The recent invasion of natural *Drosophila simulans* populations by the P-element

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The P-element is one of the best understood eukaryotic transposable elements. It invaded *Drosophila melanogaster* populations within a few decades but was thought to be absent from close relatives, including *Drosophila simulans*. Five decades after the spread in *D. melanogaster*, we provide evidence that the P-element has also invaded *D. simulans*. P-elements in *D. simulans* appear to have been acquired recently from *D. melanogaster* probably via a single horizontal transfer event. Expression data indicate that the P-element is processed in the germ line of *D. simulans*, and genomic data show an enrichment of P-element insertions in putative origins of replication, similar to that seen in *D. melanogaster*. This ongoing spread of the P-element in natural populations provides a unique opportunity to understand the dynamics of transposable element spread and the associated piwi-interacting RNAs defense mechanisms.

P-element | transposable elements | *Drosophila simulans* | population genomics | Pool-seq

The P-element, one of the best understood eukaryotic transposable elements (TEs), was originally discovered as the causal factor for a syndrome of abnormal phenotypes in *Drosophila melanogaster*. Crosses in which males derived from newly collected strains were mated with females from long established laboratory stocks produced offspring with spontaneous male recombination, high rates of sterility, and malformed gonads—that is, "hybrid dysgenesis" (1–4). Eventually it was discovered that hybrid dysgenesis was due to the presence of a TE, the P-element (5, 6), which rapidly became the workhorse of *Drosophila* transgenesis (5, 7–9). Surveys of strains collected over 70 y show that the P-element spread rapidly in natural *D. melanogaster* populations, between 1950 and 1990 (10–12), and surveys of other *Drosophila* species revealed that the P-element had been horizontally transferred (HT) from a distantly related species, *Drosophila willistoni* (13). As there could be a considerable lag time between the initial transmission of a TE and its invasion of worldwide populations, it is unclear exactly when the P-element first entered *D. melanogaster*. However, the initial HT event likely occurred somewhere between the spread of *D. melanogaster* populations into the habitat of *D. willistoni*, around 1800 (14), and the onset of the worldwide invasion of *D. melanogaster* populations, around 1950 (10). In any case, the P-element had not been found in close relatives of *D. melanogaster*, including *Drosophila simulans* (13–18). The failure of the P-element to invade *D. simulans* is surprising, as both species are cosmopolitan, are mostly sympatric, and share insertions from many TE families via horizontal transfer (19, 20). Furthermore, when artificially injected, the P-element can transpose in *D. simulans*, albeit at a reduced rate (21, 22).

Results and Discussion

The Recent Invasion of *D. simulans* Populations. Here, we show that the P-element has recently invaded natural *D. simulans* populations. We sequenced *D. simulans* collected from South Africa (in 2012) and from Florida (in 2010) as pools (Pool-seq) (23) and analyzed TE insertions in these samples using the method of Kofler et al. (24). We found P-element insertions at 624 sites in the South African sample, and at nine sites in the population from Florida (Fig. 1*A*), with most insertions segregating at low allele frequencies (<0.1; *SI Appendix*, Fig. S1). We compared these results to those from *D. melanogaster* samples collected from South Africa (in 2012) and Portugal (in 2008). In *D. melanogaster* the average number of P-element insertions per haploid genome is similar for the two populations (62 in South Africa and 60 in Portugal). In contrast, the two *D. simulans* populations are very different, with 29 P-element insertions found per haploid genome in the South African sample versus 0.4 in Florida. These differences suggest that we sampled the Florida (2010) population in the early phase of P-element invasion and the South African population (2012) at a more advanced phase. Consistent with a recent invasion of *D. simulans* populations, we did not find any P-element insertions in a pool of African (Sub-Saharan) *D. simulans* flies sampled between 2001 and 2009 (25) nor in diverse strains collected before 1998 from multiple locations including California, North America, Madagascar, New Caledonia, and Kenya (26, 27). Using sequence data from individual flies, we confirmed that the presence of the P-element in *D. simulans* is not due to a low level of contamination of the pooled flies with *D. melanogaster* or to a technical artifact. Specifically, we crossed 12 *D. simulans* Florida males to females of the sequenced *D. simulans* reference strain M252 (28), which lacks the P-element (29), and sequenced single F1 females from these crosses. Progeny of three of these crosses had P-element insertions (nine in cross 116, two in cross 174, and 12 in cross 211), a subset of which were validated using PCR (*SI Appendix*, *Results 3.1*).

