Evidence for Horizontal Transmission of the *P* Transposable Element Between *Drosophila* Species

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ABSTRACT

Several studies have suggested that *P* elements have rapidly spread through natural populations of *Drosophila melanogaster* within the last four decades. This observation, together with the observation that *P* elements are absent in the other species of the *melanogaster* subgroup, has lead to the suggestion that *P* elements may have entered the *D. melanogaster* genome by horizontal transmission from some more distantly related species. In an effort to identify the potential donor in the horizontal transfer event, we have undertaken an extensive survey of the genus *Drosophila* using Southern blot analysis. The results showed that *P*-homologous sequences are essentially confined to the subgenus *Sophophora*. The strongest *P* hybridization occurs in species from the closely related *willistoni* and *saltans* groups. The *D. melanogaster P* element is most similar to the elements from the *willistoni* group. A wild-derived strain of *D. willistoni* was subsequently selected for a more comprehensive molecular examination. As part of the analysis, a complete *P* element was cloned and sequenced from this line. Its nucleotide sequence was found to be identical to the *D. melanogaster* canonical *P*, with the exception of a single base substitution at position 32. When the cloned element was injected into *D. melanogaster* embryos, it was able to both promote transposition of a coinjected marked transposon and induce *singed-weak* mutability, thus demonstrating its ability to function as an autonomous element. The results of this study suggest that *D. willistoni* may have served as the donor species in the horizontal transfer of *P* elements to *D. melanogaster*.

VERY little is known about the origin and evolutionary history of mobile element systems. A mobile element family might originate in a species *de novo* by mutational or recombination events that alter or reorder sequences already present within the genome (*e.g.*, FINNEGAN 1985). Alternatively, mobile elements might be acquired by horizontal transmission from some other species. Such transfer events could be mediated by one or more of a number of different vectors (*e.g.*, viruses). At present, information on the mechanisms and relative frequencies of these two modes of origin is completely lacking. If horizontal transmission should prove to be other than a relatively isolated, rare event, then the implications could have a dramatic impact on many aspects of evolutionary theory.

The well-characterized *P* transposable element family represents an ideal system in which to examine a variety of evolutionary issues. *P* elements were first detected in *Drosophila melanogaster* and have been extensively studied at both the genetic and molecular levels. They are particularly interesting because they have been shown to be the causative agents of one of the two well-documented systems of hybrid dysgenesis in *D. melanogaster*. Hybrid dysgenesis is a syndrome of correlated genetic abnormalities that is induced in the germline of progeny from certain intraspecific crosses (KIDWELL, KIDWELL and SVED 1977). [For comprehensive reviews of *P* elements and P-M hybrid dysgenesis, see ENGELS (1983, 1989).]

P elements in *D. melanogaster* exhibit almost no sequence polymorphism but are heterogeneous with respect to size (O'HARE and RUBIN 1983). They can be divided into two types, complete (autonomous) elements and defective (nonautonomous) ones. Complete elements are 2.9 kb in length and encode a transposase enzyme (RIO, LASKI and RUBIN 1986). Defective elements are deletion-derivatives that are smaller and variable in size. The deletion of internal *P* element sequences occurs at a high frequency under conditions of active transposition (VOELKER *et al.* 1984; DANIELS *et al.* 1985; SEARLES *et al.* 1986; TSUBOTA and SCHEDL 1986). Both types of elements have 31-bp inverted terminal repeats and create an 8-bp target site duplication upon insertion. Defective elements can undergo transposition only in the presence of active autonomous elements.

One way to study the evolutionary origins and

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histories of mobile element families is to undertake surveys of their phylogenetic distributions. At present, it is known that *P*-homologous sequences are distributed throughout the species groups that comprise the subgenus *Sophophora* (ANXOLABÉHÈRE, NOUAUD and PÉRIQUET 1985; LANSMAN *et al.* 1985; DANIELS and STRAUSBAUGH 1986), although they are absent from the species most closely related to *D. melanogaster*, *i.e.*, the species of the *melanogaster* subgroup (BROOKFIELD, MONTGOMERY and LANGLEY 1984). This observation, together with the observation that *P* elements are completely lacking in old laboratory strains of *D. melanogaster* (BINGHAM, KIDWELL and RUBIN 1982; ANXOLABÉHÈRE, KIDWELL and PÉRIQUET 1988), suggests that the *P* transposon may have entered the *D. melanogaster* genome by horizontal transmission, perhaps as the result of some infectious process (*e.g.*, BINGHAM, KIDWELL, and RUBIN 1982). Moreover, it has been proposed that a distantly related member of the genus may have served as the donor species (DANIELS *et al.* 1984; DANIELS and STRAUSBAUGH 1986). Whether *P* elements are of recent origin in *D. melanogaster* or whether their introduction occurred at some more distant point in time has been the subject of some debate (*e.g.*, ENGELS 1986).

If the introduction were sufficiently recent to essentially preclude any appreciable base sequence divergence, then: (1) there must be at least one species other than *D. melanogaster* that contains an intact, functional *P* element, and (2) the elements (or at least a subset of them) within this species must have sequences that are virtually identical to those of the *D. melanogaster* canonical *P* (O'HARE and RUBIN 1983). The results of previous studies would suggest that the most reasonable place to start a search for such a potential donor species is within the genus itself. Although there have been a number of studies that have examined the phylogenetic distributions of *P*-homologous sequences within *Drosophila* (BROOKFIELD, MONTGOMERY and LANGLEY 1984; DANIELS *et al.* 1984; ANXOLABÉHÈRE, NOUAUD and PÉRIQUET 1985; LANSMAN *et al.* 1985; DANIELS and STRAUSBAUGH 1986; STACEY *et al.* 1986), most of these have been limited in scope to a few species or to a particular taxon within the genus. We have undertaken, therefore, a survey that is more comprehensive than any other performed to date both to screen for donor species candidates and to look for other discontinuities in the *P* element distribution that might represent potential cases of horizontal transfer.

Although our survey did not reveal any previously unknown discontinuities, it did establish the *willistoni* species group, and in particular the *willistoni* subgroup, as the only likely source of a donor species within the genus. We subsequently selected one member from this subgroup, *D. willistoni*, for a more extensive molecular examination. In the course of this analysis, we isolated, cloned and sequenced an intact, functional *D. willistoni P* element. Our results support the hypothesis that *P* elements have been transferred horizontally between *Drosophila* species.

MATERIALS AND METHODS

Fly stocks: All of the species used in this study are listed in Table 1 or in the legend to Figure 3; each listing includes one of the following designations to denote the laboratory from which the stock was obtained: BG, National Drosophila Species Resource Center, Bowling Green State University; WH, W. HEED, University of Arizona; JC, J. COYNE, University of Chicago, FA, F. AYALA, University of California at Davis; LE, L. EHRMAN, State University of New York at Purchase; MS, M. SEIGER, Wright State University.

The *D. willistoni P* element was cloned from a line established from a single female caught in Florida in 1983 by M. SEIGER. This isofemale line has been designated "willi #7."

Strains of *D. melanogaster* that were employed in this study are as follows: Harwich and π_2 are strong P strains that contain many *P* element copies. Canton S is an M strain devoid of *P* elements (*i.e.*, it is a true M strain). ry^{506} is a true M strain with a small deletion in the coding region of the *rosy* gene. *C(1)DX,y f*; ry^{506} is a compound-X, true M strain bearing the ry^{506} allele. *y* sn^{w}; ry^{506} is an M strain containing the hypermutable *singed-weak* allele (ENGELS 1979, 1989) and a rosy eye color marker. ry^{506} *P*[ry^{+} *Δ2-3*](*99B*), abbreviated *Δ2-3(99B)*, is a ry^{506} line transformed with the engineered *P* element, *P*[ry^{+} *Δ2-3*], which contains a copy of a wild type *rosy* gene inserted into a functional *P* element with a deleted third intron [for further details, see LASKI, RIO and RUBIN (1986) and ROBERTSON *et al.* (1988)]; *Δ2-3(99B)* contains two elements in reverse tandem at cytological position 99B (H. ROBERTSON, personal communication). *Δ2-3(99B)*/ry^{506} individuals were obtained from the F_1 progeny of a cross between homozygous *Δ2-3(99B)* and ry^{506} lines. See LINDSLEY and GRELL (1968) for a description of genetic symbols not described.

Plasmids: pπ25.1 contains a genomic fragment from the *D. melanogaster* P strain, π_2 (SPRADLING and RUBIN 1982). The cloned DNA consists of a complete *P* element flanked by genomic sequences from cytological region 17C; the nucleotide sequence of this *P* element has been determined (O'HARE and RUBIN 1983). The 1.7-kb *Xho*I/*Sal*I fragment from the center of the *P* element was used as probe in some of our experiments.

pπ25.7BWC ("both wings clipped"), constructed by K. O'HARE, contains a *P* element that is lacking 39 bp from its 5′ and 23 bp from its 3′ end. This plasmid contains no flanking genomic DNA, and, for this reason, is an acceptable probe for the purpose of screening different species for *P* element homologues.

The pry^{+4} plasmid carries a ry^{+} transposon that was constructed by inserting a 7.2-kb *Hin*dIII *rosy* locus fragment from ry^{+4} into the polylinker of the defective *P* element borne by the Carnegie-4 plasmid vector (RUBIN and SPRADLING 1983). The ry^{+} transposon is able to undergo transposition only in the presence of an element capable of supplying *trans*-acting transposase. pry^{+4} was used in the transformation experiments and was provided by S. CLARK.

The derivations of the *D. willistoni* plasmids, pDwP13-X30 and pDwP13F-XE4, are diagrammed in Figure 1 and described below.

