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Genetics

Sequences homologous to *P* elements occur in *Drosophila paulistorum*

(whole genome blot analysis/mobile genetic elements)

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ABSTRACT **P elements, a class of mobile genetic elements that cause hybrid dysgenesis in *Drosophila melanogaster*, have thus far been shown to occur only in this species. Using whole genome blot analysis, we present evidence which indicates that sequences homologous to *D. melanogaster P* elements also occur in *Drosophila paulistorum*, a distant relative. *D. paulistorum* is considered a species complex, consisting of six known incipient species or semispecies. All six semispecies possess *P* element homologous sequences. We further show that there is conservation of restriction enzyme recognition sites between the *D. melanogaster* and *D. paulistorum P* element sequences. The presence of *P* elements in these two species may clarify the roles of recent invasion and rapid loss in the temporal distribution of *P* elements in *Drosophila*.**

P elements are members of one of several classes of moderately repetitive, dispersed gene families in *Drosophila melanogaster* (1). They have recently become the subject of much interest because of their role in fostering a syndrome of correlated germ-line abnormalities known as P–M hybrid dysgenesis (2–4). The characteristic features of the syndrome include temperature-dependent sterility; illicit male recombination; and increased rates of mutation, chromosome aberrations, and non-disjunction. Dysgenesis occurs in the germ-line of progeny produced from certain interstrain crosses—that is, when males from a paternally contributing (P) strain are mated with females from a maternally contributing (M) strain (5–7). P strains possess at least several intact, genetically active *P* elements and manifest a P cytotype; M strains either completely lack or contain only structurally altered *P* elements and manifest an M cytotype (8–10). The *P* elements, which are stable in a P cytotype, transpose at high frequencies in an M cytotype, where they may be shown to be associated with both induced mutations and breakpoint hot spots for chromosome rearrangements (8, 11, 12). The intact *P* element is 2.9 kilobases (kb) long, contains open reading frames (which may encode transposase function), and has perfect terminal 31-base-pair inverted repeats (10). In addition to the highly conserved intact *P* elements, there are smaller elements heterogeneous in size, which presumably arise by internal deletions from the large elements (9, 10). At least some of the smaller elements are incapable of transposition unless a *trans*-acting activity is provided by intact elements (13). Experimental manipulation of *P* element transposition has been recently used to transform whole organisms (13, 14).

The population dynamics and evolutionary biology of *P* elements have been the subject of considerable experimentation and speculation. Three general observations have been made about the distribution and interactive dynamics of P and M types. First, P types were not found in strains collected from the wild before about 1950, while their frequency increases with decreasing time in laboratory cultivation (15). Second, there are distinct geographical patterns in the relative distributions of P and M types throughout the world (15). Third, *P* element activity has been shown to be highly invasive, with P types predominating in a population after only a few generations even if an excess of M types was present initially (16). This last observation is substantiated at a molecular level by the rapid acquisition of *P* elements by M type chromosomes (8).

Two general models have been proposed to explain the temporal distribution of P and M strains. The "recent invasion" hypothesis postulates that wild populations of *D. melanogaster* were recently (1940–1960) invaded by a virus-like entity containing *P* element sequences, which rapidly spread through the species by vertical transmission among members (8, 15, 17). Alternatively, the "rapid loss" hypothesis postulates that P factors were widespread in natural populations and were subsequently lost during laboratory culture (18). A critical discussion of these alternatives has been included in a recent review by Engels (3), in which he makes the important point that the rare cases of horizontal transmission of *P* elements between species will further clarify the population genetics and evolutionary biology of *P* elements. Previous attempts to find sequences homologous to *P* elements in species other than *D. melanogaster* have been unsuccessful, even when isolates of the sibling species *Drosophila simulans* have been obtained from the same population as the π_2 P strain of *D. melanogaster* (discussed in ref. 3).

