Selective Constraints on P-Element Evolution

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P elements, like mariners, inhabit eukaryotic genomes and transpose via a DNA intermediate. Mutant and wild-type elements in the same genome should be transposed with equal probability by trans-acting transposase, and so no selection should counteract the accumulation of inactivating mutations in transposase genes. Thus, copies of mariner elements diverge within a host species under no selection (Robertson and Lampe 1995). It is unknown whether or not this pattern holds for P elements, which are unrelated to mariner elements but share the same life history. Publicly available P-element sequences were analyzed for evidence of conservative selection for the function of P-element–encoded proteins. Results were compared to predictions derived from several hypotheses that could explain selection, or the lack of it. P-element protein–coding sequences do evolve under conservative selection but apparently because of more than one selective force. Of the four exons in the P-element transposase, the first three (exons 0, 1, and 2) can be translated alone into a repressor of transposition, while the last (exon 3) is only expressed as part of the full-length transposase and probably serves a transposition-specific role. As full-length P-element copies diverge from each other within a host population, selection maintains exons 0–2 but apparently not exon 3. The selection acting on exons 0–2 may act at the host level for repression of transposition (since host level selection does act on orthologous truncated elements that contain only exons 0–2). Evidence of selection on exon 3 is only found in comparisons of more diverged elements from different species, suggesting that selection for transposition acts primarily at horizontal transfer events. Thus, horizontal transfer events may be the sole source of the selection that is crucial to the maintenance of autonomous P elements in the face of mutation (as suggested by Robertson and Lampe 1995). The predictions derived here suggest a strategy for collecting sequence data that could definitively answer these questions.

Introduction

Transposable elements are complex, adapted entities created and maintained by natural selection, but the precise ways in which selection acts on them are not well understood. In the case of elements that transpose via a DNA intermediate (“cut and paste” transposition) in eukaryotic hosts, the transposase protein encoded by wild-type (autonomous) elements is presumably free to act in trans on mutant (nonautonomous) elements residing in the same genome. Therefore, wild-type and mutant elements in the same genome transpose at the same rate and, unless they are lost at different rates, mutants will increase in frequency because of mutation. It is not obvious what selective advantage wild-type elements can have under these circumstances, and in fact, the transposase genes of mariner elements from within a host species diverge under no selection (Robertson and Lampe 1995; Robertson and Martos 1997). Yet clearly some selective force must frequently favor elements that carry a functional transposase gene. Many of the possible solutions to this paradox can best be tested by analyzing the sequence evolution of orthologous and paralogous transposons from the same and different species.

P elements are a useful case, since they are among the best-characterized cut and paste transposons of eukaryotes but are unrelated to mariners. Their history within the genus Drosophila and related flies has yielded a range of cases, from recent horizontal transfer events with little or no divergence of elements within a host species, to cases in which elements within a species have diverged moderately, up to cases showing highly diverged elements in the same and in different species (Clark, Kim, and Kidwell 1998). Although several studies have suggested that some P-element sequences have evolved under selection, none of the claims known to me has been tested for statistical significance, some are not supported by the accompanying data, and the mechanism by which the proposed selection acted is frequently not specified. My intent has been to determine in what contexts selection on P-element gene sequence has and has not acted and to interpret those results in terms of the available explanations.

Hypotheses

I consider these hypothesized mechanisms of selection: (1) transposition itself may increase the host organism’s fitness (perhaps by enhancing evolutionary plasticity; Wilke and Adams 1992; Naas et al. 1995); (2) the products encoded by an element may enhance host fitness directly, not through transposition (see Sheen and Levis [1994] and Witherspoon et al. [1997] for possible examples); (3) wild-type elements may be transposed preferentially over mutant elements in the same genome (transposase may function preferentially in cis, as with some bacterial elements; Kleckner 1990); (4) wild-type elements may transpose more frequently than mutants, due to assortment across the host population (see below); (5) wild-type elements, but not mutants, are capable of infecting new host populations of the same or different species (horizontal transfer; Robertson and Lampe 1995).