The cloning of a *P* element from *D. willistoni*: Manipulations involving recombinant phages were performed by the methods described by DAVIS, BOTSTEIN and ROTH (1980). Adult genomic DNA from the *D. willistoni* line,

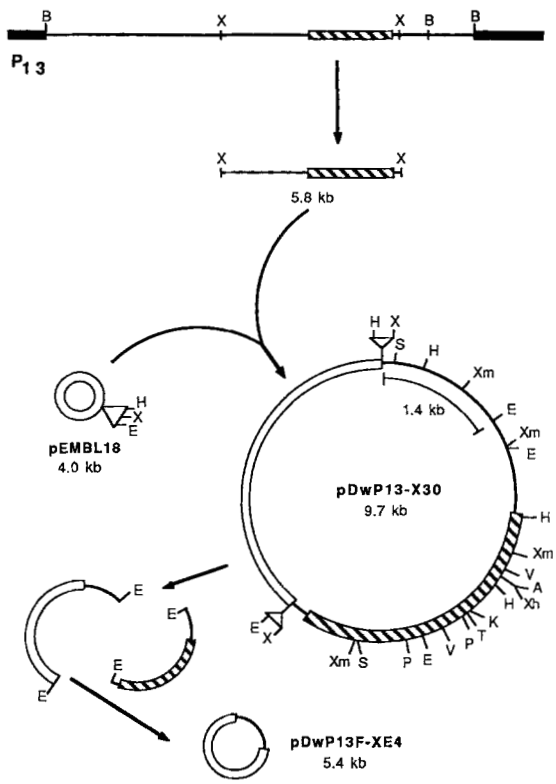

FIGURE 1.—The isolation and cloning of a full-sized *P* element from the *D. willistoni* genome. A lambda phage clone, designated P_{13}, containing a potentially intact *P* element was isolated from a "willi #7" lambda EMBL3 phage library as described in the MATERIALS AND METHODS. A 5.8-kb *Xba*I genomic fragment containing the *P* element and some flanking DNA was subsequently subcloned from P_{13} into the pEMBL18 plasmid vector to create pDwP13-X30, a limited restriction map of which is shown. Sequences derived from lambda EMBL3 are shown as fully shaded, those from pEMBL18 as unshaded and those from the *P* element as striped. The genomic sequences flanking the *P* element are depicted by thin bars. Constructs are drawn roughly to scale. The pDwP13-X30 restriction map is magnified threefold. Restriction enzyme recognition sites are designated as follows: A = *Ava*I; E = *Eco*RI; H = *Hin*dIII; K = *Kpn*I; P = *Pst*I; S = *Sal*I; T = *Sst*I; V = *Pvu*II; X = *Xba*I; Xh = *Xho*I; and Xm = *Xmn*I. The plasmid, pDwP13F-XE4, is a derivative of pDwP13-X30, and contains the 1.4-kb genomic fragment that is located approximately 1.3 kb upstream from the 5′ end of the *P* element. It was made by first digesting pDwP13-X30 DNA with *Eco*RI to remove all of the *P* element and some of the adjacent DNA and then recircularizing the plasmid by ligation.

"willi #7," was digested to completion with *Bam*HI and ligated to *Bam*HI-digested lambda EMBL3 phage DNA. The ligation mix was packaged *in vitro* and plated onto *Escherichia coli* KH802 cells. The resulting phage library was screened by plaque hybridization using nick-translated, ^{32}P-labeled BWC DNA. Phage DNAs containing *P* element sequences were further characterized by restriction enzyme and Southern blot analyses in order to identify those with potentially full-sized elements. One clone, designated P_{13}, was selected as a possible candidate. A 5.8-kb *Xba*I genomic fragment containing the *P* element and some of its flanking DNA was subsequently isolated from P_{13} and subcloned into the pEMBL18 plasmid vector (DENTE, CESARENI and CORTESE 1983) by the methods described by MANIATIS, FRITSCH and SAMBROOK (1982). A limited restriction map of this construct, which has been designated pDwP13-X30, is shown in Figure 1. The plasmid, pDwP13F-XE4, is derived from pDwP13-X30 and contains the 1.4-kb *Xba*I/*Eco*RI *D. willistoni* genomic fragment that is located approximately 1.3 kb upstream from the 5′ *P* element terminus.

DNA sequencing: *P*-containing DNA fragments from pDwP13-X30 were subcloned into pEMBL18 or pEMBL19 plasmid vectors and single-stranded template DNA was prepared as described by DENTE, CESARENI and CORTESE (1983). DNA sequences were determined by the dideoxy-chain termination method described by SANGER, NICKLEN and COULSON (1977) using T7 DNA polymerase (United States Biochemicals).

Southern blotting: Genomic DNA samples were routinely prepared from approximately 0.12 g of adult flies by the method described by DANIELS and STRAUSBAUGH (1986). DNA was quantitated by the fluorometric assay described by KISSANE and ROBINS (1958). Procedures for restriction enzyme digestion, agarose-gel electrophoresis, gel blotting and preparation of nick-translated probes are described in RUSHLOW, BENDER and CHOVNICK (1984). Gels involved in the survey of the genus were blotted to Nytran (Schleicher & Schuell); those involved in all other experiments were blotted to nitrocellulose (Schleicher & Schuell). The same blotting conditions were used for both membranes. Probe DNAs were double labeled with [^{32}P]dCTP and [^{32}P]dTTP.

Hybridizations were carried out overnight in a mixture containing 50% formamide (Fluka), 5 × SSPE [0.75 M NaCl, 0.05 M $NaHPO_4$, 0.01 M ethylenediamine-tetraacetate (EDTA), pH 7.0], 2 × Denhardt's solution [0.04% (w/v) Ficoll (type 400), 0.04% (w/v) polyvinylpyrrolidone, 0.04% (w/v) bovine serum albumin], 1% sodium dodecyl sulfate (SDS), 100 μg/ml sheared salmon sperm DNA, and 0.1 μg/ml ^{32}P-labeled probe DNA. Filters were washed for 3 hr in a series of solutions of increasing stringency, the final one of which contained 0.1 × SSPE and 0.5% SDS. Hybridizations and subsequent washes were done at either 42 or 37°. Manipulations at 42° detect sequences with ~78% identity to the probe DNA; manipulations at 37° detect those with ~73% identity, assuming a 40% G/C content, which is the content of the canonical *D. melanogaster P* element (O'HARE and RUBIN 1983).

Scanning densitometry: All autoradiograms of the *P* element survey of the genus were subjected to scanning densitometry (LKB UltroScan XL) in order to roughly quantitate relative levels of *P* hybridization under defined conditions. Each autoradiogram contained a positive (Harwich) and negative (Canton S) control lane and several lanes containing DNA from different species. Identical amounts of DNA were loaded in each lane with the exception of the Harwich control, which had 1/5 the amount of DNA of the other samples. The entire length of each lane was scanned and the density of all bands measured; a single, composite "raw score" was then calculated by integrating the area under all of the peaks. The Canton S lane provided the baseline value. So that comparisons could be made between different autoradiograms, a final, standardized score was computed for each sample by expressing its "raw score" as a percentage of the Harwich score. All samples were then placed on an arbitrarily defined, four point scale to indicate relative strength of hybridization (see Table 1).

Northern blotting: Total RNA was extracted from adult flies as described by KRAWETZ, STATES and DIXON (1986), and chromatographed on oligo(dT) cellulose (AVIV and LEDER 1972). Polyadenylated RNA was size fractionated in a 1% agarose-formaldehyde gel and transferred to a Nytran membrane (Schleicher & Schuell) by gel blotting. Hybridization and washes were performed according to the rec-

ommendations of Schleicher & Schuell for RNA transfer to Nytran.

***In situ* hybridization:** Larval salivary gland chromosomes were prepared and hybridized to biotin-labeled BWC DNA by a slight modification of the method described by ENGELS *et al.* (1986). Hybridizations were performed at 37°.

Transformation: Plasmid DNAs were introduced into the polarplasm of prepole cell embryos by the microinjection technique described by SPRADLING and RUBIN (1982).

RESULTS

The distribution of *P*-homologous sequences within the genus *Drosophila*: The primary goal in undertaking an extensive survey of the genus was to identify possible candidates that might have served as the donor species in the horizontal transfer of *P* elements to *D. melanogaster*. A secondary goal was to obtain a more precise description of the phylogenetic distribution of *P*-homologous sequences within the genus, and, to a limited extent, among several of the closely-related, derivative genera. In all, 144 species were examined, 136 from within the genus (~10% of the presently described species), representing 26 species groups and all the major radiations, and eight from outside the genus, representing five other genera. Information concerning the evolution and taxonomy of the genus *Drosophila* can be found in THROCKMORTON (1975) and BEVERLEY and WILSON (1984). The phylogenetic relationships of the groups examined in this study are depicted in Figure 2; the arrangement of subgenera, species groups and subgroups is given in Table 1.

To assay for *P*-homologous sequences, genomic DNA samples from the species of interest were digested with *Pvu*II and subjected to Southern blot analysis. In some experiments, filters were hybridized and washed under several sets of conditions, using different stringency and probe DNA combinations. Stringency conditions are described in the MATERIALS AND METHODS; the BWC plasmid and the internal *Xho*I/*Sal*I *P* fragment were used as probes. All autoradiograms were analyzed by scanning densitometry as described in the MATERIALS AND METHODS, and samples were placed on an arbitrarily defined, four point scale to indicate relative strength of hybridization. The results of the survey are shown in Table 1. The points to be made from the data are as follows:

1. Within the genus *Drosophila*, *P*-homologous sequences are essentially confined to the subgenus *Sophophora*. Of the 80 sophophoran species tested in this study, 61 showed discernible hybridization to *P* element probes. Positive results were obtained in all 10 of the *willistoni* group species, all 11 of the *obscura* group species, 6 of the 9 *saltans* group species and 34 of the 50 *melanogaster* group species tested. In addition, very weak hybridization signals were detected in 2 of the 5 species tested in the subgenus *Scaptodrosophila* and in *D. buscckii*, the sole member of the subgenus *Dorsilopha*. None of the 50 species tested from the subgenus *Drosophila* showed evidence of *P* hybridization under the conditions employed in this study. Previously, STACEY *et al.* (1986) reported that *D. nasuta*, an *immigrans* group member from the *Drosophila* subgenus, exhibited hybridization to *P* element probes under conditions of moderate stringency. We have tested the same *D. nasuta* stock under similar conditions and have found it to be negative. Moreover, we have examined 14 other species from the *immigrans* species group and have found no detectable evidence of *P* hybridization.

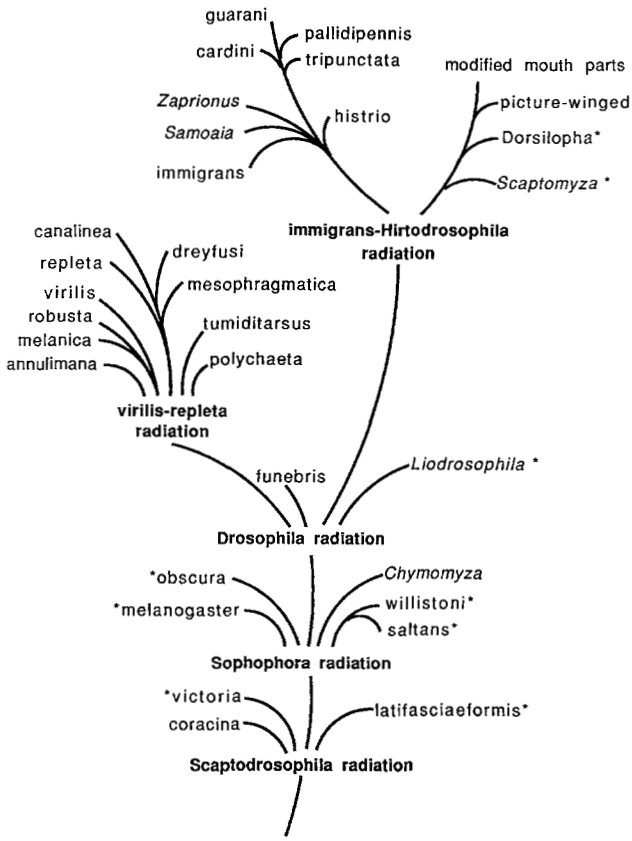

FIGURE 2.—Depiction of the evolution of the genus *Drosophila*, showing the major radiations and the present day species groups that have resulted from each (after THROCKMORTON 1975). Only subgenera and species groups screened in this study are included; several derivative genera are also shown. Those groups exhibiting evidence of *P* element hybridization in our survey are indicated by asterisks (*).

