

The *piggyBac* transposon mediates germ-line transformation in the Oriental fruit fly and closely related elements exist in its genome

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Abstract

Germ-line transformation of a white eye strain of the Oriental fruit fly, *Bactrocera dorsalis*, was achieved with the *piggyBac* vector, derived from a transposon originally isolated from the cabbage looper moth, *Trichoplusia ni*. The vector was marked with the medfly *white*⁺ gene cDNA, and three transgenic lines were identified at a frequency of approximately 2% per fertile G₀. Vector integrations were verified by Southern DNA hybridization, which also revealed the presence of endogenous genomic elements closely related to *piggyBac*. Approximately 10–20 elements per genome were evident in several *B. dorsalis* strains, and sequence analysis of 1.5 kb gene amplification products from two wild strains and the *white eye* host strain indicated 95% nucleotide and 92% amino acid sequence identity among resident elements and the *T. ni* element. *PiggyBac* was not evident by hybridization in other tephritid species, or insects previously transformed with the transposon. This is the first discovery of *piggyBac* beyond *T. ni*, and its existence in a distantly related species has important implications for the practical use of the vector and insects transformed with it.

Keywords: *piggyBac* transposon, germ-line transformation, horizontal transmission, *Bactrocera dorsalis*, Tephritidae.

Introduction

Germ-line transformation of nondrosophilid insects has recently succeeded with four transposon-based vector systems (Loukeris *et al.*, 1995; Jasinskiene *et al.*, 1998; Coates *et al.*, 1998), with the the *piggyBac* element from the cabbage looper moth, *Trichoplusia ni*, being one of the more widely used to date. *PiggyBac* has transformed the Mediterranean (Handler *et al.*, 1998) and Caribbean (Handler & Harrell, 2000) fruit flies, *Drosophila melanogaster* (Handler & Harrell, 1999), *Bombyx mori* (Tamura *et al.*, 2000), *Tribolium castaneum* (Berghammer *et al.*, 1999), and the pink bollworm (Peloquin *et al.*, 2000). The *piggyBac* transposon was first discovered as the causative agent of few polyhedra (FP) mutations in a baculovirus that was passed through the *T. ni* cell line TN-368 (Fraser *et al.*, 1983), and it was subsequently found to have originated in the genome of the insect species (Cary *et al.*, 1989). Thus far the transposon has not been found in any other species nor in any of the insects transformed with the vector, although other elements sharing the TTAA insertion site specificity of *piggyBac* have been found in other Lepidoptera (Beames & Summers, 1990). While exhaustive searches for *piggyBac* have not been made, the existence of *piggyBac* or related elements in a wide range of insects would not be unexpected given its broad range of vector function in dipterans, lepidopterans, and a coleopteran. In particular, studies in two dipterans, *Ceratitis capitata* (Handler *et al.*, 1998) and *D. melanogaster* (Handler & Harrell, 1999), showed that *piggyBac* transcription and enzymatic activity were autonomous, since an unmodified transposase helper catalysed transposition, suggesting that its mobility is not dependent on the *T. ni* cellular phenotype, or restricted by host specific factors in other insects.

A determination of the existence and function of *piggyBac* in insect species, as well as other organisms, is of particular importance for a vector that may be used for applied purposes. This information provides an indication of the potential stability of the vector in a specific host, as well as the potential for transmission into nontargeted hosts. In an effort to apply *piggyBac* transformation to additional

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insect species, we attempted transformation in the Oriental fruit fly, *Bactrocera dorsalis*. *Bactrocera dorsalis* is a highly destructive tephritid fruit fly pest whose behaviour and population size might be controlled by the molecular manipulation of transgenic strains. Furthermore, transformation of this species could be tested straightforwardly since a *white eye* strain was available (McCombs & Saul, 1992) and evidence exists for a close relationship between its *white eye* (*we*) gene (Xiao, 1997) and that of the medfly (Zwiebel *et al.*, 1995), which had previously been tested as a transgenic marker (Loukeris *et al.*, 1995; Handler *et al.*, 1998). We report here the transformation of *B. dorsalis* with the *piggyBac* vector, and the discovery of nearly identical *piggyBac* elements in the host species.