Hypothesis (4) requires further explanation, as this is the first time this effect has been made explicit. It will
be developed fully elsewhere but can be intuitively understood as follows. If the numbers of wild-type and mutant elements in individual host genomes vary independently across the host population and if the number of transposition events per host genome increases with the number of wild-type elements in that genome, then more transposition events will occur in genomes with a higher proportion of wild-type elements, and more of those transpositions will be of wild-type elements. Thus, across the host population, wild-type elements will transpose more frequently than mutant elements, resulting in selection for functional transposase even in the absence of preferential cis action of transposase. This selection will be strongest when the average number of elements per host genome is small, as the effect of an individual transposon on the composition of the elements in a genome decreases rapidly as the number of elements per genome increases. This effect, a form of “trait group selection” (Wilson 1975), is implicit in the model of Kaplan, Darden, and Langley (1985).

Predictions

These hypotheses are not mutually exclusive, and their applicability depends on parameters that are difficult to measure, such as fitness of hosts, transposition rates, cis preference of transposase, and frequency of horizontal transfers. However, analyses of sequence evolution can quickly distinguish between subsets of these hypotheses, using predictions based on the following two questions. First, has the divergence of two transposons from their most recent common ancestor (MRCA) occurred under selection for the function of the encoded transposase? Second, have the elements undergone transposition during that divergence? The first question can be answered by comparing transposase genes. As for the second, if the sequences are orthologous, they have not undergone transposition during divergence from their MRCA (though they may have given rise to copies at other loci by transposition); if they are paralogous, they probably have.

Hypotheses (1) and (2) are “host benefit” hypotheses (a type of selection at the organismal level, according to Hickey’s [1992] distinction). Under these hypotheses, wild-type elements increase in frequency by increasing the fitness of the hosts that carry them (relative to the effects of mutants). If either hypothesis is true, evidence of selection will be seen in comparisons of any sufficiently diverged elements, regardless of whether they have undergone transposition during divergence from their MRCA.

In contrast, under hypotheses (3), (4), and (5), host fitness may be unaffected by the coding capacity of transposons; instead, selection is a result of differential success in transposition (selection at the molecular level; Hickey 1992). Under these hypotheses, selection can act only when an element transposes; they do not predict that evidence of selection will be found in comparisons of elements that have diverged from their MRCA without transposing (orthologs). Thus, if (1) and (2) are false, no selection will be detected in such comparisons. If either (3) or (4) is correct, we should find evidence of selection in comparisons of elements from the same population that have undergone transposition during divergence from their MRCA (most paralogs). If the sole source of selection is due to horizontal transfers (hypothesis 5), evidence of selection will be found only in comparisons of elements from different host populations that were infected by horizontal transfers.

Different parts of the P-element transposase gene have different functions and may be subject to different selective forces. The P-element transposase gene has four exons (0 through 3), but in Drosophila somatic cells, the third intron is not spliced, and translation ends at a stop codon within that intron (Siebel and Rio 1990). The resulting truncated protein represses transposition (Misra and Rio 1990). Production of this repressor in germine cells is also important in establishing “P cytotype” (maternally inherited repression of transposition; Roche, Schiff, and Rio 1995). Thus, while exons 0–2 serve both to catalyze and repress transposition, exon 3 may have a purely transposition-specific function. If so, then we expect exons 0–2 to show the patterns of selection predicted by host benefit hypotheses (1) and (2), since repression of transposition (in the soma or the germline) directly benefits the host (Woodruff 1992; presumably, regulated transposition indirectly benefits P elements as well). Exon 3, on the other hand, should not be subject to this selection, and the patterns of selection on exon 3 could be used to distinguish between hypotheses (3), (4), and (5). I have therefore analyzed exons 0–2 and exon 3 separately.

