The *hobo* Transposable Element Excises and Has Related Elements in Tephritid Species

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ABSTRACT

Function of the *Drosophila melanogaster hobo* transposon in tephritid species was tested in transient embryonic excision assays. Wild-type and mutant strains of *Anastrepha suspensa, Bactrocera dorsalis, B. cucurbitae, Ceratitis capitata,* and *Toxotrypana curvicauda* all supported *hobo* excision or deletion both in the presence and absence of co-injected *hobo* transposase, indicating a permissive state for *hobo* mobility and the existence of endogenous systems capable of mobilizing *hobo*. In several strains *hobo* helper reduced excision. Excision depended on *hobo* sequences in the indicator plasmid, though almost all excisions were imprecise and the mobilizing systems appear mechanistically different from *hobo*. *hobo*-related sequences were identified in all species except *T. curvicauda*. Parsimony analysis yielded a subgroup including the *B. cucurbitae* and *C. capitata* sequences along with *hobo* and *Hermes*, and a separate, more divergent subgroup including the *A. suspensa* and *B. dorsalis* sequences. All of the sequences exist as multiple genomic elements, and a deleted form of the *B. cucurbitae* element exists in *B. dorsalis*. The *hobo*-related sequences are probably members of the *hAT* transposon family with some evolving from distant ancestor elements, while others may have originated from more recent horizontal transfers.

THE movement of transposable elements is subject to various levels of regulation which may vary among different species, and can vary within an individual species with respect to tissue and developmental specificities, maternal effects, and strain-specific genomic differences (see BERG and HOWE 1989). For the *hobo* transposable element, found originally in *Drosophila melanogaster* (MCGINNIS *et al.* 1983; for a review see BLACKMAN and GELBART 1989), elucidating the limits and types of movement in different species is important to understanding transposon function and mechanisms of transposition, as well as its involvement in genome organization and the interspecific flow of genetic information. From a more applied view, this information is essential to the assessment of *hobo*, or related elements, as germline transformation vectors for nondrosophilid insects, which have thus far been refractory to most other vectors and methodology (HANDLER and O'BROCHTA 1991; HANDLER 1993).

A previous study indicated that, in contrast to the *P* element, *hobo* excision is not restricted in drosophilid species, and the presence of *hobo* transposase is not required for *hobo* movement (HANDLER and GOMEZ 1995). Similar results were also reported for *hobo* excision in one nondrosophilid, *Musca domestica* (ATKINSON *et al.* 1993). Interestingly, both studies reported that for some species, the frequency of detectable *hobo* excision events actually decreased in the presence of an exogenous source of *hobo* transposase, possibly due to negative interactions with *hobo*-related elements. In *D. virilis*, which is distantly related to *D. melanogaster*, excision occurred at similar rates in the presence or absence of transposase, although precise excision was only observed in the presence of *hobo* transposase.

These results have important implications regarding the phylogenetic distribution of *hobo*, as well as the potential use of *hobo* as a vector in nondrosophilid insects. In contrast to the nearly total lack of *P* excision in nondrosophilids (O'BROCHTA and HANDLER 1988; O'BROCHTA *et al.* 1991; HANDLER *et al.* 1993), which may in part explain the failure of *P* to mediate transformation in nondrosophilids (see HANDLER and O'BROCHTA 1991), *hobo* excision and transposition (O'BROCHTA *et al.* 1994) is supportive of potential vector function. However, the mobility data also suggest that the frequency of vector integrations may be restricted by negative interactions with resident elements, which may in turn eventually act to destabilize those integrations which do occur by cross-mobilization.

In this report we describe experiments which test the frequency and types of *hobo* excision in several tephritid species and strains. This was done in an effort to extend our knowledge of *hobo* activity in nondrosophilid insects, and thus determine if these insects may also be subject to the potential limitations of *hobo* vector function. A survey of the dependence of *hobo* excision on exogenous transposase in various strains allows, as well, a determination of those strains that might be suitable hosts for stable vector integration.

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It has been suggested that autonomous *hobo* mobilization in drosophilid species and *M. domestica* is caused by *hobo*-related elements resident in the host genome. For *M. domestica* such an element, *Hermes*, has been already identified (WARREN *et al.* 1994). The possibility that *hobo*-related elements exist among a broad range of insects, and possibly organisms in general, is supported by a comparison of the *hobo* amino acid sequence with other transposable elements. General regions of homology were found among *hobo* and the plant transposons *Activator* (*Ac*) and *Tam3* (STRECK *et al.* 1986; CALVI *et al.* 1991; FELDMAR and KUNZE 1991) suggesting that, as with the *mariner* element (ROBERTSON and MACLEOD 1993), *hobo* belongs to a phylogenetically diverse transposon family. Thus, similar to the discovery of a *hobo*-related element in Musca, and *mariner*-related elements in at least ten insect orders (ROBERTSON and MACLEOD 1993), it should be possible to identify other *hobo*-related elements by polymerase chain reaction (PCR) amplification of genomic sequences using priming sites derived from conserved amino acid sequences. To give physical evidence in support of *hobo* cross-mobilization, as well as support to the notion of a diverse *hobo, Ac, Tam3* transposon family, we have identified *hobo*-related sequences in four tephritid species: *Anastrepha suspensa, Bactrocera dorsalis, Bactrocera cucurbitae,* and *Ceratitis capitata,* and show that they exist as multiple genomic elements.