Results

Transformation experiments

Germ-line transformation of the Oriental fruit fly was tested with a *piggyBac* vector marked with the medfly *white*⁺ gene cDNA under *hsp70* promoter regulation (Handler *et al.*, 1998), and a *hsp70*-regulated transposase helper (Handler & Harrell, 1999). In two experiments, 3742 preblastoderm embryos from a *we* strain were injected with vector and helper plasmids at concentrations of 500 µg/ml and 300 µg/ml, respectively (Table 1). From these injections, 243 G₀ embryos survived to adulthood and were individually back-

crossed to the *we* host strain. Of these, 157 were fertile, with three of the G₀ matings yielding progeny with pigmented eyes (Fig. 1). One of the lines, Bd1-61, yielded 119 *we*⁺ putative transformants having red-orange eye colour phenotypes, a second line, Bd1-115, yielded five G₁ offspring with yellow eyes and a third line, Bd1-137, yielded nine offspring with pale pink eyes (Table 2). Putative transformant G₁ offspring were backcrossed individually, and their G₂ *we*⁺ offspring and subsequent generations were intermated. The large number of G₁ transformants in the Bd1-61 line, affecting nearly a third of the G₁ progeny, is a clustering affect that likely represents an early integration into a germ cell chromosome.

Southern hybridization

G₂ and G₃ *we*⁺ flies were subjected to molecular analysis by Southern hybridization, which is typically performed to determine the number and general integrity of integrations for particular G₁ sublines. For integration number, genomic DNA of transformed and nontransformed flies was digested with *Bgl*II and probed with a radiolabelled *Nsi*/*Hpa* probe that spans the *piggyBac* *Bgl*II restriction site (Fig. 2A). This should result in a 1.6 kb internal vector fragment, and an individual band for each integration that spans the 5'-end junction site. In addition to the 1.6 kb fragment in putative transformant lines, numerous hybridizing fragments were detected in the transformed as well as the nontransformed

Table 1. Transformation experiments

Expt.	Vector:helper µg/µl	Eggs injected	G ₀ s mated	% Fertility	G ₁ progeny	G ₀ lines with G ₁ <i>we</i> ⁺	% Transformed
I	500 : 300	2806	205	67	12 299	3	4.5
II	500 : 300	936	38	50	1014	0	0

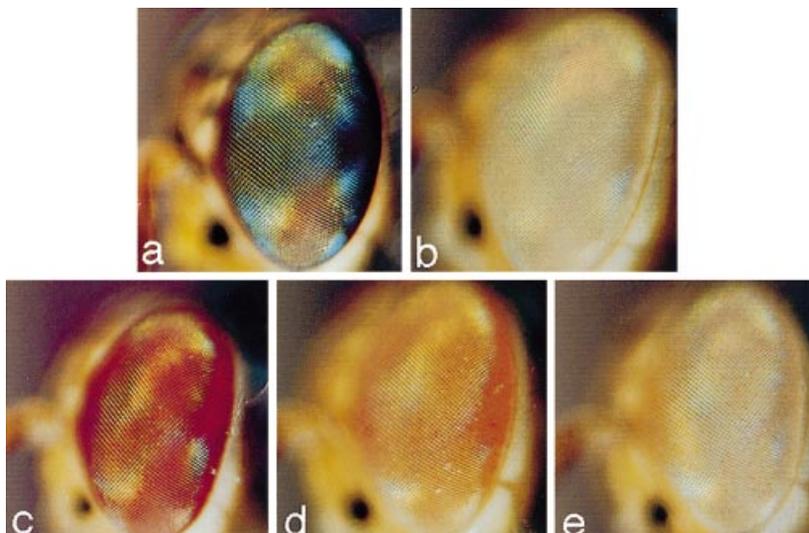


Figure 1. Eye colour phenotypes of the *B. dorsalis* strains wild-type (a), *white eye* (b), and the *Bd*[*pBCcw*] transformant lines Bd1-61 (c), Bd1-115 (d), and Bd1-137 (e). Visible descriptions are given in Table 2.