2. By far, the strongest *P* element signals were observed among the species from the closely related *willistoni* and *saltans* groups (Figure 3). Only minor differences were noted in the strength and patterns of hybridization when different probes and stringency conditions were employed. With the exception of *D. melanogaster*, the only other species that consistently demonstrated relatively strong hybridization under all conditions was *D. narragansett* from the *obscura* group. Most of the other *P*-bearing species from the *melanogaster* and *obscura* groups exhibited either no

TABLE 1

Distribution of *P* element sequences within the genus *Drosophila* and among several related genera

Source[a]	Genus	Subgenus	Species group	Subgroup	Species	Probe[b] X/S(42°)	X/S(37°)	BWC(42°)	BWC(37°)
BG 11010-0011.0	*Drosophila*	*Scaptodro-sophila*	*victoria*		*lebanonensis casteeli*			−	−
BG 11010-0021.0					*lebanonensis lebanonensis*			−	−
BG 11010-0031.0					*pattersoni*			−	−
BG 11010-0041.0					*stoni*			−	F
BG 11020-0051.0			*coracina*		*dimorpha*			−	−
BG 11020-0061.0			*latifasciaeformis*		*latifasciaeformis*			−	F
		Sophophora	*willistoni*	*willistoni*	*equinoxialis* (2)[c]	++++	++++	++++	++++
					insularis (5)	−	+	F	+
					paulistorum (20)	++++	++++	++++	++++
					pavlovskiana (1)	++++	++++	++++	++++
					tropicalis (1)	++	++	++	++
					willistoni (12)	++++	++++	++++	++++
				bocainensis	*capricorni* (2)	+	+	+	+
					fumipennis (2)	++++	++++	++++	++++
					nebulosa (7)	++++	++++	++++	++++
					sucinea (3)	+	+	+	+
			saltans	*cordata*	*neocordata* (1)	−	−	−	−
				elliptica	*emarginata* (2)	−	−	−	−
					neoelliptica (1)	−	−	−	−
				sturtevanti	*milleri* (1)	+	+	+	+
					sturtevanti (2)	++++	++++	++++	++++
				saltans	*austrosaltans* (1)	++++	++++	++++	++++
					lusaltans (1)	++	++	++	++
					prosaltans (2)	+++	+++	+++	+++
					saltans (1)	++++	++++	++++	++++
BG 14012-0141.0			*obscura*	*affinis*	*affinis*	−	F	−	+
BG 14012-0151.0					*algonquin*	−	F	−	++
BG 14012-0171.0					*azteca*	−	+	−	+
BG 14012-0181.0					*bifasciata*	+	++	+	++
BG 14012-0191.0					*narragansett*	+++	++++	+++	++++
BG 14012-0201.0					*tolteca*	−	F	−	+
BG 14011-0091.0				*obscura*	*ambigua*	−	F	−	+
BG 14011-0101.0					*miranda*	F	+	F	+
BG 14011-0111.0					*persimilis*	−	F	−	+
WH					*pseudoobscura*	−	F	−	+
BG 14011-0131.0					*subobscura*	−	F	−	+
BG 14024-0371.0			*melanogaster*	*ananassae*	*ananassae*	−	−	−	−
BG 14024-0381.0					*bipectinata*	−	F	−	F
BG 14024-0391.0					*malerkotliana*	−	F	−	F
BG 14024-0401.0					*parabipectinata*	−	F	−	F
BG 14024-0421.0					*pseudoananassae*	−	F	−	F
BG 14024-0431.0					*varians*	−	−	−	−
BG 14027-0461.0				*elegans*	*elegans*	−	−	−	−
BG 14026-0451.0				*eugracilis*	*eugracilis*	−	−	−	−
BG 14025-0441.0				*ficusphila*	*ficusphila*	F	+	F	+
BG 14021-0241.0				*melanogaster*	*mauritiana*	−	−	−	−
Various					*melanogaster*[d]	++++/−	++++/−	++++/−	++++/−
JC					*sechellia*			−	−
Various					*simulans*	−	−	−	−
BG 14021-0261.0					*yakuba*	−	−	−	−
BG 14028-0471.1				*montium*	*auraria*	F	++	++	+++
BG 14028-0481.0					*baimaii*	−	F	−	F
BG 14028-0491.0					*barbarae*	−	−	−	−
BG 14028-0501.0					*biauraria*	+	+	++	++++
BG 14028-0511.0					*bicornuta*	−	−	−	−
BG 14028-0521.0					*birchii*	−	−	−	−
BG 14028-0586.0					*diplacantha*	−	F	−	F
BG 14028-0531.0					*jambulina*	−	−	F	+
BG 14028-0541.0					*kanapiae*	−	F	−	F
BG 14028-0551.0					*khaoyana*	−	+	−	+

Table 1—Continued

Source[a]	Genus	Subgenus	Species group	Subgroup	Species	Probe[b] X/S(42°)	X/S(37°)	BWC(42°)	BWC(37°)
BG 14028-0561.0					*kikkawai*	–	F	–	F
BG 14028-0571.0					*lacteicornis*	+	++	++++	++++
BG 14028-0581.0					*lini*	–	F	–	F
BG 14028-0591.0					*mayri*	–	F	–	F
BG 14028-0601.0					*nikananu*	–	+	–	+
BG 14028-0611.0					*orosa*	–	F	–	F
BG 14028-0621.0					*parvula*	–	F	–	F
BG 14028-0631.0					*pennae*	–	F	–	F
BG 14028-0641.0					*punjabiensis*	F	F	F	F
BG 14028-0651.0					*quadraria*	F	+	++	++
BG 14028-0661.0					*rufa*	+	+	+++	+++
BG 14028-0671.0					*seguyi*	–	–	–	–
BG 14028-0681.0					*serrata*	–	F	–	F
BG 14028-0691.0					*triauraria*	+	++	++	++
BG 14028-0701.0					*tsacasi*	F	+	+	+
BG 14028-0711.0					*vulcana*	F	+	+	+
BG 14023-0331.0				*suzukii*	*lucipennis*	–	+	–	+
BG 14023-0341.0					*mimetica*	–	+	–	+
BG 14023-0351.0					*pulchrella*	–	+	–	+
BG 14023-0361.0					*rajasekari*	–	–	–	–
BG 14022-0271.0				*takahashii*	*lutescens*	–	F	–	F
BG 14022-0281.0					*paralutea*	–	–	–	–
BG 14022-0291.0					*prostipennis*	–	F	–	F
BG 14022-0301.1					*pseudotakahashii*	–	–	–	–
BG 14022-0311.0					*takahashii*	–	–	–	–
BG 14022-0321.0					*trilutea*	–	F	–	F
BG 15120-1911.0		*Drosophila*	*funebris*		*funebris*				–
BG 15120-1921.0					*macrospina limpiensis*				–
BG 15120-1931.2					*macrospina macrospina*				–
BG 15120-1941.0					*multispina*				–
BG 15120-1951.1					*subfunebris*				–
BG 15040-1171.1			*annulimana*		*aracatacas*	–			–
BG 15040-1181.0					*gibberosa*	–			–
BG 15040-1191.0					*talamancana*	–			–
WH			*melanica*		*melanica*				–
BG 15100-1711.0			*polychaeta*		*polychaeta*				–
BG 15020-1111.1			*robusta*		*robusta*				–
BG 15250-1451.0			*tumiditarsus*		*repletoides*				–
LE			*virilis*		*virilis*				–
BG 15050-1201.0			*canalinea*		*canalinea*	–			–
BG 15060-1221.2			*dreyfusi*		*camargoi*	–			–
BG 15070-1231.3					*gaucha*	–			–
BG 15070-1241.0			*mesophragmatica*		*pavani*	–			–
BG 15083-1551.0			*repleta*	*fasciola*	*ellisoni*	–			
BG 15085-1641.4				*hydei*	*hydei*	–			–
BG 15082-1511.0				*mercatorum*	*mercatorum*	–			–
WH				*mulleri*	*arizonensis*				–
WH					*mojavensis*				–
WH					*mulleri*				–
WH					*peninsularis*				–
BG 15081-1431.1					*serido*	–			–
BG 15270-2461.0			*histrio*		*sternopleuralis*				–
BG 15115-1871.0			*immigrans*	*hypocausta*	*hypocausta*				–
BG 15115-1881.0					*neohypocausta*				–
BG 15115-1891.0					*pararubida*				–
BG 15115-1901.0					*rubida*				–
BG 15111-1721.0				*immigrans*	*formosana*				–
BG 15111-1731.0					*immigrans*				–
BG 15111-1741.0					*signata*				–
BG 15114-1861.0				*lineosa*	*lineosa*				–
BG 15112-1751.0				*nasuta*	*albomicans*				–
BG 15112-1761.0					*kepulauana*				–

Source[a]	Genus	Subgenus	Species group	Subgroup	Species	Probe[b] X/S(42°)	X/S(37°)	BWC(42°)	BWC(37°)
BG 15112-1771.0					*kohkoa*				−
BG 15112-1781.0					*nasuta*				−
BG 15112-1801.0					*pulaua*				−
BG 15112-1811.0					*sulfurigaster albostrigata*				−
BG 15112-1821.0					*sulfurigaster bilimbata*				−
BG 15112-1831.0					*sulfurigaster sulfurigaster*				−
BG 15112-1841.0					*tongpua*				−
BG 15181-2181.9			*cardini*	*cardini*	*cardini*				−
BG 15181-2201.0					*neocardini*	−			−
BG 15182-2291.0				*dunni*	*dunni dunni*				−
BG 15171-2141.2			*guarani*	*guaramunu*	*guaramunu*				−
BG 15210-2331.0			*pallidipennis*		*pallidipennis pallidipennis*	−			−
BG 15220-2411.2			*tripunctata*		*unipunctata*	−			−
BG 15291-2551.0			*mod.mth.parts*	*hystricosa*	*biseriata*				−
BG 15287-2541.0			*picture-winged*	*grimshawi*	*grimshawi*				−
BG 15284-1501.0				*hawaiiensis*	*gymnobasis*				−
BG 15285-2521.0				*pilimana*	*aglaia*				−
BG 13000-0081.0		*Dorsilopha*			*busckii*			−	F
BG 20000-2621.0	*Chymomyza*				*amoena*				−
BG 20000-2631.1					*procnemis*				−
BG 60000-2751.0	*Liodrosophila*				*aerea*				F
BG 80000-2761.0	*Samoaia*				*leonensis*				−
BG 31000-2641.0	*Scaptomyza*	*Parascaptomyza*			*adusta*				+
BG 31000-2651.0					*elmoi*				+++
BG 50000-2746.0	*Zaprionus*				*inermis*				−
BG 50000-2761.0					*tuberculatus*				−

All genomic blots were analyzed by scanning densitometry (see MATERIALS AND METHODS). A plus (+) indicates that hybridization was observed; a minus (−) indicates that no hybridization was seen at the stringency employed. The number of pluses indicates the relative strength of the hybridization signal. An F indicates that one or more bands were faintly discernible on the blot but that the strength of the signal was not significantly above the negative control baseline level.

