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A *P* element-homologous sequence in the house fly, *Musca domestica*

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Abstract

Sequences homologous to the *P* transposable element have been identified in *Musca domestica*. Sequence analysis of a genomic clone (Md-P1) indicates that, although the house fly *P* element has lost its coding capacity, the basic general structure of drosophilid *P* elements is present. The house fly *P* element sequence shares a number of structural features with that from the blow fly, *Lucilia cuprina*, including a large intron separating exons 1 and 2, two additional introns interrupting exon 2 and the apparent absence of inverted repeat termini. Within a relatively well-conserved central region, the house fly sequence shows 59% similarity to the *D. melanogaster P* element, but distal regions are more diverged. Southern blot analysis of several strains indicated the presence of at least four *P* element copies.

Keywords: *P* element, transposon, Diptera, *M. domestica*.

Introduction

The *P* family of transposable elements was first discovered in *Drosophila melanogaster* (Kidwell *et al.*, 1977; Bingham *et al.*, 1982). The *P* element has subsequently become an important molecular biological tool for the manipulation of genes in this and closely related species. The ability to use *P* elements to make transgenic flies (Rubin & Spradling, 1982; Spradling & Rubin, 1982) is one of the most important of these tools. However, use of *P* element vectors in insects other than drosophilids has not yet been demonstrated because of the apparently narrow host range of the *D. melanogaster P* element (O'Brochta & Handler, 1988; O'Brochta *et al.*, 1991).

P elements are typically present in multiple copies per genome and are members of the Class II transposable elements that transpose by means of a DNA intermediate (see Engels (1989) for a review). An 8-bp duplication of host DNA is generated at the site of insertion. In most *D. melanogaster* natural populations, a minority of *P* elements are autonomous elements, and a majority are internally deleted, nonautonomous, elements. Autonomous *P* elements are 2.9 kb in length and have 31 bp perfect, inverted, terminal, repeats (O'Hare & Rubin, 1983) and 11 bp perfect, inverted, subterminal, repeats. They have four open reading frames, all of which are required to encode a transposase enzyme. Defective *P* elements are generally smaller and variable in size and are derived from complete elements by internal deletions. The induction of these deletions is considered to be associated with active transposition of *P* elements.

Although the *P* element appears to have entered the genome of *D. melanogaster* only very recently (Kidwell, 1983; Anxolabéhère *et al.*, 1988a; Daniels *et al.*, 1990), there is abundant evidence that members of this transposable element family have been present in other species for millions of years. Their distribution in the genus *Drosophila* is notably patchy, but *P* elements have been found in most species of the subgenus *Sophophora* to which *D. melanogaster* belongs (Clark & Kidwell, 1997; Daniels *et al.*, 1990; Lansman *et al.* 1985). Outside *Sophophora*, evidence for *P* elements has been reported in *Drosophila mediopunctata* (Loreto *et al.*, 1998), in *Drosophila andalusciana*, a species in the *Lordiphosa* subgenus (Anxolabéhère *et al.*, 1988b) and in three species of the immigrans radiation (Anxolabéhère *et al.*, 1988b). Outside the genus *Drosophila*, they have been found in the drosophilid *Scaptomyza pallida* (Anxolabéhère *et al.*, 1985; Simonelig & Anxolabehere, 1991; Simonelig & Anxolabéhère, 1994) and in the calliphorid *Lucilia cuprina*, the sheep blow fly (Perkins & Howells, 1992).

We report here evidence for the presence of multiple *P* elements in the genome of the house fly *Musca domestica*, together with the nucleotide sequence of one of these elements. This element has apparently been inactive for

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some time and, as expected from the species phylogeny, it is more closely related in both structure and sequence to the *P* element from *L. cuprina* than it is to that of *D. melanogaster*.

Results

P element-homologous sequences were initially detected in *M. domestica* using the polymerase chain reaction (PCR). Degenerate oligonucleotide primers, based on *P* element sequences from six species of *Drosophila*, were used in amplification reactions with *M. domestica* DNA as a template. Primers 2684 (which hybridizes to *D. melanogaster P* element positions 703–725) and 2687 (which hybridizes to *D. melanogaster P* element positions 1509–1531) yielded a distinct fragment, approximately 1000 bp in length, whereas the corresponding *P* element region amplified from *Drosophila* is about 750 bp (Fig. 1).

In order to investigate the source of the length difference identified, the house fly PCR fragment was cloned and sequenced. Within the region amplified by PCR, which includes portions of exons 1 and 2 and the intron between them, this sequence showed 59% similarity to a comparable region of the *P* element from *D. melanogaster*. However, the amplified sequence from the house fly differed in structure from the comparable region of drosophilid *P* elements in two respects. First, the fragment amplified from the house fly has an unusually long intron, separating exons 1 and 2, compared with that of *D. melanogaster*. This intron is 250 bp in *M. domestica* and only 53 bp in *D. melanogaster*. Second, a short intron (49 bp), not found in drosophilid sequences, interrupts exon 2 of the house fly sequence. (A second intron was subsequently discovered; see below.) These features account for most of the difference in size apparent in the respective PCR products.