Table 2. *Bd*[*pBCcw*] transformant lines

G ₀ line	Total G ₁	G ₁ <i>we</i> ⁺	Male	Female	Phenotype
Bd1-61	406	119	60	59	red-orange
Bd1-115	225	5	3	2	yellow
Bd1-137	83	9	5	4	pale pink

wild and we host strains (autoradiogram exposure times necessary to visualize the 1.6 kb band resulted in over-exposure of the other fragments). To better determine the molecular phenotype of these lines, hybridization was performed on the same sample DNA digested with *Nsi*I, that creates a large internal fragment of 5.6 kb (from the

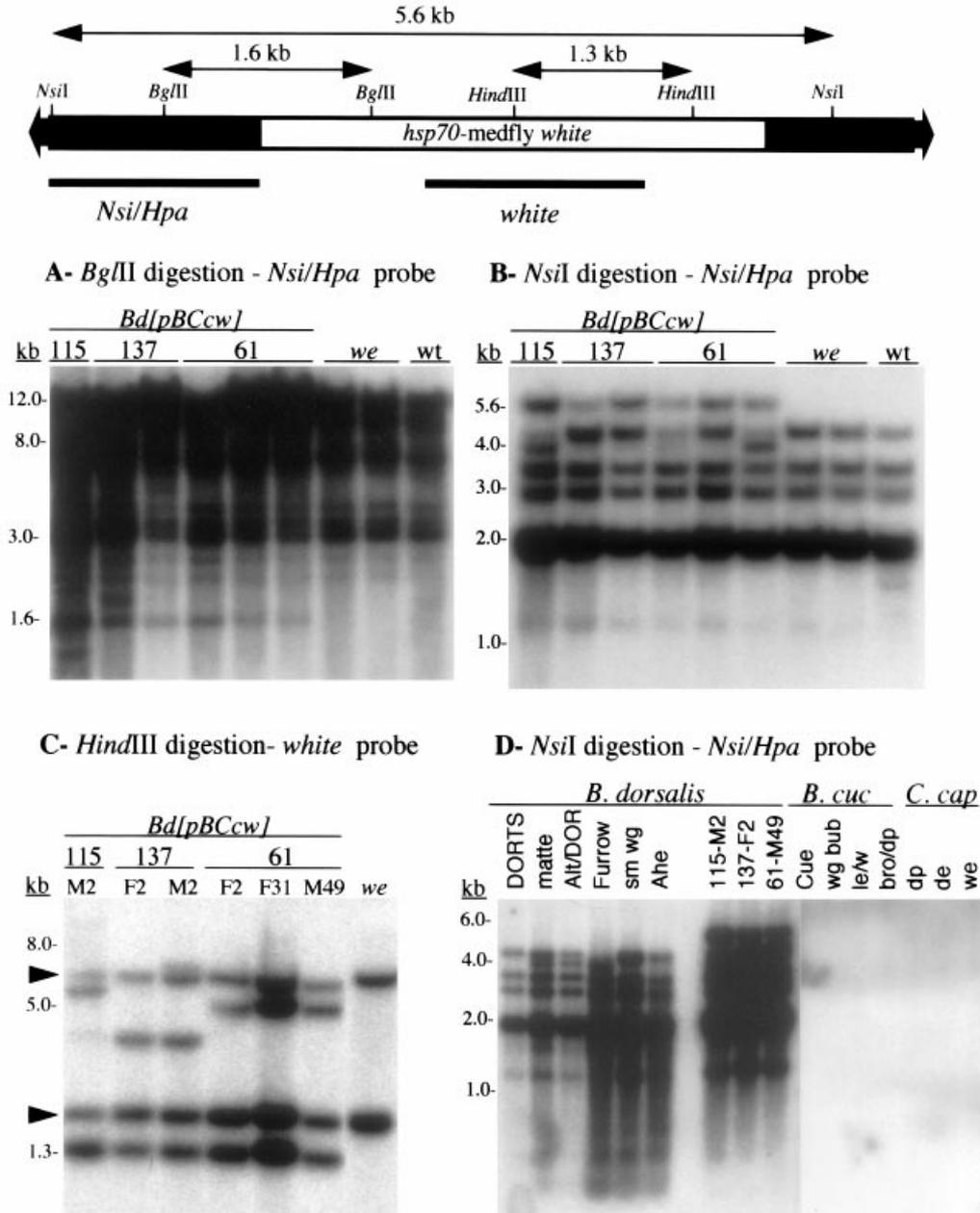


Figure 2. Southern DNA hybridization analysis of *Bd*[*pBCcw*] transformant sublines (Bd1-61, 115 and 137), *we* host strain and wild-type (*wt*) control samples, and indicated mutant and wild strains of *B. dorsalis*, *B. cucurbitae* (*B. cuc*) and *Ceratitis capitata* (*C. cap*). On top is a schematic (not to scale) of the pB[Ccw] vector showing the *Bgl*II, *Nsi*I and *Hind*III restriction sites used to digest genomic DNA, and the 1.4 kb *Nsi*I-*Hpa*I piggyBac and 1.54 kb *white* vector fragments used as hybridization probes (bars). Above the schematic are the distances used to calculate internal restriction fragment sizes. PiggyBac sequences are in black and *hsp70-white* marker sequences are in white. For each blot, the restriction enzyme used for digestion and hybridization probe are indicated. DNA size markers are shown to the left of the autoradiograms. Panel (A) shows a *Bgl*II digestion hybridized with *Nsi*I-*Hpa*I probe, (B) shows an *Nsi*I digestion hybridized with *Nsi*I-*Hpa*I probe, (C) shows a *Hind*III digestion hybridized with *white* probe (arrows indicate resident genomic *white* sequences), and (D) shows an *Nsi*I digestion hybridized with *Nsi*I-*Hpa*I probe. See Experimental procedures for details.

6.0 kb vector) (Fig. 2B). Using a *Nsi/Hpa* probe that hybridizes within this fragment, all the transformed lines yielded the 5.6 kb vector fragment, which was not detectable in the *we* or wild-type nontransformed lines. All lines, however, showed a strongly labelled 2.0 kb fragment, three to four fragments between 2.8 kb and 4.5 kb, and a single fragment at about 1.2 kb (weakly detected at the exposure shown; see Fig. 2D for better detection). Notably, the 2.0 kb fragment, which appears to represent multiple elements, is the fragment size expected from *piggyBac* unmarked by the insertion of the 3.6 kb *w⁺* marker gene construct. These hybridizations suggested the existence of *piggyBac* or closely related elements (presuming at least 80–90% identity) in the *B. dorsalis* wild Sakamoto strain and the *we* host strain. Based on the number and