MATERIALS AND METHODS

Insect species and strains: *A. suspensa* (Caribbean fruit fly) is maintained as a laboratory colony that is routinely re-established with wild flies collected in south Florida. *Toxotrypana curvicauda* (papaya fruit fly) adults were collected as wild adults near Sarasota, Florida with only first generation embryos used for experimentation (kindly provided by P. LANDOLT, USDA/ARS, Gainesville, FL). Other tephritid insects are maintained as laboratory stocks at the University of Hawaii at Manoa (kindly provided by S. SAUL and S. MCCOMBS, University of Hawaii). These included the *B. cucurbitae* (melon fly) wild Cue strain and mutant *light eye/w* strain (SAUL and MCCOMBS 1992); the *B. dorsalis* (oriental fruit fly) Kauai, Kahuku, and Sakamoto wild strains and *white eye* mutant strain (MCCOMBS and SAUL 1992); and the *C. capitata* (Mediterranean fruit fly) RÖSSLER wild strain, and *dark pupae* (RÖSSLER and KOTLIN 1976), *light eye* and *Purple* (SAUL 1985) mutant strains.

Plasmids: The pK19 (PRIDMORE 1987) and pUC19 (YANISCH-PERRON *et al.* 1985) plasmids were used in control experiments and for other plasmid constructions. Construction of the pKHFL*lacZ* indicator plasmid was described previously (HANDLER and GOMEZ 1995), but essentially it contains the complete *hobo* element from pHFL1 (CALVI *et al.* 1991) placed into the pK19 cloning site with the pUC19 *lacZ* gene inserted into a partially deleted transposase open reading frame (ORF). P[ry$^+$, HSH2] (or HSH2) was used as a heat shock regulated *hobo* transposase helper (CALVI *et al.* 1991; CALVI and GELBART 1994). Plasmid constructions and analysis followed standard cloning procedures (see SAMBROOK *et al.* 1989).

***hobo* excision assay and product analysis:** The *hobo* total excision assay involves the transient expression of plasmid-encoded genes injected into insect embryos, allowing the assay of *hobo* excision activity, and has been described previously (HANDLER and GOMEZ 1995). Briefly, kanamycin-resistant pKHFL*lacZ* indicator plasmid was either injected into embryos alone (1.0 mg/ml) or co-injected with ampicillin-resistant HSH2 helper plasmid (0.3:1.0 mg/ml, helper:indicator). After 16–20-hr incubation plasmids were harvested from surviving embryos and transformed into bacteria that were plated on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; 40 μg/ml), kanamycin (50 μg/ml) LB media, allowing only indicator plasmid-transformed bacteria to survive. Both precise and imprecise *hobo* excisions co-deleting the *lacZα* peptide gene resulted in a lack of bacterial β-galactosidase activity yielding white colonies *vs.* blue colonies having nonexcised plasmid. Excisions or deletions of *hobo* not including the internal *lacZ* gene were not detected. Putative excision plasmids were verified by *Bgl*II restriction analysis and sequenced for some experiments. Excision frequencies were computed by dividing the number of verified excision events by the total colonies. Total excision frequencies were determined from two to five independent injection-bacterial transformation experiments. Probability values (*P* values) comparing excision frequencies from pKHFL*lacZ* indicator injected alone and other plasmid injections were derived from the Fisher's Exact two-tailed test of a two-way contingency table using the Statistical Analysis System (SAS) PROC FREQ procedure (SAS Institute, Cary, NC).

PCRs: *hobo*-related sequences were amplified from genomic DNA using the PCR in 15–30 μl reactions containing Taq DNA polymerase (Boehringer-Mannheim) at 2.5 units/100 μl buffer (10 mM Tris-Cl, 1.8 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, pH 8.3), with 200 μM dNTPs, and 400 nM of each primer. Oligonucleotide primers corresponded to the nucleotide numbering system of pHFL1 (CALVI *et al.* 1991). The forward primer designated 995F corresponding to the T(V/M)DMWT amino acid sequence consensus between *hobo* and *Ac* (see CALVI *et al.* 1991) was 5′-TGG AAT TCA C(C/I)A TGG A(T/C)A TGT GGA C -3′, and the reverse primer designated as 1428R corresponding to the TRWSN consensus was 5′-GTA GTT GGA GTT CCA TCT AGT -3′. Cycling parameters were initial denaturation at 94° for 1 min, followed by 35 cycles of 1 min at 93° for denaturation, 1 min at 50° for annealing, and 2 min at 72° for extension, with a final 10-min extension. Amplified products were separated by 1% agarose gel electrophoresis and stained with ethidium bromide. Gel-purified PCR products were subcloned into ddT-tailed plasmid vectors (InVitrogen) and sequenced.

Sequence analysis: Sequencing was performed by dideoxy reactions using Sequenase (U.S. Biochemicals) or with the Sequenase Dye Terminator kit (Applied Biosystems) run on an Applied Biosystems 373A automated DNA sequencer. Sequence analysis of excision plasmids was done by alignment to the pKHFL*lacZ* indicator plasmid using the GeneWorks software (Intelligenetics).

Subcloned *hobo*-related PCR fragments were sequenced and after removal of PCR priming site sequences (since they might not precisely amplify genomic sequences), internal nucleotide sequences and their translated amino acid sequence products were aligned using ClustalV provided by the GCG (DEVEREUX *et al.* 1984) and DNAStar (DNAStar, Inc.) sequence analyses programs, with the latter software providing sequence pair distances. After slight manual adjustment, the amino acid sequence alignment was subjected to parsimony analysis using PAUP v3.1.1 (SWOFFORD 1993; kindly performed by H. ROBERTSON, University of Illinois).