Significance

Transposable elements (TEs) persist via two evolutionary strategies—in the short term, they selfishly propagate within genomes, and over the long term, they spread horizontally between species. Famously, the P-element invaded *Drosophila melanogaster* populations some time before 1950 and spread rapidly worldwide. Here, we show that it has also invaded a close relative, *Drosophila simulans*, from which it was absent until recently. The genomic tools at our disposal offer the unique opportunity to study the dynamics of a TE invasion at multiple levels and to compare the spread of the P-element in *D. simulans* with the well-investigated invasion of *D. melanogaster*.

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Data deposition: The Sanger sequenced amplicons and the *Drosophila simulans* P-element reported in this paper have been deposited in the GenBank database [accession nos. KP241673–KP241675 (amplicons) and KP256109 (P-element)]. The Illumina reads (genomic and RNA-seq) have been deposited in the Sequence Read Archive, www.ncbi.nlm.nih.gov/sra (accession no. PRJEB7936).

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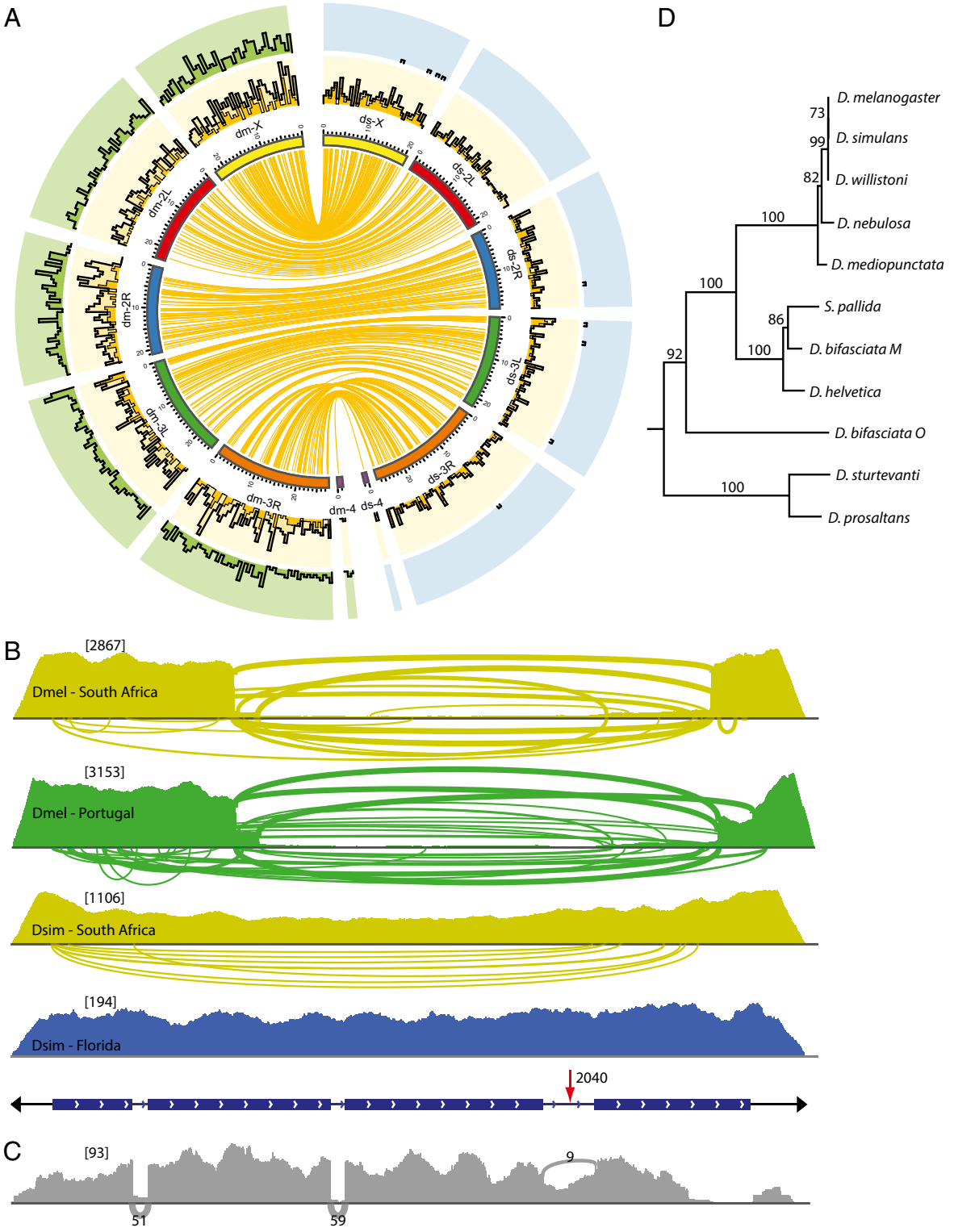