[a] Letter designations are defined in MATERIALS AND METHODS and denote the laboratories from which the stocks were obtained. The sources for the *willistoni* and *saltans* group species are given in DANIELS and STRAUSBAUGH (1986).

[b] X/S = 1.7-kb *Xho*I/*Sal*I internal fragment from pπ25.1; BWC = pπ25.7BWC (see MATERIALS AND METHODS). The hybridization and wash temperatures are in parentheses.

[c] More than one isolate was tested for many of the species in the *willistoni* and *saltans* groups; the number tested is in parentheses.

[d] *P* element-free *D. melanogaster* strains were last isolated from the wild in the early 1970s, since then all wild-derived strains have contained multiple *P* element copies (ANXOLABÉHÈRE, KIDWELL and PÉRIQUET 1988).

discernible or only weak hybridization when the internal *P* probe was used at the higher stringency. Signal intensity usually increased when either the lower stringency or the BWC probe was employed. Sometimes the changes seen under the different hybridization conditions were quite striking, as is demonstrated in Figure 4.

3. Of the five other genera surveyed, two, *Liodrosophila* and *Scaptomyza*, showed evidence of *P* element hybridization. The single *Liodrosophila* species tested, *L. aerea*, exhibited very weak signals to the BWC probe. Significantly stronger signals were observed in the two *Scaptomyza* species examined, with one, *S. elmoi*, displaying relatively strong *P* hybridization. *P* homologues have also been reported in another *Scaptomyza* species, *S. pallida* (ANXOLABÉHÈRE, NOUAUD and PÉRIQUET 1985).

Identifying potential donor species in the horizontal transfer of *P* elements to *D. melanogaster*: Under the simplest set of assumptions, the donor species in a recent transfer of *P* elements to *D. melanogaster* must fulfill one basic criterion: it must possess at least one complete *P* element with virtually the same base sequence, and hence restriction map, as the *D. melanogaster* canonical *P*. When a complete *P* element is digested with *Pvu*II, the restriction enzyme employed in our survey, it produces an internally derived, 0.9-kb fragment. This fragment shares approximately 750 bases with the *Xho*I/*Sal*I *P* fragment and produces a strong hybridization signal when the latter is used as probe. All candidates for the donor species, therefore, should show the 0.9-kb band when probed with the *Xho*I/*Sal*I fragment, even under conditions of high stringency. (It should be noted that defective elements with deletions outside the *Pvu*II/*Pvu*II interval will also produce the 0.9-kb band.)

Of all the species tested, only five, *D. equinoxialis*, *D. paulistorum*, *D. pavlovskiana*, *D. willistoni* and *D.*

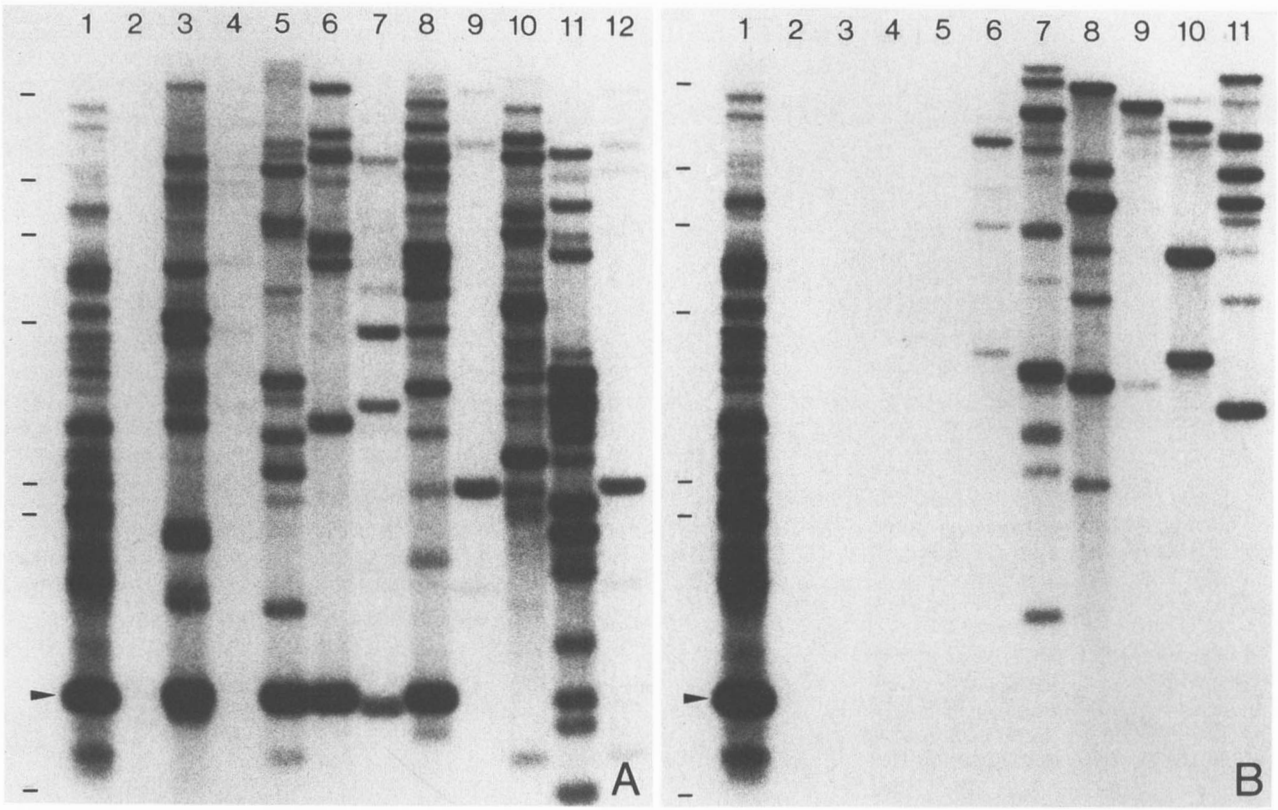

FIGURE 3.—Southern blots of the *willistoni* and *saltans* species groups. *A* and *B*, Blots were hybridized and washed at 42°; BWC was used as probe. The first two lanes in each panel represent positive and negative controls from, respectively, Harwich and Canton S, both strains of *D. melanogaster*. The source of each stock is given in parentheses; abbreviations are defined in the MATERIALS AND METHODS. An arrow indicates the position of the 0.9-kb *Pvu*II fragment. The approximate positions of the fragments generated from a *Hin*dIII digest of lambda DNA are indicated on the left. *A*, The *willistoni* species group. Samples are as follows: (3) *D. equinoxialis* (BG14030-0741.1); (4) *D. insularis* (FA); (5) *D. paulistorum* (C-2 isolate from LE); (6) *D. pavlovskiana* (FA); (7) *D. tropicalis* (BG14030-0801.0); (8) *D. willistoni* ("willi #7"); (9) *D. capricorni* (BG14030-0721.1); (10) *D. fumipennis* (BG14030-0751.1); (11) *D. nebulosa* (MS); and (12) *D. sucinea* (BG14030-0791.1). Note that *P* signals in the *D. insularis* sample (lane 4) are only weakly visible at this exposure. *B*, The *saltans* species group. Samples are as follows: (3) *D. neocordata* (BG14041-0831.0); (4) *D. emarginata* (BG14042-0841.0); (5) *D. neoelliptica* (BG14042-0851.0); (6) *D. milleri* (BG14043-0861.0); (7) *D. sturtevanti* (BG14043-0871.0); (8) *D. austrosaltans* (BG14045-0881.0); (9) *D. lusaltans* (BG14045-0891.0); (10) *D. prosaltans* (BG14045-0901.0); and (11) *D. saltans* (BG14045-0911.0).

nebulosa, possessed the diagnostic *Pvu*II fragment (see Figure 3). All of these reside within the *willistoni* species group; all but *D. nebulosa* reside within the *willistoni* subgroup (see Table 1). The field of candidates was further narrowed by focusing on those that have ranges that extend into the southern portion of North America, where the earliest *P*-activity was detected in *D. melanogaster* (ANXOLABÉHÈRE, KIDWELL and PÉRIQUET 1988). Of the five candidates, only *D. willistoni* and *D. nebulosa* have ranges that overlap this region. A more extensive screen of these two species, which included seven *D. nebulosa* and 12 *D. willistoni* lines, representing both recently caught and older laboratory strains, was subsequently undertaken (DANIELS and STRAUSBAUGH 1986). Although all of the lines contained the 0.9-kb *Pvu*II fragment, its intensity was always stronger in *D. willistoni*. Moreover, *P* element hybridization patterns were usually more varied in *D. willistoni* than in *D. nebulosa*, even among lines collected from the same locale (see Figure 6 in DANIELS and STRAUSBAUGH 1986), indicating the possible presence of active elements. Based on these considerations, *D. willistoni* was chosen for the comprehensive analysis described below. Intact, active *P* elements were not found in the one strain of *D. nebulosa* examined by LANSMAN *et al.* (1987).

Molecular characterization of the *P* elements of a *D. willistoni* strain: The strain of *D. willistoni* selected for molecular analysis has been given the designation, "willi #7" (see MATERIALS AND METHODS). This line has been inbred for approximately five years. The analysis consisted of estimating the total number of *P* elements and determining whether any were intact and transcriptionally active.