The cloned PCR fragment was used as a probe to screen a genomic DNA library from *M. domestica* and numerous positive plaques were identified. However, since an amplified library was used it was difficult to estimate the copy number for this sequence in the *M. domestica* genome (see below). Ten positive plaques were purified and mapped using restriction enzymes. The maps of the *P*-specific regions were identical for all clones and DNA from one clone, named Md-P1, was chosen for sequencing. Figure 2 shows a schematic representation of *P* element sequences from *D. melanogaster*, the blow fly *Lucilia*

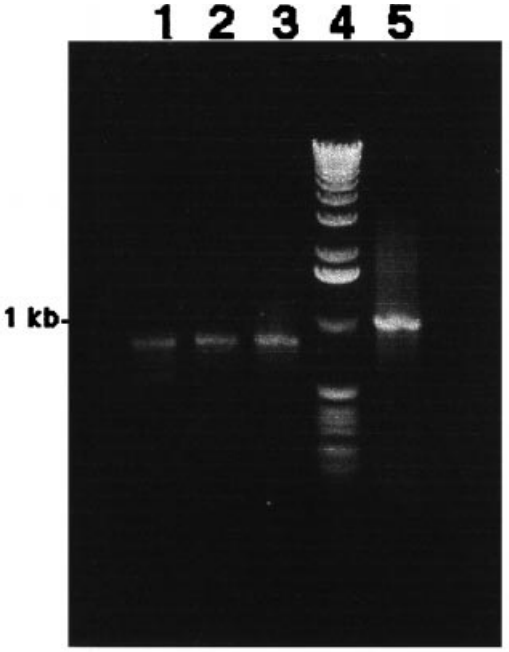

Figure 1. PCR amplification of *P* element sequences. Degenerate primers 2684 and 2687 were used in separate reactions with genomic DNA from *D. melanogaster* (lane 1), *D. simulans* (lane 2), *D. willistoni* (lane 3) and *M. domestica* (lane 5). The size marker (lane 4) is a 1-kb ladder.

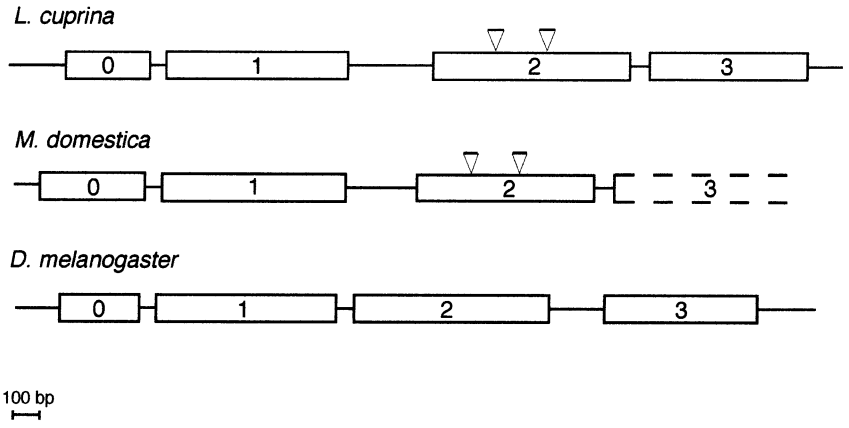

Figure 2. Schematic representation of the structures of the *P* element from *M. domestica* and those from *L. cuprina* and *D. melanogaster*. Additional introns present in exon 2 of *M. domestica* and *L. cuprina* are shown as triangular insertions. The dashed exon 3 of *M. domestica* indicates that obvious *P* element homology ends just after the beginning of this exon.

