 U of DyNAZyme II polymerase (Finnzymes Oy). Amplification was performed with a thermalcycler Hybaid Omn-E at an annealing temperature of 59°C for 1 min and making extension at 72°C for 1 min. *Hobo* sequence was completed by inverse PCR performed with the primers $F_{HOBO\text{-}I}$ (5′-CCTAGTCTCGCCACAGTAAA) and $R_{HOBO\text{-}I}$ (5′-CGGACCAGACCACTCATTGC) according to MARTIN and MOHN (1999) protocol for inverse PCR.

All the amplified fragments were gel purified and sequenced using an ABI 377 (Perkin-Elmer) at the ''BMR- University of Padova''. Sequence analysis was performed using GCG Software. *M. brassicae hobo* sequence is available at GenBank with the accession number AF487501.

Southern and dot blots were performed according to already published protocols (MANDRIOLI et al. 1999).

Chromosome preparations were obtained from spreading of *M. brassicae* CRL cells as previously described (MANDRIOLI 2002). FISH and probe la-

belling were performed as reported by MANDRIOLI et al. (2003).

RESULTS

Hobo-specific primers were initially used to amplify a 438 bp fragment from *M. brassicae* genome (Fig. 1a). Sequence analysis confirmed that this small fragment contained a *hobo*-like sequence. Inverse PCR was successively used to isolate a larger 2,800 bp fragment (Fig. 1b) that contains a complete sequence of this element. Sequencing confirmed that this fragment included both the termini of the *hobo* transposable element.

M. brassicae hobo (GenBank AF487501) was 2027 bp long and included 40 bp long inverted repeats flanking a 1935 bp open reading frame (ORF) corresponding to the putative transposase (Fig. 2). These types of repeats are unique for insect hAT elements in which the ITRs are always in a 5′ to 3′ orientation rather than a 5′ to 3′ – 3′ to 5′ as is the case with *Mbhobo*. Moreover, there is little sequence similarity between the *Mbhobo* ITRs and the ITRs of the other insect hAT elements. In addition, the *Mbhobo* ITRs are 28 bp longer than the ITRs of the *D. melanogaster hobo* element and 23 bp longer than the ITRs of the *Hermes* elements. Any *hobo*-like ITR sequence has been found even if sequencing was performed within 1 kb upstream of these 40 bp ITRs. When the nucleic acid sequence of *M. brassicae hobo* sequence has been successively aligned with the homologous sequences in GenBank from *D. melanogaster* (DMHOBOG), *Ceratitis capitata* (CCU51454) and *Bactrocera curcubitae* (BCU5142) respectively, similarity values ranging from 91.2 % to 60.5 % respectively were found. Alignment of *Mbhobo* with *Hermes* sequence from *Musca domestica* (MDOHETR) showed an average similarity of 42 %.

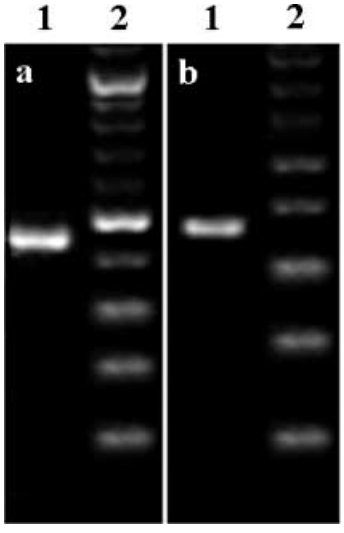

Fig. 1a–b. (**a**) A portion of *hobo* transposase has been amplified in *M. brassicae* by PCR (lane 1). The molecular weight of the amplified fragments was evaluated using a 100 bp DNA ladder (lane 2). (**b**) *Hobo* sequence has been completed by inverse PCR (lane 1). The molecular weight of the amplified fragments has been evaluated using a 500 bp DNA ladder (lane 2).

Analysis of the *M. brassicae hobo* element amplified by inverse PCR revealed that it was flanked by the sequence 5′-GTGGGTAC-3′ that conforms to the consensus insertion sequence for insect hAT elements (SAVILLE et al. 1999). This added support to the identification of the terminal nucleotides of the *Mbhobo* element as being the direct repeats shown in Fig. 2.

Southern blotting with the *hobo* probe evidenced several positive bands suggesting the presence of multiple *hobo* copies in *M. brassicae* genome (Fig. 3). Moreover, the comparison of *Msp*I and *Hpa*II restriction pattern after hybridization with *hobo* probe did not evidence any difference indicating the absence of methylation in this mobile DNA.

Dot blotting experiments showed that 4.2 ± 0.2 % of *M. brassicae* genome was constituted by *hobo* elements.

FISH experiments with *hobo* probe evidenced bright fluorescent signals on the Z and W sex chromosomes and on a telomeric region of chromosome pair 10 (Fig. 4a). All *Mbhobo* clusters resulted located into regions that were previously described as heterochromatic (MANDRIOLI 2002; MANDRIOLI et al. 2003). The identification of sex chromosomes was performed by silver staining since Z and W chromosomes are the unique NOR-bringing ones (Fig. 4b) (MANDRIOLI 2002; MANDRIOLI et al. 2003). The presence of two *hobo* clusters in FISH was consistent with the number of bands in the Southern blot.