An estimate of the total number of elements in "willi #7" was obtained by Southern blot analysis. Genomic DNA samples were simultaneously digested with *Bam*HI and *Bgl*II, restriction enzymes that lack sites in the *P* element. Such a digest yields a single band for each hemizygous and homozygous element

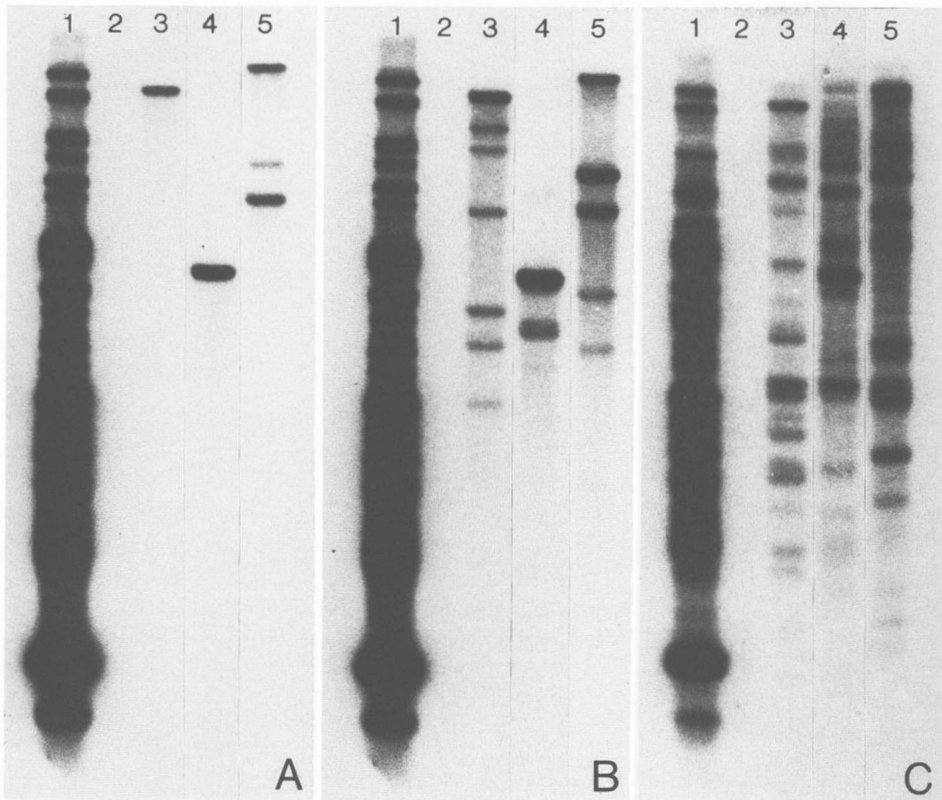

FIGURE 4.—The influence of hybridization conditions on *P* element patterns. Within the *obscura* and *melanogaster* groups, *P*-bearing species often showed marked differences in the degree of hybridization depending on probe and hybridization conditions. *A–C*, Hybridization intensities exhibited by three species from the *melanogaster* group under three different sets of conditions. In each panel, the first two lanes contain positive (Harwich) and negative (Canton S) control samples. Lanes 3–5 contain, respectively, DNA samples from *D. auraria*, *D. biauraria* and *D. lacteicornis*. The amount of DNA in the Harwich lane is only 1/5 that of the other samples. The same filter was used in (*A*) and (*B*); a different one was used in (*C*). *A*, Hybridization and subsequent washes performed at 42°; the internal *Xho*I/*Sal*I fragment was used as probe. *B*, Hybridization and washes performed at 37°, using the same probe as in (*A*). *C*, Hybridization and washes performed at 42°, using the BWC plasmid as probe.

in the genome. BWC was used as probe. DNA samples from males and females were analyzed separately. By this method, it was estimated that "willi #7" females have as many as 12, and "willi #7" males as many as 13, prominent *P* elements per haploid DNA complement (Figure 5A), although other, much weaker signals were also noted upon longer exposure. The additional signal in males suggests that there is a *P* on the *Y* chromosome in this line. *In situ* hybridization of biotinylated-BWC DNA to larval salivary gland chromosomes showed sites of hybridization in pericentric and euchromatic regions (data not shown).

By comparing male and female DNA, it is also possible to estimate the number of X-linked sites, since bands representing them are roughly twice as intense in female DNA as they are in male DNA. The number of *X*-linked sites in "willi #7" was determined by subjecting autoradiograms of male and female DNA to scanning densitometry. The results revealed that five of the 12 prominent bands in this line are *X*-linked (see Figure 5A).

An assessment of the number of potentially complete elements was made by an analysis similar to the one described by DANIELS *et al.* (1987b). Samples containing equal amounts of genomic DNA from $\Delta 2\text{-}3(99B)/ry^{506}$, $\Delta 2\text{-}3(99B)/\Delta 2\text{-}3(99B)$, "willi #7" males and "willi #7" females were digested with *Dde*I. Complete elements produce a large, 2.2-kb internal fragment when digested with this enzyme; the $P[ry^{+}\ \Delta 2\text{-}3]$ transposon yields a fragment that is about 200 bp smaller because of the deleted third intron (see MATERIALS AND METHODS). Dilutions of digested DNA from the "willi #7" samples were then compared against the $\Delta 2\text{-}3(99B)$ reference samples. The $\Delta 2\text{-}3(99B)$ line contains two tandem *P* element transposons (H. ROBERTSON, personal communication), with hemizygous [$(\Delta 2\text{-}3(99B)/ry^{506})$] and homozygous [$\Delta 2\text{-}3(99B)/\Delta 2\text{-}3(99B)$] lines containing, respectively, two and four *P* transposons per diploid DNA complement. The approximate number of complete elements is determined by selecting the 2.2-kb band in the dilution series that corresponds in intensity to the 2.0-kb band in the $\Delta 2\text{-}3(99B)$ controls. From this analysis, we estimated that the "willi #7" line has two potentially complete elements per haploid DNA complement (Figure 5B).

A Northern analysis of polyadenylated RNA from "willi #7" revealed a *P* transcript of 2.5 kb (Figure 6). This is the size of the transcript produced by an autonomous *P* element.

Isolating a full-sized *P* element from *D. willistoni*: A lambda library of genomic DNA from "willi #7" adults was prepared and screened for *P*-hybridizing plaques by one of us (L.S.) as described in the MATERIALS AND METHODS. Positive clones were further screened by restriction analysis for those containing potentially complete elements. One such clone, P_{13}, was identified. A 5.8-kb *Xba*I fragment containing the

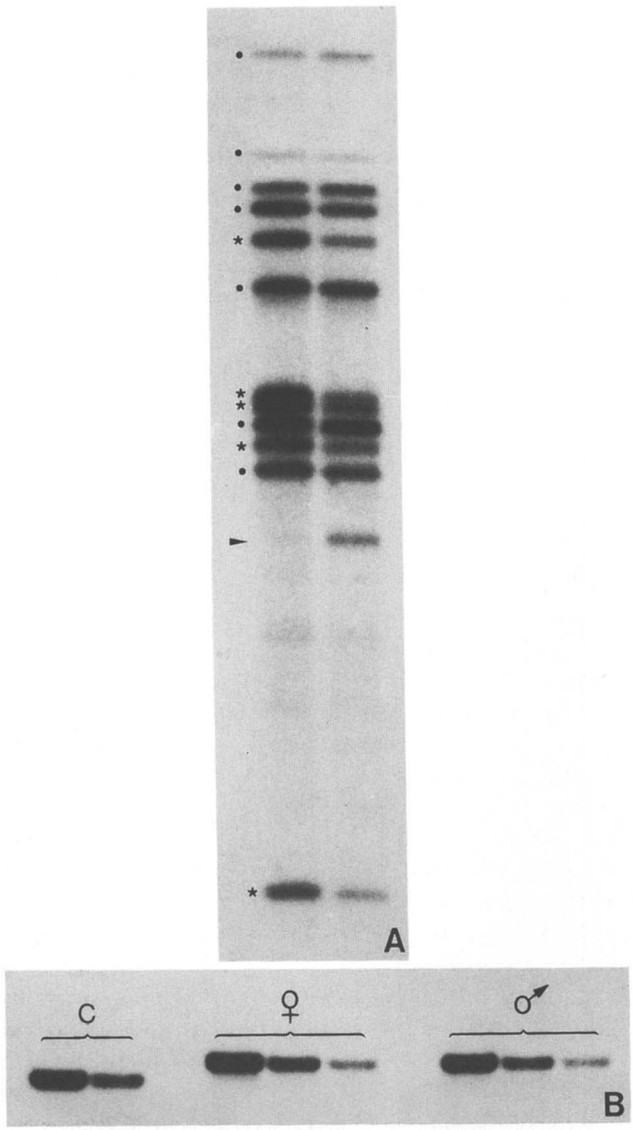

FIGURE 5.—Analysis of the "willi #7" *P* element complement. *A*, Estimate of the total number of elements per genome. Genomic DNA was digested with both *Bam*HI and *Bgl*II, enzymes that do not cut within the *P* element, and probed with BWC. The lane on the left contains DNA extracted from "willi #7" females; the lane on the right contains DNA from "willi #7" males. Each band represents a single element. It is estimated that females and males have, respectively, 12 and 13 elements per haploid DNA complement. Autosomal sites are indicated by dots, X-linked sites by stars. The male-specific site is indicated by an arrow. *B*, Estimate of the number of potentially complete elements in the "willi #7" genome. Genomic DNA was digested with *Dde*I, which produces an internal 2.2-kb fragment from a complete *P* element and a 2.0-kb fragment from the *Δ2-3(99B)* transposon (see MATERIALS AND METHODS). The BWC plasmid was used as probe. The two lanes on the left (*C*) contain equal amounts of DNA from the two reference samples, *Δ2-3(99B)/Δ2-3(99B)* and *Δ2-3(99B)/ry*^{506}, respectively. The former contains four copies, and the latter two copies, of the *P*[*ry*$^{+}$ *Δ2-3*] transposon per diploid DNA complement. The reference lanes are followed by two groups, each composed of three lanes. The groups represent dilution series of DNA from "willi #7" females and "willi #7" males. The first lane in each group contains the same amount of DNA as the reference lanes (*C*); the next two lanes contain, respectively, one-half and one-fourth the amount of DNA. Only the relevant *Dde*I fragment is shown in each lane. By comparing the dilution series to the reference samples, we estimate that males and females of the "willi #7" line contain two potentially full-sized *P* elements per haploid DNA complement.