Materials and Methods

P-element nucleotide sequences were identified in GenBank (March 1998) using TBLASTN (Altschul et al. 1990), with the peptide sequence of the Drosophila melanogaster P-element transposase (accession number X06779). Short sequences (ones that do not include all of exons 0, 1, and 2 and/or exon 3) were not used, with two exceptions (see fig. 1). Sequences too similar to others to be useful were eliminated (the most similar pair included differ at 9 of 612 sites compared in exon 3). The sequences were aligned using CLUSTAL W (Thompson, Higgins, and Gibson 1994) with default settings.

To detect evidence of selection, the divergences of these sequences from each other were analyzed as follows. The program CODEML (in PAML, version 1.4; Yang 1997) was used to estimate \( \delta_d/\delta_s \) (the ratio of per-site rates of synonymous and nonsynonymous changes) in pairwise comparisons, with the transition to transversion rate ratio set to 2.0, on the basis of the results of Moriyama and Powell (1996). Removing the effect of this mutational bias yields estimates of \( \delta_d/\delta_s \) nearer to unity, since transversions are more likely to be nonsynonymous than transitions. Frequencies of codons (for estimating codon transition probabilities) were inferred from nucleotide frequencies at first, second, and third codon positions; use of actual codon frequencies does not significantly alter the results (unpublished). Codons of an alignment that involve a gap in one or more se-
Fig. 1.—(A) Phylogeny and maps of 16 aligned P-element sequences (scale at upper right). Sequences are labeled with GenBank accession identifiers and abbreviations derived from the host species names (Dkik, Drosophila kikkawai; Ddav, D. daviidi; Dtsa, D. tsacasi; Dsub, D. subobscura; Dmad, D. madeirensis; Dgua, D. guanche; Dtris, D. tristis; Dbif, D. bifaria; Dneb, D. nebulosa; Dmel, D. melanogaster; Spal, Scaptomyza pallida.) Dsub1a and Dsub1b are adjacent sequences repeated in tandem. Alignment: Jagged lines indicate where sequences were truncated to remove nonalignable regions. Dkik and Ddav (318 and 322 bp) were included only for comparison to Dtsa, and the fragment of exon 2 sequence from Dtris in exon 2 was not analyzed. A region in exon 3 (~150 nt, represented by broken lines) could not be well aligned, due to numerous indels and high divergence in the region. Phylogeny: The phylogeny was inferred from coding sequences using maximum likelihood (DNAML in PHYLIP, version 3.5, Felsenstein 1993; a transition to transversion ratio of 1.0 maximized the likelihood of this tree; otherwise, default settings were used). Branch lengths represent numbers of expected changes per site, according to the scale bar. Bootstrap analysis using maximum parsimony (PAUP, Swoford 1991; 100 replicates, sampling variable characters only, branch and bound search) yielded a consensus tree (50% majority rule, compatible groups included) with the same topology. Solid and open squares mark nodes found in at least 99% and 75% of replicates, respectively. Certain clades are labeled (above and to the left of the basal node) for reference. (B) Scatterplot of $p_N$ versus $p_S$ (nonsynonymous or synonymous differences per nonsynonymous or synonymous sites, respectively) for pairwise comparisons among P-element sequences (Nei and Gojobori 1986; Ina et al. 1994). $p_N$ and $p_S$ were used instead of $d_N$ and $d_S$ (the number of changes per site, corrected for multiple changes at a site) to avoid large estimates of $d_S$ caused by saturation of synonymous sites ($p_S > 0.75$). In comparisons of sequences diverging under no selection but with a twofold bias against transversions (as in Drosophila nuclear DNA; Moriyama and Powell 1996), $p_S$ should be $\approx 1.15 \times p_N$ (for moderately diverged sequences, determined by calculation from the genetic code and checked by simulation). Exons 0, 1, and 2 were analyzed together (excluding the small region of the third intron that is translated in members of clades a and e, and as part of the repressor protein in full-length P elements), while exon 3 was analyzed separately. Circles represent comparisons of exon 0–2 sequences, and triangles represent exon 3 comparisons. In comparisons between elements that contain both the exon 0–2 region and exon 3, the two points are connected by a line. Open circles or triangles represent comparisons in which significant evidence of selection was detected ($p < 0.05$, see Materials and Methods), solid symbols represent comparisons in which no significant evidence of selection was found. Clusters of points are marked for reference to the phylogeny in figure 1A. Cluster b–d consists of eight open circles, comparisons between members of clade b and members of clade d; “within b, within d” refers to the seven solid circles, comparisons among members within either clade. Cluster f–Dbif1 consists of five pairs of linked circles and triangles, e–Dtsa consists of six circles (unattached to triangles), e–Dtris consists of the six unattached triangles, and e–c consists of 36 sometimes overlapping circles.
sequences are removed from the entire alignment before analysis; thus, a region of the exon 3 alignment with many indels was mostly excluded. Likelihood ratio tests were applied to each pairwise comparison to determine whether the data were better accounted for with $d_S/d_N$ estimated from the data or fixed at 1.0 (indicating no selection). All significant results ($P < 0.05$) were due to $d_S/d_N > 1$, that is, they showed evidence of conservative selection. No significant results are based on fewer than 10 nonsynonymous and 10 synonymous differences between sequences.