DISCUSSION

Hobo is a member of the hAT transposon family that includes the Ac element from *Zea mays*, *Hermes* from *M. domestica*, *Hermit* from *Lucilia cuprina* and *Tam*3 from *Antirrhinum majus* (ATKINSON et al. 1993).

M. brassicae hobo (called *Mbhobo*) evidenced a sequence similarity ranging from 60.5 % to 91.2 % with *hobo* sequences from other insects and in particular the transposase coding sequence resulted quite conservative. On the contrary, the structure of *M. brassicae hobo* ITRs is unique for insect hAT elements since they are in orientation 5′ to 3′ and 3′ to 5′ and not both in 5′ to 3′ orientation as generally reported.

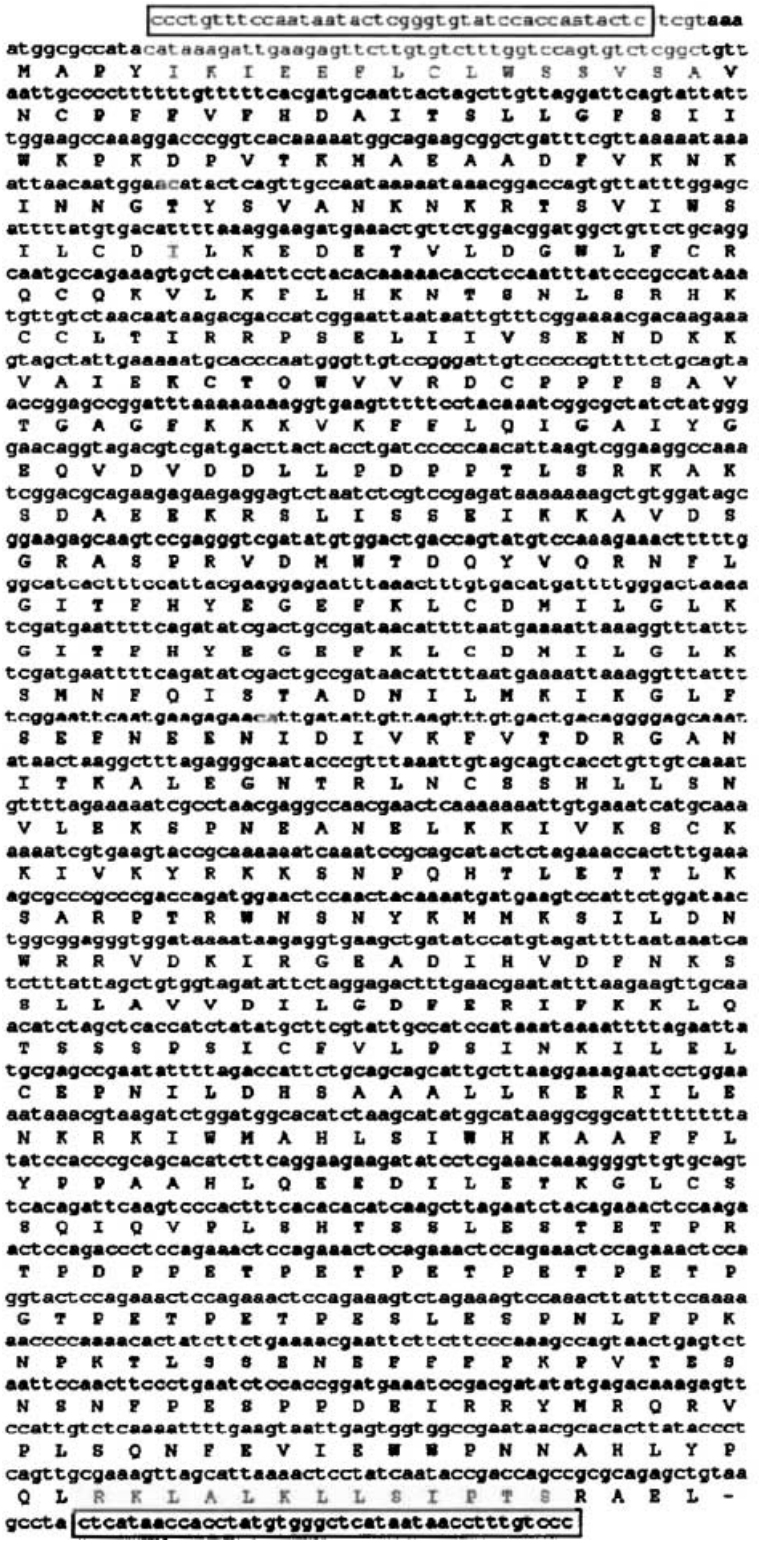

Fig. 2. *M. brassicae hobo* was constituted by 2027 bp including 40 bp long inverted repeats (boxed sequences) flanking a 1935 bp open reading frame (ORF) corresponding to the putative transposase.