In this paper, we report results that prove the existence of sequences that are homologous to *D. melanogaster P* elements in another species, *Drosophila paulistorum* (19). Members of the same subgenus as *D. melanogaster*, the neotropical *D. paulistorum* belongs to a different species group, the *willistoni* group, which is the subject of a recent comprehensive review (20). *D. paulistorum* is not a single species, but rather a species complex consisting of six semispecies or incipient species (21). The fact that these semispecies may be undergoing the transition from race to species has made the *D. paulistorum* species complex the subject of many evolutionary studies, especially those investigating reproductive isolating mechanisms (reviewed in ref. 20). For these and other reasons, we have been interested in the molecular characteristics of multigene families in the *D. paulistorum* species complex. Among the most surprising results to emerge from these studies to date is the occurrence of sequences homologous to the *D. melanogaster P* element in this rather distant relative.

MATERIALS AND METHODS

***Drosophila* Strains and Culture.** The following *D. paulistorum* strains (from the collection of L. Ehrman), represent-

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Abbreviation: kb, kilobase(s).

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ing each of the six semispecies were used in this study: Amazonian semispecies (A-28) from Belem, Brazil; Andean-Brazilian semispecies (Mesitas) from Mesitas, Colombia; Centroamerican semispecies (C-2) from Lancetilla, Honduras; interior semispecies (New Llanos or I-1) from Llanos, Colombia; Orinocan semispecies (O-11) from Georgetown, Guyana; transitional semispecies (T-1) from Santa Marta, Colombia. All except I-1 are wild-type strains that have been maintained in the laboratory between 25 and 30 yr; the I-1 strain is a derivative of a wild-type strain collected in 1958 (see ref. 20). Our standard *D. melanogaster* P strain is a derivative of the Harwich strain obtained from the collection of M. Kidwell; the M strain (*ry506*) comes from the collection of A. Chovnick. *D. simulans* (8DS) and *D. mauritiana* (Rivière Noire) come from the collection of H. Krider; both are long-established laboratory strains. Flies were grown on a standard cornmeal medium.

Genomic Blot Analysis. Plasmid DNA samples were prepared by standard cesium chloride density centrifugation methods (22), and fragments generated by restriction enzyme digestions were isolated by electroelution from agarose gels. The ^{32}P-labeled radioactive probes were prepared by standard nick-translation reactions (22). Adult flies for DNA extractions were sorted under a microscope, and genomic DNA was prepared essentially by methods published elsewhere (23). Restriction endonucleases were purchased from Bethesda Research Laboratories. In all experiments, 5 μg of DNA was digested as recommended by suppliers. Genomic DNA fragments are separated in horizontal 1% agarose gels and sequences homologous to *P* elements were visualized by autoradiography after hybridization to transferred fragments. The conditions for hybridization and rinsing of filters were exactly as described in ref. 23.

RESULTS

Fig. 1 shows a diagrammatic representation of the P-element-containing plasmid pπ25.1 (10) used in this study. It contains an intact *P* element with some flanking DNA from chromosome region 17C. Fig. 2 shows the results of whole genome blot analysis of a variety of adult *Drosophila* DNA samples digested with *Pvu* II and probed with the *Xho* I/*Sal* I fragment isolated from pπ25.1. As expected, the *D. melanogaster* P strain (lane 1) shows many bands of hybridization typical of the genomic patterns of *P* elements. There is a strong band of hybridization corresponding to a fragment size of 0.9 kb, the predicted size of a *Pvu* II/*Pvu* II fragment from a conserved intact *P* element (Fig. 1). There is no hybridization to the DNA from the *D. melanogaster* M strain. Similarly, DNA samples from the sibling species of *D. melanogaster*, *D. simulans* and *D. mauritiana*, show no detectable hybridization to the *Xho* I/*Sal* I probe (data not shown). Surprisingly, all six of the *D. paulistorum* semispecies exhibit DNA fragments that hybridize with the *P* element probe, and five of the six contain the conserved 0.9 kb internally derived *P* element sequence. The sixth semispecies, Mesitas (lane 6), has strongly hybridizing fragments but lacks the conserved 0.9 kb fragment. In addition to demonstrating the presence of *P* element sequences in *D. paulistorum*, these results suggest that there may be a lower concentration of these sequences in this species than in *D. melanogaster*.