Results and Discussion

Figure 1A lists the sequences used in this analysis and shows a map of their alignment and their inferred phylogenetic relationships. Pairs of sequences were compared, and $p_S$ and $p_N$ (synonymous and nonsynonymous differences per synonymous or nonsynonymous sites) were calculated for each pair; the results are plotted in figure 1B. Comparisons of sequences that have diverged under selection for the conservation of protein-coding function will show a lack of nonsynonymous changes, resulting in points below and to the right of the $p_S = 1.15 \times p_N$ line in figure 1B. Table 1 presents the results of maximum-likelihood analysis (see Materials and Methods) used to detect significant evidence of selection in sequence comparisons. Other studies that have addressed selection on P elements are noted in table 1.

The overarching conclusion from these analyses is that P-element gene sequences have evolved over the long term under selection for conservation of the function of their encoded proteins. To determine which of the five hypothesized mechanisms listed above could be responsible for this pattern, the results must be examined more closely. P elements and clades of elements will be referred to (in boldface) using the labels in figure 1A, and comparisons of sequences will be referred to (also in boldface) according to the clusters of points labeled in figure 1B.

Exon 0–2 Fragments (clades a and c)

The P-element fragments (containing sequences from exon 0 through exon 2) in clade a have diverged from each other under selection (table 1; comparisons...
labeled “within a” in fig. 1B). The $P$-element fragments in clade a are orthologs (Nouaud and Anxolabéhère 1997) and so must have diverged from their MRCA without undergoing transposition. Thus, the selection observed in comparisons of these elements can only be for a host benefit (hypotheses [1] and [2]). Moreover, the common ancestor of these sequences probably lacked exon 3 and the 3′ ITR of a normal $P$ element (since all members of clade a lack them) and so neither could be transposed nor encode transposase. This rules out hypothesis (1) and leaves hypothesis (2): the proteins encoded by these fragments must increase host fitness without causing transposition.

The exon 0–2 fragments in clade c are found in orthologous tandem arrays (Miller et al. 1992; Miller et al. 1995; Paricio et al. 1996). Their arrangement within the arrays is irrelevant for this analysis; the important point is that they have diverged from their MRCA without transposing. As in the previous case, their common ancestor probably lacked exon 3 and the 3′ ITR of a mobile $P$ element, so any selection observed in these comparisons supports hypothesis (2). Comparisons among the closely related members of either clade b or d (“within b, within d” in fig. 1B) do not show significant evidence of selection (table 1), but comparisons between any member of clade b and any member of clade d (b–d) do show significant evidence of selection. This confirms that at least further back in their evolutionary history, these sequences evolved under selection to provide a host benefit.