intensity of hybridizing fragments in the *NsiI* digestion blot, we estimate a copy number of 10–20 elements per genome. Assuming these are *piggyBac* elements, the fragments larger than 2.0 kb could represent either enlarged elements having insertions or internal duplications, or internally deleted elements having one or both of the *NsiI* sites deleted. Given the high frequency of internally deleted elements for other transposons existing in high copy number, the latter explanation is more likely (O'Hare & Rubin, 1983; Streck *et al.*, 1986). A 4.0 kb fragment appears in several of the transformant lines, but it is not present in the *we* or wild strains. It is also not apparent in the DORTS strain from which *we* arose, but does occur in several other mutant strains (see Fig. 2D), and this may represent an unstable element.

To more clearly determine the existence and number of *piggyBac* vector integrations, a hybridization was performed using a *white* cDNA marker gene fragment as probe to DNA digested with *HindIII* that releases an internal 1.3 kb marker gene fragment. From this hybridization, two fragments from the resident *white* gene sequence occurred in the transformed and nontransformed lines while the internal 1.3 kb vector fragment occurred only in the transformed lines (Fig. 2C). Additional fragments in the transformed lines indicated one integration in all the transformant lines except for 137-M2, which has two integrations. This further proves that the three lines were transformed at a frequency of approximately 2–3% per fertile G₀, and shows that the medfly *white⁺* gene can complement an analogous mutant gene in the Oriental fruit fly. The existence of resident *piggyBac* elements precludes the straightforward determination of vector insertion site sequences by inverse PCR, and thus the fidelity of the vector transpositions remains unknown. This will be clarified in future studies by isolation of genomic clones containing vector.

piggyBac distribution

To determine if *piggyBac*-hybridizing elements exist as well in other *B. dorsalis* strains or other tephritid species, a

Nsi/Hpa hybridization was performed on representative DNA samples, digested with *NsiI*, from *B. dorsalis* wild, mutant, and transformed strains, and DNA from strains from the melonfly, *B. cucurbitae*, and the Mediterranean fruit fly, *Ceratitis capitata* (Fig. 2D). Patterns of hybridization in transgenic and nontransgenic *B. dorsalis* samples were similar to the original hybridizations (see Fig. 2B), but *piggyBac* hybridization was not detectable after extended autoradiographic exposure in the melonfly and medfly strains.