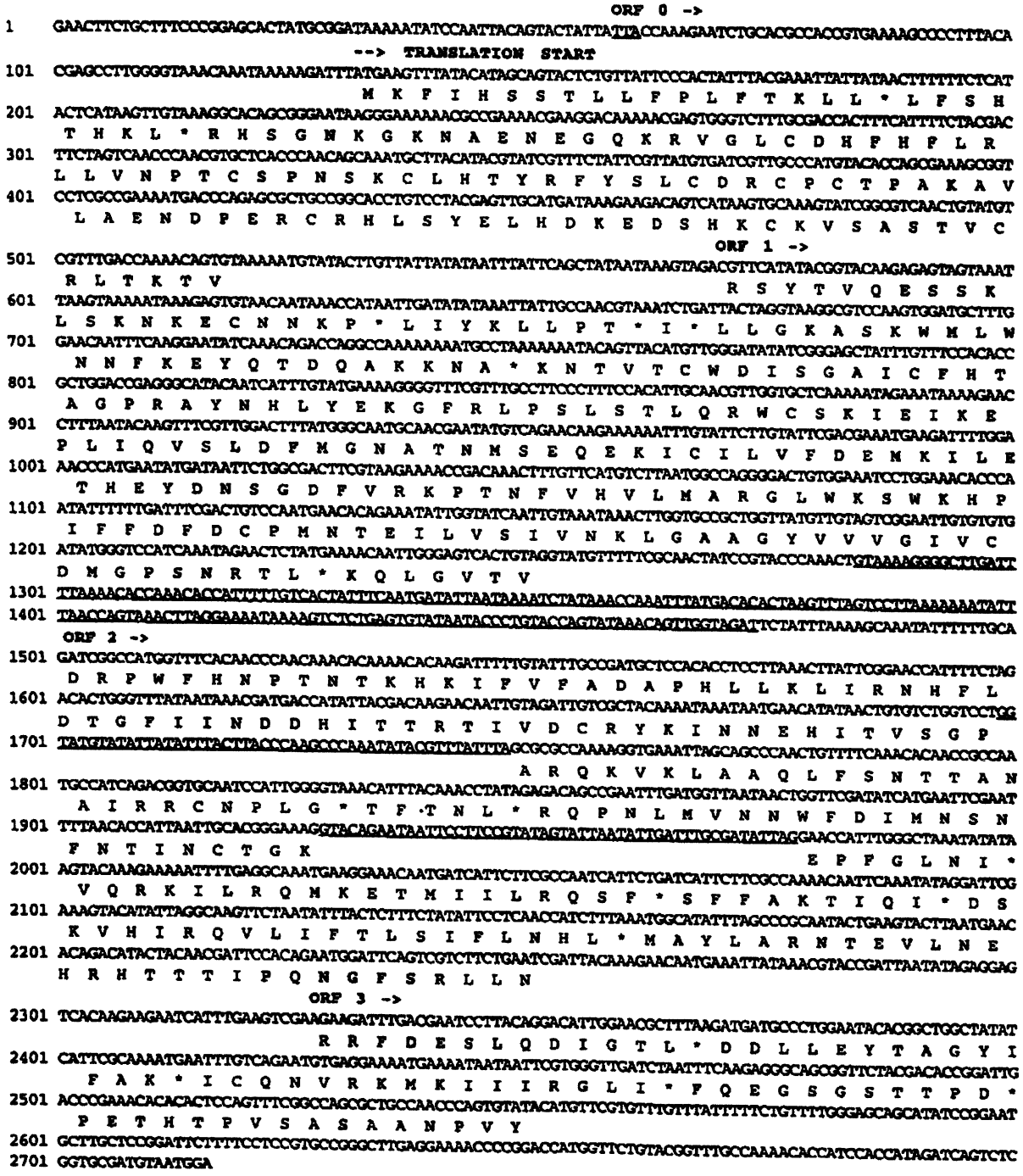

Figure 3. Nucleotide and derived amino acid sequences for the *M. domestica P* element. The underlined sequences identify the additional sequences present in the intron between exons 1 and 2 and the extra two introns present within exon 2 in the house fly. The TAT (tyrosine [Y]) codon that ends the putative exon 3 reading frame corresponds to the termination codons found at this position in *D. melanogaster* and *L. cuprina*. The precise ends of Md-P1 remain undefined.

cuprina, and from *M. domestica*. Figure 3 gives the nucleotide and derived amino acid sequences for the *M. domestica P* element and Fig. 4 shows the amino acid sequence alignments for exons 1 and 2 of *P* elements from *M. domestica*, *L. cuprina* and *D. melanogaster*.

As is the case with the Lu-P1 element from *L. cuprina*, the ends of Md-P1 are not well-defined in *M. domestica*. An additional 661 bp of this clone were sequenced in an attempt to find the inverted repeats. However, no candidate sequences were identified. Since Md-P1 is so degenerate

Figure 4. Amino acid sequence alignments for conserved regions of exons 1 and 2 of *P* elements from *M. domestica*, *L. cuprina* and *D. melanogaster*. Dashes indicate gaps introduced to optimize the alignment, and dots indicate amino acid residues shared with the *D. melanogaster* sequence. Standard amino acid abbreviations are used with stop codons indicated by asterisks.

in sequence and structure, this is not surprising. The inverted repeats of Lu-P1 from *L. cuprina* are also elusive. Thus, no good evidence for the presence of *P* element inverted repeat termini has been found in either species. In contrast, most drosophilid *P* element coding regions are flanked by short inverted repeat sequences. However, these sequences are not particularly well conserved when *P* element sequences from different *Drosophila* species are compared.