DNA hybridization: Ten to 20 μg of genomic DNA was digested with indicated restriction enzymes and separated on 0.8% agarose gels. DNA was stained with ethidium bromide,

blotted to nylon filters and immobilized by ultraviolet irradiation. Hybridization probes were subcloned *hobo*-related PCR products that were labeled with [^{32}P]dCTP by random priming (GIBCO BRL) according to the manufacturer's specifications. Hybridizations were performed either in 50% formamide at 42° or in phosphate buffer; 1% BSA; 7% SDS at 65° with an initial wash in 2× SSC; 0.5% SDS at room temperature and two washes in 1× SSC; 0.1% SDS at 60° for 30 min. Autoradiography was performed by exposure on Kodak X-Omat film at −90°.

RESULTS

***hobo* excision:** To determine the ability of tephritid species to support *hobo* mobility, transient *in vivo* excision assays were performed in embryos of various tephritid species and strains. The specificity of the *hobo* excision assay and its dependence upon transposase was previously determined for *D. melanogaster* (HANDLER and GOMEZ 1995). In these tests *hobo* excision in the *cn;ry*^{42} E strain, devoid of genomic *hobo*, was dependent upon the co-injection of transposase helper plasmid, while in various H strains containing chromosomal copies of *hobo* excision was not dependent upon exogenous helper, occurring both in its presence and absence. In general, excision frequencies were in the range of 0.4 to 1.0 x 10^{-3} excisions/pKHFL*lacZ* indicator plasmids assayed.

In the present study *hobo* excision was tested both in the presence and absence of *hobo* transposase helper in wild strains of *A. suspensa* and *T. curvicauda*, three mutant and one wild strain of *C. capitata*, four wild and one mutant strain of *B. dorsalis*, and one wild and one mutant strain of *B. cucurbitae*. In all of these tephritid species and strains excision was detected in both the presence and absence of co-injected *hobo* transposase helper, although the relative magnitude of the measured frequencies varied among the species, as well as among strains within the species (Table 1). Notably, in seven of the 12 strains tested the excision frequencies were approximately 1.5- to 6-fold higher in the presence of transposase helper, with the greatest relative difference observed in the *C. capitata dark pupae* and *B. dorsalis white eye* strains. In five strains the excision frequencies were 1.5- to 5-fold lower in the presence of *hobo* helper, with the greatest decrease observed in the *B. dorsalis* Sakamoto and *B. cucurbitae light eye* strains. In *C. capitata* all four strains tested had increased excision frequencies with helper, while the two *B. cucurbitae* strains exhibited decreased excision with helper. In *B. dorsalis* two strains exhibited increased excision, while two other strains exhibited decreased excision with helper.

To ensure that excision was dependent upon *hobo* sequences in the indicator plasmid and not random deletion of plasmid, excision was assayed in pK19, which is the host plasmid for pKHFL*lacZ*, as well as pUC19. Both pK19 and pUC19 have the *lacZ* reporter gene but lack the *hobo* sequences and adjacent chromosomal insert site DNA. No *lacZ* excision or deletion from pK19 was detected in *A. suspensa* or *B. dorsalis* Kahuku while excision from pKHFL*lacZ* occurred in these species at frequencies of 0.30 x 10^{-3} and 0.33 x 10^{-3}, respectively (Table 1). In *A. suspensa*, one putative excision/deletion was recovered from pUC19 from more than 87,000 plasmids screened. These results are consistent with previous control experiments in *D. virilis* (HANDLER and GOMEZ 1995) and *M. domestica* (ATKINSON *et al.* 1993), indicating that non-helper-mediated *lacZ* excision from the indicator plasmid is dependent on the presence of *hobo* sequences.

***hobo* excision site analysis:** Excision breakpoint sites were determined by sequencing pKHFL*lacZ* excision products using the pK19 universal or reverse priming sites adjacent to the 94E chromosomal DNA. In the *B. dorsalis* Kahuku and *C. capitata light eye* strains all the excision products, occurring in either the presence or absence of transposase (10–15 excisions sequenced for each experimental condition), resulted from imprecise excision events. Breakpoints were found within either *hobo*, 94E DNA, or the pK19 plasmid sequences. Several plasmids were found to be rearranged or had insertions of unknown (non-pKHFL*lacZ*) DNA. Approximately 10–15% of the excisions could not be sequenced, presumably due to loss of both priming sites. In *A. suspensa* all excisions in the absence of transposase were similarly imprecise (n = 18), but of the 14 excisions occurring in the presence of transposase, one was a nearly precise excision event having breakpoints at the *hobo* termini (♦) with the addition of a 6-bp inverted duplication of the proximal chromosomal insertion site DNA (underlined).

excision site

CGCAGCATCAGGA- ♦ ATCCTG ♦ ATTCAGGATATCGT

In a previous study, the same *hobo* excision site motif was predominant in excisions occurring in *D. melanogaster cn;ry*^{42} and *D. virilis* only in the presence of transposase (HANDLER and GOMEZ 1995).

Sequence analysis of *hobo*-related elements: The excision of *hobo* in the absence of an exogenous source of *hobo* transposase in all the tephritid species tested would suggest that either *hobo* itself or an element (or elements) having similar mobilizing activity exists in these species (designated as a *hobo*-related element or HRE). To identify these elements, PCR was performed using primers derived from conserved amino acid sequences in *hobo* (TVDMWT and TRWNS; positions 229–372) and the maize transposon, *Ac* (TMDMW and TRWSN; positions 299–466; KUNZE *et al.* 1987) as described by CALVI *et al.* (1991). Low-degeneracy primers yielded PCR products from both plasmid-encoded *hobo* and *Ac*, as well as genomic DNA from *D. melanogaster* H strains, *A. suspensa*, and various strains of *B. dorsalis*, *B. cucurbitae*, and *C. capitata* (data not shown). Two to three PCR products were fully sequenced from each