As suggested by Miller et al. (1992) and Nouaud and Anxolabéhère (1997), the selection in the above cases could be for repression of $P$-element transposition if horizontal transfers of mobile $P$ elements into these species are frequent enough and have dire enough consequences to prevent the loss of the functional repressor gene by mutation and drift. Such horizontal transfers might occur by occasional hybridization with related species that do carry autonomous $P$ elements. Autonomous elements could gain at most a temporary foothold in species defended by a “domesticated” (Miller et al. 1992) repressor gene, so these species would be free of full-length elements most of the time. Although the “domestication” hypothesis explains selection on these new host genes, it does not directly address the question of what selection maintains transposons, since these sequences are no longer transposable elements. Nonetheless, the same selection for host benefit that acts on these immobilized exon 0–2 fragments of $P$ elements could in principle act on exons 0–2 of full-length mobile $P$ elements, explaining the selection observed there (table 1).

Full-Length Paralogous $P$ Elements from Within a Species

Host-level selection for repression of somatic transposition could act on exons 0–2 of mobile $P$ elements, so evidence of selection in comparisons of exons 0–2 in full-length, presumably paralogous $P$ elements could be attributed to that. No orthologous elements that contain exon 3 are available, so we cannot ask whether transposase (and not just the repressor) provides some benefit to the host. Comparisons of exon 3 of paralogous elements from the same species, however, can determine whether selection for transposition acts at all within a host population, whether at the host level (hypotheses [1] and [2]) or the element level (hypotheses [3] and [4]). Selection acting on exon 3 must also act on the linked first three exons.

The accumulated data include three cases in which a pair of exon 3 sequences from within a species could be compared, along with the attached exons 0–2. The pair of elements from S. pallida (Spal1–Spal2) are only slightly diverged from each other (3.1% in exon 3 and 2.2% in exons 0–2), and no significant lack of nonsynonymous changes is detected in exon 3. However, exons 0–2 in the same pair have evolved under selection, suggesting that exons 0–2 evolve under different selective pressures than exon 3. This suggests that the $P$-element transposase gene does not evolve under selection for transposition within a species, so hypotheses (1) through (4) are incorrect in this case, as in the case of mariner (Robertson and Lampe 1995). Analysis of a recently reported sequence (note c, table 1) is weakly consistent with this interpretation.

The negative result for the exon 3 comparison does not conclusively demonstrate that no selection acts on exon 3 within a species. Exon 3 comparisons result in systematically higher $p_N$ values than exon 0–2 comparisons in the same sequence pairs (connected pairs of circles and triangles in fig. 1), suggesting that whatever selection eventually acts on exon 3 tolerates more amino acid changes and thus might not be as detectable as the selection on exons 0–2 in slightly diverged sequences. Furthermore, the shorter sequence of exon 3 reduces the power of the test, so that with the same overall divergence, a higher $d_{SD}$ ratio ($>3.5$) would be required to attain statistical significance.

The two elements from D. bifasciata (Dbif1–Dbif2) are highly diverged from each other, and selection has acted on exon 3 (and exons 0–2) during that divergence. However, it seems likely that they arrived in that species by independent horizontal transfers (Hagemann, Miller, and Pinsker 1994), so the selection observed in exon 3 could be due to repeated horizontal transfers in the history traced by the comparison of these two elements.

Similarly, a pair of elements found in L. cuprina (Perkins and Howells 1992; accession numbers M89990 and M89991) are highly diverged from each other and thus may have invaded their host by different horizontal transfers. Their divergence has occurred under selection on exons 0–2 and exon 3, and that selection may have acted at horizontal transfer events ($p_N = 0.20$ and $p_S = 0.76$, $p_N = 0.31$ and $p_S = 0.74$, respectively; $p_N$ is significantly less than $p_S$ in both cases by z tests). These sequences were not aligned with nor compared to the sequences listed in figure 1A because they are so different from them that alignment is difficult.
The selection evident in comparisons of exon 3 from more diverged P-element pairs is presumably for transposition and may act primarily at horizontal transfers (hypothesis [5]). However, comparisons among the available closely related P-element exon 3 sequences from the same species do not conclusively determine whether or not P elements evolve under selection for transposition within a host population.