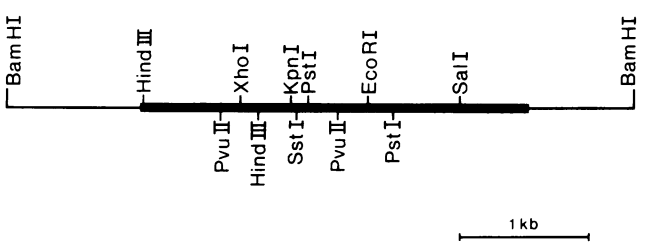

FIG. 1. Map of pertinent restriction enzyme sites of the pπ25.1 plasmid. Heavy bar represents the 2.9-kb intact *P* element; thin bars represent the flanking sequences from the 17C region. The genomic fragment was cloned into the *Bam*HI site of pBR322 (not shown). More information on this plasmid may be found in ref. 10. All restriction sites indicated here appear to be conserved in at least some of the elements present in the *D. paulistorum* transitional strain (Santa Marta, Colombia).

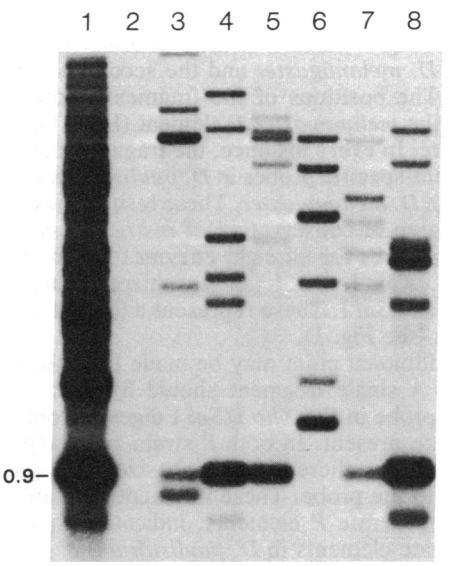

FIG. 2. Whole genome Southern blot analysis of *Pvu* II-digested DNA from *D. melanogaster* and *D. paulistorum* strains. The *Xho* I/*Sal* I fragment from the intact *P* element in pπ25.1 was used as probe. Lanes 1 and 2 contain DNA from *D. melanogaster* P and M strains, respectively. Lanes 3–8 contain DNA from representatives of the six known *D. paulistorum* semispecies as follows: lane 3, Amazonian (A-28); lane 4, Centroamerican (C-2); lane 5, interior (I-1); lane 6, Andean-Brazilian (Mesitas); lane 7, Orinocan (O-11); and lane 8, transitional (Santa Marta, T-1). The 0.9-kb fragment predicted from the map of the *D. melanogaster P* element (Fig. 1) is indicated.

Although *D. melanogaster* and *D. paulistorum* are morphologically distinct and the flies used for DNA extractions are carefully selected by microscope, we have done an additional control that eliminates any possibility of an artifactual basis for the results shown in Fig. 2. We have probed *Pvu* II digests of genomic DNA samples with the entire plasmid p6.1 (9), which contains a smaller *P* element and flanking DNA from the white locus (3C1). The results (not depicted here) of this experiment provide an important piece of information: the lanes containing *D. paulistorum* DNA samples show no hybridization to the major fragment of the conserved white locus, which are found in the *melanogaster* group sibling species. Considered with the results shown in Fig. 2, this provides a compelling argument that the homology to *P* elements detected in the *D. paulistorum* DNA samples cannot be due to any type of contamination with *D. melanogaster* sequences.