TABLE 1

***hobo* excision in tephritid species and strains**

Species	Strain	Plasmids injected	Excision frequency ($\times 10^{-3}$)[a]	*P* value[b]
Anastrepha suspensa	Wild	I[c]	0.30 (16/53,525)	
		I + HSH2	0.61 (46/74,860)	0.014
		pK19	0 (0/43,610)	0.00013
		pUC19	0.01 (1/87,400)	0.000002
Bactrocera cucurbitae	*light eye/w*	I	2.02 (7/3,460)	
		I + HSH2	0.40 (6/15,064)	0.00469
	Cue	I	0.68 (30/43,840)	
		I + HSH2	0.31 (9/28,640)	0.020
Bactrocera dorsalis	Kauai	I	0.12 (3/25,080)	
		I + HSH2	0.26 (20/77,400)	0.329
	Kahuku	I	0.33 (18/54,840)	
		I + HSH2	0.22 (26/116,580)	0.201
		pK19	0 (0/60,640)	0.000002
	Sakamoto	I	2.07 (31/15,000)	
		I + HSH2	0.59 (24/40,500)	0.000005
	white eye	I	0.09 (3/32,480)	
		I + HSH2	0.51 (17/33,060)	0.00259
Ceratitis capitata	*Purple*	I	0.43 (17/39,200)	
		I + HSH2	0.98 (26/26,545)	0.00813
	dark pupae	I	0.04 (2/46,280)	
		I + HSH2	0.23 (5/21,840)	0.038
	light eye	I	0.18 (17/97,120)	
		I + HSH2	0.76 (35/46,240)	0.0000003
	+ (RÖSSLER)	I	0.36 (5/13,970)	
		I + HSH2	0.43 (8/18,600)	0.788
Toxotrypana curvicauda	Wild	I	0.50 (36/72,600)	
		I + HSH2	0.24 (31/126,500)	0.00484

[a] Values in parentheses are the number per pKHF*lacZ* screened.
[b] Probability values for Fisher's Exact two-tailed test for excision frequencies between I and I + HSH2, pK19, or pUC19.
[c] I, pKHFL*lacZ* indicator plasmid.

species, with products from each species usually having several base pair changes. Since the analogous *hobo* sequence is part of the transposase ORF, for the purposes of this study individual PCR sequences that encoded ORFs after conceptual translation were used for further analysis. The *B. dorsalis* ORF sequence was generated as a consensus from two PCR products, and the closest complete ORF from *C. capitata* contained a single internal TAA stop signal. Thus far varying PCR conditions have not resulted in a similar PCR product from *T. curvicauda*, though products in this species, as well as additional products in the other species might have been revealed by more highly degenerate primers. The PCR DNA sequences and their translated amino acid sequences were aligned by ClustalV analysis (Figure 1; DNA alignments not shown) which were used to generate sequence pair distance matrices (Figure 2).

The *A. suspensa* PCR product (As-HRE) yielded a 468-bp sequence with a 32% nucleotide and 14.3% amino acid sequence similarity to *hobo*, while the *B. dorsalis* 471-bp product (Bd-HRE) has 31.5% nucleotide and 20.3% amino acid similarity to *hobo*. In contrast to these limited homologies, the 438-bp product (Bc-HRE) from *B. cucurbitae* has a 68.7% nucleotide and 59.4% amino acid sequence similarity to *hobo*, while the *C. capitata* 438-bp sequence (Cc-HRE) has the greatest homology to *hobo* with a 72.4% nucleotide and a 72.9% amino acid sequence similarity.

The specific pairwise distances between each nucleotide and amino acid sequence in terms of frequency of similarity and divergence are detailed further in Figure 2. Alignment of the HRE amino acid sequences, as well as the sequences of *Ac*, *hobo*, and *Hermes* bounded by the analogous primer sequences (Figure 1), was used to determine the phylogenetic relationship of these sequences by parsimony analysis using the PAUP program (SWOFFORD 1993). Two equally parismonious trees of 360 steps were generated with the *Ac* sequence defined as the outgroup. One tree had *hobo* and Bc-HRE as sister taxa, while the tree shown in Figure 3 has *hobo* and Cc-HRE as sister taxa. Bootstrap analysis with 1000 replications gives greater support for the branch grouping Bc-HRE and Cc-HRE with *hobo* and *Hermes* than for the branch grouping As-HRE and Bd-HRE, yet the latter group, nevertheless, represents elements significantly more diverged from *hobo*. The amino acid sequence pair distances indicate that for the limited region of the transposons analyzed, As-