As for Lu-P1, strong sequence similarity to the *P* element from *D. melanogaster* begins only at the end of exon 0 in *M. domestica*. However, it is possible to identify a start codon (ATG) in Md-P1 that fixes a reading frame that corresponds in length to exon 0 in *Drosophila* and *L. cuprina* Lu-P1. Over the last 40 bp of this putative exon from Md-P1 similarity increases considerably. Both the consensus 5′- and 3′-splice sites of the first intron are preserved in *M. domestica* and *L. cuprina* and there is relatively strong similarity of the intron sequences (52% identity) and lengths (51 and 53 bp, respectively) of *P* elements from these two species.

P elements from the house fly and blow fly share a number of features that distinguish them from drosophilid *P* element sequences. These include a relatively large intron separating exons 1 and 2, and two extra introns interrupting exon 2. These introns are in the exact same positions in both species, indicating a common evolutionary origin. However, while obvious *P* element sequence similarity extends through exon 3 to the end of the *P* element from *L. cuprina*, sequence similarity to the *P* element of *M. domestica* does not extend beyond the beginning of exon 3. The sequence of the 3′ end of Md-P1 remains ambiguous. Alignments using various computer programs resulted in sequence similarities of less than 50% when compared both to the Lu-P1 sequence from *L. cuprina* and various drosophilid sequences. Reasonable sequence similarity between the house fly, blow fly, and *Drosophila* sequences extends for the first 74 codons (222 bp) only. The sequence similarity picks up again only at the end of the *P* element, which represents 160 bp of noncoding sequence. Thus it appears as if exon 3 of Md-P1 has a deletion corresponding to 112 codons (336 bp). However, since the alignment in this region is uncertain, it remains a possibility that the house fly *P* element sequence has

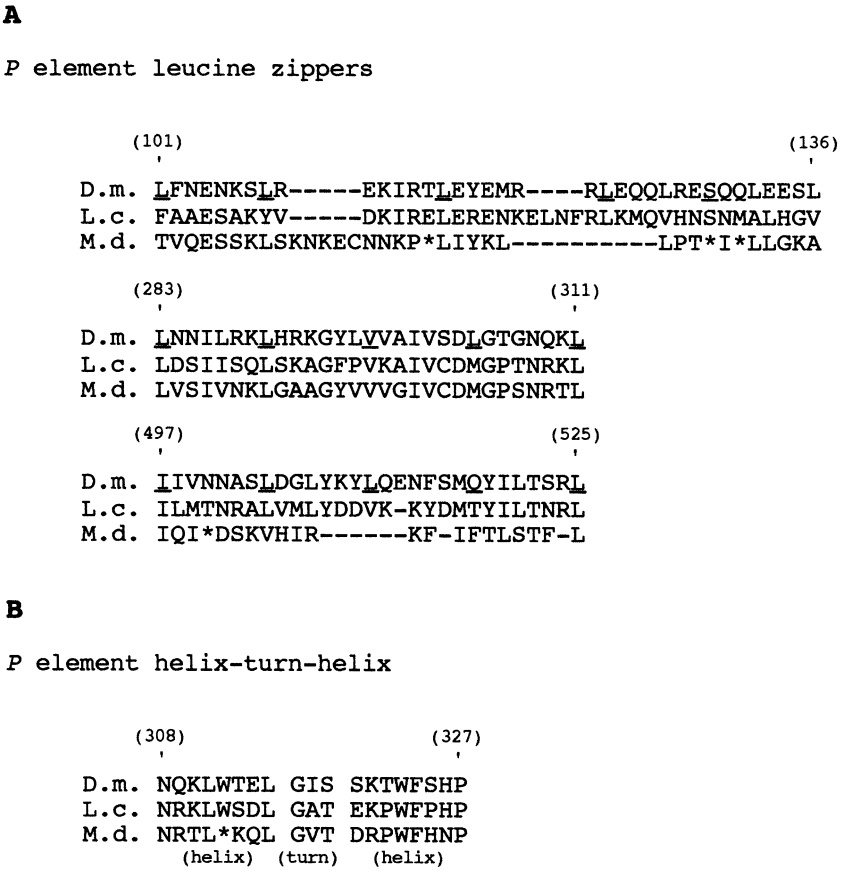

Figure 5. Amino acid sequence alignments of *P* element sequence motifs in *D. melanogaster*, *L. cuprina* and *M. domestica*. A. *P* element leucine zipper motifs. B. *P* element helix-turn-helix. Numbers in parentheses identify the nucleotide sequence position in the canonical *P* element from *D. melanogaster*. The underlined residues identify the periodic leucine, or other hydrophobic amino acids.

simply diverged to a much greater extent than exons 1 and 2 when compared to sequences from *Drosophila* and the blow fly.