To measure the level of conservation of restriction enzyme sites between the *D. melanogaster* and *D. paulistorum* elements, genomic DNA samples from the P strain of *D. melanogaster* and the Santa Marta semispecies of *D. paulistorum* have been singly or doubly digested with a number of different restriction endonucleases known to have recognition sequences within the conserved *P* element (10). These digests have been hybridized with the *Xho* I/*Sal* I fragment from the *P* element of pπ25.1. A comparison of the results from the two DNA samples for each digestion provides a measure of the level of conservation of restriction enzyme recognition sites between the *D. melanogaster* and *D. pau-*

listorum elements. The results of these experiments are shown in Fig. 3. In each of the sets of lanes, the first sample is from *D. melanogaster* and the second is from *D. paulistorum*. The positions of the fragments expected from the map of the *melanogaster P* element (Fig. 1) are indicated in the figure. In every instance, the fragment that hybridizes to *P*-element-specific probes in *D. paulistorum* is the same size as that in *D. melanogaster*. These results show that there is a high degree of conservation of restriction enzyme recognition sequences for all eight enzymes tested: *Pvu* II, *Xho* I, *Hin*dIII, *Kpn* I, *Sst* I (an isoschizomer of *Sac* I), *Pst* I, *Eco*RI, and *Sal* I. These represent a total of 11 sites in the *P* element (see Fig. 1).

An additional point may be made from the data in Fig. 3 (set 5). A single fragment should hybridize with the *Xho* I/*Sal* I probe in the *Xho* I/*Sal* I digests if only intact *P* elements are present. In both P strain and Santa Marta strain DNA samples, there are multiple sizes of fragments that hybridize to the probe. These fragments are probably derived from degenerate *P* elements, indicating that there may be degenerate elements in *D. paulistorum*.

DISCUSSION

The results demonstrate that sequences homologous to the mobile *P* elements of *D. melanogaster*, thought prior to this study to be highly species-specific, are found in the *D. paulistorum* species complex. This finding is especially striking in view of the fact that these elements are not found in the sibling species of *D. melanogaster*. The observation that *P* element sequences are not found in a cosmopolitan closely-related species in the *melanogaster* group but occur in the neotropical more distantly-related species in the *willistoni* group poses many questions and may suggest the possibility of horizontal transmission of *P* elements. In this regard, it is important to ascertain whether sequences homologous to the *D. melanogaster P* element occur in other species of the *willistoni* group. Preliminary results from genomic blots of *D. willistoni* DNA (unpublished results) indicate that *P* element sequences are present. While we realize that a more comprehensive survey of the *willistoni* group is needed, the results reported here may represent an example of the rare horizontal transmission of mobile elements between species. Alternatively, the presence of the elements in this group may reflect an initial *P* element invasion into many species at a particular place and time. The occurrence of these elements in various species of the *willistoni* group from Central and South America leads to the speculation that either invasion events into *Drosophila* or subsequent transmissions may have been widespread in this geographical area.

Regardless of the mechanisms that account for the presence of *P* element sequences in these two diverse groups, their occurrence in such evolutionarily distinct species as *D. melanogaster* and *D. paulistorum* affords us the potential to distinguish between recent invasion and rapid loss models. In a simplistic view, if *P* elements occur in these diverse groups from recent invasion, we would anticipate that the elements would be less divergent than sequences of older origin that were present when the *willistoni* and *melanogaster* groups diverged. At the limited level of restriction enzyme recognition sites, it appears that the *D. paulistorum* and *D. melanogaster P* element sequences are highly conserved. While this observation does not prove the recent invasion hypothesis, it is supportive of that model. It is important to note that the strains of *D. paulistorum* that we have tested have been in culture for 25 to 30 yr, a time consistent with the period in which the rapid invasion of *D. melanogaster* is postulated to have occurred. Determination of the precise degree of identity between the elements in the two species will certainly be required to elucidate this important aspect of *P* element biology. Furthermore, a detailed molecular comparison of the various *D. paulistorum P* element sequences will be crucial in determining the relationships between *P* element sequences in the various semispecies.