piggyBac sequences

To determine the relationship of the endogenous cross-hybridizing elements to *piggyBac*, DNA was amplified from the *B. dorsalis* host *we* strain and the wild Sakamoto and Kahuku strains using conserved primers to the *piggyBac* sequence starting at nucleotide positions 636 (forward primer) and 2123 (reverse primer). These primers encompass approximately 85% of the putative transposase transcriptional unit. The Kahuku strain was isolated from the Hawaiian island of Oahu in 1990, and the Sakamoto strain from Maui in 1990, while *we* arose as a spontaneous mutation in 1986 from a wild strain (DORTS) originally isolated on Oahu in 1956. Thus, a limited temporal and geographical isolation exists among these strains.

Strong amplification of 1.5 kb products and weaker amplification of smaller products occurred in all strains, and these were subcloned and several were sequenced. None of the smaller products were related to *piggyBac*, and it is possible that deleted *piggyBac* elements would be rarely be detected owing to deletion of one or both of the priming sites. Of the 1.5 kb products, several similar yet distinct sequences were obtained and individual sequences from each strain and their conceptual translation products were compared by alignment to each other and the *T. ni piggyBac* element. Overall nucleotide identity among the *B. dorsalis* sequences and the original *piggyBac* (3E1; Cary *et al.*, 1989) is 95%, with the closest identity of 98% occurring between the Sakamoto and Kahuku sequences (data not shown). Similarly, the overall identity of the conceptual translation products of the elements, with introduced frameshifts in Kahuku and Sakamoto to maintain homology to the *T. ni piggyBac* reading frame, resulted in an overall amino acid sequence identity of 92% by CLUSTALW analysis (Fig. 3). There are several nucleotide changes among the elements, and the Kahuku and Sakamoto sequences share common deletions of 7 bp and 10 bp that disrupt the transposase reading frame, and which do not exist in the *T. ni* or *we* elements. The *we* strain has a distinct 6 bp deletion that maintains the reading frame, although it is not known if the encoded transposase retains its functionality. Similar high levels of identity were observed in other partial sequences (data not shown), and the overall identity/similarity of the elements between strains was not significantly different than for elements within each