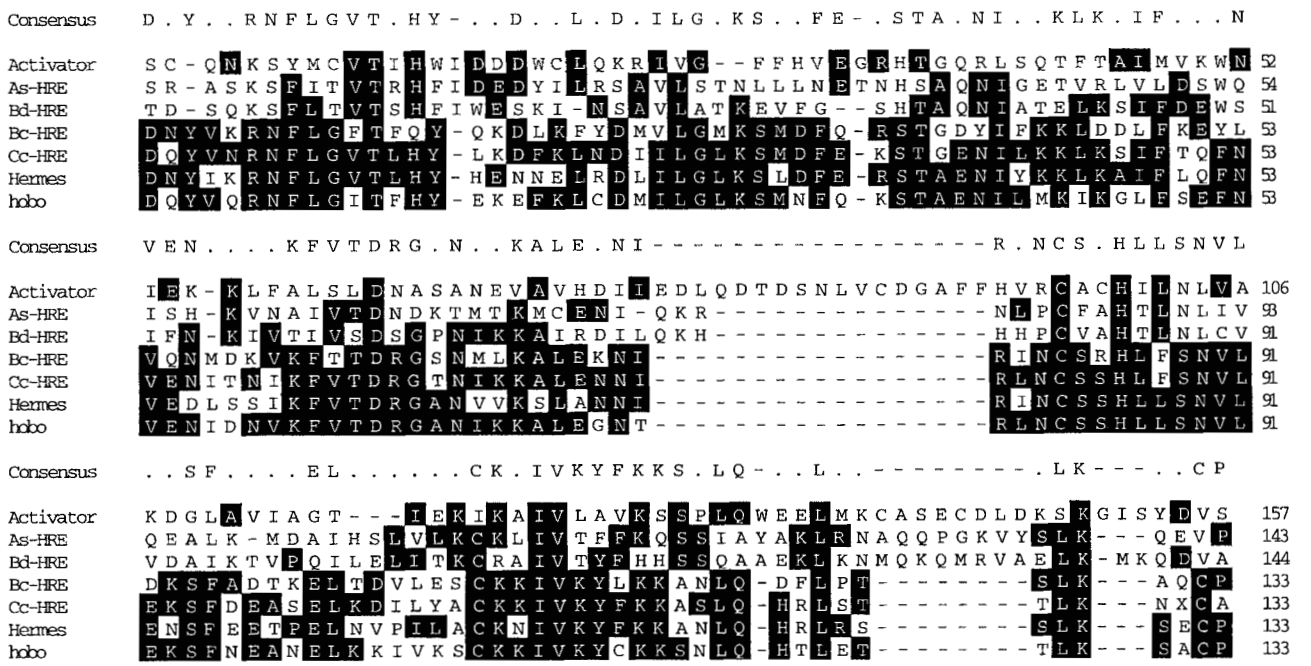

FIGURE 1.—Amino acid sequence alignments based on ClustalV analysis between the translated DNA sequence of PCR products amplified from genomic DNA from *A. suspensa* (As-HRE), *B. dorsalis* (Bd-HRE), *B. cucurbitae* (Bc-HRE), and *C. capitata* (Cc-HRE), and the corresponding sequences from *Ac* (KUNZE *et al.* 1987), *hobo* (CALVI *et al.* 1991), and *Hermes* (WARREN *et al.* 1994). Introduced gaps are shown by dashes, and consensus residues are boxed in black and shown in the consensus line when present in a majority of sequences. Residue numbers are shown at the end of the lines. The nucleotide sequences have been deposited into the GenBank under accession numbers U51451 (As-HRE), U51452 (Bc-HRE), U51453 (Bd-HRE), and U51454 (Cc-HRE).

HRE and Bd-HRE are nearly as distant from the *hobo* subgroup as is *Ac* (Figure 2).

Hybridization analysis of *hobo*-related elements: Transposons often exist as multiple copy genomic elements. To verify the genomic presence and abundance of these elements, the subcloned PCR sequences were hybridized to genomic DNA. Figure 4 shows that both the *A. suspensa* and *B. dorsalis* elements exhibited hybridization to multiple genomic sites in their own species, but did not hybridize to genomic DNA from other tephritid species or *D. melanogaster* (data not shown). The pattern of hybridization to *Hin*dIII digested DNA in several *B. dorsalis* strains was generally consistent among the strains, with some heterogeneity most apparent in the shorter restriction fragments. Approximately 5–10 copies of each *hobo*-related element per haploid genome can be deduced for both *A. suspensa* and *B. dorsalis*.

The HRE for *B. cucurbitae* hybridized to multiple genomic sites in the Cue strain (Figure 4C), but appear less abundant by inspection than As-HRE and Bd-HRE. Bc-

A

Percent Similarity

Percent Divergence	1	2	3	4	5	
1		34.8	33.1	34.2	32.0	1 As-HRE
2	39.5		32.4	32.6	31.5	2 Bd-HRE
3	52.1	51.2		67.6	68.7	3 Bc-HRE
4	51.8	49.2	32.0		72.4	4 Cc-HRE
5	52.3	50.5	31.4	26.2		5 hobo
	1	2	3	4	5	

B

Percent Similarity

Percent Divergence	1	2	3	4	5	6	7	
1		18.9	18.8	15.0	15.0	16.5	15.0	1 Activator
2	73.9		34.3	12.8	15.0	16.5	14.3	2 As-HRE
3	74.8	55.0		15.0	21.1	16.5	20.3	3 Bd-HRE
4	82.5	84.1	79.7		59.4	54.1	59.4	4 Bc-HRE
5	78.4	78.4	71.7	40.2		66.9	72.9	5 Cc-HRE
6	78.6	74.6	74.2	45.9	32.6		60.2	6 Hermes
7	82.5	79.4	73.4	40.6	26.5	39.8		7 hobo
	1	2	3	4	5	6	7	

FIGURE 2.—Sequence pair distance matrices between analogous sub-fragment DNA (A) and amino acid (B) sequences in *hobo*, *hobo*-related elements, and *hAT* transposons. Distances were calculated from DNA or amino acid sequence alignments using the Clustal method with Weighted residue weight table or PAM250 residue weight table, respectively.

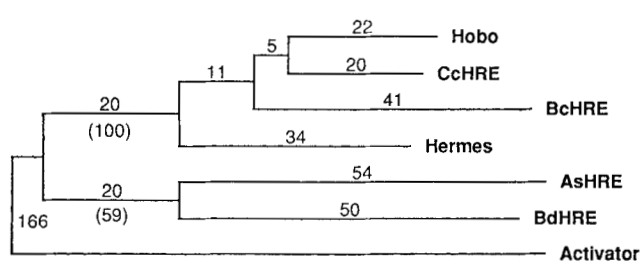

FIGURE 3.—A parsimony analysis using PAUP v. 3.1.1 based on the sequence alignment in Figure 1, using the *Ac* sequence as the outgroup. The number of amino acid changes are indicated as branch lengths with the percent support for branches after bootstrap analysis with 1000 replications given in parentheses.