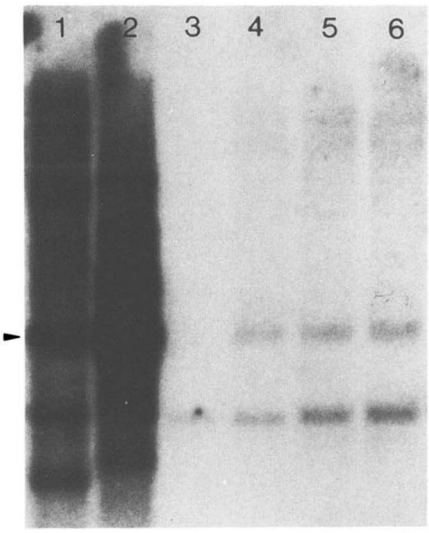

FIGURE 6.—Northern analysis of *P* element transcripts from "willi #7." Polyadenylated RNA was isolated, size fractionated in a 1% agarose-formaldehyde gel and hybridized with a ^{32}P-labeled BWC probe as described in the MATERIALS AND METHODS. Lanes 1–3 contain control samples from, respectively, π_2, Harwich and Canton S; each lane contains approximately 2 μg of poly(A^{+}) RNA. Lanes 4–6 contain, respectively, 2, 4 and 6 μg of poly(A^{+}) RNA from "willi #7." The π_2 and Harwich P strains display prominent 2.5-kb *P* element transcripts (*arrow*), which is the predominant message produced by autonomous elements. No *P* element transcripts are seen in the Canton S negative control. The "willi #7" line exhibits a single *P* element transcript of 2.5 kb, which is much less abundant than in the strong P strains of *D. melanogaster*. The low molecular weight band seen in every lane is presumed to be an artifact, perhaps resulting from nonspecific hybridization to the abundant 9S globin message, with which it comigrates.

entire element and some flanking DNA was isolated from P_{13} and subcloned into the pEMBL18 plasmid vector to make pDwP13-X30 (see MATERIALS AND METHODS), a restriction map of which is shown in Figure 1.

To verify that this element was derived from the "willi #7" genome, we performed a Southern blot analysis to determine whether genomic digests of "willi #7" DNA produce fragments of the size predicted from the pDwP13-X30 restriction map. The strategy is outlined in Figure 7A and involves restriction fragments that span both *P* element and flanking DNA sequences. The pDwP13F-XE4 plasmid, which contains the non-*P* DNA segment indicated in Figure 7A, was used as probe. Eight different digests were performed. In every case, a fragment of the expected size was generated from "willi #7" genomic DNA (Figure 7B), thus confirming that the cloned DNA fragment was derived from this line.

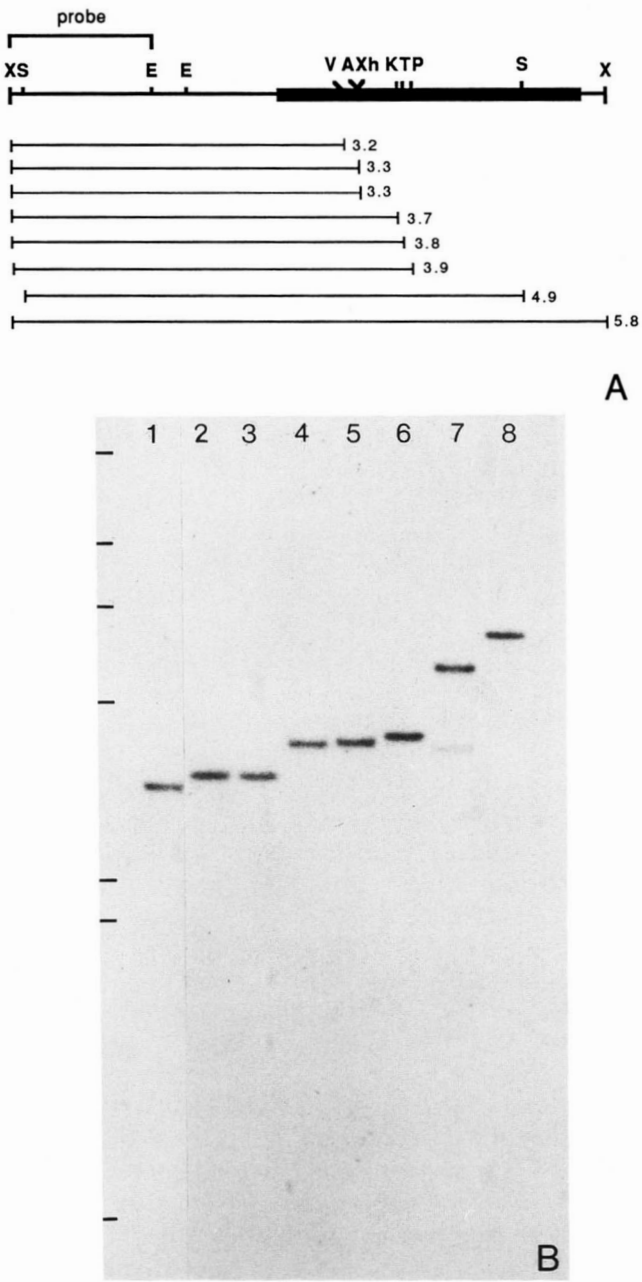

FIGURE 7.—Confirmation that the cloned *P* element is derived from the *D. willistoni* line, "willi #7." *A*, Partial restriction map of pDwP13-X30. This plasmid contains a full-sized *P* element (thick bar) flanked on both sides by genomic sequences (thin bars). The 5.8-kb genomic fragment was cloned into the *Xba*I site found in the pEMBL18 polylinker. Restriction site designations are given in the legend to Figure 1. The fragments expected when this plasmid is digested as described in (*B*) are shown below the map. The size of each (in kb) is given on the right. *B*, Southern blot of eight different restriction digests of "willi #7" genomic DNA. The pDwP13F-XE4 plasmid, which contains the piece of non-*P* DNA shown above the map in (*A*), was used as probe. DNA was digested as follows: (1) *Xba*I and *Pvu*II; (2) *Xba*I and *Ava*I; (3) *Xba*I and *Xho*I; (4) *Xba*I and *Kpn*I; (5) *Xba*I and *Sst*I; (6) *Xba*I and *Pst*I; (7) *Sal*I; and (8) *Xba*I. The second, weaker band in the *Sal*I digest (lane 7) occurs because the probe DNA overlaps the genomic fragment to the left of the *Sal*I fragment depicted in (*A*). The approximate positions of the fragments generated from a *Hin*dIII digest of lambda DNA are indicated on the left.

The *D. willistoni P* element and the *D. melanogaster* canonical *P* differ by only a single base: The base sequence of the *P* element in pDwP13-X30 was determined by one of us (K.P.) as described in the MATERIALS AND METHODS. The *D. willistoni P* element was found to be identical to the complete (and autonomous) *D. melanogaster P* element described by O'HARE and RUBIN (1983), with the single exception of an A to G transition at position 32. As has been found in *D. melanogaster*, there was an 8-bp duplication of genomic DNA (in this case GCACAACT) at the site of insertion.

The single base difference in the cloned *D. willistoni P* element results in the obliteration of a *Taq*I recognition site. This site is absent in all of the "willi #7" *P* elements that could be monitored by the method described in Figure 8A. The *Taq*I site was also found to be missing in the *P* elements of other members of the *willistoni* subgroup (Figure 8B), suggesting that the guanine base at position 32 may be common or ubiquitous in *P* elements of this taxon.

The *D. willistoni* element can promote the transposition of other *P* element transposons: The Northern and sequencing data together provide strong circumstantial evidence that the *D. willistoni* element is capable of functioning as an autonomous *P* element, since there is no existing evidence to suggest that activity is affected by a base change at position 32. To confirm its ability to function, we have used the *D. willistoni* element as a "helper" plasmid in a transformation experiment to see whether it is capable of promoting *P*-element-mediated gene transfer. The pry^{+4} plasmid (500 μg/ml), which carries a *ry*$^{+}$ transposon, was microinjected into the polarplasm of *y sn*^{w}; *ry*^{506} embryos, along with the pDwP13-X30 "helper" (150 μg/ml), by the procedure described in the MATERIALS AND METHODS. Individual G_0 male and female survivors were mated to, respectively, *C(1)DX, y f*; *ry*^{506} females and *ry*^{506} males. All G_1 progeny were screened for the presence of ry^{+} exceptions; G_1 males were also checked for mutations of the *singed-weak* allele, which is known to contain two defective *P* elements in tandem (NITASAKA and YAMAZAKI 1988; ROIHA, RUBIN and O'HARE 1988). Excision of one or the other of these elements produces two phenotypically distinguishable classes, sn$^{(+)}$ and sn^{e} (ENGELS 1979; ROIHA, RUBIN and O'HARE 1988). Of the 35 fertile G_0 adults, eight (23%) had genetically transformed ry^{+} individuals among their progeny, and three (9%) had sn$^{(+)}$ or sn^{e} exceptional progeny. All exceptions were true-breeding. In a separate experiment, only pDwP13-X30 plasmid DNA (150 μg/ml) was injected into *y sn*^{w}; *ry*^{506} embryos, and G_0 survivors were mated as above. G_1 males were screened for evidence of *sn*^{w} mutability. Of the eight fertile G_0 survivors, two (25%) produced progeny with exceptional singed phenotypes. These experiments dem-