**Full-Length P Elements from Different Species**

The recent, successful horizontal transfer of a P element into *D. melanogaster* (apparently from *D. willistoni*; Daniels et al. 1990) selected a functional element from among those available in the donor species. If selection at horizontal transfer events (hypothesis [5]) is the only mechanism by which selection acts on exon 3, then P elements within *D. willistoni* will have radiated without selection on exon 3. Even so, some lineages are expected to escape mutational inactivation for some time by chance alone. Comparisons of exon 3 in randomly chosen elements from *D. willistoni* should show no lack of nonsynonymous changes beyond that expected by chance, but comparisons of those elements with the (nonrandom) element selected by horizontal transfer should.

The comparison of Dmel with Dneb (2.7% different; Dmel–Dneb) captures a single horizontal-transfer event, since *D. nebulosa* and *D. willistoni* are closely related species that have probably inherited their P elements vertically from their common ancestor (Clark et al. 1995). This comparison reveals significant evidence of selection on exons 0–2 and a noticeable (though nonsignificant) lack of nonsynonymous changes in exon 3. (The same caveats applied to the comparison of *S. pallida* elements apply here; in addition, if horizontal-transfer events are frequent relative to the mutation rate, several transfers might be required before evidence of selection would become detectable.) Contrasting the tendency observed in this exon 3 comparison to the negative results of examining exon 3 in the *S. pallida* elements suggests that horizontal transfer is the main mechanism of selection maintaining transpositional competence in P elements. Sequencing of exon 3 in divergent elements from *D. willistoni* and *D. nebulosa* would allow tests of hypotheses (3), (4), and (5).

The remaining comparisons among full-length elements all show clear evidence of selection on exons 0–2 and exon 3 (summarized in table 1). These are comparisons of presumably paralogous elements in different species, with histories that may have involved all the forces of selection that act on these elements. Thus, exon 3 sequences (and linked exon 0–2 sequences) in these comparisons could have been under selection for transposition at horizontal transfers or within populations. At the same time, selection on exon 0–2 sequences may be due in part to host-level selection to repress transposition in germline or somatic cells.

**Conclusions**

Assuming that exon 3 functions only in transposition, the evidence of selection in comparisons of exon 3 from divergent P elements shows that P elements evolve under selection for transposition in the long term, as they must. Analysis of truncated orthologous P-element sequences shows that selection for a host-level benefit (hypothesis [2]) can act on exons 0–2. That selection (or selection for repression of transposition, if the two are different) could also be responsible for the selection detected in all comparisons of exons 0–2 from full-length paralogous elements.

If, as the analysis suggests, exon 3 actually has evolved under no selection in the two full-length *S. pallida* elements, the selection observed on the attached exons 0–2 cannot have been for transposition (since that selection would have acted on exon 3 as well). Thus, exons 0–2 and exon 3 appear to have evolved under different selective regimes. Selection for transposition at horizontal transfer events (hypothesis [5]) therefore remains as the most likely explanation for the selection observed on exon 3 in comparisons of more divergent elements from different species, although hypotheses (3) and (4) cannot be decisively rejected.

Exons 0–2 probably evolve under continual host level selection to repress transposition (hypothesis [2]) as well as the episodic selection for transposition that acts on exon 3. Insofar as repression of transposition and transposition require the same amino acid sequence in exons 0–2, host level selection for repression of transposition will maintain exons 0–2, leaving only exon 3 to be maintained by less frequent or weaker selection at the molecular level (hypotheses [3], [4], and [5]).

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