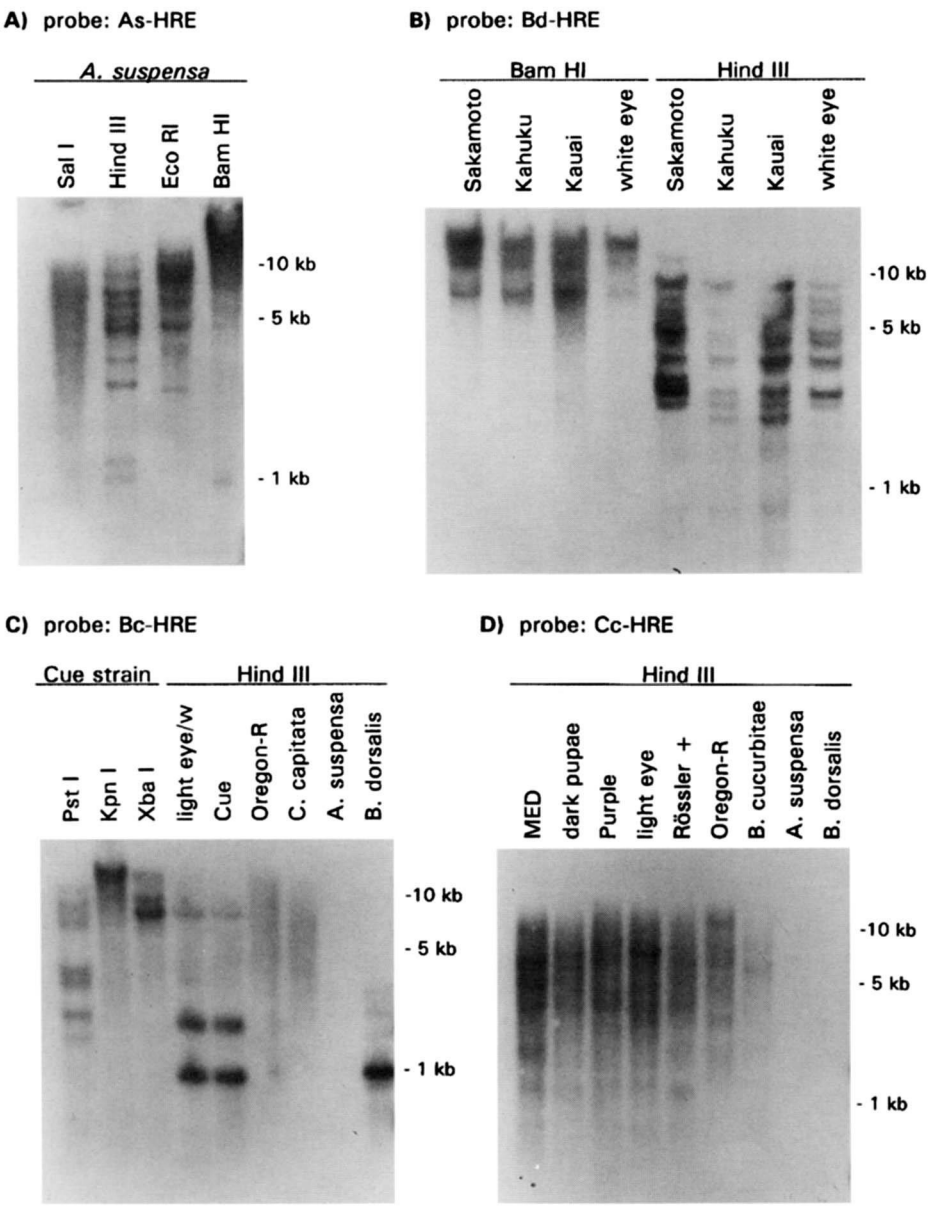

FIGURE 4.—Southern blot DNA hybridization analysis of *hobo*-related elements. (A) *A. suspensa* genomic DNA digested with indicated restriction enzymes and probed with radiolabeled As-HRE, (B) genomic DNA of *B. dorsalis* strains digested with indicated restriction enzymes and probed with radiolabeled Bd-HRE, (C) genomic DNA of the *B. cucurbitae* Cue strain digested with indicated restriction enzymes, and other indicated insect species digested with *Hin*dIII, and probed with radiolabeled Bc-HRE, and (D) genomic DNA of *C. capitata* strains and other indicated species restriction digested with *Hin*dIII and probed with radiolabeled Cc-HRE. DNA base pair sizes are indicated on the right of each autoradiogram.

HRE hybridization to *Hin*dIII-digested *B. cucurbitae* DNA resulted in strong hybridization to two bands at 1 and 1.9 kb. Since a *Hin*dIII site exists in the PCR sequence, other *Hin*dIII sites most likely exist in the element or proximal chromosomal DNA. In control hybridizations, Bc-HRE probe failed to hybridize to *Hin*dIII digested DNA from *C. capitata*, *A. suspensa*, or *D. melanogaster* (Oregon-R *hobo* containing strain), but in *B. dorsalis*, strong hybridization to a 1-kb fragment was evident, while other larger bands (including one in the 1.9-kb region) exhibited only a low level signal (additional hybridizations to DNA from several *B. dorsalis* strains yielded the same result; data not shown). This indicates that the *B. cucurbitae* element (or one closely related) exists in *B. dorsalis*, but may have an internal deletion. This deletion would almost certainly remove one of the PCR priming sites, resulting in a failure to amplify the sequence. Thus, *B. dorsalis* apparently contains two HREs, one of limited homology to *hobo* (Bd-HRE), and another more closely related to *hobo* which exists in at least one other *Bactrocera* species (Bc-HRE).