Kahuku	STRRSRVSALNIVRSQRGPTMCRNIYDPLLCFKLFFFTDEIISEIVKWTNAEISLKRRES	60
Sakamoto	STRRSRVSALNIVRSQRGPTMCRNIYDPLLCFKLFFFTDEIISEIVKWTNAEISLKRRES	60
white-eye	STRRSRVSALNIDRSQRGPTMCRNIYDPLLCFKLFFFTDEIISEIVKWTNAEISLKRRES	60
Tni	STRRSRVSALNIVRSQRGPTMCRNIYDPLLCFKLFFFTDEIISEIVKWTNAEISLKRRES	60
	***** :*****	
Kahuku	MTGATFRDTNEDEIHALFGILVMTAVRKDNHMSTDDLYDRSLSMVYVSVMSRDRFDFLIR	120
Sakamoto	MTGATFRDTNEDEIHALFGILVMTAVRKDNHMSTDDLYDRSLSMVYVSVMSRDRFDFLIR	120
white-eye	MTGATFRDTNEDVIHALFGILVMTAVRKDNHMSTDDLYDRSLSMVYVSVMSRDRFDFLIR	120
Tni	MTGATFRDTNEDEIYAFFGILVMTAVRKDNHMSTDDLFDRSLSMVYVSVMSRDRFDFLIR	120
	***** * : * :***** :*****	
Kahuku	CLRMDDKSIRPTLRGNDVFTVPRKIWDLFIHQCIHNYTPGAHLTIDEQLLGFGRGCPFRM	180
Sakamoto	CLRMDDKSIRPTLRGNDVFTVPRKIWDLFIHQCIHNYTPGAHLTIDEQLLGFGRGCPFRM	180
white-eye	CLRMDDKSIRPTLRGNDVFTVPRKIWDLFIHQCIHNYTPGAHLTIDEQLLGFGRGCPFRM	180
Tni	CLRMDDKSIRPTLRGNDVFTVPRKIWDLFIHQCIQNYTPGAHLTIDEQLLGFGRGCPFRM	180
	***** ***** :***** :*****	
Kahuku	YIPNKPSKYGIKILMDCSGTKYMINGMPYLERGTQTNQVPLGEYVVKELSKPVHGSCRN	240
Sakamoto	YIPNKPSKYGIKILMDCSGTKYMINGMPYLERGTQTNQVPLGEYVVKELSKPVHGSCRN	240
white-eye	YIPNKPSKYGIKILMDCSGTKYMINGMPYLRGTQTNQVPLGEYVVKELSKPVHGSCRN	240
Tni	YIPNKPSKYGIKILMDCSGTKYMINGMPYLRGTQTNQVPLGEYVVKELSKPVHGSCRN	240
	***** *****	
Kahuku	ITCENWFTSIPLAKX--QEPYKLTIVX---SNKREIPEVLKNSRSPVGTSMFCFDGPLT	295
Sakamoto	ITCENWFTSIPLAKN--XEPYKLTIVX---SNKREIPEVLKNSRSPVGTSMFCFDGPLT	295
white-eye	ITCDNWFTSIPLAKNLLQEPYKLTIVGTVRSNKRWIPEELKNSRSPVGTSMFCFDGPLT	300
Tni	ITCDNWFTSIPLAKNLLQEPYKLTIVGTVRSNKRWIPEVLKNSRSPVGTSMFCFDGPLT	300
	*** :***** ***** **** * * ***** :*****	
Kahuku	LVSYPKPKAKMVYLLSSCEDASINESTGPKQMIMYYNQTKGGVDTLDQMCVMSCSRKT	355
Sakamoto	LVSYPKPKAKMVYLLSSCEDASINESTGPKQMIMYYNQTKGGVDTLDQMCVMSCSRKT	355
white-eye	LVSYPKPKAKMVYLLSSC--DAPINESTGPKQMIMYYNQTKGGVDTLDQMCVMSCSRKT	358
Tni	LVSYPKPKAKMVYLLSSCEDASINESTGPKQMIMYYNQTKGGVDTLDQMCVMSCSRKT	360
	***** * :***** :***** :*****	
Kahuku	NRWPMALLYGMINIACINSFIIYSHNVSSKGEKVQSRKNFMKNLNMSLTSSFMKRLEAP	415
Sakamoto	NKWPALLYGMINIACINSFIIYSHNVSSKGEKVQSRKNFMKNLNMSLTSSFMKRLEAP	415
white-eye	NKWPALLYGMINIACINSFIIYSHNVSSKGEKVQSHKNFMKNLNMSLTSSFMGRLEAP	418
Tni	NRWPMALLYGMINIACINSFIIYSHNVSSKGEKVQSRKKFMRNLYMSLTSSFMKRLEAP	420
	* :***** ***** : * : * * ***** *****	
Kahuku	TLKRYLRDNI SNILPNEVPGTSEDSTEEPVTKKRTYCTYCPSKIIRKATASCKKCKKVIC	475
Sakamoto	TLKRYLRDNI SNILPNEVPGTSEDSTEEPVTKKRTYCTYCPSKIIRKANASCKKCKKVIC	475
white-eye	TLKRYLRDNI SNILPNEVPGTSEDSTEEPVTKKRTYCTYCPSKIIRKATASCKKCKKVIC	478
Tni	TLKRYLRDNI SNILPNEVPGTSDDSTEEPVMKKRTYCTYCPSKIIRKANASCKKCKKVIC	480
	***** :***** :***** ***** ***** :*****	
Kahuku	REHNIDICQSCF	487
Sakamoto	REHNIDICQSCF	487
white-eye	REHNIDICQSCF	490
Tni	REHNIDMCQSCF	492
	***** :*****	

Figure 3. A CLUSTALW multiple amino acid sequence alignment of the conceptual translation products of amplified genomic DNA sequences using primers to 3E1 *piggyBac*. DNA samples include the *B. dorsalis* wild strains Kahuku and Sakamoto, and the mutant *white eye* strain, whose sequences were compared to the analogous sequence from the *T. ni* 3E1 *piggyBac* element (Tni). Positions shown by an X in bold represent a frameshift to maintain the *piggyBac* reading frame. Introduced gaps are shown by hyphens in the alignment and, in the consensus line below, identical or conserved residues in all sequences are indicated by an asterisk (*), conserved substitutions indicated by a semicolon (;), and semiconserved substitutions indicated by a period (.). Residue numbers are shown at the end of the lines. The nucleotide sequences have been deposited into GENBANK under accession numbers AF289121 (Kahuku), AF289122 (Sakamoto), and AF289123 (white eye).

strain. This is consistent with previous observations of *piggyBac* elements in *T. ni* (Fraser *et al.*, 1983; Cary *et al.*, 1989).