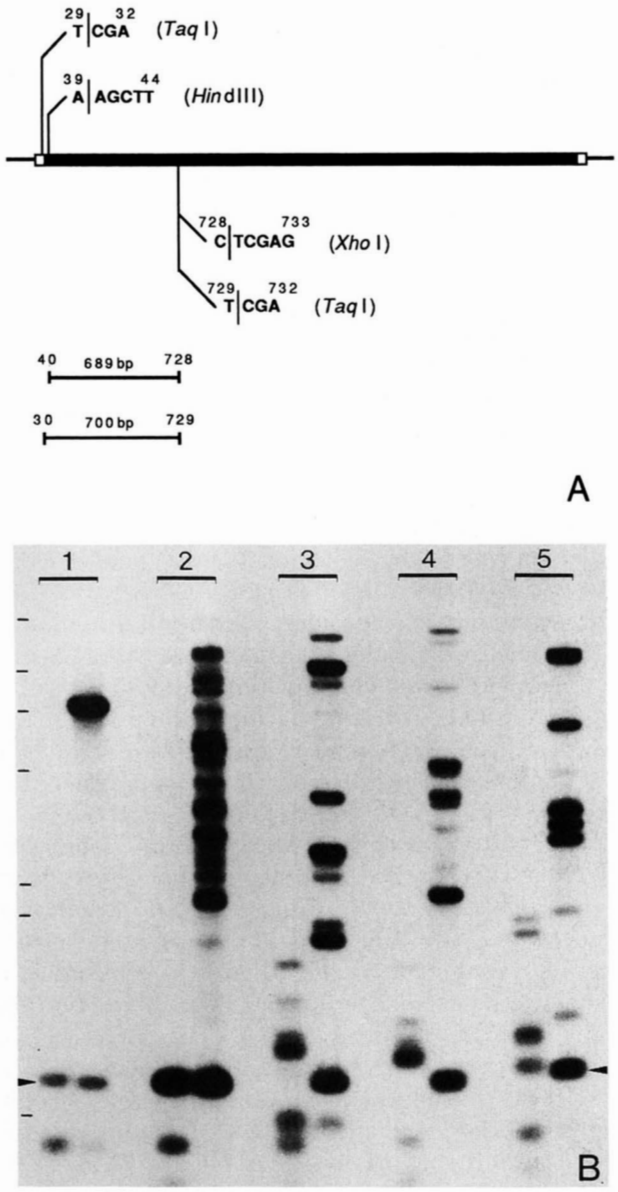

FIGURE 8.—The base difference at position 32 may be ubiquitous in *P* elements from the *willistoni* subgroup. *A*, Diagram of the *D. melanogaster P* element, showing the restriction sites pertinent to this analysis. The *Taq*I recognition sequence is a subset of the *Xho*I sequence. In *P* elements from *D. melanogaster*, the *Hin*dIII/*Xho*I and *Taq*I/*Taq*I fragments are nearly identical, spanning essentially the same piece of DNA and differing in size by only 11 bases. When the 0.7-kb *Hin*dIII/*Xho*I fragment is present, it confirms the integrity of both the DNA segment and the constituent *Taq*I recognition sequence at the *Xho*I site. If the *Taq*I recognition sequence between bases 29 and 32 is intact in all elements that yield a *Hin*dIII/*Xho*I fragment, then a *Taq*I digest should produce a fragment of about the same size and of equal intensity. *B*, Southern blot analysis of the *Taq*I polymorphism. Five pairs of lanes are shown. In each pair, the lane on the left contains DNA digested with *Taq*I, and the lane on the right contains the same DNA digested with *Hin*dIII and *Xho*I. BWC was used as probe. Samples are as follows: (1) *Δ2-3(99B)*; (2) Harwich; (3) *D. willistoni* ("willi #7"); (4) *D. equinoxialis* (BG14030-0741.1); and (5) *D. paulistorum* (C-2 strain). The arrow indicates the position of the 698-bp *Hin*dIII/*Xho*I *P* element fragment. The approximate positions of the fragments generated from a *Hin*dIII digest of lambda DNA are indicated on the left. In *D. melanogaster* (lanes 1 and 2), the two different digests produce comigrating, 0.7-kb bands that are of equal intensity, indicating that the *Taq*I site spanning base 32 is intact in most or all of the elements with intact *Hin*dIII/*Xho*I segments. On the other hand, *P* elements of three *willistoni* subgroup species produce a prominent, 0.7-kb *Hin*dIII/*Xho*I band, but lack the corresponding *Taq*I/*Taq*I band. Since a functional *Xho*I site verifies the integrity of the constituent *Taq*I recognition sequences at its 3′ end, the absence of the *Taq*I/*Taq*I band in *willistoni* subgroup species must be the result of an alteration within the *Taq*I sequence that spans bases 29 to 32 at its 5′ end.

onstrate that the *D. willistoni P* element is a functional element capable of providing the *trans*-acting product necessary for the mobilization of nonautonomous *P* element transposons.

DISCUSSION

When did *P* elements enter the genus *Drosophila*? The genus *Drosophila* arose during the Eocene age from one of several early radiations within the family Drosophilidae. It subsequently underwent extensive diversification in the form of five major radiations, the earliest of which gave rise to the subgenus *Scaptodrosophila*. This was followed by the *Sophophora* radiation, which produced the *melanogaster*, *obscura*, and closely related *willistoni* and *saltans* lineages. The complex *Drosophila* radiation ensued. This radiation is composed of three main branches, represented by the *virilis-repleta*, *immigrans* and *Hirtodrosophila* radiations [see THROCKMORTON (1975) for further details].

To identify potential donor species in the horizontal transfer of *P* elements to *D. melanogaster*, we first undertook an extensive survey of the distribution of *P*-homologous sequences within the genus *Drosophila*. Five other closely-related genera were also sampled, although much less extensively. The results showed that *P* sequences are essentially confined to the subgenus *Sophophora*, although weak hybridization was also observed in a few species from the subgenus *Scaptodrosophila* and in *D. buskii*, the only species in the subgenus *Dorsilopha*. No *P* element hybridization was detected in species from the subgenus *Drosophila*. Relatively weak *P*-hybridizing sequences were found in species from the genera *Liodrosophila* and *Scaptomyza*. In other surveys, *P* homologues have been reported in *D. phalerata* and *D. kuntzei*, both assigned to the *quinaria* species group of the subgenus *Drosophila* (ANXOLABÉHÈRE, NOUAUD and PÉRIQUET 1987), and, interestingly, in two species from the nondrosophilid families, Trixoscelididae and Opomyzidae (ANXOLABÉHÈRE and PÉRIQUET 1987). The occurrence of *P*-hybridizing sequences in species from the subgenus *Scaptodrosophila*, one of the earliest *Drosophila* lineages, suggests that *P* elements may have been present at the time of the inception of the genus about 60 million years ago.

The evolutionary history of *P* elements in *Drosophila*: To fully understand the evolutionary history of a particular transposable element within a phylogenetic lineage, it is necessary to determine its initial point of entry, its subsequent distribution and its mode of transmission between species (STACEY *et al.* 1986). If transmission has been strictly vertical (*i.e.*, mating-dependent), then descendants of an ancestral species bearing the element should also possess homologues of the element. If during evolution the element has been lost from a species, then all of its descendants should be element-free. This mode of transmission results in distribution patterns that are virtually continuous. Alternatively, if transmission has occurred horizontally between reproductively isolated species, then distribution patterns may not follow phylogenetic groupings, *i.e.*, they may be discontinuous. The fact that vertical and horizontal modes of transmission are not mutually exclusive makes it difficult to decipher the exact chain of events leading to the present day distribution of *P* sequences within *Drosophila*, since very complex patterns can result from the combination of occasional horizontal transfer and subsequent vertical transmission. Moreover, the analysis can be even further complicated by the possibility that *P* elements may have been introduced into some lineages at more than one point during their evolution.

Previous studies have uncovered two major discontinuities in the distribution of *P*-homologous sequences in the genus. With the exception of *D. melanogaster*, *P* sequences were not found in species from the *melanogaster* subgroup (BROOKFIELD, MONTGOMERY and LANGLEY 1984), nor were they found in the more primitive subgroups of the *saltans* species group (DANIELS and STRAUSBAUGH 1986). The present study confirms the results of these earlier reports. No other major discontinuities were uncovered during the course of our survey. [In an earlier study, DANIELS and STRAUSBAUGH (1986) reported that *P* sequences were conspicuously absent from one (*D. insularis*) of the species of the *willistoni* group; in this study, weak *P* hybridization was detected under conditions of reduced stringency.]

There are two basic ways to account for the current distribution of *P*-homologous sequences:

1. If horizontal transmission is assumed to be very rare, then the most parsimonious interpretation of the data favors a single introduction of *P* elements into the genus, with homologs being transmitted vertically between species. Under this set of conditions, the discontinuities in distribution can only be explained by proposing that elements have been lost in a number of species or lineages during diversification or upon subsequent evolution. There is some evidence to suggest that elements can be lost over time. The patchy distribution of predominantly weak *P* homologues in the *melanogaster* species group probably represents a situation in which sequences are gradually drifting to extinction within the lineage. This may also be the case in the other lineages in which *P* hybridization is weak. A theoretical treatment of the evolution of a hypothetical transposable element has suggested that a drift to extinction can occur under certain conditions (KAPLAN, DARDEN and LANGLEY 1985).

2. Alternatively, it can be proposed that *P* elements may have entered *Drosophila* on more than one occasion and that certain features of the present distribution may be the result of horizontal transmission (*e.g.*, LANSMAN *et al.* 1985; STACEY *et al.* 1986). This form of acquisition would perhaps explain the isolated occurrences of *P* sequences within the subgenus *Drosophila* (ANXOLABÉHÈRE, NOUAUD and PÉRIQUET 1987) and in the nondrosophilid dipterans (ANXOLABÉHÈRE and PÉRIQUET 1987), as well as the conspicuous anomalies observed in the *willistoni* and *saltans* groups (Figure 3). All of the *willistoni* group species have prominent *P* sequences, with the exception of *D. insularis*, which has elements that more closely resemble the weak *P* homologues seen in the *melanogaster* and *obscura* groups. In the *saltans* species group, *P* sequences are absent in the more primitive subgroups, but are present in those that are more derived, the reverse of what might be predicted.

The following account of the evolutionary histories of the *willistoni* and *saltans* groups, taken from THROCKMORTON (1975), may provide an understanding of these anomalies and shed some light on the possible time and location of *P* element introductions into these lineages. During the Eocene age the primary tropical disjunction separated the New and Old World tropics. As a result, the common ancestor of the *willistoni-saltans* lineage become sequestered in tropical North America where the divergence of the two groups is believed to have occurred. Thereafter, it is proposed that an ancestor of the *willistoni* lineage invaded South America by crossing the water gap that then existed between the North and South American continents. The development and diversification of the *willistoni* lineage is thought to have taken place exclusively in South America. Today, the *willistoni* species group is divided into the *willistoni* and *bocainensis* subgroups. The more primitive *willistoni* subgroup is composed of a cluster of six sibling species, one of which is *D. insularis*, a narrow island endemic confined to the Lesser Antilles (DOBZHANSKY, EHRMAN and PAVLOVSKY 1957). Analysis of allelic variation shows that it is the most genetically differentiated of the six sibling species, suggesting that it may have been the earliest to diverge from the cluster (AYALA *et al.* 1974).

The *saltans* species group, which is composed of five subgroups, shows an orderly progression in evolution from the more primitive *cordata* and *elliptica* subgroups through the *sturtevanti* and *parasaltans*

subgroups to the *saltans* subgroup, which is considered to be the most derived. Following the *willistoni-saltans* divergence in tropical North America, the *saltans* lineage continued to diversify in this region, giving rise to the ancestors of the more primitive *cordata* and *elliptica* subgroups. However, at least once prior to the closing of the water gap by the Isthmus of Panama, it is proposed that a progenitor from North America crossed the water gap and colonized the South American continent, where further diversification occurred to produce the *sturtevanti*, *parasaltans* and *saltans* subgroups.