The *P* element sequence from *M. domestica* has lost its coding capacity. The reading frame is interrupted by numerous indels and nonsense mutations. Furthermore, conserved sequences found in the *P* element sequence from *D. melanogaster* are divergent in the house fly sequence. Three leucine zipper motifs have been identified in the canonical *P* element from *D. melanogaster* (Rio, 1990). The sequence alignment of the first, located between positions 101 and 136 in the *P* element from *D. melanogaster*, is characterized by a considerable number of gaps and conserves only two of six critical hydrophobic residues in Md-P1 (Fig. 5A). In the Lu-P1 sequence from *L. cuprina*, four of six critical residues are conserved in this region. The second leucine zipper, located between *D. melanogaster* positions 283 and 311 (Fig. 5A), reveals complete conservation of five critical hydrophobic residues in both *M. domestica* and Lu-P1. In the third leucine zipper (*D. melanogaster* positions 497–525) four of five residues are conserved in both *M. domestica* and Lu-P1. However, there are deletions corresponding to a total of eight amino acid residues in the *M. domestica* sequence (Fig. 5A). The leucine zipper sequence in *D. melanogaster* is thought to be involved in dimerization of the transposase protein and DNA binding (Rio *et al.*, 1986). Also involved in DNA binding is a helix-turn-helix motif that lies between *D. melanogaster* positions 308 and 327. This sequence motif is clearly recognizable in *P* elements from both *L. cuprina* and *M. domestica*, although there are numerous substitutions, including a nonsense mutation, in the Md-P1 reading frame (Fig. 5B).

A *P* element PCR fragment isolated from *M. domestica* was used as a probe in Southern blots of genomic DNA isolated from various strains of house flies. As seen in Fig. 6, the restriction pattern is static for all strains but one. This indicates that this sequence has been inactive for a considerable length of time, a conclusion consistent with the sequence analysis above. The exception is strain Old Rutgers (R^w), which does show some variation from the static pattern observed for the other strains. It was subsequently learned that the identity of this strain is in question so work to continue investigation of this strain was not possible. The Southern analysis also permits an estimate of at least four copies of this element in the genome of *M. domestica* (Fig. 6). This compares with a single copy of the Lu-P1 sequence in the genome of *L. cuprina* and 5–10 copies of Lu-P2, another partially characterized clone (Perkins & Howells, 1992).

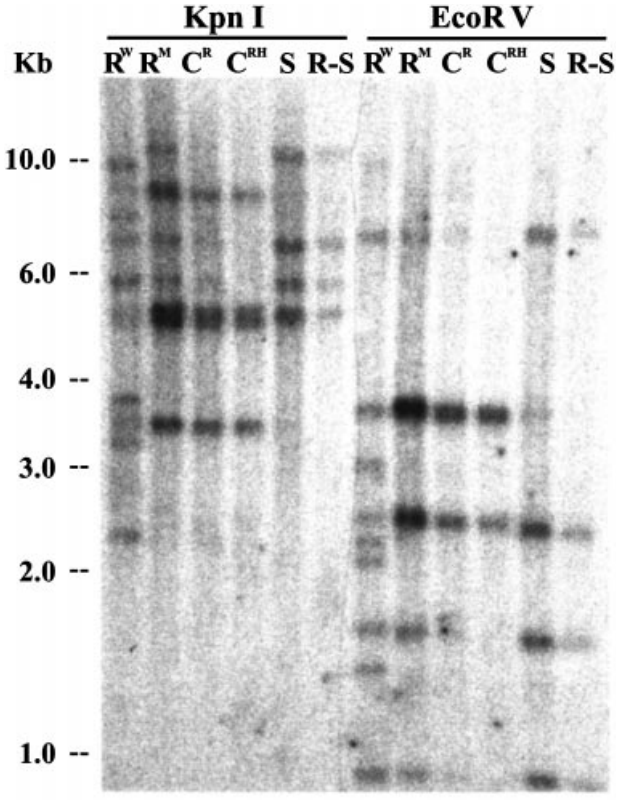

Figure 6. Southern blot analysis of genomic DNA from strains of *M. domestica* probed with the 1 kb PCR fragment generated from *M. domestica*. In lanes (1) through (6), DNA was digested with *Kpn*I. In lanes (7) through (12), DNA was restricted with *Eco* RV. Lanes: (1 and 7) Old Rutgers (2 and 8) New Rutgers (3 and 9) Cornell-R (4 and 10) Cornell-R-H (5 and 11) sbo (6 and 12) Hybrid RxS.