Discussion

Here we report germ-line transformation of the Oriental fruit fly with the *piggyBac* vector originally isolated from the cabbage looper moth, *Trichoplusia ni*. This is the third tephritid species to be transformed with *piggyBac* at a similar frequency of 2–3% per fertile G₀, further supporting the function of the transposon in dipteran species. Taken with recent data for *piggyBac* function in lepidopteran species and a coleopteran, *piggyBac* has a wide range of function that may allow its application in many insect species.

A surprising observation in this study, however, was the discovery of *piggyBac* hybridizing elements in the *white eye* host strain and a wild-type strain used for controls. These elements were found in several other wild and mutant Oriental fruit fly strains, but were not present in a survey of strains from another bactrocerid species, *B. cucurbitae*, or medfly strains, nor has it been reported for the other species transformed with *piggyBac*. Sequence analysis of genomic elements in *B. dorsalis* strains, using conserved *piggyBac* primers for gene amplification, indicated that the hybridizing fragments, indeed, represented highly conserved *piggyBac* elements in the *B. dorsalis* genome.

The fortuitous discovery of nearly identical *piggyBac* elements in a species distantly related to the original host is somewhat unique, and certainly so for the class of transposons having short inverted terminal repeat sequences. Only after comprehensive searches for *mariner*-related elements did Robertson & Lampe (1995) find nearly identical elements in two distinct orders, that being the *Himar* element originally found in the hornfly, *Haematobia irritans*, which was subsequently found in the green lacewing, *Chrysoperla plorabunda*. As theorized for the *Himar* elements, the discontinuous existence of *piggyBac* in separate orders most likely arose from a very recent horizontal transmission. It is not known if more diverged elements exist for *piggyBac*, and thus far a presumed relationship has only been made to other elements having a TTAA insertion-site specificity (Cary *et al.*, 1989; Beames & Summers, 1990), and very distantly related ancient elements in the human genome found in database searches (V. V. Kapitonov & J. Jurka, direct submission of the 'looper' sequence to REPBASE UPDATE, Genetic Information Research Institute). None of these, however, have enough similarity to make meaningful searches for related elements in specific organisms.

It is certainly of some interest to determine if *piggyBac* exists in other species and to ascertain how its movement occurred. Considering that the element was first discovered by its ability to transpose into an infecting baculovirus (Fraser

et al., 1983), and similar transposon movement is well established (Fraser *et al.*, 1985; Jehle *et al.*, 1998), it would not be surprising if cross-species transmission was virally mediated (see Fraser, 2000). It is also conceivable that transmission occurred via an intermediary species, since *B. dorsalis* and *T. ni* are thought to be geographically distinct. *T. ni* has been found in North America, Europe and Euroasia (Lindgren *et al.*, 1979), while *B. dorsalis* inhabits south-east Asia and parts of the Pacific (White & Elson-Harris, 1992).

The discovery of *piggyBac* elements in a species transformed with the element has several implications for its practical use as a vector and the organisms transformed with it. First, it is notable that reasonably efficient transformation was possible in *B. dorsalis*, which differs from the repression of *P* transposition in *P* strains (Engels, 1979; Kidwell, 1981). Whether *piggyBac* has a similar autoregulatory influence on its own mobility remains to be determined, since this may be a function of the number, structure or function of the genomic elements, or genomic silencing mechanisms within the species. If any of the resident elements are functional, then stability of introduced transgenes must be a concern. The sequence of at least one *piggyBac* element in the *we* host has the potential for function, although selected transgenic lines that have been maintained for more than two years (fifteen generations) have remained stable, based on phenotype. The potential for transgene mobilization by resident *piggyBac* elements can be assessed by transposition assays both with and without exogenous transposase, and these will be performed in the near future.