Fig. 1. Population genomics of the P-element. (*A*) Abundance of P-element insertions in natural *D. simulans* (ds) and *D. melanogaster* (dm) populations. The abundance (in insertions per 500 kbp window) is shown for populations from South Africa (yellow), Portugal (green), and Florida (blue). Insertions at similar positions in South Africa are shown in the inlay (dark yellow lines), and the abundance of these insertions is summarized in the histogram (dark yellow). All histograms have a maximum height of 18 insertions. (*B*) Diversity of P-element insertions in natural populations of *D. melanogaster* (Dmel) and *D. simulans* (Dsim). We use Sashimi plots (54), which usually indicate splicing in RNA-seq data, to visualize truncated P-element insertions in genomic DNA. Numbers in brackets are the maximum coverages. Endpoints of arches are positions of deletions, and the width of the arches scales with the logarithm of the number of reads supporting a given truncated insertion; only truncated insertions supported by at least three reads are shown. Panel at the bottom indicates the structure of the P-element (6). The four ORFs (blue), the SNP distinguishing the *D. simulans* and *D. melanogaster* P-element (red arrow), and the terminal inverted repeats (black triangles) are shown. (*C*) Expression of the P-element in *D. simulans*. RNA-seq for the population from Florida was performed, and results are visualized with a Sashimi plot (54). (*D*) Phylogeny of the P-element. The P-element of *D. simulans* most closely resembles the P-element from *D. melanogaster* (one base substitution) and *D. willistoni* (two base substitutions).

Origin of the *D. simulans* P-Element. The *D. melanogaster* and *D. simulans* P-element sequences differ by a single substitution at position 2040 (*G*→*A*; *SI Appendix*, Table S1), which occurs in all *D. simulans* P-element insertions in both populations (*SI Appendix*, Table S1). To identify the origin of the *D. simulans* P-element, we constructed a phylogeny using this sequence and that of 10 P-elements most closely related to that of *D. melanogaster* (Fig. 1*D*) (30). The *D. simulans* P-element is most similar to the *D. melanogaster* P-element (with one nucleotide difference) followed by the *D. willistoni* P-element (two nucleotide differences), suggesting that *D. melanogaster* is the likely source of the *D. simulans* P-element. If this scenario is true, the *D. simulans* allele at position 2040 might segregate in *D. melanogaster* populations. We screened several publicly available datasets of *D. melanogaster* populations (24, 29, 31) for the presence of this allele, which we found segregating at a low frequency (0.16–2%) in 5 of 13 datasets (*SI Appendix*, *Results 3.2*). This result suggests that the P-element invasion in *D. simulans* was triggered by a single horizontal transfer: Recurrent transfer would likely have resulted in the concurrent invasion of the more frequent alternative allele. Consistent with this, P-element insertions in *D. melanogaster* are 2–3.5-fold more diverse than those in *D. simulans* [sequence diversity π according to Nei and Li (32); *D. melanogaster*, South Africa $\pi = 0.00072$, Portugal $\pi = 0.00121$; *D. simulans*, South Africa $\pi = 0.00038$, Florida $\pi = 0.00035$; *SI Appendix*, Table S1). Further, in *D. melanogaster*, many independently derived truncated P-elements occur (Fig. 1*B*) (6, 7, 14, 16), whereas most *D. simulans* P-element insertions are full-length (Fig. 1*B*). The predominance of full-length insertions in *D. simulans* is consistent with a recent invasion, which requires a functional transposase not encoded by most truncated P-elements (7). In fact, some truncated P-elements repress transposition (33, 34) and may thus inhibit an invasion. The heterogeneity of P-elements in *D. melanogaster* relative to the homogeneity of P-elements in *D. simulans* further supports our hypothesis of a single horizontal transfer event, as recurrent HT probably would have resulted in higher diversity of P-elements in *D. simulans*.