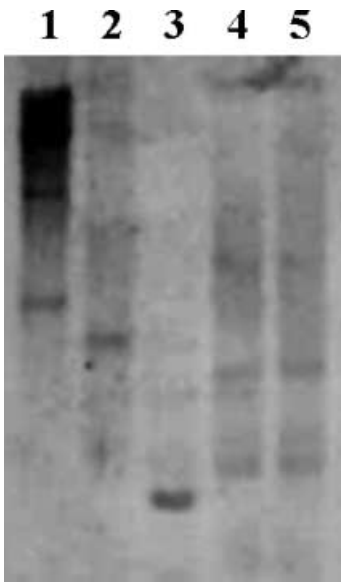

Fig. 3. *M. brassicae* genomic DNA has been digested with *Bam*HI (lane 1), *Kpn*I (lane 2), *Eco*RI (lane 3), *Msp*I (lane 4) and *Hpa*II (lane 5) and blotted with *Mbhobo* as a probe.

Mbhobo insertion site consists of the 5′-GTGGGTAC-3′ sequence that was duplicated upon transposon insertion. This result confirmed that *hobo* transposase exhibits integration site specificity as suggested by SAVILLE et al. (1999). In particular, these results reinforced the hypothesis that *hobo* integration site conforms to the NTNNNNAC consensus sequence (O'BROCHTA et al. 1994; SAVILLE et al. 1999).

Southern blotting with *hobo* probe evidenced several hybridization signals indicating that numerous *hobo* elements were present in *M. brassicae* genome. Dot blotting showed that 4.2 % of *M. brassicae* genome was due to *hobo* transposons. This amount of *hobo* elements, that is low in respect to other insects such as *M. domestica* (ATKINSON et al. 1993), could be explained by considering that these elements could have been introduced into the genome quite recently and they have not yet had sufficient time to spread throughout or taking into account the presence of transposons of the same family that could be involved in *hobo* control. This second hypothesis is particularly interesting since it suggests that other transposons of the hAT family could be present in *M. brassicae* genome.

At a cytogenetic level, transposable elements are generally not randomly distributed on chromosomes (MANUELIDIS and WARD 1984; BOYLE et al. 1990; DIMITRI and JUNAKOVIC 1999). Although chromosomal distribution of each transposable element could be peculiar, several transposons tend to be accumulated in heterochromatic regions (PIMPINELLI et al. 1995). However this heterochromatic accumulation is not related to intrinsic properties of transposases because the same elements are inserted in euchromatic regions too (DIMITRI and JUNAKOVIC 1999).

FISH evidenced a *hobo* cluster located in heterochromatic regions of *M. brassicae* Z and W chromosomes. This result could be partially explained by considering that a great amount of heterochromatin was located on the sex chromosomes and so transposons were simply located in the wider heterochromatic district of the complement. An alternative is that sex chromosomes act as a trap for transposable elements, as reported in *Drosophila* (STEINEMANN and STEINEMANN 1992). However, the last hypothesis is weakened by the presence of a large *Mbhobo* cluster in heterochromatic areas of chromosome pair 10 suggesting that transposons are preferentially clustered in heterochromatin independently from its chromosomal location.

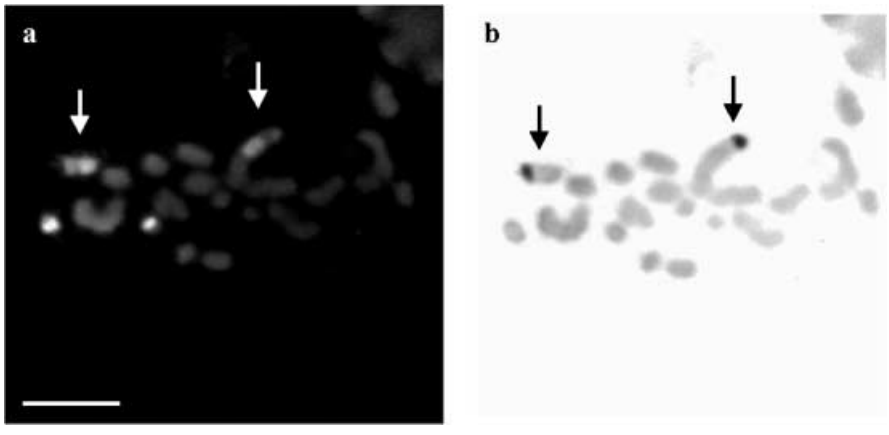

Fig. 4a–b. (**a**) FISH localized *hobo* into large clusters located on the sex chromosomes and on chromosome pair 10. (**b**) Silver staining of the same metaphase plate allowed identifying sex chromosomes. Arrows indicated Z and W chromosomes. Bar corresponds to 10 μm.

Acknowledgements – This work is supported by the grant "F.A.R." from the University of Modena and Reggio Emilia.

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