In *C. capitata*, Cc-HRE hybridization appeared to occur somewhat heterogeneously to *Hin*dIII digested DNA from various strains (Figure 4D). This might be explained by preliminary results from PCR product analysis suggesting that several different *hobo*-related elements exist in *C. capitata*, with most being closely related to *hobo*. Smeared hybridizations may also result from multiple copies in repetitive DNA. While hybridization was not detected in *A. suspensa* or *B. dorsalis*, hybridization was detected in *D. melanogaster* Oregon-R, possibly due to the similarity between *hobo* and Cc-HRE. A low-level hybridization signal was detected as well in *Hin*dIII-digested *B. cucurbitae* DNA, but apparently not to its Bc-HRE elements, which occur as 1 and 1.9-kb fragments (see Figure 4C).

DISCUSSION

The results indicate that five tephritid species are capable of supporting excision of the *D. melanogaster hobo* element in the presence of *hobo* transposase, and in its absence as well. For four of the species PCR products were generated, which are bounded by primer sites found in both *hobo* and *Ac* and which exist as repetitive elements in their respective genomes. This suggests that they belong to the superfamily of *hobo*-related, or *hAT*, transposable elements. The clustering of these elements into subgroups closely related and less related to *hobo* support the notion of both vertical evolution and horizontal transmission of *hobo*-related elements in tephritids.

***hobo* function in tephritids:** Similar to the activity of *hobo* in several drosophilid species (HANDLER and GOMEZ 1995) and *M. domestica* (ATKINSON *et al.* 1993) that do not contain *hobo* elements, excision or deletion of plasmid-borne *hobo* occurred consistently both in the presence and absence of exogenously supplied transposase helper. This indicates that unlike the *P* element, *hobo* mobility occurs among a wide range of dipteran insects and that endogenous mobilizing systems apparently exist in these species as well. The finding that *hobo*-related elements exist in at least four of the five species tested for excision gives physical evidence for these species harboring potential cross-mobilizing systems.

Consistent with the results in drosophilids, differences were observed in the frequency of *hobo* excision among species, as well as the specific influence of transposase on excision among the strains of individual species. Notably, the presence of *hobo* transposase (by coinjection of HSH2) had a negative effect on excision in two of four strains of *B. dorsalis*, both strains of *B. cucurbitae*, and the wild strain of *T. curvicauda*. Transposase caused an increase in excision in all four strains of *C. capitata* tested, as well as the wild strain of *A. suspensa*, and two strains of *B. dorsalis*. Thus, the dramatic negative effect of transposase on excision in *M. domestica* (sixfold decrease; ATKINSON *et al.* 1993) is not consistently observed in other nondrosophilid species, with the varied influence of *hobo* in tephritids being more consistent with results from drosophilid species (HANDLER and GOMEZ 1995). Furthermore, the negative interactions are apparently not strictly species-specific, since they occurred only in particular strains for some species. This is an apparent similarity to the varied activity of *hobo* in *D. melanogaster* strains such as *Uc* (HO *et al.* 1993; SHEEN *et al.* 1993) and *23.5MRF* (YANNOPOULOS *et al.* 1987). As theorized previously (ATKINSON *et al.* 1993; HANDLER and GOMEZ 1995), the negative effects of *hobo* transposase may be due to interactions with endogenous *hobo*-related systems, which result in inhibition of transposase function or an altered activity causing a lack of excision site specificity. The positive influence of exogenous *hobo* transposase was most clearly observed in the *C. capitata dark pupae* and *B. dorsalis white eye* strains where low excision frequencies without helper, close to background levels observed in *A. suspensa* with pUC19, were increased nearly sixfold with helper.

Since *hobo*-related elements were detected in all strains, their specific positive or negative influence on *hobo* excision cannot be simply attributed to their presence. The specific number of elements and their functionality in specific strains remains undetermined, and elucidation of their activity and interactions with *hobo* must await their isolation for systematic tests.

Excision process: Almost all of the excision products sequenced from *A. suspensa*, *B. dorsalis* and *C. capitata* revealed an imprecise excision event, whether they occurred in the presence or absence of *hobo* transposase, which is consistent with similar data from *M. domestica* (ATKINSON *et al.* 1993). In contrast, in a previous study with *D. virilis* precise excision events predominated in the presence of transposase (75% of excisions sequenced), occurring at a similar frequency and with the same types of excision site sequence as that found in *D. melanogaster* (HANDLER and GOMEZ 1995). In the absence of transposase all excisions in *D. virilis* were imprecise. Control experiments in all of these studies indicated that a *hobo*-dependent process was occurring and not random deletion of injected plasmid. Nevertheless, the lack of detectable precise excisions suggests that the endogenous mobilizing systems in tephritids as well as in drosophilids are at least mechanistically different from *hobo* and/or have differing target site specificities. Furthermore, it is likely that the ability of *hobo* transposase to supersede or complement the negative or competing activity of endogenous factors is limited to drosophilids or very closely related insect genera. The discovery of a single precise excision event in *A. suspensa* in the presence of *hobo* does suggest that some level of normal transposase activity is possible. Although the excision study in Musca by Atkinson *et al.* (1993) only reported imprecise excision in the presence of *hobo*, their subsequent analysis of *hobo* transposition indicated that normal excision-transposition processes do occur at lower frequencies (O'BROCHTA *et al.* 1994). Taken together, the data from these studies indicate that *hobo* excision-transposition can occur in species other than *D. melanogaster*, but at relatively low frequencies, and that *hobo* transposase is required for this process to occur normally.