Discussion

Following the earlier description of *P* elements in the blow fly, *L. cuprina* (Perkins & Howells, 1992) this report represents only the second instance of *P* element sequence identification outside of the family Drosophilidae. In neither species is there clear evidence for recent activity of *P* elements in these genomes. This is not surprising because, to date, complete sequences have been obtained from only six species in the genus *Drosophila*: *D. melanogaster* (O'Hare & Rubin, 1983), *D. willistoni* (Daniels *et al*., 1990), *D. nebulosa* (Lansman *et al*., 1987), *D. subobscura* (Paricio *et al*., 1991), *D. guanche* (Miller *et al*., 1995), and *D. bifasciata* (Hagemann *et al*., 1992) and from a single species in the drosophilid genus *Scaptomyza, S. pallida* (Simonelig & Anxolabehere, 1991). Of these, only those from *D. melanogaster, D. willistoni*, *D. bifasciata* and *S. pallida* are known to be active. The *P* element sequences characterized from *S. subobscura* are missing sequences homologous to exon 3. The remaining regions (exons 0, 1, 2) retain coding capacity and are indeed expressed (Paricio *et al*., 1991). The *P* elements from *D. nebulosa* and *D. guanche* have numerous indels and nonsense mutations (Lansman *et al*., 1987; Miller *et al*., 1995). *P* element homology ends at the beginning of exon 3 of *D. guanche*, a situation similar to that reported here for the house fly *P* element.

Similar to Lu-P1 from *L. cuprina*, homology to drosophilid *P* element sequences does not include exon 0 in Md-P1 from *M. domestica.* The overall picture is one of reasonable conservation in the central portion of the *P* elements comprising exons 1 and 2, and considerable divergence in exons 0 and 3. It is unclear why the central portion of the *P* element has been more conserved over evolutionary time than regions towards the ends, or why the pattern of conservation differs for different species. One explanation for differing degrees of sequence conservation is that some *P* elements may have acquired additional or new functions in certain genomes. For example, in *D. melanogaster*, internally deleted *P* elements serve as a repressor of transposition and it is possible that the repressor function may be maintained for a period after the transposition function has been lost (Paricio *et al*., 1991). Other authors have suggested that the insertion of TEs may provide novel *cis*-regulatory regions to preexisting host genes or that TE-derived *trans*-acting factors may undergo a molecular transition into novel host genes through a process described as molecular domestication (Miller *et al*., 1997).

On the basis of overall structure (Fig. 2) and the amino acid sequence alignment (Fig. 4), the house fly Md-P1 sequence is clearly more closely related in structure to Lu-P1 of the blow fly than it is to drosophilid *P* elements. Most striking in Md-P1 and Lu-P1 is the larger intron separating exons 1 and 2, and the presence of two small introns within exon 2. The similarities between the house fly and blow fly sequences most likely reflect their relatively close phylogenetic relationship rather than independent evolution of these features.

A depiction of the phylogeny of the main *P* element-bearing taxa is presented in Fig. 7. This provides some idea of the range of the distribution of known *P* elements within the Diptera. The house fly and blow fly belong to the families Muscidae and Calliphoridae, respectively. Drosophilidae, Muscidae and Calliphoridae all belong to the dipteran suborder Brachycera and within this suborder the division Schizophora. However, the family Drosophilidae belongs to the section Acalyptratae, while Muscidae and Calliphoridae belong to the section Calyptratae (McAlpine, 1989). Within this section, Muscidae and Calliphoridae fall into distinct superfamilies. Although sequences have not been determined, *P* elements have been detected by hybridization in two other families of flies, Opomizydae and Trixoscelididae, both closely related to the family Drosophilidae (Anxolabéhère & Périquet, 1987). The present identification of the *P* element in another dipteran family (Muscidae) suggests that