The *D. simulans* and *D. melanogaster* P-Element Behave Similarly. To investigate whether the P-element behaves similarly in *D. simulans*, we analyzed two well-known features of the *D. melanogaster* P-elements: regulation by alternative splicing and insertion site preferences. In *D. melanogaster* the P-element produces active transposase only in the germ line (16), with this tissue specificity controlled posttranscriptionally by alternative splicing of the third intron (35). In the soma, transcripts retain the third intron, producing a truncated, inactive version of the transposase protein; in the germ line, this intron is spliced out, yielding a functional transposase (35). Host genes responsible for alternative splicing of the third intron [P-element somatic inhibitor (*Psi*), heterogeneous nuclear ribonucleoprotein at 27C (*Hrb27C*)] are highly conserved between *D. melanogaster* and *D. simulans* (36), and so we anticipated that the same pattern of alternative splicing occurs in *D. simulans*. We therefore analyzed RNA-seq data from the Florida *D. simulans* flies for evidence of alternative splicing of the third intron (Fig. 1*C*). We found low levels of spliced transcripts producing transposase, with the splicing of the third intron being supported by nine reads. However, most reads (38; average of both splice sites) support retention of the third intron, suggesting that the P-element is expressed and regulated somatically in *D. simulans* as in *D. melanogaster* (Fig. 1*C*).

In *D. melanogaster*, the P-element shows a strong preference for insertion into the promotor regions of genes (37) and a further bias for origin recognition complex (ORC) binding sites (38). We found that a substantial fraction of P-element insertions were at similar positions (±1,000 bp) in both *D. melanogaster* and *D. simulans* (428 of 1,466 insertions in *D. melanogaster*, where 15 are expected due to chance; $\chi^2 = 11{,}488$; df = 1; $P < 2.2e{-}16$; Fig. 1*A* and *SI Appendix*, Fig. S2). In principle, these insertions could have been inherited from the ancestor of the two species (2–3 million years ago) (39, 40), but this seems unlikely as it would be counter to the evidence showing that the element was absent from both species until recently (13–16). Further, P-element insertions typically occur at low frequency; it is implausible that the shared insertions would have segregated at low frequencies without being lost by genetic drift since the split of the two species. Instead, the presence of insertions at similar sites is likely due to insertion biases: De novo P-element insertions tend to occur in a few hotspots, with 30–40% of all P-element insertions occurring in just 2–3% of the genome (37, 38). To investigate whether the same insertion bias occurs in *D. simulans*, we identified 1-kb windows that contained at least two independent insertions generated in the course of the *Drosophila* Gene Disruption Project (18,214 insertions) (37). In this way, 2.3% of the genome was identified as potential P-element hotspots (2,826 1-kb windows). In the *D. melanogaster* sample, 63.5% of P-element insertions from the population from South Africa lie in these regions, representing a significant enrichment ($\chi^2 = 24{,}226$; df = 1; $P < 2.2e{-}16$; Table 1). We next identified the homologous regions in the *D. simulans* genome using sequence similarity; 54.3% of the *D. simulans* insertions occur at these sites (Table 1), again a significant enrichment ($\chi^2 = 9{,}955$; df = 1; $P < 2.2e{-}16$). As P-element insertions at similar positions in the two species are significantly more enriched in hotspots (81%) than other P-element insertions (based on *D. melanogaster* insertions; 56.6%; $\chi^2 = 107$; df = 1; $P < 2.2e{-}16$), we suggest that the insertion bias accounts for the large fraction of insertions at similar positions. In fact, the target site specificity of P-elements may enhance its invasive properties. That is, P-elements transpose

Table 1. Insertion bias of the P-element and other TEs in a natural population of *D. melanogaster* (Dmel) and *D. simulans* (Dsim) from South Africa

			Hotspots, ~2%		ORC, ~1.6%		Promotor, ~11%	
Element	Genus	Total	*n*	Enrichment	*n*	Enrichment	*n*	Enrichment
P-element	Dmel	1,466	931	27.1*	672	27.3*	789	4.5*
	Dsim	624	339	31.6*	319	34.3*	312	5.2*
	Similar	428	347	34.5*	255	35.4*	234	4.6*
Other TEs	Dmel	19,362	392	0.9	290	0.9	1,791	0.8
	Dsim	13,503	143	0.6	147	0.7	734	0.6
	Similar	1,017	22	0.9	6	0.4	116	1.0

Insertions at similar sites in the two species are shown as a distinct category (similar; subset of *D. melanogaster* insertions). The counts (*n*) and the relative enrichment relative to a random distribution of insertions in the genome are shown for P-element insertion hotspots, ORC binding sites (ORCs), and putative promotor regions (regions within 500 bp of a transcription start site). Approximate proportions of genomic features are given. Annotations were obtained for *D. melanogaster*, and homologous regions in *D. simulans* were identified by sequence similarity. An asterisk indicates highly significant enrichment ($P < 0.001$) relative to other TEs.