***hobo*-related elements:** *hobo*-related element subfragments were isolated as PCR products in *A. suspensa*, *B. dorsalis*, *B. cucurbitae*, and *C. capitata* through the use of priming sites that encode homologous amino acid sequences in *hobo* and *Ac* (CALVI *et al.* 1991). The PCR sequences identified generally represent complete ORFs, which exist as multiple repetitive elements in their respective genomes. As with the *M. domestica Hermes* element, these elements may be responsible for the cross-mobilization of *hobo* and give support to the

existence of the *hobo*, *Ac* and *Tam3* (*hAT*) superfamily of short inverted terminal repeat transposons. The limited sequence comparisons and hybridization studies presented here indicate disparate homologies among the *hobo*-related elements, with parsimony analysis revealing at least two subgroups of closely and more distantly related elements to *hobo*. Since the tephritid species in this study diverged from Drosophila ~120–140 Myr ago and diverged from each other ~20–30 Myr ago (BEVERLEY and WILSON 1984), the PAUP sister taxa relationships do not support direct vertical evolution for all of these elements from a common ancestor.

The previously identified insect transposons within the *hAT* family are closely related, suggesting a limited divergence of these elements in the Insecta (WARREN *et al.* 1994). This is supported by the Bc-HRE and Cc-HRE sequences, which are comparably similar to *hobo* and *Hermes* as well as to one another. A wider divergence is apparent, however, by comparisons to As-HRE and Bd-HRE, which are nearly as diverged from the *hobo* subgroup as is *Ac*. If these limited sequence comparisons are maintained for the complete elements, then As-HRE and Bd-HRE may have evolved from an ancient *hobo* or *hAT* predecessor (or predecessors), possibly branching close to the time of divergence of their host species (this comparison is maintained for a complete Bd-HRE; A. M. HANDLER and S. P. GOMEZ, unpublished data). In contrast, Bc-HRE and Cc-HRE, as well as *Hermes* and *hobo*, appear to have arisen from a much more recent common ancestor, which may have been horizontally transmitted. Horizontal transmission for *hobo* has already been suggested within the Drosophilidae (DANIELS *et al.* 1990a; BOUSSY and DANIELS 1991; PASCUAL and PERIQUET 1991; SIMMONS 1992), as well as for *P* (DANIELS *et al.* 1990b) and *mariner* (MARUYAMA and HARTL 1991; ROBERTSON 1993; ROBERTSON and MACLEOD 1993; ROBERTSON and LAMPE 1995), though alternative explanations for these apparently close relationships also have been presented (CAPY *et al.* 1994; CUMMINGS 1994). The formal possibility exists as well that the more diverged elements also arose from recent horizontal transfers, but from more distantly related organisms.

Comparisons of *mariner* elements by ROBERTSON and MACLEOD (1993) reveal considerable diversity of *mariner* among insects and the existence of multiple elements in the same species, possibly resulting from both vertical evolution and horizontal transfers. Similarly, we found two *hobo*-related elements in *B. dorsalis*. Bd-HRE, which is distantly related to *hobo*, may have evolved vertically in the species, while Bc-HRE, originally found in *B. cucurbitae*, is more closely related to *hobo*, suggesting that it originated from a more recent horizontal transfer.

Implications for *hobo* gene-vector function: Taken together, results on the presence and activity of *hobo*-related systems in both drosophilid (HANDLER and GOMEZ 1995) and tephritid species have important implications for the possible use of *hobo* as a vector for gene transfer in nondrosophilids. Of primary importance is that, unlike *P* (O'BROCHTA and HANDLER 1988; HANDLER *et al.* 1993), *hobo* has a broad range of function in dipteran insects and may successfully mediate germline transformation in these insects as well as others. However, presuming that efficient transformation requires accurate excision and transposition, relatively low rates of precise excision and transposition would indicate a significantly decreased rate of *hobo* transformation for nondrosophilids. Only a single precise *hobo* excision event in *A. suspensa* was detected in this study, and O'Brochta *et al.* (1994) observed *hobo* plasmid-to-plasmid transposition in nondrosophilids at four- to 10-fold lower levels relative to *D. melanogaster*. A decreased level of *hobo* transformation in species distantly related to *D. melanogaster* is supported in part by recent *hobo*-mediated transformations of *D. virilis*, which diverged approximately 40 Myr ago (RUSSO *et al.* 1995). Transformation was observed by us (S. P. GOMEZ and A. M. HANDLER, unpublished data) and LOZOVSKAYA *et al.* (1996) at frequencies of 0.5%, in contrast to frequencies approaching 30% in *D. melanogaster* (BLACKMAN *et al.* 1989).

A more daunting consideration for the use of *hobo* and related gene vectors is the cross-mobilizing interactions, which may destabilize integrations. *P* and *hobo* vector stability in *D. melanogaster* is maintained in large part by the use of M or E host strains, respectively, which are devoid of the vector transposon (RUBIN and SPRADLING 1982; BLACKMAN *et al.* 1989). Thus far, on the basis of cross-mobilization and, for most, PCR detection, all nonmelanogaster species appear to have *hobo*-related systems. While we cannot directly infer how these systems function and interact, it is likely that systems capable of promoting *hobo* excision from plasmids will have similar effects on chromosomal integrations of *hobo* or their own related element. On the basis of their limited cross-mobilization of *hobo*, excision and transposition assays may reveal optimal host strains, such as *C. capitata dark pupae* and *B. dorsalis white eye*, but it is likely that more direct approaches will be required for vector stability, including constructs that can be altered post-integration to limit their mobility.

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