The conclusion that the *P* elements of *D. melanogaster* and *D. paulistorum* are conserved is subject to two qualifications. First, the conservation of restriction enzyme recognition sites does not necessarily mean that there has been a conservation of base sequences throughout the element. It is possible that the relatively small number of nucleotides assayed by this property are not representative of the entire *P* element and, therefore, divergence may not be reflected in the results. This objection does not seem likely based on an

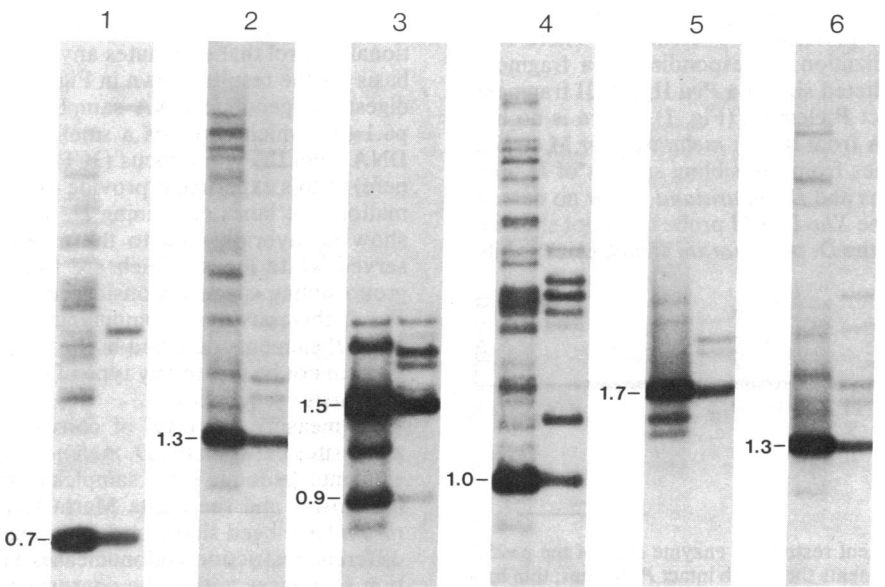

FIG. 3. Whole genome Southern blot analysis showing conservation of restriction enzyme sites between *D. melanogaster* and *D. paulistorum P* elements. Filters are probed with the *Xho* I/*Sal* I fragment. Pairs of lanes contain *D. melanogaster* P strain DNA on the left and *D. paulistorum* Transitional semispecies (Santa Marta) DNA on the right. Each pair of DNA samples was digested with restriction enzymes as follows: lane 1, *Pst* I; lane 2, *Kpn* I and *Sal* I; lane 3, *Hin*dIII and *Sal* I; lane 4, *Eco*RI and *Xho* I; lane 5, *Xho* I and *Sal* I; lane 6, *Sst* I and *Sal* I. In each instance, a single internally derived P fragment is generated, except for the *Hin*dIII and *Sal* I digestion, which generates two internal fragments (see Fig. 1). Sizes of expected fragments are indicated in kilobases.

analysis of divergence in another repeated gene family, the histone repeats. In studies of restriction enzyme recognition sites in *D. paulistorum* histone DNA, we have found many differences from the *D. melanogaster* histone repeats (unpublished results). In this case, a relatively small number of restriction enzyme recognition sites is more than adequate to detect divergence, even in genes that encode highly conserved proteins. A second and more important caution is that the enzyme recognition sequences that we have mapped span the region from the leftmost *Hin*dIII site to the *Sal* I site. The majority of these sites cluster in the central region of the *D. melanogaster P* element. We know nothing about the ends of the elements from *D. paulistorum*, and information about these will be required before any conclusions about the identity of the elements will be definitive. The results of DNA sequencing of these elements will provide information concerning the relationships between the elements in *D. paulistorum* and those in *D. melanogaster*.

If conserved *P* elements are in fact present in *D. paulistorum*, it will be important to learn whether these elements are functionally equivalent to those in *D. melanogaster*. To ascertain this, cloned *P* elements from *D. paulistorum* will be injected into embryos from various M cytotype *D. melanogaster* strains. These experiments will determine whether *D. paulistorum* elements are capable of transposition in the "heterologous" *D. melanogaster* system and whether these elements can produce destabilization and/or transposition of native *D. melanogaster* elements, such as those at the sn^w locus (24). The results of these experiments are likely to contribute significantly to our understanding of the evolutionary and population biology of this class of mobile elements.

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