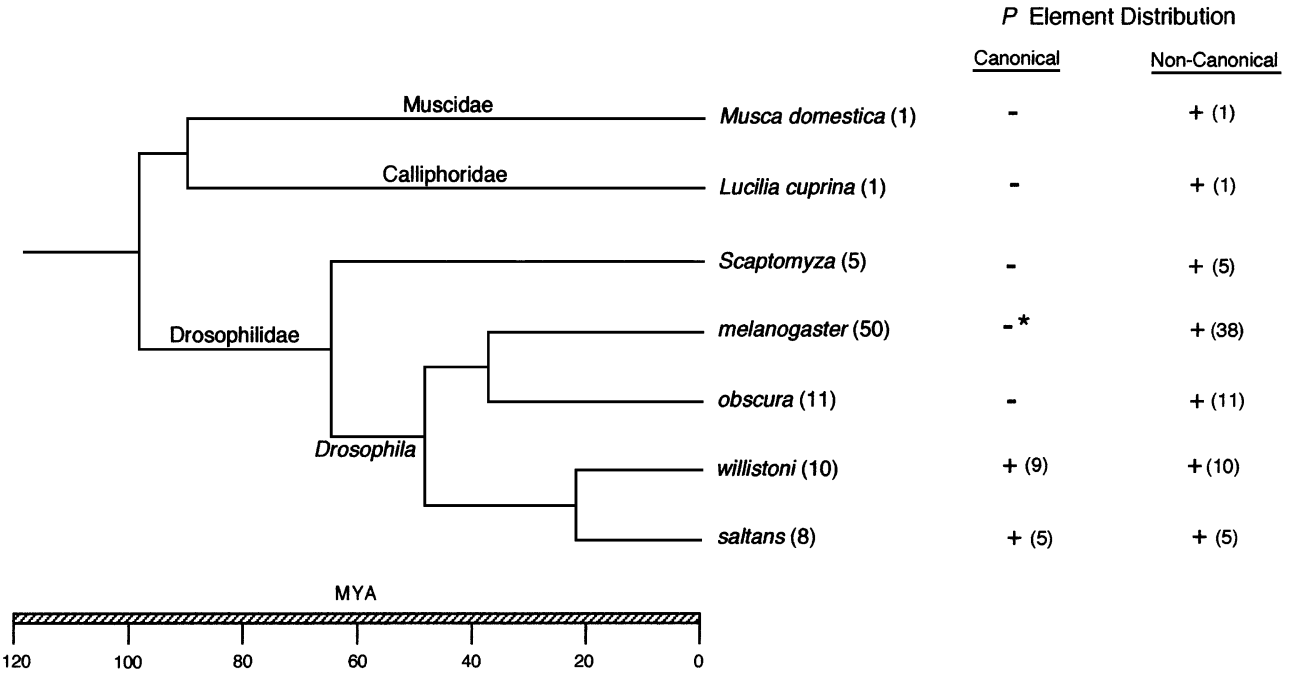

Figure 7. Phylogeny of selected species in the order Diptera that carry *P* element sequences. The phylogeny is based on information in McAlpine (1989) and shows approximate divergence times. The number of species examined for each terminal group is shown in parentheses. *P* element distribution identifies canonical and noncanonical *P* elements. The term canonical refers to those *P* elements that are similar to the active *P* element from *D. melanogaster*; noncanonical elements are all other sequences (see Clark *et al.*, 1995). Numbers in each column represent the total number of the species examined that possess *P* elements of one kind or another. The four *Drosophila* lineages refer to the four principle species groups in the subgenus *Sophophora*. Within each species group, multiple species (as shown in parentheses) were examined. The asterisk denotes the canonical *P* element that was transferred horizontally from *D. willistoni* (*willistoni* species group) to *D. melanogaster* (*melanogaster* species group).

this TE may be more widely distributed than previously thought. The use of additional PCR primers, designed to reflect the diversity of known sequences, may extend this distribution to other flies and perhaps other insects.

With the early success of *P* element germline transformation in *D. melanogaster* (Spradling & Rubin, 1982) and the development of efficient *P* element-based gene vectors (Steller & Pirrotta, 1985), there was initially considerable optimism that this element could be used as the basis of a generalized gene transformation system in a wide variety of insects. The development of *P* vectors carrying dominant selectable markers (Steller & Pirrotta, 1985) allowed this notion to be tested. In contrast to positive results in a number of Drosophilid species, negative results were obtained from several nondrosophilid species (Handler & O'Brochta, 1991). Furthermore, O'Brochta & Handler (1988) and O'Brochta *et al.* (1991) adapted an excision assay developed by Rio *et al.* (1986) to assess *P* functionality in the soma of both drosophilids and nondrosophilids. They found that although *P* elements could be mobilized in all the drosophilids tested, the *P* excision frequency decreased as a function of relatedness to *D. melanogaster* and *P* element mobility was not detected in tephritids, sphaerocerids, muscids or phorids (Handler & O'Brochta, 1991).

In light of the present results demonstrating that members of the *P* element family were active at some time in the past in *M. domestica*, it is interesting to speculate on the nature of the possible reason for the failure of previous assays to detect *P* element movement in muscids. A number of possible reasons for the failure of *P* elements to be mobilized in nondrosophilids have been advanced (Handler & O'Brochta, 1991), including the absence of requisite host-encoded cofactors and the existence of repressors of transposition in the genomes of these species. However, in species such as *L. cuprina*, in which *P* elements were earlier reported (Perkins & Howells, 1992), and now *M. domestica*, knowledge that the ancestral genomes of these species clearly supported *P* element movement in the past, should make it possible to narrow down the options for present day failure, particularly when the sequences are available.