Based on the Southern blot data and the biogeographical inferences of THROCKMORTON (1975), we have formulated the following hypothetical scenario to account for the present day *P* distributions: *P* elements initially entered the genus early in its radiation and were transmitted vertically through one or more of the early *Scaptodrosophila* lineages. During the sophophoran radiation, they were passed to at least the *protomelanogaster* lineage, which later gave rise to the *melanogaster* and *obscura* groups. Whether *P* elements were transmitted to the ancestors of the *willistoni-saltans* lineage during this period is more problematic, but the presence of weak *P* homologues in *D. insularis*, similar to those of the *melanogaster* and *obscura* groups, suggests that *P* elements may have entered at least the *willistoni* lineage at this time. Whether they also entered the *saltans* lineage is more difficult to determine, since there is no evidence of their occurrence in the primitive subgroups as they exist today. It is possible that elements once existed in these subgroups, but subsequently have drifted to extinction, as is probably the case for many of the *melanogaster* group species that now completely lack *P*-hybridizing sequences. During this phase, *P* sequences may also have been transmitted vertically to a very limited number of lineages that arose during the *Drosophila* radiation. The remnants of the initial invasion are perhaps manifested today by the very weak *P* homologs seen in the *Scaptodrosophila* species, in the *melanogaster* and *obscura* groups, and in *D. insularis*.

At some point following both the establishment of the *saltans* lineage on the South American continent and the physical isolation of the *D. insularis* ancestor, a second invasion occurred, with *P* elements being introduced, or perhaps reintroduced, independently into the extant *willistoni* and *saltans* lineages then present on the South American continent. This introduction could have been mediated by an infectious process that occurred exclusively on the continent during this time. Such an event would explain the conspicuous absence of *P* homologs in the more primitive *saltans* subgroups, which at the time were geographically isolated in tropical North America, and the absence of prominent *P* sequences in *D. insularis*, the ancestor of which may have become separated from South America on an island mass destined to become the Lesser Antilles. This second introduction most likely would have taken place before the complete blossoming of sibling clusters and at a time prior to the formation of the Isthmus of Panama about 5 million years ago. The independent introduction into *D. melanogaster* probably occurred at some later time, following its divergence from *D. simulans*. We emphasize that the above scenario is hypothetical and does not rule out other plausible interpretations of the data.

Did *D. willistoni* serve as the donor species in the proposed horizontal transfer of *P* elements to *D. melanogaster*? The results of our survey showed that *D. melanogaster P* elements more closely resemble those of the *willistoni* species group, and, in particular, the *willistoni* subgroup, than those of the *melanogaster* and *obscura* species groups. Within the *willistoni* subgroup, four species were initially considered as potential donor species. From these, *D. willistoni* was singled out for further analysis because: (1) different isolates exhibit very different restriction patterns, a possible indication of active transposition; and (2) it is the only species in the subgroup whose range overlaps the geographic region where the earliest *P* element activity was detected in *D. melanogaster*.

Subsequently, an intact *P* element was cloned and sequenced from a *D. willistoni* iso-female line. It was found that this element differs from the *D. melanogaster* canonical *P* by only a single base, an A to G transition at position 32. This base substitution creates 32-bp terminal inverted repeats instead of the 31-bp repeats typical of *D. melanogaster* elements. Whether this difference has any influence on the transposability of the *D. willistoni P* element is not known. We further note that a guanine base was also found at position 32 in the two *P* elements cloned from *D. nebulosa* (LANSMAN *et al.* 1987) and in a defective *P* element, referred to as KP′, from *D. melanogaster* (ITOH and HORI 1988). This element is identical to the KP element described by BLACK *et al.* (1987), with the exception of the single base substitution. This raises the possibility that there may be a subset of intact *P* elements within *D. melanogaster* that are completely identical to the cloned *D. willistoni P* element.

Northern blot analysis of poly(A^+) RNA from the *D. willistoni* line from which the cloned *P* element was derived revealed a *P* transcript of the size expected from an autonomous element, suggesting the presence of at least one potentially active element. The functionality of this element was subsequently confirmed by the demonstrations of its ability to promote ry^+ transformation when co-injected with a ry^+ transposon and its ability to induce sn^w mutability.

The similarity of the *D. willistoni* element to the *D. melanogaster* canonical *P*, together with the proof of

its functionality, strongly suggests that *D. willistoni* may have served as the donor species in the interspecific transfer event, although other species from the *willistoni* subgroup, namely *D. equinoxialis*, *D. paulistorum*, *D. pavlovskiana* and even *D. tropicalis*, of which only a single isolate was tested, cannot be completely ruled out at this time. All have broad distributions within Central and South America, with the exception of *D. pavlovskiana*, which is a rare, narrow endemic (SPASSKY *et al.* 1971).

A comparison of *P* element dynamics in *D. melanogaster* and *D. willistoni*: Whether *P* elements behave in *D. willistoni* as they do in *D. melanogaster* is yet to be determined. There is accumulating evidence to suggest that certain host properties influence the expression of *P* elements (DANIELS *et al.* 1987a; O'BROCHTA and HANDLER 1988; RIO *et al.* 1988; RIO and RUBIN 1988). It is not yet known whether *D. willistoni* possesses the host functions that permit the phenotypic manifestations of P-M hybrid dysgenesis in *D. melanogaster*. If *P* elements are of relatively recent origin in *D. melanogaster*, then this species may not have had sufficient time to adapt to the effects of this element system. It seems possible, then, that the expression of hybrid dysgenesis in *D. melanogaster* may show interesting differences when compared to *D. willistoni*, which apparently has harbored active *P* elements for a longer period of time. Such a comparison may shed light on the question of whether hybrid dysgenesis is an early, transient phase of a *P* element invasion.

Certain differences have been noted between the two species. We have examined 12 lines of *D. willistoni*, representing older laboratory strains and recent isolates from the wild. *P* element copy number was found to be consistently lower in these lines than in most *P*-bearing strains of *D. melanogaster*. Additional lines collected from different locales have been screened by L. STRAUSBAUGH and J. POWELL (personal communication) with the same results. In the *D. willistoni* line from which the cloned element was derived, low copy number was accompanied by far fewer *P* element transcripts than are found in strong P strains of *D. melanogaster*. Limiting the number of elements within the genome could constitute one way of minimizing the deleterious phenotypic effects of hybrid dysgenesis at the level of the organism.

The recent-invasion hypothesis: The recent-invasion hypothesis, sometimes referred to as the rapid-invasion hypothesis, proposes that *P* transposable elements have spread rapidly through global populations of *D. melanogaster* within the last half century (KIDWELL 1979, 1983). This model was inspired by temporal and geographic surveys that showed that P strains were absent in collections made before 1950, but increasingly common in those made after that time (KIDWELL 1983). The hypothesis generally assumes that initial *P* element entry was by horizontal transmission.

It is important to note that the recent-invasion hypothesis does not directly address the issue of time of *P* element introduction into the *D. melanogaster* genome; it only addresses the time of *P* element spread through natural populations. KIDWELL (1986) has cautioned that "recent invasion does not necessarily imply recent origin." *P* elements may have been acquired at a time much prior to the global invasion, persisting at low frequencies in isolated subpopulations until very recently. The rapid spread of *P* elements within the last half century could have been triggered by man's recent global activities, which have produced drastic changes in *D. melanogaster* demography (KIDWELL 1986; ENGELS 1989). Support for such a scenario comes from simulation studies (UYENOYAMA 1985) that show that a transposable element, such as *P*, can exist within a population for a long period of time at very low frequencies, thereby avoiding detection by limited sampling. However, once the element has reached a certain critical frequency, it can quickly sweep to fixation. Under this set of conditions, *P* elements could have entered the *D. melanogaster* genome as long ago as the time immediately following the divergence from *D. simulans*, which is estimated to have occurred anywhere from 1 to 7 million years ago, depending on the method used to measure the rate of evolutionary separation (SCAVARDA and HARTL 1984). There are at least two pieces of evidence, however, that would suggest that *P* elements may have entered *D. melanogaster* more recently than this. First, all *D. melanogaster P* elements thus far examined show almost complete sequence homogeneity, even when elements from geographically diverse strains are compared (*e.g.*, O'HARE and RUBIN 1983; SAKOYAMA *et al.* 1985; BLACK *et al.* 1987). Second, the *D. willistoni* element is nearly identical to the *D. melanogaster* canonical *P* (this report). This observation is especially relevant if *D. willistoni* served as the donor in the postulated horizontal transfer event. If *P* elements have existed in *D. melanogaster* for a very long time (*i.e.*, millions of years), then we would expect more sequence divergence among its elements, as well as between them and the *D. willistoni P*. In fact, this is what was observed in a study of two *P* elements from *D. nebulosa* (LANSMAN *et al.* 1987), a *willistoni* group species that has presumably harbored elements for a relatively long period of time. These elements had diverged 10% from each other and 4–6% from the *D. melanogaster* canonical *P*.

Since we know almost nothing about the rates of sequence divergence for multicopy, mobile DNAs in *Drosophila*, it is impossible to pinpoint precisely the time of *P* element entry into *D. melanogaster*. All that may be said currently is that *P* elements have not been present in this species long enough to accumulate

appreciable sequence divergence. Based upon theoretical considerations of the rate at which new transposable elements are likely to originate within the *D. melanogaster* genome, ENGELS (1986, 1989) has argued that it is improbable that *P* elements have arisen in *D. melanogaster* within the last half century. These arguments are not in conflict with the present data. Whether *P* elements arose in *D. melanogaster* just prior to their worldwide spread during the last few decades or whether they were extant in the organism for some period of time before the global invasion is still a matter of conjecture.

The role of horizontal transmission in eukaryotic evolution: Cross-species gene transfer is well known among prokaryotes, especially in environments where selective pressures have been intensified (*e.g.*, by antibiotics). However, the extent to which such events occur in higher organisms remains unknown. Thus far, only a very few solid candidates for interspecific gene transfer involving eukaryotic species have been described (LEWIN 1982, 1985), although there is increasing speculation that this form of inheritance may play a more significant role in eukaryotic evolution than previously thought (*e.g.*, SYVANEN 1986). Of the possible mechanisms to shuttle DNA between species, viruses are perhaps considered the most promising candidates. It is known that certain insect viruses are able to incorporate foreign DNA into their genomes without affecting their ability to replicate or form infectious particles (FRASER 1986). A significant proportion of these DNA insertion events involve host transposable elements (FRASER 1986; MILLER and MILLER 1982). If the transfer of transposable elements between species should prove to be other than a rare event, then the case for the involvement of these genetic entities in eukaryotic evolution becomes even more compelling.

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