via a cut-and-paste mechanism (41, 42), which does not inherently lead to an increase of copy numbers. An increase in copy numbers is achieved by postreplication repair of double-strand breaks resulting from P-element excisions using the sister chromatid as a template, thus preserving the insertion at the excision site (41). Copy numbers may be further increased by preferential insertion of P-elements into unreplicated regions, which could be mediated by a bias for insertion into ORC binding sites (38). Consistent with results for *D. melanogaster*, we find the strongest insertion site bias is for ORCs (Table 1); assuming conservation of ORC sites, we find a 34-fold enrichment of *D. simulans* P-element insertions in ORC binding sites (χ^2 = 9,703; df = 1; $P < 2.2e{-}16$; Table 1). Although insertion bias into ORCs or promotor regions explains the majority of P-element insertions at similar positions in *D. melanogaster* and *D. simulans*, we also found that 85 (20%) of these insertions do not overlap with known ORCs or promotors, suggesting either an unaccounted bias or incomplete annotations.

Reasons for the Delayed Invasion of *D. simulans*. Why did it take the P-element almost 50 y longer to invade *D. simulans* than to invade *D. melanogaster*? The two hypotheses put forward to explain this phenomenon (17) invoked either genomic factors that prevent the establishment of the P-element in *D. simulans* (21, 43) or the rarity of horizontal transfer (18). Genomic barriers to the establishment of P-element in *D. simulans* might have been overcome by adaptation of the TE to *D. simulans*. The single substitution distinguishing the *D. melanogaster* and *D. simulans* P-element seems unlikely to confer a functional advantage: It occurs in an intron and does not coincide with characterized splicing motifs (6, 44) or with the 9-bp motif responsible for maternal transmission (45). Instead, our observations suggest that successful horizontal transmission of the P-element is rare. The data here suggest that *D. simulans* P-elements have a single origin: Recurrent invasion from *D. melanogaster* would result in *D. simulans* insertions with a subset of the diversity of the insertions in *D. melanogaster*, or at least the concurrent invasion of the wild-type allele of the *D. melanogaster* P-element. Instead, we find that *D. simulans* insertions are fixed for a rare *D. melanogaster* variant.

Conclusions

Our observation of a recent, ongoing invasion of TEs into a previously uninfected species provides a unique opportunity to study the dynamics of TE spread in natural populations. We note that the insertion bias of the P-element has led to a form of parallel molecular evolution in the two species. Such mutational biases may promote parallel phenotypic evolution, thus enhancing the repeatability of evolution (46, 47). In *D. melanogaster*, the host has adapted to the P-element via the production of piwi-interacting RNAs (piRNA) with sequence similarity to the P-element, which acts to suppress transposition (48, 49); for example, Khurana et al. (50) showed that the fertility of females suffering from hybrid dysgenesis can be restored by the formation of new piRNA producing loci, which result from transposition of P-elements into piRNA clusters. Therefore, it will be particularly important to link the ongoing spread with the buildup of piRNAs controlling the spread of P-elements (49) and their relative dynamics in different environments given the strong temperature dependence of hybrid dysgenesis (16). The invasion of *D. simulans* may lead to the P-element rapidly invading the rest of the *melanogaster* subgroup; both P-element–free relatives, *Drosophila mauritiana* (where we did not find any P-element insertions; *SI Appendix*, *Results 3.3*) and *Drosophila sechellia* (13, 15), are known to hybridize with *D. simulans* (51, 52).

Materials and Methods

We measured TE abundance in two populations of *D. melanogaster* and two populations of *D. simulans* using Pool-seq data and PoPoolation TE (24), as described in ref. 29. We used three previously published datasets [*D. melanogaster* from South Africa (29), *D. melanogaster* from Portugal (24), and *D. simulans* from South Africa (29)] and additionally sequenced a *D. simulans* population from Florida as a pool using Illumina paired-end sequencing. To confirm the presence of the P-element in *D. simulans*, we crossed several males from Florida with the *D. simulans* strain M252 (28) and sequenced some F1 progeny individually with Illumina paired-end sequencing. PCR primers were designed to confirm some insertions in the progeny, and amplicons were sequenced using the Sanger technology. We used RNA-seq to measure expression of the P-element. RNA was extracted from whole adults of *D. simulans* females from Florida that were kept in the laboratory for two generations at 15 °C. Insertion bias of P-elements was measured using publicly available data of 18,214 independent P-element insertions (37) and ORC binding sites (38), and regions 500 bp within transcription start sites were used as putative promotor sequences (annotation of *D. melanogaster* v5.57; flybase.org/). The programming language R (53) was used for all statistical analyses. See *SI Appendix* for more details.

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