The results of phylogenetic analyses of *P* elements in *Sophophora* (Clark *et al.*, 1995, 1998; Clark & Kidwell, 1997) have provided evidence for the existence of multiple *P* element subfamilies in single species lineages that apparently must have entered the genome at different times during the past. This strongly suggests that *P* element-encoded repressors have not been successful in preventing repeated introductions of new *P* element subfamilies

in these lineages. Such evidence provides another reason for confidence that overcoming the barriers to movement may not be as difficult as might have been thought. Despite several recent successes in developing new vector systems based on transposable elements other than *P* (e.g. Coates *et al*., 1998; Loukeris *et al*., 1995a, b; Jasinskiene *et al*., 1998), it may be necessary to develop an array of transformation systems with varying properties for use in different insects and under different conditions.

Experimental procedures

House fly strains

The five *M. domestica* strains used are listed in Table 1. They were kindly supplied by F. W. Plapp Jr, Department of Entomology, University of Arizona.

Primer design/PCR

Genomic DNA was prepared from wild-type *M. domestica* larvae as described (Cockburn & Seawright, 1988) and used as a template in PCR amplification. Degenerate primers (2684:GCTATTTGNY-TNCAYACCGCNGG, 2687:CCCAATGNATWGCANCGTCTKAT) were designed to correspond to two regions of the most conserved nucleic acid sequences located between nucleotides 703 (exon 1) and 1530 (exon 2) of the *P* elements. Primer 2684 is 256-fold degenerate; primer 2687 is 64-fold degenerate. Amplification reactions were carried out in 50 µL volumes, with 100 ng template DNA and 0.25 units of *Taq* polymerase (Gibco-BRL, Gaithersburg, MD). The reaction conditions were template denaturation for 1 min, 94 °C; primer annealing for 1 min, 50 °C; and primer extension for 1 min, 72 °C (with 2 s. added for each cycle), for a total of thirty cycles.

Southern hybridization

Genomic DNA was extracted from ten adult flies of each strain and 10 µg DNA was used for restriction digestion. Gel electrophoresis, Southern blotting and filter hybridizations were performed as described in (Sambrook *et al*., 1989). The filters were probed with the 1 kb PCR fragment generated from *M. domestica.* Filters were probed and washed under conditions of high stringency (65 °C, 0.1 × SSC final wash).

Library screening

A genomic DNA library of the *M. domestica* R-Diazinon (Rutgers) strain was kindly supplied by Rene Feyereisen. 150 000 plaques from the genomic library were screened by standard protocols (Sambrook *et al*., 1989) using the PCR fragment as a probe under stringent hybridization conditions.

Cloning and sequencing

Lambda DNA was isolated by the high yield method for isolation of lambda DNA (Lee & Clark, 1997). After mapping, the DNA fragment that was selected by Southern hybridization was subcloned into pBluscript vectors (Stratagene) by standard ligation and transformation techniques, using *E. coli* host strain DH5-alpha. The *P* element DNA sequence was obtained from both strands using both manual and automated sequencing techniques (the latter employed an ABI 377 automated DNA sequencer in the Laboratory of Molecular Systematics and Evolution, University of Arizona).

Sequence alignment

The conserved portion of the *P* element was aligned by eye. The more divergent ends of the *P* element sequence were aligned with the aid of computer programs Clustal W (Thompson *et al*., 1994) and GeneStream <http://eerie.fr/bin/align-guess.cgi>. Discrepancies between the programs were resolved by eye to maximize sequence similarity. The sequence of the *P* element from *M. domestica* has been submitted to GenBank (accession number AF 183396).

Acknowledgements

We thank Rene Feyereisen for supplying the *M. domestica* genomic DNA library and F. W. Plapp Jr for supplying

Table 1. Description of *Musca domestica* strains used.

Name of strain	Abbreviation	Mutants	Origin	Description
Old Rutgers	R^W	Wild-type	Unknown	Has metabolic resistance to insecticides
New Rutgers	R^M	Wild-type	From New Jersey dairy barns in the early 1960s	Has metabolic resistance to insecticides
Cornell-R	C^R	Wild-type	Mid 1960s, NY state poultry houses	Combines metabolic and target site resistance to organophosphate insecticide
Cornell-R-H	C^{RH}	Wild-type	Mid 1960s, NY state poultry houses	Unknown
sbo	S	Stubby wing, brown body, ochre eye, visible recessive mutations on chromosomes 2, 3 and 5, respeitively	Unknown	
Hybrid R × S	R-S		Cross between DDT-R (resistant to cyclodienes and DDT) and sbo	

M. domestica strains. We are indebted to Jake Tu, Becky Wattam, Joana Silva and three anonymous reviewers for comments on the manuscript. This work was supported by a postdoctoral fellowship to J.B.C. and a grant from the John D. and Catherine T. MacArthur Foundation for the sport of research on vector biology at the University of Arizona.

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