Of greater consequence for the widespread use of *piggyBac* as a vector in economically and medically important insects is its ability, possibly in concert with another vector system, to move horizontally into other species. This may be of less concern for defective nonautonomous vectors, but movement may still be catalysed by similar elements in targeted and nontargeted hosts. Cross-mobilization has already been observed between *hobo* and *Hermes* (Sundararajan *et al.*, 1999), and this potential exists as well for other related transposons. Possibilities for the horizontal transmission of *piggyBac* vectors must be resolved by further investigation into the element's function and distribution before it can be widely used for applied purposes, and these concerns should also extend to other vector systems.

Experimental procedures

Insect strains and rearing

The *Bactrocera dorsalis we*, wild-type, and transformed strains were maintained in a quarantine facility at the Department of Entomology, University of Hawaii. Standard larval and adult rearing methods were employed (McCombs & Saul, 1992).

Plasmids

The *piggyBac* vector, pB[Ccw], marked with the medfly *white*⁺ cDNA gene (Handler *et al.*, 1998) and the helper plasmid, phspBac, having the transposase gene under *hsp70* regulation (Handler & Harrell, 1999), were described previously. Briefly, medfly *w*⁺ regulated by *hsp70* (Zwiebel *et al.*, 1995) was isolated as a 3.6 kb *EcoRI* fragment that was blunted and ligated into the *HpaI* site of the *piggyBac* plasmid p3E1.2 (Cary *et al.*, 1989). The phspBac helper has the 457 bp *XbaI-XmnI* 5' nontranslated sequence from the *hsp70* gene ligated into the *SacI* deletion site of p3E1.2.

Injections

Embryo injections used procedures that were developed for the Mediterranean fruit fly (Handler *et al.*, 1998), and were modified from standard *Drosophila* procedures. Eggs were dechorionated in 1.6% hypochlorite solution followed by several washes in 0.02% Triton-X100. Eggs were placed on double-stick tape, desiccated in room-air and injected under Halocarbon 700 oil. DNA mixtures had vector:helper concentrations of 500 : 300 µg/ml in injection buffer (5 mM KCl; 0.1 sodium phosphate pH 6.8). Injected G₀ eggs were placed in an oxygenated and humidified tissue culture chamber at 23–25 °C and heat shocked at 37 °C for one hour at 16–20 h after injection. Eclosed G₀ adults were backcrossed individually to *we* host flies.

Southern hybridization

Five to 10 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 generated from the 5'-end *piggyBac* *Nsil-HpaI* restriction fragment (1.4 kb) isolated from p3E1.2 and the *XhoI-KpnI* fragment (1.54 kb) from the medfly *white* gene cDNA. Probe DNA was radiolabelled with [³²P]-dCTP by random priming (Gibco BRL) according to the manufacturer's specifications. Hybridizations were performed in phosphate buffer pH 7.5; 1% BSA; 7% SDS at 65 °C with an initial wash in 2× SSC; 0.2% SDS at room temperature and two washes in 1× SSC; 0.1% SDS at 55 °C for 30 min. Autoradiography was performed by exposure on Kodak X-Omat film at –70 °C.

Polymerase chain reaction and sequence analysis

PCR was performed on ≈ 100 ng of genomic DNA using the forward primer (636F) 5'-GTCCACGAGCGGTAGCCGAG-3' and the reverse primer (2123R) 5'-GTCAGTCCAGAAACAACCTTTGGC-3', with nucleotide positions relative to the 3E1 *piggyBac* sequence. PCR products were observed on agarose gel electrophoresis, subcloned directly into the pGEM T-Easy vector (Promega), and sequenced using M13 forward and reverse primers. Sequence alignments were performed using GENEWORKS 2.5 (Oxford Molecular Group) (Lipman & Pearson, 1985) and CLUSTALW (EMBL, European Bioinformatics Institute) software.

Acknowledgements

We thank David O'Brochta and Bettina Moser for comments on the manuscript. This work was supported by the USDA-APHIS-PPQ Mediterranean Fruit Fly Project, the

USDA-NRI Competitive Grants Program #99-35302-8138 (A.M.H.), and the California Department of Food and Agriculture #94-0615 (S.D.M.).

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